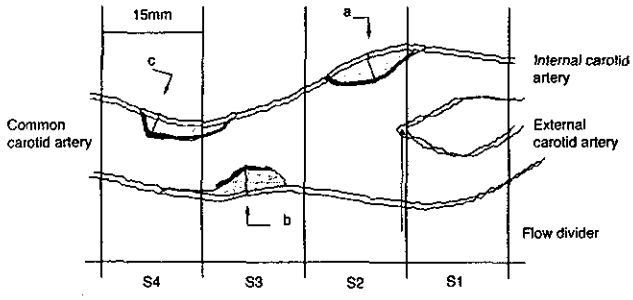

Figure 1. Diagram of carotid bifurcation and plaque score measurement obtained from B-mode ultrasonography. Plaque score was calculated by summing all plaque thicknesses in millimeters in each segment on both sides (a+b+c+contralateral plaques). Carotid artery was divided into 4 parts of 15 mm in length each from the flow divider (S1 to S4).

patients without follow-up carotid ultrasonography were deleted from the analysis. Ultimately, 179 patients were enrolled in the previous study, of whom 129 patients were hypertensives being treated with drug therapy or lifestyle modification. Information about antihypertensive therapy was missing for 5 patients, so this study presents data on the remaining 124 patients.

Risk Factors

Blood pressure was measured in the right arm with the patient in the seated position after a 5-minute rest, following recommendations of the American Heart Association.[10] The average of 2 consecutive blood pressure measurements was calculated. Hypertension was defined as an SBP of ≥140 mm Hg, a diastolic blood pressure (DBP) of ≥90 mm Hg, or current use of antihypertensive medications. The other traditional risk factors for CVD were classified as follows. Hypercholesterolemia was defined as a total cholesterol level of ≥220 mg/dL (5.69 mmol/L) or current cholesterol-lowering therapy. Diabetes mellitus was defined as a glycosylated hemoglobin A_{1c} concentration of >5.8% or current use of oral hypoglycemic agents. Body mass index was the weight in kilograms divided by the square of the height in meters. Patients were categorized as smokers if they were current smokers or had stopped smoking <1 month before entry into the study. Cigarette pack years were calculated for each patient as a measure of cumulative smoking exposure. Patients were categorized as having CVD if there was a history of cerebrovascular disease, ischemic heart disease, aortic aneurysm, or peripheral vascular disease.

Evaluation of Carotid Atherosclerosis

To evaluate the progression of carotid atherosclerosis, high-resolution B-mode ultrasonography using a 7.5-MHz duplex probe (EUB-525, Hitachi) was performed repeatedly over a period of ≥2 years. Baseline and follow-up ultrasound images were recorded on VHS videotape, and the changes of each plaque were evaluated in a blinded manner. The method was similar to that used by us in another prospective study.[3,11] On the basis of our previous findings, the upper limit of normal for the intima-media thickness (IMT) was set at 1.0 mm, and areas with an IMT of ≥1.1 mm were defined as atheromatous plaques. The plaque score was calculated by summing the thickness of all plaques measured in both carotid arteries (Figure 1),[12] and we used the number of plaques and the plaque score to estimate the severity of carotid atherosclerosis. The progression of atherosclerosis was estimated by inserting each parameter into the following formula: Δ value/year=(final value−baseline value)/years of follow-up. Advanced carotid atherosclerosis was defined as a plaque score of >10,[11] and such patients were not enrolled in this study.

Measurement of the Circulating hs-CRP Concentration

Blood samples were collected in tubes containing citric acid and stored at −80°C after centrifugation. The stored serum for each patient was thawed in April 1998 for hs-CRP measurement using an automatic immunonephelometer with a sensitivity of 0.02 mg/dL (Behring NA latex CRP; Behring Institute).

Statistical Analysis

Natural log transformation of the hs-CRP data achieved a normal distribution, so log-transformed hs-CRP values were used. All hs-CRP concentrations below the detection limit were assigned a log-transformed value of −4.605 (ie, an hs-CRP value of 0.01 mg/dL). The relationship between measured risk factors, including log-transformed CRP values, and the parameters of carotid atherosclerosis was evaluated by calculation of Pearson correlation coefficients. Spearman rank correlation coefficients were used for the skewed distribution of cigarette pack years. Student *t* test was used to evaluate the difference between the parameters in relation to the presence and absence of categorized traditional risk factors, including treatment with statins, aspirin, or angiotensin-converting enzyme (ACE) inhibitors. Multiple linear regression analyses were performed to assess the contribution of CRP to the prediction of annual changes of each parameter compared with the contribution of hypertension and other traditional risk factors. Two-way ANOVA with Newman-Keuls test was used to estimate between-group differences of parameters of carotid atherosclerosis in relation to hs-CRP and blood pressure. Probability values (2-tailed) of <0.05 were considered significant. For 2-way ANOVA test, Statistica for Windows R 5.5 (StatSoft) was used. The other statistical analyses were performed with SPSS for Windows version 9.0J.

Results

The baseline characteristics of the 124 subjects are summarized in Table 1. The follow-up period was 35±10 months. With regard to the relationships between hs-CRP and traditional risk factors, there was a significant association of the hs-CRP level with age (r=0.22; P<0.05), fasting blood glucose (r=0.19; P<0.05), and high-density lipoprotein cholesterol (r=−0.19; P<0.05). The relationship of CRP with cigarette pack years (r=0.17; P=0.059), pulse pressure (r=0.17; P=0.060), and SBP (r=0.15; P=0.086) was also positive but showed no statistical significance. There was no significant relationship between the hs-CRP level and the other traditional risk factors. Sex and the presence or absence of risk factors and treatment with ACE inhibitors, statins, or aspirin had no significant influence on the hs-CRP levels.

Among categorized risk factors, men had further progression than women (0.76±1.18 versus 0.43±1.00 in annual change of plaque score; P<0.05). The relationships between hs-CRP, pulse pressure, SBP, DBP, and the parameters of carotid atherosclerosis are shown in Table 2. Pulse pressure, SBP, and hs-CRP were correlated with the annual changes of plaque number and plaque score in simple regression analysis, and the correlations remained significant after adjusting for the effect of other traditional risk factors and for the baseline severity of carotid atherosclerosis. No other traditional risk factors (including DBP) were significantly correlated with the parameters of carotid atherosclerosis in simple regression analysis. When analysis was limited to the patients without hypercholesterolemia, diabetes mellitus, or current smoking, the results were similar to those in the total patient population, except that there was no significant association with SBP in the nonhypercholesterolemic or nondiabetic subgroups (Table 2). The progression of carotid atherosclerosis in relation to pulse pressure/SBP and hs-CRP is shown in Figures 2 and 3, respectively. Patients were divided into 2

TABLE 1. Baseline Characteristics of the Patients (n=124)

Age, y	62.7±8.7
Male	66 (53)
Antihypertensives medication	102 (82)
ACEI/CCB/β-blocker	33 (27)/74 (60)/37 (30)
α-blocker/diuretics	11 (9)/5 (4)
SBP/DBP, mm Hg	139±16/83±11
Pulse pressure, mm Hg	56±15
Hypercholesterolemia/statin medication	48 (39)/26 (21)
Total/HDL cholesterol, mg/dL (mmol/L)	205±31/58±15 (5.3±0.8/1.5±0.4)
Diabetes mellitus/oral hypoglycemic agents	21 (17)/4 (3)
Fasting blood glucose, mg/dL (mmol/L)	104±29 (5.8±1.6)
Hemoglobin A_{1c}, %	5.3±0.8
Body mass index, kg/m^2	23.7±2.8
Current smoker	14 (11)
Cigarette pack years	0 (0, 6.0) [10.0]
History of CVD	36 (29)
Antiplatelet medication/aspirin medication	25 (20)/7 (6)
CRP, mg/dL	0.07 (0.04, 0.15)
Plaque No.	1.0 (0, 3.0) [2.4]
Plaque score	2.4 (0, 4.5) [3.9]

The age, blood pressure, cholesterol, fasting blood glucose, hemoglobin A_{1c}, and body mass index are shown as mean±SD. Data on the blood pressure, cholesterol, fasting blood glucose, and hemoglobin A_{1c} are shown for all 124 patients. Cigarette pack years, CRP, plaque no., and plaque score are shown as the median and interquartile range. The mean values of cigarette pack years for past and current smokers and the mean plaque no. and plaque score for the patients with carotid atherosclerosis are shown in square brackets. Other values are the no. of patients, along with the proportion in parentheses.

ACEI indicates ACE inhibitor; CCB, calcium channel blocker.

