Table 1. The prevalence of diabetic retinopathy by fasting and 2-h plasma glucose levels defined by the 1997 ADA criteria, the Hisayama Study 1998

	Population at risk	Mild retinopathy *n* (%)	Moderate retinopathy *n* (%)	Proliferative retinopathy *n* (%)	Any retinopathy *n* (%)
FPG (mmol/l)					
<6.1	1383	4 (0.3)	0 (0.0)	0 (0.0)	4 (0.3)
6.1–6.9	152	9 (5.9)	0 (0.0)	0 (0.0)	9 (5.9)
≥7.0	104	14 (13.5)	5 (4.8)	5 (4.8)	24 (23.1)
2-h PG (mmol/l)					
<7.8	1201	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
7.8–11.0	280	5 (1.8)	0 (0.0)	0 (0.0)	5 (1.8)
≥11.1	156	22 (14.1)	5 (3.2)	5 (3.2)	32 (20.5)

FPG, fasting plasma glucose; 2-h PG, 2-hour plasma glucose

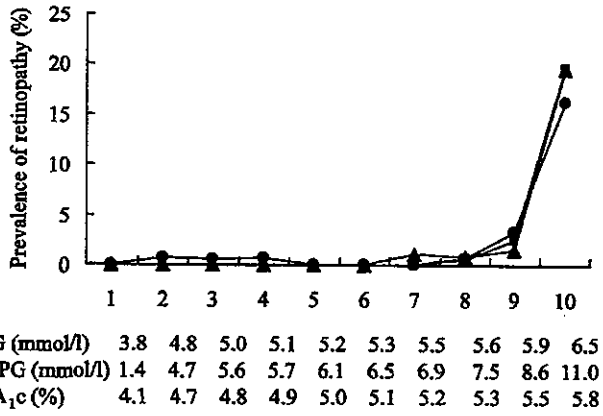

Fig. 1. Prevalence of retinopathy by deciles of the distribution of FPG, 2-h PG and HbA_1c levels in the Hisayama study. The *x* axis labels indicate the lower limit of each decile group. Black circles, FPG; black squares, 2-h PG; black triangles, HbA_1c

Table 2. Optimal cut-off points defined by maximising the sensitivity and specificity to identify diabetic retinopathy, the Hisayama Study 1998

	FPG	2-h PG	HbA_1c
Cut-off point	6.4 mmol/l	11.1 mmol/l	5.7%
Sensitivity (%)	86.5	86.5	86.5
Specificity (%)	87.3	89.6	90.1
ROC curve area (%)	90.0	96.1	94.5

FPG, fasting plasma glucose; 2-h PG, 2-hour plasma glucose; ROC, receiver operating characteristic

maximum of sensitivity and specificity was 6.4 mmol/l for FPG, 11.1 mmol/l for 2-h PG, and 5.7% for HbA_1c (Table 2). The sensitivities of these cut-off points for the three measurements were identical (86.5%), and the specificities were similar (FPG 87.3%; 2-h PG 89.6%; HbA_1c 90.1%). The specificity for the FPG level of 7.0 mmol/l was higher (91.3%), but its sensitivity (70.3%) was lower than that for the FPG of 6.4 mmol/l.

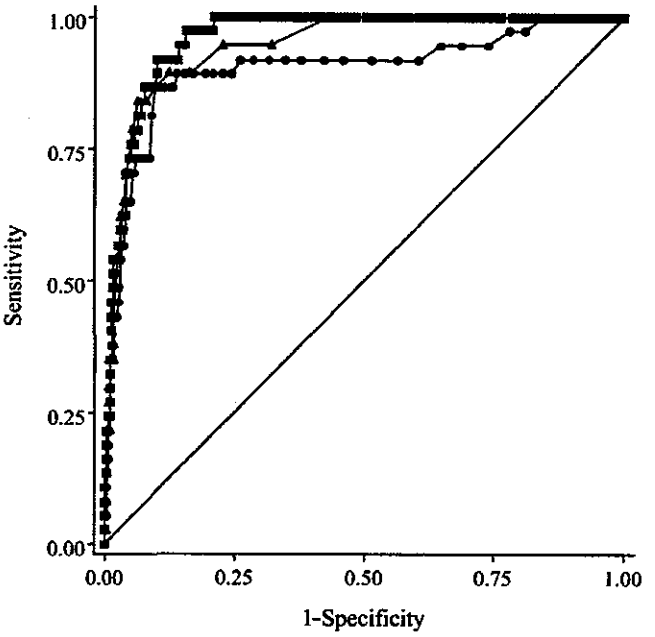

Fig. 2. Receiver operating characteristics (ROC) curves for FPG, 2-h PG and HbA_1c measures for predicting the presence of diabetic retinopathy. Black circles, FPG (ROC area 90.0%); black squares, 2-h PG (ROC area 96.1%); black triangles, HbA_1c (ROC area 94.5%); black line, reference

Discussion

The current guidelines for the diagnosis of diabetes are based on several population studies examining the relationship between the measures of glycaemia and retinopathy [1]. The Hisayama study has allowed us to collect data on a large number of Japanese individuals with a range of glucose levels. We compared the efficacy of tests measuring FPG, 2-h PG and HbA_1c levels in predicting diabetic retinopathy, and found no significant difference in the ability to predict retinopathy among the three measures of glycaemia. In our subjects, the optimal cut-off levels defined by the ROC curves maximising the sensitivity and specificity

to identify diabetic retinopathy were 6.4 mmol/l for FPG, 11.1 mmol/l for 2-h PG, and 5.7% for HbA_1c. In addition, according to the prevalence of retinopathy examined by deciles of the distribution of the FPG, 2-h PG and HbA_1c levels, a threshold was evident between the ninth and tenth deciles of each variable, below which retinopathy was almost absent and above which a distinct increase in retinopathy was observed. The cut-off point between the ninth and tenth deciles of each measure of glycaemia was very similar to that determined by the ROC curve. In contrast, the cut-off level of 2-h PG was consistent with the current ADA and WHO criteria. However, the cut-off level of FPG was slightly lower than that of the diagnostic criteria.

All three measures of glycaemia (FPG, 2-h PG and HbA_1c) were strongly associated with retinopathy in our Japanese population, which was similar to the findings in Pima Indians [3] and Egyptians [4], and to the results of the NHANES III [1]. The Egyptian study [4] reported that the area under the ROC curve for 2-h PG was similar to that for FPG, but that the area under the curve for HbA_1c was significantly smaller than that for FPG and 2-h PG. The authors concluded that the FPG and 2-h PG were each strongly and equally associated with retinopathy for diagnostic purposes. In contrast, in the Pima Indian study [3], ROC analysis showed that the area under the curve for 2-h PG was slightly but not significantly larger than that for FPG and that for HbA_1c. This result is consistent with that of the present study. Our findings, together with those of the Pima Indian study, suggest that all measures are equally effective for diagnostic purposes, and that the FPG or HbA_1c alone are also acceptable alternatives to 2-h PG, which is complicated to measure by OGTT.

Optimal cut-off levels of plasma glucose for defining diabetes vary between populations. In the Pima Indian study [3], the ROC curve analysis identified the optimal FPG cut-off level as 6.8 mmol/l. The NHANES III [1] also reported that the FPG cut-off level equivalent to the 2-h PG criterion of 11.1 mmol/l was 6.7 mmol/l. In contrast, we found that the optimal cut-off level of 2-h PG for diagnosis of diabetes, 11.1 mmol/l, was consistent with current ADA and WHO criteria. However, the optimal cut-off level of FPG, 6.4 mmol/l, was slightly lower than that of the diagnostic criteria [18]. Other Asian population studies [19, 20] have also reported that the optimal cut-off levels of FPG for diagnosis of diabetes ranged from 5.6 to 6.0 mmol/l in the sensitivity and specificity analysis for retinopathy. These findings suggest that the optimal level of diagnostic FPG is lower in Asian populations, including the Japanese population, than in western populations. In addition to the lower incidence of obesity in Asian populations, racial, genetic or environmental factors could contribute to this discrepancy.

This study has several limitations. Firstly, our results could be biased by the low participation rate. To ascertain the possibility of this bias, we compared the mean values of age, FPG, 2-h PG and HbA_1c levels as well as the proportion of men and women between the subjects who did participate in ophthalmic examination and those who did not. However, no significant differences in these parameters were observed between the groups, suggesting that this limitation does not invalidate the findings of the present study to a large extent. Secondly, our study population included individuals with diabetes who were taking oral anti-hyperglycaemic agents, resulting in a possible bias in the distribution of glycaemia. Although we performed analyses in which we excluded subjects on anti-hyperglycaemic medication, we could not draw any definitive conclusions due to the relatively low number of diabetic subjects not on anti-hyperglycaemic medication. However, it is to be expected that anti-hyperglycaemic medication has the same impact on FPG, 2-h PG and HbA_1c levels, and thus medication was unlikely to have affected our findings on the ability of these measurements to predict diabetic retinopathy. In addition, we computed the FPG level equivalent of the 2-h PG criterion of 11.1 mmol/l among Hisayama residents who underwent OGTT in 1988 and found it to be 6.2 mmol/l (data not shown). This value was similar to the optimal FPG cut-off level of 6.4 mmol/l in the present study. It was therefore suggested that this type of bias did not distort the conclusions of our study. Thirdly, because of the cross-sectional design of this study, it is still unclear how the onset of retinopathy is related to the three measures of glycaemia. Further prospective investigation would help to clarify this issue.

In conclusion, after consideration of the risk of diabetic retinopathy, our population-based study suggests that measuring FPG or HbA_1c is just as useful as measuring 2-h PG for the diagnosis of diabetes. Furthermore, there is a possibility that diabetic retinopathy occurs in patients with FPG levels lower than those currently used for the diagnosis of diabetes in the Japanese population.

References

1. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1997) Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 20:1183–1197
2. World Health Organization (1999) Definition, diagnosis and classification of diabetes mellitus and its complications. Department of Noncommunicable Disease Surveillance, WHO, Geneva
3. McCance DR, Hanson RL, Charles MA et al. (1994) Comparison of test for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. BMJ 308:1323–1328
4. Engelgau MM, Thompson TJ, Herman WH et al. (1997) Comparison of fasting and 2-h glucose and HbA_{1c} levels for diagnosing diabetes: diagnostic criteria and performance revisited. Diabetes Care 20:785–791

