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

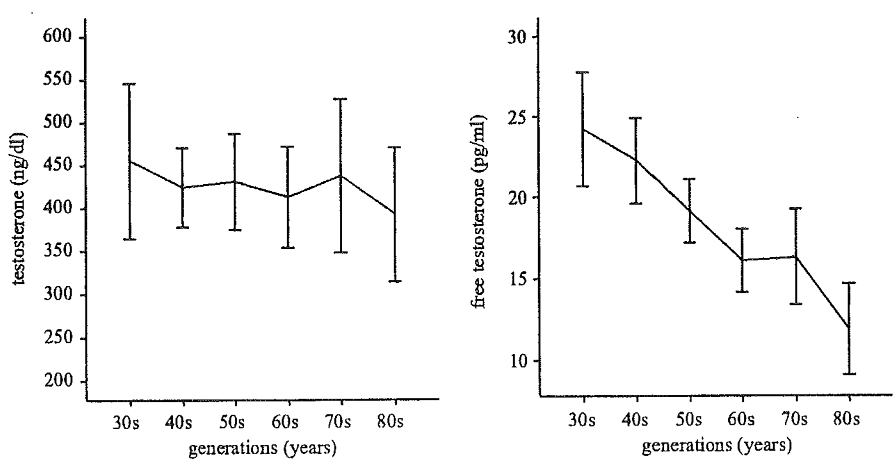

Figure 3 Mean plasma testosterone and free testosterone levels in males for each generation. Numbers of male subjects: 30s, $n = 19$; 40s, $n = 21$; 50s, $n = 21$; 60s, $n = 21$; 70s, $n = 21$; 80s, $n = 20$. Conversion factors: testosterone, nM = ng/dl × 0.03467; free testosterone, pM = pg/ml × 3.467.

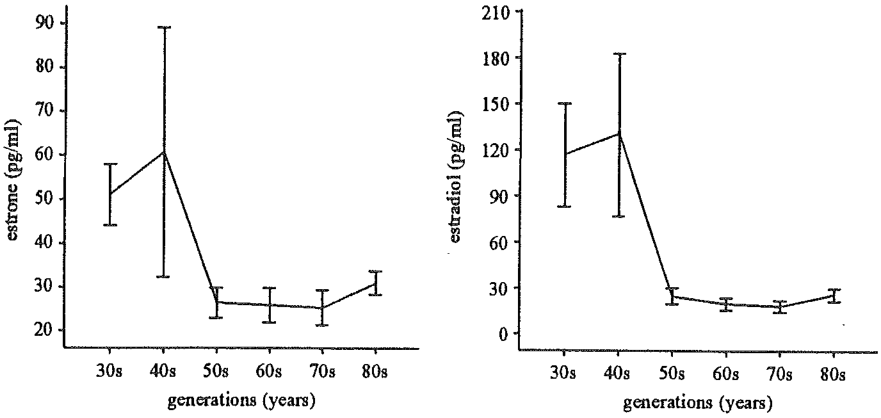

Figure 4 Mean plasma estrone (E1) and estradiol (E2) levels in females for each generation. Numbers of female subjects: 30s, $n = 21$; 40s, $n = 21$; 50s, $n = 21$; 60s, $n = 21$; 70s, $n = 21$; 80s, $n = 9$. Conversion factors: estrone, pM = pg/ml × 3.699; estradiol, pM = pg/ml × 3.671.

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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ORIGINAL ARTICLE

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

Takeshi Isobe, Shigeyuki Saitoh, Satoru Takagi, Hirofumi Ohnishi, Junichi Ohhata, Hiroshi Takeuchi, Yu Chiba, Nobuo Katoh, Tadashi Fujiwara, Hiroshi Akasaka and Kazuaki Shimamoto

Second Department of Internal Medicine, Sapporo Medical University, School of Medicine, Sapporo, Japan

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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Correspondence: Dr Takeshi Isobe, MD, Second Department of Internal Medicine, Sapporo Medical University, School of Medicine, S-1, W-16, Sapporo 060-8543, Japan. Email: isobet@sapmed.ac.jp

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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-dimethoxyanilinehydroxy-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

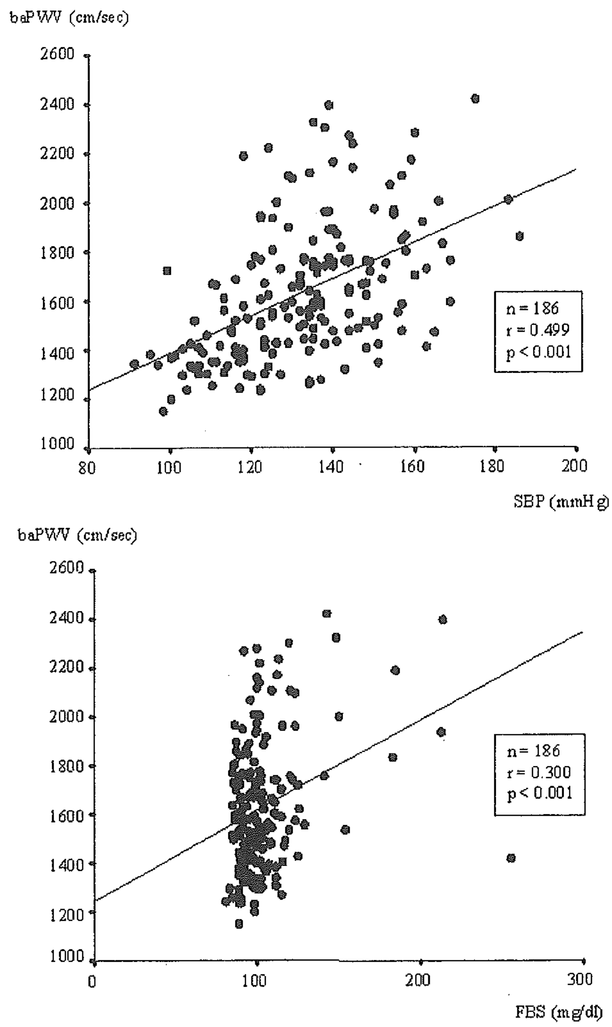

Figure 1 Correlations between systolic blood pressure (SBP) and brachial–ankle pulse wave velocity (baPWV) and between fasting blood sugar (FBS) and baPWV.

Table 1 Background of subjects

Group *n*	NGT-NT 78	NGT-HT 65	IFG-NT 8	IFG-HT 13	DM-NT 15	DM-HT 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.

