**Table 4.** Linear regression analysis using HOMA-IR as dependent variable

| | | β | 95%CI | | Standardized β | p value |
|---|---|---|---|---|---|---|
| Current smoking - 0 cig./day (never smoker) | | | | | | |
| 0 g/day (never drinker) | BMI | 0.20 | 0.16 | 0.24 | 0.33 | <0.001 |
| | GGT | 0.07 | 0.03 | 0.11 | 0.12 | 0.001 |
| 1-19 g/day | BMI | 0.22 | 0.19 | 0.24 | 0.42 | <0.001 |
| | GGT | 0.04 | 0.02 | 0.05 | 0.12 | <0.001 |
| 20-39 g/day | BMI | 0.21 | 0.19 | 0.22 | 0.49 | <0.001 |
| | GGT | 0.03 | 0.02 | 0.05 | 0.13 | <0.001 |
| 40-59 g/day | BMI | 0.18 | 0.16 | 0.20 | 0.47 | <0.001 |
| | GGT | 0.04 | 0.03 | 0.04 | 0.20 | <0.001 |
| ≥60 g/day | BMI | 0.26 | 0.23 | 0.30 | 0.57 | <0.001 |
| | GGT | 0.02 | 0.01 | 0.04 | 0.13 | 0.001 |
| Current smoking - 1-9 cig./day | | | | | | |
| 0 g/day (never drinker) | BMI | 0.44 | 0.21 | 0.67 | 0.49 | <0.001 |
| | GGT | 0.13 | −0.02 | 0.28 | 0.22 | 0.084 |
| 1-19 g/day | BMI | 0.24 | 0.17 | 0.32 | 0.47 | <0.001 |
| | GGT | 0.07 | 0.01 | 0.12 | 0.18 | 0.016 |
| 20-39 g/day | BMI | 0.18 | 0.13 | 0.23 | 0.42 | <0.001 |
| | GGT | 0.01 | −0.02 | 0.04 | 0.05 | 0.396 |
| 40-59 g/day | BMI | 0.23 | 0.18 | 0.27 | 0.50 | <0.001 |
| | GGT | 0.01 | 0.00 | 0.03 | 0.11 | 0.049 |
| ≥60 g/day | BMI | 0.27 | 0.20 | 0.34 | 0.57 | <0.001 |
| | GGT | 0.04 | 0.02 | 0.06 | 0.26 | <0.001 |
| Current smoking - 10-19 cig./day | | | | | | |
| 0 g/day (never drinker) | BMI | 0.16 | 0.10 | 0.22 | 0.34 | <0.001 |
| | GGT | 0.27 | 0.19 | 0.36 | 0.40 | <0.001 |
| 1-19 g/day | BMI | 0.22 | 0.18 | 0.26 | 0.49 | <0.001 |
| | GGT | 0.08 | 0.05 | 0.12 | 0.22 | <0.001 |
| 20-39 g/day | BMI | 0.18 | 0.15 | 0.21 | 0.44 | <0.001 |
| | GGT | 0.00 | −0.01 | 0.01 | 0.02 | 0.583 |
| 40-59 g/day | BMI | 0.25 | 0.22 | 0.27 | 0.57 | <0.001 |
| | GGT | 0.02 | 0.01 | 0.03 | 0.16 | <0.001 |
| ≥60 g/day | BMI | 0.22 | 0.18 | 0.26 | 0.54 | <0.001 |
| | GGT | 0.03 | 0.01 | 0.04 | 0.17 | <0.001 |
| Current smoking - 20-39 cig./day | | | | | | |
| 0 g/day (never drinker) | BMI | 0.27 | 0.18 | 0.37 | 0.42 | <0.001 |
| | GGT | 0.17 | 0.04 | 0.30 | 0.20 | 0.010 |
| 1-19 g/day | BMI | 0.12 | 0.06 | 0.18 | 0.30 | <0.001 |
| | GGT | 0.07 | 0.00 | 0.14 | 0.16 | 0.036 |
| 20-39 g/day | BMI | 0.22 | 0.18 | 0.26 | 0.47 | <0.001 |
| | GGT | 0.04 | 0.02 | 0.07 | 0.16 | <0.001 |
| 40-59 g/day | BMI | 0.28 | 0.18 | 0.37 | 0.22 | <0.001 |
| | GGT | 0.02 | −0.02 | 0.06 | 0.03 | 0.395 |
| ≥60 g/day | BMI | 0.22 | 0.19 | 0.26 | 0.57 | <0.001 |
| | GGT | 0.01 | 0.00 | 0.01 | 0.15 | <0.001 |
| Current smoking - ≥40 cig./day | | | | | | |
| 0 g/day (never drinker) | BMI | −0.07 | −0.29 | 0.16 | −0.12 | 0.551 |
| | GGT | 0.41 | 0.18 | 0.64 | 0.80 | 0.002 |
| 1-19 g/day | BMI | −0.07 | −0.29 | 0.16 | −0.12 | 0.551 |
| | GGT | 0.41 | 0.18 | 0.64 | 0.80 | 0.002 |
| 20-39 g/day | BMI | 0.15 | 0.02 | 0.28 | 0.42 | 0.028 |
| | GGT | 0.10 | −0.15 | 0.34 | 0.17 | 0.425 |
| 40-59 g/day | BMI | 0.18 | 0.06 | 0.30 | 0.37 | 0.003 |
| | GGT | 0.01 | −0.02 | 0.03 | 0.07 | 0.559 |
| ≥60 g/day | BMI | 0.28 | 0.18 | 0.38 | 0.47 | <0.001 |
| | GGT | 0.05 | 0.03 | 0.07 | 0.42 | <0.001 |

Standardized β values are estimates resulting from analysis performed on into standardized variables. For the calculation of β values, BMI was subdivided into 1 kg/m$^2$ increments, and GGT into 10 IU/L increments. Age, BMI, and GGT were used as independent variables. BMI, body mass index; GGT, gamma-glutamyl transpeptidase.

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ditions, such as smoking, obesity, and hepatic steatosis[23, 24]. Evidence is accumulating that higher serum GGT levels may be associated with an increased incidence of cardiovascular events[5], metabolic syndrome and diabetes[8, 25, 26]; therefore, more attention has been paid recently to this liver enzyme. It is possible that the association between GGT and various disorders observed in previous studies may be mediated, in part, by enhanced insulin resistance in subjects with increased GGT levels.

Although mild to moderate alcohol consumption may increase GGT, it may improve insulin sensitivity[18, 27], leading to a reduction in the prevalence of metabolic syndrome[17]. This finding is in contrast to the observation that cigarette smoking will not improve insulin resistance, even in light smokers[14]. As alcohol consumption has opposite effects on GGT and insulin resistance, the mode of association between GGT and HOMA-IR might differ according to the drinking status; however, only a few studies have analyzed the relationship between GGT and insulin resistance in various drinking conditions. Yokoyama and colleagues reported that GGT is associated with increased insulin resistance in non-drinkers[28] and light drinkers, but not in heavy drinkers[29], a finding that supports the notion that the mode of association between GGT and HOMA-IR differs according to the drinking status. Yamada *et al.* have reported that HOMA-IR rose with increasing serum GGT in both alcohol consumers and non-consumers, and HOMA-IR values corresponding to all serum GGT levels were lower in alcohol consumers than in non-consumers[30]. A recent study indicated that cigarette smoking may also affect both GGT and insulin resistance independent of the drinking status, and cigarette smoking and alcohol intake may have a synergistic impact on GGT[13]. Smoking status should also be considered when assessing the impact of alcohol intake on the association between GGT and insulin resistance; however, to our knowledge, no previous studies have investigated the relationship between GGT and insulin resistance after stratifying both the drinking status and smoking status, as in the current study.

We found that in 19 of the 25 subgroups divided according to smoking and drinking status, GGT was found to be a positive predictive value of HOMA-IR, which indicates that increased GGT is associated with enhanced insulin resistance regardless of the smoking and drinking status. From this type of cross-sectional study, we cannot conclude whether there is any causal or resultant relationship between GGT and HOMA-IR. A recent study showed that GGT may play a causal role in promoting insulin resistance, presumably by enhancing oxidative stress[31, 32] and hepatic steatosis[33]. Whether a change in HOMA-IR would result in a predicted change in GGT should be investigated in future longitudinal studies.

Our study has some limitations. First, we did not take into account coffee intake, which might affect GGT level[2]. Second, as the prevalence of smokers was low, we did not analyze the data of female subjects. Third, the number of daily cigarettes and alcohol consumption solely reflected the amount that was being consumed at one time, and disregarded the frequency of smoking or drinking consumption. Therefore, this estimation of smoking and drinking quantity was not equal to the mean daily number of cigarettes smoked and the amount of alcohol consumption, except in every-day smokers and drinkers, respectively. We performed such an analysis because the frequency of smoking (or drinking) was reported as a category, two or three times per week, for example; therefore, it was technically difficult to estimate the mean daily number of cigarettes smoked or the alcohol consumption. In the future, however, the frequency of drinking and smoking should also be considered in such an analysis. Fourth, we did not exclude individuals who were taking antihypertensive and/or antidiabetic drugs, which may have affected serum GGT and HOMA-IR values.

In summary, alcohol consumption showed a graded positive association with GGT and a U-shaped negative association with HOMA-IR. Cigarette smoking may further increase GGT levels in individuals who are current drinkers and drink 20 g or more per day. In 19 of the 25 drinking × smoking categories, GGT was found be a positive predictive value of HOMA-IR, and GGT was not a significant negative predictor of HOMA-IR, regardless of the drinking or smoking status. These data indicate a positive association between GGT and insulin resistance also in current drinkers.

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ORIGINAL ARTICLE
Renal Disease

# Relationship Between Renal Dysfunction and Severity of Coronary Artery Disease in Japanese Patients

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**Background:** The relationship between renal dysfunction and the severity of coronary artery disease (CAD) was examined.

