Report

Report of the Working Group for Dietary Salt Reduction of the Japanese Society of Hypertension: (2) Assessment of Salt Intake in the Management of Hypertension

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Restriction of dietary salt is widely recommended in the management of hypertension, but assessment of individual salt intake has drawn little attention. The understanding of salt intake is important as a guide for optimizing salt-restriction strategies. However, precise evaluation of salt intake is difficult. More reliable methods are more difficult to perform, whereas easier methods are less reliable. Thus, the method to assess salt intake should be determined as the situation demands. The Working Group for Dietary Salt Reduction of the Japanese Society of Hypertension recommends the assessment of individual salt intake using one of the following methods in the management of hypertension. 1) The measurement of the sodium (Na) excretion from 24-h urine sampling or nutritionist's analysis of the dietary contents, which are reliable but difficult to perform, are suitable for facilities specializing in the treatment of hypertension. 2) Estimation of the Na excretion from the Na/creatinine (Cr) ratio in spot urine is less reliable but practical and is suitable for general medical facilities. 3) Estimation using an electronic salt sensor equipped with a calculation formula is also less reliable but is simple enough that patients can use it themselves. The patients are considered to be compliant with the salt-restriction regimen if salt intake measured by whichever method is less than 6 g (100 mmol)/day. (*Hypertens Res* 2007; 30: 887–893)

Key Words: **salt intake, food weighing, food questionnaire, urinary sodium excretion, hypertension**

Introduction

Excessive salt or sodium (Na) intake causes hypertension, and restriction of salt intake is widely recommended for the management of hypertension. In the 2004 version of the Japanese Society of Hypertension (JSH) Guidelines for the Management of Hypertension (JSH 2004), the target of salt restriction was tightened from 7 g/day or less to less than 6 g/day (*1*). On the other hand, while the salt intake in Japan is decreasing, it is still high, being about 11 g/day (*2*). Also, salt intake shows considerable individual variation and daily fluctuation in the same individual.

An understanding of individual salt intake is considered to be important for successful salt reduction, because it leads to appropriate guidance and judgement of whether the target of salt restriction has been attained. However, there are several problems with the assessment of salt intake, and its imple-

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Table 1. Evaluation Methods of Salt Intake

Evaluation method	Reliability	Convenience
Evaluations based on dietary contents		
Weighing method	◎	×
Questionnaire method	○	△
Measurement before intake	◎	×
Evaluation using test paper or salt sensor	×	◎
Evaluations based on the measurement of urinary Na excretion		
24-h pooled urine	◎	×
Nighttime or early morning urine	○	△
The second urine sample after waking	○	△
Spot urine	△ (○*)	○
Evaluation using test paper or salt sensor	× (△**)	◎

◎, excellent; ○, good; △, fair; ×, poor. *When a formula for the estimation of the daily creatinine (Cr) excretion is used. **When a salt sensor installed with the formula is used.

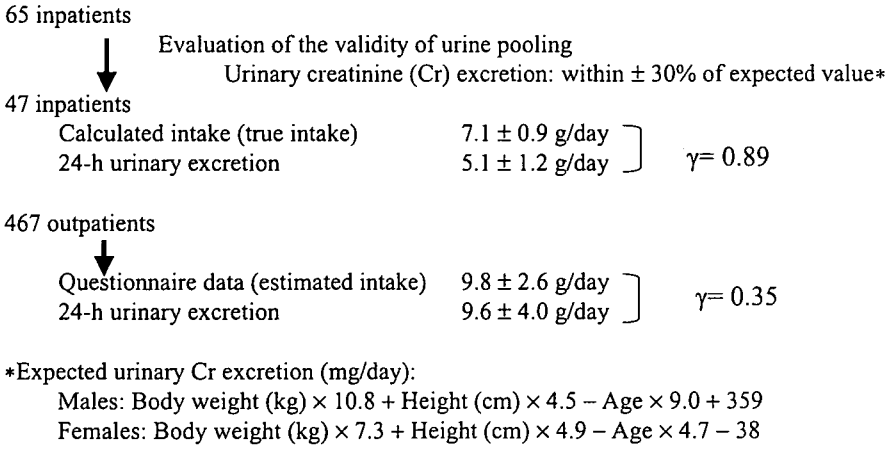

Fig. 1. *Calculated dietary salt intake and 24-h urinary excretion in inpatients and estimated salt intake based on a questionnaire and 24-h urinary excretion in outpatients (from data of Fukumoto et al. (9))*

mentation is often difficult. Most of the current guidelines for the management of hypertension do not mention the methodological aspect of assessing salt intake. While the guidelines of the World Health Organization and International Society of Hypertension (WHO/ISH) state that counseling by a skilled nutritionist and monitoring of the urinary Na level are necessary in most cases, they do not mention specific methods for these purposes (*3*).

This report describes variations and characteristics of salt intake–assessment methods and proposes the guidelines for the assessment of salt intake for the management of hypertension as part of the activities of the Working Group for Dietary Salt Reduction of the JSH. The Japanese version of the working group report has been published previously (*4*).

Methods to Assess Salt Intake

There are several methods for the assessment of salt intake. In general, however, the choice of method involves a compromise between accuracy and ease-of-use, with relatively precise methods being difficult to perform, and simpler methods being less reliable (Table 1). Also, because salt intake is not fixed in each person, its assessment is naturally subject to limitations in accuracy (*5*). Sodium, which is important in the occurrence and progression of hypertension, is primarily ingested as salt (NaCl). Since 1 g of salt is equivalent to 17 mmol (17 mEq) of Na, 6 g of salt is about 100 mmol (100 mEq) of Na. In terms of relative weight, a given amount of Na in salt would weigh 2.5 times more than the equivalent amount as pure Na (for example, 400 mg of Na is equal to 1 g of salt).

Assessment Based on Dietary Contents

Weighing Method

This method, by which salt intake is estimated by weighing

Table 2. Formula for the Estimation of the 24-h Sodium (Na) Excretion from Nighttime Urine Data and Estimated Cr Excretion (*18*)

24-h Na excretion (mmol/day)	
Male	$0.634 \times (Na_n/Cr_n) \times Pr.UCr_{24} + 104.7$
Female	$0.682 \times (Na_n/Cr_n) \times Pr.UCr_{24} + 62.6$
	Na_n: Na concentration in nighttime urine (mEq/L)
	Cr_n: Cr concentration in nighttime urine (g/L)
	$Pr.UCr_{24}$: estimated 24-h urinary Cr excretion (g/day)
	Male $0.027 \times$ LBM
	Female $0.022 \times$ LBM
	LBM = Body weight (kg) − Body fat mass (kg)

Cr, creatinine; LBM, lean body mass.

Table 3. Formula for the Estimation of the 24-h Na Excretion from Data in the Second Urine Sample after Waking and Estimated Cr Excretion (*19*)

24-h Na excretion (mmol/day) $= 16.3 \times \sqrt{(Na_{SMU}/Cr_{SMU}) \times Pr.UCr_{24}}$
Na_{SMU}: Na concentration in 2nd urine sample after waking (mEq/L)
Cr_{SMU}: Cr concentration in 2nd urine sample after waking (mg/L)
$Pr.UCr_{24}$: estimated 24-h urinary Cr excretion (mg/day)
Male Body weight (kg) × 15.1 + Height (cm) × 7.4 − Age × 12.4 − 80
Female Body weight (kg) × 8.6 + Height (cm) × 5.1 − Age × 4.7 − 75

Cr, creatinine.

the food ingested by each subject, is highly reliable (*6*). Concerning Na, the values estimated from the food weight based on the Standard Tables of Food Composition in Japan (*7*) have been shown to be close to, and strongly correlated with, the actual values measured in the ingested food. However, this method is complicated and requires calculation by a nutritionist. Also, a 1-day survey is considered to be insufficient for accurate assessment of salt intake, which changes from day to day.

Questionnaire Method

By this method, dietary salt intake is estimated from data obtained by a questionnaire or interview performed over one to several days. While it is easier than the weighing method, this method still requires calculation by a nutritionist. Although there has been a report suggesting that its reliability is comparable to that of the weighing method (*8*), its accuracy is considered to be slightly inferior. Also, while the mean salt intake estimated by interview has been reported to agree with the value based on the 24-h urinary Na excretion, its correlation to actual salt intake was not high, and actual salt intake may be underestimated by this method (*9*) (Fig. 1).

Measurement before Intake

In the measurement-before-intake method, daily salt intake is determined by measuring or estimating the salt content of food to be eaten before ingestion. If performed precisely, this method is highly reliable. Hospital meals and test meals for clinical research are examined by this method. Since the salt intake is revealed before ingestion of the meal, this method is useful for the practice of salt reduction. However, it is inconvenient to measure the salt content before each meal. Moreover, accurate determination requires calculation by a nutritionist, although rough calculation can be performed by untrained individuals.

