Center. Genomic DNA was isolated from peripheral blood leukocytes with an NA-3000 nucleic acid isolation system (Kurabo, Osaka, Japan).[14]

Sequencing and genotyping of klotho SNP

Sequencing and genotyping have been described previously.[15] Briefly, we attempted to sequence the promoter region and all exons of *klotho* in 96 Japanese patients with hypertension. All exons with their flanking sequences and approximately 1.6 kb of the promoter region were directly sequenced with an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA) using four sets of primers. Information about the primers and polymerase chain reaction (PCR) conditions is available on request. The obtained sequences were examined for the presence of variations using sequencer software (Gene Codes, Ann Arbor, MI, USA), followed by visual inspection. The A of the ATG of the initiator Met codon was designated nucleotide +1. The nucleotide sequence (GenBank accession ID NT_004671) was used as a reference sequence. We selected and genotyped four SNP (rs7323281; intron1, rs5644481; exon4, rs3752472; exon3, rs650439; intron4 on website, db SNP http://www.ncbi.nlm.nih.gov/projects/SNP/) selected as representative SNP of haplotype blocks and a minor allele frequency (MAF) of more than 0.05 obtained by direct sequencing. These four SNP genotyped in the present study were registered in the National Center for Biotechnology Information (NCBI) database, but other SNP identified by direct sequencing were novel (Supporting Information Table S1). We checked the Tag SNP on the conditions of r^2 more than 0.5 and MAF more than 0.05 from 45 Japanese in the HapMap database. In consequence, eight SNP were hit. In the present study, we directly sequenced the promoter, all exons and its adjacent intron in 96 Japanese. On the other hand, the HapMap database consists of full sequencing data. The difference of SNP number between HapMap and the present study for the covering area of the klotho gene would be mainly due to this reason. We think that the coding region would be more important than the intron area. The TaqMan PCR method was used for genotyping as previously described.[16] All clinical data, sequencing and genotyping results were anonymous.

Evaluation of carotid atherosclerosis

Carotid ultrasonography was used to measure mean IMT (m-IMT) to evaluate atherosclerosis as previously described.[17,18] Briefly, ultrasonography of both carotid arteries was performed with a high-resolution Duplex scanner (SSA-250A; probe, SMA-736S mechanical sector scanner; Toshiba, Tokyo, Japan) for B-scans. All measurements were performed by two trained sonographers who were unaware of the subjects' clinical data. We defined carotid atherosclerosis as m-IMT of 1.1 mm or more.

Statistical analysis

Values are expressed as the mean ± standard deviation The distribution of genotypes between groups with an m-IMT of 1.1 mm or more and groups with an m-IMT of less than 1.1 mm was analyzed by χ^2-test analysis. Differences in variables between hypertensive patients and the general population were also assessed by Student's *t*-test and χ^2-test analysis. Hardy–Weinberg equilibrium (HWE) was assessed by χ^2-test analysis.

In multivariable models, we considered atherosclerosis-associated risk factors and drug treatment. Multiple logistic analysis and ANCOVA were performed with confounders including age, sex, BMI, diabetes, hyperlipidemia, hypertension, chronic kidney disease (CKD), smoking status and information about taking drugs. CKD was defined as an estimated glomerular filtration rate of less than 60 mL/min per 1.73 m^2. Information about taking drugs included antihypertensive, lipid-lowering and hypoglycemic drugs. The adjusted odds ratios (OR) are given with the 95% confidence intervals (CI). All analyses except ANCOVA were performed with JMP statistical software ver. 8 and ANCOVA was performed with Dr SPSS II statistical software ver. 11.0.1. Statistical significance was established at $P < 0.05$.

Using HapMap database

We used HapMap database (http://hapmap.jst.go.jp) to assess SNP located in the same haplotype block with rs650439. Our data sources was Japanese, HapMap Data Rel 24/phaseII Nov08, on NCBI B36 assembly, dbSNP b126.

Results

Study population

The characteristics of the subjects are shown in Table 1. Hypertensive patients had higher risk factors than the general population, for example, rate of male sex, BMI, blood pressure, prevalence of diabetes, prevalence of hyperlipidaemia, lower HDL cholesterol and CKD. The average m-IMT was higher in hypertensive patients than in the general population. In hypertensive patients, 184 subjects (21.6%) were classified as carotid atherosclerosis, and 90.6%, 30.1% and 8.1% subjects of this group were taking antihypertensive, lipid-lowering and antidiabetic drugs, respectively. On the other hand, in there general population there were only 70 subjects (3.9%) with carotid atherosclerosis, and 24.8%, 13.6%

Table 1 Characteristics of the hypertensive patients and general population

	Hypertensive (n = 853)	General (n = 1783)	P-value
Age, mean ± SD	65.2 ± 10.4	64.8 ± 11.2	0.3357
Sex (% male)	54.5	46.0	<0.0001
BMI, mean ± SD (kg/m^2)	23.9 ± 4.49	22.7 ± 3.1	<0.0001
SBP, mean ± SD (mmHg)	139.5 ± 17.5	130.1 ± 19.7	<0.0001
DBP, mean ± SD (mmHg)	82.4 ± 10.6	78.1 ± 10.4	<0.0001
Hypertension (%)	100	42.4	<0.0001
Diabetes (%)	24.2	8.64	<0.0001
HbA1c, mean ± SD (%)	5.68 ± 0.83	5.5 ± 0.7	<0.0001
Hyperlipidaemia (%)	62.1	42.1	<0.0001
HDL-C, mean ± SD (mg/dl)	51.9 ± 15.8	60.1 ± 15.6	<0.0001
Smoking Status (%)			<0.0001
Current	11.8	17.3	0.0002
Past	35.2	24.4	<0.0001
Never	53.0	58.3	0.0128
CKD (%)	30.7	14.1	<0.0001
eGFR, mean ± SD (mL/min per 1.73 m^2)	69.2 ± 27.1	77.8 ± 19.2	<0.0001
mean IMT, mean ± SD (mm)	0.84 ± 0.18	0.82 ± 0.13	0.0033
Number of subjects with IMT thickening (%)	184 (21.6%)	70 (3.9%)	<0.0001

SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high density lipoprotein cholesterol; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; IMT, intima-media thickness.

and 4.5% subjects were taking antihypertensive, lipid-lowering and antidiabetic drugs, respectively.

Association of klotho SNP with carotid atherosclerosis in hypertensive populations

The genotype frequencies of all analyzed polymorphisms were consistent with HWE. The relations between genotypes and atherosclerosis for the hypertensive group were analyzed in additive, dominant and recessive models. There were some significant associations in this analysis (Supporting Information Table S2). The rs650439 in *klotho* was significantly associated with carotid atherosclerosis (TT vs TA vs AA; $\chi^2 = 7.49$, $P = 0.02$, TT + TA vs AA [recessive model]; $\chi^2 = 6.33$, $P = 0.01$). The rs3752472 SNP was also associated with carotid atherosclerosis (CC + TC vs TT [dominant model]; $\chi^2 = 4.41$, $P = 0.04$). The other two SNP were not significantly associated with carotid atherosclerosis (data not shown).

The relationships between the two SNP (rs650439, rs3752472) and carotid atherosclerosis analyzed by multiple logistic analysis with confounders including age, sex, BMI, diabetes, hyperlipidaemia, CKD, smoking status and taking drugs are shown in Table 2. In this analysis, rs650439 was significantly associated with carotid atherosclerosis (TT vs TA vs AA, $P < 0.01$; TT + TA vs AA; $P < 0.01$), but rs3752472 was not significant (CC + TC vs TT, $P = 0.26$). ANCOVA was performed to assess the associations between SNP and the m-IMT value. rs650439 was only significantly associated with the m-IMT value (TT + TA vs AA, $P = 0.04$; Table 3). The other SNP were not significantly associated with the m-IMT.

Association of klotho polymorphisms with carotid atherosclerosis in the general population

Unlike the hypertensive group, rs650439 and rs3752472 were not significantly associated with carotid atherosclerosis on χ^2-test analysis (Supporting Information Table S2) and multiple logistic regression analysis adjusted by age, sex, BMI, diabetes, hyperlipidaemia, hypertension, smoking status, CKD and drug treatment (data not shown). The other two SNP, rs7323281 and rs564481, were not associated with carotid atherosclerosis either (data not shown). There were no significant associations between the m-IMT value and *klotho* SNP analyzed by ANCOVA (Table 4). We performed the subgroup analysis by ANCOVA in normotensive and hypertensive subjects of the general population. There were no significant associations between *klotho* SNP and m-IMT values, even in rs650439 (Table 5).

Discussion

Cardiovascular diseases (CVD) such as myocardial infarction and stroke are the main cause of human

Table 2 Multiple logistic regression analysis† for carotid atherosclerosis and klotho gene polymorphism in the hypertensive patients

	Odds ratio	95% confidence interval	*P*-value
rs650439(TT + TA vs AA)			<0.01
TT + TA/AA	1.688	1.178–2.442	
rs650439 (TT vs TA vs AA)			<0.01
TT/AA	1.221	0.665–2.176	
TA/AA	1.825	1.254–2.677	
rs3752472 (CC + CT vs TT)			0.26
CC + CT/TT	0.347	0.044–2.263	
rs3752472 (CC vs CT vs TT)			0.18
CC/TT	0.332	0.042–2.173	
TC/TT	0.474	0.057–3.232	

†Adjusted for age, sex, body mass index, smoking, diabetes, hyperlipidaemia, chronic kidney disease (estimated glomerular filtration rate <60mL/min per 1.73 m^2) and drug treatments.

Table 3 ANCOVA† of mean IMT and klotho gene polymorphism in the hypertensive patients

SNP	Genotype	Mean IMT	95% confidence interval	*P*-value
rs650439	TT	0.844 ± 0.017	0.811–0.877	0.11
	TA	0.853 ± 0.009	0.836–0.870	
	AA	0.827 ± 0.009	0.809–0.844	
rs650439	TT + TA	0.851 ± 0.008	0.836–0.866	0.04
	AA	0.826 ± 0.009	0.809–0.844	
rs3752472	CC	0.837 ± 0.006	0.825–0.850	0.31
	CT	0.855 ± 0.015	0.825–0.885	
	TT	0.924 ± 0.076	0.776–1.074	
rs3752472	CC + CT	0.840 ± 0.006	0.828–0.852	0.26
	TT	0.925 ± 0.076	0.776–1.074	

IMT, intima-media thickness; SNP, single nucleotide polymorphism. †Adjusted for age, sex, body mass index, smoking, diabetes, hyperlipidaemia, chronic kidney disease (estimated glomerular filtration rate <60 mL/min per 1.73 m^2), and drug treatments.

mortality. Atherosclerosis plays a pivotal role in the pathogenesis of CVD, so prevention of atherosclerosis is the main goal of both clinicians and researchers. Mice deficient in klotho gene expression exhibit a syndrome resembling premature human aging, including atherosclerosis.[1] Previous studies have suggested an association of the klotho gene polymorphisms with atherosclerotic disease in white and Japanese populations.[6] However, these previous studies did not measure atherosclerosis parameters such as carotid IMT.