**Methods and Results:** The severity of CAD in 572 patients was graded according to the number of stenotic coronary arteries, and the estimated glomerular filtration rate (eGFR) was monitored for 3 years. Patients were stratified into 3 eGFR groups: normal (>75 $ml \cdot min^{-1} \cdot 1.73 m^{-2}$), mild reduction (60–75) and chronic kidney disease (CKD: <60). There were 161 patients in the CKD group. The average number of stenotic coronary arteries was larger in the CKD group than in the other groups (normal vs mild reduction vs CKD=1.35±0.07 (SE) vs 1.22±0.08 vs 1.69±0.08 vessel disease (VD), P<0.001). During the 3-year follow-up, the renal function of 13.8% of the patients worsened. Those who showed more deterioration of eGFR had more severe CAD than those who did not (1.20±0.06 vs 1.61±0.06 VD, P<0.001). Multivariate analysis revealed that the severity of CAD was independently and significantly associated with the deterioration of eGFR.

**Conclusions:** Patients with CKD had more severe CAD, which may explain the high rate of cardiovascular events in these patients. Moreover, the prognosis of renal function was poor in patients with severe CAD, and CAD was found to be an independent risk factor for worsening of renal dysfunction. (*Circ J* 2010; **74:** 786–791)

**Key Words:** Chronic kidney disease; Coronary artery disease; Glomerular filtration rate; Renal function

It is well established that decreased renal function is associated with an increased frequency of cardiovascular disease, so patients with end-stage renal disease have a very high risk for cardiovascular events. However, this is the case even in patients with mildly reduced renal function. In fact, Go et al reported that among the American population patients with mild chronic kidney disease (CKD), such as those whose glomerular filtration rate (GFR) is between 45 and 59 $ml \cdot min^{-1} \cdot 1.73 m^{-2}$, already showed substantial increases in the frequency of cardiovascular events.[1] This has been confirmed not only in population-based epidemiological studies, but also in clinical trials.[2–6] However, the mechanisms of the involvement of renal dysfunction in the occurrence of cardiovascular events remain unclear, although several possibilities have been suggested. It is also unclear which cardiovascular events are likely to occur in patients with renal damage. According to previous reports, coronary artery disease (CAD), including acute myocardial infarction (AMI), is the most frequent type of cardiovascular event in patients with CKD.[3–6] Furthermore, the prognosis of such patients is worse than in those without CKD.[1,2] A Japanese population study recently showed that the risk of cardiovascular events increased as renal function decreased.[7] In that study the leading etiology of the cardiovascular events was cerebral vascular accidents rather than CAD. In Asian countries, particularly in Japan, the occurrence of stroke is twice that of CAD.[8] Nonetheless, the prevalence of AMI is higher in patients with decreased renal function.[7]

In the present study, we explored the relationship between renal dysfunction and the severity of CAD by counting the number of stenotic coronary arteries in Japanese patients. Furthermore, as cardiovascular damage has been suggested to aggravate renal dysfunction,[9–11] we followed patients with CAD for 3 years to examine the influence of CAD on renal function.

## Methods

For this study, data from 572 consecutive Japanese patients who underwent scheduled coronary angiography (CAG) at the University of Tokyo Hospital under the suspected diagnosis of CAD from August 1999 to February 2004 were analyzed retrospectively.

Scheduled CAG was performed using a transradial, trans-

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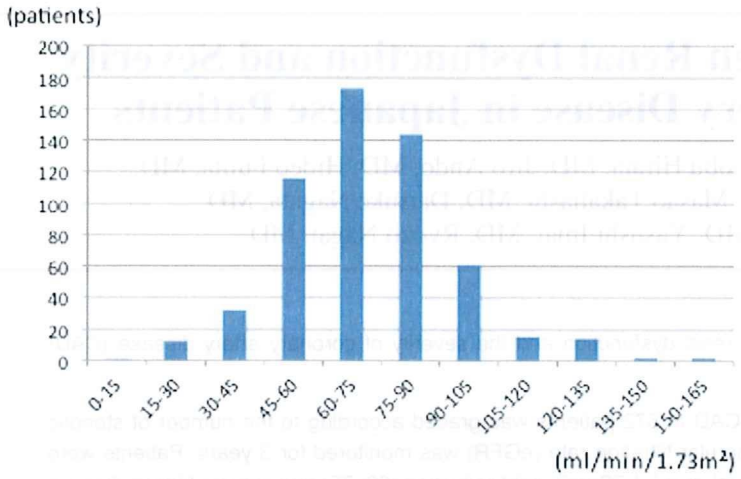


**Figure 1.** Distribution of the estimated glomerular filtration rate.

**Table 1. Clinical Profile of the Patients in Each Renal Function Group**

| | Total (eGFR) | Normal (>75) | Mild reduction (60–75) | CKD (<60) | P value (ml·min$^{-1}$·1.73 m$^{-2}$) |
|---|---|---|---|---|---|
| n | 572 | 238 | 173 | 161 | |
| Sex, M/F | 412/160 | 174/64 | 127/46 | 111/50 | 0.589 |
| Age (years) | 66.4±0.4 | 63.1±0.6 | 67.1±0.7 | 70.7±0.7 | <0.001 |
| BMI | 23.9±0.1 | 24.0±0.2 | 23.5±0.2 | 24.2±0.3 | 0.151 |
| Coexisting coronary risk factors | | | | | |
| Hypertension, % | 92.7 | 91.6 | 92.5 | 94.4 | 0.570 |
| Diastolic BP, mmHg | 77.0±12.9 | 77.5±13.5 | 77.8±13.1 | 75.4±11.7 | 0.178 |
| Systolic BP, mmHg | 135.6±20.7 | 134.9±20.5 | 135.9±20.9 | 136.2±20.9 | 0.824 |
| Diabetes mellitus, % | 36.1 | 37.0 | 35.0 | 36.0 | 0.852 |
| Dyslipidemia, % | 63.1 | 63.4 | 64.2 | 61.0 | 0.872 |
| Smoking habit, % | 61.2 | 61.3 | 62.4 | 60.0 | 0.870 |
| No. of coronary risk factors/patient | 2.53±0.04 | 2.54±0.06 | 2.54±0.07 | 2.52±0.07 | 0.967 |
| Serum Cr, mg/dl | 0.84±0.01 | 0.64±0.01 | 0.82±0.01 | 1.14±0.03 | <0.001 |
| eGFR, ml·min$^{-1}$·1.73 m$^{-2}$ | 72.1±0.9 | 91.1±1.0 | 68.0±0.3 | 48.5±0.8 | <0.001 |
| LVEF, % | 66.0±12.1 | 66.4±12.2 | 66.7±12.1 | 64.7±11.8 | 0.294 |
| Plasma BNP, pg/ml | 85±205 | 67±142 | 59±92 | 140±328 | <0.001 |

Data are mean±SE or percentage.
eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; BMI, body mass index; BP, blood pressure; Cr, creatinine; LVEF, left ventricular ejection fraction; BNP, B-type natriuretic peptide.

brachial or transfemoral approach. All angiographic reports were reviewed by at least 2 operators. The severity of coronary artery stenosis was assessed in the worst view projection and the percentage of luminal narrowing was recorded according to the American Heart Association criteria.[12] Luminal narrowing >51% was considered as significant stenosis. The left anterior descending, left circumflex and right coronary arteries were evaluated, and the number of stenotic arteries was recorded (0 to 3-vessel disease (VD)). A significant stenosis in the left main trunk was scored as 2VD.

Patients were admitted 1–3 days before the day CAG was to be performed. Body weight and blood pressure were measured in the morning of the day of admission. Blood samples were obtained from the antecubital vein, while the patient was supine, in the morning after an overnight fast. The plasma B-type natriuretic peptide (BNP) concentration was measured by enzymatic immunoassay,[13] and that of serum creatinine (Cr) by an enzymatic method using a standard autoanalyzer. Echocardiographic parameters were measured within 1 month after diagnostic CAG. The left ventricular (LV) dimension was measured on the long-axis view of the left ventricle taken with the patient in the left lateral position. LV ejection fraction (LVEF) was obtained by the following formula: LVEF=(LV end-diastolic volume−end-systolic volume)/LV end-diastolic volume. To evaluate cardiovascular risk factors, the numbers of smokers and patients with hypertension, diabetes mellitus or dyslipidemia were determined. Hypertension was defined as blood pressure >140/90 mmHg or use of antihypertensive agents; diabetes mellitus by fasting blood glucose ≥126 mg/dl or use of hypoglycemic agents or insulin; dyslipidemia by low-density lipoprotein cholesterol level ≥140 mg/dl, high-density lipoprotein cholesterol level ≤40 mg/dl or use of lipid lowering agents; and smoking by present or past smoking.

The patients were divided into 3 groups according to their estimated GFR (eGFR) calculated by the Modification of

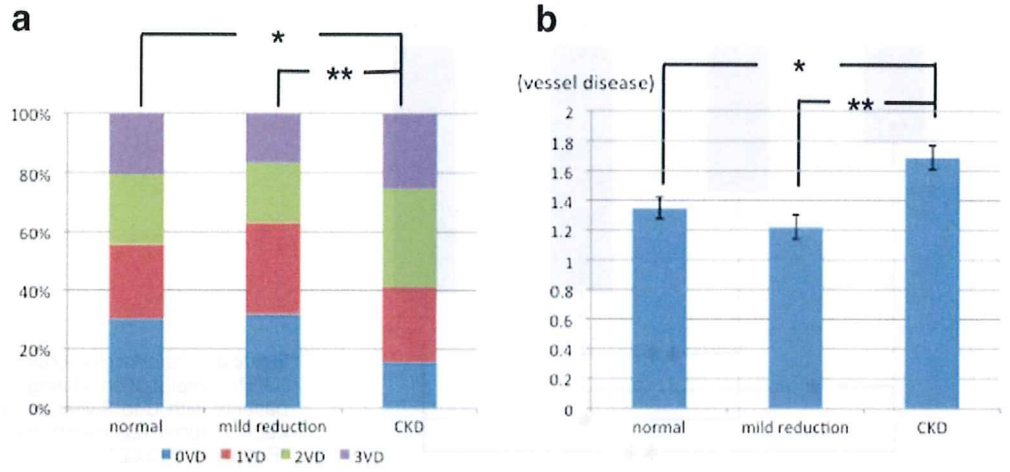


**Figure 2.** (a) Percentages of patients with 0 to 3-vessel disease (VD) in each group. (b) Number of stenotic coronary arteries in each group. Each column represents the mean±SE. The percentages of patients with multivessel stenosis (2VD+3VD) were significantly greater in the chronic kidney disease (CKD) group (*P<0.01, **P<0.001).