Measurement of the Urinary Na Excretion

Measurement by 24-h Urine

In this method, urine is collected for 24 h, and salt intake is assessed by determining the urinary Na excretion. This method is considered to be reliable and is used in many clinical and epidemiological studies, including the international cooperative Intersalt study (*10*). However, it is relatively difficult to perform because of the necessity of 24-h urine sampling, and inadequate urine pooling leads to underestimation of salt intake. The nuisance of 24-h urine collection is slightly mitigated by the use of a portable urine sampler (Urinmate®), which allows fractionated partial urine sampling (*11*). For accurate assessment of salt intake, even the 24-h urine sampling method is insufficient if performed over only 1 day, and thus measurement over several days is considered necessary (*11*, *12*). In addition, while most of the ingested Na is excreted in urine, part of it is contained in feces or sweat. Salt intake determined from the Na excretion in 24-h urine has been shown to be 0.5–3 g/day lower than the true intake, and

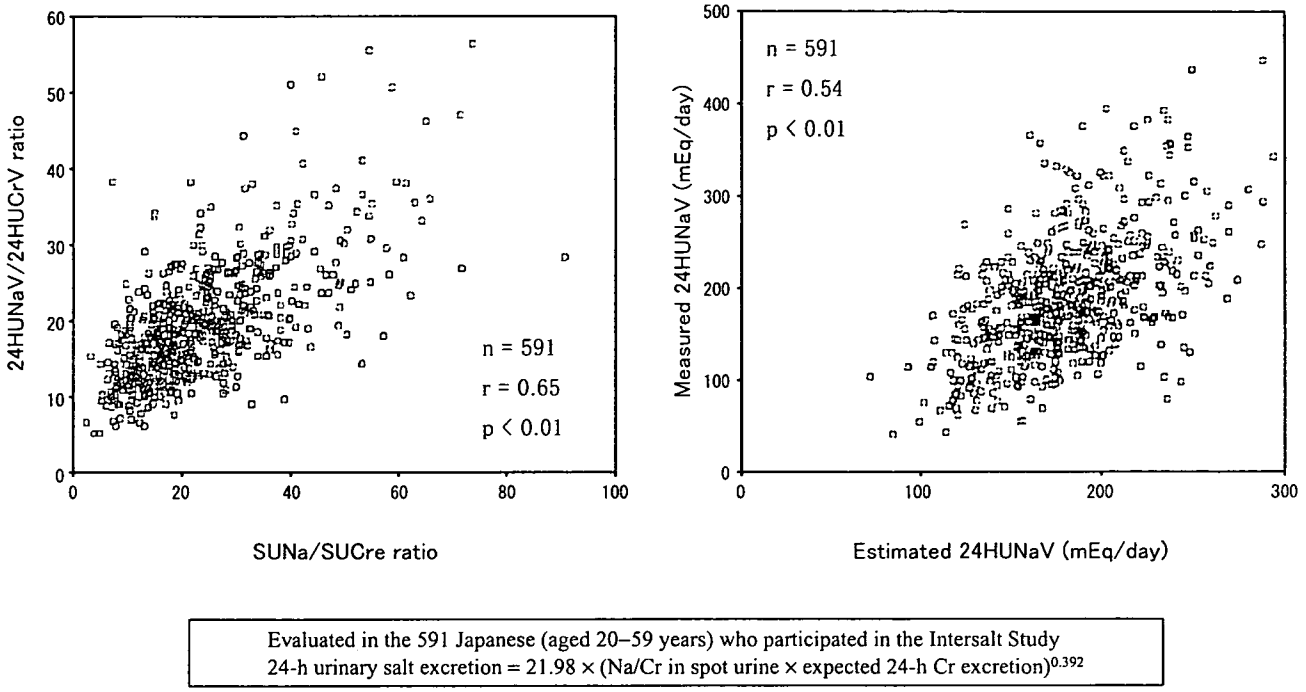

Fig. 2. *Evaluation of salt intake by spot urine. The left plot shows the relationship between the sodium (Na)/Cr ratio in spot urine (SUNa/SUCr ratio) and Na/Cr ratio in 24-h urine (24HUNaV/24HUCrV ratio). The right plot shows the relationship between the estimated 24-h urinary Na excretion by the calculation formula based on spot urine data (Estimated 24HUNaV) and measured 24-h urinary Na excretion (Measured 24HUNaV) (from Tanaka et al. (21) with modification).*

Table 4. Formula for the Estimation of the 24-h Na Excretion from Spot Urine Data and Estimated Cr Excretion (*21*)

24-h Na excretion (mmol/day) = $21.98 \times \{(Na_S/Cr_S) \times Pr.UCr_{24}\}^{0.392}$
Na_S: Na concentration in spot urine (mEq/L)
Cr_S: Cr concentration in spot urine (mg/L)
$Pr.UCr_{24}$: estimated 24-h urinary Cr excretion (mg/day)
$Pr.UCr_{24} = -2.04 \times$ Age $+ 14.89 \times$ Body weight (kg) $+ 16.14 \times$ Height (cm) $- 2244.45$

Cr, creatinine.

it is underestimated even with complete urine collection (*9*, *12*, *13*) (Fig. 1).

Measurement by Nighttime and Overnight Urine

Sampling of nighttime or early morning (overnight) urine, which consists of nighttime urine, is often employed, because it is easier than 24-h urine sampling and still provides a relatively long-term sample. In addition, Na excretion in nighttime urine is well correlated with that in 24-h urine (*14*, *15*). However, Na excretion exhibits diurnal fluctuation, being about 20% lower during the nighttime than the daytime (*16*, *17*). Therefore, simple estimation of salt intake from the Na excretion in nighttime urine is considered to result in greater underestimation than that in 24-h collected urine. However, the 24-h Na excretion estimated by the following calculation using Na excretions in nighttime urine has been reported to be in relatively close agreement with the value determined in 24-h sampled urine (*18*) (Table 2). In this method, 24-h Na excretion was estimated by applying Na and creatinine (Cr) excretions in nighttime urine and estimated 24-h urinary Cr excretion, calculated using the lean body mass from the height, body weight, and body fat mass.

Measurement by the Second Urine Sample after Waking

In another previously reported method to estimate the daily urinary Na excretion, the Na and Cr concentrations in the second urine sample after waking, and the 24-h urinary Cr excretion estimated from height, body weight, and age, are applied to a calculation formula (*19*) (Table 3). The Na excretion estimated by this method is closely correlated with the value determined in 24-h pooled urine. However, its clinical use may be limited by the condition that the urine must be col-

Table 5. Guidelines for the Evaluation of Salt Intake

Evaluation method	Recommendability	Major application target
Measurement of the Na excretion in 24-h pooled urine, or Weighing or questionnaire survey by a nutritionist	Although highly reliable and recommendable, these methods are complicated. Recommended if the patients' cooperation and the facility's ability are secured	Special facilities for hypertension treatment
Estimation as Na/Cr ratio based on measurement of Na and Cr in spot urine samples*	Although the reliability is relatively low, the method is simple and recommended as a practical evaluation procedure	Medical facilities in general
Estimation in early morning urine (nighttime urine) using an electronic salt sensor installed with calculation formula**	Although the reliability is relatively low, the method is recommendable. It is convenient and can be performed by the patients themselves	Patients themselves

*Early morning urine (nighttime urine) may also be used; the reliability is increased by the use of the calculation formula incorporating the estimated 24-h Cr excretion (Tables 2–4). **Methods using test paper or a simple salt sensor are convenient but unreliable, and quantitative evaluation is difficult. Cr, creatinine.

lected as the second urine sample after waking and before breakfast.

Measurement by Spot Urine

Evaluation of salt intake using a spot urine sample collected at any time would be easy to perform. The Na excretion per amount of Cr in spot urine correlates relatively well with the Na excretion per amount of Cr in 24-h urine sampling (*20, 21*) (Fig. 2), but the correlation between the Na excretion in spot urine and that in 24-h pooled urine is not very high (*15, 20*). However, the estimated Na excretion calculated using a formula incorporating the estimated 24-h urinary Cr excretion (Table 4) is reportedly close to the actually measured 24-h urinary Na excretion (*21*) (Fig. 2). The method to estimate the daily Na intake from the Na excretion per gram of Cr calculated from the Na and Cr concentrations in spot urine is not very reliable but is simple and considered to be clinically useful.

Assessment Using Test Paper or a Salt Sensor

This method, by which salt intake is estimated by measuring the salt concentration in spot urine or overnight urine using test paper or an electronic salt sensor, is the simplest (*22, 23*). The test paper or salt sensor usually detects chloride (Cl) rather than Na, and the results of examination of overnight urine using a test paper have been shown to be correlated with salt intake estimated by a nutritional survey (*23*). However, these should be regarded as unreliable and semi-quantitative methods. Recently, a urinary salt sensor, which estimates salt intake by analyzing data in overnight urine using a preinstalled calculation formula, has become available and is expected to increase the reliability (*24*).

The salt concentration in food can be determined using test paper or a salt sensor. In one previous report, however, the salt concentration of miso soup was found to be unrelated to the urinary salt level (*23*). The estimation of daily salt intake from the salt concentration of a single food item is thus considered to be difficult.