In the present study, we evaluated the relationship between representative SNP in *klotho* and carotid atherosclerosis directly evaluated by ultrasonography using two different subjects: patients with hypertension and subjects from a general population in Japan. We genotyped four SNP (rs7323281; intron1, rs5644481; exon4, rs3752472; exon3, rs650439; intron4) selected as representative SNP from haplotype blocks obtained by the direct sequencing for *klotho* in 96 individuals. Arking *et al.* reported a functional variant of the klotho gene polymorphism (KL-VS) associated with aging[4] and early onset of CAD.[5] Imamura *et al.* reported that G-395A in a promoter region in *klotho* may be associated with CAD in Japan.[9] However, in the present study, we did not identify F352V or C370S (KL-VS) in exon2 or G-395A in the *klotho* promoter region by direct sequencing for human *klotho*.

In our multivariate logistic analysis, rs650439 (intron4) was strongly associated with carotid atherosclerosis in hypertensive patients ($P < 0.01$). In ANCOVA,

Table 4 ANCOVA† of mean IMT and klotho gene polymorphism in the general population

SNP	Genotype	Mean IMT	95% confidence interval	*P*-value
rs650439	TT	0.827 ± 0.007	0.813–0.841	0.19
	TA	0.816 ± 0.004	0.809–0.824	
	AA	0.825 ± 0.004	0.818–0.833	
rs650439	TT + TA	0.819 ± 0.003	0.812–0.826	0.21
	AA	0.825 ± 0.004	0.818–0.833	
rs3752472	CC	0.823 ± 0.003	0.818–0.829	0.35
	CT	0.813 ± 0.007	0.801–0.827	
	TT	0.802 ± 0.030	0.744–0.861	
rs3752472	CC + CT	0.822 ± 0.003	0.817–0.827	0.52
	TT	0.802 ± 0.030	0.744–0.861	

IMT, intima-media thickness; SNP, single nucleotide polymorphism. †Adjusted for age, sex, body mass index, smoking, hypertension, diabetes, hyperlipidaemia, chronic kidney disease (estimated glomerular filtration rate <60 mL/min per 1.73 m^2) and drug treatments.

Table 5 ANCOVA† of mean IMT and rs650439 in the hypertensive patients and the general population

SNP	Genotype	Mean IMT	95% confidence interval	*P*-value
Hypertensive	TT + TA	0.851 ± 0.008	0.836–0.866	0.04
	AA	0.826 ± 0.009	0.809–0.844	
General with hypertension	TT + TA	0.860 ± 0.006	0.849–0.871	0.11
	AA	0.874 ± 0.006	0.861–0.887	
General without hypertension	TT + TA	0.788 ± 0.004	0.780–0.796	0.76
	AA	0.790 ± 0.005	0.781–0.800	

IMT, intima-media thickness; SNP, single nucleotide polymorphism. †Adjusted for age, sex, body mass index, smoking, hypertension, diabetes, hyperlipidaemia, chronic kidney disease (estimated glomerular filtration rate <60 mL/min per 1.73 m^2) and drug treatments.

rs650439 was also significantly associated with the m-IMT value (TT + TA vs AA, $P = 0.04$; Table 3). On the other hand, in these analyses, the significant association of rs650439 was not observed in the general population. We have performed a linear regression analysis between genotypes and IMT as a continuous variable. There was a significant correlation of genotypes in rs650439 with IMT after adjusted confounding factors, only in hypertensive patients (TT + TA vs AA; $P = 0.04$). These results suggested that SNP of *klotho* may affect the progression of carotid atherosclerosis in the subjects with hypertension. However, there was no significant association of SNP of *klotho* with carotid atherosclerosis in the general population. In order to assess this discrepancy, we performed the subgroup analysis in normotensive and hypertensive subjects of a general population, however, *klotho* rs650439 was also not associated with IMT in this subgroup analysis (Table 5). The number of subjects with IMT of 1.1 or more was small ($n = 41$) even in hypertensive subjects of the general population. Although HWE were not significantly different among number of genotypes in subjects with or without IMT of 1.1 or more, allele frequency was quite different from the NCBI database. We consider these may be reasons that *klotho* rs650439 was not associated with IMT in hypertensive subjects of the general population.

Comparing the backgrounds in hypertensive patients and the general population, the hypertensive group clearly had higher risk factors such as SBP, prevalence of diabetes, hyperlipidaemia and CKD than the general population (Table 1). Moreover, risk factors for atherosclerosis such as diabetes, dyslipidemia and CKD were obviously of higher prevalence in hypertensive patients compared to the general population with hypertension (Supporting Information Table S3). Thus, we suppose

that *klotho* gene polymorphisms may be influencing additively on progression of atherosclerosis induced by classical risk factors.

Previous studies reported that patients with multiple risk factors have elevated oxidative stress. For example, patients with metabolic syndrome may have elevated oxidative stress[19] in their cardiovascular systems. Total body fat and waist circumference have been demonstrated to be positively associated with oxidative stress-mediated endothelial dysfunction[20] and vascular endothelial cell nicotinamide adenine dinucleotide phosphate oxidase activity.[21] Furthermore, several studies demonstrated that human endothelial and smooth muscle cells incubated with high glucose concentrations upregulate oxidative stress.[22,23] From these aspects, hypertensive patients might have higher oxidative stress than general population in this study. We have previously reported that *klotho* gene delivery upregulates manganese superoxide dismutase protein expression and suppresses the oxidative stress *in vivo*.[10] We have also reported that klotho protein reduced H_2O_2-induced apoptosis and senescence in vascular cells,[11] suggesting that it can protect endothelial cells from oxidative stress. Kuro-o *et al.* reported that klotho protein inhibits insulin signals and promotes FOXO activity, inducing superoxide dismutase production.[24,25] These previous reports suggest that klotho may have an antioxidative stress function. Therefore, we can speculate that the protective effect of klotho protein would be strongly expressed in a hyper-oxidative stress state, and that hypertensive patients with multiple risk factors may be more sensitive to reduction of klotho protein function. This may be the reason why the *klotho* rs650439 was associated with carotid atherosclerosis only in hypertensive patients.

Klotho protein is known to regulate calcium and phosphate metabolism,[26] and the abnormality of calcium and phosphate level affects progression of carotid atherosclerosis. Therefore, we investigated the relationship between SNP in *klotho* and serum level of calcium and phosphate. However, there were no significant differences in serum calcium and phosphorus levels among each genotype (data not shown). Thus, we suppose that carotid atherosclerosis may not be due to systemic abnormality of calcium and phosphorus metabolism.

The SNP in *klotho*, rs650439, is located in intron 4. According to this study, we speculate that rs650439 may modulate klotho protein function. One possible mechanism is that rs650439 may induce a splicing abnormality leading to a change in klotho protein construction or function. Another possible mechanism is that other functional SNP affecting *klotho* expression or function may be located in the same haplotype block as rs650439. We assessed the SNP located in the same haplotype block as rs650439 using the HapMap database. Almost all SNP were in intron1, and the rest of the SNP closely linked with rs650439 were in intron3, exon4 and near the 3'-terminal. There were no SNP located in the promoter region. rs648202, the only SNP located in a coding region (exon4), was synonymous (Ala to Ala). The influence of rs650439 on *klotho* function could not be elucidated in this study. Further studies are needed to clarify the functional role of rs650439 in *klotho*.

Although the difference of genetic backgrounds plays a key role in the pathophysiology of atherosclerosis, the effect of only one SNP would be limited. Thus, the other gene SNP which were associated with the progression of atherosclerosis might affect the results of the present study, and the interaction between *klotho* rs650439 and gene polymorphisms in other atherosclerosis-related genes may be leading to the multiplier effects.

In conclusion, this study is the first to reveal that the SNP in *klotho* is associated with carotid atherosclerosis in patients with hypertension. Further studies are needed to clarify the functional role of *klotho* rs650439 on the progression of atherosclerosis.

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References

1 Kuro-o M, Matsumura Y, Aizawa H *et al.* Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 1997; **390**: 45–51.
2 Shimada T, Takeshita Y, Murohara T *et al.* Angiogenesis and vasculogenesis are impaired in the precocious-aging klotho mouse. *Circulation* 2004; **110**: 1148–1155.
3 Saito Y, Yamagishi T, Nakamura T *et al.* Klotho protein protects against endothelial dysfunction. *Biochem Biophys Res Commun* 1998; **248**: 324–329.
4 Arking DE, Krebsova A, Macek M *et al.* Association of human aging with a functional variant of klotho. *Proc Nat Acad Sci USA* 2002; **99**: 856–861.
5 Arking DE, Becker DM, Yanek LR *et al.* KLOTHO allele status and the risk of early-onset occult coronary artery disease. *Am J Hum Genet* 2003; **72**: 1154–1161.
6 Arking DE, Atzmon G, Arking A, Barzilai N, Dietz HC. Association between a functional variant of the KLOTHO gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity. *Circ Res* 2005; **96**: 412–418.
7 Kawano K, Ogata N, Chiano M *et al.* Klotho gene polymorphisms associated with bone density of aged postmenopausal women. *J Bone Miner Res* 2002; **17**: 1744–1751.
8 Shimokata H, Fukukawa Y, Nishita Y. Klotho gene promoter polymorphism and cognitive impairment. *Geriatr Gerontol Int* 2006; **6**: 136–141.

9 Imamura A, Okumura K, Ogawa Y *et al.* Klotho gene polymorphism may be a genetic risk factor for atherosclerotic coronary artery disease but not for vasospastic angina in Japanese. *Clin Chim Acta* 2006; **371**: 66–70.
10 Ohta J, Ishikawa K, Yang J *et al.* Klotho gene delivery suppresses oxidative stress in vivo. *Geriat Gerontol Int* 2007; **7**: 293–299.
11 Ikushima M, Rakugi H, Ishikawa K *et al.* Anti-apoptotic and anti-senescence effects of Klotho on vascular endothelial cells. *Biochem Biophys Res Commun* 2006; **339**: 827–832.
12 Maekawa Y, Ishikawa K, Yasuda O *et al.* Klotho suppresses TNF-alpha-induced expression of adhesion molecules in the endothelium and attenuates NF-kappaB activation. *Endocrine* 2009; **35**: 341–346.
13 Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999; **340**: 115–126.
14 Okuda T, Fujioka Y, Kamide K *et al.* Verification of 525 coding SNP in 179 hypertension candidate genes in the Japanese population: identification of 159 SNP in 93 genes. *J Hum Genet* 2002; **47**: 387–394.
15 Yang J, Kamide K, Kokubo Y *et al.* Genetic variations of regulator of G-protein signaling 2 in hypertensive patients and in the general population. *J Hypertens* 2005; **23**: 1497–1505.
16 Tanaka C, Kamide K, Takiuchi S *et al.* An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms. *Hypertens Res* 2003; **26**: 301–306.
17 Takiuchi S, Mannami T, Miyata T *et al.* Identification of 21 single nucleotide polymorphisms in human hepatocyte growth factor gene and association with blood pressure and carotid atherosclerosis in the Japanese population. *Atherosclerosis* 2004; **173**: 301–307.
18 Mannami T, Baba S, Ogata J. Potential of carotid enlargement as a useful indicator affected by high blood pressure in a large general population of a Japanese city: the Suita study. *Stroke* 2000; **31**: 2958–2965.
19 Armutcu F, Ataymen M, Atmaca H, Gurel A. Oxidative stress markers, C-reactive protein and heat shock protein 70 levels in subjects with metabolic syndrome. *Clin Chem Lab Med* 2008; **46**: 785–790.
20 Perticone F, Ceravolo R, Candigliota M *et al.* Obesity and body fat distribution induce endothelial dysfunction by oxidative stress: protective effect of vitamin C. *Diabetes* 2001; **50**: 159–165.
21 Silver AE, Beske SD, Christou DD *et al.* Overweight and obese humans demonstrate increased vascular endothelial NAD(P)H oxidase-p47(phox) expression and evidence of endothelial oxidative stress. *Circulation* 2007; **115**: 627–637.
22 Ceriello A, dello Russo P, Amstad P, Cerutti P. High glucose induces antioxidant enzymes in human endothelial cells in culture. Evidence linking hyperglycemia and oxidative stress. *Diabetes* 1996; **45**: 471–477.
23 Du XL, Edelstein D, Rossetti L *et al.* Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Nat Acad Sci USA* 2000; **97**: 12222–12226.
24 Kurosu H, Yamamoto M, Clark JD *et al.* Suppression of aging in mice by the hormone Klotho. *Science* 2005; **309**: 1829–1833.
25 Yamamoto M, Clark JD, Pastor JV *et al.* Regulation of oxidative stress by the anti-aging hormone klotho. *J Biol Chem* 2005; **280**: 38029–38034.
26 Nabeshima Y, Imura H. alpha-Klotho: a regulator that integrates calcium homeostasis. *Am J Nephrol* 2008; **28**: 455–464.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Identified single nucleotide polymorphisms of *klotho* by direct sequencing.
Table S2. Genotype distributions of each polymorphism in the hypertensive and general population.
Table S3. Comparison of subjects' backgrounds in the hypertensive group and the general population with hypertension.