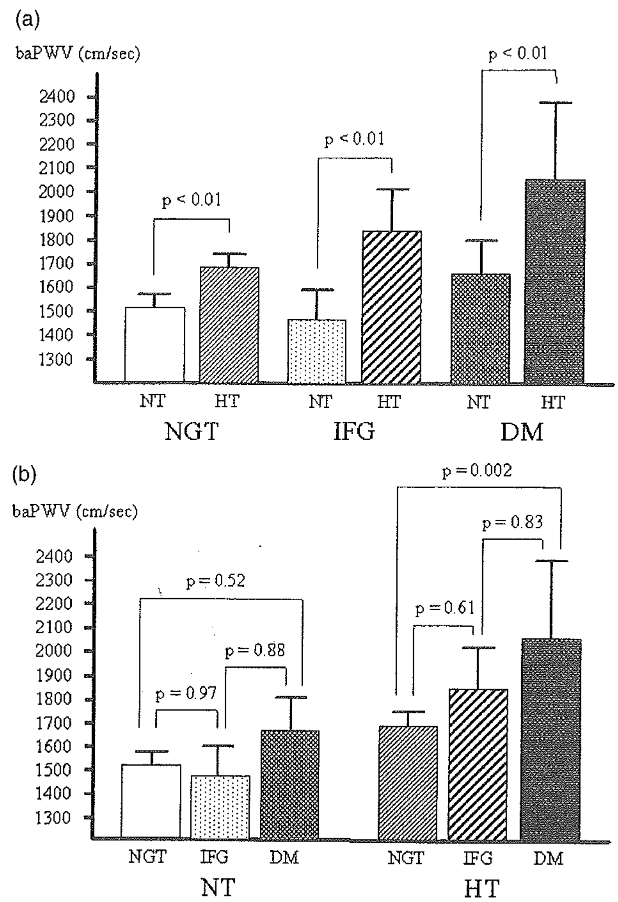

Figure 2 (a) Brachial–ankle pulse wave velocity (baPWV) for three groups classified by plasma glucose level. (NT, normal blood pressure; HT, hypertension, systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or therapy for hypertension; NGT, normal fasting glucose, fasting blood 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 arteriosclerosis using PWV measurement, this measurement will become an extremely useful means for the prevention of cardiovascular diseases in the elderly. It has been reported that both the frequency of calcification of the aorta, as seen on CT images, and carotid intima-media thickness, as measured on echoaortographic images, increase as PWV increases.[11] It has also been reported that cfPWV is significantly higher in patients with hypertension and/or diabetes mellitus.[2,3,12] Blacher *et al.* found by comparison of values of cfPWV in subjects with and without atherosclerosis that PWV in subjects with atherosclerosis was significantly higher than that in subjects without atherosclerosis, and they reported that the risk of cardiovascular disease, even in subjects without atherosclerosis, increased with increases in cfPWV.[13]

In a previous study, we examined baPWV and impaired glucose tolerance in middle-aged and elderly male subjects of a general population, who participated in the medical examination in the same towns in 2000, and we showed that baPWV is a useful indicator of the progression of arteriosclerosis and that baPWV starts to increase not from the DM stage but from the borderline blood sugar stage.[3] The results of that study also suggested that the blood sugar level is likely to promote the progression of arteriosclerosis in a manner dependent on their values.

In the present investigation, we measured SBP, DBP, BMI, FBS, TC, TG, HDL and baPWV in elderly male subjects of a general population. The values of BMI, TC, TG and HDL in those subjects are comparable to mean values for age-matched Japanese, and there were no significant intergroup differences (Table 1). Therefore, it may be possible to directly assess the relationships of baPWV with blood pressure and blood sugar levels without the effects of other risk factors. In the present study, subjects with ABI ≤ 0.9, indicating arteriosclerosis obliterans,[5] were excluded. Subjects with a past history of arteriosclerotic diseases such as angina pectoris, myocardial infarction and cerebral infarction were also excluded. The subjects in the present study can therefore be considered to be relatively healthy.

In the elderly subjects in the present investigation, as was also previously reported for middle-aged subjects, baPWV increased as blood pressure and blood sugar level increased, and positive correlations were found between blood pressure and baPWV and between blood sugar level and baPWV (Fig. 1). In the NT group, baPWV did not increase significantly with an increase in the level of blood sugar. We have already examined the relationship between baPWV and impaired glucose tolerance in middle-aged people and found that baPWV was already starting to increase at the borderline blood sugar stage.[3] Although further study is needed to determine the reason for the difference between baPWV in middle-aged people and that in elderly people, the results of the present study suggest that the combination of hypertension and impaired glucose tolerance in elderly people promotes the progression of arteriosclerosis. It is also thought that maintenance of normal blood pressure until an elderly age delays the progression of arteriosclerosis by impaired glucose tolerance.

A comparison of the NT and HT groups showed that baPWV increased significantly regardless of glucose tolerance impairment, and baPWV in the HT group increased as the level of blood sugar increased. A tendency for baPWV to be high was found even at the IFG stage, and baPWV was significantly higher in the DM group than in the NGT group ($P = 0.002$). The results suggest that baPWV is elevated in people with HT even at the stage of IFG and that, conversely, arteriosclerosis strongly progresses in people with IFG if complicated by hypertension. By a multiple regression analysis using baPWV as a dependent variable, age, SBP and FBS level were selected as independent variables. It is conceivable that SBP and FBS level independently contribute to the elevation of baPWV. The above-described results suggest that strict control of blood pressure and blood sugar levels in the elderly might be necessary for the prevention of arteriosclerosis.

It is well known that PWV increases as age increases. This is thought to reflect the development of arteriosclerosis due to aging. However, to date, it has been difficult to detect differences in the degree of progression of arteriosclerosis by the presence or absence of risk factors. There have been very few studies in which the relationship between risk factors and arteriosclerosis in elderly subjects was investigated using change in PWV as an end point. Mackey *et al.* recently reported results of a 4-year follow-up study on the relationship between arteriosclerosis and risk factors in elderly subjects in whom cfPWV had been measured.[14] They used measurements of cfPWV to estimate the degree of arteriosclerosis in 356 male and female subjects aged 70–96 years, and they reported that the degree of arteriosclerosis was positively correlated with systolic blood pressure, age, fasting blood sugar level and blood sugar level 2 h after a meal, insulin level 2 h after a meal, triglyceride, girth of the abdomen and heart rate. After adjustment for age and blood pressure, heart rate in men and heart rate as well as girth of the abdomen in women remained positively correlated with degree of arteriosclerosis. Their results suggest that the degree of arteriosclerosis in the elderly is related to states of risk factors, particularly heart rate and insulin resistance, during the past several years. Although our investigation was a cross-sectional study on baPWV and current risk factors, the finding of a significant correlation between elevation of blood sugar level and baPWV suggests that there is a link between insulin resistance and baPWV. Our results are considered to be compatible with the results reported by Mackey *et al.*

In our previous cohort study, we investigated the relationships between FBS and plasma glucose concentrations 2 h after load (2h-PG) in 1991 and 1992 and baPWV in 2001 and 2002.[15] Significant positive correlations were found between 2h-PG in 1991 and 1992 and baPWV in 2001 and 2002. Multiple regression analysis was performed on baPWV with regard to sex, BMI, TC and 2h-PG, and then 2h-PG was selected as an independent variable. In that cohort study, we suggested that 2h-PG plays a key role in the increase in baPWV after 10 years. The DECODE study group recently reported results of a 7-year follow-up study on the relationships of FBS and 2h-PG with risk of death.[16] They suggested that 2h-PG as well as FBS identified individuals at increased risk of death. In our cohort study, baPWV was higher in both the IFG group and the IGT group than in the NGT group. It was not clarified whether baPWV was affected more by an increase in FBS or by an increase in 2h-PG. In our cohort study, baPWV, FBS and 2h-PG were not measured at the same time. Further investigation is therefore need.