Assessment of Salt Intake for the Management of Hypertension

As mentioned above, there are several problems with the assessment of salt intake. Even measurement of the dietary salt content and the 24-h urine sampling method, which are considered to be highly reliable, are not sufficiently accurate and are difficult to perform (Table 1). Although the examination of the Na/Cr ratio in spot urine and the test paper method are easier to perform, they are less reliable. Calculation using a formula and the data of nighttime or spot urine is more reliable but more complicated. Also, it should be noted that salt intake determined from the urinary Na excretion or by the questionnaire method tends to be underestimated.

Despite these problems, the assessment of salt intake in individual patients is useful for motivating patients to reduce their salt intake, as well as for guiding their progress and evaluating the results. Such assessment is strongly recommended for the management of hypertension, because it provides patients with concrete numerical values of their salt intake. The use of more reliable methods is desirable, if possible, but even less reliable methods are of clinical value.

The Working Group for Dietary Salt Reduction of the JSH proposes the guidelines shown in Table 5 for the assessment of salt intake for the management of hypertension. In the management of hypertensive patients, salt intake should be evaluated individually using one of the following methods whenever possible.

1) The measurement of the Na excretion in 24-h pooled urine or a nutritionist's analysis of the dietary contents: These

methods are reliable but often difficult to perform. They are recommended depending on the patients' cooperativeness and the facility's competence and are suited for facilities specializing in hypertension.

2) Estimation of the Na excretion from the Na/Cr ratio in spot urine: This method is less reliable, but it is easy to perform and is considered to be practical. Since the daily Cr excretion of Japanese is about 1 g (about 10 mmol) (*10*), salt intake is estimated to be about 6 g if the Na excretion per gram of Cr is 100 mmol. Therefore, this method is considered to be useful for salt reduction guidance. However, the urinary Cr excretion varies considerably according to the physique, age, and gender of patients. Therefore, note that true salt intake is lower in small females and higher in large males than the value estimated from the Na/Cr ratio. Overnight urine (nighttime urine) may also be used. The reliability can be increased by the use of a calculation formula (Tables 2–4).

3) Estimation using an electronic salt sensor equipped with a calculation formula in early morning urine (overnight urine): Although this method is less reliable, it can be recommended, because it is simple and can be performed by the patients themselves. However, the patient must purchase a salt sensor, or the medical facility must lend one to the patient, for home monitoring.

According to the guidelines for lifestyle modifications in the management of hypertension, the patient is considered to be compliant with the salt restriction regimen if the salt intake measured by whichever method is less than 6 g (100 mmol Na)/day and not if it is higher.

Conclusions

Although there are several methods for the assessment of salt intake, the precise determination of salt intake in individual patients is difficult. Reliable methods are difficult to perform, and simpler methods are less reliable. However, the assessment of salt intake is strongly recommended, because it is useful for informing patients of their salt intake and conducting salt restriction.

In the management of hypertension, it is desirable to assess salt intake by one of the following three methods whenever possible: 1) Measurement of the Na excretion in 24-h pooled urine or a nutritionist's analysis of the dietary contents: Although these are desirable methods because of their reliability, they are difficult to perform and are suited for facilities specializing in hypertension. 2) Estimation of the Na excretion from the Na/Cr ratio in spot urine: This is a less reliable but practical method and is suited for general medical facilities. Overnight urine (nighttime urine) may also be used, and the reliability is increased by the use of a calculation formula. 3) Estimation using an electronic salt sensor equipped with a calculation formula in overnight urine: While this method is less reliable, it can be recommended, because it is simple and can be performed by the patients themselves. The patient is judged to be compliant with the salt restriction regimen if salt intake (excretion) estimated by any of the methods is less than 6 g (100 mmol)/day but not if it is higher.

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Original Article

C-Reactive Protein, Left Ventricular Mass Index, and Risk of Cardiovascular Disease in Essential Hypertension

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We examined the association between C-reactive protein (CRP) and left ventricular mass index (LVMI), and investigated prospectively the incidence of cardiovascular disease (CVD) in asymptomatic subjects with essential hypertension. A total of 629 subjects (mean age 62 years, 51% female) free of prior CVD were included in this study. In cross-sectional analysis at baseline, patients were divided into three groups according to serum CRP levels: <1, 1 to 2, and >2 mg/L. In multivariate analysis, LVMI increased in a stepwise fashion with increasing CRP levels in both men (127.2±2.9, 138.7±4.1, 141.8±3.5 g/m^2, respectively; F=6.85, p=0.001) and women (119.5±3.6, 129.2±4.9, 130.2±4.8 g/m^2; F=4.23, p=0.031). During follow-up (mean 32 months), 52 subjects (19 female) developed CVD. Kaplan-Meier analysis with log-rank tests showed a significantly poorer event-free survival rate in the group with elevated CRP levels (≥1 mg/L) (χ^2=8.22, p<0.01) and that with left ventricular hypertrophy (LVH) (χ^2=19.91, p<0.01). When participants were divided into four groups on the basis of CRP level (<1 or ≥1 mg/L) and the absence or presence of LVH, the group with LVH/CRP≥1 mg/L showed markedly poorer event-free survival (χ^2=28.02, p<0.01), and the adjusted hazard ratio by multivariate Cox regression analysis was 2.65 (95% confidence interval [CI]=1.55–5.46, p<0.01). In the subgroup with LVH (n=362), a significantly lower event-free survival rate of CVD was also observed in the group with CRP≥1 mg/L (hazard ratio [HR] 1.37, 95% CI: 1.02–1.85, p=0.025). Our findings demonstrate that the CRP level is independently associated with LVMI, and suggest that measurement of CRP may provide clinically important prognostic information to supplement LVH. (*Hypertens Res* 2007; 30: 1177–1185)

Key Words: **C-reactive protein, hypertrophy, cardiovascular disease, echocardiography, follow-up study**

Introduction

Hypertension is a central risk factor for cardiovascular disease (CVD), and the cardiovascular prognosis in patients with hypertension depends not only on the level of blood pressure (BP), but also on the presence of associated risk factors. Inflammatory processes are now recognized to play a fundamental role in atherogenesis (*1*). In addition, basic and clinical data suggest the possible involvement of inflammation in the genesis of hypertension and that hypertension in turn induces a proinflammatory response (*2–4*). C-reactive protein (CRP), a marker of the reactant plasma protein component of the inflammatory response, has been found to be a robust predictor of the development of CVD in several large epidemiological studies (*5–10*). The guidelines from the European Society of Hypertension and the European Society of Cardiology (ESH-ESC) (*11*), and the Centers for Disease Control

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and Prevention/American Heart Association (CDC/AHA) (*12*) also stress the importance of measuring CRP in primary prevention. On the other hand, an elevated CRP level is affected by or linked to hypertension and many metabolic factors such as insulin resistance (*13*, *14*), all of which are also associated with left ventricular hypertrophy (LVH). Previous reports have examined the association between CRP and left ventricular (LV) mass, but the results obtained are controversial (*15–19*). Thus, the clinical importance of CRP in LVH has not been fully elucidated. In this study, we investigated the potential interrelationships between CRP and LV mass index (LVMI) in patients with essential hypertension by using the clinical cut-off levels of CRP. We further sought to evaluate the interrelationships between CRP, LVMI, and incidence of CVD in asymptomatic hypertensive patients. In addition, we attempted to determine whether CRP might provide useful prognostic information to enhance that by provided by LVH.

Methods

Study Subjects

From March 1997 to March 2004, a total of 629 essential hypertensive patients who had good-quality echocardiographic recordings were consecutively enrolled in this study and monitored for 31.6±0.8 months. All subjects were selected from patients who were admitted and underwent medical investigation including a general check-up at the National Cardiovascular Center in Osaka, Japan. Hypertension was defined as systolic BP (SBP) ≥140 mmHg and/or diastolic BP (DBP) ≥90 mmHg on repeated measurements, or receipt of antihypertensive treatment. Diabetes mellitus was defined according to the American Diabetes Association criteria (*20*). Smoking status was determined by interview, and defined as currently smoking or not. Ischemic heart disease was defined as a 75% or greater organic stenosis of at least one major coronary artery as confirmed by coronary angiography, or a history of myocardial infarction or percutaneous transluminal coronary angioplasty. Exclusion criteria included ischemic heart disease, acute coronary syndrome, congestive heart failure (CHF) (New York Heart Association [NYHA] class II or greater), old cerebral infarction, history of transient ischemic attack, secondary hypertension, receipt of hormone replacement therapy and/or an anti-inflammatory drug (aspirin or nonsteroidal anti-inflammatory drug [NSAID]), chronic infection, and cancer. Participants with moderate or severe aortic or mitral regurgitation or a heart rate >100 bpm were also excluded. All procedures in the present study were carried out in accordance with institutional and national ethical guidelines for human studies. All subjects enrolled in this study were Japanese, and all gave informed consent to participate in this study.

Baseline Clinical Characteristics

After fasting overnight, BP was measured with an appropriate arm cuff and a mercury column sphygmomanometer on the left arm after a resting period of at least 10 min in the supine position. After BP measurement, venous blood sampling was performed in all subjects. Height and body weight were measured, and body mass index was calculated. The following parameters were also determined: total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-chol), and CRP levels. High-sensitivity CRP was measured by a validated latex-enhanced immunonephelometric assay (sensitivity 0.1 mg/L; Roche Diagnostics, Indianapolis, USA). If the CRP level was >10 mg/L, the test was repeated.