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REVIEW

Physio-pathological effects of alcohol on the cardiovascular system: its role in hypertension and cardiovascular disease

Yuhei Kawano

Alcohol has complex effects on the cardiovascular system. The purpose of this article is to review physio-pathological effects of alcohol on cardiovascular and related systems and to describe its role in hypertension and cardiovascular disease. The relationship between alcohol and hypertension is well known, and a reduction in the alcohol intake is widely recommended in the management of hypertension. Moreover, alcohol has both pressor and depressor actions. The latter actions are clear in Oriental subjects, especially in those who show alcohol flush because of the genetic variation in aldehyde dehydrogenase activity. Repeated alcohol intake in the evening causes an elevation in daytime and a reduction in nighttime blood pressure (BP), with little change in the average 24-h BP in Japanese men. Thus, the hypertensive effect of alcohol seems to be overestimated by the measurement of casual BP during the day. Heavy alcohol intake seems to increase the risk of several cardiovascular diseases, such as hemorrhagic stroke, arrhythmia and heart failure. On the other hand, alcohol may act to prevent atherosclerosis and to decrease the risk of ischemic heart disease, mainly by increasing HDL cholesterol and inhibiting thrombus formation. A J- or U-shaped relationship has been observed between the level of alcohol intake and risk of cardiovascular mortality and total mortality. It is reasonable to reduce the alcohol intake to less than 30 ml per day for men and 15 ml per day for women in the management of hypertension. As a small amount of alcohol seems to be beneficial, abstinence from alcohol is not recommended to prevent cardiovascular disease.
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Keywords: alcohol; blood pressure; cardiovascular disease

INTRODUCTION

Alcohol has complex effects on the cardiovascular system. The relationship between alcohol and hypertension is well known, and a restriction of alcohol intake is widely recommended as a part of lifestyle modifications in the management of hypertension.[1–6] Alcohol has both pressor and depressor actions, however, and the genetic susceptibility regarding alcohol metabolism influences the cardiovascular effect of alcohol.[3,7] The effect of alcohol on blood pressure (BP) is also modified by several factors, such as the level of consumption, time period after the last drink and overall drinking behavior.

Alcohol consumption is associated with several cardiovascular diseases, such as brain hemorrhage, heart failure and arrhythmia, as well as with other disorders.[3,8–11] Heavy drinking and alcoholism not only lead to medical problems but are also serious social concerns. However, alcohol also seems to have beneficial effects, including the prevention of ischemic heart disease. It has been shown that cardiovascular and all-cause mortality is lower in light drinkers compared with nondrinkers.[3,8–12]

The purpose of this article is to review physio-pathological effects of alcohol on cardiovascular and related systems and to describe its role in hypertension and cardiovascular disease. I will outline the cardiovascular actions of alcohol, the effects of alcohol on BP and hypertension, including changes in 24-h BP, and the relationship between alcohol and cardiovascular diseases.

CARDIOVASCULAR ACTIONS OF ALCOHOL

Acute effect on BP

The effect of a single intake of alcohol on BP in normal subjects is not consistent among studies. Some studies have shown an increase in BP,[13,14] whereas it decreased[15,16] or remained unchanged[17,18] in others. In hypertensive patients, the BP also became elevated[19] or fell[20] after a single ingestion of alcohol.

In studies showing the pressor effect of alcohol, a BP increase was observed within 1 h after drinking; however, the increase was not sustained.[3] On the other hand, BP usually fell or remained unchanged after alcohol consumption in studies with prolonged observation periods. Stott *et al.*[21] reported that BP levels increased at 1 h after drinking but tended to decrease over the next 7 h in normotensive subjects.

We examined the effect of a single intake of alcohol (1 ml kg^{-1}) on BP using ambulatory BP monitoring (ABPM) in hypertensive

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Japanese men.[7] As shown in Figure 1, the BP decreased and the heart rate increased for several hours after drinking alcohol. This alcohol-induced hypotension was marked in subjects showing facial flush identified by visual inspection after drinking, and was mild in those who did not show such flush. A transient pressor response to alcohol consumption was not observed in our study, and BP values the day after drinking were comparable to those on the control day.

Minami *et al.*[22] also studied the effect of a single intake of alcohol on ambulatory BP in normotensive and hypertensive Japanese men in relation to the genotype of aldehyde dehydrogenase 2 (ALDH2). In their study, BP decreased significantly after alcohol ingestion in the inactive ALDH2 group (heterozygotes and a homozygote for ALDH2*2), whereas the reduction in BP was small but significant only for diastolic BP in the active ALDH2 group (homozygotes for ALDH2*1).

A single intake of alcohol therefore mainly acts to lower the BP, at least in Japanese men. As this depressor effect varies according to race and the presence and absence of alcohol flush, genetic variation in ALDH2 activity seems to have a major function. It has been shown that subjects with the ALDH2*2 genotype, which is common in Mongoloids but rare in Caucasians and Africans, show facial flush after drinking alcohol because of the accumulation of vasodilative acetaldehyde.[3,22–24] The mechanisms causing a transient pressor response to alcohol are not clear. An emotional change or gastric irritation rather than a direct effect of alcohol, however, may be involved in the pressor response because it takes several hours for blood alcohol to disappear after a single intake.[3,21]

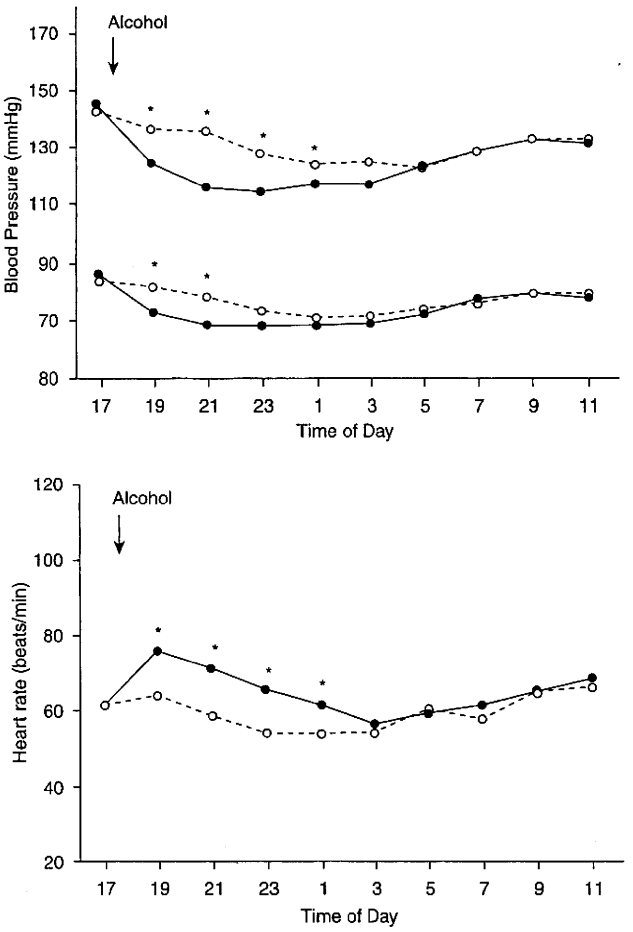

Figure 1 Ambulatory blood pressure (BP) and heart rate during the alcohol intake day (●) and the control day (○). *: $P<0.05$ between the two periods (adopted from Kawano *et al.*[7] with permission).

Vascular actions

The action of alcohol on the vasculature is variable according to its concentration and the kind of blood vessel.[25,26] High concentrations of alcohol constrict most blood vessels. This vasoconstriction depends on calcium ions and is inhibited by calcium channel blockers. Alcohol also acts to augment the vasoconstriction caused by catecholamines and vasopressin and inhibits endothelium-dependent vasodilation.[27,28] It has been suggested that endothelin and nitric oxide are involved in alcohol-induced vasoconstriction.[29] Soardo *et al.*[30] observed that alcohol increased the levels of endothelin-1, nitric oxide, plasminogen activator inhibitor-1 and oxidative stress both *in vivo* and *in vitro*. As the scavengers of oxidants prevented those changes, oxidative stress may have a role in the alcohol-induced endothelial dysfunction.[30] It was, however, reported that the flow-mediated dilation of the brachial artery and blood markers of endothelial function were similar between the usual drinking period and the alcohol restriction period in healthy men.[31]

On the other hand, low concentrations of alcohol usually dilate blood vessels.[25,26] This effect also seems to be mediated by calcium ions and endothelium-derived nitric oxide. It has been shown that low doses of alcohol increase the release of nitric oxide and augment endothelium-dependent vasodilation.[32] Criscione *et al.*[27] reported that ethanol inhibits norepinephrine-induced vasoconstriction in the rat mesenteric artery. They also observed that norepinephrine-induced vasoconstriction is enhanced after the withdrawal of alcohol. These results seem to be consistent with the time-dependent BP changes after alcohol consumption in humans.

Acetaldehyde, a metabolite of alcohol, acts as a vasodilator.[17] Subjects with low-active aldehyde dehydrogenase (ALDH2*2) show facial flush after alcohol ingestion because of the accumulation of acetaldehyde in the blood. Such subjects, especially those homozygous for the ALDH2*2 genotype, show marked tachycardia and hypotension after alcohol consumption.[3,23]

In our study, the alcohol-induced BP reduction in hypertensive patients was due to a decrease in peripheral vascular resistance (Table 1).[7] We also observed that the intracellular sodium concentration in red blood cells decreases after alcohol ingestion.[33] This change may also act to dilate blood vessels through a decrease in the intracellular calcium concentration.