Rachel *et al.* recently reported that excess body weight is associated with higher aortic stiffness in whites and African Americans.[17] They used measurements of cfPWV in 186 young adults (20–40 years old) and 177 older adults (41–70 years old), and the subjects were divided into three groups on the basis of BMI: a normal weight group (BMI < 25 kg/m^2), an overweight group ($25 \leq$ BMI ≤ 30 kg/m^2) and an obese group (BMI > 30 kg/m^2). They found a significant positive correlation between BMI and cfPWV. In the obese group, cfPWV was higher than that in the normal weight group. Although cfPWV in the overweight group was not higher than in the normal weight group in 20–30 year old subjects, it increased in parallel with increases in age and finally reached the same level as that in the obese group in 41–59 year old subjects. Their results suggest that BMI is the strongest independent predictor of cfPWV. Nakanishi *et al.* recently reported that BMI affects cfPWV after 9 years in 2186 Japanese males.[18] In our previous cohort study, we also found that BMI affects baPWV after 10 years in 530 Japanese subjects. In the present cross-sectional study, there was no correlation between BMI and baPWV, and BMI was not selected as an independent variable in multiple regression analysis performed on baPWV. The discrepancy between the results for white and African Americans and those for Japanese might be due to differences in races. This was because very few Japanese have a BMI greater then 30 kg/m^2. A further cross-sectional study is needed to determine whether visceral fat accumulation or waist to hip ratio affects baPWV in Japanese people. In our present cross-sectional study, BMI did not affect baPWV. On the other hand, in the previous longitudinal study, it was suggested that BMI has an influence on PWV after about 10 years in Japanese. Further study is needed to determine the time at which a high BMI starts to affect PWV in Japanese people.

Hypertension and diabetes mellitus are risk factors for arteriosclerosis, and their combination exerts a synergistic effect on the progression of arteriosclerosis and on the development of arteriosclerotic disease. The results of the present study suggest that strict control of blood pressure and blood sugar level might be necessary in order to prevent the development of arteriosclerotic diseases even in the elderly if mild impairment of glucose tolerance is combined with hypertension.

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Editor's comment

This article was selected by the Japan Geriatric Society for its outstanding contribution to geriatrics.

Original Article

Development and Progression of Atherosclerotic Disease in Relation to Insulin Resistance and Hyperinsulinemia

Tadashi FUJIWARA, Shigeyuki SAITOH, Satoru TAKAGI, Hiroshi TAKEUCHI, Takeshi ISOBE, Yu CHIBA, Tetsuji MIURA, and Kazuaki SHIMAMOTO

It is unclear whether the role of insulin resistance in the development of atherosclerotic cardiovascular disease is similar in populations in which the incidence of atherosclerotic diseases significantly differs from that in Western countries. The aim of this study was to determine the relationship between insulin resistance and the development of cardiovascular disease in the Japanese population. We conducted 75 g-oral glucose tolerance tests (OGTTs) on 1,928 inhabitants of two towns in Hokkaido, Japan. Subjects using antihypertensive agents and known diabetic patients were excluded from the study. Data from the remaining 1,227 subjects (540 males and 687 females; mean age 56.0±10.8 years) were used for the analysis, and 1,051 subjects were seen in a follow-up care setting for a period of 8 years. The presence of insulin resistance was defined according to the guidelines reported our previous study: insulin levels of 64.0 mU/l or higher 2 h after the 75 g-OGTT. The insulin-resistant (IR) group had several risk factors such as hypertension, diabetes, treated or untreated hypercholesterolemia, hypertriglyceridemia, low high-density-lipoprotein (HDL) cholesterol levels, and obesity. During the follow-up period of 8 years, the incidence of coronary artery disease, which was adjusted for age, body mass index, sex, systolic blood pressure, fasting plasma glucose, total cholesterol, triglyceride, and HDL cholesterol was significantly (3.2 times) higher in the IR group than in the insulin non-resistant group. The results suggested that insulin resistance is an independent risk factor for coronary artery disease in Japanese subjects, as has also been demonstrated in the case of individuals in Europe and USA. (*Hypertens Res* 2005; 28: 665–670)

Key Words: **insulin resistance, cardiovascular disease, risk factors**

Introduction

The incidence of cardiovascular disease is high in patients with multiple risk factor syndrome (or metabolic syndrome). Although the criteria for the diagnosis of multiple risk factor syndrome have not been standardized, there is a consensus that insulin resistance is an important factor underlying the association of this syndrome with atherosclerotic cardiovascular diseases. Insulin resistance is known to cause multiple pro-atherosclerotic effects on the hemostatic system as well as on vascular endothelial function (*1*, *2*). Furthermore, several studies using meta-analysis have indicated that hyperinsulinemia is associated with various atherosclerotic cardiovascular diseases, including the coronary artery disease (*3*–*7*). However, the incidence of atherosclerotic vascular diseases differs among different races; epidemiological studies conducted to date have revealed racial differences in the relationship between insulin resistance and atherosclerotic diseases (*8*, *9*). No study has yet been conducted to clarify the impact of insulin resistance on the development and progress of atherosclerotic cardiovascular disease in the general Japa-

From the Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan.
Address for Reprints: Tadashi Fujiwara, M.D., Second Department of Internal Medicine, Sapporo Medical University School of Medicine, S-1, W-16, Chuo-ku, Sapporo 060–8543, Japan. E-mail: tfujiwar@sapmed.ac.jp
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Table 1. Baseline Characteristics

	Male	Female
Age (year)	57.2±11.3	55.1±10.3
BMI (kg/m^2)	23.2±3.0	23.3±2.9
SBP (mmHg)	125.2±15.8	123.3±16.5
DBP (mmHg)	75.4±9.0	73.4±9.0
FPG (mg/dl)	89.9±12.0	87.3±10.9
TC (mg/dl)	184.3±30.2	196.3±34.6
TG (mg/dl)	148.0±110.4	111.1±62.9
HDL-C (mg/dl)	52.6±14.0	57.7±13.2

Values are expressed as means±SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density-lipoprotein cholesterol.

nese population.

