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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 $\mu\text{mol/L}$; $P=0.0001$). Among the CVD types, both the cerebral infarction cases (2.98 versus 3.59 $\mu\text{mol/L}$; $P=0.001$) and cerebral hemorrhage cases (2.51 versus 3.43 $\mu\text{mol/L}$; $P=0.0027$) 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 $\mu\text{mol/L}$; $P=0.36$) and myocardial infarction (3.65 versus 3.77 $\mu\text{mol/L}$; $P=0.69$), 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, 0.41; 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 degrades 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 malignancies⁶ 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

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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 al¹³ at the Saga Research Institute of Ohtsuka Pharmaceutical Co, Ltd, with no awareness of the case-control status or of clinical information. Plasma vitamin B₆ concentrations were also determined using high-performance liquid chromatography with fluorescence detection. A chemiluminescent immunoassay was used to measure plasma folate and vitamin B₁₂. 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/m²) 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 B₆ and higher vitamin B₁₂ levels than the latter. The mean total homocysteine levels were significantly higher in

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

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

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 B₆ levels, whereas plasma folate and vitamin B₁₂ 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 B₆, vitamin B₁₂, 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

Factors	Quartiles of Glutathione (μ mol/L)				P for trend
	<2.53 (n=142)	2.53–3.41 (n=143)	3.41–4.4 (n=143)	>4.4 (n=141)	
Age, y	70 \pm 9	69 \pm 9	70 \pm 9	67 \pm 9	0.02
Sex, % male	61	52	53	56	0.39
Systolic blood pressure, mm Hg	142 \pm 2	141 \pm 2	135 \pm 2	133 \pm 2	0.0001
Diastolic blood pressure, mm Hg	78 \pm 1	79 \pm 1	77 \pm 1	74 \pm 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 \pm 0.3	22.4 \pm 0.3	22.3 \pm 0.3	21.4 \pm 0.3	0.13
Cholesterol, mmol/L	5.1 \pm 0.1	5.2 \pm 0.1	5.3 \pm 0.1	5.4 \pm 0.1	0.004
Total protein, g/L	70 \pm 0.4	72 \pm 0.4	73 \pm 0.4	72 \pm 0.4	0.0004
Folate, nmol/L	6.4 \pm 0.3	6.4 \pm 0.3	6.5 \pm 0.3	6.5 \pm 0.3	0.64
Vitamin B ₆ , nmol/L	68.2 \pm 2.2	74.1 \pm 2.2	92.9 \pm 2.2	82.3 \pm 2.2	0.008
Vitamin B ₁₂ , pmol/L	728 \pm 26	649 \pm 23	674 \pm 24	692 \pm 24	0.55
Total homocysteine, μ mol/L	11.7 \pm 0.4	11.4 \pm 0.4	11.7 \pm 0.4	11.0 \pm 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 \pm 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 ($\mu\text{mol/L}$)				<i>P</i> for trend
		<2.53 OR	2.53–3.41 OR (95% CI)	3.41–4.4 OR (95% CI)	>4.4 OR (95% CI)	
		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 B₆, vitamin B₁₂, 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

Subtype of Cerebral Infarction		Tertile of Glutathione ($\mu\text{mol/L}$)			<i>P</i> for trend
		<2.9 OR	2.9–4.1 OR (95% CI)	>4.1 OR (95% CI)	
		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 B₆, vitamin B₁₂, 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 al¹⁵ 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 al¹⁶ 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 B₁₂ 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.

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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. Toyooka 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. Orłowski 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.