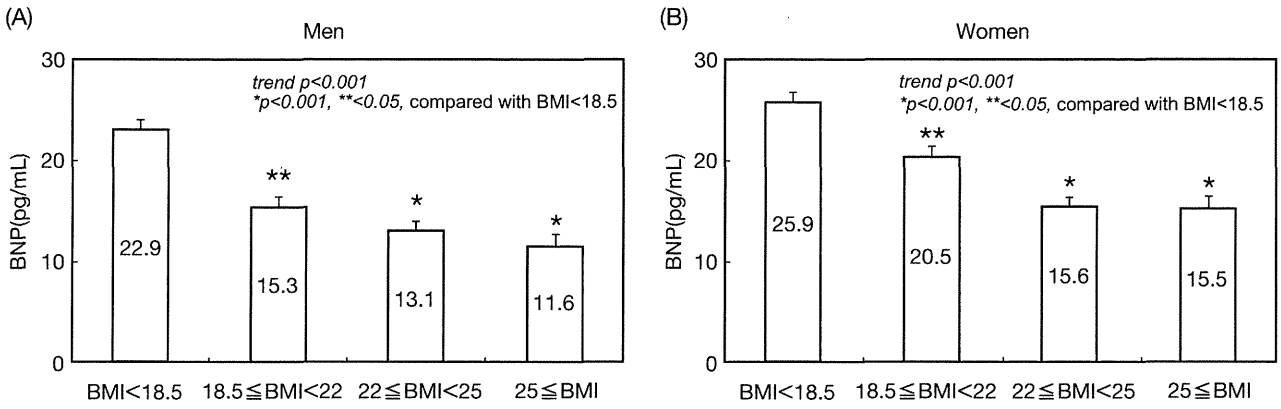

Fig. 1 Adjusted BNP levels stratified by BNP categories. Mean levels and SE of BNP for men (A) and women (B) are shown. Covariates used for adjustment are listed in Table 2.

mal) and ≧ 18.4 pg/mL (abnormal); the same covariates were evaluated in these models as in the linear regression models described above. The adjusted odds ratios of having normal BNP levels are shown in Table 3. After multivariable adjustment, highest BMI (25 kg/m^2 <BMI) was associated with having a 2.1- to 2.3-fold increase in the odds of having normal BNP levels (p<0.001). Overall, for each 1 unit increase in BMI, there was a 11% to 16% greater chance of having normal BNP (p<0.01).

Association between Body Composition and BNP Levels

Results of multivariable regression models relating plasma BNP levels with various obesity related factors are shown in Table 4. Model 1 used BMI as a measure of obesity, and model 2 replaced BMI with percent of body fat. In model 3, percent body fat was replaced by body fat mass and lean body mass. Model 4 replaced BMI with skin fold thickness, and Model 5 replaced BMI with waist circumferences. After adjustment for the same covariates as in Table 2, inverse associations were confirmed between percent of body fat, body fat mass, skin fold thickness and waist circumferences and BNP levels in both sexes (all p<0.01). However, the inverse association with lean body mass was not significant for BNP (p=0.188 in men and p=0.079 in women).

Discussion

In the present study, we showed that higher BMI was significantly associated with lower plasma BNP levels in a general Japanese population. The finding is not attributable to underlying differences in cardiovascular risk factors between obese and non-obese subjects. We also showed that the inverse association between body fat mass, skin fold thickness and waist circumferences and BNP. This is the first report that analyzes the relations between BNP levels and various obesity related factors.

Several studies, including large, population-based cohorts [7, 8], have demonstrated that BMI was inversely correlated with BNP levels in patients with heart failure [9-12]. In the Dallas Heart Study [8], they focused on the body composition instead of BMI and showed an inverse association between plasma BNP and lean mass. However, these studies were conducted mostly in Western countries, where BMI is much higher than in other parts of the world.

In this study, where the average BMI levels (around 23 kg/m^2) was much lower in comparison with the general Western population (around 28 kg/m^2) [8], the association between higher BMI and lower BNP levels was observed after multivariable adjustment. Furthermore, we divided adiposity into its fat and lean mass components and found that fat mass was responsible for the association between higher BMI and lower BNP levels.

As it was already suggested, the natriuretic peptide system and adiposity are closely linked [18, 19]. Natriuretic peptide clearance receptors (NPR-C) are abundant in adipose tissue [18], and thus, it is suggested that adipocytes participate in a removal of BNP from circulation, which leads to the lower plasma BNP levels in obese patients. Furthermore, the Framingham Heart Study [7] showed that obese in-

Table 2 Multivariable linear models of plasma log BNP.

Models	Men		Women	
	β-coefficient (SE)	*p* value	β-coefficient (SE)	*p* value
Continuous BMI, per 1kg/m^2	-0.022 (0.005)	<0.001	-0.021 (0.004)	<0.001
BMI categories				
BMI < 18.5	Referent	-	Referent	-
18.5≦BMI<22	-0.087 (0.033)	0.009	-0.050 (0.019)	0.008
22≦BMI<25	-0.122 (0.032)	<0.001	-0.109 (0.019)	<0.001
25≦BMI	-0.148 (0.034)	<0.001	-0.112 (0.021)	<0.001

The multiplicative effect on plasma BNP levels can be estimated by exponentiating the β-coefficient. For instance, highest BMI (BMI ≦ 25) is associated with a 29% reduction in BNP levels in men, because $10^{(-0.148)} = 0.71$. All models are adjusted age, systolic blood pressure, pulse rate, serum creatinine, and left ventricular hypertrophy.

Table 3 Influence of BMI on odds of having normal plasma BNP levels (< 18.4 pg/mL).

BMI categories	Men		Women	
	Odds ratio (95% CI)	*p* value	Odds ratio (95% CI)	*p* value
BMI < 18.5	1.00 (referent)	-	1.00 (referent)	-
18.5≦BMI<22	1.66 (1.10-2.56)	0.019	1.33 (1.03-1.74)	0.033
22≦BMI<25	2.08 (1.38-3.19)	0.001	1.99 (1.52-2.63)	<0.001
25≦BMI	2.25 (1.47-3.51)	<0.001	2.13 (1.59-2.88)	<0.001
BMI (continuous), per 1kg/m^2	1.11 (1.04-1.18)	0.001	1.16 (1.11-1.23)	<0.001

Multivariable logistic regression models for low BNP levels are adjusted for age, systolic blood pressure, pulse rate, serum creatinine, and left ventricular hypertrophy.

Table 4 Multivariable associations between obesity related factors and BNP.

Models	Men		Women	
	β-coefficient (SE)	*p* value	β-coefficient (SE)	*p* value
Model 1				
BMI	-0.002 (0.005)	<0.001	-0.021 (0.004)	<0.001
Model 2				
Percent of body fat	-0.012 (0.003)	<0.001	-0.011 (0.002)	<0.001
Model 3				
Body fat mass	-0.014 (0.003)	<0.001	-0.013 (0.003)	<0.001
Lean body mass	0.004 (0.003)	0.188	0.007 (0.004)	0.079
Model 4				
Skinfold thickness	-0.007 (0.002)	<0.001	-0.004 (0.001)	0.001
Model 5				
Waist circumference	-0.006 (0.002)	<0.001	-0.006 (0.001)	<0.001

The multiplicative effect on plasma BNP levels can be estimated by exponentiating the β-coefficient. All models are adjusted for age, systolic blood pressure, pulse rate, serum creatinine, and left ventricular hypertrophy.

dividuals had higher odds of having low plasma N-terminal proANP. In the Dallas Heart Study [8], the association between higher BMI and lower NT-proBNP was observed. Since both N-terminal proANP and N-terminal proBNP are not cleared by clearance receptors, the findings of reduced N-terminal proANP levels and N-terminal proBNP levels in obese individuals indicate the mechanism other than the adipocyte clearance of the peptides exists. Recent investigations have raised the possibility that the relation between fat and BNP is bidirectional. Adipocytes also express natriuretic peptide receptor-A (NPR-A), which mediate the biologic effects of ANP and BNP [18]. Investigators have demonstrated activation of NPR-A on adipocytes induces lipolysis [19]. Thus, low BNP levels may lead to reduced lipolysis, additionally perpetuating the obese state.