5. Harris MI, Rowland M, Klein R, Byrd-Holt DD, Cowie CC (1998) Is the risk of diabetic retinopathy greater in non-Hispanic Blacks and Mexican Americans than in non-Hispanic Whites with type 2 diabetes? Diabetes Care 85:1230–1235
6. Harris EL, Sherman SH, Georeopoulos A (1999) Black-white differences in risk of developing retinopathy among individuals with type 2 diabetes. Diabetes Care 22:779–783
7. West SK, Klein R, Rodriguez J et al. (2001) Diabetes and diabetic retinopathy in a Mexican-American population. Diabetes Care 24:1204–1209
8. Ito C, Maeda R, Ishida S, Harada H, Inoue N, Sasaki H (2000) Importance of OGTT for diagnosing diabetes mellitus based on prevalence and incidence of retinopathy. Diabetes Res Clin Pract 49:181–186
9. Ohmura T, Ueda K, Kiyohara Y et al. (1993) Prevalence of type 2 (non-insulin-dependent) diabetes mellitus and impaired glucose tolerance in the Japanese general population: the Hisayama Study. Diabetologia 36:1198–1203
10. Ohmura T, Ueda K, Kiyohara Y et al. (1994) The association of the insulin resistance syndrome with impaired glucose tolerance and NIDDM in the Japanese general population: the Hisayama Study. Diabetologia 37:897–904
11. Miyazaki M, Nakamura H, Kubo M et al. (2003) Risk factors for age related maculopathy in a Japanese population: the Hisayama study. Br J Ophthalmol 87:469–472
12. Rajala U, Qiao Q, Laakso M, Keinänen-kiukaanniemi S (1998) Prevalence of retinopathy in people with diabetes, impaired glucose tolerance, and normal glucose tolerance. Diabetes Care 21:1664–1669
13. Diabetic Retinopathy Study Research Group (1981) Report 7: a modification of the Airlie House classification of diabetic retinopathy. Invest Ophthalmol Vis Sci 21:210–226
14. Klein R, Klein BEK, Magli YL et al. (1986) An alternative method of grading diabetic retinopathy. Ophthalmology 93:1183–1187
15. Early Treatment of Diabetic Retinopathy Study Research Group (1991) Report 10: grading diabetic retinopathy from stereoscopic fundus photographs: an extension of the Airlie House classification. Ophthalmology 98:786–806
16. Hanley JA, McNeil BJ (1982) The meaning and use of area under a receiver operating characteristic (ROC) curve. Radiology 143:29–36
17. Zweig MH, Campbell G (1993) Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 39:561–577
18. The Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus (2002) Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. Diabetes Res Clin Pract 55:65–85
19. Lee C, Fook-Chong S (1997) Evaluation of fasting plasma glucose as a screening test for diabetes mellitus in Singaporean adults. Diabet Med 14:119–122
20. Nitiyanant W, Ploybutr S, Sriussadaporn S, Yamwong P, Vannasaeng S (1998) Evaluation of the new fasting plasma glucose cut-off level of 7.0 mmol/l in detection of diabetes mellitus in the Thai population. Diabetes Res Clin Pract 41:171–176

Relationship Between Plasma Glutathione Levels and Cardiovascular Disease in a Defined Population

The Hisayama Study

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Background and Purpose—Glutathione (GSH) appears to have marked antioxidant activities and therefore may prevent cardiovascular disease (CVD). However, there are very few reports on this subject. In a community-based case–control study, we tested the hypothesis that low levels of plasma GSH are closely associated with CVD and its clinical types.

Methods—The association between fasting plasma total GSH (tGSH) levels and CVD were assessed using conditional logistic regression analysis among 134 CVD cases and 435 age- and sex-matched healthy control subjects.

Results—Mean tGSH concentrations were lower in all CVD cases than in the control subjects (3.06 versus 3.71 μmol/L; $P=0.0001$). Among the CVD types, both the cerebral infarction cases (2.98 versus 3.59 μmol/L; $P=0.001$) and cerebral hemorrhage cases (2.51 versus 3.43 μmol/L; $P=0.0027$) had significantly lower tGSH levels than the corresponding control groups had. The same tendency was observed for cases of subarachnoid hemorrhage (3.45 versus 3.83 μmol/L; $P=0.36$) and myocardial infarction (3.65 versus 3.77 μmol/L; $P=0.69$), but these differences were not statistically significant. After adjustment for other confounding factors, the risk of CVD was significantly lower in the third (adjusted odds ratio, 041; 95% CI, 0.21 to 0.77) and the fourth quartiles (adjusted odds ratio, 0.25; 95% CI, 0.12 to 0.51) than in the first. This association was most prominent in patients with lacunar infarction or cerebral hemorrhage.

Conclusions—These findings suggest that reduced plasma tGSH levels are a risk factor for CVD, especially for cerebral small vessel disease. (***Stroke.*** **2004;35:2072-2077.)**

Key Words: cardiovascular diseases ■ cerebral hemorrhage ■ lacunar infarction ■ risk factors

Oxidative stress appears to play a major role in the development of cardiovascular disease (CVD).[1] Several endogenous substances, including homocysteine, which may be involved in the production of oxygen radicals in vessel walls, are reported to promote atherosclerotic disease by causing oxidative vascular injury.[2] Conversely, antioxidants such as vitamin C, vitamin E, and carotene may have protective effects against the development of CVD.[3]

Glutathione (GSH), a sulfhydryl (SH)-containing tripeptide, has several major physiological functions: it maintains SH groups of proteins in a reduced state, participates in amino acid transport, detoxifies foreign compounds, enzymatically degenerates endogenous peroxides, forms bioactive molecules, and acts as a coenzyme in several enzymatic reactions.[2] GSH has also been demonstrated to play a role in detoxifying oxygen radicals and therefore may prevent cellular damage from oxidative stress.[2] Several clinical case–control studies have shown that patients under chronic disease states such as heart disease,[4] arthritis,[4,5] diabetes,[4,5] and malignancies[6] have lower plasma levels of GSH than control subjects, suggesting that GSH has a protective role against such diseases. As for CVD, only a few studies have associated GSH levels in plasma or red blood cells with coronary heart disease.[7,8] Thus far, no study has shown an association with stroke.

Since 1961, we have been performing a cohort study of CVD in the town of Hisayama, a suburban community of ≈7500 residents on Kyushu Island in Japan. The present report describes this population-based retrospective case–control study, which was designed to investigate the relationship between plasma total GSH (tGSH) levels and clinical types of CVD (namely, type-specific stroke and myocardial infarction) in the community of Hisayama.

Subjects and Methods

Patients and Control Subjects

Throughout the course of the Hisayama study, information concerning newly developed cases of CVD among residents was collected through weekly visits to local practitioners and major hospitals in

Received February 5, 2004; final revision received June 4, 2004; accepted June 22, 2004.

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Stroke is available at http://www.strokeaha.org

DOI: 10.1161/01.STR.0000138022.86509.2d

and around town.[9] Regular health checks were performed biennially to residents aged 40 years or older to obtain information about any new cardiovascular events missed by the monitoring network. Whenever a new cardiovascular event was suspected, one of the study physicians neurologically and physically examined the subject (ultimately including the majority of subjects) and collected clinical information, including that regarding the course of the disease, as soon as possible.

Stroke was defined as a sudden onset of nonconvulsive and focal neurological deficit persisting for >24 hours and was classified as cerebral infarction, cerebral hemorrhage, subarachnoid hemorrhage, or an undetermined type.[10] Morphological examinations by several imaging techniques or autopsy, or both, were performed on almost all stroke cases encountered.[11] Cerebral infarction was further subdivided into 4 clinical categories: lacunar infarction, atherothrombotic infarction, cardioembolic infarction, and undetermined subtypes, according to the criteria established previously and described in detail elsewhere.[11]

Diagnosis of myocardial infarction was based on detailed clinical information and at least one of the following findings: electrocardiographic evidence of myocardial infarction; elevated cardiac enzymes; or a morphological finding including echocardiographic, scintigraphic, and angiographic abnormalities compatible with myocardial injury.

From June to October 1996, we enrolled all of the town's prevalent cases of CVD, for a preliminary total of 176 patients with a history of stroke or myocardial infarction.[12] Excluding cases with severe disability or with undetermined stroke type, a total of 134 cases (69 men and 65 women; mean age, 72.0±4.6 years; range, 46 to 91 years) were eligible for the present study. The mean interval from the onset of CVD to blood sampling for plasma tGSH measurement was 7.5 years (range, 3 months to 30 years). The patient group included 75 cases of cerebral infarction, 28 cases of cerebral hemorrhage, 14 cases of subarachnoid hemorrhage, 21 cases of myocardial infarction, and 4 cases of simultaneous cerebral and myocardial infarctions. The 75 cerebral infarction cases were subdivided into 43 cases of lacunar infarction, 24 of atherothrombotic infarction, and 8 of cardioembolic infarction.

As a control group, Hisayama residents who were healthy and free from both stroke and myocardial infarction, and who had participated in the 1996 health checkup, were randomly selected. For each CVD case, there were 1 to 5 sex- and age-matched (±2 years) controls. The control group consisted of 435 individuals (246 men and 189 women; mean age, 67.9±2.4 years; range, 46 to 91 years).

Laboratory Measurement

During the screening period in 1996, blood samples were obtained from all cases and control subjects in an overnight fasting state. Plasma tGSH and total homocysteine levels in the collected samples of CVD cases and controls were measured, using the high-performance liquid chromatography method described previously by Toyo'oka et al[13] at the Saga Research Institute of Ohtsuka Pharmaceutical Co, Ltd, with no awareness of the case–control status or of clinical information. Plasma vitamin B_6 concentrations were also determined using high-performance liquid chromatography with fluorescence detection. A chemiluminescent immunoassay was used to measure plasma folate and vitamin B_{12}. Serum cholesterol levels were measured enzymatically, and total protein levels were determined by the Biuret method. Diabetes mellitus was determined by either a 75-g oral glucose tolerance test (the 1998 WHO criteria), casual blood glucose levels (>11.1 mmol/L), or a medical history of diabetes. Height and weight were measured in light clothes without shoes, and the body mass index (kg/m^2) was calculated. Sitting blood pressure was measured 3 times on the right upper arm using a sphygmomanometer after a rest of at least 5 minutes. The average of the 3 measurements was used for the analysis. Hypertension was defined as a systolic blood pressure reading ≥140 mm Hg, a diastolic blood pressure reading ≥90 mm Hg, or the current use of antihypertensive drugs. Questions on personal smoking habits and alcohol consumption were asked, and the subjects were categorized as either current users or not.

TABLE 1. Clinical Characteristics of the Study Subjects

Factors	Cases (n=134)	Controls (n=435)
Age, y	72±9*	68±9
Sex, % male	51†	57
Systolic blood pressure, mm Hg	145±3†	136±1
Diastolic blood pressure, mm Hg	80±2	77±1
Hypertension, %	56†	50
Diabetes, %	11†	9
Body mass index, kg/m^2	21.3±0.3†	22.2±0.2
Cholesterol, mmol/L	5.0±0.08*	5.3±0.04
Total protein, g/L	71±0.4*	72±0.2
Folate, nmol/L	6.1±0.3	6.6±0.2
Vitamin B_6, nmol/L	64.6±2.1*	83.8±1.5
Vitamin B_{12}, pmol/L	754±27†	666±13
Total homocysteine, μmol/L	12.8±0.3*	11.1±0.2
Drinking, %	30†	36
Smoking, %	21	25

All variables except for age and sex were adjusted for age and sex.
Values are expressed as means±SE (for age, SD) and percentages.
*P<0.01, †P<0.05 vs controls.

Statistical Analysis

The mean age was compared using the Student t test, as was the frequency of male gender using the χ^2 test. Age- and sex-adjusted mean values of relevant factors were calculated using the covariance method. Differences in the parameters between CVD cases and controls were assessed by the Student t test, and trends in the parameters among the tGSH quartiles were assessed by multiple linear regression analysis. The age- and sex-adjusted frequencies were calculated by the direct method, then compared by the Cochran–Mantel–Haenszel χ^2 test using 10-year age groupings with the total subjects as a standard.

The odds ratio (OR) and 95% CI of CVD and its clinical types were calculated by the distribution of tGSH tertiles or quartiles using conditional logistic regression analysis. A value of P<0.05 was considered statistically significant.

Ethical considerations

This study was conducted with the approval of the Human Ethics Review Committee of the Kyushu University Graduate School of Medical Sciences. Written informed consent for medical research was obtained from all participants.