Table 1 Hemodynamic variables after alcohol intake in hypertensive patients (adopted from Kawano *et al.* with permission)

	Control day		*Alcohol intake day*	
Variables	*5 PM*	*7 PM*	*5 PM*	*7 PM*
MBP (mm Hg)	109±4	100±4	106±5	89±3*†
HR (b.p.m.)	54±2	56±2	52±3	68±5*†
CI ($l\,min^{-1}\,m^{-1}$)	2.6±0.2	2.9±0.3	2.6±0.2	3.3±0.3*†
PVR ($dyn\,s^{-1}\,cm^{-5}$)	2061±208	1791±225	2096±268	1305±114*†
LVFS (%)	34±2	37±2	35±1	43±2*†
LVESWS ($10^3\,dyn\,cm^{-2}$)	67±10	56±7	62±5	37±5*†

Abbreviations: b.p.m., beats per minute; CI, cardiac index; HR, heart rate; LVESWS, left ventricular end-systolic wall stress; LVFS, left ventricular fractional shortening; MBP, mean blood pressure; PVR, peripheral vascular resistance.
*$P<0.05$ *vs.* the control day, †$P<0.05$ *vs.* 5 PM on the alcohol intake day.

Taken together, alcohol has both constrictive and dilative actions on blood vessels, and these effects may be dependent on race, the dose and timing of alcohol consumption.

Cardiac actions

The effects of alcohol on the heart are also complex.[3,8,34] It has been shown that alcohol directly inhibits the contractility of cardiac muscle in a dose-dependent manner.[18,26] This negative inotropic action is apparent in the isolated heart or after blocking of the autonomic nervous system.[18]

Cardiac function, however, does not often change or even increase after the administration of alcohol in normal humans and animals. Kupari *et al.*[23] reported that both the heart rate and cardiac output increased whereas systemic vascular resistance decreased after alcohol ingestion in healthy volunteers.[15] They also observed that those changes were small in subjects who did not show facial flush, but were marked in subjects who showed flush after drinking. In our study, the heart rate and cardiac output also increased significantly after alcohol ingestion in hypertensive patients[7] (Table 1). The activation of the sympathetic nervous system seems to mask the direct inhibitory action of alcohol on the heart. Indeed, we have observed that the alcohol-induced increases in heart rate and cardiac output are attenuated after the administration of a beta blocker.[35]

The adverse influence of alcohol on the heart is clear after the consumption of large amounts for many years. It has been shown that the total alcohol intake is positively related to the left ventricular mass and negatively related to the left ventricular ejection fraction.[36] Structural changes in cardiac muscle were also observed in heavy drinkers [37] as well as in ethanol-fed rats.[38] These changes may be involved in alcohol-induced cardiomyopathy, heart failure and arrhythmia.

Alcohol withdrawal syndrome

Chronic heavy drinkers, such as alcoholic patients, show alcohol withdrawal syndrome, which is characterized by psycho-neurological symptoms and signs after the sudden cessation of alcohol consumption. This syndrome includes elevation of the BP and heart rate because of activation of the sympathetic nervous system.[26] The pressor response during alcohol withdrawal reaches a peak the day after cessation.[39] In this case, the BP decreased to a lower level compared with baseline within several days after alcohol withdrawal in heavy drinkers.[40] As habitual drinkers experience a mild degree of repeated alcohol withdrawal in daily life, it is possible that this withdrawal phenomenon contributes to alcohol-related hypertension.

NEUROHORMONAL ACTIONS OF ALCOHOL

Actions on the autonomic nervous system

It has been shown that alcohol activates the sympathetic nervous system.[3,26] Van de Borne *et al.*[41] observed an increase in muscle sympathetic nerve activity after a single intake of alcohol in normal men. In their study, BP did not change, although the heart rate increased significantly. In our study, plasma catecholamines increased but BP decreased after alcohol ingestion in hypertensive patients, and the increase in plasma catecholamines was more pronounced in subjects with a large BP reduction.[7,42] These results suggest that the activation of the sympathetic nervous system occurs in response to BP change and acts to compensate for any further BP reduction.

Experimental studies have shown that alcohol suppresses the baroreceptor reflex.[43] Narkiewicz *et al.*[44] reported that alcohol enhances the hypotension induced by lower body negative pressure. The combination of impairment of the baroreceptor reflex and systemic vasodilation acts to potentiate orthostatic hypotension and may induce syncope after drinking in susceptible subjects.

Actions on the endocrine system

It is known that alcohol increases plasma renin activity.[7,26,42] As the increase in renin activity was suppressed by pretreatment with propranolol in our study,[35] it seems to be mediated by the sympathetic nervous system. Alcohol also stimulated the release of adrenocorticotropic hormone, and increases in plasma cortisol and aldosterone were observed after drinking.[26] It has been reported that dexamethasone inhibited the BP elevation and sympathetic activation after alcohol ingestion.[45] We and others, however, have failed to observe significant changes in adrenocorticotropic hormone, cortisol or aldosterone.[21,42]

Alcohol suppresses the release of vasopressin; however, this change does not seem to mediate the acute depressor effect of alcohol.[21,26,42] Several depressor hormones and substances, such as atrial natriuretic peptide, prostaglandin E_2, beta endorphin and cyclic GMP, did not change after alcohol ingestion.[42] Although data relating alcohol intake to plasma atrial natriuretic peptide have been inconsistent, Djousse *et al.*[46] observed a positive relationship after adjusting for several confounding factors.

The level of plasma insulin increases after alcohol intake; however, the change is less than that induced by an isocaloric control drink.[42] It has been shown that a light-to-moderate intake of alcohol enhances insulin sensitivity[47] and reduces the risk of type 2 diabetes mellitus.[48] Alcohol therefore seems to have a beneficial effect on insulin and glucose metabolism.

Taken together, alcohol causes various changes in the autonomic nervous system and endocrine system, but these changes do not seem to have a major role in the pressor or depressor effect of alcohol except in the case of alcohol withdrawal syndrome.

ACTIONS OF ALCOHOL ON WATER AND ELECTROLYTE METABOLISM

Alcohol also exhibits actions on water and electrolyte metabolism. Urinary excretion increases after alcohol ingestion.[49,50] The increase in urine volume seems to be caused by fluid intake and the suppression of vasopressin.[21,27]

We have studied the effect of repeated alcohol intake for 7 days on the urine volume and sodium excretion in hypertensive patients.[51] Urine volume increased on days 3–5 but not on day 1. Urinary sodium excretion decreased in the early phase but increased in the late phase. The average BP also decreased in the early phase and then returned toward the baseline levels. The initial BP reduction may mask the alcohol-induced diuresis and causes sodium retention, which may be involved in subsequent BP elevation.

It has been shown that urinary potassium excretion decreases after alcohol ingestion.[48,49] In our study, the serum potassium level decreased after a single intake of alcohol.[7,35] This change in serum potassium seems to be mediated by the sympathetic nervous system, as propranolol attenuated alcohol-induced hypokalemia.[35] Conversely, alcohol increases the urinary excretion of magnesium and calcium.[49,50,52] It is possible that magnesium and calcium are depleted in habitual drinkers, and the alcohol-induced changes in these minerals may contribute to BP elevation and arrhythmia.[3,53]

ALCOHOL AND HYPERTENSION

Epidemiological studies

Numerous epidemiological observational studies have examined the relationship between alcohol consumption and BP or hypertension. Almost all of them have shown that habitual drinkers have a higher BP

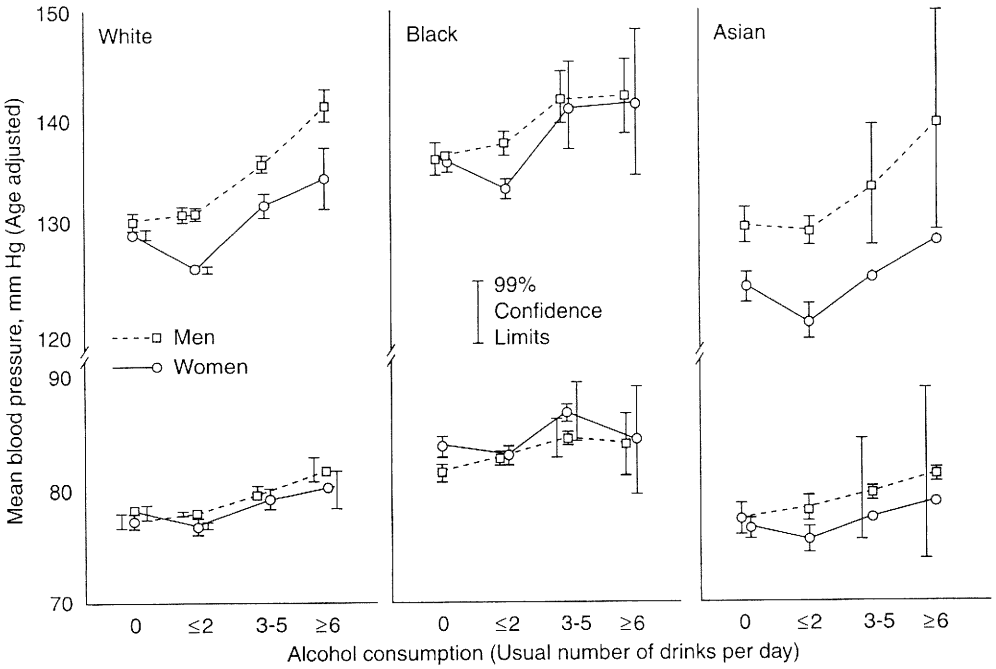

Figure 2 Mean systolic and diastolic BPs for White, Black or Asian men and women for known drinking habits (adopted from Klatsky *et al.*[54] with permission).

and higher prevalence of hypertension than nondrinkers.[1–3,54–58] These associations have been observed regardless of race, gender, age and the type of alcohol (Figure 2). Although some studies suggest the presence of a threshold regarding the pressor effect of alcohol,[59,60] the relationship between the level of alcohol consumption and BP is usually linear. In cross-sectional studies, the systolic BP increased by 3–4 mm Hg and diastolic BP increased by 1–2 mm Hg per three drinks per day (one drink contained 10–15 ml, or 8–12 g of alcohol).[1] Intake of 10 ml per day of alcohol therefore seems to elevate the systolic BP by about 1 mm Hg in humans. It has been estimated that about 10% of hypertension in the general population can be attributed to alcohol.[1–3]

The relationship between alcohol and BP seems to be independent of confounding variables. Increases in the body weight and abdominal fat associated with alcohol consumption, however, may have a role in alcohol-related hypertension. Suter *et al.*[61] observed that both the BP and waist/hip ratio increased with the level of alcohol intake, and there was a positive association between changes in body weight and alcohol consumption.

The hypertensive effect of alcohol has also been shown in longitudinal studies.[62–64] Tsuruta *et al.*[62] reported that the probability of the development of hypertension in heavy drinkers (alcohol consumption ≧46 g per day) was about twice that of the rest of the population after a 12-year follow-up among normotensive men. Fuchs *et al.*[63] showed that the consumption of alcohol at ≧30 g per day was an independent risk factor among participants in the Atherosclerosis Risk in Communities (ARIC) study. Nakanishi *et al.*[64] also observed that the risk for hypertension increased in a dose-dependent manner with increases in alcohol intake among Japanese men in a longitudinal study.