Several limitations of our study deserve comment. First, since our study was cross-sectional study, we cannot demonstrate the cause-effect relation between low plasma BNP and obesity related factors. Second, plasma BNP levels are under the calculable levels of the assay detection limits in 9.0% of all subjects. Misclassification of BNP levels above and below the detection limit would be expected to cause a conservative bias. To overcome the potential bias, we also used logistic regression analyses to account for the left censoring of the BNP distribution. Finally, we cannot exclude the possibility that obese individuals might have had better cardiac function. However, since many previous studies suggest that obesity has been consistently associated with left ventricular hypertrophy [20, 21], dilatation [22] and the increase in the risk of overt heart failure [23], the possibility is highly unlikely.

In conclusion, higher BMI was associated with lower BNP levels in a general Japanese population, even after adjusted for relevant factors. Body fat mass was responsible for this relationship. Further studies will be needed to explore the underlying mechanism.

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ORIGINAL ARTICLE

The combined impact of blood pressure category and glucose abnormality on the incidence of cardiovascular diseases in a Japanese urban cohort: the Suita Study

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Few prospective studies have examined the combined impact of blood pressure (BP) categories and glucose abnormalities on the incidence of cardiovascular disease (CVD) in the general Asian population. This study aimed to examine the effect of the combined risks of these factors on the incidence of CVD in a general Japanese population. We studied 5321 Japanese individuals (aged 30–79 years), without CVD at baseline, who received follow-up for an average of 11.7 years. Serum fasting glucose categories were defined according to the 2003 American Diabetes Association recommendations. BP categories were defined by the 2009 Japanese Society of Hypertension Guidelines for the Management of Hypertension. The Cox proportional hazard ratios (HRs) for CVD according to the serum glucose and BP categories were calculated. In 62 036 person-years of follow-up, we documented 364 CVD events (198 stroke and 166 coronary heart disease (CHD)). Compared with normoglycemic subjects, the multivariable HRs (95% confidence intervals (CIs)) for CVD, CHD and stroke were 1.25 (1.00–1.58), 1.46 (1.04–2.04) and 1.11 (0.81–1.52), respectively, in individuals with impaired fasting glucose (IFG), whereas these values were 2.13 (1.50–3.03), 2.28 (1.34–3.88) and 2.08 (1.29–3.35), respectively, in individuals with diabetes mellitus (DM). Compared with normoglycemic and optimal blood pressure (BP) subjects, increased risks of CVD were observed in the normoglycemic subjects with high-normal BP or hypertension, the IFG subjects with normal or higher BP, and the DM subjects regardless of BP category (*P*-value for interaction=0.046). In conclusion, the high-normal BP subjects in all glucose categories and the normal BP subjects with IFG showed increased risk of CVD in this Japanese population. Further investigation of larger cohorts of DM subjects should be conducted to better understand this phenomenon.
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Keywords: blood pressure category; cardiovascular disease; cohort study; diabetes mellitus; impaired fasting glucose

INTRODUCTION

Hypertension is one of the strongest risk factors for increased incidence of cardiovascular disease (CVD) worldwide.[1–3] Recently, high-normal blood pressure (BP)[1,2] and prehypertension[3] have also been recognized as risk factors for CVD.[4–6] Increased BP is the most likely precipitator of CVD and stroke.[5,7,8] Furthermore, the prevalence of glucose intolerance and obesity has increased greatly in recent years.[9,10] Diabetes mellitus (DM) has become a major public health problem[11,12] as well as a risk factor for all-cause mortality[11] and CVD.[10,13–15] Recently, prediabetic hyperglycemia has been recognized to confer an increased risk for CVD.[16] However, a few population studies[17] have reported a positive association between CVD and impaired fasting glucose (defined as blood glucose of 5.6–6.9 mmoll^{-1} according to the 2003 American Diabetes Association definition).[18]

Evaluation of the combined impact of these two major borderline risk factors is essential in preventing CVD because elevated BP is the highest population attributable fraction (PAF) of CVD incidence, and the incidence of hyperglycemia is increasing in Asian and Western countries. There have been a few population studies on the association between the occurrence of hypertension together with DM and the risk of stroke[19–21] and coronary heart disease (CHD).[22] However, few population cohort studies have evaluated the impact of the combination of BP categories (optimal BP, normal BP, high-normal BP (or prehypertension) and hypertension) and fasting glucose categories (normoglycemia, impaired fasting glucose (IFG) and DM) on the risk

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of CVD. Thus, the aim of this study was to examine the combined impact of BP categories and blood glucose abnormalities on the incidence of CVD in a general urban Japanese population.

METHODS

Study subjects

The Suita Study, a cohort study for CVD in urban residents, was established in 1989. The details of this study have been described elsewhere.[5,23–29] Briefly, 6485 individuals (aged 30 to 79 years) underwent regular health checkups between September 1989 and March 1994. Some cohort members were excluded for the following reasons: past or present history of CVD at baseline (n=208); missing data (n=170); nonfasting blood collections (n=173); or lost from follow-up (n=613). After applying these exclusions, a total of 5321 subjects (aged 30 to 79 years) participated in the baseline examination. Informed consent was obtained from all participants. This study was approved by the institutional review board of the National Cardiovascular Center.

Measurement of BP and fasting glucose

Measurement of BP has been described elsewhere.[5] In brief, well-trained physicians measured the BP of each individual three times in a seated position using a mercury column sphygmomanometer, an appropriately sized cuff and a standard protocol. Before the initial BP reading was obtained, participants were seated at rest for at least 5 min. Systolic (SBP) and diastolic (DBP) blood pressures were recorded as the average of the second and third measurements, which were taken more than 1 min apart.

At the time of the baseline examination, subjects were classified into one of the following BP categories based on the 2009 Japanese Society of Hypertension Guidelines for the Management of Hypertension:[2] optimal BP (SBP, $<$120 mm Hg and DBP, $<$80 mm Hg); normal BP (SBP, 120 to 129 mm Hg and DBP, 80 to 84 mm Hg); high-normal BP (SBP, 130 to 139 mm Hg and DBP, 85 to 89 mm Hg); and hypertension (SBP, $\geqslant$140 mm Hg or DBP, $\geqslant$90 mm Hg or antihypertensive drug use). If the SBP and DBP readings for a subject were in different categories, then the subject was categorized into the higher of the two categories.

We performed routine fasting blood collection and immediately measured serum glucose and total cholesterol levels using the same autoanalyzer (Toshiba TBA-80). Fasting serum glucose categories were defined as follows:[18] DM (fasting serum glucose $\geqslant$7.0 mmol l^{-1} (126 mg per 100 ml) or medications for DM); IFG (fasting serum glucose levels 5.6 to 6.9 mmol l^{-1} (100 to 125 mg per 100 ml)); and normoglycemia (fasting serum glucose levels $<$5.6 mmol l^{-1} ($<$100 mg per 100 ml)). Hypercholesterolemia was defined as total serum cholesterol levels $\geqslant$5.7 mmol l^{-1} (220 mg per 100 ml) or current use of antihyperlipidemic medications. Physicians or nurses administered questionnaires addressing personal habits and present illness at the baseline examination. Body mass index was calculated as weight (kg) divided by height (m) squared.

Confirmation of stroke and coronary heart disease and end point determination

Medical records were reviewed by registered hospital physicians or research physicians who were blinded to the baseline data. Strokes were defined according to the US National Survey of Stroke criteria.[30] For each stroke subtype (that is, cerebral infarction (thrombotic or embolic infarction), intracerebral hemorrhage and subarachnoid hemorrhage), a definite diagnosis was established based on examination of computed tomographic scans, magnetic resonance images or autopsies. Definite and probable myocardial infarctions were defined according to the criteria set out by the MONICA project.[31] The criteria for a diagnosis of CHD included first ever acute myocardial infarction, sudden cardiac death within 24 h after the onset of acute illness or coronary artery disease followed by coronary artery bypass surgery or angioplasty. In this study, CVD was defined as stroke or CHD.

To detect CHD and stroke occurrences, each participant's health status was checked during clinical visits to the National Cardiovascular Center every 2 years. Yearly questionnaires by mail or telephone were also completed by all participants. In addition, to complete our surveillance for fatal strokes and CHD, we conducted a systematic search for death certificates. All data were checked against medical records to confirm the incidence of CVD. When informed consent could not be obtained for a medical records survey (19.5%), we identified possible strokes or CHD using information from (1) questionnaires for present illness of stroke and CHD at the health examination and/or (2) death certificates bearing a diagnosis of probable stroke or CHD. The end point of the follow-up period for each participant was, whichever of the following options occurred first: (1) date of the first diagnosis of CHD or stroke event; (2) date of death; (3) date of leaving Suita; or (4) 31 December, 2005.