Results

The clinical characteristics of CVD cases and control subjects are demonstrated in Table 1. Because there were fewer control subjects in the elderly than in the younger case–control sets, especially in the case of females, the mean age and proportion of women were higher in the CVD group than in the control group. Thus, comparisons for other variables were performed after adjusting for age and sex. Mean systolic blood pressure and the frequency of hypertension and diabetes were significantly higher among CVD cases than among control subjects. CVD patients had lower body mass index, serum cholesterol, and total protein levels. Although the plasma folate concentration was the same between CVD patients and controls, the former presented lower plasma vitamin B_6 and higher vitamin B_{12} levels than the latter. The mean total homocysteine levels were significantly higher in

TABLE 2. Comparison of Age- and Sex-Adjusted Mean Values±SE of Fasting Total Plasma Glutathione Concentrations Between Cases With Cardiovascular Disease and Controls

	Cases and Controls	Plasma Glutathione (μmol/L)	P
Cardiovascular disease	Case (n=134)	3.06±0.12	
	Control (n=435)	3.71±0.06	0.0001
Cerebral infarction	Case (n=75)	2.98±0.16	
	Control (n=248)	3.59±0.08	0.001
Cerebral hemorrhage	Case (n=28)	2.51±0.27	
	Control (n=121)	3.43±0.13	0.0027
Subarachnoid hemorrhage	Case (n=14)	3.45±0.37	
	Control (n=67)	3.83±0.17	0.36
Myocardial infarction	Case (n=21)	3.65±0.29	
	Control (n=95)	3.77±0.13	0.69

CVD cases than in the control subjects. Alcohol consumption was significantly less frequent in CVD patients than in the control subjects, whereas the frequency of smoking habits was the same between the 2 groups.

The age- and sex-adjusted mean values of plasma tGSH levels were significantly lower among CVD cases overall than among the control subjects (Table 2). Among CVD types, cases of cerebral infarction or hemorrhage had significantly lower tGSH levels than those of the respective corresponding control groups. A similar tendency was observed in cases of subarachnoid hemorrhage or myocardial infarction, although the differences were not statistically significant.

CVD patients and control subjects were combined into 1 group, then divided into quartiles based on their tGSH levels. The mean value or frequency of each relevant factor was then compared among the 4 groups (Table 3). Individuals who were included in the fourth quartile of tGSH were younger, but the proportion of men did not differ among the quartiles. The levels of systolic and diastolic blood pressures decreased with increasing tGSH levels, whereas the frequency of hypertension did not significantly differ among the 4 groups. The frequency of diabetes significantly decreased with elevating tGSH levels. Although the body mass index was the same across tGSH levels, serum cholesterol levels significantly increased with elevating tGSH. Individuals who were included in the first quartile of tGSH had low mean serum total protein and vitamin B_6 levels, whereas plasma folate and vitamin B_{12} levels were the same across all tGSH levels. There was no correlation between tGSH and total homocysteine levels. The frequency of alcohol consumption significantly decreased with increasing tGSH levels, although no such trend was seen in the frequency of smoking habits.

To further evaluate the association of CVD with tGSH levels, crude and multivariate-adjusted ORs were calculated by quartiles of tGSH levels (Table 4). Compared with the first quartile, in the third and fourth quartiles the risk of CVD decreased with elevating tGSH and was significantly lower in the third (crude OR, 0.41; 95% CI, 0.23 to 0.72) and the fourth (crude OR, 0.24; 95% CI, 0.12 to 0.46) quartiles. A similar pattern was observed for cerebral infarction and cerebral hemorrhage, but not for subarachnoid hemorrhage or myocardial infarction. The magnitude of the effect of tGSH on each type of CVD, except for cerebral hemorrhage in the fourth quartile, was not found to be attenuated substantially from quartile to quartile, even after adjustment for other confounding factors such as systolic blood pressure, diabetes, body mass index, cholesterol, total protein, folate, vitamin B_6, vitamin B_{12}, total homocysteine, smoking habits, and alcohol consumption.

TABLE 3. Age- and Sex-Adjusted Mean Values or Frequencies of Cardiovascular Risk Factors According to Quartiles of Total Glutathione Levels

	Quartiles of Glutathione (μmol/L)				
Factors	<2.53 (n=142)	2.53–3.41 (n=143)	3.41–4.4 (n=143)	>4.4 (n=141)	P for trend
Age, y	70±9	69±9	70±9	67±9	0.02
Sex, % male	61	52	53	56	0.39
Systolic blood pressure, mm Hg	142±2	141±2	135±2	133±2	0.0001
Diastolic blood pressure, mm Hg	78±1	79±1	77±1	74±1	0.0001
Hypertension, %	54	62	47	49	0.07
Diabetes, %	15	8	8	6	0.02
Body mass index, kg/m^2	22.0±0.3	22.4±0.3	22.3±0.3	21.4±0.3	0.13
Cholesterol, mmol/L	5.1±0.1	5.2±0.1	5.3±0.1	5.4±0.1	0.004
Total protein, g/L	70±0.4	72±0.4	73±0.4	72±0.4	0.0004
Folate, nmol/L	6.4±0.3	6.4±0.3	6.5±0.3	6.5±0.3	0.64
Vitamin B_6, nmol/L	68.2±2.2	74.1±2.2	92.9±2.2	82.3±2.2	0.008
Vitamin B_{12}, pmol/L	728±26	649±23	674±24	692±24	0.55
Total homocysteine, μmol/L	11.7±0.4	11.4±0.4	11.7±0.4	11.0±0.4	0.12
Drinking, %	41	34	29	29	0.008
Smoking, %	27	19	25	24	0.59

Age and sex were not age- and sex-adjusted. Values are expressed as means±SE (for age, SD) and percentages.

TABLE 4. Crude and Adjusted Odds Ratios of Cardiovascular Disease and its Types in Each Quartile of Total Glutathione Distribution

		Quartiles of Glutathione (μmol/L)				
		<2.53 OR	2.53–3.41 OR (95% CI)	3.41–4.4 OR (95% CI)	>4.4 OR (95% CI)	*P* for trend
		n=142	n=143	n=143	n=141	
Cardiovascular disease	Crude	1.0	0.54 (0.31–0.92)	0.41 (0.23–0.72)	0.24 (0.12–0.46)	0.0001
	Adjusted*	1.0	0.57 (0.31–1.05)	0.41 (0.21–0.77)	0.25 (0.12–0.51)	0.0001
		n=89	n=85	n=78	n=71	
Cerebral infarction	Crude	1.0	0.59 (0.29–1.2)	0.31 (0.14–0.68)	0.22 (0.15–0.32)	0.0001
	Adjusted*	1.0	0.55 (0.24–1.25)	0.29 (0.12–0.69)	0.19 (0.07–0.52)	0.0002
		n=46	n=45	n=29	n=29	
Cerebral hemorrhage	Crude	1.0	0.36 (0.13–1.02)	0.08 (0.01–0.63)†	0.24 (0.07–0.90)	0.006
	Adjusted*	1.0	0.37 (0.10–1.30)	0.05 (0.01–0.58)†	0.37 (0.08–1.69)	0.06
		n=17	n=12	n=28	n=24	
Subarachnoid hemorrhage	Crude	1.0	0.97 (0.14–6.88)	1.26 (0.29–5.55)	0.69 (0.13–3.82)	0.77
	Adjusted*	1.0	0.97 (0.14–6.88)	1.26 (0.29–5.55)	0.69 (0.13–3.82)	0.77
		n=22	n=28	n=33	n=33	
Myocardial infarction	Crude	1.0	1.97 (0.43–8.94)	1.81 (0.42–7.85)	0.45 (0.06–3.39)	0.52
	Adjusted*	1.0	3.51 (0.60–20.5)	2.39 (0.43–13.4)	0.43 (0.04–4.09)	0.40

OR indicates odds ratio.

*Adjusted for age, sex, systolic blood pressure, diabetes, body mass index, cholesterol, total protein, folate, vitamin B_6, vitamin B_{12}, total homocysteine, smoking, and drinking.

We further divided the combined group of patients with cerebral infarction and the corresponding control subjects into tertiles by tGSH levels and estimated the OR of each subtype of cerebral infarction (Table 5). The risk of lacunar infarction was significantly lower in the second and third tertiles than in the first. In the case of atherothrombotic infarction or cardioembolic infarction, however, the risk decreased with elevating tGSH levels. However, these trends were not statistically significant. Because there were no cases of cardioembolic infarction in the second tertile, we could not estimate OR for this tGSH level.

Discussion

The major new finding of the present study is that CVD cases had much lower levels of plasma tGSH than control subjects did. The risk of CVD continuously decreased with increasing

TABLE 5. Crude and Adjusted Odds Ratios of Subtypes of Cerebral Infarction in Each Tertile of Total Glutathione Distribution

		Tertile of Glutathione (μmol/L)			
Subtype of Cerebral Infarction		<2.9 OR	2.9–4.1 OR (95% CI)	>4.1 OR (95% CI)	*P* for trend
		n=68	n=66	n=69	
Lacunar infarction	Crude	1.0	0.35 (0.14–0.86)	0.33 (0.14–0.76)	0.009
	Adjusted*	1.0	0.22 (0.07–0.66)	0.23 (0.09–0.65)	0.02
		n=43	n=43	n=37	
Atherothrombotic infarction	Crude	1.0	0.49 (0.16–1.52)	0.46 (0.15–1.38)	0.15
	Adjusted*	1.0	0.45 (0.14–1.48)	0.47 (0.14–1.59)	0.21
		n=15	n=7	n=9	
Cardioembolic infarction	Crude	1.0	NA	0.30 (0.03–2.8)	0.25
	Adjusted	1.0	NA	0.30 (0.03–2.8)	0.25

OR indicates odds ratio; NA, not available.

*Adjusted for age, sex, systolic blood pressure, diabetes, body mass index, cholesterol, total protein, folate, vitamin B_6, vitamin B_{12}, total homocysteine, smoking, and drinking.

tGSH levels and was not attenuated even after adjustment for other confounding factors. Thus, the reduced level of plasma tGSH may be an independent risk factor for the development of CVD.

Among the clinical types of CVD, the risk of lacunar infarction and cerebral hemorrhage significantly decreased with elevating tertiles of tGSH. A similar tendency was observed for atherothrombotic, cardioembolic, and myocardial infarctions, although for these groups the difference was not statistically significant. It is well-known that arteriosclerotic lesions of the perforating intracerebral arteries induced mainly by chronic arterial hypertension contribute to the development of lacunar infarction and cerebral hemorrhage. However, both atherothrombotic infarction and myocardial infarction are the consequences of atherosclerosis of large cerebral and coronary arteries, and rupture of an intracranial saccular aneurysm is the most common cause of subarachnoid hemorrhage. γ-glutamyl transpeptidase, produced in the first step of the breakdown of GSH, is contained in larger quantities with much higher enzyme activity in the endothelium of capillaries than in that of larger vessels in the brain.[14] This suggests that the concentration of GSH in the brain is apt to decrease more in capillaries than in large arteries; consequently, cerebral small arteries may be more sensitive to fluctuation in levels of plasma GSH. However, atherothrombotic and myocardial infarctions are associated with major risk factors—such as hypertension, diabetes, and smoking—that carry greater exposure to oxidative stresses and therefore may be associated with tGSH deficiency. In addition, the sample size of atherothrombotic, cardioembolic, and myocardial infarction was insufficient to draw a conclusion. Thus, our findings imply that plasma tGSH offers a strong defense mechanism at least against arteriosclerosis of small cerebral arteries, whereas its preventive effects on atherosclerosis of large vessels are inconclusive.