**Table 2. Clinical Background of Patients Whose Renal Function Remained Unchanged (Upper Half) or Decreased (Lower Half) During Follow-up**

| | Total | Upper half | Lower half | P value |
|---|---|---|---|---|
| n | 572 | 286 | 286 | |
| Age, (years) | 66.4±0.4 | 65.5±0.6 | 67.3±0.5 | 0.020 |
| Sex, M/F | 412/160 | 211/75 | 201/85 | 0.352 |
| BMI | 23.9±0.1 | 24.0±0.2 | 23.8±0.2 | 0.599 |
| Coexisting coronary risk factors | | | | |
| Hypertension, % | 92.7 | 91.3 | 94.1 | 0.200 |
| Systolic BP, mmHg | 135.6±0.9 | 131.6±1.1 | 139.5±1.3 | <0.001 |
| Diastolic BP, mmHg | 77.0±0.5 | 76.0±0.7 | 78.0±0.8 | 0.076 |
| Diabetes mellitus, % | 36.2 | 31.1 | 41.3 | 0.012 |
| Dyslipidemia, % | 63.1 | 62.2 | 64.0 | 0.665 |
| Smoking habit, % | 61.2 | 58.4 | 64.0 | 0.170 |
| No. of coronary risk factors/patient | 2.53±0.04 | 2.43±0.05 | 2.63±0.05 | 0.008 |
| Baseline serum Cr, mg/dl | 0.84±0.01 | 0.85±0.01 | 0.82±0.02 | 0.253 |
| Serum Cr after 3 years, mg/dl | 1.01±0.03 | 0.83±0.01 | 1.19±0.02 | 0.001 |
| Baseline GFR, $ml \cdot min^{-1} \cdot 1.73 m^{-2}$ | 72.1±0.9 | 69.5±1.0 | 74.6±1.4 | 0.003 |
| GFR after 3 years, $ml \cdot min^{-1} \cdot 1.73 m^{-2}$ | 62.4±0.8 | 69.9±1.0 | 54.9±1.2 | <0.001 |
| Change in GFR after follow-up, % | −12.8±0.8 | 1.4±0.7 | −27.0±0.8 | <0.001 |
| LVEF, % | 66.0±0.5 | 66.4±0.7 | 65.3±0.7 | 0.271 |
| Plasma BNP, pg/ml | 85.1±8.6 | 69.3±8.5 | 100.1±14.7 | <0.001 |
| Administration of ACEI or ARB, % | 49.8 | 48.9 | 51.7 | 0.358 |
| No. of stenotic coronary arteries, VD | 1.41±0.05 | 1.20±0.06 | 1.61±0.06 | <0.001 |
| No. of CAG and PCI/patient during follow-up | 2.81±0.09 | 2.57±0.12 | 3.05±0.14 | 0.012 |

Data are mean±SE or percentage.
GFR, glomerular filtration rate; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; VD, vessel disease; CAG, coronary angiography; PCI, percutaneous coronary intervention. Other abbreviations see in Table 1.

Diet in Renal Disease (MDRD) equation[14] with coefficients modified for Japanese patients:[15] eGFR ($ml \cdot min^{-1} \cdot 1.73 m^{-2}$) = $194 \times Cr^{-1.094} \times age^{-0.287}$ (×0.739 if female). The normal group had an eGFR >75 $ml \cdot min^{-1} \cdot 1.73 m^{-2}$; the mild reduction group had an eGFR between 60 and 75 $ml \cdot min^{-1} \cdot 1.73 m^{-2}$; the CKD group had an eGFR <60 $ml \cdot min^{-1} \cdot 1.73 m^{-2}$. The patients were excluded because of unstable renal function if they had overt congestive heart failure or AMI, or were on hemodialysis.

The study was approved by the institutional ethical committee (No. 2252).

All study subjects visited hospital regularly as outpatients after discharge. eGFR was monitored for 3 years after diagnostic CAG. To evaluate the effect of contrast media admin-

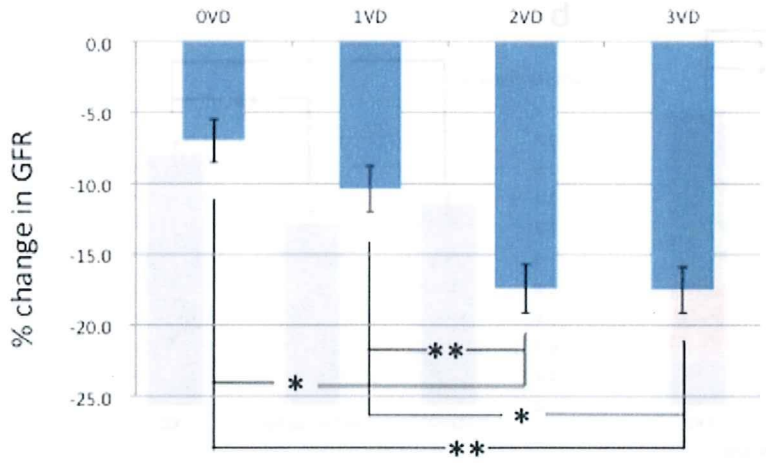


**Figure 3.** Percent glomerular filtration rate (GFR) deterioration during follow-up in patients with 0 to 3-vessel disease (VD). Each column represents the mean±SE. *P<0.01, **P<0.001.

**Table 3. Correlation Between the Percent GFR Deterioration During Follow-up and Clinical Parameters**

| | Univariate analysis | | Multivariate analysis ($r^2$=0.1417) | | |
|---|---|---|---|---|---|
| | $r^2$ | P value | $\beta$ | F value | P value |
| Age | 0.0031 | 0.1828 | 0.0739 | 1.5479 | 0.1223 |
| Sex | 0.0041 | 0.1244 | – | – | – |
| BMI | 0.0001 | 0.8558 | – | – | – |
| Coexisting coronary risk factors | | | | | |
| Hypertension | 0.0001 | 0.7903 | −0.0539 | −1.2641 | 0.2068 |
| Systolic BP | 0.0351 | <0.0001 | 0.1542 | 2.9390 | 0.0034 |
| Diastolic BP | 0.0069 | 0.0488 | 0.0265 | 0.5088 | 0.6111 |
| Diabetes mellitus | 0.0209 | 0.0005 | 0.0875 | 2.0253 | 0.0434 |
| Hyperlipidemia | 0.0021 | 0.2795 | 0.0395 | 0.9055 | 0.3656 |
| Smoking habit | 0.0021 | 0.2736 | −0.0068 | −0.1638 | 0.8700 |
| Baseline eGFR | 0.0135 | 0.0053 | 0.1812 | 4.0747 | 0.0001 |
| LVEF | 0.0045 | 0.1092 | −0.0157 | −0.3480 | 0.7280 |
| Plasma BNP | 0.0468 | <0.0001 | 0.1953 | 4.2209 | <0.0001 |
| No. of stenotic coronary arteries | 0.0399 | <0.0001 | 0.1140 | 2.4282 | 0.0155 |
| No. of CAG and PCI/patient during follow-up | 0.0118 | 0.0094 | 0.0379 | 0.8365 | 0.4033 |

Abbreviations see in Tables 1,2.

istered during CAG or percutaneous coronary intervention (PCI), the frequency of exposure of each patient to contrast media during those 3 years was recorded.

### Statistical Analysis

Values are expressed as the mean±SE. Statistical analyses were performed using SPSS version 17.0 (Chicago, IL, USA). Unpaired Student's t-test was used for comparisons between 2 groups. Tukey's multiple comparison of means following ANOVA was used for comparisons among more than 2 groups. A multiple linear regression analysis of independent predictors of renal prognosis was also performed. The level of statistical significance was set at P<0.05.

## Results

The baseline eGFR of the 572 patients showed a normal distribution (Figure 1), and the mean was 72.1±0.9 ml·min$^{-1}$·1.73 m$^{-2}$ (median, 71.7; interquartile range, 58.0–84.7). There were 173 (30.2%) patients with a normal eGFR, 238 (41.6%) with a mildly reduced eGFR, and 161 (28.1%) with CKD. The clinical profile of the patients in each group is shown in Table 1. Although CKD patients were slightly older than those in the other groups and had decreased renal function, the prevalence of risk factors for CAD was similar among the 3 groups.

As for the severity of CAD, 151 patients (26.4%) had 1VD, 145 (25.3%) had 2VD and 123 (21.5%) had 3VD (Figure 2a). No significant stenotic lesions were detected in 153 (26.3%) patients. The percentages of patients with multivessel stenosis (2VD+3VD) were significantly greater in the CKD group (P<0.001). The CKD group had a significantly higher number of stenotic coronary arteries then the normal and the mild reduction groups (Figure 2b). Although blood pressure and LVEF did not differ significantly among the 3 groups, the CKD group had a significantly higher plasma level of BNP than the other 2 groups (Table 1).

At the end of the 3-year follow-up eGFR had decreased from 72.1±0.9 to 62.4±0.8 ml·min$^{-1}$·1.73 m$^{-2}$ (median, 63.4; interquartile range, 50.1–73.9; P<0.001). The rate of decline was 3.2±0.2 ml·min$^{-1}$·1.73 m$^{-2}$·year$^{-1}$ and showed a normal distribution; 79 (13.8%) patients were newly diagnosed with

CKD during the follow up. On the other hand, no CKD patient showed improvement of eGFR during the same period.

We examined the factors related to the deterioration of renal function. Table 2 compares the clinical background of patients with unchanged renal function (ie, patients included in the upper half of the percent deterioration of eGFR) with that of patients with worsened renal dysfunction (the lower half). Age, systolic blood pressure, prevalence of diabetes mellitus, baseline eGFR, plasma BNP, number of coronary risk factors and number of CAG and PCI during follow-up per patient were found to be significantly higher in the lower half group (ie, the group showing a greater reduction of eGFR). There was no significant difference in the medications nor in the frequency of administration of angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) between the 2 groups. The number of stenotic coronary arteries was significantly greater in patients with decreased renal dysfunction compared with patients with unchanged renal function. The percent eGFR deterioration during follow up was significantly higher in the patients whose diagnostic CAG revealed multivessel disease (Figure 3). Stepwise multiple regression analysis was performed to evaluate the independent determinants of the percent eGFR deterioration (Table 3) and it was found that the number of stenotic coronary arteries, systolic blood pressure, prevalence of diabetes mellitus, baseline eGFR and plasma BNP, but not the number of CAG and PCI, showed an independent and significant association with the percent eGFR deterioration during follow-up.