Statistical analysis

Analyses of variance and χ^2-tests were used to compare mean values and frequencies. The Cox proportional hazard ratios (HRs) and 95% confidence intervals (95% CIs) were fitted to each glucose category (normoglycemia, IFG and DM) after adjusting for sex and age in 5-year increments as stratified variables and other potential confounding factors at baseline, including BP category (optimal, normal, and high-normal BP and hypertension), hypercholesterolemia (positive or negative), body mass index (continuous variable), smoking status (never, ex-smoker and current smoker) and drinking status (never, ex-drinker and current drinker). Test for effect modification by glucose category was conducted with an interaction term generated by multiplying BP category by glucose category. We conducted tests for trend across the BP categories and tested the significance of this variable.

To express the combined impact of glucose and BP categories on the incidence of CVD in these participants, we estimated the PAF as follows:

$$PAF = Pe \times (HR - 1)/HR,$$

where Pe is the proportion of incident cases in the combination of glucose and BP categories, and HR is the multivariable-adjusted hazard ratio.[32] All statistical analyses were conducted using the SAS statistical software package (release version 8.2; SAS Institute, Cary, NC, USA).

RESULTS

The frequencies of IFG and DM increased with age in both men and women (Figure 1). Table 1 shows the distribution of CVD risk factors at baseline according to fasting glucose categories at baseline. Both men and women with DM were older and had a higher body mass index as well as a higher prevalence of hypertension, hypercholesterolemia and medication for hypertension than those without DM. Men with DM had a lower frequency of never drinking than men without DM.

In 62 036 person-years of follow-up (an average of 11.7 years of follow-up), we documented 364 CVD (198 strokes and 166 CHD) events. Table 2 shows the age- and sex-adjusted HRs and multivariable-adjusted HRs for incidence of CVD according to glucose categories in men and women. Compared with normoglycemic subjects, the multivariable HRs (95% CIs) for CVD, CHD and stroke were 1.25 (1.00–1.58), 1.46 (1.04–2.04) and 1.11 (0.81–1.52), respectively in IFG subjects, whereas these values were 2.13 (1.50–3.03), 2.28 (1.34–3.88) and 2.08 (1.29–3.35), respectively in DM subjects. Compared with normoglycemic subjects, IFG and DM were risk factors for CVD and CHD in women, and DM was a risk factor for CVD and stroke in men.

Figure 2 shows the multivariable HRs of CVD for the combined impact of the fasting glucose and BP categories. Compared with normoglycemic subjects with optimal BP, the following groups showed increased risk of CVD: the normoglycemic subjects with high-normal BP or hypertension (P-value for trend of BP category$<$0.001); the IFG subjects with normal or higher BP (P-value for trend of BP category=0.001); and the DM subjects in any BP category (P-value for trend of BP category=0.41). After excluding subjects taking diabetic medication, the P-value for the BP category trend was not statistically significant in the DM subjects.

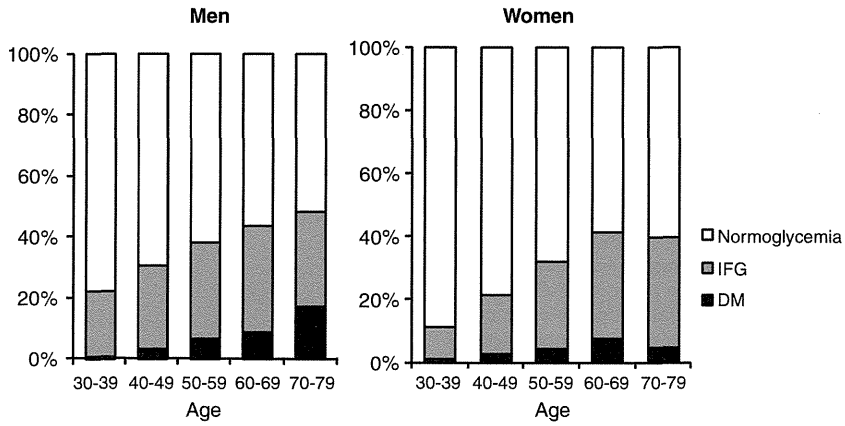

Figure 1 Frequency of type 2 diabetes mellitus according to sex and age.

Table 1 Baseline characteristics of study subjects according to fasting glucose categories at baseline

	Men				*Women*			
	Normoglycemia	*IFG*	*DM*	P-*value*	*Normoglycemia*	*IFG*	*DM*	P-*value*
Number of subjects, *n*	1458	874	154	—	2126	611	98	—
Age, in years	54±14	57±12	60±10	<0.001	52±13	59±11	60±10	<0.001
Body mass index, kg m^{-2}	22.5±2.8	23.3±2.9	23.3±3.2	<0.001	21.8±3.0	23.1±3.4	24.5±4.2	<0.001
Blood pressure category, %[a]				<0.001				<0.001
Optimal blood pressure	37	24	20		49	23	17	
Normal blood pressure	19	19	17		16	16	17	
High-normal blood pressure	16	19	14		13	18	15	
Hypertension	28	39	49		21	43	51	
Hypercholesterolemia, %[b]	26	33	36	<0.001	38	54	59	<0.001
Medication, %								
Hypertension	10	12	18	0.002	8	16	22	<0.001
Diabetes	—	—	36	—	—	—	38	—
Smoking status, %				0.156				0.325
Current	55	51	50		13	10	11	
Quit	25	29	32		3	3	4	
Never	19	20	18		84	87	85	
Drinking status, %				<0.001				0.330
Current	76	77	76		34	32	24	
Quit	2	2	9		1	1	2	
Never	22	20	15		65	67	74	

Abbreviations: DM, diabetes mellitus; DBP, diastolic blood pressure; IFG, impaired fasting glucose; SBP, systolic blood pressure.
Normoglycemia: fasting glucose levels <5.6 mmol l^{-1}; IFG: fasting glucose levels 5.6 to 6.9 mmol l^{-1}; DM: fasting glucose levels ⩾7.0 mmol l^{-1} or medication for diabetes.
[a]Blood pressure category was based on the ESH–ESC 2007 guidelines: optimal (SBP <120 mm Hg and DBP <80 mm Hg), normal blood pressure (SBP 120–129 mm Hg and DBP 80–84 mm Hg), high-normal blood pressure (SBP 130–139 mm Hg and DBP 85–89 mm Hg) and hypertension (SBP ⩾140 mm Hg or DBP ⩾90 mm Hg or antihypertensive drug use).
[b]Hypercholesterolemia: antilipidemic drug user or total cholesterol ⩾5.7 mmol l^{-1} ± values are the means±s.d.'s.

The significant interaction terms between fasting blood glucose and BP categories were observed in CVD (P=0.046); however, the interaction term was not significant after exclusion of DM subjects.

Q3 Using the HRs, we estimated the PAF of CVD to exposure to the combined impact of fasting glucose and BP categories at baseline (Figure 3). The population-attributable risk percentage for CVD incidence was estimated at 3.7% for subjects with normoglycemia and high-normal BP, 5.7% for subjects with IFG and normal or high-normal BP group and 8.2% for subjects with DM and any BP category group, when comparing these groups with the normoglycemic and optimal BP group.