In the present study, we aimed to determine the relationship of insulin resistance with established risk factors and with the incidence of cardiovascular events in the general Japanese population. We performed mass screening examinations, including a 75 g-oral glucose tolerance test (OGTT), for the inhabitants of two towns in Hokkaido, Japan, and followed these individuals for 8 years for cardiovascular events and mortality. Insulin resistance was diagnosed on the basis of blood insulin levels 2 h after administration of the 75 g-OGTT, according to the methods described in our previous study (*10*).

The present results support the hypothesis that insulin resistance is an important independent risk factor in coronary artery disease and its occurrence in the Japanese population.

Methods

Study Subjects

The subjects of this study were 2,138 inhabitants of two towns in Hokkaido, who participated in a health examination program in 1991 and 1992 (*9*). The subjects inhabited a largely agricultural area. Subjects using anti-hypertensive agents and those undergoing medical treatment for diabetics were excluded from the analysis, because these morbid states exert an effect on insulin resistance. Data obtained from the remaining 1,227 subjects (540 males and 687 females; mean age 56.0±10.8 years) were used for analysis.

Parameters

Blood samples were obtained from the subjects in the early morning after an overnight fast, and the following factors were measured: fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG) and high-density-lipoprotein (HDL) cholesterol levels, and blood pressure (systolic and diastolic blood pressure [*i.e.*, SBP and DBP]), which was measured twice with the subject in a sitting position after a 5-min rest to calculate the mean blood pressure. A 75 g-OGTT was then performed on each subject to determine the blood insulin level 2 h after administration of the test (120 IRI). The cutoff point of 120 IRI≥64.0 mU/l was used to determine insulin resistance according to the report by Oimatsu *et al.* (*10*).

The following definitions of risk factors and cardiovascular diseases were employed: obesity was defined as a body mass index (BMI) of 25 kg/m^2 or higher (*11*) and according to the criteria of JNC-VI of the International Hypertension Society, hypertension was defined as a SBP of 140 mmHg or higher, or a DBP of 90 mmHg or higher (*12*). The 1997 criteria of the American Diabetes Society was used for the diagnosis of diabetes mellitus, namely, a FPG level of 126 mg/dl or higher, or a plasma glucose level 2 h after the administration of the 75 g-OGTT of 200 mg/dl or higher (*13*). Hypercholesterolemia was defined as a plasma cholesterol level of 220 mg/dl or higher. Hypertriglyceridemia was defined as plasma TG levels of 150 mg/dl or higher, and low HDL cholesterol was defined as blood HDL cholesterol levels of less than 40 mg/dl (*14*). Coronary artery disease was defined as myocardial infarction or angina pectoris. Cerebral vascular disease was defined as cerebral infarction or cerebral hemorrhage. We excluded subjects with subarachnoid hemorrhage and unclassified stroke from the analysis. Public health nurses, who were continuously engaged in local public health services, performed the follow-up examinations. The diagnosis of cases involving morbidity was confirmed by answers to questionnaires mailed to the doctors in charge of the cases in hospitals or in outpatient clinics, and electrocardiographies and brain CT scans were also reviewed in as many cases as possible. The nurses checked the death certificates in cases of mortality.

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. Informed, written consent was obtained from all subjects after they had been provided with a complete explanation of the purpose, nature, and risks of all procedures used.

The data are shown as mean±SD. An unpaired t-test was used to test the differences between mean values in the two groups, and a p value of less than 0.05 was considered statistically significant. TG values were logarithmically transformed before the analysis. A simple correlation analysis was applied to test the relationship between the onset of disease and insulin resistance using a significance level of $p<0.05$. The χ^2 test was used to examine the relationship between insulin resistance and risk factors for atherosclerosis. Because there were significant differences in age between the insulin non-resistant (NR) group and the insulin-resistant (IR) group, we used the Mantel-Haenszel test to assess differences in the onset of cardiovascular disease and insulin resistance. Multiple logistic regression analysis was used to test the relation-

Table 2. Baseline Characteristics in the NR Group and the IR Group

	Male			Female		
	NR group	IR group	*p*-value	NR group	IR group	*p*-value
Age (year)	57.1±11.4	58.8±10.1	0.381	54.9±10.3	58.5±9.4	0.041
BMI (kg/m^2)	23.0±2.8	26.7±2.8	<0.001	23.1±2.8	26.4±2.6	<0.001
SBP (mmHg)	125±15.7	128.4±17.0	<0.001	122.6±16.2	137.7±15.6	<0.001
DBP (mmHg)	75.2±9.2	78.4±6.4	0.008	73.1±8.9	79.1±9.1	<0.001
FPG (mg/dl)	89.6±11.9	94.8±12.9	0.011	87.1±11.0	91.8±7.0	<0.001
TC (mg/dl)	183.4±29.3	196.5±38.6	0.049	195.3±34.2	214.3±37.3	0.002
log TG (mg/dl)	4.788±0.528	5.056±0.528	0.659	4.578±0.428	5.008±0.537	<0.001
HDL-C (mg/dl)	52.9±13.9	48.4±14.6	0.074	58.0±13.2	51.4±13.2	0.004

Values are means±SD. NR, insulin non-resistant; IR, insulin-resistant; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density-lipoprotein cholesterol.

Table 3. Frequency of Each Risk Factor in the IR Group and the NR Group

	NR group	IR group	*p*-value
Number	1,155	72	
Obesity (%)	24.1	74.6	<0.001
Hypertension (%)	18.0	33.8	0.002
Diabetes mellitus (%)	1.0	5.6	0.012
High total cholesterol (%)	19.0	36.1	0.001
High triglyceride (%)	22.8	44.4	<0.001
Low high-density-lipoprotein cholesterol (%)	12.9	29.6	<0.001

Values are expressed as %. NR, insulin non-resistant; IR, insulin-resistant.

ship between the onset of cardiovascular disease and insulin resistance and/or other risk factors. The commercially available statistical package SPSS Version 12.0J was used for the statistical analyses.

Results

A cross-sectional investigation was conducted in the first year of the current study. The baseline characteristics of subjects of each gender are shown in Table 1. As regards risk factors, the levels of the following factors were higher in men than in women: age, FPG, DBP, and TG. On the other hand, TC and HDL cholesterol levels were higher in women. There were no significant gender-related differences in SBP. The subjects were divided into an NR group (control group) and an IR group. In the IR group, 6.5% of the subjects were males and 4.4% were females. Among males, the IR group had significantly higher BMI, SBP, DBP, FPG, and TC values than the NR group. However, no significant difference was observed in terms of age, log TG, or HDL cholesterol between the two groups. Among females, the IR group had significantly higher age, BMI, SBP, DBP, FPG, TC, and log TG values than the NR group. Moreover, females in the IR group had lower HDL cholesterol levels than the NR group (Table 2). The prevalence of female or male subjects with obesity, hypertension, diabetes, or abnormalities in plasma lipid levels were significantly higher in the IR group than in the NR group (Table 3).