## Discussion

The incidence of cardiovascular disease increases in patients with reduced renal function. Although the exact mechanisms by which impaired renal function relates to cardiovascular disease remain unclear, many possibilities have been suggested; for example, renal dysfunction activates the renin–angiotensin system and sympathetic nervous system, elevates blood pressure, causes anemia and vascular stiffness and calcification, and so on.[16,17] In the present study, the average number of stenotic coronary arteries was significantly bigger in the CKD group compared with the other 2 groups, which may explain at least in part the poor prognosis of CKD patients. CKD patients were older than patients in the other groups, as reported in previous studies,[1,2,16,17] and this may also have affected the severity of CAD because age has been reported to be a risk factor for CAD.[18] In addition, the CKD group had a significantly higher plasma level of BNP, even though blood pressure and LVEF did not differ among the 3 groups. This finding suggests that patients in the CKD group have a larger cardiac overload, although decreased renal clearance of BNP may explain its high plasma level.

On diagnostic CAG, 26.2% of the patients had CKD. In previous general population studies, 17.5% of subjects in the United States, and 10.3% of subjects in Japan were reported to have CKD.[1,19] Compared with those reports, the percentage of patients with CKD found in the present study was very high and may be because they already had a substantial number of coronary risk factors. Furthermore, patients in the CKD group were older than those in the other 2 groups.

During follow-up, the eGFR rate of decline was approximately $3\,ml \cdot min^{-1} \cdot 1.73\,m^{-2} \cdot year^{-1}$. Although eGFR is a function of age, 3 years is too short a period to explain this deterioration. eGFR decreases by only $0.1\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$ from $60\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$ if serum Cr in a patient aged 60 years is 1.0 mg/dl. It has been reported that the eGFR rate of decline is approximately $1\,ml \cdot min^{-1} \cdot 1.73\,m^{-2} \cdot year^{-1}$ in Western countries,[20] or $0.36\,ml \cdot min^{-1} \cdot 1.73\,m^{-2} \cdot year^{-1}$ in the Japanese general population.[21] Compared with previous data, the patient groups we analyzed had a very poor prognosis regarding renal function. There was no difference between the 2 groups in the usage of ACEI or ARB, which are known to have a renal protective effect. Multivariate analysis showed that the number of stenotic coronary arteries was significantly associated with the percent eGFR deterioration, whereas age, the total number of CAG and PCI during follow-up, and LVEF were not. This finding suggests that CAD can independently affect the prognosis of renal function.

It has been suggested that atherosclerosis causes renal dysfunction. Our study confirms that common mechanisms promote CAD and CKD. O'Hare et al showed that the frequency of increased Cr was significantly higher in those with a reduced ankle–brachial blood pressure index among subjects who participated in the Atherosclerosis Risk in Communities (ARIC) Study.[9] Elsayed et al[10] monitored renal function for 9.3 years on average in subjects from the ARIC Study and the Cardiovascular Health Study. In patients with cardiovascular disease, the odds ratio for worsening of renal failure was significantly high. Furthermore, in the Framingham Heart Study the new onset of renal disease was closely related to the coexistence of coronary risk factors.[11] These findings imply that the presence of atherosclerosis is a risk factor for worsening of renal dysfunction.

Prevalence of diabetes mellitus and a high systolic blood pressure also showed a significant association with reduced eGFR. It has been demonstrated that renal dysfunction worsens more rapidly in diabetic patients[22] and hypertensive patients.[23] Moreover, multivariate analysis revealed a significant relation between baseline eGFR and the rate of reduction in eGFR, which means that the reduction in eGFR in 3 years was greater in patients with a greater baseline eGFR. The reason for this cannot be clarified from the data obtained in the present study. However, it is possible that the decrease in eGFR in diabetic patients in the state of glomerular hyperfiltration may be even greater.

Another possible explanation for the worsening of renal dysfunction in patients with severe CAD is that they may be more exposed to contrast medium. Contrast medium-induced acute kidney injury (AKI) is a serious iatrogenic complication after CAG or PCI. In previous studies, contrast medium-induced AKI was reported as an increased risk of death or late cardiovascular events.[24,25] CKD, diabetes mellitus, and larger volumes of contrast medium administered in a single procedure were demonstrated to be independent risk factors for contrast medium-induced AKI.[26] There have been no reports regarding whether procedural times of CAG and PCI affect the long-term prognosis of renal dysfunction. However, in the present study contrast medium did not seem to cause the eGFR deterioration observed in the patients with multivessel CAD because the procedural times of CAG and PCI were not independent determinants. We examined the effect of the cumulative amount of contrast media administered in 3 years and did not find a significant relationship between eGFR deterioration and the amount of contrast media (r=0.06, NS, n=318). We could not collect information regarding whether any patient developed AKI after the first CAG or not. AKI itself may have an effect on the long-term prognosis of renal function.

### Study Limitations

In the original definition by the K/DOQI,[27] CKD is defined

as GFR <60 ml·min$^{-1}$·1.73 m$^{-2}$ or having markers of kidney damage even without GFR decrease. Proteinuria has been sometimes referred as a marker of kidney damage, and in the previous studies, proteinuria has been reported to be a possible marker predicting prognosis of renal function.[21] In the present study, we did not include proteinuria in the definition of CKD in order to concentrate on the change in GFR of the patients with CAD over the 3-year period, but this may give some weakness to our data.

There is a common tendency to refrain from catheter examinations of patients with decreased GFR because of the possibility of inducing an acute deterioration by the use of contrast media. This might have brought some bias to the present study because patients with a more severe clinical presentation tended to undergo CAG even if they had decreased GFR. However, the eGFR was normally distributed in the present study, as reported for the Japanese general population,[28] so the bias, if present, may be small.

## Conclusion

Patients with CKD have more severe CAD, which may be why there is a high rate of cardiovascular events in CKD patients, that is, the so-called cardiorenal association. Moreover, patients with more severe CAD had a poor prognosis for renal function itself. CAD seemed to be an independent risk factor for worsening of renal dysfunction.

## Acknowledgment

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Review

# Diagnosis and Treatment of Endothelial Dysfunction in Cardiovascular Disease

## A Review

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**SUMMARY**

Vascular endothelial dysfunction reflected by reduced nitric oxide (NO) availability is certainly the causative factor or promoting mechanism of arteriosclerosis. It is necessary to detect endothelial dysfunction at an early stage using appropriate methods, and to choose a treatment for the recovery of endothelial function. There are nonpharmacological and pharmacological therapies to attain endothelial repair. The former includes body weight reduction, aerobic exercise, and restriction of salt intake, while the latter includes the use of renin-angiotensin system inhibitors, calcium antagonists, some types of $\beta$ blockers, statins, erythropoietin, tetrahydrobiopterin, and antioxidants. These therapies are intended to increase NO synthase activity and NO release, inhibit NO degradation, and enhance the activity of endothelial progenitor cells. (Int Heart J 2010; 51: 1-6)

**Key words:** Nitric oxide, Asymmetric dimethyl arginine, Endothelial protection, Endothelial progenitor cells, Angiotensin, Statins

It has been shown that vascular endothelial dysfunction precedes the development of arteriosclerosis, and thereafter plays an important role in its progression. Therefore, preservation or recovery of endothelial function is important to inhibit the development of arteriosclerosis and thereby prevent the occurrence of cardiovascular events. In this review, we discuss the diagnosis and treatment of cardiovascular disease from the viewpoint of endothelial function (Figure).

**Endothelial function and NO:**

*1) Endothelial cells and NO synthase.* NO is synthesized from L-arginine by NO synthase (NOS) in vascular endothelial cells. NOS catalyzes a guanidino nitrogen of L-arginine by oxidation, and in turn releases NO and L-citrulline. All three NOS isoforms, neuronal, inducible, and endothelial NOS, share a carboxyl terminal domain homologous to cytochrome P-450 reductase, have binding sites for NADPH, FMN, tetrahydrobiopterin (BH4), and calmodullin, and need these as coenzymes to be activated.

*2) NO release mechanism.* Endothelial NOS is usually activated by an increase of the intracellular concentration of $Ca^{2+}$. The release of NO increases when endothelial cells are under shear stress caused by blood flow increase, which is the most physiological stimulus for NO release. This mechanism is the principle basis why flow-mediated dilation (FMD) at the time of reactive hyperemia is useful to clinically evaluate vascular endothelial function. Experimentally, acetylcholine (ACh) administered into the arteries causes vasodilation because the intracellular $Ca^{2+}$ concentration in endothelial cells is increased by ACh. Furthermore, it has been clarified that various stimuli such as insulin, estrogen, VEGF, and adrenomedullin activate the phosphatidyl inositol-3 kinase (PI3K)/Akt system, and that in turn Akt phosphorylates 1177$^{th}$ serine of NOS.[1)]

*3) Role of NO in development of arteriosclerosis.* There is a large body of evidence on the role of hyperlipidemia in the onset of arteriosclerosis. However, it is also known that the

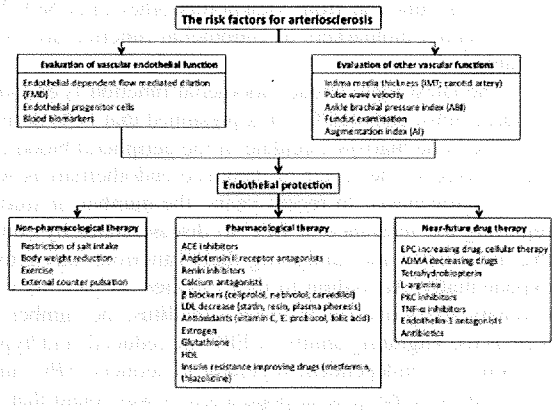


**Figure.** Strategies to improve endothelial function.

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onset of arteriosclerosis is associated with other risk factors, including hypertension, diabetes mellitus, and smoking, with a common mechanism of action. Namely, all these risk factors initially damage the endothelial cells. Such endothelial cell damage results in a reduced NO activity. NO exerts various antiarteriosclerotic actions other than endothelial-dependent vasodilation: NO suppresses the secretion of vasoconstrictive factors such as endothelin, and suppresses the aggregation of platelets as well as the expression of adhesive molecules, inhibiting adhesion of monocytes to the endothelial cells. Furthermore, it decreases oxidation of LDL. Through these actions NO eventually reduces the proliferation or migration of smooth muscle cells, and suppresses the development of atherosclerosis. In fact, NO synthesis inhibitors cause an elevation of blood pressure and marked vascular damage, as observed in endothelial NOS knockout (KO) mice.[2]

**Indicator of endothelial function:** Because it is difficult to evaluate all endothelial functions, the activity of endogenous NO is measured representatively. Among the methods available, measurement of endothelial-dependent vasodilatation in the forearm arteries using ACh is the golden standard in clinical practice. Nevertheless, NO is not the only factor involved in this reaction; but as a whole it can be said that it reflects the state of vascular endothelial function. Vascular echography or peripheral arterial tonometry (PAT) is employed to measure FMD in clinical settings in a noninvasive manner. The Framingham Heart Study found that the hyperemic response (PAT ratio) at 90-120 seconds after 5-minute forearm cuff occlusion correlated with cardiovascular risk factors in Framingham Third Generation Cohort participants; that is, 1957 subjects aged 40 years on average.[3] This hyperemic response was attenuated by NOS inhibition, thus it may reflect the state of endothelial function. Recently, there have been many attempts to investigate endothelial function from viewpoints other than NO. The new potential biomarkers of endothelial function are listed in Table I.