Although epidemiological studies have clearly shown the hypertensive effect of alcohol, most studies did not consider the time-related effect of alcohol on BP. This fact may be important because BP measurement has been carried out during the daytime, whereas alcohol is usually consumed at night. Moreira *et al.*[65] reported that the BP in habitual drinkers was higher at 13–24 h after the last drink compared with that within 3 h or at more than 24 h. Kawabe *et al.*[66] observed that the evening home BP was lower but the morning home BP was higher on drinking compared with nondrinking days in Japanese volunteers. These findings suggest that the BP in habitual drinkers is overestimated by casual BP measurement taken during the day.

Clinical studies

Clinical intervention studies have also revealed an increase in BP with alcohol consumption and a BP decrease with alcohol restriction. Howes and Reid,[67] observed a BP increase after repeated alcohol consumption for 7 days in normotensive subjects. Potter and Beevers,[68] reported a BP increase after alcohol intake for 4 days in hypertensive patients. Using a crossover design, Puddey *et al.*[69,70] compared BP values during a 6-week period of unrestricted alcohol consumption and that of alcohol restriction in normotensive and hypertensive subjects. The average level of alcohol consumption was 50 ml per day during the unrestricted period and 10 ml per day during the restricted period, and the BP was 3/2 mm Hg higher in the former than in the latter period in normotensive subjects.[69] In hypertensive subjects, the BP was 5/3 mm Hg higher in the unrestricted than during the restricted period.[70] Ueshima *et al.*[71] also examined the effect of a 2-week period of alcohol intake and restriction using a crossover method in Japanese hypertensive patients and observed a similar BP elevation with alcohol consumption.

According to a meta-analysis of 15 randomized controlled trials, BP significantly decreased with alcohol restriction[72] (Figure 3). The mean BP reduction was 3.3/2.0 mm Hg, and there was a dose–response relationship between alcohol reduction and decrease in BP. The effects of intervention were enhanced in studies with a higher baseline BP. The results of this meta-analysis support the importance of alcohol reduction in the management of hypertension among heavy drinkers.

Most clinical studies have not considered the timing of alcohol intake and BP measurement. We studied the effect of repeated episodes of alcohol consumption on BP with ABPM under standar-

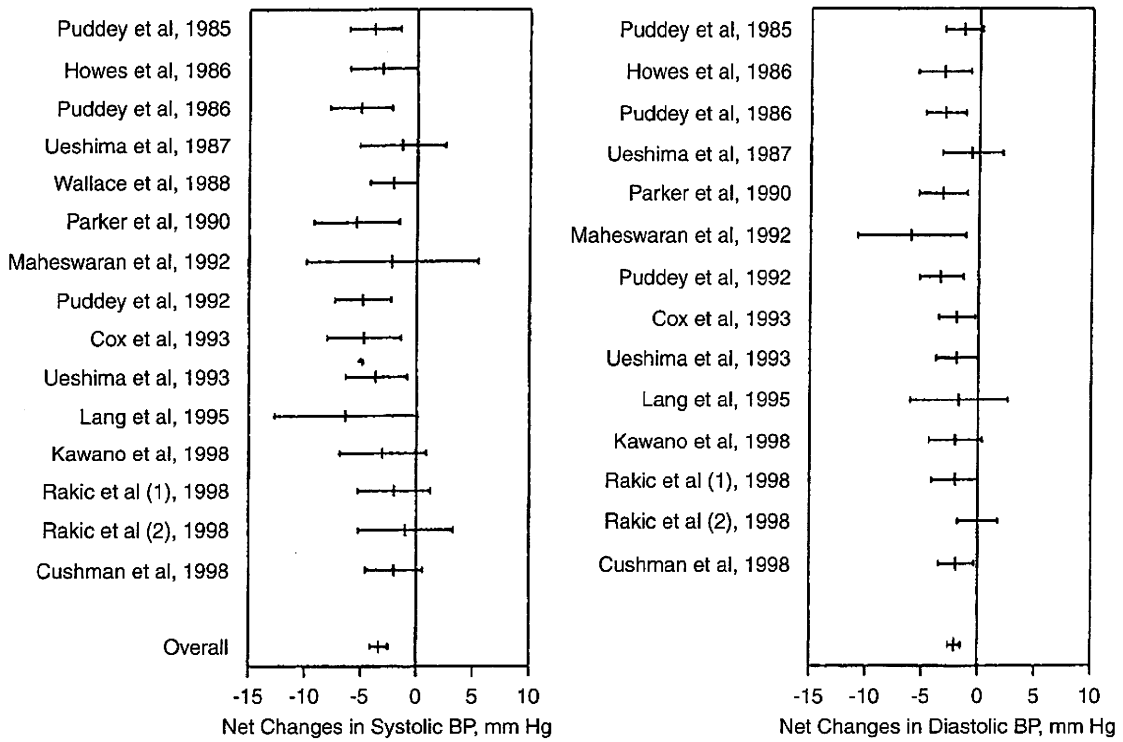

Figure 3 Average net changes in systolic and diastolic BPs and corresponding 95% CIs related to alcohol reduction intervention in 15 randomized controlled trials (adopted from Xin *et al.*[72] with permission).

dized conditions in Japanese men with hypertension.[73] After several days of the control period, the subjects consumed 1 ml kg^{-1} of alcohol with an evening meal for 7 days. Evening BP values decreased for several hours after alcohol consumption on both days 1 and 7, whereas morning BP was unchanged on day 1 but increased on day 7. The average 24-h BP was lower on day 1 and was the same on day 7 compared with the control period. A short-term repeated intake of alcohol therefore causes biphasic changes in BP without altering the average 24-h BP, at least in Japanese men.

We also examined the effect of a 4-week period of unrestricted alcohol consumption and that of alcohol restriction on the 24-h BP in hypertensive patients in a randomized crossover study.[74] The average level of daily alcohol intake was 66 ml in the unrestricted period and 11 ml in the restricted period. The daytime BP was 3/2 mm Hg higher in the unrestricted period than in the restricted period, but the nighttime BP was 4/2 mm Hg lower in the former (Figure 4). The average 24-h BP was comparable between the two periods. These effects of alcohol resulted in changes in the dipping pattern of the 24-h BP. Half of those who did not show a dip in BP during the restricted period changed and showed a dip in the unrestricted period, and half of those that showed a dip showed an extreme dip during this period.

On the other hand, Minami *et al.*[75] observed reductions in the daytime (−3.4 mm Hg) and 24-h (−3.2 mm Hg) systolic BPs after 3 weeks of alcohol restriction in Japanese men. In their study, daytime, nighttime and 24-h diastolic BPs did not change with alcohol reduction (−1.1, +2.1 and −0.3 mm Hg, respectively).

The effect of alcohol on the 24-h BP may differ between Orientals and Caucasians. Howes *et al.*[76] observed that short-term alcohol intake increased BP variability without changing the average BP in Australian subjects. However, Rakic *et al.*[77] showed that the average 24-h BP increased significantly after 4 weeks of alcohol consumption in Australian men. In their study, the average 24-h systolic BP was 2–3 mm Hg higher and the nighttime BP was not lower in the unrestricted compared with the restricted period. It was also reported that the average 24-h BP decreased after abstinence in alcoholic patients.[78]

In a systematic review, McFadden *et al.*[79] analyzed clinical trials that examined the BP after a period of sustained alcohol intake. In this review, the pressor effect of alcohol was evident in non-ABPM studies, but not in ABPM studies. An early effect of reducing the BP and a later effect of raising the BP led to smaller differences in the net effect of alcohol on BP values in ABPM studies.

We also studied changes in morning and evening home BP measurements during each of the 4 weeks of unrestricted consumption and restriction in hypertensive patients.[80] In this study, the morning BP increased by 4.4 mm Hg but late evening BP decreased by 7.4 mm Hg at the end of the unrestricted alcohol intake period. The pressor effect was significant from week 2, whereas the depressor effect was evident from day 1. These results indicate that the status of alcohol intake influences the morning–evening BP difference, and that slow pressor mechanism(s) are involved in alcohol-induced BP elevation.

It is therefore clear that alcohol consumption contributes to hypertension, and alcohol restriction decreases the daytime BP. It should be noted, however, that the effects of alcohol on BP vary according to the level and duration of consumption and the time from the last drink. Alcohol seems to exert a marked influence on circadian BP variation, whereas its influence on the average 24-h BP seems to be small. The mechanisms of the pressor effects of alcohol have not been fully clarified; however, changes in vascular reactivity and sympathetic nerve activity related to intermittent alcohol withdrawal seem to be more important than the direct actions of alcohol. Deficiencies in

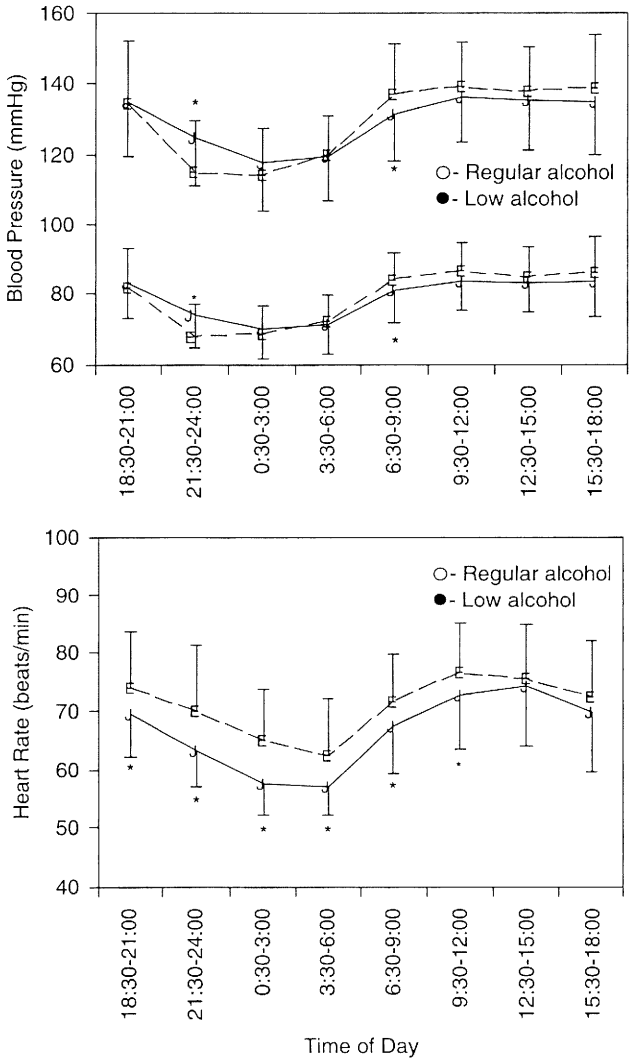

Figure 4 Profile of 24-h BP and heart rate at the end of the regular-alcohol period and the low-alcohol period in hypertensive patients. $^*P<0.05$ between the two periods (adopted from Kawano *et al.*[74] with permission).

magnesium and calcium may contribute to BP elevation after chronic alcohol consumption. Increases in the caloric intake through consuming alcoholic beverages and elevated salt intake associated with drinking may also be involved in alcohol-related hypertension.

Experimental studies

Many experimental studies have examined the effect of chronic administration of alcohol on BP; however, the results have been inconsistent.