DISCUSSION

In this population cohort study, we found that DM was a risk factor for CVD, stroke and CHD, whereas an IFG of 5.6 to 6.9 mmol l^{-1} was a risk factor for CVD and CHD only. A combined effect of IFG

Table 2 Age- and multivariable-adjusted hazard ratios (95% confidential intervals) for cardiovascular disease according to blood glucose category

	Blood glucose category			
	Normoglycemia	*IFG*	*Diabetes*	*P-value for trend*
Men and women, number	3584	1485	252	
Person-years, in years	42701	16741	2594	
Cardiovascular disease				
Case	184	139	41	
Age and sex-adjusted	1	1.34 (1.07–1.68)	2.45 (1.73–3.45)	<0.001
Multivariable-adjusted	1	1.25 (1.00–1.58)	2.13 (1.50–3.03)	<0.001
Coronary artery disease				
Case	78	70	18	
Age and sex-adjusted	1	1.54 (1.10–2.13)	2.53 (1.51–4.25)	<0.001
Multivariable-adjusted	1	1.46 (1.04–2.04)	2.28 (1.34–3.88)	0.001
Stroke				
Case	106	69	23	
Age and sex-adjusted	1	1.21 (0.89–1.65)	2.51 (1.58–3.96)	<0.001
Multivariable-adjusted	1	1.11 (0.81–1.52)	2.08 (1.29–3.35)	0.016
Men, number	1,458	874	154	
Person-years, years	16,901	9844	1560	
Cardiovascular disease				
Case	107	91	25	
Age-adjusted	1	1.19 (0.90–1.58)	1.93 (1.25–2.99)	0.007
Multivariable-adjusted	1	1.13 (0.85–1.51)	1.75 (1.12–2.73)	0.032
Coronary artery disease				
Case	50	50	11	
Age-adjusted	1	1.39 (0.93–2.06)	1.89 (0.98–3.64)	0.027
Multivariable-adjusted	1	1.31 (0.87–1.96)	1.69 (0.86–3.31)	0.077
Stroke				
Case	57	41	14	
Age-adjusted	1	1.01 (0.68–1.52)	2.00 (1.11–3.61)	0.103
Multivariable-adjusted	1	0.97 (0.64–1.46)	1.78 (1.00–3.12)	0.216
Women, number	2,126	611	98	
Person-years, in years	25,800	6897	1033	
Cardiovascular disease				
Case	77	48	16	
Age-adjusted	1	1.62 (1.12–2.33)	3.70 (2.14–6.40)	<0.001
Multivariable-adjusted	1	1.49 (1.02–2.16)	3.07 (1.73–5.45)	<0.001
Coronary artery disease				
Case	28	20	7	
Age-adjusted	1	1.86 (1.04–3.25)	4.62 (1.99–10.72)	<0.001
Multivariable-adjusted	1	1.83 (1.01–3.32)	4.32 (1.81–10.31)	<0.001
Stroke				
Case	49	28	9	
Age-adjusted	1	1.53 (0.96–2.45)	3.54 (1.71–7.29)	<0.001
Multivariable-adjusted	1	1.36 (0.84–2.19)	2.66 (1.22–5.80)	0.018

Abbreviations: DM, diabetes mellitus; IFG, impaired fasting glucose.
Multivariate analyses were adjusted for age, body mass index, hypertension, hyperlipidemia and smoking and drinking status.
Blood glucose categories: Normal, fasting glucose levels <5.6 mmol l^{-1}; IFG, fasting glucose levels 5.6–6.9 mmol l^{-1}; DM, fasting glucose levels ⩾7.0 mmol l^{-1} or medication for diabetes.

and prehypertension on the incidence of CVD was observed. The high-normal BP subjects in any glucose category and the normal BP subjects with IFG in the Japanese population showed increased risks of CVD. To our knowledge, this study is the first on the combined impact of these borderline risk factors, IFG and prehypertension on the incidence of CVD in a general Asian population.

Previous cohort studies have shown that DM is a risk factor for CVD, stroke[14,15] and CHD.[13] The results of our study are also essentially compatible with the previous cohort studies in Japan. The Hisayama Study demonstrated that glucose intolerance for 2421 participants was a risk factor for increased incidence of stroke and CHD.[15] Iso *et al.*[20] reported that glucose abnormalities were a risk factor for ischemic stroke in a Japanese population by using nonfasting glucose levels. The NIPPON DATA 80 Study indicated that DM, defined by nonfasting blood glucose levels, was a risk factor for CVD mortality.[33] In the Funagata Diabetes Study, IFG was not a risk factor

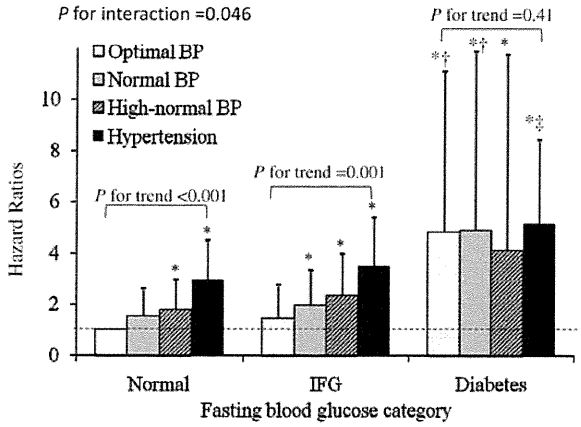

Figure 2 The influence of fasting glucose and BP categories on multivariable-adjusted hazard ratios and 95% confidence intervals for the incidence of cardiovascular disease. $^{*}P<0.05$, compared with normoglycemic subjects with normal BP. $^{\dagger}P<0.05$, compared with normoglycemic subjects in the same BP category. $^{\ddagger}P<0.05$, compared with normoglycemic subjects with hypertension.

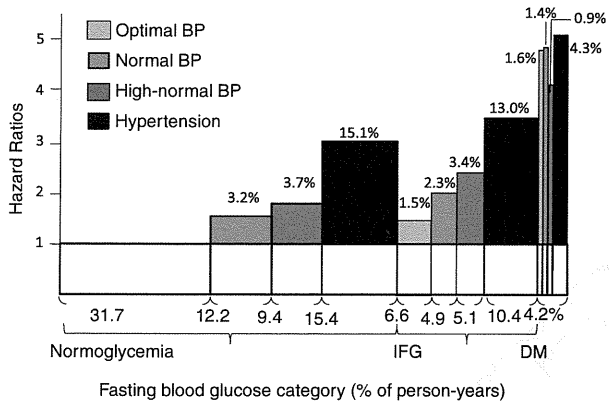

Figure 3 The hazard ratios and positive fractions attributable to exposure to the combined impact of glucose (normoglycemia, impaired fasting glucose and diabetes) and blood pressure categories (optimal, normal, and high-normal blood pressures and hypertension) at baseline for CVD were estimated. The gray and black areas represent excessive incidence of CVD in the high blood glucose and high blood pressure categories compared with the subjects with normoglycemia and optimal blood pressure as a reference.

for CVD mortality, although impaired glucose tolerance was a risk factor for CVD.[34]

Compared with previous studies, our study has several methodological strengths. First, our cohort population was relatively large and was selected at random from an urban population in contrast to most other cohort populations in Asia, which were selected from rural populations.[15,20,34] Second, all of our cohort participants were examined at one place and measured using the same autoanalyzer at one laboratory. Finally, our study examined the risk of CVD incidence, not CVD mortality.

In our study, we used the definitions of IFG and CVD/CHD set forth by the 2003 American Diabetes Association recommendations. In the Framingham Heart Study, the 2003 IFG definition was predictive of CHD in women but not in men,[17] a finding which was similar to our results. However, fewer studies have examined the association of the 2003 IFG definitions for CHD and stroke. Kanaya *et al.*[35] showed that the 2003 defination for IFG was not associated with increased risk of CHD or stroke among postmenopausal women with coronary artery disease.Kim *et al.*[36] reported that one-third of the population has IFG according to the 2003 definition. However, many of these individuals do not have increased prevalence of CHD.

Hu *et al.*[19] reported that hypertension and DM increased stroke risk independently and that their combination additively increased stroke risk. In our study, the risks of CVD in the normoglycemic and IFG groups were linearly related to the BP category (*P*-value for trend <0.001). However, the risks of CVD in the DM group did not change with BP category (*P*-value for trend=0.4), which was compatible with a previous result for trends between glucose category and hypertension status.[20] Recently, the ACCORD BP Study has shown that targeting an SBP <120 mm Hg, as opposed to an SBP <140 mm Hg, did not reduce the rate of a composite outcome of fatal and nonfatal major cardiovascular events in patients with type 2 diabetes.[37] Although present studies suggest that decreasing BP may be an effective way to prevent CVD in normoglycemic or IFG subjects, further investigations are required to clarify the interaction between the BP categories of DM subjects at risk for CVD in other large cohorts.

The percentage of the PAF for CVD incidence in normoglycemic subjects with high-normal BP or IFG subjects with normal or high-normal BP (PAF=12.6%) was 1.5 times higher than that in the DM subjects in any BP category (PAF=8.2%). Also, the PAF suggested that 12.6% of CVD cases would be preventable if the borderline glucose and blood pressure levels were controlled to within normoglycemic and optimal BP ranges.