The association between insulin resistance and the aggregation of risk factors is shown in Fig. 1. The prevalence of insulin resistance (0.7, 4.4, 10.6, and 19.2%: $p<0.001$) was found to increase with the number of risk factors present (0, 1, 2, and 3 or more). In addition, 70.2% of the subjects in the IR group had two or more risk factors, whereas 70.6% of the subjects in the NR group had none or only one risk factor (data not shown).

During the follow-up period, coronary artery diseases developed in 43 subjects (acute myocardial infarction in 15 subjects and angina pectoris in 28 subjects), and cerebral vascular accidents occurred in 15 subjects (cerebral infarction in 11 subjects and cerebral hemorrhage in four subjects). The incidence of coronary artery disease in the IR group (16.1%) was significantly higher than that in the NR group (3.4%), but no significant difference was found between these two groups in terms of the incidence of cerebral vascular disease (3.6 *vs.* 1.3%, $p=0.188$) (Table 4).

Table 5 shows the results of the multiple logistic analyses of the determinants of coronary artery disease-associated morbidity during an 8-year follow-up period. The IR group had a 3.2-fold higher incidence of coronary artery disease than the NR group, even after corrections were made for age, gender, BMI, SBP, FPG, and TC. SBP, age, and sex were also shown to be significant risk factors in the present dataset.

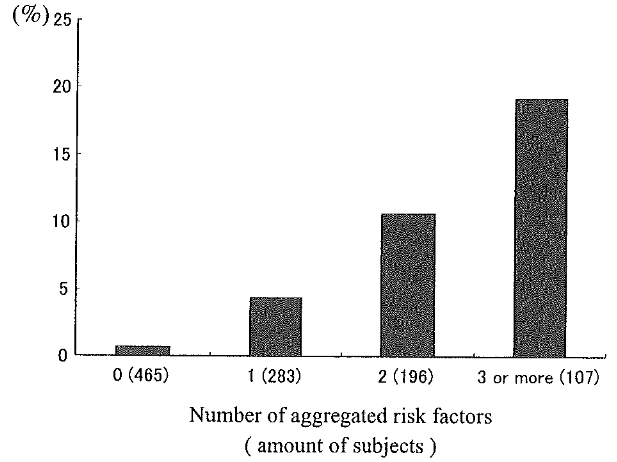

Fig. 1. *Percentages of IR and NR subjects according to the aggregation of risk factors. The percentage of IR subjects tended to be higher than that of NR subjects with respect to an increasing number of risk factors. Risk factors: obesity, hypertension, diabetes mellitus, high total cholesterol, high triglyceride, low high-density-lipoprotein cholesterol.*

Discussion

It is known that the following are potential markers of insulin resistance: the product of insulin levels and plasma glucose levels, as determined by the 75 g-OGTT (Σ BS/IRI, sum of the 0–120 min values), and the fasting insulin and FPG levels (HOMA values; 104/Ip×Gp, where Gp and Ip are maximum blood glucose level and maximum insulin level, respectively). A cutoff point of IRI≥64.0 mU/l at 120 min after the administration of the 75 g-OGTT was used as a marker of insulin resistance in the present study. This cutoff point has been demonstrated as a valid marker of insulin resistance, as the insulin level observed 120 min after glucose loading in the context of the 75 g-OGTT is known to correlate negatively with the *M* value obtained by the glucose clamp method, thus indicating that insulin secretion has been maintained (*10*). Age, gender, and medication potentially exert effects on insulin sensitivity, and diuretic agents, α and β blockers, calcium channel blockers, and angiotensin converting enzyme inhibitors have been shown to affect insulin resistance. However, since the subjects of the present study were primarily inhabitants of rural communities (excluding those subjects administered anti-hypertensive agents and diabetes medications), it is expected that the above factors had only limited effects on the results. The findings of the above mentioned meta-analysis revealed that the fasting insulin level or a cutoff value in the 80th percentile could be used for the determination of insulin resistance. According to the criteria for diagnosis used in the present study, 5.3% of the subjects suffered from insulin resistance. However, the criteria used in our study were stricter than those used in the studies conducted in Western countries, and it is therefore difficult to draw conclusions regarding differences in the effects of insulin resistance on the development of coronary artery disease in the Western samples.

Zavaroni *et al.* examined subjects living in a workers' community; in that study, a glucose intolerant non-obese group suffering from hyperinsulinemia was compared to a group with normal insulin levels, and the results showed that before the onset of diabetes, the former group had higher levels of TG, low-density-lipoprotein (LDL) cholesterol, and blood pressure, as well as a higher incidence of coronary artery disease, than the latter group (*15*). Moreover, on the basis of the results of a follow-up study carried out over a period of 8 years, the San Antonio Heart Study revealed associations between fasting hyperinsulinemia and the onset of diabetes, low HDL cholesterol, and hypertension (*16*). It has also been reported that subjects with multiple risk factors had significantly higher fasting insulin levels than did those presenting with only a single risk factor. The results of the cross-sectional investigation in the first year of the present study showed that in the IR group, the incidence of hypertension, diabetes, and abnormal lipid metabolism was significantly higher than that in the NR group. The present results also suggested that insulin resistance is associated with atherosclerotic risk factors and also with the accumulation of these risk factors. These results are thus consistent with the findings of recent studies conducted in Western countries.

Several studies have been conducted to investigate the relationship between insulin resistance and the onset of coronary artery disease; differences between such studies were considered in terms of baseline characteristics such as age, male-to-female ratio, drug usage, and length of follow-up period, as well as in terms of the criteria used for the diagnosis of insulin resistance by determination of the insulin level. Ruige *et al.* carried out a meta-analysis of 17 past major epidemiological studies in Western countries, and they showed that insulin resistance and hyperinsulinemia are independent risk factors in ischemic heart disease, and that race influences the relationship between the onset of coronary artery disease and insulin resistance (*7*). Racial differences have been shown to exist in the relationship between insulin resistance and atherosclerotic diseases. The IRAS study showed that the progression of atherosclerosis differs among races (*17*). Pima Indians, who have high insulin resistance, have a low incidence of coronary artery disease (*8*). A comparison of Japanese and Western samples revealed that the latter group had the capacity to secrete more insulin, and most of the Japanese diabetes patients studied did not have hyperinsulinemia (*18*). In Westerners, pancreatic insulin secretion function is maintained after the onset of type II diabetes. The condition of diabetes associated with hyperinsulinemia and insulin resistance differs greatly from that observed among Japanese. The results of our follow-up study of Japanese subjects showed that a significant relationship existed between insulin resistance and the incidence of coronary artery disease, even after the correction of risk factors for atherosclerosis, such as age,

Table 4. Cardiovascular Event in the IR Group and the NR Group

	NR group	IR group	Odds ratio	p-value
Follow up	995	56		
Coronary artery disease	34	9	5.4 (4.6*)	<0.001 (<0.001*)
(Morbidity (%))	(3.4)	(16.1)		
Stroke	13	2	2.8 (2.1*)	0.188 (0.348*)
(Morbidity (%))	(1.3)	(3.6)		

Values are expressed as % or number. NR, insulin non-resistant; IR, insulin-resistant. *Adjusted for age.