Echocardiographic Methods and Calculation of Derived Variables

Imaging and Doppler echocardiography were performed in all participants in this study, as previously described (*21*, *22*). Studies were performed using phased-array echocardiography with M-mode, 2-dimensional, pulsed, and color-flow Doppler capabilities. LV internal dimension and septal and posterior wall thickness were measured at end-diastole and end-systole according to the American Society of Echocardiography recommendations (*23*). Color-flow Doppler recordings were used to check for aortic and mitral regurgitation, as previously described (*24*). End-diastolic dimensions were used to calculate LV mass by a previously reported formula (*25*). LV mass was considered an unadjusted variable, and was normalized by body surface area and expressed as LVMI.

The LV diastolic filling pattern was recorded from the apical transducer position with the subject in the left lateral decubitus position, with the sample volume situated between the mitral leaflet tips. The leading edge of the transmitral Doppler flow pattern was traced to derive the peak of early diastolic and atrial phase LV filling, their ratio (E-A ratio), and the deceleration time of early diastolic LV filling (DcT).

Pulmonary venous flow velocities were recorded by placing a sample volume of about 1 cm into the right superior pulmonary vein (*26*). The variables derived were the peak pulmonary venous flow during ventricular systole (PVs), that during ventricular diastole, and their ratio (S-D ratio). When a biphasic PVs was detected, the highest peak velocity was used (*26*). All measurements were performed by one trained investigator who was blinded to the clinical data of the subjects.

Clinical End Points

For survival analysis, observation began on the day of echocardiography, and all subjects were followed at the National Cardiovascular Center in Osaka, and treated by implementation of standard lifestyle and pharmacologic mea-

Table 1. Baseline Clinical Characteristics of Study Subjects

Variables	Male (n=309)	Female (n=320)
Age (years)	61.4±0.7	62.4±0.6
Body mass index (kg/m^2)	24.4±0.2	24.2±0.2
Duration of hypertension (years)	15.4±0.6	15.1±0.6
Current smoker (%)	25.6	8.2†
Systolic BP (mmHg)	143.8±0.9	143.7±0.9
Diastolic BP (mmHg)	81.9±0.6	79.7±0.6†
Heart rate (bpm)	61.9±0.8	67.3±0.5*
Diabetes (%)	28.5	19.7†
Total cholesterol (mmol/L)	5.07±0.05	5.32±0.04†
Triglycerides (mmol/L)	1.73±0.08	1.31±0.04†
HDL-chol (mmol/L)	1.18±0.02	1.40±0.02†
Hyperlipidemia treatment (%)	22.7	34.7†
CRP# (mg/L)	0.70 (0.30, 1.90)	0.50 (0.30, 1.40)†
<1.0 mg/L (%)	56.3	64.4
1.0–2.0 mg/L (%)	19.1	17.8
>2.0 mg/L (%)	24.6	17.8

Data are means±SEM or percentages. #Values were log-transformed for analysis and presented as median (first quartile and third quartile). *p<0.05 and †p<0.01 *vs.* male subjects. BP, blood pressure; HDL-chol, high density lipoprotein cholesterol; CRP, C-reactive protein.

Table 2. Cross-Sectional Analysis of Echocardiographic Parameters According to CRP Category

Variables	CRP		
	<1.0 mg/L	1.0–2.0 mg/L	>2.0 mg/L
Male			
LVMI (g/m^2)	127.7±2.3	138.8±4.0*	143.0±3.4†
E-A ratio	0.93±0.02	0.81±0.04*	0.81±0.03†
DcT (ms)	225.5±3.5	245.8±6.0†	243.0±5.3*
S-D ratio	1.49±0.03	1.73±0.05†	1.66±0.05†
Female			
LVMI (g/m^2)	114.7±2.1	126.0±4.0*	132.3±4.2†
E-A ratio	0.90±0.02	0.77±0.03†	0.81±0.03*
DcT (ms)	224.5±3.0	245.9±5.8†	241.0±5.8*
S-D ratio	1.64±0.03	1.77±0.05*	1.76±0.05*

Data are mean±SEM. *p<0.05 and †p<0.01. CRP, C-reactive protein; LVMI, left ventricular mass index; E-A ratio, the ratio of the peak velocity of early diastolic to atrial phase left ventricular filling; DcT, the deceleration time of early diastolic left ventricular filling; S-D ratio, the ratio of the peak pulmonary venous flow during ventricular systole to the during ventricular diastole.

sures. All subjects were periodically referred to our institution for BP control and other diagnostic procedures. CVD events of interest in this study were: myocardial infarction confirmed by electrocardiographic changes, coronary angiography and/or myocardial scintigraphy findings, stroke confirmed by clinical symptoms, computed tomography and magnetic resonance angiography and/or cerebrovascular angiography findings, peripheral arterial occlusive disease confirmed by clinical symptoms, magnetic resonance angiography and/or peripheral angiography (the presence of ≥1 stenosis of ≥50% in the iliac, femoral, popliteal, or crural arteries), and CHF requiring hospitalization. CHF was defined by the Framingham Heart Study criteria (*27*). These criteria require the simultaneous presence of at least two major criteria, or one major criterion in conjunction with two minor criteria, to establish a diagnosis of CHF, and have been validated previously (*28*). The cause of death was classified as CVD if there was sudden death from CVD, by an independent review panel of physicians who were unaware of the echocardiographic and clinical findings. Events that were more equivocal, such as unrecognized myocardial infarction, were not included as CVD for this analysis. Furthermore, patients with clinical evi-

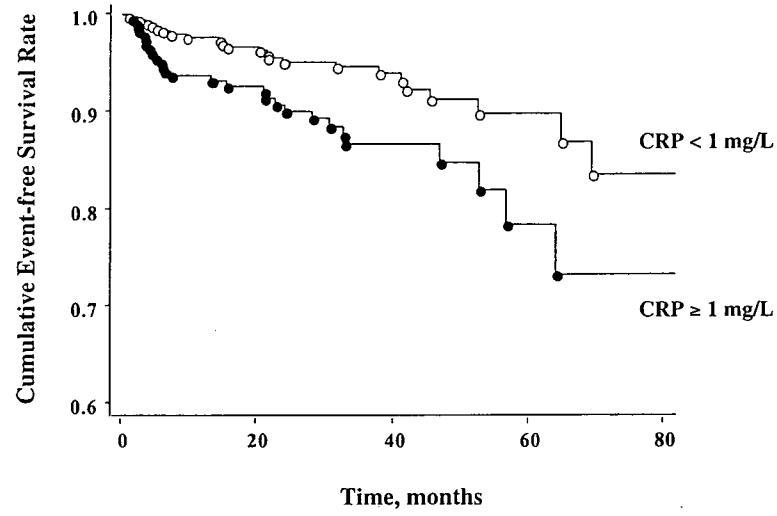

Fig. 1. *Cardiovascular event-free survival in two groups divided by baseline CRP level: < 1.0 or ≥ 1.0 mg/L (log-rank $\chi^2 = 8.22$, $p < 0.004$).*

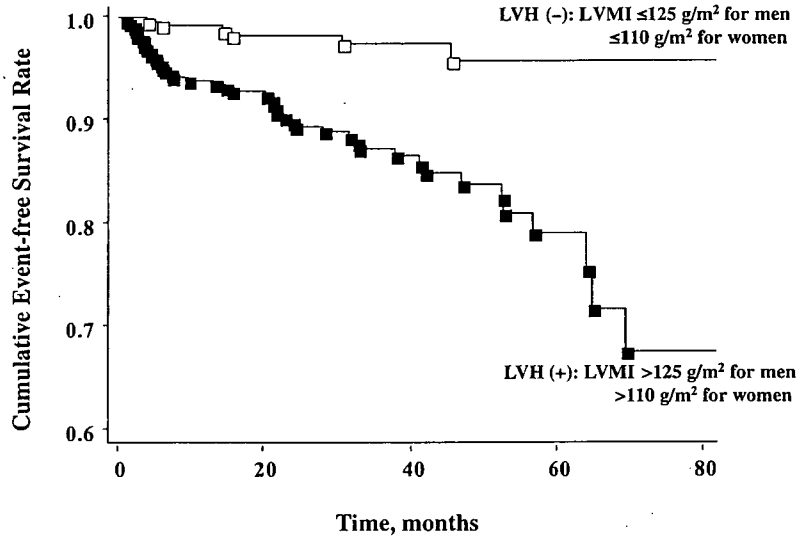

Fig. 2. *Cardiovascular event-free survival in two groups divided by the absence or presence of LVH (log-rank $\chi^2 = 19.91$, $p < 0.001$).*

dence of pneumonia or uremia were excluded. For patients who experienced multiple non-fatal episodes of CVD, the analysis included only the first event.