TABLE 2. Association Between hs-CRP, Blood Pressure, and Parameters Associated With the Development of Carotid Atherosclerosis

		Simple Regression		Multivariate Regression* (Standardized)	
		r	*P*	β	*P*
In total patients (n=124)					
ΔPN/yr	hs-CRP	0.314	<0.001	0.343	0.001
	Pulse pressure	0.281	0.002	0.267	0.006
	SBP	0.290	0.001	0.299	0.001
	DBP	0.054	0.557	0.000	0.999
ΔPS/yr	hs-CRP	0.328	<0.001	0.376	<0.001
	Pulse pressure	0.287	0.001	0.268	0.005
	SBP	0.238	0.008	0.227	0.014
	DBP	−0.031	0.736	−0.104	0.299
ΔPS/yr per subgroup					
Patients without hypercholesterolemia (n=76)					
hs-CRP		0.343	0.002	0.452	0.001
Pulse pressure		0.274	0.017	0.282	0.034
SBP		0.210	0.068	0.148	0.235
Patients without diabetes mellitus (n=103)					
Hs-CRP		0.283	0.004	0.424	<0.001
Pulse pressure		0.256	0.009	0.326	0.004
SBP		0.180	0.070	0.185	0.080
Noncurrent smoker (n=110)					
Hs-CRP		0.258	0.006	0.310	0.005
Pulse pressure		0.316	0.001	0.331	0.002
SBP		0.268	0.005	0.391	0.002

ΔPN/yr indicates annual change of plaque number; ΔPS/yr, annual change of plaque score.

*Each parameter of blood pressure, together with hs-CRP and other traditional risk factors, was used as an independent variable in each multivariate regression model. The standardized β and *P* values of hs-CRP in Table 2 are adjusted for pulse pressure, age, sex, total cholesterol, hemoglobin A_{1c} cigarette pack years, body mass index, the severity of carotid atherosclerosis, and uses of ACE inhibitor, statin, and aspirin. When SBP or DBP was used instead of pulse pressure as a parameter of blood pressure, the standardized β and *P* values of hs-CRP were similar to those in Table 2. hs-CRP indicates high sensitivity C-reaction protein; ΔPS/y, annual change of plaque score.

groups at the median pulse pressure (53 mm Hg), an SBP of 140 mm Hg, and an hs-CRP value of 0.12 mg/dL. We reported previously that annual rate of increase in carotid atherosclerosis was accelerated in patients with an hs-CRP value of ≥0.12 mg/dL.[3] Patients with higher hs-CRP levels had greater progression of atherosclerosis than those with lower hs-CRP levels in both the lower and higher pulse pressure groups and even in patients with an SBP of <140 mm Hg on antihypertensive therapy. When analysis was limited to the 64 patients with blood pressure of <140/90 mm Hg on antihypertensive therapy, the relationship between hs-CRP and carotid atherosclerosis was stronger than that for pulse pressure. There were no significant relationships between the other traditional risk factors (including SBP and DBP) and the annual changes of plaque number or plaque score, except for body mass index (Table 3).

Discussion

This is the first study to demonstrate that evaluation of CRP could be equal or superior for predicting the development of carotid atherosclerosis to measurement of the pulse pressure or SBP in hypertensives and that its predictive value is independent of blood pressure. With respect to the association between blood pressure and carotid atherosclerosis, to the best of our knowledge, there have been few longitudinal studies focused on the middle-aged and elderly population.[13,14] These studies have emphasized an elevated pulse pressure and SBP as risk factors for atherosclerosis. Similar to the results of such studies, our findings suggested that pulse pressure and SBP are related to the progression of carotid atherosclerosis. It is thought that an elevated pulse pressure causes greater stretching of the arteries, which induces fatigue and fracture of the elastic elements and thus is likely to hasten the development of intimal damage that leads to atherosclerosis.[15] The Framingham study demonstrated a link between cardiovascular mortality and pulse

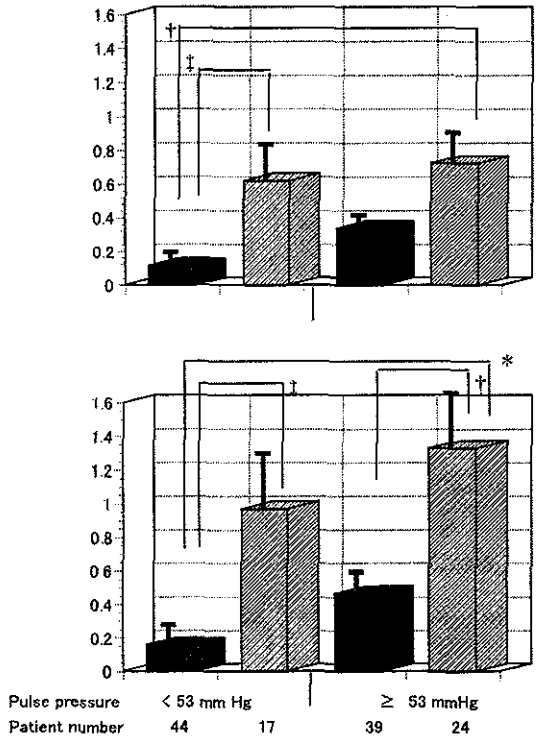

Figure 2. The annual changes of plaque number (top) and plaque score (bottom) in relation to pulse pressure and hs-CRP. ▨hs-CRP ≥0.12 mg/dL. ■hs-CRP< 0.12 mg/dL. Bars represent mean values and lines represent the SEM. *P<0.001; †P<0.01; ‡P<0.05.

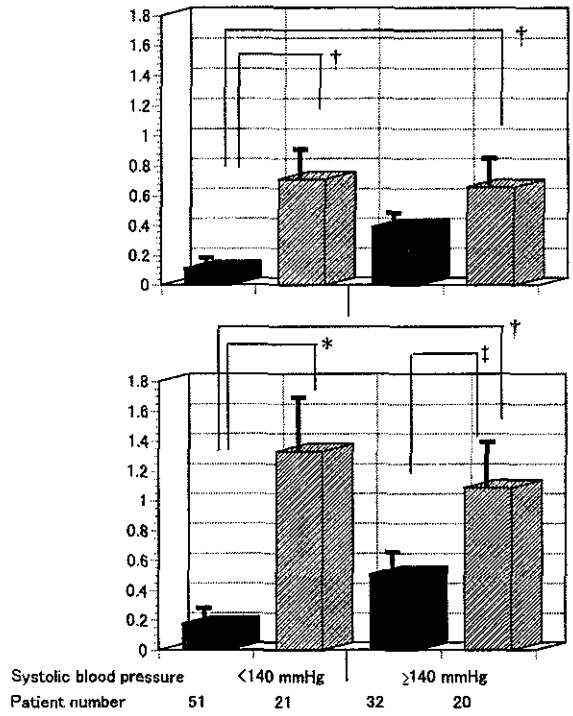

Figure 3. The annual changes of plaque number (top) and plaque score (bottom) in relation to SBP and hs-CRP. ▨hs-CRP ≥0.12 mg/dL. ■hs-CRP< 0.12 mg/dL. Bars represent mean values and lines represent the SEM. *P<0.001; †P<0.01; ‡P<0.05.

pressure by longitudinal follow-up of persons >50 years old.[16] Another large-scale study revealed similar results in male subjects aged 40 to 69 years,[17] whereas the age of the present study population was similar. We found that there was no significant relationship between the other traditional risk factors (including DBP) and the progression of carotid atherosclerosis. The lack of an association with these risk factors in the present study can be partly explained by the influence of drug therapy and lifestyle modification or the low statistical power of our analysis. The age of the present study population may also help to explain the lack of an association between DBP and carotid atherosclerosis.

Multivariate analysis revealed that CRP was one of the independent predictors of the progression of carotid atherosclerosis. Subset analysis excluding each traditional risk factor showed a similar result. One possible reason that a high CRP level is associated with carotid atherosclerosis indepen-

TABLE 3. Association Between hs-CRP Concentration, Blood Pressure, and Parameters Associated With the Development of Carotid Atherosclerosis in 64 Hypertensive Patients With Blood Pressure of <140/90 mm Hg on Antihypertensive Therapy

	Simple Regression			
	ΔPN/yr		ΔPS/yr	
	r	P	r	P
hs-CRP	0.404 (0.23, 0.91)	0.002	0.436 (0.48, 1.80)	<0.001
Pulse pressure	0.264 (0.015, −0.34)	0.044	0.310 (0.05, −1.8)	0.016
SBP	0.225	0.086	0.237	0.069
DBP	−0.007	0.958	−0.040	0.761
Age	−0.198	0.134	−0.183	0.162
Total cholesterol	0.078	0.558	0.011	0.931
Hemoglobin A_{1c}	−0.021	0.882	−0.016	0.907
Cigarette pack years	0.004	−0.977	0.012	0.930
Body mass index	0.334 (0.048, −0.73)	0.010	0.272 (0.12, −2.36)	0.035

ΔPN/yr indicates annual change of plaque number; ΔPS/yr, annual change of plaque score. The values in parentheses show the regression coefficient and intercept.

dently of blood pressure and other traditional risk factors may be the tight linkage of CRP with atherosclerotic processes. For example, CRP may contribute to monocyte recruitment in atherogenesis[18] and to induction of tissue factor release by monocytes, which is potentiated by interferon-γ and lipopolysaccharide.[19] CRP has a direct influence on atherosclerotic vessels by activation of the complement system, thereby promoting inflammation and thrombosis.[20] A recent clinical study showed that CRP was significantly correlated with the calculated 10-year Framingham coronary heart disease risk (FCHDR) but was weakly correlated with most individual components of the FCHDR score.[21] This suggested that CRP may capture different components than the traditional components of coronary risk reflected in the FCHDR score. Thus, monitoring of the blood pressure is important but not enough to predict the development of atherosclerosis in hypertensives. The average of 2 consecutive office blood pressure measurements at 1 time point was representative of the blood pressure value in the present study. A recent study suggested that circadian SBP variability is the best independent predictor of the development of carotid atherosclerosis,[13] whereas a cross-sectional study revealed that target organ damage caused by hypertension is more closely related to the home blood pressure than the office blood pressure.[22] The serum level of CRP may partly reflect the circadian blood pressure pattern or home blood pressure, or may be an indicator of a step in the process of atherosclerosis itself,[9] making it equal or superior to office blood pressure measurement for the prediction of atherosclerosis.