Several mechanisms by which GSH may prevent cerebrovascular damage have been suggested. Harlan et al[15] showed that depletion of GSH by buthionine sulfoximine, an inhibitor of glutathione synthesis, augmented the endothelial damage caused by hydrogen peroxide released from activated neutrophils. Thus, GSH may have marked protective effects against oxidative damage by means of its direct antioxidative effects.[2] GSH has been reported also to play a role in the maintenance of SH groups and other cellular antioxidants in a reduced state, thereby maintaining their antioxidant effects.[2] In addition, Thomas et al[16] showed that both GSH and GSH-dependent selenoperoxidase protect cells against the damage induced by oxidized low-density lipoprotein. Presumably, this protection may be the result of detoxification of lipid hydroperoxides and the reduced formation of free radical intermediates with greater reactivity.[16]

Several limitations of our study should be discussed. The primary limitation is that our data were derived from a retrospective case–control study. Thus, we cannot exclude the possibility that decreased tGSH was a consequence of CVD or related conditions. Vegetarians were reported to have higher plasma levels of tGSH than nonvegetarians,[17] and healthy men receiving ascorbic acid-deficient diets had lower plasma tGSH levels than control subjects.[18] Thus, it is possible that changes in lifestyle after CVD onset, such as decreased dietary intake of vegetables and vitamins, may be related to or contribute to the decreased plasma tGSH levels in our patients. We did not examine dietary intake in this case–control study. However, the plasma concentrations of vitamin B_{12} and folate in our CVD patients were higher than or approximately equal to those of the controls, suggesting that the CVD patients did not have vitamin-deficient diets. The secondary limitation is that our study lacked information on drug use, which could affect plasma tGSH levels. Although the effects of drug use on tGSH levels have been scarcely studied, it has been reported that antihypertensive agents, long-acting nitrates, and aspirin, which are frequently used in CVD patients, did not affect plasma tGSH levels.[7] Thus, a bias from this source is unlikely. The third limitation is that our sample size of CVD patients is relatively small for subtype analysis, especially for myocardial infarction and the subtypes of stroke. Further study with a larger sample size is needed to establish more definitive conclusions.

In conclusion, a reduced level of tGSH may be an important risk factor for the development of CVD, and especially of lacunar infarction and cerebral hemorrhage. There is evidence that orally administered GSH increases its plasma concentrations in animals and humans.[19] Thus, it is anticipated that oral administration of GSH is a possible therapeutic strategy for the prevention of CVD, although further studies, including randomized, double-blind, and placebo-controlled trials, are essential to confirm the preventive effects of GSH against CVD.

Acknowledgments

The authors thank the residents of Hisayama Town, for their participation in the survey, and the staff of the Division of Health and Welfare of Hisayama Town, for their cooperation in this study. This study was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

1. Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, Mccord JM, Harman D. Oxygen radicals and human disease. *Ann Intern Med.* 1987;107:526–545.
2. Stamler JS, Slivka A. Biological chemistry of thiols in the vasculature and in vascular-related disease. *Nutr Rev.* 1996;54:1–30.
3. Jha P, Flather M, Lonn E, Farkouh M, Yusuf S. The antioxidant vitamins and cardiovascular disease. *Ann Intern Med.* 1995;123:860–872.
4. Julius M, Lang CA, Gleiberman L, Harburg E, DiFranceisco W, Schork A. Glutathione and morbidity in a community-based sample of elderly. *J Clin Epidemiol.* 1994;47:1021–1026.
5. Nuttall SL, Martin U, Sinclair AJ, Kendall MJ. Glutathione: in sickness and in health. *Lancet.* 1998;351:645–646.
6. Beutler E, Gelbart T. Plasma glutathione in health and in patients with malignant disease. *J Lab Clin Med.* 1985;105:581–584.
7. Bridges AB, Scott NA, Pringle TH, McNeill GP. Relationship between the extent of coronary artery disease and indicators of free radical activity. *Clin Cardiol.* 1992;15:169–174.
8. Gu M, Love H, Schofield D, Turkie W, Odom N, Braganza JM. A pilot study of blood antioxidant and free radical marker profiles in patients awaiting coronary artery bypass grafting. *Clin Chim Acta.* 1996;252:181–195.
9. Kiyohara Y, Kato I, Iwamoto H, Nakayama K, Fujishima M. The impact of alcohol and hypertension on stroke incidence in a general Japanese population. *Stroke.* 1995;26:368–372.

10. World Health Organization. Cerebrovascular diseases: prevention, treatment, and rehabilitation. Technical report series No. 469. Geneva: World Health Organization; 1971.
11. Tanizaki Y, Kiyohara Y, Kato I, Iwamoto H, Nakayama K, Shinohara N, Arima H, Tanaka K, Ibayashi S, Fujishima M. Incidence and risk factors for subtypes of cerebral infarction in a general population: the Hisayama Study. *Stroke.* 2000;31:2616–2622.
12. Shimizu S, Kiyohara Y, Kato I, Tanizaki Y, Ueno H, Kimura Y, Iwamoto H, Kubo M, Arima H, Ibayashi S, Fujishima M. Plasma homocyst(e)ine concentrations and the risk of subtypes of cerebral infarction: the Hisayama Study. *Cerebrovasc Dis.* 2002;13:9–15.
13. Toyo'oka T, Uchiyama S, Saito Y. Simultaneous determinant of thiols and disulfides by high-performance liquid chromatography with fluorescence detection. *Anal Chim Acta.* 1988;205:29–41.
14. Orlowski M, Sessa G, Green JP. γ-Glutamyl transpeptidase in brain capillaries: possible site of a blood–brain barrier for amino acids. *Science.* 1974;184:66–68.
15. Harlan JM, Levine JD, Callahan KS, Schwartz BR. Glutathione redox cycle protects cultured endothelial cells against lysis by extracellularly generated hydrogen peroxide. *J Clin Invest.* 1984;73:706–713.
16. Thomas JP, Geiger PG, Girotti AW. Lethal damage to endothelial cells by oxidized low density lipoprotein: role of selenoperoxidases in cytoprotection against lipid hydroperoxide- and iron-mediated reaction. *J Lipid Res.* 1993;34:479–490.
17. Flagg EW, Coates RJ, Jones DP, Eley JM, Gunter EW, Jackson B, Greenberg RS. Plasma total glutathione in humans and its association with demographic and health-related factors. *Br J Nutr.* 1993;70: 797–808.
18. Henning SM, Zhang JZ, Mckee RW, Swendseid ME, Jacob RA. Glutathione blood levels and other oxidant defense indices in men fed diets low in vitamin C. *J Nutr.* 1991;121:1969–1975.
19. Hagen TM, Jones DP. Role of glutathione transport in extrahepatic detoxication. In: Sakamoto Y, Higashi T, Taniguchi N, Meister A, eds. *Glutathione Centennial — Molecular Perspectives and Clinical Implications.* San Diego, CA: Academic Press; 1989:423–433.

Hyperhomocysteinemia and the Development of Chronic Kidney Disease in a General Population: The Hisayama Study

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• ***Background:*** **Hyperhomocysteinemia has been linked with various atherosclerotic diseases, but has not been evaluated sufficiently as a risk factor for the development of chronic kidney disease (CKD) in the general population.** ***Methods:*** **To clarify this issue, we followed up 1,477 community-dwelling individuals without CKD, aged 40 years or older, for 5 years and examined the effects of baseline serum total homocysteine (tHcy) levels on the development of CKD.** ***Results:*** **During follow-up, 88 subjects experienced CKD. Baseline tHcy levels were greater in men than women (1.35 versus 1.04 mg/L [10.0 versus 7.7 μmol/L]; $P < 0.01$). Age-adjusted 5-year incidences were 2.2% in the low tertile, 5.4% in the middle tertile, and 8.6% in the high tertile of tHcy levels for men and 3.3%, 6.0%, and 6.9% for women, respectively. The difference between the low and high tertiles was statistically significant for both sexes ($P < 0.05$). In multivariate analysis, these relationships remained substantially unchanged, even after adjustment for other confounding factors, such as systolic blood pressure, antihypertensive medication, hemoglobin A_{1c} level, total cholesterol level, high-density lipoprotein cholesterol level, habitual smoker status, regular alcohol intake, proteinuria, and baseline kidney function (odds ratio [OR] in the high tertile of tHcy levels, 2.09; 95% confidence interval [CI], 0.66 to 6.61 for men; OR, 2.86; 95% CI, 1.10 to 7.43 for women). Furthermore, baseline tHcy level showed a significantly inverse association with rate of change in kidney function during the 5 years after being adjusted for confounding factors, including baseline kidney function.** ***Conclusion:*** **Our findings suggest that elevated serum tHcy levels are a significant risk factor for the development of CKD in the general population.** ***Am J Kidney Dis*** **44:437-445.**

INDEX WORDS: Homocysteine; chronic kidney disease (CKD); glomerular filtration rate (GFR); prospective study; general population.

CHRONIC KIDNEY DISEASE (CKD) is a worldwide public health problem; there is an increasing incidence and prevalence of renal failure, with poor outcomes and high cost.[1] Thus, treatment of CKD at earlier stages is of significance in preventing the progression toward renal failure.[2-4]

Identification of factors responsible for the progression toward renal failure is an ongoing area of interest. Several previous studies reported that age, blood pressure, diabetes, proteinuria, such dyslipidemia as apolipoprotein B or high-density lipoprotein (HDL) cholesterol level abnormalities, and smoking were associated with the subsequent decline in glomerular filtration rate (GFR).[5-9] However, regardless of the treatment and prevention of these factors, patients with renal failure are increasing in number, suggesting that other factors also must be evaluated.

Homocysteine is a sulfur-containing amino acid formed during metabolism of the essential amino acid methionine.[10] In 1969, elevated plasma total homocysteine (tHcy) levels were first linked with vascular disease by McCully.[11] Recent studies have confirmed that elevated tHcy levels are associated with atherosclerotic disease in coronary, cerebral, and peripheral blood vessels in the general population,[12-23] similar to the tendency observed in patients with renal failure.[24,25] Thus, it is reasonable to hypothesize that hyperhomocysteinemia may lead to intrarenal arteriosclerotic lesions and decline in GFR. However, previous prospective studies examining subjects without diabetes with reduced GFR could not find a significant relationship between hyperhomocysteinemia and decline in renal function.[26,27] Furthermore, this issue has not yet been examined in any prospective cohort studies of subjects with normal renal function.

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Received January 8, 2004; accepted in revised form May 20, 2004.

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0272-6386/04/4403-0006$30.00/0
doi:10.1053/j.ajkd.2004.05.024

To clarify whether hyperhomocysteinemia has a significant role in deterioration in renal function, we conducted a prospective cohort study in a Japanese community and investigated the relationship between moderate hyperhomocysteinemia and the development of CKD in subjects with normal renal function.

METHODS

Study Population

The Hisayama study, an epidemiological study of cerebrovascular and cardiovascular diseases, was established in 1961 in Hisayama Town, a suburban community adjacent to Fukuoka City, a metropolitan area of Kyushu Island in southern Japan. The population of the town is approximately 7,500, and full community surveys of the residents have been repeated since 1961.[28]

In 1988, a screening survey for the present study was performed in Hisayama Town. A detailed description of this survey was published previously.[29] Briefly, 2,736 Hisayama residents aged 40 years or older (80.7% of the total population of this age group) consented to participate in the examination and underwent a comprehensive assessment, including estimation of GFR. After excluding 1 subject for whom no blood sample was obtained, 110 subjects with frozen blood samples inadequate for measuring serum tHcy levels, and 324 subjects with moderate or severe CKD (GFR < 60 mL/min/1.73 m^2), the remaining 2,301 individuals were enrolled in this study. Of those, 1,477 subjects (596 men; 881 women) who participated in the health examination in 1993 were finally determined to be the cohort for the present study (reparticipation rate, 64.1%).

Risk Factors

At baseline examination, a self-administered questionnaire concerning current antihypertensive treatment, habitual smoker status, and regular alcohol intake was completed in advance by each participant and checked by trained interviewers at the screening. These variables were classified as being either habitually used or not. Blood pressure was measured 3 times after subjects had rested for at least 5 minutes by using a standard mercury sphygmomanometer with subjects in a sitting position. The mean of the 3 measurements was used for the analysis. Body height and weight were measured in light clothing without shoes, and body mass index was calculated as weight in kilograms divided by height in meters squared. Study physicians performed physical examinations on all participants and rechecked their medical histories.