Our results showed that hyperglycemia conferred a slightly higher risk of CVD incidence in women than in men, although men had greater absolute event rates for CVD. Previous studies have shown that the impact of DM on the risk of CVD is significantly greater in women than in men.[13,17,38] Lee *et al.* reported that the HRs of coronary heart disease for DM were 2.6 for women and 1.9 for men. In the Framingham Heart Study, [17] IFG was associated with increased CHD risk only in women (HR=1.7; 95% CI, 1.0–3.0). The reason for these sex differences in the association between DM and CVD remains unclear.

Our study has several limitations. The primary limitation is the regression dilution bias; this study was based on a single day measurement of serum glucose and BP levels.[39] That is, the fasting serum glucose and BP levels might have been misclassified. Second, as we did not perform glucose tolerance tests, we may have missed subjects with impaired glucose tolerance. Finally, we did not examine the combined effect of BP categories and glucose abnormalities after stratification by CVD subtypes, such as stroke and CHD because of the small sample size.

In conclusion, DM is a risk factor for CVD, stroke, and CHD, whereas an IFG of 5.6 to 6.9 mmoll^{-1} is a risk factor for CVD and CHD in women. The risks of CVD in the normoglycemic and IFG groups were linearly related to the BP category. The high-normal BP subjects in any glucose categories and the normal BP subjects with IFG showed increased risks of CVD in this Japanese population. Further investigations of larger cohorts of DM subjects are needed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Original Article

Impact of Statin Treatment on the Clinical Fate of Heterozygous Familial Hypercholesterolemia

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Aim: **Familial hypercholesterolemia (FH) patients are at particular risk for premature coronary artery disease (CAD) caused by high levels of low density lipoprotein (LDL). Administration of statins enabled us to reduce LDL-C levels in heterozygous FH patients. To evaluate the impact of statins on the clinical fate of heterozygous FH, a retrospective study was performed.**
Methods: **We analyzed the clinical influence of statins on age at the first clinical onset of CAD in 329 consecutive FH patients referred to the lipid clinic of the National Cardiovascular Center. Among 329 heterozygous FH patients, the onset of CAD was identified in 101.**
Results: **The age at onset of CAD was 58.8 ± 12.5 years in the 25 patients on statins at onset, significantly higher than that in the 76 patients not on statins (47.6 ± 10.5 years) ($p<0.001$). The average age at CAD onset was significantly higher after widespread use of statins (54.2 ± 13.2 years in 48 patients; Group 1) compared to before October 1989 when statins were approved in Japan (46.9 ± 9.6 years in 53 patients; Group 2, $p=0.002$). A significant difference was seen between Groups 1 and 2 in the variables, including sex, prevalence of smoking habit, LDL-C, and the use of statins, aspirin and probucol. After adjusting for these variables, only statin use was independently associated with the difference in age at CAD onset by multivariable analysis.**
Conclusion: **Statins have improved the clinical course of patients with heterozygous FH.**

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Key words; Familial hypercholesterolemia, Statin, Coronary artery disease, LDL cholesterol

Introduction

Familial hypercholesterolemia (FH) is a heritable disease of high prevalence with an autosomal-dominant mode of transmission and is linked to mutations in the low-density lipoprotein (LDL) receptor or its related gene. It is characterized by phenotypes of the elevation of plasma LDL, cutaneous and tendenous xanthomas, arcus corneae, and coronary artery disease (CAD) due to premature atherosclerosis[1)]. The earliest clinical sign of heterozygous FH is an elevation of plasma LDL cholesterol (LDL-C), noted as early as at birth[2)]. All other clinical manifestations seem due to an increase of LDL-C in plasma. CAD is the most serious clinical manifestation and determines the prognosis of FH. According to a previous report, Japanese FH heterozygotes generally develop the first CAD event in their 40s or later for men and 50s or later for

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women[3].

To reduce plasma LDL-C in FH heterozygotes, bile acid-sequestering resins have been used since the 1970s to upregulate the LDL receptor, but their effect is limited to a 10 to 20% decline because of the concomitant induction of hepatic cholesterol synthesis[4]. Statins, competitive inhibitors of a rate-limiting enzyme of cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, were introduced onto the market in the late 1980s. Pravastatin, the first approved statin in Japan, became commercially available at the beginning of October 1989 and simvastatin one year later[5]. Synthetic analogues became available in the late 1990s, including several "strong" statins, which lower the level of LDL-C by more than 40%[6]. Many large-scale clinical trials of statins worldwide, including Japan, showed that they reduced the risk of cardiac events or stroke in hypercholesterolemic populations[7-10]. Effective reduction of LDL-C by statins was also shown in FH heterozygotes[11, 12]; however, their clinical benefits in FH patients have not been clearly demonstrated with fixed clinical endpoints. This is partly because of the extremely high risk for CAD in FH patients, thus making controlled clinical trials of sufficient size to yield significant outcomes unethical.

Aim

Substantial numbers of FH patients have been referred to and regularly treated at the lipid clinic of the National Cardiovascular Center (NCVC) since it was founded in 1977. We therefore retrospectively analyzed the clinical records of these patients to assess the impact of the introduction of statins on the clinical prognosis of FH heterozygous patients, using patient age at the development of CAD. This parameter is specific and solid for each patient and the analysis is less influenced or biased by other factors. In addition, Mabuchi and colleagues used the same parameter in their study of Japanese FH reported before statin availability[13].

Methods

Subjects

Of the patients referred to the lipid clinic at NCVC from 1977 to 2007, 329 consecutive patients (139 men, 190 women) were diagnosed as FH heterozygotes using the criteria previously described[14]. Most of the FH patients analyzed in the present paper were referred to our lipid clinic by their general practitioner because of hypercholesterolemia. The medical records of patients were examined according to the analysis protocol approved by our institutional ethics committee (ID#M20-25-2). Of the 329 FH patients, 101 were identified as having CAD, specifically, coronary artery stenosis (more than 75%) on angiography, including 53 patients who had CAD at the first clinic visit. The other 228 patients did not have clinical or angiographic evidence of CAD. For each patient, the age at onset of CAD was determined by the first sign, ascertained by a standardized questionnaire, which included fixed clinical endpoints of CAD, administered by attending physicians at the clinic. The compliance with statins was evaluated from the medical records.

Clinical Risk Factors

Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared (kg/m^2). Hypertension was defined as the use of antihypertensive drugs or a blood pressure level higher than 140 mmHg systolic or 90 mmHg diastolic or both at the first clinic visit (the criteria for hypertension of the Japanese Society of Hypertension Guidelines)[15]. Diabetes mellitus was defined according to the 2002 Guideline for the Treatment of Diabetes Mellitus of the Japan Diabetes Society[16]. A family history of CAD was identified by the standardized questionnaire. Smoking was identified from patients' self-reporting. Achilles tendon thickness was measured as previously described[17].

Analysis of Serum Lipids

Fasting plasma lipid concentration was measured before any lipid-lowering treatment. Total cholesterol (TC), triglycerides (TG), and HDL cholesterol (HDL-C) levels were measured enzymatically using an automated system in the clinical laboratory of the NCVC. LDL-C level was calculated by the Friedewald formula when the TG level was less than 400 mg/dL; three patients with TG level more than 400 mg/dL were omitted from this particular analysis. TG values were expressed as the median, (range), and logarithmically transformed before analysis.

Statistical Analysis

Statistical analysis was performed using the SPSS 15.0 (SPSS Inc., Chicago, IL) program. Parametric values are expressed as the mean ± standard deviation (SD). The statistical significance of differences in continuous variables was evaluated by Student's t test for unpaired data or ANOVA. The Pearson's χ^2 test was used to assess differences in the distribution of categorical traits.

Table 1. Clinical characteristics of heterozygous FH patients with or without coronary artery disease (CAD) at first visit to our center.