Chronic inflammation may induce endothelial dysfunction, which is followed by further elevation of blood pressure (pulse pressure and SBP)[23] and the onset of cardiovascular disease.[24] Several studies have shown that CRP is an independent risk factor for hypertension,[25,26] so CRP, inflammation, and hypertension appear to be linked in the process of atherosclerosis. A recent study suggested that inflammation is important for accelerated progression of atherosclerosis, particularly in hypertensives.[8] Although the relationship of CRP with pulse pressure and SBP was positive in the present study, it did not reach statistical significance. This lack of a significant association might be attributable to the low statistical power of our analysis or use of antihypertensive medication by the subjects,[27] or it may indicate that the actual association is weak.[21]

It could be argued that our results were influenced by a selection bias of the patient population because most of them were on antihypertensive therapy and some had other traditional risk factors. However, the relationship of pulse pressure, SBP, and CRP with carotid atherosclerosis remained significant after adjusting for antihypertensive therapy and other traditional risk factors, and stratified analysis showed similar results. Recent guidelines have proposed that the entire adult population should not be screened for CRP measurement for purposes of cardiovascular risk assessment but that the measurement may be useful in selected patients, such as those estimated to have a moderate risk on the basis of the 10-year FCHDR.[9] The risk management in the present study population was similar to the FCHDR concept of moderate risk, and we demonstrated that CRP was equal or superior to the office blood pressure for predicting the progression of carotid atherosclerosis, with these parameters being independent of each other. In conclusion, measurement of CRP may be valuable for predicting the progression of carotid atherosclerosis in selected hypertensive patients who are already being treated by drug therapy or lifestyle modification.

Acknowledgments

This study was supported in part by a Japan Heart Foundation/Pfizer grant for research on hypertension and vascular metabolism, health and labor sciences research grants for clinical research for evidenced-based medicine, the Smoking Research Foundation of Japan, and a research grant for cardiovascular diseases (15-5C) from the Japanese Ministry of Health, Labor, and Welfare.

References

1. Manson JE, Tosteson H, Ridker PM, Satterfield S, Hebert P, O'Connor GT, Buring JE, Hennekens CH. The primary prevention of myocardial infarction. *N Engl J Med.* 1992;326:1406–1416.
2. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med.* 1999; 340:115–126.
3. Hashimoto H, Kitagawa K, Hougaku H, Shimizu Y, Sakaguchi M, Nagai Y, Iyama S, Yamanishi H, Matsumoto M, Hori M. C-reactive protein is an independent predictor of the rate of increase in early carotid atherosclerosis. *Circulation.* 2001;104:63–67.
4. Van Der Meer IM, De Maat MP, Hak AE, Kiliaan AJ, Del Sol AI, Van Der Kuip DA, Nijhuis RL, Hofman A, Witteman JC. C-reactive protein predicts progression of atherosclerosis measured at various sites in the arterial tree: the Rotterdam Study. *Stroke.* 2002;33:2750–2755.
5. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med.* 1997;336:973–979.
6. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med.* 2000;342:836–843.
7. Kervinen H, Palosuo T, Manninen V, Tenkanen L, Vaarala O, Manttari M. Joint effects of C-reactive protein and other risk factors on acute coronary events. *Am Heart J.* 2001;141:580–585.
8. Engstrom G, Lind P, Hedblad B, Stavenow L, Janzon L, Lindgarde F. Long-term effects of inflammation-sensitive plasma proteins and systolic blood pressure on incidence of stroke. *Stroke.* 2002;33:2744–2749.
9. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice. A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation.* 2003;107:499–511.
10. Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M, Morgenstern BZ. Human blood pressure determination by sphygmomanometry. *Circulation.* 1993;88:2460–2470.
11. Handa N, Matsumoto M, Maeda H, Hougaku H, Kamada T. Ischemic stroke events and carotid atherosclerosis. Results of the Osaka Follow-up Study for Ultrasonographic Assessment of Carotid Atherosclerosis (the OSACA Study). *Stroke.* 1995;26:1781–1786.
12. Handa N, Matsumoto M, Maeda H, Hougaku H, Ogawa S, Fukunaga R, Yoneda S, Kimura K, Kamada T. Ultrasonic evaluation of early carotid atherosclerosis. *Stroke.* 1990;21:1567–1572.
13. Sander D, Kukla C, Klingelhofer J, Winbeck K, Conrad B. Relationship between circadian blood pressure patterns and progression of early carotid atherosclerosis: a 3-year follow-up study. *Circulation.* 2000;102: 1536–1541.
14. Lakka TA, Salonen R, Kaplan GA, Salonen JT. Blood pressure and the progression of carotid atherosclerosis in middle-aged men. *Hypertension.* 1999;34:51–56.
15. Nichols WW, O'Rourke MF. *McDonald's Blood Flow in Arteries.* 4th ed. London, UK: Arnold; 1998.
16. Franklin SS, Khan SA, Wong ND, Larson MG, Levy D. Is pulse pressure useful in predicting risk for coronary heart disease? The Framingham Heart Study. *Circulation.* 1999;100:354–360.

17. Benetos A, Rudnichi A, Safar M, Guize L. Pulse pressure and cardiovascular mortality in normotensive and hypertensive subjects. *Hypertension.* 1998;32:560–564.
18. Torzewski M, Rist C, Mortensen RF, Zwaka TP, Bienek M, Waltenberger J, Koenig W, Schmitz G, Hombach V, Torzewski J. C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. *Arterioscler Thromb Vasc Biol.* 2000;20:2094–2099.
19. Nakagomi A, Freedman SB, Geczy CL. Interferon-gamma and lipopolysaccharide potentiate monocyte tissue factor induction by C-reactive protein: relationship with age, sex, and hormone replacement treatment. *Circulation.* 2000;101:1785–1791.
20. Lagrand WK, Visser CA, Hermens WT, Niessen HW, Verheugt FW, Wolbink GJ, Hack CE. C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation.* 1999;100:96–102.
21. Albert MA, Glynn RJ, Ridker PM. Plasma concentration of C-reactive protein and the calculated Framingham coronary heart disease risk score. *Circulation.* 2003;108:161–165.
22. Yarows SA, Julius S, Pickering TG. Home blood pressure monitoring. *Arch Intern Med.* 2000;160:1251–1257.
23. Engstrom G, Janzon L, Berglund G, Lind P, Stavenow L, Hedblad B, Lindgarde F. Blood pressure increase and incidence of hypertension in relation to inflammation-sensitive plasma proteins. *Arterioscler Thromb Vasc Biol.* 2002;22:2054–2058.
24. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol.* 1999;19:972–978.
25. Rohde LE, Hennekens CH, Ridker PM. Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. *Am J Cardiol.* 1999;84:1018–1022.
26. Bautista LE, Lopez-Jaramillo P, Vera LM, Casas JP, Otero AP, Guaracao AI. Is C-reactive protein an independent risk factor for essential hypertension? *J Hypertens.* 2001;19:857–861.
27. Kranzhofer R, Schmidt J, Pfeiffer CA, Hagl S, Libby P, Kubler W. Angiotensin induces inflammatory activation of human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol.* 1999;19:1623–1629.

J Cardiol 2004 Jul; 44(1): 13–20

Effect of Short-Term Administration of High Dose L-Arginine on Restenosis After Percutaneous Transluminal Coronary Angioplasty

Teruo SHIRAKI, MD
Toshiyuki TAKAMURA, MD
Akio KAJIYAMA, MD
Takefumi OKA, MD
Daiji SAITO, MD

Abstract

Background. A single and local administration of L-arginine after balloon angioplasty enhances nitric oxide (NO) generation and inhibits lesion formation in animals.

Objectives. The present study assessed the effect of increasing NO to inhibit restenosis after percutaneous transluminal coronary angioplasty (PTCA) in humans by local and systemic administration of L-arginine, a precursor of NO in humans.

Methods. L-arginine was administered to 34 consecutive patients with angina pectoris or old myocardial infarction via a cardiac catheter (500 mg/4 min) before PTCA, and via a peripheral vein (30 g/4 hr, for 5 days) after PTCA. Patients were treated between December 1998 and December 2000. Plasma concentrations of L-arginine, NO (as nitrite + nitrate) and cyclic guanosine monophosphate (cGMP) were measured before and after L-arginine administration. The control group consisted of 90 patients who underwent PTCA successfully without L-arginine administration in the period between July 1996 and November 1998. Baseline clinical and angiographic characteristics were compared between the two groups. All patients were followed by coronary angiography for 3 months after PTCA. Quantitative coronary angiography and restenosis rate were studied.