Table 5. Multiple Logistic Analysis for Coronary Artery Disease Morbidity

	Odds ratio	95.0% CI	p-value
IR/NR	3.203	1.260–8.142	0.014
SBP	1.023	1.004–1.042	0.020
Age	1.040	1.003–1.077	0.033
Sex (male)	1.976	0.995–3.925	0.052
TC	1.006	0.966–1.016	0.234
HDL-C	0.991	0.996–1.016	0.458
TG	0.999	0.995–1.003	0.611
FPG	0.994	0.963–1.026	0.709
BMI	1.015	0.903–1.141	0.801

Independent variables: coronary artery disease morbidity. Dependent variables: insulin resistance (IR/NR), age, sex, BMI, SBP, FPG, TC, TG, HDL-C. Odds ratios for continuous risk factors expressed for single value higher. NR, insulin non-resistant; IR, insulin-resistant; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density-lipoprotein cholesterol.

gender, blood pressure, blood glucose, and total cholesterol. Insulin resistance causes atherosclerosis by giving rise to risk factors for atherosclerosis. Moreover, insulin itself directly promotes atherosclerosis. Insulin resistance is classified as decreased tissue sensitivity to insulin or compensatory hyperinsulinemia. The pathological states caused by low insulin sensitivity include glucose intolerance and lipid metabolism disorders, such as hypertriglyceridemia, elevated very low-density-lipoprotein cholesterol, and low HDL cholesterol. Hyperinsulinemia can cause hypertension *via* an increase in sympathetic nerve activity, increased renin-angiotensin activity, sodium retention in the kidneys, increased Na^+/H^+ pump activity, activation of insulin-like growth factor (IGF) receptors, and impaired endothelium-dependent vasodilation. We believe that insulin resistance is a marked risk factor for the development of coronary artery disease, even after subtraction of the influence of other risk factors, because increased Na^+/H^+ pump activity, IGF receptor activation, and impaired endothelium-dependent vasodilation can be direct causes of atherosclerotic disease, and because plasminogen-activator-inhibitor-1 (PAI-1) can increase blood coagulability to accelerate atherosclerosis. PAI-1 levels are known to be elevated in patients in an insulin-resistant state. However, these pathways are known to differ among races. Nonetheless, the results of the present study indicated that insulin resistance in Japanese, as well as in Westerners, is involved in the development of coronary artery disease, independent of the other risk factors for atherosclerosis.

Our results did not show a significant correlation between insulin resistance and the incidence of stroke. However, the Helsinki Policemen Study showed that insulin resistance is a predictor not only of coronary artery disease, but also of stroke (*19*). In our study, only a small number of subjects experienced cerebrovascular disorders. The following potential reasons for this were considered: the mean age of the subjects included in the analysis was 56, which is otherwise associated with a relatively low prevalence of cerebrovascular disorder; patients who were under treatment for hypertension and diabetes were excluded from the study, resulting in a sample with only a low risk of cerebrovascular disorder; and mild transient ischemic attack might have been missed in the present study. Moreover, in a previous study of Japanese subjects, it was found that the percentage of subjects with insulin resistance was significantly high but only in the atherothrombotic infarction group, and not in groups with other types of cerebrovascular disorder (*20*).

Studies performed in Western countries have reported that insulin resistance leads to the development of hypertension, diabetes, and abnormal lipid metabolism, all of which are known to lead to the progression of atherosclerosis and the development of cardiovascular diseases. As has been noted in these reports, insulin resistance leads to hypertension in Japanese subjects (*21*). In the present study, we investigated the relationship between insulin resistance and the development of cardiovascular disease in Japanese subjects who had undergone a number of screening examinations. Our results demonstrated that insulin resistance is associated with the development of coronary artery disease; this finding is similar to those of studies conducted in Europe and in USA. According to the present study, the expected frequency of patients suffering from cerebral vascular disease while also having insulin resistance was low, *i.e.*, 0.8, and therefore it was impossible to arrive at a conclusion regarding the relationship between insulin resistance and the onset of cerebral vascular disease (*22*). In our study, the stroke incidence was 1.4%, which was also low compared with that expected for Japanese people in general. It is thought that the reason for this relatively low value was that we analyzed a group from which patients with a previous history of hypertension or diabetes were excluded.

The number of Japanese with risk factors for atherosclerosis (*e.g.*, hypertension, diabetes, hyperlipidemia, and obesity) has been increasing in recent years due to changes in diet and habitual exercise. The incidence of either hyperinsulinemia or insulin resistance is also believed to be increasing in Japan. Therefore, the evaluation of insulin resistance will be important in the future for the prevention of atherosclerotic disease. An investigation of changes in insulin resistance should enable the determination of the risk for atherosclerotic disease in the Japanese population, and would contribute to the elucidation of racial differences in the relationship between insulin resistance and the development of atherosclerotic disease.

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Adiponectin and other Adipocytokines as Predictors of Markers of Triglyceride-Rich Lipoprotein Metabolism

Dick C. Chan,[1] Gerald F. Watts,[1*] Theodore W.K. Ng,[1] Yoshiaki Uchida,[2] Naohiko Sakai,[3] Shizuya Yamashita,[3] and P. Hugh R. Barrett[1]

Background: Adipocytokines are bioactive peptides that may play an important role in the regulation of glucose and lipid metabolism. In this study, we investigated the association of plasma adipocytokine concentrations with markers of triglyceride-rich lipoprotein (TRL) metabolism in men.

Methods: Fasting adiponectin, leptin, resistin, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), apolipoprotein (apo) B-48, apo C-III, and remnant-like particle (RLP)-cholesterol concentrations were measured by immunoassays and insulin resistance by homeostasis assessment (HOMA) score in 41 nondiabetic men with a body mass index of 22–35 kg/m^2. Visceral and subcutaneous adipose tissue masses (ATMs) were determined by magnetic resonance imaging and total ATM by bioelectrical impedance.

Results: In univariate regression, plasma adiponectin and leptin concentrations were inversely and directly associated with plasma apoB-48, apoC-III, RLP-cholesterol, triglycerides, VLDL-apoB, and VLDL-triglycerides (P <0.05). Resistin, IL-6, and TNF-α were not significantly associated with any of these variables, except for a direct correction between apoC-III and IL-6 (P <0.05). In multivariate regression including HOMA, age, nonesterified fatty acids, and adipose tissue compartment, adiponectin was an independent predictor of plasma apoB-48 (β coefficient = −0.354; P = 0.048), apoC-III (β coefficient = −0.406; P = 0.012), RLP-cholesterol (β coefficient = −0.377; P = 0.016), and triglycerides (β coefficient = −0.374; P = 0.013). By contrast, leptin was not an independent predictor of these TRL markers. Plasma apoB-48, apoC-III, RLP-cholesterol, and triglycerides were all significantly and positively associated with plasma insulin, HOMA, and visceral, subcutaneous, and total ATMs (P <0.05).