Strickland and Wooles,[81] reported an elevation of BP after ethanol administration (5–20% in drinking water) for 4 weeks in rats. In their study, the plasma level of norepinephrine was decreased in ethanol-fed animals. Vasdev *et al.*[82] observed BP elevation after 1 week during the administration of ethanol (5–10%) to Wister Kyoto rats. They noted increases in the concentration of platelet intracellular calcium ions and the uptake of calcium in the aorta. Hsieh *et al.*[53] identified increases in BP and intracellular calcium ions and a decrease in intracellular magnesium ions after 4 weeks of ethanol administration (15%) to rats. They suggested a role of magnesium deficiency in ethanol-induced hypertension as the BP elevation was attenuated by magnesium supplementation. Puddey *et al.*[83] reported a BP elevation of ~10 mm Hg and decreases in the level of phospholipids and the ratio of unsaturated/saturated fatty acids in the aorta and kidney after the chronic administration of alcohol to rats. Harada *et al.*[84] also observed increases in the BP and platelet-free calcium concentration with ethanol consumption (15%) in Wister Kyoto rats.

In some studies, the BP did not change after the chronic administration of alcohol to animals. Abdel-Rahman[85] reported that the BP increase was not different between ethanol-fed (5–20% in drinking water) spontaneously hypertensive (SH) rats and control SH rats during a 13-week observation period. The depressor effect of clonidine, however, was reduced in the ethanol-fed SH rats, suggesting a change in the neural regulation of BP.

Several studies have shown that chronic alcohol intake decreases BP in animals. Howe *et al.*[86] reported that BP values in alcohol-fed (5–20% in drinking water) Wister Kyoto, SH and stroke-prone SH rats were lower than those of respective control rats during a 6-month observation period. Hatton *et al.*[87] observed a BP decrease during chronic ethanol administration (36% in a liquid diet) for 18 weeks in Wistar rats. The vasoconstrictor response of resistant arteries to norepinephrine was enhanced and the vasodilator response to alcohol was attenuated in their study. Beilin *et al.*[88] reported a decrease in the resting BP of Wister Kyoto and SH rats after 12 weeks of ethanol administration (20% in drinking water), although cardiovascular reactivity to noise-related stress was augmented in the ethanol-fed SH rats. El-Mas and Abdel-Rahman[89] also observed a lower BP in freely moving, ethanol-fed (5% in a liquid diet) rats compared with control rats based on telemonitoring of the BP.

The reasons for the inconsistent results in experimental studies are not clear, but cannot be explained by differences in daily doses of alcohol administration. The periods of alcohol administration, however, are generally longer in studies showing BP reduction than those showing BP elevation. The timing of BP measurement may be important, such as in clinical studies. Crandall *et al.*[90] administered 30% alcohol twice daily (7–8 g kg^{-1}) for 10 weeks to rats and examined the levels of BP and blood alcohol. In their study, BP was normal at the time of the peak blood alcohol level but was elevated at 24 h after alcohol consumption, when alcohol was not detected in the plasma. Their results suggest that alcohol-induced hypertension is not because of its direct action but to alcohol withdrawal.

The harmful effects of large doses of alcohol, such as cardiac dysfunction, have been shown in experimental studies.[91] Schlicht *et al.*[92] however, reported that the lifespan of SH rats was prolonged by the chronic administration of ethanol. These observations are important, as both the adverse effects of a large amount of alcohol and the beneficial effects of a moderate amount on cardiovascular disease and total mortality have been shown in large-scale epidemiological studies.[8–12]

Interaction with antihypertensive drugs

Alcohol interacts with several antihypertensive agents. Experimental studies have shown that alcohol attenuates the effect of centrally acting antihypertensive drugs such as clonidine.[86] Heavy drinking is recognized as one of the factors responsible for resistant hypertension. The interaction between alcohol and antihypertensive drugs and the hypertensive effect of alcohol may have a role in alcohol-related resistant hypertension. In addition, heavy drinkers often show poor adherence to both pharmacological treatment and lifestyle modifications. Habitual drinkers taking antihypertensive drugs are also prone to morning hypertension.[93]

Table 2 Moderation of alcohol consumption recommended by hypertension treatment guidelines

JNC-7 (2003)	*ESH-ESC 2007*	*JSH 2009*
Men: ≦2 drinks per day (≦30 ml per day)	Men: ≦20–30 g per day	Men: ≦20–30 ml per day
Women, light weight person: ≦1 drink per day (≦15 ml per day)	Women: ≦10–20 g per day	Women: ≦10–20 ml per day

Expressed as amount of ethanol. ESH-ESC 2007, European Society of Hypertension–European Society of Cardiology guidelines;[5] JNC-7, Joint National Committee 7th report (Chobanian *et al.*[4]); JSH 2009, Japanese Society of Hypertension guidelines (Ogihara *et al.*[6]).

The combination of alcohol and antihypertensive drugs may also lead to a marked BP reduction. This phenomenon has been known; however, few clinical studies have addressed this interaction. It is possible that sympatholytic drugs augment the depressor effect of alcohol, as alcohol-induced hypotension is associated with the reflex activation of the sympathetic nervous system.[7] In our studies, alcohol and a beta blocker, propranolol, additively lowered the nighttime BP,[35] whereas alcohol and an alpha blocker, prazosin, synergistically acted to lower the BP in hypertensive patients.[94] It has also been reported that alcohol enhances the depressor effect of the calcium antagonist felodipine.[95] Changes in the type and timing of antihypertensive medication along with the moderation of alcohol consumption should be considered to treat hypertensive patients with a drinking habit.

Hypertension treatment guidelines

As an excess consumption of alcohol is a risk factor for hypertension, all hypertension treatment guidelines recommend the moderation of alcohol intake as a part of lifestyle modifications for the management of hypertension (Table 2). The 7th report of the Joint National Committee in the United States (JNC-7) recommends the limitation of daily alcohol consumption to no more than two drinks (30 ml) for most men and to no more than one drink (15 ml) for women and light-weight men.[4] According to the European guidelines (European Society of Hypertension–European Society of Cardiology guidelines, ESH–ESC 2007), the upper limit is 20–30 g per day for men and 10–20 g per day for women. The Japanese guidelines provide similar recommendations (20–30 ml per day for men and 10–20 ml per day for women). Of note, 600 ml of beer or 250 ml of wine contains about 30 ml of ethanol.

These recommendations put forward by the guidelines are appropriate because small doses of alcohol exert little adverse effects on BP and the cardiovascular system. There are, however, some concerns regarding the efficacy of alcohol restriction on BP because the effect of alcohol on average 24-h BP levels seems to be very small. In our studies, salt restriction and weight reduction substantially decreased the BP for 24 h, but the effect of alcohol restriction on average 24-h BP was not significant.[96–98] As light drinking has beneficial effects on the cardiovascular system, as described later, abstinence from alcohol should not be imposed on hypertensive individuals except for patients with special conditions.

ALCOHOL AND CARDIOVASCULAR DISEASE

Cardiac disease

Heart failure. A heavy alcohol intake is associated with cardiac hypertrophy and the risk of cardiomyopathy and heart failure.[8,36,37] It has been shown that the total consumption of alcohol is positively related to a left ventricular mass and is negatively associated with the ejection fraction in asymptomatic alcoholic subjects.[37] Recent epidemiological studies, however, have shown that moderate alcohol consumption is associated with a lower risk of heart failure.[99,100] Klatsky *et al.*[101] reported that heavy drinkers had an increased risk of heart failure because of noncoronary artery disease, whereas alcohol drinking was inversely related to the risk of heart failure because of coronary artery disease. Heavy drinking therefore seems to increase the risk of heart failure but light-to-moderate drinking may decrease the risk, probably because of its favorable association with coronary artery disease.

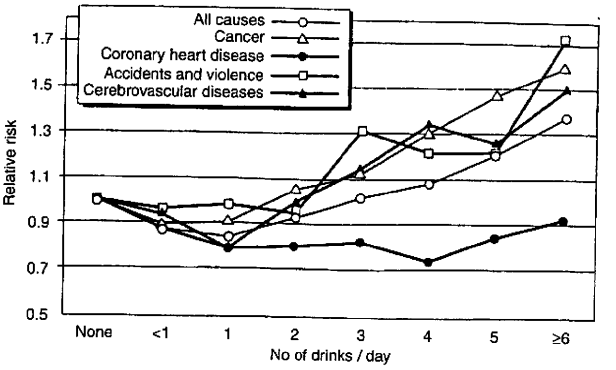

Figure 5 Alcohol consumption and relative risk of death over 12 years in American Cancer Society prospective study of 276 802 men aged 40–59 (adopted from Boffetta *et al.*[105] with permission).

Arrhythmia. Alcohol intake is associated with the risk of tachyarrhythmia, such as ventricular and supraventricular premature contractions and atrial fibrillation.[8,102–104] In the Danish Diet, Cancer and Health study, moderate-to-heavy consumption of alcohol was associated with an increased risk of atrial fibrillation.[103,104] Such alcohol-induced arrhythmia often occurs after binge drinking. Activation of the sympathetic nervous system and a decrease in the serum potassium level after drinking may trigger this arrhythmia.[3] Cardiac functional and structural changes because of chronic alcohol consumption also seem to have a role in arrhythmia.[37]

Coronary heart disease. Alcohol seems to have a beneficial effect on coronary heart disease.[8,9] It has been shown that the risk of myocardial infarction is 20–50% lower in habitual compared with nondrinkers.[9,105–108] This risk reduction is dose-dependent up to the level of moderate drinking, but further risk reduction has not been observed in heavy drinkers (Figure 5). A U-shaped relationship has been observed between the level of alcohol consumption and degree of coronary calcification in a general population.[109] In some studies, such as the Japan Collaborative Cohort Study,[110] the beneficial effect of alcohol on coronary heart disease was modest and not significant (Table 3).

The mechanisms behind the inverse association of alcohol with coronary heart disease have not been fully clarified. The alcohol-induced increase in HDL cholesterol, however, seems to be the most important mechanism.[107,111] The inhibitory effect of alcohol on blood coagulation also contributes to the lower risk of myocardial infarction.[107] In addition, it has been shown that moderate alcohol consumption is associated with a lower plasma level of C-reactive protein, suggesting an anti-inflammatory action of alcohol.[112] The weak effect of alcohol on the average 24-h BP may also have a role as a high BP is a strong risk factor for coronary heart disease.[74,79]

Red wine contains polyphenols that act to prevent atherosclerosis because of their antioxidant effect. Several studies have shown that

Table 3 Mortality from stroke, coronary heart disease and total cardiovascular disease by alcohol consumption category in men in the Japan Collaborative Cohort Study (adopted from Ikehara *et al.*[110] with modification)

			Ethanol intake, g per day			
	Nondrinkers	*Ex-drinkers*	*0.1–22.9*	*23.0–45.9*	*46.0–68.9*	*≧69.0*
Person–years	96423	25919	78478	101256	90000	41588
Total stroke	200	126	114	168	173	83
Age-adjusted HR	1.00	1.93[a]	0.91	0.98	1.46[a]	1.89[a]
Multivariable HR	1.00	1.90[a]	0.95	0.96	1.39[a]	1.71[a]
Hemorrhagic stroke	55	31	41	52	60	37
Age-adjusted HR	1.00	1.80[a]	1.09	1.02	1.51[a]	2.30[a]
Multivariable HR	1.00	1.79[a]	1.16	1.02	1.47	2.16[a]
Ischemic stroke	126	88	60	101	95	37
Age-adjusted HR	1.00	2.12[a]	0.80	0.99	1.44[a]	1.60[a]
Multivariable HR	1.00	2.11[a]	0.81	0.94	1.34[a]	1.39
Coronary heart disease	116	56	71	90	65	33
Age-adjusted HR	1.00	1.50[a]	0.94	88	0.87	1.16
Multivariable HR	1.00	1.35	0.96	0.82	0.76	0.95
Total cardiovascular disease	487	282	269	379	342	162
Age-adjusted HR	1.00	1.77[a]	0.88	0.90	1.16[a]	1.47[a]
Multivariable HR	1.00	1.66[a]	0.90	0.87	1.07	1.28[a]

Abbreviation: HR, hazard ratio.
[a]Significant *vs.* nondrinkers.

people who mainly drink red wine have a lower risk of cardiovascular disease than those who drink other kinds of alcoholic beverage.[113,114] It is suggested, however, that the low incidence of myocardial infarction in habitual drinkers is largely attributed to the effect of alcohol itself.[115]

Cerebrovascular disease

The relationship between alcohol consumption and total cerebrovascular disease is generally J-shaped, although it differs according to subtypes of stroke [105,116] (Figure 5). It is clear that alcohol is a risk factor for hemorrhagic stroke. A positive linear relationship has been observed between the level of alcohol consumption and risk of brain or subarachnoid hemorrhage.[8,110,116,117] Actions on the BP and blood coagulation system seem to be underlying mechanisms for this adverse influence of alcohol.