The latest topic about endothelial function is endothelial progenitor cells (EPC). It is presumed that EPC derived from the bone marrow circulate in the peripheral blood and are involved in the repair of damaged endothelium as well as in angiogenesis. In recent years, the number of studies reporting the relevance of EPC to disease states by counting these cells and analyzing their functions has grown exponentially. According to these studies, in patients with coronary artery disease or diabetes mellitus, the numbers as well as the migrating ability of EPC are reduced, and hypertension is an independent regulator of reduced EPC function.[4] Even in the general population, it was found that the larger the number of risk factors for cardiovascular disease, the smaller the number of EPC was. In addition, in patients with reduced endothelial function as assessed by measuring FMD of forearm arteries, the number of EPC was smaller than in those with preserved endothelial function. Therefore, in investigations involving the general population, the number of EPC will become an excellent alternative marker of vascular endothelial function or accumulation of cardiovascular risks.

**Which diseases affect endothelial function?:** Panza, *et al*[5] measured FMD by infusing ACh into the forearm arteries and found that in hypertensive patients, vasodilation was decreased compared with that in normotensive individuals. Ludmer, *et al*[6] infused ACh into the coronary arteries and demonstrated contraction of the coronary arteries in patients with coronary artery disease. Later it was clarified that in patients with coronary artery disease, endothelial-dependent vasodilation was reduced not only in the coronary arteries but also in the forearm arteries. In fact, the degree of FMD of the forearm arteries in patients with coronary artery disease was a predictor of cardiac events. Furthermore, it is known that endothelial-dependent vasodilation is reduced in patients with risk factors such as diabetes mellitus, dyslipidemia, and smoking. It is also known that in humans with or without coronary artery disease, the number of classical risk factors is inversely correlated with the state of endothelial function. Namely, the degree of reduction of endothelial-dependent vasodilation runs parallel to the number of conventional risk factors.[7]

**Table I.** New Biomarkers of Vascular Endothelial Dysfunction

| |
|---|
| Insulin resistance |
| Homocystinemia |
| Lipoprotein (a) |
| Endogenous NO synthesis inhibitors (ADMA) |
| Adiponectin |
| Inflammatory factors (CRP, IL-1, IL-6, TNF-$\alpha$, MCP-1) |
| Endothelial progenitor cells (EPC) |
| Vasodilators (nitrite and nitrate, 6-keto PGF1$\alpha$) |
| Vasoconstrictors (endothelin, thromboxan A2, ROS) |
| Adhesion molecules (VCAM-1, ICAM-1, P & E-selectin) |
| Thrombotic hemostatic factors (PAI-1, TPA, von Willebrand factor, thrombomodulin) |

**Factors that directly affect endothelial function:**

*1) Oxidative stress.* Oxidative stress by various reactive oxygen species (ROS) promotes the development of arteriosclerosis. NO is closely involved in this mechanism. The ROS react with and not only deactivate NO, but also produce a strongly oxidative substance, peroxynitrite. Oxidative stress also reduces synthesis of NOS at the protein level. Angiotensin II (AII), a known arteriosclerosis promoting hormone, produces ROS through the stimulation of NADP/NADPH oxidase in vascular smooth muscle cells. Physiologically, there is a balance between NO and AII, but in patients with arteriosclerosis NO is reduced and AII is increased, thus arteriosclerosis is worsened even further.

The evidence that oxidative stress affects endothelial function is provided by the effect of antioxidant drugs. For example, tempol reduces oxidative stress markers and exerts a marked antihypertensive effect in a number of experimental models of hypertension.[8] Also in humans, the usefulness of vitamins C and E, allopurinol, flavonoid, and folic acid, which reduces the concentration of homocysteine, has been demonstrated. Vitamin C strongly inhibits the oxidation of lipids, particularly LDL. So far it has been shown that oral administration or intra-arterial infusion of vitamin C improves endothelial-dependent FMD in patients with various disease states such as hypertension, ischemic heart disease, and dyslipidemia, as well as in smokers. However, there is no evidence that the long-term administration of these antioxidants always contributes to suppress the occurrence of

cardiovascular events.

*2) Increase of inhibitors of endogenous NO synthesis.* Protein arginine methyltransferase acts on proteins containing methylated arginine to produce asymmetric dimethylarginine (ADMA), which inhibits the synthesis of NO similarly to L-NMMA. Physiologically, dimethylaminohydrolase (DDAH) exists in endothelial cells, and it degrades these methyl arginines. However, in arteriosclerosis, the blood concentration of ADMA is increased. It has been shown from the analysis of genetically-engineered mice expressing increased or decreased levels of DDAH that accumulation of ADMA attenuates endothelial function, and may cause vascular disorders including coronary lesions. Thus, drugs that could reduce ADMA are expected; in fact, ACE inhibitors and ARBs are known to have that effect.[9] ADMA promotes the expression of ACE, most likely through p38 MAP kinase, increasing AII and oxidative stress, thus, the effect of renin-angiotensin system inhibitors may be potentiated. Regarding statins, several studies have been conducted, but no remarkable effect has been identified. Yet, it is known that the effect of statins is decreased in the state of increased ADMD.

*3) Inflammation.* It is known that bacterial infection attenuates endothelial function. Furthermore, administration of inflammatory cytokines also attenuates endothelial function. The study by Fichtlscherer, *et al*,[10] which examined the relationship between inflammatory markers and endothelial function, showed that vasodilation caused by ACh in the forearm arteries significantly correlated with CRP values in 60 patients with coronary artery disease. More directly, anti-inflammatory drugs such as COX inhibitors improve endothelial function in patients with arteriosclerosis. Whether antibacterial drugs improve vascular endothelial function is contentious, but recently comorbidity of periodontal disease and cardiovascular disease has drawn attention, as FMD is significantly improved by intensive treatment including administration of antibiotics for periodontal disease.[11] It was shown that when porphyromonas gingivalis, which is a major bacterial pathogen of periodontal disease, was present, the endothelial thickness of the carotid artery increased or that the bacterium was found in the intimal tissues of the resected carotid artery.

*4) Adipocytokines derived from visceral fat.* Among the adipocytokines playing an important role in the pathogenesis of metabolic syndrome, TNF-$\alpha$ is known to reduce endothelial function by increasing insulin resistance or its inflammatory effect, and endothelial function recovers after the reduction of TNF-$\alpha$.[12] Adiponectin is a protein with antiarteriosclerotic activity whose receptor is located in vascular endothelial cells; thus, NO is released upon stimulation of endothelial cells by adiponectin. As for the releasing mechanism, adiponectin activates endothelial NOS through AMP activating kinase and the PI3K/Akt system. Ouchi, *et al*[13] found a positive correlation between adiponectin and FMD in hypertensive patients. In patients with diabetes mellitus in whom adiponectin concentration is reduced, endothelial function is attenuated.

*5) Aldosterone.* The involvement of aldosterone in the onset of cardiovascular complications in hypertensive patients has recently attracted attention. Aldosterone is known to be involved in organ fibrosis independently from its action at the level of renal tubules or in hypertension, but there are many unknown issues concerning its mechanism of action. NO synthesis inhibition is associated with marked increases of aldosterone. At the same time, fibrosis of the renal interstitium is particularly increased, and these changes are all suppressed by an aldosterone receptor antagonist.[14] FMD is reduced in hypertensive patients in the presence of a hyperaldosterone state, and it is reported that administration of spironolactone for 3 months significantly improves endothelium-dependent vasodilation.[15] Therefore, it is possible that endothelial disorder is involved in the development of hypertension or cardiovascular disorder through an increase of aldosterone. Oxidative stress, which enhances the effect of aldosterone, may also be involved.[16]

*6) Depletion of tetrahydrobiopterin (BH4).* BH4 is a cofactor of NOS, and plays an important role in stabilizing dimer formation of NOS. NOS starts to produce ROS when BH4 is reduced (NOS uncoupling). When the aorta of mouse GTP cyclohydrolase 1 (GTPCH1), which is the rate-limiting enzyme for BH4 synthesis, is inhibited, endothelial-dependent vasodilation is decreased, and concurrently blood pressure, ROS, or adhesive molecules increase.[17] In the state of hypertension, arteriosclerosis and ischemia,[18] which are states of insufficient utilization of BH4, endothelial function is improved by supplying BH4.

**Usefulness of endothelial-dependent FMD measurement as a predictor of prognosis and cardiovascular events:** Vascular changes that become the primary cause of cardiovascular events start early in childhood, and it is important to detect them at an early stage. Even in the Framingham study, prediction of a cardiovascular event was possible only in 60-70% of the cases. If it were possible to detect asymptomatic patients who have such risk factors at the earliest possible stage by other methods, the advantages of such methods would be remarkable. As one of such methods, direct measurement of vascular endothelial function has gained significance.