Statistical Analysis

Parametric data are presented as the means±SEM. Total subjects were divided into three groups for each sex using the clinical cut-off levels of CRP of < 1, 1 to 2, and >2 mg/L, and then the significance of any differences among groups was evaluated using one-way analysis of variance (ANOVA) with Dunnett's multiple comparison posttest. Multiple regression models were used to assess the relationship between LVMI and CRP level categories after adjustment for potential confounding factors affecting LVMI. Because of the right skew in CRP distribution, levels of CRP were log-transformed to examine the significance of any difference among groups.

Event-free survival analysis was performed with the Kaplan-Meier method to plot the cumulative incidence of CVD according to CRP level (< 1 or ≥ 1 mg/L) or the absence or presence of LVH (LVMI > 125 g/m^2 for men and > 110 g/m^2 for women) (*11*, *29*), and the groups were compared by the Mantel log rank test. The cut-off level of CRP of 1 mg/L was based both on the AHA/CDC (*12*) and the ESH-ESC (*11*) rec-

Table 3. Baseline Clinical Characteristics of Study Subjects

Variables	Non-LVH		LVH	
	CRP <1 mg/L (n=175)	CRP ≥1 mg/L (n=92)	CRP<1 mg/L (n=204)	CRP ≥1 mg/L (n=158)
Male (%)	44.0	55.0	47.1	53.8
Age (years)	57.9±0.9§	62.7±1.2†	63.3±0.8†	64.0±0.9†
Body mass index (kg/m^2)	23.7±0.3	24.8±0.4*	24.2±0.2*	24.7±0.3*
Duration of hypertension (years)	13.0±0.8‡	15.6±1.1	15.9±0.7*	16.8±0.8†
Current smoker (%)	14.4	23.1‡	11.3	22.8‡
Systolic BP (mmHg)	142±1‡	139±2§	146±1*	146±1*
Diastolic BP (mmHg)	82±1	81±1	80±1	80±1
Heart rate (bpm)	67.8±0.6‡	68.5±0.9‡	65.4±0.6*	65.8±0.7
Diabetes (%)	14.3	33.0†,‡	17.7	38.0†,§
Total cholesterol (mmol/L)	5.29±0.06	5.38±0.09	5.15±0.06	5.05±0.06*
Triglycerides (mmol/L)	1.38±0.08	1.95±0.11†,§	1.35±0.07	1.63±0.08§
HDL-chol (mmol/L)	1.41±0.03	1.20±0.04†,‡	1.34±0.03	1.17±0.03†,§
CRP¶ (mg/L)	0.32 (0.30, 0.50)	1.90 (1.40, 3.30)†,§	0.33 (0.28, 0.52)	2.30 (1.30, 3.73)†,§
LVMI (g/m^2)	98.8±1.8§	98.2±2.5§	146.0±1.7†	153.1±1.9†
E-A ratio	0.97±0.02§	0.84±0.03†	0.86±0.02†	0.80±0.02†,‡
DcT (ms)	220.4±3.6‡	237.7±5.0*	234.6±3.3*	244.0±3.8†
S-D ratio	1.54±0.03‡	1.73±0.04†	1.65±0.03*	1.69±0.03†
Hyperlipidemia treatment (%)	28.0	29.7	26.0	32.9
Antihypertensive medication (%)				
Calcium channel blocker	59.4	63.7	67.7	81.0†,‡
β-Blocker	22.9	20.9	25.0	39.2†,§
ACEI or ARB	30.9	38.5	33.3	37.3*
Diuretic	8.6§	16.5	21.1†	26.0†
Number of CVD events	3	4	20	25

Data are means±SEM or percentage. ¶Values were log-transformed for analysis and presented as median (first quartile and third quartile). *p<0.05 and †p<0.01 *vs.* non-LVH/CRP <1 mg/L. ‡p<0.05 and §p<0.01 *vs.* LVH/CRP ≥1 mg/L. LVH, left ventricular hypertrophy; CRP, C-reactive protein; BP, blood pressure; HDL-chol, high density lipoprotein cholesterol; LVMI, left ventricular mass index; E-A ratio, the ratio of the peak velocity of early diastolic to atrial phase left ventricular filling; DcT, the deceleration time of early diastolic left ventricular filling; S-D ratio, the ratio of the peak pulmonary venous flow during ventricular systole to the during ventricular diastole; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CVD, cardiovascular disease.

ommendations in which CRP levels of ≥1 mg/L were defined as average- and high-risk groups. Cox proportional hazard analysis was used to examine the association between variables and the cumulative incidence of CVD in crude models. These effects were measured by hazard ratios (HR) and their 95% confidence intervals (CI) based on Cox regression models.

To evaluate combined effects, we divided the total subjects into four groups according to the baseline level of CRP (<1 or ≥1 mg/L) and the absence or presence of LVH. ANOVA with Dunnett's multiple comparison posttest was also used to analyze data among the four groups. Kaplan-Meier curves for event-free survival according to the level of CRP and LVH category were also constructed. The relative risk of CVD events in Cox proportional-hazard analysis was assessed in crude and multivariate models, after accounting for relevant variables using a p value of less than 0.05 as the selection criterion. The cumulative incidence of CVD was calculated using the group with non-LVH/CRP <1 mg/L as a reference for the others. A p value less than 0.05 was considered statistically significant. All calculations were performed using a standard statistical package (JMP 4.0; SAS Institute, Cary, USA).

Results

Association between CRP and LVMI

Baseline clinical and biochemical characteristics of the study subjects are shown in Table 1. At baseline, diuretics, β-blockers, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, and calcium-channel blockers were used alone or in various combinations in 18.1%, 27.3%, 34.5%, and 68.2% of the study patients, respectively. Male subjects had significantly higher LVMI levels as compared with female subjects (135.6±1.9 *vs.* 120.1±1.9 g/m^2; p<0.01).

Table 4. Combined Effects of CRP and LVMI as Predictors of CVD Events

LVH and CRP	Crude		Risk factor–adjusted model#		
	HR (95% CI)	*p*	HR (95% CI)	χ^2	*p*
Non-LVH/CRP <1 mg/L	1 (reference)		1 (reference)		
Non-LVH/CRP ≥1 mg/L	1.63 (0.77–3.68)	0.199	1.40 (0.65–3.18)	0.772	0.380
LVH/CRP <1 mg/L	2.42 (1.42–4.98)	<0.001	2.212 (1.29–4.57)	9.166	0.003
LVH/CRP ≥1 mg/L	3.37 (1.99–6.90)	<0.001	2.648 (1.55–5.46)	15.095	<0.001

#Risk factor–adjusted model adjusts for the effects of age, sex, diabetes, systolic blood pressure, and HDL-chol. CRP, C-reactive protein; HR, hazard ratios; CI, confidence intervals; LVMI, left ventricular mass index; LVH, left ventricular hypertrophy; HDL, high-density lipoprotein.

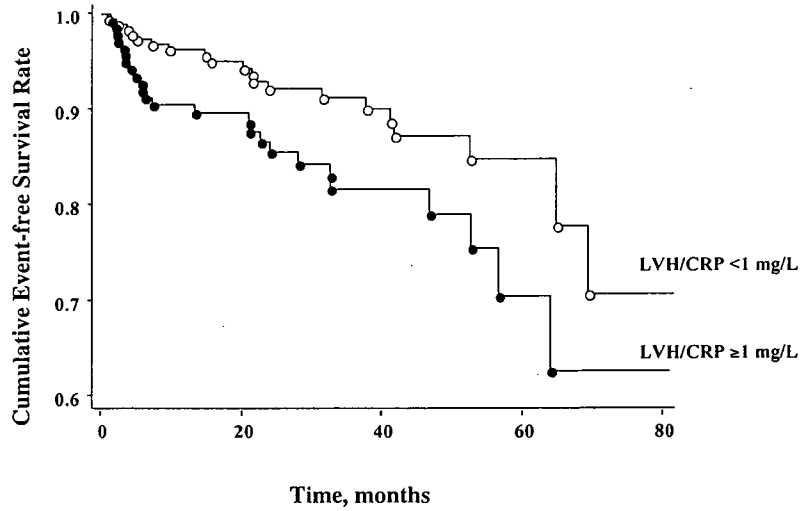

Fig. 3. *Kaplan-Meier survival curves for CVD events in the subgroup with LVH (n=362) (log-rank $\chi^2=5.13$, $p=0.024$).*

There was a linear increase in LVMI levels as the CRP categories increased; median LVMI levels for those with a CRP level of <1, 1 to 2, and >2 mg/L were 128.5, 133.9, and 139.7 in males, and 111.3, 125.1, and 121.8 g/m^2 in females, respectively (both, *p* for trend <0.01). Table 2 shows the crude mean levels and SEM ranges of echocardiographic parameters according to CRP category at enrollment. A similar linear increase in the levels of the S-D ratio and DcT, and decrease in the levels of the E-A ratio, were also seen as the levels of CRP category increased. Multivariate analysis including age, body mass index, diabetes, duration of hypertension, SBP and DBP, heart rate, total cholesterol, triglycerides, HDL-chol, and smoking status was performed, and indicated that the CRP category was independently associated with LVMI both in male (CRP [mg/L] <1: 127.2±2.9; 1–2: 138.7±4.1; >2: 141.8±3.5 g/m^2; $F=6.85$, $p=0.001$) and female subjects (CRP [mg/L] <1: 119.5±3.6; 1–2: 129.2±4.9; >2: 130.2±4.8 g/m^2; $F=4.23$, $p=0.031$). In addition, even after adjustment for taking antihyperlipidemia medication such as statins, fibrates, and niacin, and antihypertensive medication, increasing categories of CRP were still independent predictors of LVMI (male, $F=5.12$, $p=0.007$; female, $F=4.20$, $p=0.032$). To exclude the effect of drugs on CRP level, we further examined the association between CRP category and LVMI after excluding subjects receiving antihyperlipidemia medication (male, $n=233$; female, $n=209$). Even after excluding these subjects, increasing categories of CRP were significant predictors of LVMI in both male (CRP [mg/L] <1: 129.7±2.6; 1–2: 133.5±4.6; >2: 145.8±4.0 g/m^2 ($p<0.01$ *vs.* CRP<1 mg/L)) and female subjects (CRP [mg/L] <1: 116.2±2.8; 1–2: 124.9±5.5; >3: 127.8±5.8 g/m^2 ($p<0.01$ *vs.* CRP<1 mg/L)).