Blood samples were collected from an antecubital vein after an overnight fast for determination of serum creatinine, urea nitrogen, albumin, lipid, and plasma glucose levels. These specimens were assayed within 24 hours. Part of the serum was stored at -20°C until measurement of tHcy. Fresh-voided urine samples were collected at the examination, and proteinuria is defined as 1^+ or more by using a reagent strip. Serum creatinine concentration was measured by Jaffé's method, and plasma fasting glucose concentration was measured by means of the glucose oxidase method. Hemoglobin A_{1c} level was measured by means of high-performance liquid chromatography. Total cholesterol and HDL cholesterol levels were determined enzymatically. Frozen serum samples were thawed and assayed for serum tHcy using the high-performance liquid chromatography method in 2002.

Definition of CKD and GFR Slope

GFR was estimated using the simplified prediction equation derived from the Modification of Diet in Renal Disease (MDRD) study[30] and derived using the following equation:

$$\text{GFR (mL/min/1.73 m}^2) = 170 \times (\text{serum creatinine [mg/dL]})^{-0.999} \times (\text{age [years]})^{-0.176} \times (\text{serum urea nitrogen [mg/dL]})^{-0.170} \times (\text{serum albumin [g/dL]})^{+0.318} \times (0.762 \text{ if female})$$

GFR less than 60 mL/min/1.73 m^2 (<1.00 mL/s/1.73 m^2) is defined as CKD according to the National Kidney Foundation–Kidney Disease Outcomes Quality Initiative guidelines.[31]

The rate of change in GFR was calculated as GFR slope by using the following equation:

$$\text{GFR slope (mL/min/1.73 m}^2\text{/y)} = (\text{GFR in 1993 [mL/min/1.73m}^2] - \text{GFR in 1988 [mL/min/1.73 m}^2])/5$$

Statistical Analysis

The SAS computer package (SAS Institute, Cary, NC)[32] was used to perform all statistical analyses. To analyze serum tHcy levels as categorical variables, they were divided into tertiles according to sex. The median value of tHcy levels in each tertile was used as the categorical value for each level. Relationships between tHcy levels and relevant factors or GFR slope were tested by means of linear regression or logistic regression analysis, as appropriate. In these analyses, serum tHcy and creatinine levels were transformed into logarithms to improve the skewed distribution. Age-adjusted cumulative incidences of CKD were calculated by means of the direct method using the World Health Organization standard population in 1998 and compared by means of Mantel-Haenszel chi-square test using 10-year age groupings. Age- and multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) also were calculated by means of logistic regression analysis. P less than 0.05 is considered statistically significant in all analyses.

RESULTS

Baseline characteristics of subjects according to sex are listed in Table 1. Mean age was 58 years for men and 57 years for women. Mean GFR, serum urea nitrogen level, and creatinine level and frequency of proteinuria were greater

Table 1. Clinical Characteristics of Study Population According to Sex in 1988

Variables	Men (n = 596)	Women (n = 881)
Age (y)	58 ± 10	57 ± 10
GFR (mL/min/1.73 m^2)	78.7 ± 11.0	73.0 ± 8.7
Serum urea nitrogen (mg/dL)	16 ± 4	15 ± 3
Creatinine (mg/dL)	1.1 ± 0.1	0.9 ± 0.1
Proteinuria (%)	7.7	3.2
Albumin (g/dL)	4.3 ± 0.2	4.3 ± 0.2
Systolic blood pressure (mm Hg)	134 ± 20	130 ± 20
Diastolic blood pressure (mm Hg)	80 ± 11	76 ± 11
Antihypertensive medication (%)	12.8	11.5
Regular alcohol intake (%)	60.9	8.7
Habitual smokers (%)	46.5	5.1
Fasting blood glucose (mg/dL)	106 ± 23	102 ± 21
Hemoglobin A_{1c} (%)	5.6 ± 0.7	5.5 ± 0.7
Total cholesterol (mg/dL)	199 ± 40	214 ± 40
HDL cholesterol (mg/dL)	49 ± 11	52 ± 11
Body mass index (kg/m^2)	22.9 ± 2.8	23.1 ± 3.2
tHcy (μmol/L)	10.0 ± 3.6	7.7 ± 2.2

NOTE. Values expressed as mean ± SD or frequencies. To convert GFR in mL/min to mL/s, multiply by 0.01667; serum urea nitrogen in mg/dL to mmol/L, multiply by 0.357; serum creatinine in mg/dL to μmol/L, multiply by 88.4; albumin in g/dL to g/L, multiply by 10; glucose in mg/dL to mmol/L, multiply by 0.05551; total or HDL cholesterol in mg/dL to mmol/L, multiply by 0.02586; tHcy in μmol/L to mg/L, divide by 7.397.

in men than women, whereas mean values for albumin were not different between sexes. Mean systolic and diastolic blood pressures and frequency of antihypertensive medication use also were greater in men. Regular alcohol intake and habitual smoker status were much more frequent in men than women. Male subjects had greater mean fasting plasma glucose and hemoglobin A_{1c} levels. Mean values for total cholesterol were greater in women, whereas mean HDL cholesterol level and body mass index were similar for both sexes. Men had greater serum tHcy levels than women.

Mean values or frequencies of potentially relevant factors are listed by tertiles of serum tHcy levels for men in Table 2. Mean age was not significantly different among serum tHcy levels. Mean values for GFR significantly decreased with increasing tHcy levels, but opposite effects were observed for mean creatinine and albumin levels and frequency of proteinuria. Mean serum urea nitrogen levels did not change with changing tHcy levels. Mean systolic blood pressure showed a tendency to increase with increasing serum tHcy levels, but mean diastolic blood pressure and frequency of antihypertensive medication did not. The frequency of regular alcohol intake significantly decreased with increasing tHcy levels. No dose-response relationships were observed between tHcy levels and frequency of habitual smoker status or mean values for fasting blood glucose, hemoglobin A_{1c}, total cholesterol, and HDL cholesterol, whereas mean body mass index significantly increased with increasing tHcy levels.

Women showed the same tendencies as men for all except 6 relevant factors: mean age and frequency of antihypertensive medication significantly increased with elevating tHcy levels, whereas the opposite effect was observed for mean HDL cholesterol level. Frequency of proteinuria, alcohol intake, and mean body mass index did not change with changing tHcy levels (Table 3).

During the 5-year follow-up, 88 subjects (39 men, 49 women) experienced CKD. Age-adjusted 5-year cumulative incidence rates of CKD according to tertiles of serum tHcy levels are shown according to sex in Fig 1. The incidence was 2.2% in the low tertile, 5.4% in the middle tertile, and 8.6% in the high tertile of tHcy levels for men and 3.3%, 6.0%, and 6.9% for women, respectively. The difference between low and high tertiles was statistically significant for both sexes ($P < 0.05$). However, mean values for GFR at the end of follow-up in subjects who developed CKD were not significantly different among tHcy tertiles in each sex (mean GFR, 55 ± 4 [SD] mL/min/1.73 mm^2 [0.92 ± 0.06 mL/s/1.73 mm^2] in the low tertile, 55 ± 5 mL/min/1.73 mm^2 [0.91 ± 0.09 mL/s/1.73 mm^2] in the middle tertile, and 53 ± 11 mL/min/1.73 mm^2 [0.88 ± 0.18 mL/s/1.73 mm^2] in the high tertile of tHcy levels for men; 56 ± 4 mL/min/1.73 mm^2 [0.94 ± 0.07 mL/s/1.73 mm^2], 55 ± 5 mL/min/1.73 mm^2 [0.91 ± 0.09 mL/s/1.73 mm^2], and 57 ± 2 mL/min/1.73 mm^2 [0.95 ± 0.04 mL/s/1.73 mm^2] for women, respectively).

As shown in model 1 in Table 4, age-adjusted logistic analysis showed that risk for CKD increased with increasing tHcy levels in both men (middle tertile: OR, 2.27; 95% CI, 0.78 to 6.63; high tertile: OR, 3.68; 95% CI, 1.32 to 10.23) and women (middle tertile: OR, 2.34; 95% CI,

Table 2. Mean Values or Frequencies of Potential Risk Factors and Laboratory Variables According to Tertiles of tHcy Levels for 596 Men in 1988

Variables	Tertiles of tHcy Levels (μmol/L) ≤8.3 (n = 187)	8.4-10.5 (n = 210)	≥10.6 (n = 199)	*P* for Trend
Age (y)	57 ± 10	59 ± 10	59 ± 11	NS
GFR (mL/min/1.73 m^2)	82 ± 11	78 ± 11	76 ± 10	<0.01
Serum urea nitrogen (mg/dL)	15 ± 4	16 ± 4	16 ± 3	NS
Creatinine (mg/dL)	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	<0.01
Proteinuria (%)	4.8	6.7	11.6	<0.05
Albumin (g/dL)	4.2 ± 0.3	4.3 ± 0.2	4.3 ± 0.2	<0.05
Systolic blood pressure (mm Hg)	131 ± 19	134 ± 17	135 ± 23	<0.05
Diastolic blood pressure (mm Hg)	79 ± 11	81 ± 11	80 ± 12	NS
Antihypertensive medication (%)	9.1	13.8	15.1	NS
Regular alcohol intake (%)	69.0	58.1	56.3	<0.05
Habitual smokers (%)	54.0	41.0	45.2	NS
Fasting blood glucose (mg/dL)	106 ± 24	107 ± 24	105 ± 19	NS
Hemoglobin A_{1c} (%)	5.7 ± 0.7	5.6 ± 0.7	5.6 ± 0.7	NS
Total cholesterol (mg/dL)	196 ± 37	204 ± 39	195 ± 42	NS
HDL cholesterol (mg/dL)	49 ± 11	50 ± 11	47 ± 11	NS
Body mass index (kg/m^2)	22.5 ± 2.8	23.1 ± 2.8	23.2 ± 2.9	<0.01

NOTE. Values expressed as mean ± SD or frequencies. To convert GFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, multiply by 0.01667; serum urea nitrogen in mg/dL to mmol/L, multiply by 0.357; serum creatinine in mg/dL to μmol/L, multiply by 88.4; albumin in g/dL to g/L, multiply by 10; fasting blood glucose in mg/dL to mmol/L, multiply by 0.05551; total or HDL cholesterol in mg/dL to mmol/L, multiply by 0.02586; tHcy in μmol/L to mg/L, divide by 7.397.

Abbreviation: NS, not significant.

0.91 to 6.05; high tertile: OR, 2.93; 95% CI, 1.16 to 7.39). These relationships remained substantially unchanged, even after adjustment for other confounding factors, such as systolic blood pressure, antihypertensive medication, hemoglobin A_{1c} level, total cholesterol level, HDL cholesterol level, serum albumin level, regular alcohol intake, habitual smoker status, and proteinuria (model 2). ORs in the high tertile were 3.42 (95% CI, 1.15 to 10.20) for men and 3.20 (95% CI, 1.25 to 8.22) for women. When baseline inverse serum creatinine was added to the independent variables used in model 2, risk for CKD in the high tertile of tHcy levels tended to increase for men, but not significantly, probably because of overadjustment (model 3). These relationships remained unchanged after adjusting for GFR calculated using the MDRD formula or for creatinine clearance calculated using the Cockcroft-Gault formula, rather than inverse serum creatinine.