Schachinger, *et al*[19] investigated endothelial-dependent vasodilation of the coronary arteries in 147 patients with coronary artery disease, and followed them up for 7.7 years. In patients with decreased vasodilation, cardiovascular events occurred with significant frequency, and decreased endothelial function was an additional predictor, independent of the other risk factors for coronary lesions. On the other hand, Perticone, *et al*[20] investigated the vascular reactivity of the forearm arteries induced by ACh in 225 hypertensive patients, and followed them for a mean of 31.5 months. They found that even in those with essential hypertension, the endothelial function of forearm arteries was a predictor of cardiovascular events because the incidence of cardiovascular events was the highest in the group with reduced endothelial-dependent FMD.

The relation between the number of EPC, which is correlated with endothelial-dependent FMD and prognosis of cardiovascular diseases, has also been reported. Werner, *et al*[21] measured the number of EPC in patients with ischemic heart disease, and analyzed its relation with the frequency of cardiovascular events one year later. They showed that the frequency of cardiovascular death, onset of primary major cardiovascular events, revascularization, and hospitalization increased in relation to the decrease in the number of

**Table II.** Reports on the Effect of Irbesartan on Human Vascular Endothelial Function

| Authors | Subjects | Number of cases | Administered drug (mg) | Administration period | Endothelial-dependent vasodilation (evaluation method) | Other effects |
|---|---|---|---|---|---|---|
| Warnholtz A | Coronary artery disease | 72 | Irbesartan 300 | 6 months | Brachial arteries FMD ↑ Intracoronary acetylcholine → | ADMA → |
| Morawietz H | CABG patients | 49 | Irbesartan 150<br>Pravastatin 40 concomitantly used | 4 weeks | Removed internal mammary artery acetylcholine ↑ ↑<br>↑<br>↑ ↑ ↑ | |
| Bahlmann FH | Type 2 diabetes mellitus | 18 | Olmesartan 40<br>Irbesartan 300 | 12 weeks | EPC ↑<br>↑ | |
| Ceriello A | Type 2 diabetes mellitus | 20 | Irbesartan 300<br>Atorvastatin 40 concomitantly used | 4 days | Brachial artery FMD ↑<br>↑<br>↑ | Nitrotyrosine ↓<br>ICAM-1 ↓<br>IL-6 ↓ |
| Sola S | Metabolic syndrome | 20 | Irbesartan 150<br>Lipoic acid 300 concomitantly used | 4 weeks | Brachial artery FMD ↑<br>↑<br>↑ | 8-isoprostane ↓<br>PAI-1 ↓<br>IL-6 ↓ |
| Bragulat E | Essential hypertension | 58 | Irbesartan 150-300 | 6 months | Plethysmography ↑ | Vascular constriction by L-NMMA ↑<br>Blood endothelin-1 ↓ |
| Schiffrin EL | Essential hypertension | 32 | Irbesartan 235<br>Atenolol 73 | 12 months | Removed gluteal artery acetylcholine ↑<br>→ | Ratio of media thickness/lumen ↓<br>→ |
| Von zur Muhlen B | Essential hypertension | 22 | Irbesartan 150-300<br>Atenolol 50-100 | 3 months | Plethysmography ↑<br>↑ | |
| Bragulat E | Essential hypertension | 34 | Irbesartan 150-300 | 6 months | Plethysmography ↑ | Vascular constriction by L-NMMA ↑<br>Blood endothelin-1 ↓ |
| Bragulat E | Essential hypertension | 32 | Irbesartan 150-300 | 6 months | Plethysmography ↑ | Vascular constriction by L-NMMA ↑<br>Blood endothelin-1 ↓ |

↑ : Increase, → : No change, ↓ : Decrease.

circulating EPC.

The incidence of cardiovascular events in our country is particularly low, therefore, to evaluate endothelial function as a surrogate marker of cardiovascular disorder may be extremely useful to diagnose severity, determine therapeutic strategy, or estimate the prognosis.

**Can endothelial function be improved by treatment?:** It is known that in a number of arteriosclerotic diseases endothelial function is improved by appropriate treatment. However, the treatment effect and the improvement of endothelial function do not always run in parallel, and the effect of drugs *per se* on endothelial function cannot be disregarded.

***1) Nonpharmacological therapy***

*a) Reduction of body weight:* It has been shown that endothelial function recovers when obese patients with coronary disease lose weight.[22] It is presumed that concurrent improvement of a variety of risk factors may be involved, but decreases in circulating cytokines, especially inflammatory cytokines released from visceral fat, may contribute to this.

*b) Restriction of salt intake:* Intake of sodium elevates blood pressure, and in case of salt-dependent hypertension, endothelial dysfunction is remarkable. It was recently clarified *in vitro* that in the presence of aldosterone, stiffness of cultured endothelial cells increased when the extracellular sodium concentration was increased from 135mM to 145mM.[23] That is, NO release decreases because of the reduced capacity of endothelial cells to transform. The vascular protective effects of NO are observed independent of the fall in blood pressure by limiting salt intake.

*c) Exercise:* It has been confirmed that even in human coronary arteries, endothelial function is improved by exercise.

An increase in blood flow during exercise increases shear stress on the vascular walls, which increases NO release. Exercise has also been reported to reduce oxidative stress and increase the number of EPC.

***2) Pharmacological therapy***

*a) Calcium antagonists:* The vasodilatory effect of calcium antagonists decreases after removal of endothelial cells or administration of NOS inhibitors, guanylate cyclase inhibitors, or bradykinin (BK) receptor antagonists. A number of reports have found that NO production is increased by calcium antagonists. In clinical cases, endothelial-dependent FMD is improved by their long-term administration. Reduction in blood pressure, increase in blood flow, antioxidant effect, increase in VEGF, increase in superoxide dismutase (SOD), and BK increasing effect have been suggested as possible mechanisms of action.

*b) β blockers:* In general, the endothelial protective effect of β blockers is not very strong. However, the involvement of NO in the vasodilatory mechanism of action of some β blockers has recently been pointed out. An increase of intracellular $Ca^{2+}$ by activation of phospholipase C and an increase of NO release by acting on the estrogen receptor of the endothelial cells have been postulated as the mechanism of NO release by nebivolol.[24] Concerning celiprolol, NO release was reported to take place through an increase in intracellular calcium by stimulation of serotonergic receptors.[25] Furthermore, celiprolol contributed to NO release by activating the PI3K/Akt pathway system.[26] On the other hand, some β blockers such as carvedilol have been shown to have an endothelial protective effect based on their antioxidant effect.

*c) ACE inhibitors:* There have been several reports describing a strong improvement of endothelial function with ACE inhibitor administration. Initially, the mechanism by which ACE inhibitors protect the endothelium was explained as follows: They exclusively inhibit the degradation of BK, increase BK, and stimulate BK type 2 receptors of endothelial cells to release NO. However, in several cardiovascular diseases, production of ROS by AII is increased. Therefore, it is believed that when AII is reduced by ACE inhibitors, the production of ROS decreases and NO activity increases.

*d) ARBs:* ARBs improve endothelial function in a number of arteriosclerotic diseases. The effects of irbesartan, which are frequently reported in humans, are summarized in Table II. Endothelial-dependent FMD improved in coronary disorder,[27] diabetes mellitus,[28] and essential hypertension[29] after long-term administration of irbesartan. The primary mechanism of action of ARBs is an AII-receptor antagonistic action; thus, blood and local concentrations of AII increase stimulating AII type 2 receptors. As a result, vasodilation and an antiapoptotic effect occur; this is considered to be an NO release mechanism of action. Similarly, an active metabolite of AII, AII (1-7), which is known to have an NO release effect, increases. In various arteriosclerotic diseases, ARBs improve endothelial function in a relatively short period of time, and concurrently exert anti-inflammatory and antioxidant effects. Furthermore, ARBs were reported to increase the number of EPC in patients with type 2 diabetes.[28]

*e) Renin inhibitors:* Similarly to the above 2 drugs, a renin inhibitor improves endothelial function in experimental animals. In Watanabe hyperlipidemic rabbits, the renin inhibitor aliskiren enhanced both the increase in blood NO concentration induced by ACh and the decrease in the NO release after treatment with L-NMMA to a degree similar to that obtained with valsartan; furthermore, it concurrently reduced the area of plaques in the aorta. These effects were additively enhanced by combination use of aliskiren and valsartan,[30] suggesting an AII-independent action of aliskiren. Reports of their effects in humans are expected soon.

*f) Statins:* Statins (HMG CoA reductase inhibitors) are cholesterol lowering drugs that inhibit the onset of ischemic heart disease and cerebral stroke, as demonstrated by numerous large-scale clinical studies. It is known that administration of statins for 6 months improves endothelial-dependent vasodilation in coronary arteries or forearm arteries of patients with hypercholesterolemia. The endothelium-dependent vasodilation in the forearm arteries improves in 2-12 weeks, and this effect does not run parallel to the lowering effect on LDL cholesterol, thus this is considered to be a pleiotropic effect. However, LDL apheresis or cholestyramine also improves endothelial function, thus the improvement of endothelial function must also be due to the cholesterol-lowering effect of the drug. Activation of the PI3-K/Akt pathway, a decrease in caveolin-1, an increase of eNOS and Hsp90 interaction, stabilization of eNOS mRNA by inhibition of the Rho/Rho kinase pathway, and a decrease of adhesion molecules are involved in the mechanism of direct enhancement of NO release by statins. In addition, statins increase the number of EPC in patients with coronary disease or cardiac failure.[31] Nevertheless, this action on EPC is not observed when NOS does not work, thus this may also occur due to an NO increasing effect of statins.

*g) Insulin-resistance improving drugs:* Patients with hypertension, diabetes, obesity, and dyslipidemia are not only in an insulin resistance state, but also have endothelial damage. This may be explained by a reduction of endothelial-dependent vasodilation by insulin. Insulin-induced NO release due to activation of NOS through the PI3-K/Akt pathway. Thiazolidinediones, which increase insulin sensitivity, lower CRP and ADMD values and improve endothelial-dependent vasodilation in diabetic patients. These drugs also prevented the occurrence of cardiovascular events in a large-scale clinical study.

*h) Erythropoietin (EPO):* EPO activates the PI3-K/Akt pathway and promotes NO release. Furthermore, there is a report indicating that blood EPO concentration is directly proportional to the number of EPC in patients with coronary artery disease.[32] Also, administration of EPO increased the number of EPC. These findings suggest that endogenous EPO may play an important role in the production of EPC.