Results. Baseline clinical and angiographic characteristics were not different between the two study groups. Despite a significant elevation in plasma L-arginine concentration after L-arginine administration, NO and cGMP did not increase significantly. After PTCA, the difference in restenosis rates between L-arginine and control subjects (34% vs 44%) was not significantly different.

Conclusions. Short-term administration of high dose L-arginine did not significantly change the restenosis rate after PTCA.

J Cardiol 2004 Jul; 44(1): 13–20

Key Words

■ **Myocardial infarction, treatment** ■ **Angioplasty (PTCA)** ■ **Restenosis**
■ **Nitric oxide (L-arginine administration)**

INTRODUCTION

Percutaneous transluminal coronary angioplasty (PTCA) is commonly used as a nonsurgical treatment for occlusive or stenotic atherosclerotic coronary artery disease. Despite an initial success rate greater than 90%, patency does not continue in the long-term because of restenosis. Indeed, restenosis affects as many as 30–40% of successfully dilated lesions[1]. Experimental and necrotic tissue studies suggest that restenosis is secondary to balloon-induced injury to proliferating vascular smooth

国立岩国病院 内科: 〒740–8510 山口県岩国市黒磯町2–5–1
Department of Cardiology, Iwakuni National Hospital, Yamaguchi
Address for correspondence: SHIRAKI T, MD, Department of Cardiology, Iwakuni National Hospital, Kuroiso-cho 2–5–1, Iwakuni, Yamaguchi 740–8510
Manuscript received December 17, 2003; revised March 11 and May 7, 2004; accepted May 7, 2004

muscle cells[2]. To address this problem, various techniques, including anticoagulants and angiotensin converting enzyme inhibitors and new devices have been tried to decrease restenosis, but most have failed[1-3]. Only stents have decreased restenosis[4,5]. The rate of in-stent restenosis remains about 20%, judging by neointimal formation consisting of migration and proliferation of smooth muscle cells with deposition of extracellular matrix[6]. Despite this significant reduction in the restenosis rate in primary studies of intracoronary brachytherapy[7], edge restenosis and late coronary occlusion remain unsolved[8,9]. Drug coated stents decrease the restenosis rate even further[10,11]. However, the long-term results and side effects remain unknown.

Recently, it was established that nitric oxide (NO) has many biological effects. For example, NO can interfere with monocyte adhesion and chemotaxis, platelet adherence and aggregation, and vascular smooth muscle cell proliferation[12-18]. Thus, NO may reduce restenosis after PTCA. A single intramural delivery of L-arginine, the precursor of NO, improved vasomotion and attenuated neointimal lesion formation after balloon angioplasty in previous reports[19,20]. Therefore, the present study examined the potential of L-arginine administration to reduce restenosis after PTCA in humans.

SUBJECTS AND METHODS

Study population

This study included 35 consecutive patients admitted to our hospital with angina pectoris or old myocardial infarction in the period between December 1998 and December 2000. All patients had angiographical coronary artery stenosis greater than 50% and exercise thallium myocardial scintigraphy indicated that they had myocardial ischemia. This study excluded patients with recent myocardial infarction (< 3 weeks), recent unstable angina, restenosis, left main trunk lesion, chronic total occlusion, severe left ventricular dysfunction (ejection fraction < 40%), uncontrolled diabetes mellitus (hemoglobin A_{1c} > 8.0%), bronchial asthma, renal failure (serum creatinine level > 2.0 mg/dl), acidosis, amino acid metabolic dysfunction, or other disease using steroid hormone. The protocol for administration of L-arginine was approved by the hospital ethics committee and written informed consent according to the Helsinki Declaration was obtained from all patients. The control subjects consisted of 90 patients successfully treated with PTCA without L-arginine administration at our hospital in the period between July 1996 and November 1998. They underwent follow-up coronary angiography on average 3 months after PTCA. All patients enrolled in this study received isosorbide dinitrate (60 mg/day), an angiotensin converting enzyme inhibitor (enarapril 5 mg/day or temocapril 2 mg/day), a calcium antagonist (long acting nifedipine 30 mg/day or long acting diltiazem 300 mg/day), acetylsalicylic acid (81 mg/day) and ticlopidine (100 mg/day). Treatment was started at least 2 weeks before PTCA and was continued for 3 months.

Percutaneous transluminal coronary angioplasty procedure

PTCA was performed by the femoral approach with a bolus dose of 10,000 U of heparin. A guiding catheter (8F Brite Tip; Cordis), guide wire (0.014 High torque floppy; ACS) and semi-compliant balloon catheters (Bandit; Boston Scientific) were used in all patients. Balloon inflation for 1 min was repeated until the residual stenosis became less than 25%. Patients who underwent emergent coronary artery bypass grafting or stent implantation due to acute occlusion after PTCA with flow-limiting dissection were excluded from this study.

Quantitative analysis of coronary angiography

Angiography was recorded from multiple projections for all patients before and after PTCA. Quantitative analysis of the lesions was carefully performed based on standard criteria for preprocedural lesion morphology, and the lesions were also categorized according to the modified American College of Cardiology/American Heart Association (ACC/AHA) classification system. Quantitative coronary angiography (edge detection method; MAC HEART DATABASE SYSTEM, Baxter) was used to evaluate the initial and long-term results of PTCA. Cinefilm was used as a medium. One experienced cardiologist not involved in this study measured the diameter of the coronary artery three times and the mean value was used for analysis. The minimal lumen diameter of the stenotic lesion, and the proximal and distal sites of the lesion were measured five times: immediately before L-arginine administration, after L-arginine administration via guiding catheter, after isosorbide dinitrate

administration, immediately after PTCA, and 3 months after PTCA. A guiding catheter filled with contrast medium was used as the scaling device.

Initial success was defined as percentage diameter stenosis (%DS) of $< 25\%$ after PTCA without major complications (death, emergent coronary artery bypass grafting, stent implantation, Q-wave infarction). Restenosis was defined as %DS $> 50\%$ at follow-up angiography.

L-arginine administration

i) L-arginine (500 mg/4 min; Hoechst Marion Roussel) was initially administered into the coronary artery via guiding catheter before PTCA.

ii) PTCA was performed after intracoronary infusion of 5 mg of isosorbide dinitrate.

iii) Systemic administration of L-arginine via peripheral vein was started from the beginning of PTCA and was repeated once a day for the following 4 days at the same rate (30 g/4 hr).

Measurement of plasma L-arginine concentration

A 5-French NIH catheter was inserted into the coronary sinus through the internal jugular vein during PTCA in nine patients. Plasma L-arginine concentrations in the coronary sinus and peripheral veins were measured before and after intracoronary L-arginine administration. Plasma L-arginine concentrations in the peripheral vein were measured before, immediately after, 6 hr after, and 12 hr after the systemic administration of L-arginine in 10 patients. All samples were measured with an amino acid analyzer at a commercial laboratory (SRL).

Measurement of nitric oxide and cyclic guanosine monophosphate

The production of NO in blood was evaluated by the measurement of nitrite ion (NO_2^-) + nitrate ion (NO_3^-) by the Gries method using a chemiluminescence NO analyzer (SPD-10A, Shimazu Industries) at a commercial laboratory (SRL). Cyclic guanosine monophosphate (cGMP) was measured by radioimmunoasssay with a gamma counter (ARC-950, AROKA) at a commercial laboratory (SRL) according to the manufacturer's protocol. The same blood sample collected for the measurement of L-arginine was also used for the measurements of NO and cGMP.

Table 1 Baseline patient characteristics

	Arginine (+) ($n=34$)	Arginine (−) ($n=90$)	p value
Age (yr)	67±10	63±10	NS
Male (%)	71 (24/34)	71 (64/90)	NS
Risk factors (%)			
Hypertension	26 (9/34)	29 (26/90)	NS
Diabetes mellitus	18 (9/34)	16 (14/90)	NS
Hypercholesterolemia	12 (4/34)	14 (13/90)	NS
Smoking	24 (8/34)	22 (20/90)	NS
Previous myocardial infarction	44 (15/34)	48 (43/90)	NS
Total cholesterol (mg/dl)	199±44	193±39	NS
HDL-cholesterol (mg/dl)	52±10	44±14	NS
Triglyceride (mg/dl)	169±136	159±119	NS
Uric acid (mg/dl)	5.6±1.7	5.9±1.6	NS
Blood sugar (mg/dl)	120±31	119±32	NS

Continuous values are mean±SD.
HDL=high-density lipoprotein.

Statistical analysis

Data was expressed as mean ± standard deviation. The chi-square test was used to assess differences in categorical variables. The paired Student's t-test was used to assess differences in continuous variables between the two groups. p values of less than 0.05 were considered significant.