Finally, we performed a slope analysis in which the association between continuous values of tHcy and GFR slope was examined by using a multiple regression model after adjusting for age, systolic blood pressure, antihypertensive medication, hemoglobin A_{1c} level, total cholesterol level, HDL cholesterol level, serum albumin level, habitual smoker status, regular alcohol intake, proteinuria, and inverse serum creatinine. This analysis showed a significantly negative association between tHcy levels and rates of change in GFR for both sexes (for GFR slope by an increment of 1 log of tHcy concentration: β, −0.63; F value, 5.91; $P < 0.05$ for men; β, −0.73; F value, 8.18; $P < 0.01$ for women).

DISCUSSION

To our knowledge, this is the first population-based cohort study showing that serum tHcy levels are positively associated with the development of CKD. This association remained unchanged, even after adjustments were made for other confounding factors, such as age, sex, systolic blood pressure, antihypertensive medication, hemoglobin A_{1c} level, total cholesterol level, HDL cholesterol level, regular alcohol intake, habitual smoker status, proteinuria, and baseline renal function, suggesting that moderately el-

Table 3. Mean Values or Frequencies of Potential Risk Factors and Laboratory Variables According to Tertiles of tHcy Levels for 881 Women in 1988

Variables	Tertiles of tHcy Levels (μmol/L) ≤6.6 (n = 276)	6.7-8.2 (n = 311)	≥8.3 (n = 294)	*P* for Trend
Age (y)	55 ± 10	57 ± 10	60 ± 11	<0.01
GFR (mL/min/1.73 m^2)	75 ± 9	73 ± 8	72 ± 9	<0.01
Serum urea nitrogen (mg/dL)	14 ± 3	14 ± 3	15 ± 3	NS
Creatinine (mg/dL)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	<0.01
Proteinuria (%)	3.6	3.2	2.7	NS
Albumin (g/dL)	4.2 ± 0.2	4.2 ± 0.2	4.3 ± 0.2	<0.05
Systolic blood pressure (mm Hg)	127 ± 20	129 ± 19	134 ± 20	<0.01
Diastolic blood pressure (mm Hg)	75 ± 12	76 ± 10	76 ± 10	NS
Antihypertensive medication (%)	8.3	11.3	14.6	<0.05
Regular alcohol intake (%)	9.8	8.7	7.8	NS
Habitual smokers (%)	5.4	4.2	5.8	NS
Fasting blood glucose (mg/dL)	102 ± 25	101 ± 21	101 ± 17	NS
Hemoglobin A_{1c} (%)	5.5 ± 0.9	5.5 ± 0.6	5.5 ± 0.6	NS
Total cholesterol (mg/dL)	212 ± 40	215 ± 40	217 ± 40	NS
HDL cholesterol (mg/dL)	53 ± 12	51 ± 11	51 ± 11	<0.05
Body mass index (kg/m^2)	23.0 ± 3.2	22.9 ± 3.1	23.3 ± 3.2	NS

NOTE. Values expressed as mean ± SD or frequencies. To convert GFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, multiply by 0.01667; serum urea nitrogen in mg/dL to mmol/L, multiply by 0.357; serum creatinine in mg/dL to μmol/L, multiply by 88.4; albumin in g/dL to g/L, multiply by 10; fasting blood glucose in mg/dL to mmol/L, multiply by 0.05551; total or HDL-cholesterol in mg/dL to mmol/L, multiply by 0.02586; tHcy in μmol/L to mg/L, divide by 7.397.

Abbreviation: NS, not significant.

evated serum tHcy levels are an independent risk factor for CKD in the general population.

Previously, 2 prospective studies investigated the relationships between hyperhomocysteinemia and progression of kidney disease. Samuelsson et al[26] did not find a significant relationship between tHcy level and GFR decline in a follow-up study of 83 patients without diabetes with reduced GFR. Similar findings were observed in another follow-up study of 804 patients without diabetes with moderate or severe decline in GFR.[27] These inconsistent results may be caused by such method limitations as inadequate statistical power because of the small number of subjects and short follow-up periods (within 3.5 years) in these previous studies. Another possible reason for the discrepancy between the previous studies and ours is that effects of hyperhomocysteinemia may differ between subjects with normal renal function and those with reduced renal function; presumably relevant factors, such as blood pressure and proteinuria, affect the progression of kidney disease more strongly than tHcy level in subjects with a moderate or severe decline in GFR.

Several mechanisms by which tHcy might cause vascular damage have been suggested. Elevated tHcy levels promote the proliferation of vascular smooth muscle cells by stimulation of the mitogen-activated protein kinase signal transduction pathway and DNA synthesis.[10] They also impair endothelial vasodilatation by inhibiting the generation of endothelial mediators, including nitric oxide, and they promote adhesion between neutrophil and endothelial cells.[10] tHcy generates superoxide radicals, which inhibit the endothelial-dependent relaxation of vessels.[33] Furthermore, tHcy oxidizes low-density lipoprotein and thus may promote the cellular uptake of modified low-density lipoprotein.[34] Through these mechanisms, elevated tHcy levels might cause intrarenal arteriosclerosis or arteriolar hyalinosis, resulting in a chronic reduction in renal perfusion pressure.[10,35] Chronic hypoperfusion leads to focal or global glomerulosclerosis, tubular atrophy, and interstitial fibrosis.[35] In our previous autopsy-based population survey, intrarenal arteriosclerosis, arteriolar hyalinosis, and glomerulosclerosis were associated closely with reduced GFR.[36] Thus, it is conceivable that el-

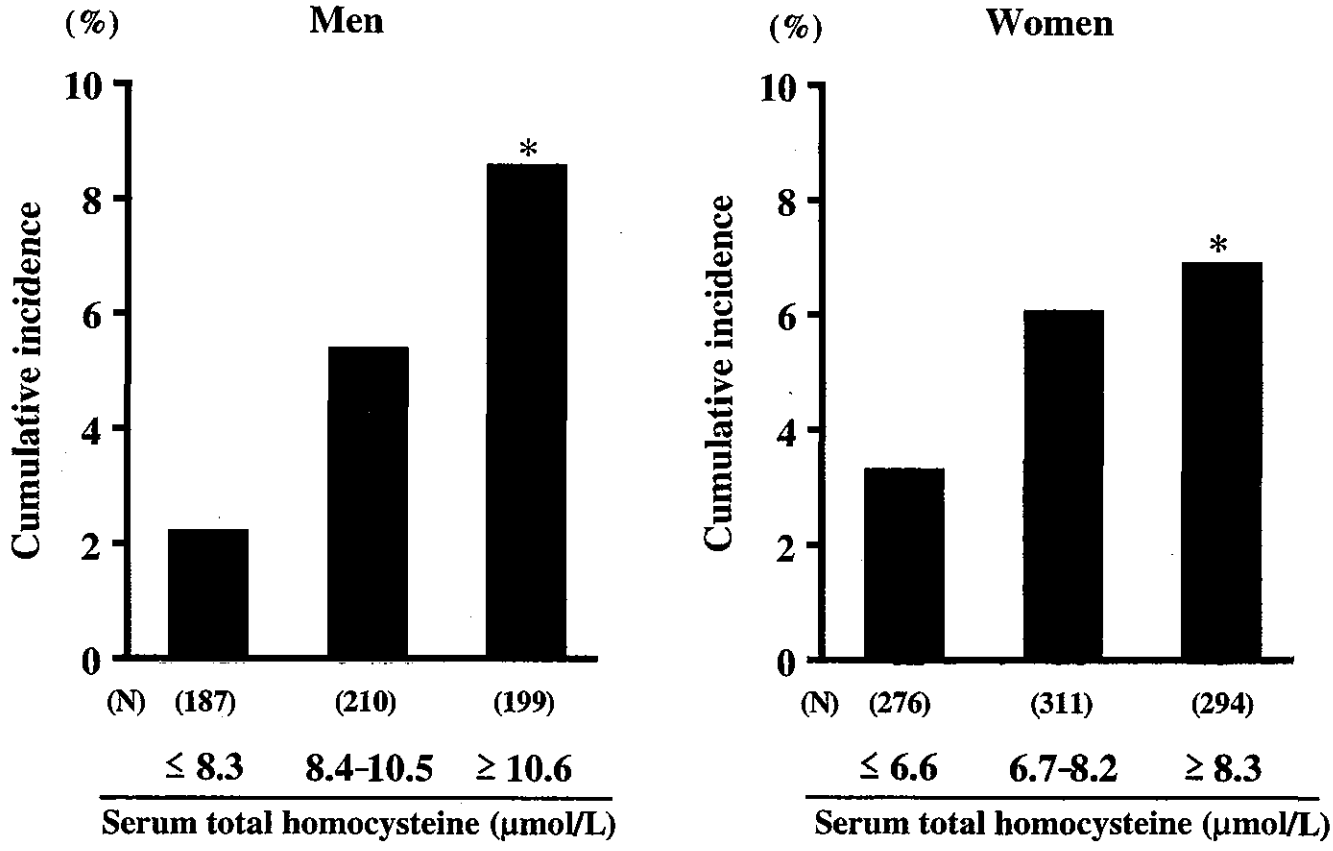

Fig 1. Age-adjusted 5-year cumulative incidence rates of CKD according to tertiles of tHcy levels by sex. *$P < 0.05$ versus low tertile. To convert tHcy in μmol/L to mg/L, divide by 7.397.

evated tHcy levels induce the progression of intrarenal arteriosclerotic vascular lesions, resulting in the development of CKD.

Previous prospective and cross-sectional studies have shown clear associations between tHcy level and the development of coronary heart disease and stroke,[12-23] as well as the anatomic extent of systemic atherosclerosis.[37,38] Furthermore, in recent cross-sectional and prospective studies of general populations, elevated tHcy

Table 4. Multivariate-Adjusted Analysis for the Development of CKD According to Tertiles of tHcy Levels by Sex During a 5-Year Follow-Up

	Tertiles of tHcy Levels (μmol/L)			
	≤8.3 (n = 187)	8.4-10.5 (n = 210)	≥10.6 (n = 199)	*P* for Trend
Men				
Model 1	1.00	2.27 (0.78-6.63)	3.68 (1.32-10.23)*	<0.01
Model 2	1.00	2.28 (0.74-7.02)	3.42 (1.15-10.20)*	<0.05
Model 3	1.00	1.68 (0.52-5.42)	2.09 (0.66-6.61)	0.23
	≤6.6 (n = 276)	6.7-8.2 (n = 311)	≥8.3 (n = 294)	
Women				
Model 1	1.00	2.34 (0.91-6.05)	2.93 (1.16-7.39)*	<0.05
Model 2	1.00	2.52 (0.96-6.61)	3.20 (1.25-8.22)*	<0.05
Model 3	1.00	2.37 (0.90-6.25)	2.86 (1.10-7.43)*	<0.05

NOTE. Values expressed as OR (95% CI). Model 1, adjusted for age; model 2, adjusted for age, systolic blood pressure, antihypertensive medication, hemoglobin A_{1c} level, total cholesterol level, HDL cholesterol level, serum albumin level, habitual smokers, regular alcohol intake, and proteinuria; model 3, adjusted for confounding factors used in model 2 and inverse serum creatinine. To convert tHcy in μmol/L to mg/L, divide by 7.397.

*$P < 0.05$ versus low tertile.

levels were independently associated with microalbuminuria,[9,40] a marker of endothelial dysfunction and a predictor of future cardiovascular disease and renal dysfunction.[41-44] It is well recognized that there are histological and immunohistochemical similarities between the evolving fatty streak in the atherosclerotic vessel wall and progressive glomerular lesions leading to glomerulosclerosis.[45,46] These findings may give additional support to the hypothesis that tHcy leads to glomerulosclerosis by inducing vascular damage.