Predictive Value of CRP and LVMI for CVD

During the follow-up period, 52 patients (8.3%, 19 female) developed CVD. There were 27 subjects with CHF, 12 with stroke, 5 with myocardial infarction, and 2 with peripheral arterial occlusive disease, and 6 patients died of CVD causes. The CRP level and LVMI were significantly higher in patients who developed CVD during the follow-up period than in event-free subjects (CRP: 3.87±0.98 *vs.* 1.93±0.29 mg/L; LVMI: 156.4±4.7 *vs.* 125.2±1.4 g/m^2; $p<0.01$, respectively). The results of the life table analysis of CVD

events throughout the follow-up period according to the two groups on the basis of the CRP level (<1 or ≥1 mg/L) and the absence or presence of LVH are plotted in Figs. 1 and 2, respectively. These curves show significantly poorer event-free survival in the group with high CRP and that with LVH, respectively. A univariate Cox proportional-hazard model showed that high CRP (HR 1.51; 95% CI, 1.15–2.01; $p<0.01$) and LVH (HR 2.45; 95% CI, 1.66–3.96; $p<0.01$) were significant predictors of CVD events. Other variables in this study that significantly predicted CVD events included age (HR 1.04 for each 1 year increase; 95% CI: 1.01–1.07; $p<0.01$), sex (HR 1.40 for males; 95% CI: 1.06–1.88; $p<0.02$), diabetes (HR 1.61 for diabetes; 95% CI: 1.21–2.12; $p<0.01$), and SBP (HR 1.03 for each 1.0 mmHg increase; 95% CI: 1.01–1.04; $p<0.01$), and HDL-chol (HR 0.87 for each 10 mmol/L increase; 95% CI: 0.80–0.95; $p<0.01$).

Incidence of CVD Jointly with CRP and LVH

To assess the combined effects of CRP and LVH, we constructed survival curves after dividing the total subjects into four groups on the basis of CRP level (<1 or ≥1 mg/L) and the absence or presence of LVH: non-LVH/CRP <1, non-LVH/CRP ≥1, LVH/CRP <1, and LVH/CRP ≥1. The baseline clinical and biochemical characteristics of the study subjects are shown in Table 3. The group with LVH/CRP ≥1 showed a significantly higher prevalence of current smokers and diabetes, significantly lower HDL-chol and E-A ratio than that with LVH/ CRP <1. Kaplan-Meier curves in the four groups showed significantly poorer event-free survival in subjects with LVH/CRP ≥1 (log-rank $\chi^2=28.02$, $p<0.001$). In Cox regression analysis (Table 4), the risk for CVD was markedly increased in the group with LVH/CRP ≥1 (HR 3.37) compared with the non-LVH/CRP <1 group. In multivariate Cox regression analysis including age, sex, diabetes, SBP, and HDL-chol, the combination of LVH and CRP ≥1 mg/L was an independent predictor of CVD (HR 2.65).

When the analysis was restricted to the subgroup with LVH ($n=362$), 45 CVD events occurred during the follow-up period. Even in these subjects, the CRP level was significantly higher in patients who developed CVD during the follow-up period than in event-free subjects (3.82±0.92 *vs.* 2.02±0.35 mg/L, $p<0.01$). The results of the life table analysis of CVD throughout the follow-up period according to the two groups on the basis of CRP level (<1 or ≥1 mg/L) are plotted in Fig. 3, and these curves show significantly poorer event-free survival in the group with CRP ≥1 mg/L. In addition, in Cox regression analysis, CRP ≥1 mg/L was associated with a 1.37-fold higher risk of CVD events in the subgroup with LVH (HR 1.37; 95% CI: 1.02–1.85; $p=0.025$).

Discussion

Our cross-sectional study revealed a linear relationship between categories of CRP and LVMI, and the relationship was evident even after adjustment for confounding factors by multivariate analysis. In a follow-up study, both high CRP and LVH were significant determinants of CVD events, and the combination of elevated CRP and LVH was a powerful predictor for CVD events. In addition, the present study also demonstrated that, in patients with LVH, CRP had additive predictive value for CVD risk.

The present observation of an independent association between categories of CRP level and LVMI is consistent with previous findings (*15*, *19*), and the present study extended these observations for CRP to essential hypertension. A raised baseline CRP value has been associated with inflammation, endothelial dysfunction (*14*), obesity (*14*), the metabolic syndrome (*30*, *31*), diabetes mellitus (*32*), insulin resistance (*13*), and severity of hypertension (*33*), and thus, various metabolic disorders may occur with increasing CRP level, and simultaneously promote an increase in LV mass. On the other hand, local CRP synthesis and secretion by smooth muscle cells, including those of the human coronary artery, have been suggested (*34*). It is possible to speculate that CRP may play a more direct role in promoting LVH, including 1) increasing phosphatidylinositol-3 kinase activity (*35*); 2) upregulating inducible nitric oxide synthase, certain cell signal transduction pathways including the mitogen-activated protein kinase pathway, and nuclear factor κ-B; 3) upregulating angiotensin II type 1 receptor in vascular smooth muscle cells, and directly quenching the production of nitric oxide by endothelial cells (*2*, *4*), resulting in increased production of endothelin-1 (*34*, *36*); and 4) elevation of von Willebrand factor (*37*), which is known to be associated with endothelial dysfunction. Thus, cardiac hypertrophy may be, at least in part, attributable to an increase in CRP itself, *via* activated transcriptional regulatory mechanisms, proinflammatory and proatherogenic effects, and stimulation of endothelial dysfunction.

Previous studies have demonstrated that CRP evaluation adds prognostic value to metabolic syndrome (*38*), higher fibrinogen (*39*), or higher BP (*3*) in terms of CVD risk. The present prospective cohort of initially asymptomatic essential hypertensive patients revealed the importance of measuring CRP in addition to LVMI by echocardiography, and also showed that the combined evaluation of both CRP and LVMI was superior as a method of risk detection compared to measurement of either biologic marker alone. In addition, the present findings suggest that, in the presence of LVH, CRP adds important and independent prognostic information in terms of CVD risk. This result may have been due to the fact that our subjects with higher LVMI and CRP had more severe dyslipidemia, as well as a higher prevalence of current-smoking and diabetes, which are established risk factors for CVD. On the other hand, several mechanisms have been proposed to account for the association between CRP and CVD, including: 1) induction of loss of vasoreactivity, expression of adhesion molecules and secretion of chemoattractants in the endothelium; 2) increases in tissue factor secretion, promo-

tion of monocyte chemotaxis and adhesion to endothelial cells, reactive oxygen species release, matrix metalloproteinase-1 induction, and promotion of oxidized low-density lipoprotein uptake, which leads to increased foam cell formation; 3) increased expression of angiotensin II type 1 receptor mRNA and protein expression as well as increased vascular smooth muscle cell proliferation and migration; 4) binding of CRP to enzymatically modified low density lipoprotein, with the resulting complex showing increased ability to convert C3 and activate its complement (*34*, *36*); and 5) attenuation of the production of nitric oxide and prostacyclin by endothelial cells (*2*, *4*). Furthermore, our results showed that more severe impairment of relaxation was observed in subjects with high LVMI and high CRP, and this "impaired relaxation" is known to be associated with increased risk of CVD (*40*). In addition, a significant association between the categories of CRP and the E-A ratio, DcT, and S-D ratio, markers of relaxation impairment, was observed in this study, and a high CRP level may contribute to the progression of LV dysfunction. Consequently, we propose that, in subjects with LVH, activation of the renin-angiotensin system, activation of proatherogenic and proinflammatory responses in cardiovascular cells, and more impaired relaxation may occur with increasing CRP level, and enhance the risk for CVD.