RESULTS

Table 1 shows the clinical characteristics of patients in both groups. There were no statistically significant differences between the two groups with respect to age, sex, total cholesterol, triglyceride, high-density lipoprotein-cholesterol, uric acid, fasting blood sugar, coronary risk factors and prior myocardial infarction. **Table 2** shows the angiographic characteristics of the two groups. Lesion vessels and ACC/AHA classification types showed no difference. PTCA was successfully performed in 34 patients (success rate of 99%). One patient was defined as failed PTCA because the residual stenosis was 50% after PTCA and he was excluded from the study.

L-arginine was administered to all 34 patients via the coronary artery and peripheral vein. One patient had severe headache and the infusion speed of L-arginine was decreased (30 g/4 hr → 30 g/8 hr). Plasma L-arginine concentration after intracoronary and systemic administration of L-arginine signifi-

cantly increased (**Tables 3** and **4**). The diameter of the coronary artery did not change significantly after intracoronary administration of L-arginine. NO and cGMP did not increase significantly in the coronary sinus or the peripheral vein after intracoronary administration of L-arginine ($n=9$; **Table 3**). After systemic administration of L-arginine, NO did not increase significantly ($n=10$; **Table 4**). Follow-up angiography was performed in 32 patients (94%). The parameters of quantitative coronary angiography and the restenosis rate in the L-arginine group after PTCA were not significantly different from those of the control group (**Tables 5** and **6**).

Table 2 Baseline lesion angiographic characteristics

	Arginine (+) (n=34)	Arginine (−) (n=90)	p value
Vessel (%)			NS
LAD	50 (17/34)	67 (60/90)	
LCX	15 (5/34)	7 (6/90)	
RCA	35 (12/34)	26 (24/90)	
ACC/AHA lesion type (%)			NS
A	24 (8/34)	18 (16/90)	
B	62 (21/34)	79 (71/90)	
C	14 (5/34)	3 (3/90)	
Maximal inflation pressure (atm)	9.7±3.1	8.3±2.2	NS
Balloon-artery ratio	1.04±0.14	1.05±0.15	NS
Total inflation time (sec)	360±180	300±144	NS

Continuous values are mean±SD.
LAD=left anterior descending artery; LCX=left circumflex coronary artery; RCA=right coronary artery; ACC/AHA=American College of Cardiology/American Heart Association.

DISCUSSION

The restenosis rate after PTCA is 30−40% and is still the biggest limitation of PTCA. The mechanism of restenosis consists of platelet aggregation within 48 hr after PTCA, followed by intimal hyperplasia, proliferation and migration of smooth muscle cells for 3 or 6 months, and vascular remodeling by proliferating extracellular matrix[1-3]. Intimal hyperplasia and vascular remodeling are the most important processes. Many experimental studies have shown that NO produced from L-arginine by constitutive NO synthetase in endothelial cells regulates intimal hyperplasia[16,21,22]. Although endothelial cells are injured after PTCA and regenerate quickly, NO production remains disturbed, due to the decrease in constitutive NO synthetase in the regenerated endothelium[23,24]. However, NO synthetase is induced in the vascular smooth muscle cells of injured arteries in response to the cytokines produced at the injured site. Then, NO is produced by the smooth muscle cells[25]. The improvement in endothelial function due to the administration of L-arginine to levels that exceed the Km is called the arginine paradox, and may be explained by a relative intracellular deficiency in L-arginine caused by the competition of asymmetric

Table 3 Changes in plasma concentration of L-arginine, nitric oxide and cyclic guanosine monophosphate during intracoronary administration of L-arginine

	L-arginine (nmol/ml)		NO (μmol/l)		cGMP (pmol/ml)	
	Before	After	Before	After	Before	After
Coronary sinus	72±19	1,670±1,801*	31±11	32±12	8.2±4.3	8.3±4.2
Peripheral vein	64±16	326±57*	32.7±11.1	30.8±10.8	7.9±4.0	8.2±4.3

Values are mean±SD ($n=9$). *$p<0.05$ vs control before L-arginine administration.
NO=nitric oxide; cGMP=cyclic guanosine monophosphate.

Table 4 Serial changes of L-arginine, nitric oxide, and cyclic guanosine monophosphate during systemic administration of L-arginine

	Before	Just after	6 hr later	14 hr later
L-arginine (nmol/ml)	108±97	2,417±1,733*	340±381	151±36
NO (μmol/l)	33±4	28±1	39±7	36±7

Values are mean±SD ($n=10$). *$p<0.05$ vs control before L-arginine administration.
Abbreviation as in Table 3.

Table 5 Results of quantitative coronary angiography analysis

	Arginine (+) (n=32)	Arginine (−) (n=90)	p value
Reference diameter (mm)			
Before PTCA	2.80±0.54	2.78±0.52	NS
After PTCA	2.81±0.55	2.79±0.52	NS
Follow up	2.81±0.54	2.78±0.53	NS
MLD (mm)			
Before PTCA	0.77±0.34	0.76±0.32	NS
After PTCA	2.15±0.47	2.13±0.48	NS
Follow up	1.64±0.58	1.55±0.57	NS
Changes in MLD (mm)			
Acute gain	1.39±0.50	1.38±0.49	NS
Late loss	0.52±0.58	0.58±0.57	NS
Net gain	0.87±0.60	0.80±0.59	NS
Loss index	0.38±0.76	0.42±0.74	NS
Percentage of stenosis (%)			
Before PTCA	72.5±10.3	72.7±10.6	NS
After PTCA	23.5±7.3	23.7±7.6	NS
Follow up	41.6±18.6	44.2±19.2	NS

Values are mean±SD.
MLD=minimal lumen diameter; PTCA=percutaneous transluminal coronary angioplasty.

dimethyl L-arginine with L-arginine and the dysfunction of the cationic acid transporter of L-arginine[26-29]. Therefore, the administration of a high dose of L-arginine may increase production of NO and reduce restenosis after PTCA.

The rate and the dose of intracoronary administration of L-arginine in our study were similar to those in experimental studies in animal or humans[30,31]. The rate of systemic administration of L-arginine was also designed to be similar to that of intracoronary administration to maintain the same L-arginine concentration in the coronary artery. The daily dose of systemic infusion was also similar to that of oral administration in animals[22]. Ethical limitations in Japan did not permit us to use L-arginine for oral administration at that time. The plasma concentration of L-arginine at the end of the period of systemic administration was equal to that at the end of the period of intracoronary administration. The use of a high dose of L-arginine was safe in our patients with intracoronary or systemic administration. Only one patient (3%) experienced headache during systemic administration and deceleration of the rate of administration improved the symptom.

Table 6 Restenosis rate at follow up

	L-arginine (+) (n=32)	L-arginine (−) (n=90)	p value
Restenosis rate (%)	34 (11/32)	44 (40/90)	NS
Follow up rate (%)	94 (32/34)	100 (90/90)	NS

We used plasma concentration of nitrate and nitrite as an indicator of NO production. After 12 hr of fasting, as much as 90% of the circulating nitrite is derived directly from the L-arginine: NO pathway[32]. However, the circulating nitrate concentration is usually influenced by dietary intake, especially by nitrate-rich foods like lettuce[33]. All the patients enrolled in this study received their usual diet except for lunch just before PTCA. Therefore, the fact that the plasma concentration of nitrate and nitrite did not change after L-arginine administration in both the coronary sinus and the peripheral vein may have been due to dietary influences. The plasma cGMP concentration is also an indicator of NO production. However, local cGMP elevations in the endothelial cells or in the fibroblasts in small vessels like the coronary artery may not have affected plasma cGMP levels.

Follow-up angiography showed that the minimal lumen diameter after PTCA was slightly bigger and the restenosis rate was slightly lower in patients with L-arginine administration than in control subjects. However, the difference was not statistically significant. Recently, it has been reported that vascular remodeling is the main determinant of lumen size after arterial injury, rather than intimal hyperplasia, in atherosclerotic rabbits. However, L-arginine supplementation did not decrease the in-stent reocclusion rate, which suggests that positive or negative vascular remodeling can be excluded[34]. Furthermore, neither L-arginine nor N^G-nitro-L-arginine methyl ester (L-NAME) administration after balloon angioplasty in hypercholesterolemic rabbits significantly changed lumen size, as L-arginine inhibited whereas L-NAME stimulated intimal hyperplasia and vascular enlargement[35]. In contrast, oral L-arginine administration from 2 days prior to 4 weeks following catheter-induced injury to the rabbit thoracic aorta and iliac artery attenuated the development of intimal hyperplasia in experimental studies[21,22]. Furthermore, a single and small dose of L-arginine administration through a Dispatch catheter decreased intimal hyperplasia.

The catheter was shown to maintain a high concentration of drug in the coronary artery for a long time after a single administration[19,20]. These results may depend on the bifunctional regulation of apoptosis according to the NO concentration. Physiologically relevant levels of NO seem to suppress apoptosis of endothelial cells. However, higher levels of NO induction may overwhelm cellular protective mechanisms and exert proapoptotic and cytotoxic effects on endothelial and smooth muscle cells[36].