	Total subjects	CAD (+)	CAD (−)	*p value*
n	329	101	228	
Age (years)	43.8±16.0	48.9±10.2	41.6±17.6	<0.001
Sex				
Men	139 (42.2%)	66 (65.3%)	73 (32.0%)	<0.001
BMI (kg/m^2)	22.0±3.2	23.0±2.7	22.6±3.3	<0.001
Total cholesterol (mg/dL)	319±70	333±85	313±61	0.039
Triglyceride (mg/dL)	(114) 80-176	(147) 96-193	(109) 76-162	0.263
HDL cholesterol (mg/dL)	50±17	42±14	54±17	<0.001
LDL cholesterol (mg/dL)	241±72	259±84	232±65	<0.001
Hypertension (*n*, %)	54 (16.4%)	33 (32.7%)	21 (9.2%)	<0.001
Diabetes Mellitus (*n*, %)	13 (4%)	8 (7.9%)	5 (2.2%)	0.014
Family history of CAD (*n*, %)	121 (36.8%)	46 (45.5%)	75 (32.9%)	0.028
Smoking habits (*n*, %)	127 (38.6%)	72 (71.3%)	55 (24.1%)	<0.001
Achilles tendon thickness (mm)	13.5±5.4	16.2±5.7	12.1±4.6	<0.001
CAD present at first visit (*n*, %)	53 (16.1)	53 (52.5)	0 (0)	<0.001
Statin treatment at first clinic visit	39 (11.9)	18 (17.8)	21 (9.2)	0.541

Values are shown as the mean ± SD except for triglyceride. For triglyceride, the median (range) is shown.
BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; CAD, coronary artery disease

Results

Patient Background

The baseline clinical characteristics of the 329 heterozygous FH patients analyzed in this study are shown in **Table 1**. Their plasma lipid and lipoprotein profiles are similar to patients in previous reports of Japanese FH[3, 18]. Patients with CAD were older, had higher levels of BMI, TC, and LDL-C, lower HDL-C, and a higher incidence of diabetes mellitus, hypertension, a family history of CAD, and smoking habit, compared to patients without CAD.

Onset of CAD

In the 101 patients with CAD, age by decade at the first onset of CAD is illustrated in **Fig. 1**. The average age was 45.8±10.6 years in men and 59.0±9.5 years in women, and this is consistent with a previous report of Japanese FH patients[13]. Analysis of CAD onset in relation to the presence (+) or absence (−) of statin treatment showed that in the 66 FH men with CAD, 13 did and 53 did not have statin treatment, and in the 35 FH women with CAD, 12 did and 23 did not have statin treatment. The age distribution at the first onset of CAD in statin (+) or statin (−) patients is shown in **Fig. 2**. The peak was at an older age in statin (+) men and women (Panels A and B, respectively) compared to statin (−). The lipid profile at the time of first onset of CAD in statin (+) and statin (−)

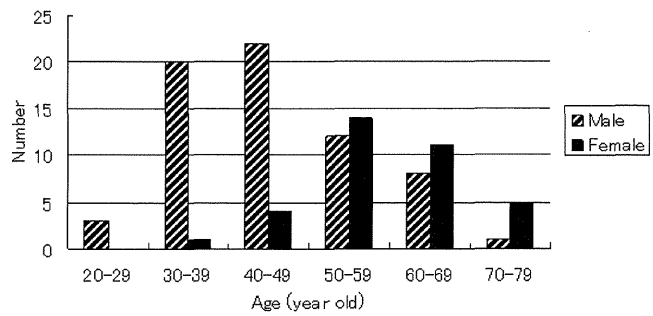

Fig. 1. Distribution of age when CAD was first identified in 101 men and women with heterozygous familial hypercholesterolemia (FH) and coronary artery disease (CAD), for the study period of 1969 to June 2007

patients is shown in **Table 2**. Statin (+) patients were older when CAD was identified and had lower TC and LDL-C levels than statin (−) patients.

To identify the factors that may influence the age at which CAD developed in statin (+) and statin (−) patients, we analyzed covariates (ANCOVA; **Table 3**), which included sex, smoking, BMI, hypertension, diabetes mellitus, family history of CAD, thickness of Achilles tendon, LDL-C levels, and the use of aspirin, probucol, and cholestyramine. We found that statin (+) patients were older when CAD developed, about 10 years older for each variable compared to statin (−) patients, which may be due to the use of statins and

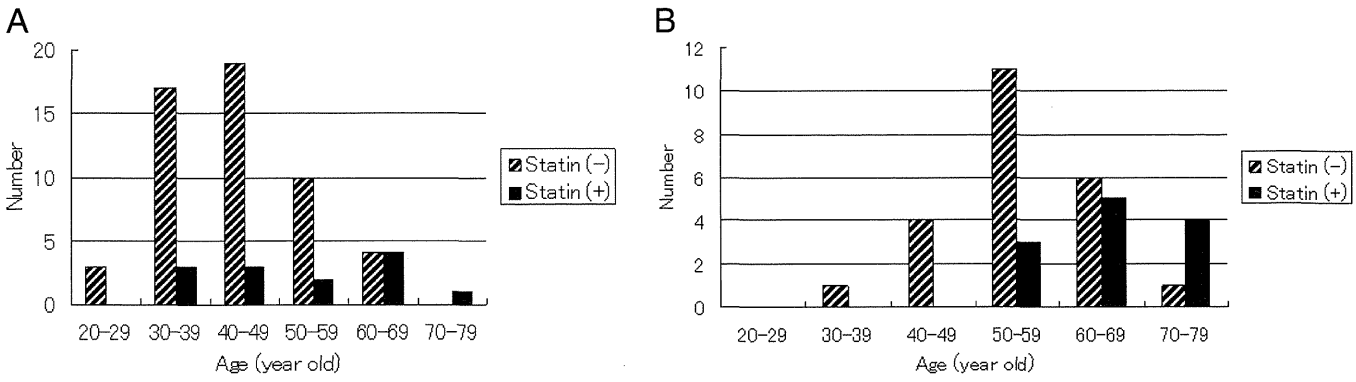

Fig. 2. Distribution of age when CAD was first identified in men (Panel A) and women (Panel B) with CAD taking a statin (+) or not (−)

Table 2. Age, lipid and lipoprotein profiles of FH at the onset of CAD in relation to statin use.

	Statin (+)	Statin (−)	*p value*
n	25	76	
Age of onset of CAD	57.8 ± 12.5	47.6 ± 10.5	<0.001
Lipid and lipoprotein profile at the event			
Total cholesterol (mg/dL)	242 ± 55	315 ± 108	<0.001
Triglycerides (mg/dL)	(127) 93-171	(115) 91-153	0.922
HDL cholesterol (mg/dL)	40 ± 12	38 ± 13	0.569
LDL cholesterol	167 ± 35	250 ± 108	<0.001

Values are shown as the mean ± SD except for triglyceride. For triglyceride, the median (range) is shown.

Table 3. Onset age of CAD adjusted by each variable.

Variables	Age (95% CI) in Statin (+)	Age (95% CI) in Statin (−)	*p value*
Overall	57.8 (55.3-60.3)	47.6 (46.4-48.8)	<0.001
Smoking habit	58.2 (54.1-62.3)	47.3 (44.8-49.7)	<0.001
Sex	57.2 (53.3-61.0)	48.1 (45.9-50.3)	<0.001
BMI	58.9(54.4-63.3)	47.5 (45.0-50.1)	<0.001
Hypertension	59.4 (54.8-64.4)	47.4 (44.8-49.9)	<0.001
Diabetes mellitus	58.7 (54.3-63.1)	47.7 (45.2-50.3)	<0.001
Family history of CAD	58.8 (54.4-63.2)	47.1 (44.6-49.7)	<0.001
Achilles tendon thickness	58.7 (54.3-63.2)	46.7 (44.0-49.4)	<0.001
LDL cholesterol	58.4 (53.9-63.0)	47.6 (45.0-50.3)	<0.001
Aspirin	57.2 (52.9-61.5)	48.2 (45.7-50.7)	0.001
Probucol	56.0 (51.0-61.0)	48.6 (46.0-51.3)	0.017
Cholestyramine	58.2 (53.0-63.3)	47.9 (45.2-50.6)	0.001

the reduction of LDL-C.