Conclusions: These data suggest that the plasma adiponectin concentration may not only link abdominal fat, insulin resistance, and dyslipidemia, but may also exert an independent role in regulating TRL metabolism.
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Obesity is strongly associated with insulin resistance and dyslipidemia and increased risk of cardiovascular disease (CVD)[4] *(1, 2)*. Hypertriglyceridemia attributable to increased plasma concentrations of VLDL apolipoprotein (apo) B-100 and chylomicron apoB-48 is the most consistent lipid disorder in obesity *(3, 4)*. The precise underlying mechanism for this lipid abnormality remains unclear but may relate to oversecretion, reduced hydrolysis, and/or impaired clearance of triglyceride-rich lipoproteins (TRLs) *(3, 5)*. We have previously shown that obesity in men is associated with increased hepatic secretion and delayed catabolism of TRLs and that these kinetic defects are in part related to accumulation of abdominal fat and insulin resistance *(3, 5)*. We also demonstrated

[1] School of Medicine and Pharmacology, Western Australian Institute for Medical Research, University of Western Australia, Perth, Western Australia, Australia.
[2] Fujirebio Inc., Research and Development Division, Tokyo, Japan.
[3] Department of Internal Medicine and Molecular Science, Osaka University Graduate School of Medicine, Osaka, Japan.
*Address correspondence to this author at: School of Medicine and Pharmacology, University of Western Australia, Royal Perth Hospital, GPO Box X2213, Perth Western Australia 6847, Australia. Fax 61-8-9224-0246; e-mail gfwatts@cyllene.uwa.edu.au.
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[4] Nonstandard abbreviations: CVD, cardiovascular disease; apo, apolipoprotein; TRL, triglyceride-rich lipoprotein; RLP, remnant-like particle; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; MRI, magnetic resonance imaging; HOMA, homeostasis assessment; BMI, body mass index; ATM, adipose tissue mass; FFM, fat-free mass; SAATM, subcutaneous abdominal adipose tissue mass; IPATM, intraperitoneal adipose tissue mass; RPATM, retroperitoneal adipose tissue mass; NEFA, nonesterified fatty acid; and SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

that obese individuals have insulin resistance and increased concentrations of markers of TRL metabolism, including apoB-48, apoC-III, remnant-like particle (RLP)-cholesterol, non-HDL cholesterol, and triglycerides *(3)*.

The precise relationships between dyslipoproteinemia, adiposity, and insulin resistance are complex and undefined *(2, 4, 6)*. Adipose tissue has recently been shown to secrete a variety of bioactive peptides, called adipocytokines, that can potentially impact on glucose and lipid metabolism *(7–9)*. These adipocytokines include adiponectin, leptin, resistin, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). Adiponectin, also known as adipocyte complement-related protein of 30 kDa (ACRP30), adipoQ, and gelatin-binding protein of 28 kDa (GBP28), is a protein present at relatively high concentrations in the circulation *(9)*. Unlike other adipocytokines, plasma adiponectin concentrations are decreased in obese and insulin-resistant individuals, including those with type 2 diabetes *(10, 11)*. Experimental and clinical evidence suggests that other adipocytokines may exert their effects on insulin sensitivity by influencing adipocyte expression and secretion of adiponectin *(12)*. Hypertriglyceridemia, low HDL-cholesterol concentrations, and decreased LDL particle size have recently been shown in humans to be correlated with low plasma adiponectin concentrations independent of the amount of intraabdominal fat and degree of insulin resistance *(13, 14)*. Moreover, a recent intervention trial reported that changes in adiponectin concentrations after weight loss are correlated with improvements in plasma lipid concentrations independent of changes in adiposity and insulin sensitivity *(13)*. However, the association between plasma adiponectin and specific markers of TRLs in relation to insulin resistance and body fat distribution has not been investigated previously.

In the present study, we hypothesized that, in men, adiponectin would be the adipocytokine most closely associated with changes in TRLs, namely apoB-48 and RLP-cholesterol, and that this association would be independent of body fat compartments and insulin resistance. Our principal aims were (*a*) to examine the association of markers of TRL metabolism, as reflected by plasma concentrations of apoB-48, apoC-III, and RLP-cholesterol, with plasma adiponectin, leptin, resistin, TNF-α, and IL-6 concentrations; and (*b*) to explain these associations in relation to variations in body fat compartments and insulin resistance, measured by magnetic resonance imaging (MRI) and homeostasis assessment (HOMA) score, respectively.

Materials and Methods

STUDY POPULATION

We studied 41 Caucasian men with a body mass index (BMI) ranging from 22 to 35 kg/m^2 and no history of familial dyslipidemia, intercurrent illness, or use of medications affecting lipid metabolism. All were nonsmokers and were consuming ad libitum weight-maintenance diets, as described previously *(15)*. Participants were selected from the community. The study was approved by the Royal Perth Hospital Ethics Committee.

CLINICAL PROTOCOLS

Clinical tests. Body weight, height, and waist and hip circumference were recorded as described previously *(15)*; BMI and waist-to-hip ratio were calculated. Blood pressure was recorded semiautomatically by use of a Dinamap recorder (Critilzon). Body composition was estimated, with participants at rest in the supine position after emptying their bladders, by use of a Holtain Body Composition Analyser (Holtain Ltd.) from which total adipose tissue mass (ATM), fat mass, and fat-free mass (FFM) were derived; FFM was calculated by use of the formula of Lukaski et al. *(16)*: FFM = $(0.85 \times H^2/Z) + 3.04$, where H is the height (cm) of the individual and Z is impedance. For this measure, participants were also asked to refrain from alcoholic beverages for 24 h; they were then studied in the morning, 15 min after emptying their bladder and in a temperature-controlled room. The technical error for FFM was <3%.

Dietary and energy expenditure records. Participants completed a 7-day food intake diary that recorded all dietary, alcohol, and energy intake; data were analyzed by use of DIET4 Nutrient Calculation Software (Xyris Software) based on the Australian Food Composition Database (NUTTAB 95; Australian Government Nutrient Database).