Additionally, the administration of L-arginine improved endothelial dysfunction in the microcirculation but not in the macrocirculation in animal and human experiments[30,31]. The minimal and reference vessel diameter after a single intracoronary injection of L-arginine did not change in our study. Thus, the endothelial dysfunction of the coronary artery in the epicardium might not be improved enough to inhibit the growth of smooth muscle cells that result from the administration of L-arginine in humans.

Study limitations

The present study included a small number of patients. A larger population study may be required to clarify whether NO can reduce restenosis after PTCA or not. Although the baseline clinical and angiographic characteristics of both groups were similar and without significant differences, our study was not a randomized study. As stated above, we could not use purified L-arginine for oral supplementation because of ethical limitations in Japan. Furthermore, we could not use an infusion catheter because of the small stock available in Japan at that time. The long-lasting elevation of plasma L-arginine levels after low dose but not high dose administration of L-arginine using these products may affect the restenosis rate after PTCA.

CONCLUSIONS

This study showed that the short-term administration of high dose L-arginine did not significantly change the restenosis rate after PTCA.

要　約

冠動脈形成術後再狭窄に及ぼすL-アルギニン高用量短期投与の効果

白木　照夫　高村　俊行　梶山　晃雄　岡　岳文　斎藤　大治

背景・目的：動物実験においてアルギニンの局所単回投与により，一酸化窒素(NO)の産生が増強され，冠動脈形成術後再狭窄病変の予防効果が証明されている．本研究ではヒトにおいて，NOの前駆物質であるアルギニンの局所および全身投与により，冠動脈形成術後の再狭窄が抑制されるか否かを検討した．

方　法：対象は1998年12月－2000年12月の間に当院に入院した，狭心症ないし陳旧性心筋梗塞患者連続34例である．カテーテルを通して，4分間で500mgのL-アルギニンを投与したのち，冠動脈形成術を行い，終了後30gのL-アルギニンを経静脈的に1日1回4時間かけて投与し，これを5日間行った．冠静脈洞および末梢血血漿中のL-アルギニン，NO(亜硝酸および硝酸イオン)，サイクリックGMPを，L-アルギニンの投与前後で測定した．対照には1996年7月－1998年11月にL-アルギニン非投与下に冠動脈形成術を行い，成功した90症例とした．対照群，L-アルギニン投与群の臨床指標および病変形態を比較した．両群ともに3ヵ月後に冠動脈造影を行い，術前後の冠動脈径の定量的評価を行い，再狭窄率を求めた．

結　果：両群間で臨床指標および病変形態には差はみられなかった．L-アルギニン投与後の血漿アルギニン濃度は有意に上昇したが，NOおよびサイクリックGMP濃度は，冠静脈洞ならびに末梢血中ともに増加しなかった．3ヵ月後の再狭窄率は，アルギニン投与群，非投与群の間で有意差がなかった(再狭窄率：投与群34%，非投与群44%)．

結　論：NOの前駆物質であるL-アルギニンの短期投与は，冠動脈形成術後の再狭窄率を有意には変化させなかった．

J Cardiol 2004 Jul; 44(1): 13－20

References

1) Pompa JJ, Califf RM, Topol EJ: Clinical trials of restenosis after coronary angioplasty. Circulation 1991; **84**: 1426–1436
2) Liu MW, Roubin GS, King SBI Ⅲ: Restenosis after coronary angioplasty: Potential biologic determinants and role of intimal hyperplasia. Circulation 1989; **79**: 1374–1387
3) Lafont A, Faxon D: Why do animal models of post-angioplasty restenosis sometimes poorly predict the outcome of clinical trials? Cardiovasc Res 1998; **39**: 50–59
4) Serruys PW, de Jaegere P, Kiemenneij F, Macaya C, Rutsch W, Heyndrickx G, Emanuelsson H, Marco J, Legrand V, Materne P, Belardi J, Sigwart U, Colombo A, Goy JJ, Heuvel P, Delcan J, Morel M, for the Benestent Study Group: A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. N Engl J Med 1994; **331**: 489–495
5) Fishman DL, Leon MB, Baim DS, Schatz RA, Savage MP, Penn I, Detre K, Veltri L, Ricci D, Nobuyoshi M, Cleman M, Heuser R, Almond D, Teirstein PS, Fish D, Colombo A, Brinker J, Moses J, Shaknovich A, Hirshfeld J, Bailey S, Ellis S, Rake R, Godberg S, for the Stent Restenosis Study Investigators: A randomized comparison of coronary-stent placement and balloon angioplasty in the treatment of coronary artery disease. N Engl J Med 1994; **331**: 496–501
6) Hoffmann R, Mintz GS, Dussaillant GR, Pompa JJ, Pichard AD, Satler LF, Kent KM, Griffin J, Leon MB: Patterns and mechanism of in-stent restenosis: A serial intravascular ultrasound study. Circulation 1996; **94**: 1247–1254
7) Teirstein PS, Massulllo V, Jani S, Pompa JJ, Mintz GS, Russo RJ, Schatz RA, Guarneri EM, Steuterman S, Morris NB, Leon MB, Tripuraneni P: Catheter-based radiotherapy to inhibit restenosis after coronary stenting. N Engl J Med 1997; **336**: 1697–1703
8) Albiero R, Nishida T, Adamian M, Amato A, Vaghetti M, Corvaja N, Di Mario C, Colombo A: Edge restenosis after implantation of high activity ^{32}P radioactive β-emiting stents. Circulation 2000; **101**: 2454–2457
9) Costa MA, Sabat M, van der Giessen WJ, Kay IP, Cervinka P, Ligthart JMR, Serrano P, Coen VL, Levendag PC, Serruys PW: Late coronary occlusion after intracoronary brachytherapy. Circulation 1999; **100**: 789–792
10) Sousa JE, Costa MA, Abizaid A, Abizaid AS, Feres F, Pinto IM, Seixas AC, Staico R, Mattos LA, Sousa A, Falotico R, Jaeger J, Pompa JJ, Serruys PW: Lack of neointimal proliferation after implantation of sirolimus-coated stents in human coronary arteries: A quantitative coronary angiography and three-dimensional intravscular ultrasound study. Circulation 2001; **103**: 192–195
11) Liistro F, Stankovic MD, Di Mario C, Takagi T, Chieffo A, Moshiri S, Montorfano M, Carlino M, Briguori C, Pagnotta P, Albiero R, Corvaja N, Colombo A: First clinical experience with a paclitaxel redivate-eluting polymer stent system implantation for in-stent restenosis: Immediate and long-term clinical and angiographic outcome. Circulation 2002; **105**: 1883–1886
12) Wang BY, Candipan RC, Arjomandi M, Hsiun PT, Tsao PS, Cooke JP: Arginine restores nitric oxide activity and inhibits monocyte accumulation after vascular injury in hypercholesterolemic rabbits. J Am Coll Cardiol 1996; **28**: 1573–1579
13) Goves PH, Lewis MJ, Cheadle HA, Penny WJ: SIN-1 reduces platelet adhesion and platelet thrombus formation in a porcine model of balloon angioplasty. Circulation 1993; **87**: 590–597
14) Furlong B, Henderson AM, Lewis MJ, Smith JA: Endothelium-derived-relaxing factor inhibits in vitro platelet aggregation. Br J Pharmacol 1987; **90**: 687–692
15) Kubes P, Suzuki M, Granger DN: Nitric oxide: An endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci U S A 1991; **88**: 4651–4655
16) Garg UC, Hassid A: Nitric-oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. J Clin Invest 1989; **83**: 1774–1777
17) Clancy RM, Leszczynska-Piziak J, Abramson SB: Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production via a direct action on the NADPH oxidase. J Clin Invest 1992; **90**: 1116–1121
18) Gibbons GH, Dzau VJ: The emerging concept of vascular remodeling. N Engl J Med 1994; **330**: 1431–1438
19) Schwarzacher SP, Lim TT, Wang B, Kernoff RS, Niebauer J, Cooke JP, Yeung AC: Local intramural delivery of L-arginine enhances nitric oxide generation and inhibits lesion formation after balloon angioplasty. Circulation 1997; **95**: 1863–1869
20) Niebauer J, Schwarzacher SP, Hayase M, Wang B, Kernoff RS, Cooke JP, Yeung AC: Local L-arginine delivery after balloon angioplasty reduces monocyte binding and induces apoptosis. Circulation 1999; **100**: 1830–1835
21) McNamara DB, Bedi B, Aurora H, Tena L, Ignarro LJ, Kadowitz PJ, Akers DL: L-arginine inhibits balloon catheter-induced intimal hyperplasia. Biochem Biophys Res Commun 1993; **193**: 291–296
22) Greenless C, Wadsworth RM, Martorana PA, Wainwright CL: The effects of L-arginine on neointimal formation and vascular function following balloon injury in heritable hyperlipidaemic rabbits. Cardiovasc Res 1997; **35**: 351–359
23) Myer RP, Webel R, Thondapu V, Xu VT, Amann J, Tanner MA, Jenkins JS, Pollock JS, Jaughlin MH: Restenosis associated with decreased coronary artery nitric syntase. Int J Cardiol 1995; **55**: 183–191
24) Bosmans JM, Bult H, Vrints CJ, Kockx MM, Herman AG: Balloon angioplasty and induction of non-endothelial nitric oxide synthase in rabbit carotid arteries. Eur J Pharmacol 1996; **310**: 163–174
25) Joly GA, Schini VB, Vanhoutte PM: Balloon injury and interleukin-1β induce nitric oxide synthase activity in rat carotid arteries. Circ Res 1992; **71**: 331–338
26) Lerman A, Burnett JC Jr, Higano ST, McKinley LJ, Holmes DR Jr: Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. Circulation 1998; **97**: 2123–2128
27) Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP: Asymmetric dimethylarginine (ADMA): A novel risk factor for endothelial dysfunction: Its role in hypercholesterolemia. Circulation 1998; **98**: 1842–1847
28) Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP: Novel mechanism for endothelial dysfunction:

Dysregulation of dimethylarginine dimethylaminohydrolase. Circulation 1999; **99**: 3092–3095
29) Kaye DM, Ahlers BA, Autelitano DJ, Chin-Dusting PF: In vivo and in vitro evidence for impaired arginine transport in human heart failure. Circulation 2000; **102**: 2707–2712
30) Egashira K, Hirooka Y, Kuga T, Mohri M, Takeshita A: Effect of L-arginine supplementation on endothelium-dependent coronary vasodilation in patients with angina pectoris and normal coronary arteriograms. Circulation 1996; **94**: 130–134
31) Drexler H, Zeiher AM, Meinzer K, Just H: Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. Lancet 1991; **338**: 1546–1550
32) Rhodes PM, Leone AM, Francis PL, Struthers AD, Moncada S, Rhodes P: The L-arginine: Nitric oxide pathway is the major source of plasma nitrite in fasted humans. Biochem Biophys Res Commun 1995; **209**: 590–596
33) Lundberg JON, Weitzberg E, Lundberg JM, Alving K: Intragastric nitric oxide production in humans : Measurements in expelled air. Gut 1994; **35**: 1543–1546
34) Dudek D, Heba G, Bartus S, Partyka U, Dembinska-Kiec A, Huk J, Legutko J, Dibiel JS: Effects of L-arginine supplementation on endothelial function after stent implantation. Kardiol Pol 2002; **57**: 389–398
35) Le Tourneau T, Van Belle E, Corseaux D, Vallet B, Lebuffeg G, Dupuis B, Lablanche JM, McFadden E, Bauters C, Bertrand M: Role of nitric oxide in restenosis after experimental balloon angioplasty in the hypercholesterolemic rabbit: Effects on neointimal hyperplasia and vascular remodeling. J Am Coll Cardiol 1999; **33**: 876–882
36) Kim YM, Bombeck CA, Billar TR: Nitric oxide as a bifunctional regulator of apoptosis. Circ Res 1999; **84**: 253–256

Original Article

Relationship between the Awareness of Salt Restriction and the Actual Salt Intake in Hypertensive Patients

Yuko OHTA, Takuya TSUCHIHASHI, Michio UENO, Tomoko KAJIOKA, Uran ONAKA, Mitsuhiro TOMINAGA, and Kimika ETO

A 24-h home urine collection was conducted to estimate accurate salt intake in hypertensive outpatients. Using 24-h urinary creatinine excretion as a criterion for success, urine samples were obtained from 534 hypertensive patients. The urinary salt excretion of hypertensive outpatients ranged widely from 1.5 to 23.4 g/day (mean value 9.7±3.9 g/day). Urinary salt excretion was higher in males than in females (10.6±4.0 *vs.* 9.2±3.7 g/day, $p<0.01$). Based on the questionnaires, the patients were divided into salt-conscious patients, or those who were careful to reduce their daily salt intake, and non-salt-conscious patients. It was found that urinary salt excretion was lower in the salt-conscious group than in the non-salt-conscious group (9.4±3.8 *vs.* 10.6±4.0 g/day, $p<0.01$), but that urinary salt excretion adjusted for body weight was not significantly different between the two groups (0.16±0.06 *vs.* 0.17±0.07 g/kg/day). Our results suggest that there was no obvious reduction in the actual salt intake in salt-conscious patients, suggesting the importance of monitoring salt intake by 24-h home urine collection and informing patients of their actual salt intake as a means of encouraging the achievement of salt restriction. (*Hypertens Res* 2004; 27: 243–246)

***Key Words*: salt restriction, 24-h home urine collection, urinary salt excretion, hypertension, salt intake**

Introduction

Extensive epidemiological literature has already documented the correlation between salt intake and blood pressure (BP) or the prevalence of hypertension (*1*, *2*). Salt restriction is now also widely promoted as an effective non-pharmacological approach to managing mild hypertension, as well as an important adjunct to pharmacological treatment in moderate and severe hypertension (*3–7*). The seventh report of the Joint National Committee (JNC 7) recommends sodium reduction to a level of no more than 100 mmol/day in hypertensive patients (*8*). Thus, it is recommended that physicians advise patients to reduce their salt intake, but the efficacy of this advice is questionable if patients' actual salt intake is not monitored. The aim of this study was to investigate urinary salt excretion and the relationship between the awareness of salt restriction and the actual salt intake in hypertensive outpatients.

Methods

We undertook 24-h home urine collection at first visit in 652 outpatients between January, 1998 and December, 1999. Twenty four-hour urine samples were collected using a partition cup (proportional sampling method (*9*)), which collects a 1/50 portion of the 24-h urine. If the 24-h creatinine excretion was within ±30% of the estimated values, the urine collection was considered successful. If the urine collection was judged to be unsuccessful, the patients were asked to try again. Patients who failed to complete the 24-h urine collection, in spite of possible repeated collection, were excluded from further analysis. BP was measured with a sphygmomanometer by the doctors while the patients were seated. Hypertension was considered to be present in patients with systolic blood pressure (SBP)≥140 mmHg and/or diastolic

From the Division of Hypertension and Nephrology, Clinical Research Institute, National Kyushu Medical Center, Fukuoka, Japan.
Address for Reprints: Yuko Ohta, M.D., Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3–1–1, Higashi-ku, Fukuoka 812–8582, Japan. E-mail: yukoo@intmed2.med.kyushu-u.ac.jp
Received September 22, 2003; Accepted in revised form January 9, 2004.

Table 1. Characteristics in Male and Female Patients

	All	Males	Females
Number of patients	534	211	323
Age (years)	58.3±11.6	58.0±12.9	58.5±10.6
BW (kg)	61.3±11.2	68.1±11.1**	56.9±8.9
BMI (kg/m^2)	24.3±3.5	24.6±3.3	24.1±3.6
SBP (mmHg)	144.0±12.5	144.3±13.0	143.9±12.2
DBP (mmHg)	87.4±7.7	87.9±7.8	87.0±7.7
Serum creatinine (mg/dl)	0.9±0.7	1.1±0.9**	0.7±0.4
Urinary salt excretion (g/day)	9.7±3.9	10.6±4.0**	9.2±3.7
Urinary salt excretion adjusted for BW (g/kg/day)	0.16±0.06	0.16±0.06	0.16±0.07
Antihypertensive drug (%)	49.6	52.1	48.0
Family history of hypertension (%)	47.6	51.7	44.9
Diabetes mellitus (%)	12.2	13.3	11.5

Values are means±SD. ** $p<0.01$ *vs.* females. BW, body weight; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

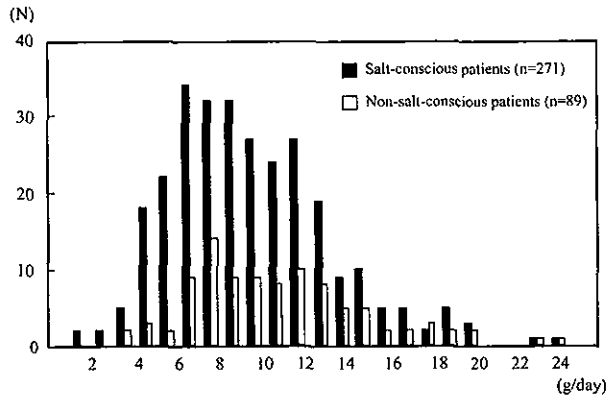

Fig. 1. *The distribution of 24-h urinary salt excretion in salt-conscious and non-salt-conscious patients.*

blood pressure (DBP)≥90 mmHg, or those patients on antihypertensive medication. The patients were asked, by questionnaire, about aspects of their lifestyle, such as habitual alcohol intake, smoking, and exercise, and were also asked whether they were conscious of salt, calorie and fat restrictions. The protocol was explained in detail, and informed consent was obtained from each patient.

Statistical Analysis

Values are presented as the means±SD. The differences in the variables were compared by one-way ANOVA. A χ^2 test was also utilized when appropriate. P values less than 0.05 were considered statistically significant.

Results

Among the 652 patients enrolled in this study, 534 patients successfully collected 24-h urine samples.