*i) Treatments that affect the number of EPC:* The existence of risk factors leads to endothelial dysfunction, which in turn becomes the stimulus to enhance the mobilization of EPC, their accumulation at the damaged site, and angiogenesis. Exercise increases the number and function of EPC in humans. The number of EPC decreases in the presence of high levels of ADMA and LDL cholesterol, low HDL cholesterol, hypertension, diabetes mellitus, smoking, and hyperhomocysteinemia. On the other hand, the number of

EPC is increased by drugs such as statins, ARBs, ACE inhibitors, PDE5 inhibitors, rosiglitazone, or erythropoietin. The population of EPC is proportional to the plasma concentration of estrogen. Rapamycin decreases the number of EPC.[33] Circulating EPC may contribute to the repair of the endothelium.

**Conclusion:** There is no doubt that in the treatment of arteriosclerotic disease, it is essential to consider the improvement and repair of endothelial function. However, it is desirable to establish a simple method to measure endothelial function and to develop a more specific treatment to improve it.

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ORIGINAL ARTICLE
Ischemic Heart Disease

# Effects of Nicorandil on Cardiovascular Events in Patients With Coronary Artery Disease in The Japanese Coronary Artery Disease (JCAD) Study

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***Background:*** Nicorandil has cardioprotective effects in the ischemic myocardium, mimicking ischemic preconditioning, and is thus expected to improve the prognosis of ischemic heart disease (IHD). As part of the Japanese Coronary Artery Disease (JCAD) Study, a multicenter collaborative prospective observational study of a large cohort of coronary artery disease patients, the effect of nicorandil on outcome was examined.

***Methods and Results:*** In total, 2,558 patients with nicorandil treatment and controls subjected to propensity score matching were eligible among 13,812 patients registered in the JCAD study. The mean follow-up interval was 2.7 years. The primary endpoint, death from all causes, was significantly lower, by 35% (hazard ratio 0.65, P=0.0008), in the nicorandil group than in the control group. There were also significant reductions in secondary endpoints, including cardiac death (56%), fatal myocardial infarction (56%), cerebral or vascular death (71%), and congestive heart failure (33%) in the nicorandil group, with no excess of deaths from other non-cardiovascular causes. Treatment with nicorandil reduced the number of deaths from all causes to a similar extent with or without treatment with sulfonylureas.

***Conclusions:*** The reduction in cardiovascular death with nicorandil was large in patients with IHD, which has important implications for treatment. (*Circ J* 2010; **74:** 503–509)

**Key Words:** Cardiovascular events; Coronary artery disease; Japanese Coronary Artery Disease (JCAD) Study; Nicorandil

Nicorandil has a potassium channel-opening effect that activates adenosine triphosphate-sensitive potassium (K-ATP) channels and induces nitric oxide in the same fashion as nitrates, resulting in increased intracellular cyclic GMP.[1,2] Nicorandil also has cardioprotective effects in the ischemic myocardium, as demonstrated by reduced myocardial necrosis and improvement of myocardial stunning following coronary artery reperfusion, and thus mimics ischemic preconditioning.[3–7] These characteristics are expected to improve the prognosis of ischemic heart disease (IHD).

The Impact of Nicorandil in Angina (IONA) study, a randomized placebo-controlled trial, demonstrated significant reductions in the primary composite endpoint of coronary heart disease death, nonfatal myocardial infarction (MI) or unplanned hospital admission for cardiac chest pain after nicorandil administration in patients with stable angina.[8] However, it has never been clearly determined in a large-scale study whether nicorandil is useful in high-risk patients with IHD such as acute MI and unstable angina. Another important issue remaining from the IONA study is whether K-ATP channel blockers used to treat diabetes eliminate the effects of nicorandil in patients with IHD. Furthermore, it is uncertain which dosage of nicorandil is most appropriate in the treatment of IHD.

The Japanese Coronary Artery Disease (JCAD) Study, which featured a period of observation longer than in IONA, was a multicenter collaborative prospective observational study of a large cohort of coronary artery disease patients performed to investigate risk factors and current status of medication use and examine differences in outcomes of coronary artery disease.[9–11] As a part of this study, we exam-

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**Table 1. Patient Characteristics, Angiographic Findings, and Treatments in the Nicorandil and Control Groups After Propensity Score Matching**

| | Control | Nicorandil | P value |
|---|---|---|---|
| N | 2,558 | 2,558 | |
| Age (years) | 67.0 (27–93) | 67.0 (30–94) | 0.8362 |
| Male | 74.9% | 75.2% | 0.8211 |
| SBP | 132.9±19.7 | 132.8±20.3 | 0.6938 |
| DBP | 74.7±11.7 | 73.9±11.6 | 0.0060 |
| Hypertension | 53.4% | 55.1% | 0.2066 |
| Hyperlipidemia | 50.4% | 53.0% | 0.0609 |
| Impaired glucose tolerance | 41.4% | 41.5% | 0.9096 |
| BMI >25 kg/m$^2$ | 34.2% | 31.0% | 0.0133 |
| Smoker | 37.1% | 37.6% | 0.7072 |
| Family history | 13.6% | 15.9% | 0.0199 |
| Heart failure | 10.3% | 11.1% | 0.3659 |
| Left main coronary artery stenosis | 3.5% | 4.3% | 0.1472 |
| No. of coronary artery stenoses | 1.87±0.81 | 1.86±0.82 | 0.7606 |
| Medications | | | |
| Statins | 35.8% | 35.7% | 0.9303 |
| $\beta$-blockers | 19.9% | 22.0% | 0.0740 |
| Diuretics | 18.3% | 18.3% | 0.9423 |
| ACEIs | 33.3% | 29.2% | 0.0012 |
| ARBs | 14.3% | 14.9% | 0.5530 |
| Sulfonylureas | 10.2% | 10.3% | 0.8901 |

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

ined whether nicorandil improved the outcome of coronary artery disease in actual clinical practice.

## Methods

The JCAD study included consecutive patients with IHD who exhibited at least 75% organic stenosis of a major coronary on coronary angiography. All patients gave written informed consent to participate in this study. A total of 15,628 patients were registered and of them 13,812 could be followed and analyzed (follow-up rate 88.4%; mean follow-up interval 2.7 years). All medications and risk factors for coronary artery disease were recorded. In total, 2,558 patients were treated with nicorandil. In this subgroup analysis, primary and secondary endpoints were compared between patients with and without nicorandil treatment. The primary endpoint was all-cause death. Secondary endpoints were cardiac death, fatal MI, nonfatal MI, cerebral and vascular death, other causes of death, all events, cardiac events, congestive heart failure, and cardiopulmonary arrest on arrival (CPAOA). In this study, in-hospital deaths because of acute MI or CPAOA were defined as fatal MI.

Another subgroup analysis examined whether all-cause death or cardiac death differed depending on sulfonylurea treatment (glibenclamide, gliclazide or glimepiride) in patients being treated with nicorandil.

Clinical events were defined as in a previous report.[9] In brief, all-cause death included cardiac, cerebral, vascular, and other deaths. Cerebral events included cerebral hemorrhage, cerebral infarction, and transient ischemic attack. Cardiac events consisted of fatal and nonfatal MI, unstable angina, congestive heart failure, coronary bypass graft surgery, resuscitated cardiac arrest, and CPAOA. Angiographic restenosis when incidentally detected during routine follow-up coronary angiography without clinical symptoms was excluded from events. Aortic dissection and rupture of aortic aneurysm were classified as vascular events.

### Statistical Analysis

Intention-to treat analysis was performed, with the assumption that the medications used at discharge remained unchanged during the follow-up period.

In the observational study, covariates between the control group and treatment group could not be adjusted for, so bias regarding determination of the effects of medication was thus possible. However, propensity score analysis including the conditional probability of taking a medication using covariates may produce a good balance of covariates between 2 groups and is able to reduce this type of bias. A propensity score for each patient indicating the probability of taking medication was therefore calculated by multiple logistic regression analysis.[12] All clinical characteristics and medications except nicorandil were used in this calculation. The log-rank procedure and Cox's proportional hazards model were used to calculate confidence intervals. Cumulative incidence curves were generated by the Kaplan-Meier method for endpoints in the nicorandil and control groups. All calculated values are expressed as the mean±standard deviation. P values for background characteristics, all medications, and risk factors were calculated by the chi-square test. Statistical analysis was performed with SAS version 9.1 (SAS Institute Inc, Cary, NC, USA). Statistical significance was accepted at $P<0.05$.

## Results

After propensity score matching, there were 2,558 patients in each group. Baseline covariates likely to influence differ-