On the other hand, the relationship between alcohol consumption and the risk of ischemic stroke has been found to be J- or U-shaped.[110,116–118] The low risk in light drinkers seems to be due to the lower degree of atherosclerosis and the inhibition of blood coagulation, as in the case of ischemic heart disease. The increased risk in heavy drinkers is probably related to increases in the level and variability of the BP, hemoconcentration because of dehydration and thromboembolism associated with alcohol-induced atrial fibrillation. Regarding alcoholic beverage types, wine drinkers seem to have a lower risk of ischemic stroke.[119,120]

The favorable association of light-to-moderate drinking with the risk of ischemic stroke seems to be more apparent in Caucasians than in Japanese, although the results of epidemiological studies have been inconsistent in both populations. The racial differences may be related to variation in the frequencies of stroke subtypes. Atherothrombotic brain infarction is common in Caucasians, whereas lacunar stroke is more common in Japanese.

Several studies have examined the relationship between alcohol intake and subclinical findings on magnetic resonance imaging of the brain in general populations. In the Cardiovascular Study, a U-shaped relationship was observed between alcohol consumption and white matter abnormalities. Moreover, moderate drinking was also associated with a lower risk of lacunar infarction compared with abstainers.[121] Such risk reduction with moderate drinking, however, was not observed in the ARIC study, and an increased level of alcohol intake was associated with brain atrophy.[122]

Peripheral arterial disease and atherosclerosis

As light-to-moderate consumption of alcohol seems to act to suppress the progression of atherosclerosis, it may also have a favorable influence on peripheral arterial disease. The Edinburgh Artery Study supported the protective effect of alcohol, as there was a positive association between the level of alcohol intake and the ankle brachial index.[123] In the Physicians' Health Study, habitual drinkers showed a 26% lower incidence of peripheral arterial disease compared with nondrinkers after adjustment for confounding factors.[124] Similar results were also shown in the Framingham Heart Study and the ARIC study.[125,126]

Regarding the association of alcohol and carotid atherosclerosis, an inverse relationship was noted in the Lausanne Stroke Registry.[127] On the other hand, there was no significant association between alcohol intake and the carotid artery thickness in the ARIC study.[128] A U- or J-shaped relationship was observed between the level of alcohol intake and severity of carotid atherosclerosis in the Bruneck Study and the Study of Health in Pomerania.[129,130] Although the results of epidemiological studies have been inconsistent, light-to-moderate consumption seems to inhibit the development of carotid atherosclerosis.

Several studies examined the relationship between alcohol consumption and arterial stiffness by measuring the pulse wave velocity.

Sierksma *et al.*[131] identified a U-shaped relationship between alcohol consumption and the aortic pulse wave velocity. van den Elzen *et al.*[132] observed an inverse relationship between the alcohol intake and pulse wave velocity in young men and women. On the other hand, Kurihara *et al.*[133] reported that the brachial–ankle pulse wave velocity was elevated in heavy drinkers. These studies also support the favorable effect of moderate and the harmful effect of heavy drinking on large arteries.

Cardiovascular mortality and total mortality

As described earlier, alcohol seems to exert both beneficial and adverse effects on cardiovascular diseases. The relationship between alcohol consumption and total cardiovascular mortality has been shown to be J-, U- or L-shaped. A J-shaped relationship was observed in the Japan Collaborative Cohort Study; however, the beneficial effect of light-to-moderate drinking was not significant.[110] On the other hand, in very large longitudinal studies conducted by the American Cancer Society, a U-shaped relationship was observed in the original study,[105] and the relationship was L-shape (nondrinkers showed the highest cardiovascular mortality) in Study II.[10] In a meta-analysis conducted by Di Castelnuovo *et al.*,[114] a light-to-moderate consumption of wine or beer was associated with lower cardiovascular risk. A drinking habit, particularly wine consumption, has been shown as a part of a lifestyle associated with low cardiovascular risk.[134,135] It has also been suggested that the risk reduction associated with alcohol consumption is low in individuals without cardiovascular risk factors but is high in those with a marked cardiovascular risk. Taken together, light-to-moderate alcohol consumption seems to decrease cardiovascular mortality, whereas heavy drinking may result in poor cardiovascular outcomes compared with abstainers.

Alcohol is also related to several cancers, liver disease, psychiatric and neurological disorders and injury, and it seems to influence total mortality. A J- or U-shaped relationship has been observed between the level of alcohol intake and total mortality.[10,11,105,136,137] It has been suggested that all-cause mortality is the lowest among subjects who consume about one drink per day. In the American Cancer Society Prospective Study II, total mortality was lower in drinkers than in nondrinkers.[10] It has also been shown that wine drinkers have a lower mortality rate than drinkers who avoid wine.[136] In a meta-analysis of 34 studies including more than one million individuals, a J-shaped relationship was found between alcohol consumption and total mortality.[11] In this analysis, low levels of alcohol intake (one to two drinks per day for women and two to four drinks per day for men) were inversely associated with total mortality, although higher levels of alcohol increased mortality. Those findings suggest that a light-to-moderate intake of alcohol decreases but heavy consumption increases total mortality compared to nondrinking.

CONCLUSIONS

Alcohol has complex effects on the cardiovascular system. It is clear that alcohol consumption is related to hypertension, and therefore the restriction of alcohol intake is recommended in the management of hypertension. Alcohol and its metabolites, however, also exhibit a vasodilatory action, and the BP usually decreased after alcohol ingestion, especially in Orientals who show alcohol flush. Mechanisms for the pressor action of alcohol have not been completely clarified; however, an increase in the vascular sensitivity, activation of the sympathetic nervous system and depletion of magnesium and calcium may be involved. The depressor action of alcohol is due to a decrease in systemic vascular resistance that may be related to the attenuation of vascular sensitivity and production of nitric oxide. The pressor effect of alcohol consumed in the evening is apparent during the day, but its effect on average 24-h BP seems to be very small. It should be mentioned that casual BP measurement may lead to overestimating the hypertensive effect of alcohol.

Alcohol seems to exert both harmful and beneficial effects on cardiovascular disease. An excessive intake of alcohol is associated with increased risks of heart failure, arrhythmia and hemorrhagic stroke and causes an increase in total mortality. Light-to-moderate drinkers, however, show lower rates of atherosclerosis and lower risks of coronary heart disease, heart failure, ischemic stroke, peripheral artery disease and cardiovascular and total mortality compared with nondrinkers. As the aim of the management of hypertension is the prevention of cardiovascular disease and premature death, moderation of alcohol intake is to be recommended to hypertensive patients, but abstinence from alcohol should not be insisted on unless there are specific indications for it.

1 McMahon S. Alcohol consumption and hypertension. *Hypertension* 1987; **9**: 111–121.
2 Beilin LJ, Puddey IB. Alcohol and hypertension: an update. *Hypertension* 2006; **47**: 1035–1038.
3 Kawano Y. Alcohol and hypertension: state of the art. *Clin Hypertens* 2000; **6**: 14–28.
4 Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL, Jones DW, Materson BJ, Oparil S, Wright Jr JT, Roccela ET. the National High Blood Pressure Education Program Coordinating Committee: the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003; **42**: 1206–1252.
5 The task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC): 2007 guidelines for the management of arterial hypertension. *J Hypertens* 2007; **25**: 1105–1187.
6 Ogihara T, Kikuchi K, Matsuoka H, Fujita T, Higaki J, Horiuchi M, Imai Y, Imaizumi T, Ito S, Iwao H, Kario K, Kawano Y, Kim-Mitsuyama S, Kimura G, Matsubara H, Matsuura H, Naruse M, Saito I, Shimada K, Shimamoto K, Suzuki H, Takishita S, Tanahashi N, Tsuchihashi T, Uchiyama M, Ueda S, Ueshima H, Umemura S, Ishimitsu T, Rakugi H. The Japanese Society of Hypertension guidelines for the management of hypertension (JSH 2009). *Hypertens Res* 2009; **32**: 3–107.
7 Kawano Y, Abe H, Kojima S, Ashida T, Yoshida K, Imanishi M, Yoshimi H, Kimura G, Kuramochi M, Omae T. Acute depressor effect of alcohol in patients with essential hypertension. *Hypertension* 1992; **20**: 219–226.
8 Regan JT. Alcohol and the cardiovascular system. *JAMA* 1990; **264**: 377–381.
9 Marmot M, Brunner E. Alcohol and cardiovascular disease: the status of the U shaped curve. *Br Med J* 1991; **303**: 565–568.
10 Thun MG, Peto R, Lopez AD, Monaco JH, Henley SJ, Hearth Jr CW, Doll R. Alcohol consumption and mortality among middle-aged and elderly US. Adults. *N Engl J Med* 1997; **337**: 1705–1714.
11 Di Castelnuovo A, Costanzo S, Bagnaldi V, Donati MB, Iacoviello L, de Gaetano G. Alcohol dosing and total mortality in men and women: an updated meta-analysis of 34 prospective studies. *Arch Intern Med* 2006; **166**: 2437–2445.
12 O'Keefe JH, Bybee KA, Lavie CJ. Alcohol and cardiovascular health: the razor-sharp double-edged sword. *J Am Coll Cardiol* 2007; **50**: 1009–1014.
13 Ireland MA, Vandongen R, Davidson L, Beilin LJ, Rouse IL. Acute effects of moderate alcohol consumption on blood pressure and plasma catecholamines. *Clin Sci* 1984; **66**: 643–648.
14 Grassi GM, Somers VK, Renk WS, Abboud FM, Marl AL. Effects of alcohol intake on blood pressure and sympathetic nerve activity in normotensive humans: a preliminary report. *J Hypertens* 1989; **7**(Suppl 6): S20–S21.
15 Kupari M. Acute cardiovascular effects of alcohol: a controlled non-invasive study. *Br Heart J* 1983; **49**: 174–182.
16 Puddey IB, Vandongen R, Beilin L, Rouse IL. Alcohol stimulation of renin release in man: its relation to the hemodynamic, electrolyte, and sympatho-adrenal responses to drinking. *J Clin Endocrinol Metab* 1985; **61**: 37–41.
17 Riff DR, Jain AC, Doyle JT. Acute hemodynamic effects of ethanol on normal human volunteers. *Am Heart J* 1969; **78**: 592–597.
18 Child JS, Kovick RB, Levisman JA, Pearce ML. Cardiac effects of acute ethanol ingestion unmasked by autonomic blockade. *Circulation* 1979; **59**: 120–125.
19 Potter JF, MacDonald IA, Beevers DG. Alcohol raises blood pressure in hypertensive patients. *J Hypertens* 1986; **4**: 435–441.
20 Carretta R, Fabris B, Bardelli M, Fischetti F, Muiesan S, Vran F, Bellinin G, Campanacci L. Hemodynamic effects of intravenous infusion of alcohol in essential hypertension. *J Hypertens* 1987; 5(Suppl 5): S505–S507.
21 Stott DJ, Ball SG, Inglis GC, Davies DL, Fraser R, Murray GD, McInnes GT. Effects of a single moderate dose of alcohol on blood pressure, heart rate and associated metabolic and endocrine changes. *Clin Sci* 1987; **73**: 411–416.