It is not possible to conclude whether CRP stimulates higher LVMI or whether CRP is increased before the development of LVH. Indeed, it is possible that both these effects work in tandem in CVD. In addition, this study had missing baseline data and information on other potentially important characteristics, such as alcohol intake and physical activity, which are also associated with a lower CRP level. Finally, we did not account for the duration of hypertension prior to treatment at our institution. Because our data were obtained in subjects with already-treated essential hypertension at the start of the study, these results could underestimate the involvement of BP itself in the development of LVH and CVD events.

In conclusion, in essential hypertensive subjects initially free of CVD, CRP showed a significant association with LVMI. In those with LVH, the baseline CRP level added clinically relevant prognostic information concerning CVD risk. In hypertensive as well as LVH subjects, assessment of CRP levels may help to refine CVD risk stratification.

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and Prevention/American Heart Association (CDC/AHA) (*12*) also stress the importance of measuring CRP in primary prevention. On the other hand, an elevated CRP level is affected by or linked to hypertension and many metabolic factors such as insulin resistance (*13*, *14*), all of which are also associated with left ventricular hypertrophy (LVH). Previous reports have examined the association between CRP and left ventricular (LV) mass, but the results obtained are controversial (*15–19*). Thus, the clinical importance of CRP in LVH has not been fully elucidated. In this study, we investigated the potential interrelationships between CRP and LV mass index (LVMI) in patients with essential hypertension by using the clinical cut-off levels of CRP. We further sought to evaluate the interrelationships between CRP, LVMI, and incidence of CVD in asymptomatic hypertensive patients. In addition, we attempted to determine whether CRP might provide useful prognostic information to enhance that by provided by LVH.

Methods

Study Subjects

From March 1997 to March 2004, a total of 629 essential hypertensive patients who had good-quality echocardiographic recordings were consecutively enrolled in this study and monitored for 31.6±0.8 months. All subjects were selected from patients who were admitted and underwent medical investigation including a general check-up at the National Cardiovascular Center in Osaka, Japan. Hypertension was defined as systolic BP (SBP) ≥140 mmHg and/or diastolic BP (DBP) ≥90 mmHg on repeated measurements, or receipt of antihypertensive treatment. Diabetes mellitus was defined according to the American Diabetes Association criteria (*20*). Smoking status was determined by interview, and defined as currently smoking or not. Ischemic heart disease was defined as a 75% or greater organic stenosis of at least one major coronary artery as confirmed by coronary angiography, or a history of myocardial infarction or percutaneous transluminal coronary angioplasty. Exclusion criteria included ischemic heart disease, acute coronary syndrome, congestive heart failure (CHF) (New York Heart Association [NYHA] class II or greater), old cerebral infarction, history of transient ischemic attack, secondary hypertension, receipt of hormone replacement therapy and/or an anti-inflammatory drug (aspirin or nonsteroidal anti-inflammatory drug [NSAID]), chronic infection, and cancer. Participants with moderate or severe aortic or mitral regurgitation or a heart rate >100 bpm were also excluded. All procedures in the present study were carried out in accordance with institutional and national ethical guidelines for human studies. All subjects enrolled in this study were Japanese, and all gave informed consent to participate in this study.

Baseline Clinical Characteristics

After fasting overnight, BP was measured with an appropriate arm cuff and a mercury column sphygmomanometer on the left arm after a resting period of at least 10 min in the supine position. After BP measurement, venous blood sampling was performed in all subjects. Height and body weight were measured, and body mass index was calculated. The following parameters were also determined: total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-chol), and CRP levels. High-sensitivity CRP was measured by a validated latex-enhanced immunonephelometric assay (sensitivity 0.1 mg/L; Roche Diagnostics, Indianapolis, USA). If the CRP level was >10 mg/L, the test was repeated.

Echocardiographic Methods and Calculation of Derived Variables

Imaging and Doppler echocardiography were performed in all participants in this study, as previously described (*21*, *22*). Studies were performed using phased-array echocardiography with M-mode, 2-dimensional, pulsed, and color-flow Doppler capabilities. LV internal dimension and septal and posterior wall thickness were measured at end-diastole and end-systole according to the American Society of Echocardiography recommendations (*23*). Color-flow Doppler recordings were used to check for aortic and mitral regurgitation, as previously described (*24*). End-diastolic dimensions were used to calculate LV mass by a previously reported formula (*25*). LV mass was considered an unadjusted variable, and was normalized by body surface area and expressed as LVMI.

The LV diastolic filling pattern was recorded from the apical transducer position with the subject in the left lateral decubitus position, with the sample volume situated between the mitral leaflet tips. The leading edge of the transmitral Doppler flow pattern was traced to derive the peak of early diastolic and atrial phase LV filling, their ratio (E-A ratio), and the deceleration time of early diastolic LV filling (DcT).

Pulmonary venous flow velocities were recorded by placing a sample volume of about 1 cm into the right superior pulmonary vein (*26*). The variables derived were the peak pulmonary venous flow during ventricular systole (PVs), that during ventricular diastole, and their ratio (S-D ratio). When a biphasic PVs was detected, the highest peak velocity was used (*26*). All measurements were performed by one trained investigator who was blinded to the clinical data of the subjects.

Clinical End Points

For survival analysis, observation began on the day of echocardiography, and all subjects were followed at the National Cardiovascular Center in Osaka, and treated by implementation of standard lifestyle and pharmacologic mea-

Table 1. Baseline Clinical Characteristics of Study Subjects

Variables	Male (n=309)	Female (n=320)
Age (years)	61.4±0.7	62.4±0.6
Body mass index (kg/m^2)	24.4±0.2	24.2±0.2
Duration of hypertension (years)	15.4±0.6	15.1±0.6
Current smoker (%)	25.6	8.2†
Systolic BP (mmHg)	143.8±0.9	143.7±0.9
Diastolic BP (mmHg)	81.9±0.6	79.7±0.6†
Heart rate (bpm)	61.9±0.8	67.3±0.5*
Diabetes (%)	28.5	19.7†
Total cholesterol (mmol/L)	5.07±0.05	5.32±0.04†
Triglycerides (mmol/L)	1.73±0.08	1.31±0.04†
HDL-chol (mmol/L)	1.18±0.02	1.40±0.02†
Hyperlipidemia treatment (%)	22.7	34.7†
CRP# (mg/L)	0.70 (0.30, 1.90)	0.50 (0.30, 1.40)†
<1.0 mg/L (%)	56.3	64.4
1.0–2.0 mg/L (%)	19.1	17.8
>2.0 mg/L (%)	24.6	17.8

Data are means±SEM or percentages. #Values were log-transformed for analysis and presented as median (first quartile and third quartile). *p<0.05 and †p<0.01 *vs.* male subjects. BP, blood pressure; HDL-chol, high density lipoprotein cholesterol; CRP, C-reactive protein.

Table 2. Cross-Sectional Analysis of Echocardiographic Parameters According to CRP Category

Variables	CRP		
	<1.0 mg/L	1.0–2.0 mg/L	>2.0 mg/L
Male			
LVMI (g/m^2)	127.7±2.3	138.8±4.0*	143.0±3.4†
E-A ratio	0.93±0.02	0.81±0.04*	0.81±0.03†
DcT (ms)	225.5±3.5	245.8±6.0†	243.0±5.3*
S-D ratio	1.49±0.03	1.73±0.05†	1.66±0.05†
Female			
LVMI (g/m^2)	114.7±2.1	126.0±4.0*	132.3±4.2†
E-A ratio	0.90±0.02	0.77±0.03†	0.81±0.03*
DcT (ms)	224.5±3.0	245.9±5.8†	241.0±5.8*
S-D ratio	1.64±0.03	1.77±0.05*	1.76±0.05*

Data are mean±SEM. *p<0.05 and †p<0.01. CRP, C-reactive protein; LVMI, left ventricular mass index; E-A ratio, the ratio of the peak velocity of early diastolic to atrial phase left ventricular filling; DcT, the deceleration time of early diastolic left ventricular filling; S-D ratio, the ratio of the peak pulmonary venous flow during ventricular systole to the during ventricular diastole.

sures. All subjects were periodically referred to our institution for BP control and other diagnostic procedures. CVD events of interest in this study were: myocardial infarction confirmed by electrocardiographic changes, coronary angiography and/or myocardial scintigraphy findings, stroke confirmed by clinical symptoms, computed tomography and magnetic resonance angiography and/or cerebrovascular angiography findings, peripheral arterial occlusive disease confirmed by clinical symptoms, magnetic resonance angiography and/or peripheral angiography (the presence of ≥1 stenosis of ≥50% in the iliac, femoral, popliteal, or crural arteries), and CHF requiring hospitalization. CHF was defined by the Framingham Heart Study criteria (*27*). These criteria require the simultaneous presence of at least two major criteria, or one major criterion in conjunction with two minor criteria, to establish a diagnosis of CHF, and have been validated previously (*28*). The cause of death was classified as CVD if there was sudden death from CVD, by an independent review panel of physicians who were unaware of the echocardiographic and clinical findings. Events that were more equivocal, such as unrecognized myocardial infarction, were not included as CVD for this analysis. Furthermore, patients with clinical evi-