To determine the impact of statin treatment on the age at which CAD developed, we analyzed the same data for the pre- and post-statin eras. Pravastatin was the first statin approved in Japan. Patients were divided into two groups: Group 1 developed CAD before the end of September 1989 (n=53) and Group 2 developed CAD from October 1989 (to June 2007; n=48). Of the 66 men with CAD, 39 were in Group 1 and 27 in Group 2, and of the 35 women with CAD, 14 were in Group 1 and 21 in Group 2. The men and women whose CAD developed after the beginning of October 1989 were older than those who developed CAD before that date (**Fig. 3A, B**). At the

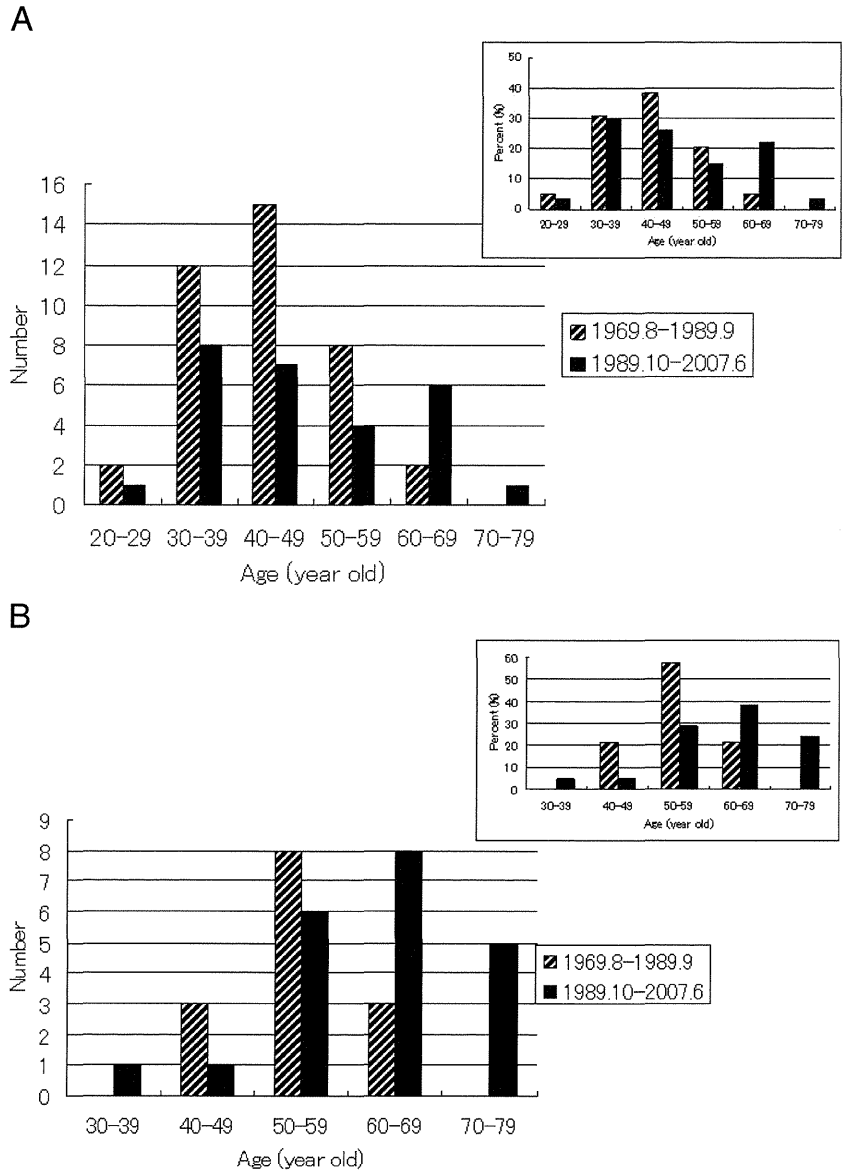

Fig. 3. Distribution of age at CAD onset in men (Panel A) and women (Panel B) who developed CAD before the end of September 1989 from October 1989

Each inset figure shows the percent of distribution, respectively.

first clinic visit, no clinical differences were seen in these patients in average age, BMI, plasma lipid and lipoprotein profile, Achilles tendon thickness and the incidence of hypertension, diabetes mellitus, and family history of CAD (**Table 4**); however, significantly more of the patients who developed CAD before the end of September 1989 were smokers. Assessment of clinical parameters obtained at the time CAD was identified shows that patients who developed CAD after the beginning of October 1989 were older (**Table 5**), reflecting the influence of statins on the onset age of CAD (**Fig. 3A, B**), and that TC and LDL-C levels were lower, reflecting that more of these patients were receiving lipid-lowering treatment than patients who developed CAD before this date.

Analysis of Factors that Affect Age at the First Onset of CAD

Age at the development of CAD in Groups 1 and 2 was analyzed using analysis of covariance (AN-

Table 4. Clinical characteristics (at first visit) of FH Patients depending on the onset date of CAD

	Group 1 1969–Sept. 1989	Group 2 Oct. 1989–June 2007	*p value*
n	53	48	
Age	48.4 ± 9.1	49.5 ± 11.4	0.584
Sex			
Male	39 (73%)	27 (56%)	0.068
BMI (kg/m^2)	22.6 ± 2.8	23.5 ± 2.6	0.288
Total cholesterol (mg/dL)	343 ± 84	321 ± 85	0.195
Triglycerides (mg/dL)	(114) 103–193	(148) 82–208	0.785
HDL cholesterol (mg/dL)	40 ± 15	44 ± 13	0.127
LDL cholesterol (mg/dL)	268 ± 80	250 ± 87	0.279
Hypertension (*n*, %)	21 (39.6%)	12 (25.0%)	0.118
Diabetes Mellitus (*n*, %)	2 (4%)	4 (8.3%)	0.535
Family history of CAD (*n*, %)	23 (43.4%)	25 (52.1%)	0.317
Smoking habits (*n*, %)	41 (83.7%)	31 (64.6%)	0.036
Achilles tendon thickness (mm)	16.0 ± 5.3	16.5 ± 6.1	0.710

Values are shown as the mean ± SD except for triglyceride. For triglyceride, the median (range) is shown.

Table 5. Age, lipid and lipoprotein profiles and medication of FH at the onset of CAD.

	Group 1 1969–Sept. 1989	Group 2 Oct. 1989–June 2007	*p value*
n	53	48	
Age of onset of CAD	46.9 ± 9.6	54.2 ± 13.2	0.002
Lipid and lipoprotein profile at the event			
Total cholesterol (mg/dL)	323 ± 100	267 ± 95	0.011
Triglycerides (mg/dL)	(119) 96–162	(121) 79–152	0.427
HDL cholesterol (mg/dL)	36 ± 13	41 ± 12	0.088
LDL cholesterol	257±100	199 ± 95	0.011
Medication, *n* (%)			
Statin	1 (2.0)	24 (50.0)	< 0.0001
Probucol	6 (11.8)	17 (35.4)	0.005
Cholestyramine	3 (5.7)	11 (22.9)	0.015
Aspirin	1 (2.0)	7 (14.6)	0.021
No medication	44 (83.0)	22 (45.8)	< 0.001

Values are shown as Mean ± SD except for triglyceride. For triglyceride, median (range) is shown.

Table 6. Onset age of CAD adjusted by each variable.

Variables	Age (95% CI) in Group 1	Age (95% CI) in Group 2	*p value*
Overall	46.9 (44.2–50.0)	54.2 (50.3–58.0)	0.002
Smoking habits	46.9 (43.7–50.0)	53.4 (50.2–56.5	0.005
Sex	47.9 (45.2–50.7)	53.1 (50.2–55.9)	0.013
LDL cholesterol	48.2 (44.2–52.3)	54.5 (50.8–58.2)	0.029
Statin	49.1 (45.8–48.3)	51.8 (48.3–55.4)	0.325
Aspirin	47.9 (44.8–51.0)	53.2 (50.0–56.4)	0.021
Probucol	48.1 (45.0–51.2)	53.0 (49.8–56.2)	0.034
Cholestyramine	47.6 (44.4–50.8)	53.6 (50.2–56.9)	0.013

COVA; **Table 6**). Significant differences between groups were seen for sex, prevalence of smoking, LDL-C, and the use of statins, aspirin and probucol. After adjusting for these variables, statin use was independently associated with age at the onset of CAD.

Discussion

The mortality rate for CAD is 11 times higher in heterozygous FH patients than in the general population; thus, prevention of CAD is the key therapeutic goal for these patients[14]. Treatment to reduce high levels of LDL-C in FH patients was limited before statins became available, and a clinically meaningful decrease in LDL-C levels was difficult to obtain. Pravastatin was first introduced onto the Japanese market at the beginning of October 1989 and thereafter, LDL-C reductions of 20% to 30%, even in FH heterozygous patients, became possible[19]. Recently, the risk of myocardial infarction in heterozygous FH was reported to be reduced by 76%, similar to the general population of the Netherlands[20]. In the present paper, we assessed the impact of statin use on the clinical prognosis of Japanese FH patients visiting our lipid clinic by retrospectively analyzing their clinical records. The use of statins delayed the first CAD event by about 7 years in FH patients whose first event occurred after the introduction of statins, compared to FH patients whose first event occurred prior to the introduction of statins.