In our study, the frequency of regular alcohol intake decreased with increasing tHcy levels. Although chronic alcoholism clearly increases serum tHcy levels, possibly because of vitamin deficiencies,[47,48] effects of mild to moderate alcohol consumption are debated in the epidemiological studies.[49,50] In our subjects, alcohol consumption was associated positively with serum tHcy level ($r = 0.19$; $P < 0.01$). The majority of our subjects who consumed alcoholic beverages were mild and moderate alcohol drinkers. Thus, our findings suggest that mild to moderate alcohol intake exerts a favorable influence on tHcy levels.

Several limitations of our study should be discussed. The primary limitation is that our results might be biased by the exclusion of 111 subjects for whom serum tHcy levels could not be determined because of the lack of a serum sample or inadequate serum samples. These subjects might have had high serum tHcy levels because their mean age and systolic blood pressure levels were higher than those of the subjects in the present study. Additionally, it is possible that our results are biased by the exclusion of subjects who did not return for the follow-up examination. Among 824 subjects without a follow-up examination (431 men, 393 women), 103 patients died during the follow-up period: 45 of these patients died of cancer; 23 patients, cardiovascular disease; and the remaining subjects, other diseases. At baseline, mean age for men was significantly older in subjects with a follow-up examination than in those without it (58 versus 56 years), but this trend did not hold for women. However, mean values for tHcy, GFR, systolic and diastolic blood pressure, and frequency of proteinuria were not significantly different between the 2 groups for both sexes. Thus, this bias has the potential to alter our findings, but is not likely to do so.

A second limitation is that our GFR estimates made using the simplified prediction equation derived from the MDRD study and based on a single blood sample might not be sufficiently correct, although this prediction equation, among other equations of its type, is considered to be the most precise estimate of GFR.[30] In addition, a recent report showed that repeated serum creatinine measurements were necessary to correct within-person measurement variations in serum creatinine level (1% to ~10%),[51] suggesting that some nondifferential misclassifications of cases with CKD may have occurred in our study. Given that these limitations can reduce the impact of tHcy, the true association may be stronger than that shown in our findings.

A third limitation is that we have no data for vitamin status, including the status of folic acid, vitamin B_6, and vitamin B_{12}, or methylenetetrahydrofolate reductase (MTHFR) polymorphism, which affects serum tHcy levels.[10,52] Sarnak et al[27] reported that lower serum folate, pyridoxal 5-phosphate (active form of vitamin B_6), and vitamin B_{12} levels were not associated with the decline in GFR in multivariable analysis. To our knowledge, no previous study evaluated the relationship between MTHFR polymorphism and progression of kidney disease. Thus, it is not clear that vitamin status and MTHFR polymorphism influence the association between tHcy level and CKD. Additional examination on this issue is needed.

A fourth limitation is that tHcy concentrations were determined by using serum samples that had been frozen for 12 years. In theory, use of frozen samples can result in incorrect values because of either sample breakdown or hemoconcentration caused by dehydration of the sample. However, several studies have documented the stability of tHcy samples frozen for as long as 10 years,[53,54] and other studies also used frozen samples to assay tHcy.[16,17,52] Additionally, distributions of serum tHcy levels in our population were similar to those in other Japanese populations.[38] Thus, this limitation seems not to invalidate the association of tHcy level with the development of CKD found in our subjects.

The final limitation is that we have no information about type of underlying renal disease. Such

information could be obtained by detailed clinical examination, including renal biopsy and ultrasonography, but these diagnostic procedures are not considered feasible for a cohort study recruited from a general population, such as ours.

In conclusion, the findings of this study suggest that elevated tHcy levels are a significant risk factor for the development of CKD in the general population. At present, the extent to which tHcy-lowering treatment can attenuate the risk for CKD is not known. Thus, a tHcy-lowering clinical trial is needed to clarify whether the reduction in tHcy concentrations will result in an improved renal prognosis.

REFERENCES

1. Obrador GT, Pereira BJ, Kausz AT: Chronic kidney disease in the United States: An underrecognized problem. Semin Nephrol 22:441-448, 2002

2. Modification of Diet in Renal Disease Study Group: Effects of dietary protein restriction on the progression of moderate renal disease in the Modification of Diet in Renal Disease Study. J Am Soc Nephrol 7:2616-2626, 1996

3. Giatras I, Lau J, Levey AS: Effect of angiotensin-converting enzyme inhibitors on the progression of nondiabetic renal disease: A meta-analysis of randomized trials. Angiotensin-Converting-Enzyme Inhibition and Progressive Renal Disease Study Group. Ann Intern Med 127:337-345, 1997

4. Pereira BJ: Optimization of pre-ESRD care: The key to improved dialysis outcomes. Kidney Int 57:351-365, 2000

5. Klahr S, Levey AS, Beck GJ, et al: The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. N Engl J Med 330:877-884, 1994

6. Hunsicker LG, Adler S, Caggiula A, et al: Predictors of the progression of renal disease in the Modification of Diet in Renal Disease Study. Kidney Int 51:1908-1919, 1997

7. Fox CS, Larson MG, Leip EP, et al: Predictors of new-onset kidney disease in a community-based population. JAMA 291:844-850, 2004

8. Samuelsson O, Mulec H, Knight-Gibson C, et al: Lipoprotein abnormalities are associated with increased rate of progression of human chronic renal insufficiency. Nephrol Dial Transplant 12:1908-1915, 1997

9. Tozawa M, Iseki K, Iseki C, et al: Influence of smoking and obesity on the development of proteinuria. Kidney Int 62:956-962, 2002

10. Haynes WG: Hyperhomocysteinemia, vascular function and atherosclerosis: Effects of vitamins. Cardiovasc Drugs Ther 16:391-399, 2002

11. McCully KS: Vascular pathology of homocysteinemia: Implications for the pathogenesis of arteriosclerosis. Am J Pathol 56:111-128, 1969

12. Boers GH, Smals AG, Trijbels FJ, et al: Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. N Engl J Med 313:709-715, 1985

13. Clarke R, Daly L, Robinson K, et al: Hyperhomocysteinemia: An independent risk factor for vascular disease. N Engl J Med 324:1149-1155, 1991

14. Israelsson B, Brattstrom LE, Hultberg BL: Homocysteine and myocardial infarction. Atherosclerosis 71:227-233, 1988

15. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 274:1049-1057, 1995

16. Bostom AG, Silbershatz H, Rosenberg IH, et al: Nonfasting plasma total homocysteine levels and all-cause and cardiovascular disease mortality in elderly Framingham men and women. Arch Intern Med 159:1077-1080, 1999

17. Kark JD, Selhub J, Adler B, et al: Nonfasting plasma total homocysteine level and mortality in middle-aged and elderly men and women in Jerusalem. Ann Intern Med 131:321-330, 1999

18. Ueland PM, Refsum H, Beresford SA, Vollset SE: The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr 72:324-332, 2000

19. Vollset SE, Refsum H, Tverdal A, et al: Plasma total homocysteine and cardiovascular and noncardiovascular mortality: The Hordaland Homocysteine Study. Am J Clin Nutr 74:130-136, 2001

20. Perry IJ, Refsum H, Morris RW, et al: Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 346:1395-1398, 1995

21. Shimizu H, Kiyohara Y, Kato I, et al: Plasma homocyst(e)ine concentrations and the risk of subtypes of cerebral infarction. The Hisayama study. Cerebrovasc Dis 13:9-15, 2002

22. Matsui T, Arai H, Yuzuriha T, et al: Elevated plasma homocysteine levels and risk of silent brain infarction in elderly people. Stroke 32:1116-1119, 2001

23. Fallon UB, Virtamo J, Young I, et al: Homocysteine and cerebral infarction in Finnish male smokers. Stroke 34:1359-1363, 2003

24. Clarke R, Lewington S, Landray M: Homocysteine, renal function, and risk of cardiovascular disease. Kidney Int Suppl 84:S131-S133, 2003

25. Prichard S: Risk factors for coronary artery disease in patients with renal failure. Am J Med Sci 325:209-213, 2003

26. Samuelsson O, Lee DM, Attman PO, et al: The plasma levels of homocysteine are elevated in moderate renal insufficiency but do not predict the rate of progression. Nephron 82:306-311, 1999

27. Samak MJ, Wang SR, Beck GJ, et al: Homocysteine, cysteine, and B vitamins as predictors of kidney disease progression. Am J Kidney Dis 40:932-939, 2002

28. Ueda K, Omae T, Hirota Y, et al: Epidemiological and clinico-pathological study on renal diseases observed in the autopsy cases in Hisayama population, Kyushu Island, Japan. J Chronic Dis 29:159-173, 1976

29. Ohmura T, Ueda K, Kiyohara Y, et al: Prevalence of type 2 (non-insulin-dependent) diabetes mellitus and impaired glucose tolerance in the Japanese general population: The Hisayama study. Diabetologia 36:1198-1203, 1993

30. Levey AS, Bosch JP, Lewis JB, et al: A more accurate method to estimate glomerular filtration rate from serum

creatinine: A new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 130:461-470, 1999

31. National Kidney Foundation: K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, classification, and stratification. Am J Kidney Dis 39:S17-S31, 2002 (suppl 2)

32. SAS Institute: SAS Procedure Guide, version 6 (ed 3). Cary, NC, SAS Institute, 1990

33. Emsley AM, Jeremy JY, Gomes GN, Angelini GD, Plane F: Investigation of the inhibitory effects of homocysteine and copper on nitric oxide-mediated relaxation of rat isolated aorta. Br J Pharmacol 126:1034-1040, 1999

34. Heinecke JW, Rosen H, Suzuki LA, Chait A: The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. J Biol Chem 262:10098-10103, 1987

35. Meyrier A, Hill GS, Simon P: Ischemic renal diseases: New insights into old entities. Kidney Int 54:2-13, 1998

36. Kubo M, Kiyohara Y, Kato I, et al: Risk factors for renal glomerular and vascular changes in an autopsy-based population survey: The Hisayama study. Kidney Int 63:1508-1515, 2003

37. Selhub J, Jacques PF, Bostom AG, et al: Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med 332:286-291, 1995

38. Adachi H, Hirai Y, Fujiura Y, et al: Plasma homocysteine levels and atherosclerosis in Japan: Epidemiological study by use of carotid ultrasonography. Stroke 33:2177-2181, 2002

39. Hoogeveen EK, Kostense PJ, Jager A, et al: Serum homocysteine level and protein intake are related to risk of microalbuminuria: The Hoorn Study. Kidney Int 54:203-209, 1998

40. Jager A, Kostense PJ, Nijpels G, et al: Serum homocysteine levels are associated with the development of (micro)albuminuria: The Hoorn study. Arterioscler Thromb Vasc Biol 21:74-81, 2001

41. Dinneen SF, Gerstein HC: The association of microalbuminuria and mortality in non-insulin-dependent diabetes mellitus. A systematic overview of the literature. Arch Intern Med 157:1413-1418, 1997

42. Yudkin JS, Forrest RD, Jackson CA: Microalbuminuria as predictor of vascular disease in non-diabetic subjects. Islington Diabetes Survey. Lancet 2:530-533, 1988

43. Gerstein HC, Mann JF, Yi Q, et al, HOPE Study Investigators: Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. JAMA 286:421-426, 2001

44. Nelson RG, Knowler WC, Pettitt DJ, Saad MF, Charles MA, Bennett PH: Assessment of risk of overt nephropathy in diabetic patients from albumin excretion in untimed urine specimens. Arch Intern Med 151:1761-1765, 1991

45. Keane WF, Kasiske BL, O'Donnell MP: Lipids and progressive glomerulosclerosis. A model analogous to atherosclerosis. Am J Nephrol 8:261-271, 1988

46. Diamond JR: Analogous pathobiologic mechanisms in glomerulosclerosis and atherosclerosis. Kidney Int Suppl 31:S29-S34, 1991