MRI. MRI of eight transaxial segments (field of view, 40–48 cm; 10-mm thickness) at intervertebral disc positions from T11 to S1 was carried out with a 1.0-Tesla Picker MR scanner (Picker International) and a T1-weighted fast-spin-echo sequence with a high fat-to-water signal ratio. Subcutaneous abdominal ATM (SAATM), intraperitoneal ATM (IPATM), and retroperitoneal ATM (RPATM) areas were calculated by summing the relevant adipose tissue pixel units with purpose-designed software, as used previously *(15)*. Fat anterior to the posterior peritoneum and anterior abdominal wall was defined as IPATM, and fat posterior to the posterior peritoneum was defined as RPATM. Anterior and posterior subcutaneous abdominal compartments was separated by drawing a perpendicular line at the midpoint of the anterior–posterior line passing through midpoints of the vertebral bodies in the MRI images. Anterior SAATM was obtained by subtracting posterior SAATM from total SAATM. The imaging protocol has a technical error of <10% and is highly correlated (R^2 = 99%) with measurements obtained from imaging of the abdominal region by contiguous transaxial slices. On the basis of phantom studies using oil/water emulsions, the accuracy of our method for delineating regional adipose tissue was 100.1 (0.01)%. The reproducibility of duplicate in vivo measures of

IPATM and SAATM had a CV <3.5%. Further details are described elsewhere *(15)*.

BIOCHEMICAL ANALYSES

Fasting plasma cholesterol, triglycerides, and HDL-cholesterol were determined by standard enzymatic methods (interassay CVs <3%). LDL-cholesterol was calculated by use of the Friedewald equation. Non-HDL cholesterol was derived as total cholesterol minus HDL-cholesterol. The VLDL fraction was isolated from 3 mL of plasma by ultracentrifugation (Kontron Instruments) at 147 000*g* for 16 h at 4 °C, and the triglyceride concentration was measured as described above. VLDL-apoB concentrations were determined by a modified Lowry method (interassay CV <5%) *(5)*. Plasma nonesterified fatty acids (NEFAs) and glucose were measured by enzymatic methods and insulin by an immunosorbent assay. Insulin resistance was estimated by the HOMA score *(17)*. Plasma apoB-48 concentrations were measured by a sandwich ELISA using anti-human apoB-48 monoclonal antibodies (designated B-48-151) as reported previously (interassay CV <5%) *(18)*. This direct ELISA measurement of apoB-48 in plasma was highly correlated with the traditional method using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) coupled with immunoblotting and enhanced chemiluminescence in frozen plasma samples ($n = 30$; $r = 0.805$; $P < 0.001$) with a wide range of triglyceride concentrations (0.4–5.0 mmol/L) *(19)*. Values were 0.3–17.7 mg/L [mean (SD), 5.5 (4.8) mg/L] for the ELISA method and 9.51–54.40 mg/L [52.3 (11.6) mg/L] for the SDS-PAGE method. Plasma apoC-III was measured by immunoturbidimetric assay (Daiichi). Plasma RLP-cholesterol was determined with a JIMRO-II (Japan Immunoresearch Laboratories) assay using an immunoseparation technique (interassay CV <5%) *(20)*. Plasma adiponectin, leptin, IL-6, TNF-α, and resistin concentrations were measured by enzyme immunoassays (R & D Systems and Phoenix Pharmaceuticals); the interassay CV for these methods were <7%.

STATISTICAL ANALYSES

All analyses were performed with SPSS 11.5 (SPSS). The data are reported as the mean (SD). Skewed data were log-transformed where appropriate. Associations were examined by simple and stepwise linear regression methods. Because we carried out multiple comparisons, we considered that only $P < 0.01$ was of major importance in univariate analysis, but we also considered the conventional $P < 0.05$ as being statistically significant. Multiple regression models were used to determine the variables that best predicted plasma apoB-48, apoC-III, RLP-cholesterol, triglyceride, and VLDL-apoB concentrations. The adipose tissue compartment the most significantly correlated with corresponding dependent variable in stepwise regression analysis was included in the regression models.

Table 1. Anthropometric and ATM characteristics of the 41 men studied.

Characteristics	Mean (SD)	Range
Age, years	47 (9)	25–61
Systolic blood pressure, mmHg	127 (16)	96–164
Diastolic blood pressure, mmHg	76 (10)	53–96
Weight, kg	97 (12)	67–117
BMI, kg/m^2	30 (3)	22–35
Waist-to-hip ratio	1.0 (0.1)	0.9–1.1
FFM, kg	63 (8)	40–83
IPATM, kg	3.7 (1.5)	1.2–8.3
RPATM, kg	0.5 (0.06)	0.1–3.7
SAATM, kg	4.0 (1.4)	1.4–6.9
Anterior SAATM, kg	1.4 (0.6)	0.2–2.9
Posterior SAATM, kg	2.6 (0.9)	0.8–4.4
Total ATM, kg	33 (10)	13–56

Results

The anthropometric and ATM characteristics of the 41 men are shown in Table 1. They were generally middle aged and normotensive. Thirteen were nonobese, and 28 were obese, defined as BMI ≥30 kg/m^2. The mean proportions of total adipose tissue as IPATM, RPATM, and SAATM were 11%, 1.5%, and 12.1%, respectively. Of total SAATM, 35% was in the anterior and 65% in the posterior compartment.

The biochemical characteristics in the individuals studied are shown in Table 2. On average, the group was dyslipidemic, with increased triglycerides and low HDL-cholesterol, and insulin resistant. Four had impaired fasting glucose (6.1–6.9 mmol/L). Mean (SD) dietary intake per day was as follows: energy, 9276 (2030) kJ; total fat, 36 (7)%; carbohydrates, 38 (8)%; protein, 21 (4)%; alco-

Table 2. Biochemical characteristics of the men studied.

Characteristics	Mean (SD)	Range
Cholesterol, mmol/L	5.7 (0.9)	3.8–8.3
Triglycerides, mmol/L	2.7 (1.9)	0.5–8.8
HDL-cholesterol, mmol/L	1.0 (0.3)	0.6–1.8
Non-HDL cholesterol, mmol/L	4.7 (1.0)	3.1–7.4
LDL-cholesterol, mmol/L	3.6 (0.9)	1.5–5.8
RLP-cholesterol, mg/L	367 (34)	52–1670
ApoB-48, mg/L	8.9 (6.1)	1.0–24
ApoC-III, mg/L	163 (98)	29–410
VLDL-triglycerides, mmol/L	1.3 (1.3)	0.1–6.1
VLDL-apoB, mg/L	194 (167)	34–865
VLDL-triglycerides/apoB	13.2 (9.3)	0.36–63.9
NEFAs, mmol/L	0.90 (0.27)	0.45–1.67
Glucose, mmol/L	5.4 (0.6)	4.1–6.9
Insulin, mIU/L	11.7 (8.5)	2.6–41.8
HOMA score	2.9 (2.3)	0.6–10.8
Adiponectin, mg/L	4.4 (2.4)	1.4–11.4
Leptin, μg/L	18 (4)	10–23
Resistin, μg/L	204 (69)	53–366
TNF-α, ng/L	17 (7)	6–39
IL-6, ng/L	12 (5)	4–30