Characteristics in male and female patients are shown in Table 1. The patients had a mean age of 58.3±11.6 (26–90) years, and a mean BP of 144.0±12.5/87.4±7.7 mmHg. There were no differences in age, body mass index (BMI), BP, or prevalence of patients receiving antihypertensive drugs between males and females. Body weight and urinary salt excretion were significantly higher in males than in females. However, urinary salt excretion adjusted for body weight was similar between the two groups.

The questionnaire on the awareness of salt restriction was obtained from 360 patients. As shown in Fig. 1, the values of the 24-h urinary salt excretion were distributed widely, ranging from 1.5 to 23.4 g/day. Comparisons of the characteristics between the patients who were careful to reduce their daily salt intake (salt-conscious group, $n=271$) and the non-salt-conscious group ($n=89$) are presented in Table 2. There were no differences in BMI and frequency of family history of hypertension between the two groups. The salt-conscious group was older than the non-salt-conscious group, and there was a higher prevalence of females. This group also showed a lower BP and a higher prevalence of being on antihypertensive medication. Urinary salt excretion was significantly lower in the salt-conscious group than in the non-salt-conscious group (9.4±3.8 *vs.* 10.6±4.0 g/day, $p<0.01$), but urinary salt excretion adjusted for body weight was not significantly different between the groups.

Table 2. Characteristics in the Salt-Conscious and Non-Salt-Conscious Groups

	Salt-conscious group	Non-salt-conscious group
Number of patients	271	89
Men (%)	36.9††	55.0
Age (years)	59.7±11.3*	54.6±11.9
BW (kg)	60.5±11.6	63.8±12.2
BMI (kg/m^2)	24.2±3.6	24.5±3.7
SBP (mmHg)	141.0±10.4**	144.6±11.9
DBP (mmHg)	85.5±6.9**	87.9±7.1
Serum creatinine (mg/dl)	0.9±0.7	0.9±0.9
Urinary salt excretion (g/day)	9.4±3.8**	10.6±4.0
Urinary salt excretion adjusted for BW (g/kg/day)	0.16±0.06	0.17±0.07
Antihypertensive drug (%)	77.9**	60.7
Family history of hypertension (%)	73.1	62.9
Diabetes mellitus (%)	11.1	13.5

Values are means±SD. * $p<0.05$, ** $p<0.01$ *vs.* non-salt-conscious by ANOVA, †† $p<0.01$ *vs.* non-salt-conscious by χ^2 test. BW, body weight; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Discussion

The present study demonstrated that in hypertensive outpatients, there was no relationship between the awareness of salt restriction and the actual salt intake evaluated by 24-h urinary collection, which has been widely used to estimate dietary salt intake in epidemiological studies (*10–13*).

In the present study, urinary salt excretion was higher in males than in females, which may be attributable to the greater energy intake in males than females (*14*). In fact, males showed a higher body weight, and when urinary salt excretion was adjusted for body weight, urinary salt excretion was comparable between males and females.

It seems reasonable that BP and urinary salt excretion were significantly lower in the salt-conscious group than in the non-salt-conscious group. The characteristics of the two groups differ in sex and age. Thus, we may speculate that elderly and female individuals could be more aware than others of the importance of lifestyle modifications, such as salt restriction. However, no significant difference was found in urinary salt excretion adjusted for body weight between the salt-conscious and non-salt-conscious groups. This observation indicates that awareness of the necessity of salt restriction may not lead to an actual reduction of salt intake. Salt intake by the Japanese population has traditionally been high, although it has been decreasing in recent years. The National Nutrition Survey in Japan showed that the salt intake was 11.5 g in 2001 (*15*). The difficulty in achieving long-term dietary salt restriction might be attributable to the difficulty in changing the dietary habits of the Japanese. It has also been pointed out that although there has been an increase in variety in the Japanese diet, there is now a greater reliance on dining out, and the consumption of fast foods is increasing (*12*, *16*). These trends in the dietary habits of Japanese may also make it difficult to reduce salt intake.

With regard to seasonal variation, urinary salt excretion tended to decrease in summer (*17*). In the present study, 24-h urine collection was performed throughout the year in both groups. Thus, it seems unlikely that the seasonal variation of urinary salt excretion influenced the principal results of this study.

Doctors advise all hypertensive patients to reduce their salt intake, but it is important to evaluate whether patients follow this advice. Some methods have been proposed to improve compliance with dietary salt restriction. One study indicated that group management, in which feedback is provided to patients on their urinary salt excretion, was more effective in decreasing dietary salt intake than advice given without this support, or through an intensive educational effort by doctors and clinics (*18*). Another study showed that self-monitoring of urinary salt excretion at home, using chloride titrator strips, could, in conjunction with dietary counseling, facilitate compliance with a reduced salt intake (*19*). However, another report indicated that short counseling sessions with advice on salt restriction were not successful in producing dietary changes (*5*). Taken together, these results suggest that repeated monitoring of urinary salt excretion, along with providing feedback to patients, is the most important and practical way to achieve the reduction of salt intake in individual hypertensives.

In conclusion, there was no obvious reduction in actual salt intake in salt-conscious patients in the present study, suggesting the importance of monitoring salt intake and informing patients of their actual salt intake as a means of encouraging the achievement of salt restriction.

Acknowledgements

We greatly appreciate the technical assistance we received from the staff of the Division of Nutrition, National Kyushu Medical Center. We would also like to thank Ms. T. Shimada for her technical and secretarial assistance.

References

1. Alli C, Avanzini F, Bettelli G, *et al*: Feasibility of a long-term low-sodium diet in mild hypertension. *J Hum Hypertens* 1992; **6**: 281–286.
2. Rio AD, Villamil JLR: Metabolic effects of strict salt restriction in essential hypertensive patients. *J Intern Med* 1993; **233**: 409–414.
3. Haddy FJ, Pamnani MB: Role of dietary salt in hypertension. *J Am Coll Nutr* 1995; **14**: 428–438.
4. Korhonen MH, Jarvinen RMK, Sarkkinen ES, Uusitupa MIJ: Effects of a salt-restricted diet on the intake of other

nutrients. *Am J Clin Nutr* 2000; **72**: 414–420.
5. Korhonen MH, Litmanen H, Rauramaa R, Vaisanen SB, Niskanen L, Uusitupa MIJ: Adherence to the salt restriction diet among people with mildly elevated blood pressure. *Eur J Clin Nutr* 1999; **53**: 880–885.
6. Appel LJ, Espeland MA, Easter L, Wilson AC, Folmar S, Lacy CR: Effects of reduced sodium intake on hypertension control in older individuals. *Arch Intern Med* 2001; **161**: 685–693.
7. Kumagai H, Onoyama K, Fujishima M: Effects of salt restriction on blood volume, hemodynamics and humoral factors in hypertensive patients with chronic glomerulonephritis. *Am J Hypertens* 1989; **2**: 669–674.
8. Chobanian AV, Bakris GL, Black HR, *et al*: The seventh report of the joint national committee on prevention, detection, evaluation, and treatment on high blood pressure. *JAMA* 2003; **289**: 2560–2572.
9. Tochikubo O, Uneda S, Kaneko Y: Simple portable device for sampling a whole day's urine and its application to hypertensive outpatients. *Hypertension* 1983; **5**: 270–274.
10. Pietinen P: Estimating sodium intake from food consumption data. *Ann Nutr Metab* 1982; **26**: 90–99.
11. Luft FC, Fineberg NS, Sloan RS: Estimating dietary sodium intake in individuals receiving a randomly fluctuating intake. *Hypertension* 1982; **4**: 805–808.
12. Daniels SD, Meyer RA, Loggie JMH: Determinants of cardiac involvement in children and adolescents with essential hypertension. *Circulation* 1990; **82**: 1243–1248.
13. Luft FC, Sloan RS, Fineberg NS, Free AH: The utility of overnight urine collections in assessing compliance with a low sodium intake diet. *JAMA* 1983; **249**: 1764–1768.
14. Nestel PJ, Clifton PM, Noakes M, McArthur R, Howe PR: Enhanced blood pressure response to dietary salt in elderly women, especially those with small waist:hip ratio. *J Hypertens* 1993; **11**: 1387–1394.
15. Ministry of Health, Labour and Welfare, Japan: The National Nutrition Survey in Japan, 2001. Tokyo, Dai-ichi Shuppan Press, 2003, p182.
16. Liu L, Mizushima S, Ikeda K, *et al*: Comparative studies of diet-related factors and blood pressure among Chinese and Japanese: results from the China-Japan cooperative research of the WHO-Cardiac study. *Hypertens Res* 2000; **23**: 413–420.
17. Hashimoto J, Imai Y, Minami N, *et al*: Compliance with long-term dietary salt restriction in hypertensive outpatients. *Clin Exp Hypertens* 1994; **16**: 729–739.
18. Nugent CA, Carnahan JE, Seehan ET, Myers C: Salt restriction in hypertensive patients: comparison of advice, education, and group management. *Arch Intern Med* 1984; **144**: 1415–1417.
19. Luft FC, Sloan RS, Lang CL, *et al*: Influence of home monitoring on compliance with a reduced sodium intake diet. *Arch Intern Med* 1984; **144**: 1963–1965.