In this study, 101 of 329 (30.6%) consecutive heterozygotes of FH had clinical evidence of CAD. The profile of CAD patients is similar to previous reports, that is, more men than women[3, 21, 22], and higher BMI, higher TC and LDL-C levels, lower HDL-C levels, and a higher incidence of hypertension, diabetes mellitus, family history of CAD, and smoking[3, 13, 23, 24].

The time span of our study allowed us to assess the impact on the development of CAD of the introduction of statins onto the Japanese market at the beginning of October 1989. Comparing clinical parameters at the first clinic visit in the patients whose CAD developed before the end of September 1989 with after that date, revealed that only smoking was different, perhaps reflecting the social trend against smoking (**Table 4**). In contrast, interesting differences between these groups were seen in relation to when they developed CAD. Patients who developed CAD prior to the introduction of statins were younger on average (46.9 years old) and had higher levels of TC and LDL-C (323 and 257 mg/dL, respectively). Two other prominent differences were the improved lipid-lowering drug regimens, including statins, cholestyramine, probucol, and aspirin, and a decline in the number of smokers. Notably, statin use was independently and significantly associated with age at CAD onset in the 101 FH patients on covariate analysis of factors known to affect the age of developing CAD. Besides these factors, many other factors should be considered for the potential influence on the onset age of CAD, such as the widespread recognition of FH and the regimen for the treatment of other risk factors, such as hypertension and diabetes mellitus. Nevertheless, we should conclude from this analysis that the use of statins is a major factor contributing to the improvement of the clinical prognosis of FH patients in Japan.

More recently, "strong" statins have become available, making it possible to reduce LDL-C levels to much lower levels compared to conventional statins in FH patients[25-27]. The possible impact of these stronger statins on delaying the development of CAD in FH patients will be of interest.

One diagnostic criterion for heterozygous FH in the existing guidelines is a family history of premature CAD[28-30]. However, our results suggest that this criterion may need to be reconsidered because of the proven ability of statin treatment to delay the development of CAD to an age similar to that in persons who do not have heterozygous FH.

We showed in this retrospective analysis that the development of CAD was delayed by about 7 years in FH patients whose CAD developed after the introduction of statins in Japan compared to those whose CAD developed before the current statin era.

Acknowledgments

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Original Article

Effect of Exercise Intervention on Endothelial Function and Incidence of Cardiovascular Disease in Patients with Type 2 Diabetes

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Aim: The effects of exercise intervention and to assess its long-term efficacy in preventing subsequent cardiovascular events in patients with type 2 diabetes were little known on randomized controlled trial.
Methods: Thirty-eight type 2 diabetic patients (21 men and 17 women) were assigned to either the exercise group ($n=21$) or the control group without exercise training ($n=17$) by simple randomization. The exercise training group was scheduled for aerobic and resistance exercise programs for 3 months. After the 3-month, we investigated endothelial function, insulin resistance, adipocytokines and inflammatory markers. The endothelial function was evaluated by examining a flow-mediated endothelium-dependent vasodilatation (FMD). Furthermore, we followed the incidence of cardiovascular events for 24 months.
Results: After 3-month, HbA1c was decreased significantly in both groups. FMD was increased from $7.3\pm4.7\%$ to $10.9\pm6.2\%$ only in the exercise group ($p<0.05$). Long-term follow-up data showed that the control group developed cardiovascular events more frequently than did the exercise group ($p<0.05$).
Conclusions: Exercise improves endothelial dysfunction independently of glycemic control and insulin sensitivity in patients with type 2 diabetes. The beneficial effects of 3-month exercise to reduce cardiovascular events persist for 24 months.

J Atheroscler Thromb, 2010; 17:828-833.

Key words; Exercise, Type 2 diabetes, Endothelial function, Cardiovascular events

Introduction

Physical exercise has been reported to reduce the incidence of diabetes[1, 2], and its modification has long been recommended as one of the three main components of diabetic treatments in addition to diet modification and medication[3]. It is well known that regular exercise produces beneficial effects on risk factors of atherosclerosis by improving glycemic control[4], insulin resistance[5], and dyslipidemia and hypertension[6], and contributes to reduce cardiovascular morbidity and mortality not only in the general population[7, 8] but also in patients with type 2 diabetes[9, 10]; however, the mechanisms by which exercise training prevents the progression of atherosclerotic diseases in type 2 diabetes are still unclear, and the effect of exercise on the prognosis of patients with type 2 diabetes remains uncertain.

The purpose of the present study was to investigate the effects of exercise intervention on endothelial function, insulin resistance, adipocytokines and inflammatory markers in patients with type 2 diabetes in a randomized controlled trial. To assess the long-term efficacy of exercise intervention on the prevention of atherosclerotic disorders, we examined the fol-

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low-up data of the participants after the exercise intervention period.

Methods

Study subjects and intervention protocol

Thirty-eight Japanese patients (21 men and 17 women) with type 2 diabetes, who were admitted to our hospital for treatment of diabetes from August 2002 to January 2004, participated in this study. Patients who had symptomatic coronary artery disease, proliferative diabetic retinopathy, overt proteinuria, autonomic disorder or orthopedic disorders were excluded from this study. The study design was approved by the ethics committee of the National Cardiovascular Center. All participants gave written informed consent.

Study participants were assigned to either the exercise training group (exercise group, $n=21$) or the group without exercise training (control group, $n=17$) by simple randomization without the permuted block method. Patients in the exercise group were scheduled for exercise programs 3–5 times weekly for 3 months, supervised by physiotherapists. Each session consisted of 10-min warming-up, 20-min aerobic dance, 20-min stationary bicycle riding, 20-min resistance training and 5-min cool-down. The training heart rate was determined according to Karvonen's equation ($k=0.6$)[11]. Patients in the control group did not take part in the exercise programs. Both groups received comparable dietary and medical intervention after registration for 3 months.

Clinical examination and measurement of exercise capacity and insulin sensitivity

Clinical and metabolic parameters, including exercise capacity and insulin sensitivity, were measured before and after the intervention period. Systolic and diastolic blood pressures were examined after a minimum of 10-min rest in a sitting position. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Blood samples were collected in the morning after a 12-hour overnight fast, and fasting plasma glucose, HbA1c, lipid profiles, serum adiponectin, leptin, and high-sensitivity C-reactive protein (CRP) levels were analyzed. Exercise capacity was determined by cardiopulmonary exercise testing, the details of which have been published elsewhere[11]. Insulin sensitivity was evaluated by the modified steady-state plasma glucose method (SSPG)[12].

Assessment of endothelial function by flow-mediated brachial artery dilatation

Endothelial function was studied by examining the brachial artery response to flow-mediated endothelium-dependent vasodilatation (FMD), according to the method described previously[13]. Briefly, participants were examined after 15-min rest in the fasting state. After baseline diameter of the right brachial artery was measured by ultrasound images, the pneumatic cuff placed around the right forearm was inflated to 220 mmHg to occlude the brachial artery. The cuff was kept inflated for 270 seconds, and then the diameter of the brachial artery was measured for 120 seconds after cuff deflation. FMD was calculated from baseline and maximum diameters of the brachial artery.

Long-term follow-up of clinical events of the diabetic patients

As a post-hoc study, most of the participants (exercise group, $n=21$; control group, $n=17$) were followed in our outpatient unit for 24 months after randomization. We accumulated the follow-up data for 24 months and determined new-onset cardiovascular events, including acute myocardial infarction, angina pectoris, cerebral infarction, and cerebral hemorrhage.

Statistical analysis

Analysis was based on the intention-to-treat principle. Data was expressed as the means ± S.D. To compare the two groups, we used two-tailed unpaired t tests for continuous variables. The differences between the baseline and after 3 months were evaluated by the two-tailed paired t test in each group. Additionally, we analyzed the incidence of cardiovascular events during 24 months after randomization by Kaplan-Meier analysis of the time to cardiovascular events according to exercise intervention. All analysis was conducted using JMP version 6.0 software (SAS Institute Inc., USA). *p values* below 0.05 were considered statistically significant.

Results

The clinical backgrounds of the two groups are shown in **Table 1**. The clinical and metabolic parameters at baseline except for SSPG were not significantly different between the two groups (**Table 2**), while SSPG at baseline in both groups indicated an insulin-resistant state. After the 3-month intervention, HbA1c and serum LDL cholesterol levels were decreased and serum HDL cholesterol and serum adiponectin levels were increased in both groups. BMI was significantly