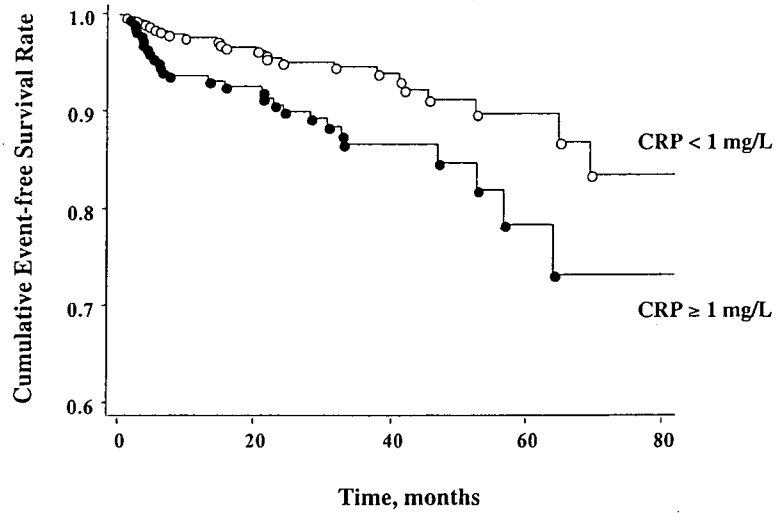

Fig. 1. *Cardiovascular event-free survival in two groups divided by baseline CRP level: < 1.0 or ≥ 1.0 mg/L (log-rank $\chi^2 = 8.22$, $p < 0.004$).*

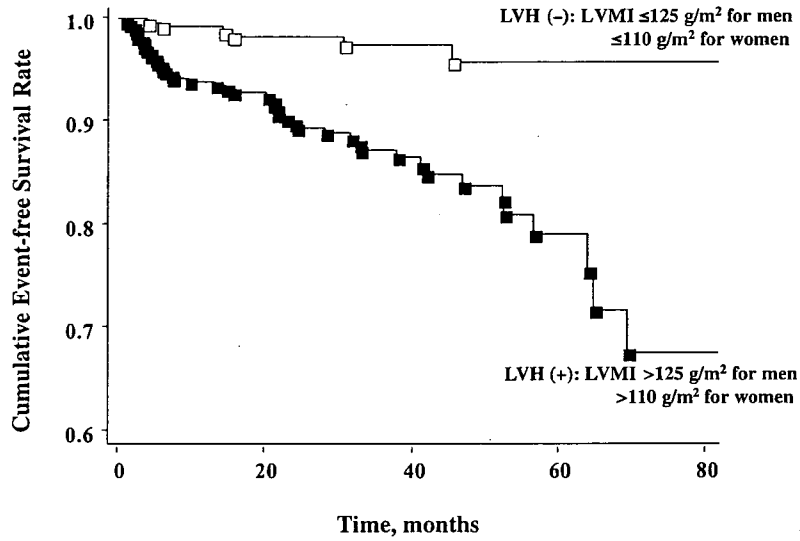

Fig. 2. *Cardiovascular event-free survival in two groups divided by the absence or presence of LVH (log-rank $\chi^2 = 19.91$, $p < 0.001$).*

dence of pneumonia or uremia were excluded. For patients who experienced multiple non-fatal episodes of CVD, the analysis included only the first event.

Statistical Analysis

Parametric data are presented as the means±SEM. Total subjects were divided into three groups for each sex using the clinical cut-off levels of CRP of < 1, 1 to 2, and > 2 mg/L, and then the significance of any differences among groups was evaluated using one-way analysis of variance (ANOVA) with Dunnett's multiple comparison posttest. Multiple regression models were used to assess the relationship between LVMI and CRP level categories after adjustment for potential confounding factors affecting LVMI. Because of the right skew in CRP distribution, levels of CRP were log-transformed to examine the significance of any difference among groups.

Event-free survival analysis was performed with the Kaplan-Meier method to plot the cumulative incidence of CVD according to CRP level (< 1 or ≥ 1 mg/L) or the absence or presence of LVH (LVMI > 125 g/m² for men and > 110 g/m² for women) (*11*, *29*), and the groups were compared by the Mantel log rank test. The cut-off level of CRP of 1 mg/L was based both on the AHA/CDC (*12*) and the ESH-ESC (*11*) rec-

Table 3. Baseline Clinical Characteristics of Study Subjects

Variables	Non-LVH		LVH	
	CRP < 1 mg/L (n=175)	CRP ≥ 1 mg/L (n=92)	CRP < 1 mg/L (n=204)	CRP ≥ 1 mg/L (n=158)
Male (%)	44.0	55.0	47.1	53.8
Age (years)	57.9±0.9§	62.7±1.2†	63.3±0.8†	64.0±0.9†
Body mass index (kg/m²)	23.7±0.3	24.8±0.4*	24.2±0.2*	24.7±0.3*
Duration of hypertension (years)	13.0±0.8‡	15.6±1.1	15.9±0.7*	16.8±0.8†
Current smoker (%)	14.4	23.1‡	11.3	22.8‡
Systolic BP (mmHg)	142±1‡	139±2§	146±1*	146±1*
Diastolic BP (mmHg)	82±1	81±1	80±1	80±1
Heart rate (bpm)	67.8±0.6‡	68.5±0.9‡	65.4±0.6*	65.8±0.7
Diabetes (%)	14.3	33.0†,‡	17.7	38.0†,§
Total cholesterol (mmol/L)	5.29±0.06	5.38±0.09	5.15±0.06	5.05±0.06*
Triglycerides (mmol/L)	1.38±0.08	1.95±0.11†,§	1.35±0.07	1.63±0.08§
HDL-chol (mmol/L)	1.41±0.03	1.20±0.04†,‡	1.34±0.03	1.17±0.03†,§
CRP¶ (mg/L)	0.32 (0.30, 0.50)	1.90 (1.40, 3.30)†,§	0.33 (0.28, 0.52)	2.30 (1.30, 3.73)†,§
LVMI (g/m²)	98.8±1.8§	98.2±2.5§	146.0±1.7†	153.1±1.9†
E-A ratio	0.97±0.02§	0.84±0.03†	0.86±0.02†	0.80±0.02†,‡
DcT (ms)	220.4±3.6‡	237.7±5.0*	234.6±3.3*	244.0±3.8†
S-D ratio	1.54±0.03‡	1.73±0.04†	1.65±0.03*	1.69±0.03†
Hyperlipidemia treatment (%)	28.0	29.7	26.0	32.9
Antihypertensive medication (%)				
Calcium channel blocker	59.4	63.7	67.7	81.0†,‡
β-Blocker	22.9	20.9	25.0	39.2†,§
ACEI or ARB	30.9	38.5	33.3	37.3*
Diuretic	8.6§	16.5	21.1†	26.0†
Number of CVD events	3	4	20	25

Data are means±SEM or percentage. ¶Values were log-transformed for analysis and presented as median (first quartile and third quartile). *p<0.05 and †p<0.01 *vs.* non-LVH/CRP < 1 mg/L. ‡p<0.05 and §p<0.01 *vs.* LVH/CRP ≥ 1 mg/L. LVH, left ventricular hypertrophy; CRP, C-reactive protein; BP, blood pressure; HDL-chol, high density lipoprotein cholesterol; LVMI, left ventricular mass index; E-A ratio, the ratio of the peak velocity of early diastolic to atrial phase left ventricular filling; DcT, the deceleration time of early diastolic left ventricular filling; S-D ratio, the ratio of the peak pulmonary venous flow during ventricular systole to the during ventricular diastole; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CVD, cardiovascular disease.

ommendations in which CRP levels of ≥ 1 mg/L were defined as average- and high-risk groups. Cox proportional hazard analysis was used to examine the association between variables and the cumulative incidence of CVD in crude models. These effects were measured by hazard ratios (HR) and their 95% confidence intervals (CI) based on Cox regression models.

To evaluate combined effects, we divided the total subjects into four groups according to the baseline level of CRP (< 1 or ≥ 1 mg/L) and the absence or presence of LVH. ANOVA with Dunnett's multiple comparison posttest was also used to analyze data among the four groups. Kaplan-Meier curves for event-free survival according to the level of CRP and LVH category were also constructed. The relative risk of CVD events in Cox proportional-hazard analysis was assessed in crude and multivariate models, after accounting for relevant variables using a p value of less than 0.05 as the selection criterion. The cumulative incidence of CVD was calculated using the group with non-LVH/CRP < 1 mg/L as a reference for the others. A p value less than 0.05 was considered statistically significant. All calculations were performed using a standard statistical package (JMP 4.0; SAS Institute, Cary, USA).

Results

Association between CRP and LVMI

Baseline clinical and biochemical characteristics of the study subjects are shown in Table 1. At baseline, diuretics, β-blockers, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, and calcium-channel blockers were used alone or in various combinations in 18.1%, 27.3%, 34.5%, and 68.2% of the study patients, respectively. Male subjects had significantly higher LVMI levels as compared with female subjects (135.6±1.9 *vs.* 120.1±1.9 g/m²; p<0.01).