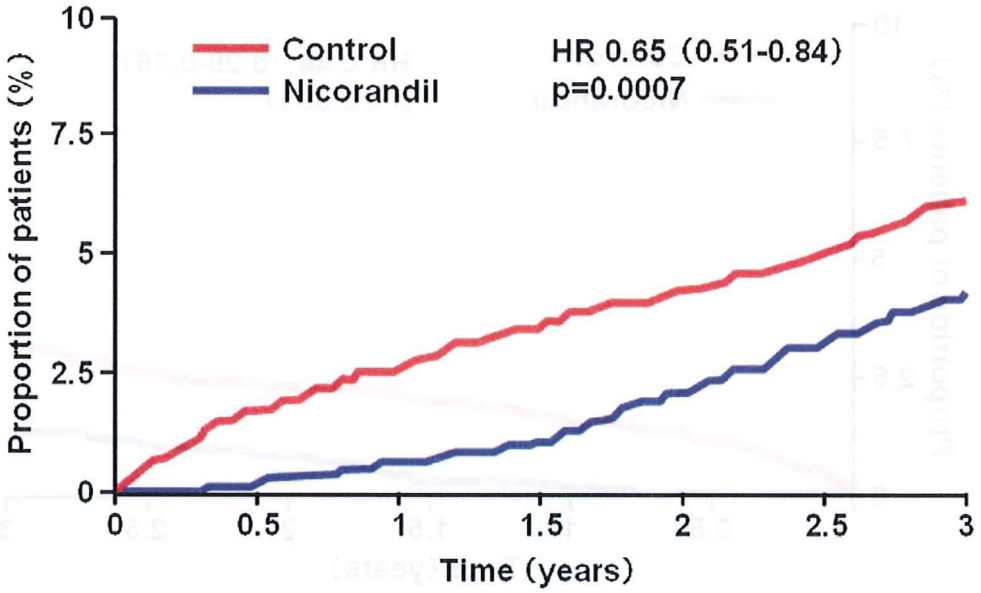


**Figure 1.** Cumulative incidence of the primary endpoint, deaths from all causes. HR, hazard ratio.

**Table 2. Incidences of Primary and Secondary Endpoints and All Events in the Control and Nicorandil Groups**

| | Control (n=2,558) | Nicorandil (n=2,558) | HR (95%CI) | P value |
|---|---|---|---|---|
| | Rate per 1,000 patient-years | Rate per 1,000 patient-years | | |
| **Primary endpoint** | | | | |
| All-cause deaths | 22.61 | 15.23 | 0.65 (0.51–0.84) | 0.0008 |
| **Secondary endpoints** | | | | |
| Cardiac deaths | 11.23 | 4.93 | 0.44 (0.29–0.66) | 0.0001 |
| Fatal MI | 6.29 | 2.77 | 0.44 (0.26–0.76) | 0.0022 |
| Nonfatal MI | 5.74 | 7.19 | 1.22 (0.80–1.86) | 0.3525 |
| Cerebral or vascular death | 1.90 | 0.58 | 0.29 (0.09–0.89) | 0.0299 |
| Non-CVD deaths | 9.48 | 9.72 | 0.98 (0.69–1.38) | 0.9000 |
| **All events** | 70.69 | 63.47 | 0.88 (0.77–1.01) | 0.0730 |
| Cardiac events | 53.65 | 48.26 | 0.89 (0.76–1.04) | 0.1375 |
| CHF | 14.23 | 9.71 | 0.67 (0.49–0.92) | 0.0140 |
| CPAOA | 4.38 | 1.60 | 0.36 (0.18–0.73) | 0.0042 |

HR, hazard ratio; CI, confidence interval; MI, myocardial infarction; CVD, cardiovascular disease; CHF, congestive heart failure; CPAOA, cardiopulmonary arrest on arrival.

ences in the rate of occurrence of cardiovascular events between the nicorandil-treated and control groups were adjusted for using propensity score matching (Table 1). Although there was a minimal difference between the control and nicorandil groups in median diastolic blood pressure, the median systolic blood pressure did not differ between the 2 groups. However, the frequencies of body mass index >25 kg/m$^2$ and angiotensin-converting enzyme inhibitor use were higher in the control than in the nicorandil group. Family history of cardiovascular disease was more common in the nicorandil than in the control group. The mean dosage of nicorandil was 15.04±4.74 mg.

The primary endpoint of all-cause death was significantly lower, by 35% (hazard ratio (HR)=0.65 [0.51–0.84], P=0.0008), in the nicorandil group than in the control group (Figure 1, Table 2).

In the nicorandil group, there were also significant reductions in 5 of the 9 secondary endpoints, some of which were incorporated in the primary endpoint: cardiac death (56%) (Figure 2), fatal MI (56%) (Figure 3), cerebral or vascular death (71%), congestive heart failure (33%), and CPAOA (64%). In contrast, nonfatal MI slightly increased (22%), though not to a significant extent (Figure 3). The number of all events tended to be reduced, by 12%, with non-significantly fewer cardiac events and no excess of deaths from other non-cardiovascular causes (9.48 vs 9.72/1,000 patient-

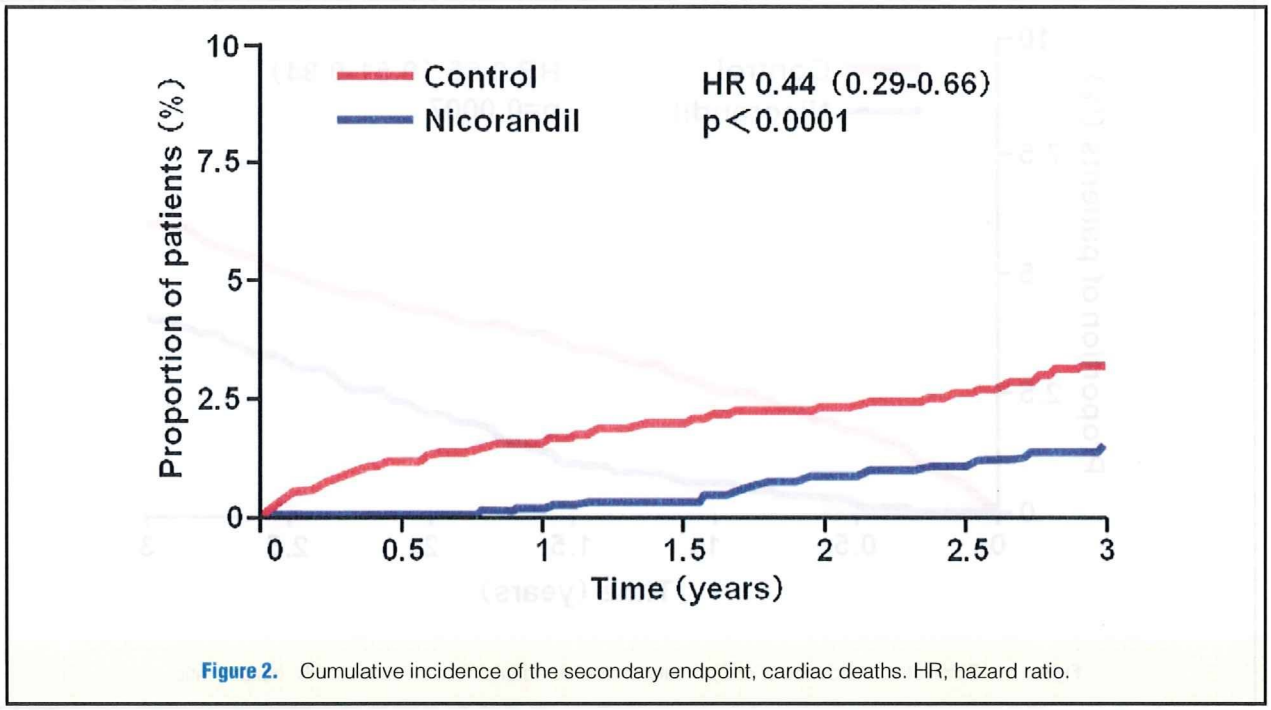


**Figure 2.** Cumulative incidence of the secondary endpoint, cardiac deaths. HR, hazard ratio.

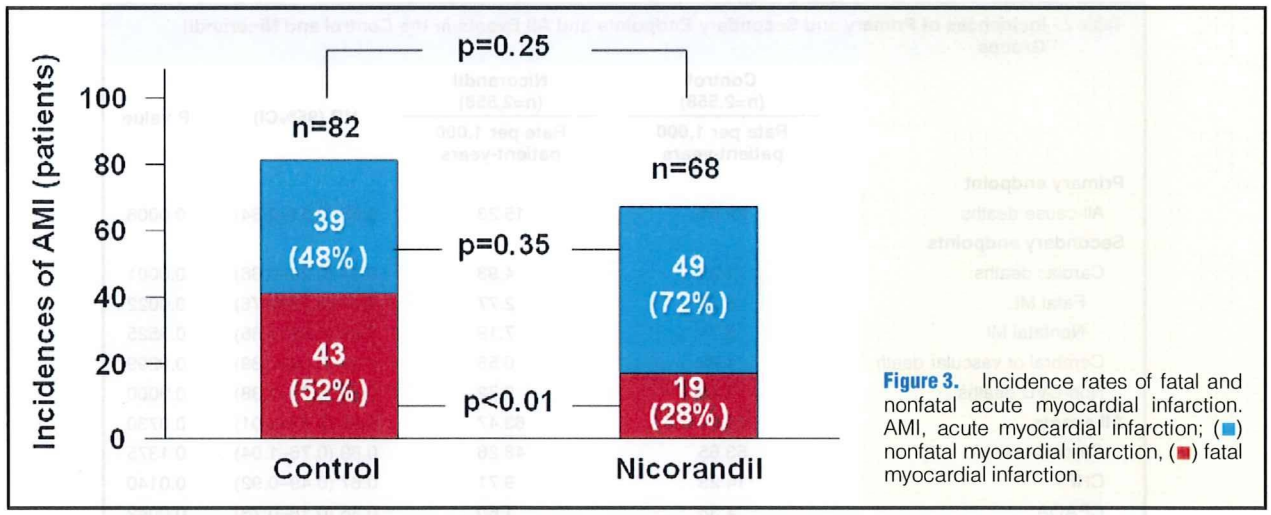


**Figure 3.** Incidence rates of fatal and nonfatal acute myocardial infarction. AMI, acute myocardial infarction; (■) nonfatal myocardial infarction, (■) fatal myocardial infarction.

year) (Table 2). Interestingly, cardiac death was almost completely eliminated by nicorandil during the first year of treatment (Figure 2).

In the group without sulfonylurea treatment, treatment with nicorandil significantly reduced all-cause deaths and cardiac deaths compared with the controls. Similarly, nicorandil treatment with sulfonylureas tended to reduce all-cause deaths compared with the control group, although this difference was not significant (P=0.0719). However, there was no significant difference in the proportional incidence of cardiac deaths between the control group and the group with nicorandil treatment and sulfonylureas, though cardiac deaths, which occurred in relatively few patients, did not increase in the group with nicorandil treatment with sulfonylureas compared with the controls (6.10 vs 4.49/1,000 patient-year) (Table 3).

The cardiac mortality rate was significantly higher in diabetic patients than in non-diabetic patients with or without nicorandil (control group: HR=1.65, P=0.035; nicorandil group: HR=2.72, P=0.007), and cardiac mortality rate was thus higher in patients with diabetes than in those without in this study. Sulfonylureas were used by 29.0% of all diabetic patients, and 28.8% of the patients not taking sulfonylureas had diabetes. Both total and cardiac mortalities were fewer in the sulfonylurea group than in the non-sulfonylurea group, though not to a significant extent (HR=0.72, P=0.132; HR=0.62, P=0.171, respectively). Moreover, the cardiac mortality rates in the analysis of only the diabetic patients in the non-sulfonylurea groups were higher (control group: 15.94/1,000 patient-year (33/766), nicorandil group: 8.50/1,000 patient-year (17/740)) than in all patients in the non-sulfonylurea group (Table 3).