47. Cravo ML, Camilo ME: Hyperhomocysteinemia in chronic alcoholism: Relations to folic acid and vitamins B(6) and B(12) status. Nutrition 16:296-302, 2000

48. Cravo ML, Gloria LM, Selhub J, et al: Hyperhomocysteinemia in chronic alcoholism: Correlation with folate, vitamin B-12, and vitamin B-6 status. Am J Clin Nutr 63:220-224, 1996

49. Mennen LI, de Courcy GP, Guilland JC, et al: Homocysteine, cardiovascular disease risk factors, and habitual diet in the French Supplementation with Antioxidant Vitamins and Minerals Study. Am J Clin Nutr 76:1279-1289, 2002

50. Ganji V, Kafai MR: Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the Third National Health and Nutrition Examination Survey, 1988-1994. Am J Clin Nutr 77:826-833, 2003

51. Hsu CY, Chertow GM, Curhan GC: Methodological issues in studying the epidemiology of mild to moderate chronic renal insufficiency. Kidney Int 61:1567-1576, 2002

52. Ma J, Stampfer MJ, Hennekens CH, et al: Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. Circulation 94:2410-2416, 1996

53. Stabler SP, Marcell PD, Podell ER, Allen RH: Quantitation of total homocysteine, total cysteine, and methionine in normal serum and urine using capillary gas chromatography-mass spectrometry. Anal Biochem 162:185-196, 1987

54. Israelsson B, Brattstrom L, Refsum H: Homocysteine in frozen plasma samples. A short cut to establish hyperhomocysteinaemia as a risk factor for arteriosclerosis? Scand J Clin Lab Invest 53:465-469, 1993

RAPID COMMUNICATION
Clinical

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J Dent Res 83(6):485-490, 2004

The Severity of Periodontal Disease is Associated with the Development of Glucose Intolerance in Non-diabetics: The Hisayama Study

ABSTRACT

Inflammation is hypothesized to play a significant role in the development of type 2 diabetes; however, reports on clinical inflammatory conditions are limited. Studies have suggested that periodontitis affects glucose control in diabetics. This community-based study examined the relationship between periodontitis and glucose tolerance status, including changes in status. The relationship between periodontal condition and the results of a 75-g oral glucose tolerance test was examined in 961 adults in 1998. Deep pockets (mean pocket depth > 2.0 mm) were significantly associated with impaired glucose tolerance and with diabetes as compared with shallow pockets (< 1.3 mm). In the subgroup with normal glucose tolerance 10 years previously, subjects who subsequently developed impaired glucose tolerance were significantly more likely to have deep pockets. Deep pockets were closely related to current glucose tolerance status and the development of glucose intolerance.

KEY WORDS: periodontal disease, diabetes, glucose tolerance, risk factor, epidemiology.

Received May 21, 2003; Last revision March 28, 2004; Accepted April 9, 2004

INTRODUCTION

Recent evidence suggests that chronic subclinical inflammation plays an intermediary role in the pathogenesis of type 2 diabetes (Festa *et al.*, 2000; Pradhan *et al.*, 2001). Elevated levels of inflammatory markers, such as C-reactive protein (CRP) and interleukin-6 (IL-6), are reported to be significant risk indicators of type 2 diabetes (Pradhan *et al.*, 2001). However, few studies have examined the clinical conditions that cause chronic inflammation. Periodontal disease is a very common chronic subclinical inflammation, which the majority of affected people do not notice, even if they have been affected for years. In the US, about 30% of adults have periodontal disease with periodontal pockets ≥ 4 mm deep, which are a hotbed of subgingival anaerobes, such as *Porphyromonas gingivalis* (Brown *et al.*, 1996; Albandar *et al.*, 1999). Many studies have long recognized that periodontitis is more prevalent in diabetic patients and worsens with diabetes (Page *et al.*, 1997). Moreover, studies have indicated that treating periodontitis in diabetic patients has a beneficial effect on their glucose control (Williams and Mahan, 1960; Miller *et al.*, 1992; Grossi *et al.*, 1997; Collin *et al.*, 1998; Grossi, 2001; Iwamoto *et al.*, 2001; Stewart *et al.*, 2001). A randomized clinical trial in Pima Indians with type 2 diabetes demonstrated that periodontal treatment with doxycycline reduced *P. gingivalis* in periodontal pockets and also reduced hemoglobin A1c (HbA1c) after 3 mos (Grossi *et al.*, 1997; Grossi, 2001). The HbA1c level deteriorated in type 2 diabetic patients with, but not in patients without, severe periodontitis (Collin *et al.*, 1998). Elevated serum CRP (Wakai *et al.*, 1999; Loos *et al.*, 2000; Slade *et al.*, 2000; Wu *et al.*, 2000; Noack *et al.*, 2001; Glurich *et al.*, 2002; Saito *et al.*, 2003) and IL-6 (Loos *et al.*, 2000) levels have been reported in subjects with periodontitis. A recent study reported that serum IgG titers against *P. gingivalis* were correlated with CRP in Japanese type 2 diabetic subjects (Nishimura *et al.*, 2002). Therefore, we hypothesized that periodontal disease is a risk factor for type 2 diabetes. However, no studies have examined the relationship between periodontal disease and longitudinal changes in glucose control in non-diabetic subjects. In 1998, we conducted a periodontal examination as part of the Hisayama Study (Kubo *et al.*, 1999). In this article, we examine the relationship between periodontal condition and glucose tolerance using a cross-sectional, retrospective cohort design that considers past glucose tolerance.

MATERIALS & METHODS

The Hisayama Study began in 1961 and is an ongoing prospective cohort study of cardiovascular diseases. As part of the survey, between July and September, 1998, a total of 2180 Hisayama residents aged 40 to 79 yrs (52.1% of the total population in that age group) underwent a comprehensive health examination that included a fasting 75-g oral glucose tolerance test (OGTT). In all, 1111 of these residents underwent a periodontal examination. We excluded the 150

Table 1. Comparison of Subjects' Characteristics According to Glucose Tolerance Status in 1998 (n = 961)

Characteristics	Normal Glucose Tolerance (NGT, n = 669, 69.6%)	Impaired Glucose Tolerance (IGT, n = 191, 19.9%)	Diabetes (n = 101, 10.5%)	P[a]
Periodontal Condition		*Mean ± SD*		
Mean pocket depth *(mm)*	1.6 ± 0.5	1.7 ± 0.5[c]	1.8 ± 0.6[d]	
Maximum pocket depth (mm)	3.5 ± 1.4	3.9 ± 1.5[b]	4.0 ± 1.7[c]	
Mean attachment loss (mm)	1.9 ± 0.8	2.0 ± 0.9	2.3 ± 0.9[de]	
Maximum attachment loss (mm)	4.1 ± 1.71	4.5 ± 2.1	4.9 ± 2.1[c]	
Number of teeth	25.4 ± 4.0	24.9 ± 3.9	23.6 ± 4.0[de]	
Dental plaque index	1.0 ± 0.6	1.2 ± 0.7[c]	1.3 ± 0.7[d]	
Mean pocket depth (mm)		*No. of subjects (%)*		
Low (< 1.3)	230 (78.0)	47 (15.9)	18 (6.1)	0.0001
Intermediate (1.3-2.0)	322 (69.0)	94 (20.1)	51 (10.9)	
High (> 2.0)	117 (58.8)	50 (25.1)	32 (16.1)	
Mean attachment loss (mm)				
Low (< 1.5)	184 (71.0)	57 (22.0)	18 (6.9)	< 0.0001
Intermediate (1.5-2.5)	366 (73.1)	90 (18.0)	45 (9.0)	
High (> 2.5)	119 (59.2)	44 (21.9)	38 (18.9)	
General condition		*Mean ± SD*		
Age (yrs)	55.6 ± 8.8	57.0 ± 8.9	60.5 ± 6.9[df]	
BMI (kg/m^2)	22.8 ± 2.9	24.2 ± 3.6[d]	24.4 ± 3.6[d]	
HbA1c (%)	5.0 ± 0.2	5.2 ± 0.4[d]	6.4 ± 1.1[df]	
Triglyceride (mg/dL)	120 ± 90	136 ± 78	164 ± 94[de]	
Total cholesterol (mg/dL)	205 ± 35	208 ± 37	215 ± 38[b]	
HDL cholesterol (mg/dL)	59 ± 14	55 ± 13[c]	55 ± 14[b]	
LDL cholesterol (mg/dL)	122 ± 32	126 ± 33	128 ± 38	
Systolic blood pressure (mm Hg)	126 ± 20	133 ± 18[d]	142 ± 22[df]	
Diastolic blood pressure (mm Hg)	77 ± 11	80 ± 10[c]	82 ± 12[d]	
Smoking habits		*No. of subjects (%)*		
Never	466 (72.5)	121 (18.8)	56 (8.7)	0.049
Past	89 (62.7)	32 (22.5)	21 (14.8)	
Current	114 (64.8)	38 (21.6)	24 (13.6)	
Alcohol consumption (g/month)				
0	408 (72.2)	109 (19.3)	48 (8.5)	0.059
1-399	116 (69.9)	30 (18.1)	20 (12.0)	
400-1199	87 (65.4)	31 (23.3)	15 (11.3)	
≥ 1200	58 (59.8)	21 (21.6)	18 (18.6)	
Exercise frequency (times/wk)				
0	495 (70.2)	143 (20.3)	67 (9.5)	0.20
1-2	90 (73.2)	19 (15.4)	14 (11.4)	
≥ 3	84 (63.2)	29 (21.8)	20 (15.0)	
Sex				
Men	233 (61.8)	83 (22.0)	61 (16.2)	< 0.0001
Women	436 (74.7)	108 (18.5)	40 (6.8)	

[a] Actual probability by Pearson's chi-square test to compare the proportion of subjects.
[b-f] Student's *t* test or Welch's *t* test in case of unequal variances was applied with Bonferroni's correction of p-values for type 1 errors in multiple comparisons.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$, compared with NGT.
[e] $p < 0.05$.
[f] $p < 0.001$, comparison between diabetes and IGT.

subjects with fewer than 10 teeth from this study, because of the inherent difficulties in properly assessing periodontal health in these patients. Ultimately, 961 subjects (377 men and 584 women) with at least 10 teeth were analyzed.

In 1988, 2480 Hisayama residents aged 40 to 79 yrs underwent a similar health examination without a periodontal examination (Kubo *et al.*, 1999). Of the 961 subjects examined in 1998, 244 subjects under 50 yrs of age were excluded, since they were under 40 yrs of age in 1988. The 591 subjects for whom OGTT results in 1988 were available (82.4%) were enrolled in this study. Of these, 415 subjects had normal glucose tolerance in 1988 (152 men and 263 women, 50-79 yrs old in 1998), and we analyzed the relationship between periodontal conditions and the development of glucose intolerance between 1988 and 1998.

In the 1998 examination, following the method of the Third National Health and Nutrition Examination Survey (NHANES III) (Brown *et al.*, 1996), a periodontal examination was performed on 2 randomly selected quadrants, 1 maxillary and 1 mandibular, by four trained dentists, using a normal dental chair. Mean periodontal pocket depth and attachment loss were analyzed. The subjects were divided into 3 categories with respect to each of the 2 periodontal measurements, mean pocket depth and mean attachment loss: 'Low' (< 1.3 mm), 'Intermediate' (1.3-2.0 mm), and 'High' (> 2.0 mm) mean pocket depth; and 'Low' (< 1.5 mm), 'Intermediate' (1.5-2.5 mm), and 'High' (> 2.5 mm) mean attachment loss. For both measurements, the 'High' categories corresponded to the highest 20% of the measurements and the 'Low' to the lowest 30%. A report on NHANES III (Albandar *et al.*, 1999) showed that about 20% of