22 Minami J, Todoroki M, Ishimitsu T, Tamamoto H, Abe S, Fukunaga T, Matsuoka H. Effects of alcohol intake on ambulatory blood pressure, heart rate, and heart rate variability in Japanese men with different ALDH2 genotypes. *J Hum Hypertens* 2002; **16**: 345–351.
23 Kupari M, Eriksson CJP, Heikkila J, Ylikahri R. Alcohol and the heart: intense hemodynamic changes associated with alcohol flush in Orientals. *Acta Med Scand* 1983; **213**: 91–98.
24 Kupari M, Lindros K, Hillbom M, Heikkila J, Ylikahri R. Cardiovascular effects of acetaldehyde accumulation after ethanol ingestion: their modification by β-adrenergic blockade and alcohol dehydrogenase inhibition. *Alcohol Clin Exp Res* 1983; **7**: 283–288.
25 Altura BM, Altura BT. Microvascular and vascular smooth muscle actions of ethanol, acetaldehyde, and acetate. *Fed Proc* 1982; **41**: 2447–2451.
26 Howes LG, Reid JL. The effects of alcohol on local, neural and humoral cardiovascular regulation. *Clin Sci* 1986; **71**: 9–15.
27 Criscione L, Powell JR, Burdett R, Engesser S, Schlager F, Schoepfer A. Alcohol suppresses endothelium-dependent relaxation in rat mesenteric vascular beds. *Hypertension* 1989; **13**: 964–967.
28 Brizzolara AL, Morris DG, Burnstock G. Ethanol affects sympathetic cotransmission and endothelium-dependent relaxation in the rat. *Eur J Pharmacol* 1994; **254**: 175–181.
29 Oshita M, Takei Y, Kawano S, Yoshihara H, Hijioka T, Fukui H, Goto M, Masuda E, Nishimura Y, Fusamoto H, Kamada T. Roles of endothelin-1 and nitric oxide in the mechanism for ethanol-induced vasoconstriction in rat liver. *J Clin Invest* 1993; **91**: 1337–1342.
30 Soardo G, Donnini D, Varutti R, Moretti M, Milocco C, Basan L, Esposito W, Casaccio D, Stel G, Catena C, Curcio F, Sechi LA. Alcohol-induced endothelial changes are associated with oxidative stress and are rapidly reversed after withdrawal. *Alcohol Clin Exp Res* 2005; **29**: 1889–1898.
31 Zilkens RR, Pich L, Burke V, Beilin LJ, Watts GF, Puddey IB. Effects of alcohol intake on endothelial function in men: a randomized controlled trial. *J Hypertens* 2003; **21**: 97–103.
32 Puddey IB, Zilkens RR, Croft KD, Beilin LJ. Alcohol and endothelial function: a brief review. *Clin Exp Pharmacol Physiol* 2001; **28**: 1020–1024.
33 Kojima S, Kawano Y, Abe H, Sanai T, Yoshida K, Imanishi M, Ashida T, Kimura G, Yoshimi H, Matsuoka H, Omae T. Acute effects of alcohol ingestion on blood pressure and erythrocyte sodium concentration. *J Hypertens* 1993; **11**: 185–190.
34 Lucas DL, Brown RA, Wassef M, Giles TD. Alcohol and the cardiovascular system: research challenges and opportunities. *J Am Coll Cardiol* 2005; **45**: 1916–1924.
35 Kawano Y, Abe H, Kojima S, Takishita S, Omae T. Effects of propranolol on cardiovascular and neurohumoral actions of alcohol in hypertensive patients. *Blood Press* 1999; **8**: 37–42.
36 Manolio TA, Levy D, Garrison RJ, Castelli WP, Kannel WB. Relation of alcohol intake to left ventricular mass: the Framingham Study. *J Am Coll Cardiol* 1991; **17**: 717–721.
37 Urbano-Marquez A, Estruch R, Navarro-Lopez F, Grau JM, Mont L, Rubin E. The effects of alcoholism on skeletal and cardiac muscle. *N Engl J Med* 1989; **320**: 409–415.
38 Capasso JM, Li P, Guideri G, Malhotra A, Cortese R, Anversa P. Myocardial mechanical, biochemical, and structural alterations induced by chronic ethanol ingestion in rats. *Circ Res* 1992; **71**: 346–356.
39 Bannan LT, Potter JF, Beevers DG, Saunders JB, Waters JRF, Ingram MC. Effect of alcohol withdrawal on blood pressure, plasma renin activity, aldosterone, cortisol and dopamine beta-hydroxylase. *Clin Sci* 1984; **66**: 659–663.
40 Soardo G, Donnini D, Varutti R, Milocco C, Basan L, Esposito W, Casaccio D, Isola M, Soldano F, Sechi LA. Effects of alcohol withdrawal on blood pressure in hypertensive heavy drinkers. *J Hypertens* 2006; **24**: 1493–1498.
41 van de Borne P, Mark AL, Montano N, Mion D, Somers VK. Effects of alcohol on sympathetic activity, hemodynamics, and chemoreflex sensitivity. *Hypertension* 1997; **29**: 1278–1283.
42 Kawano Y, Abe H, Imanishi M, Yoshimi H, Takishita S, Omae T. Pressor and depressor hormones during alcohol-induced blood pressure reduction in hypertensive patients. *J Hum Hypertens* 1998; **10**: 595–599.
43 Abdel-Rahman AA, Dar MS, Wooles WR. Effects of chronic ethanol administration on artrial baroreceptor function and pressor and depressor responsiveness in rats. *J Pharmacol Exp Ther* 1985; **232**: 194–201.
44 Narkiewicz K, Cooley RL, Somers VK. Alcohol potentiates orthostatic hypotension: implications for alcohol-related syncope. *Circulation* 2000; **101**: 398–402.
45 Randin D, Vollenweider P, Tappy L, Jequier E, Nicod P, Scherer U. Suppression of alcohol-induced hypertension by dexamethasone. *N Engl J Med* 1995; **332**: 1733–1737.
46 Djousse L, Hunt SC, Eckfeldt JH, Arnett DK, Province MA, WEllison RC. Alcohol consumption and plasma atrial natriuretic peptide (from the HyperGEN study). *Am J Cardiol* 2006; **98**: 628–632.
47 Goude G, Fagerberg B, Hulthe J. Alcohol consumption, the metabolic syndrome and insulin resistance in 58-year-old clinically healthy men (AIR study). *Clin Sci* 2002; **102**: 345–352.
48 Wannamethee SG, Camargo Jr CA, Manson JE, Willett WC, Rimm EB. Alcohol drinking patterns and risk of type 2 diabetes mellitus among younger women. *Arch Intern Med* 2003; **163**: 1329–1336.
49 Rubini ME, Kleeman CR, Lamdin E. Studies on alcohol diuresis. 1. The effect of ethyl alcohol ingestion on water, electrolyte and acid-base metabolism. *J Clin Invest* 1955; **34**: 439–447.
50 Kalbfleisch JM, Lindeman RD, Ginn HE, Smith WO. Effects of ethanol administration on urinary excretion of magnesium and other electrolytes in alcoholic and normal subjects. *J Clin Invest* 1963; **42**: 1471–1475.
51 Kawano Y, Abe H, Kojima S, Takishita S, Matsuoka H. Effects of repeated alcohol intake on blood pressure and sodium balance in Japanese men with hypertension. *Hypertens Res* 2004; **27**: 167–172.
52 Hsieh ST, Saito K, Miyajima T, Lin CM, Yokoyama M. Effects of alcohol moderation on blood pressure and intracellular cations in mild essential hypertension. *Am J Hypertens* 1995; **8**: 696–703.
53 Hsieh ST, Sano H, Saito K, Kubota Y, Yokoyama M. Magnesium supplementation prevents the development of alcohol-induced hypertension. *Hypertension* 1992; **19**: 175–182.
54 Klatsky AL, Friedman GD, Siegelaub AB, Gerald M. Alcohol consumption and blood pressure: Kaiser-Permanente multiphasic health examination data. *N Engl J Med* 1977; **296**: 1120–1194.
55 Marmot MG, Elliott P, Shipley MJ, Dyer AR, Ueshima H, Beevers DG, Stamler R, Kesteloot H, Rose G, Stamler J. Alcohol and blood pressure: the INTERSALT study. *Br Med J* 1994; **308**: 1263–1267.
56 Okamura T, Tanaka T, Yoshita K, Chiba N, Takebayashi T, Kikuchi Y, Tamaki J, Tamura U, Minai J, Kadowaki T, Miura K, Nakagawa H, Tanihara S, Okayama A, Ueshima H. Specific alcoholic beverage and blood pressure in a middle-aged Japanese population: the High-risk and Population Strategy for Occupational Health Promotion (HIPOP-OHP) study. *J Hum Hypertens* 2004; **18**: 9–16.
57 Stranges S, Wu T, Dorn JM, Freudenheim JL, Muti P, Farinaro P, Russel M, Nochajski TH, Trevisan M. Relationship of alcohol drinking pattern to risk of hypertension: a population-based study. *Hypertension* 2004; **44**: 813–819.
58 Nakamura K, Okamura T, Hayakawa T, Hozawa A, Kadowaki T, Murakami Y, Kita Y, Okayama A, Ueshima H. The proportion of individuals with alcohol-induced hypertension among total hypertensives in a general Japanese population: NIPPON DATA 90. *Hypertens Res* 2007; **30**: 663–668.
59 Panagiotakos DB, Kourlaba G, Zeinbekis A, Toutouzas P, Polychronopoulos E. The J-shaped association of alcohol consumption on blood pressure levels in elderly people from Mediterranean Islands (MEDIS epidemiological study). *J Hum Hypertens* 2007; **21**: 585–587.
60 Sesso HD, Cook NR, Buring JE, Manson JE, Gaziano JM. Alcohol consumption and the risk of hypertension in women and men. *Hypertension* 2008; **51**: 1080–1087.
61 Suter PM, Maire R, Vetter V. Is an increased waist:hip ratio the cause of alcohol-induced hypertension? The AIR 94 study. *J Hypertens* 1995; **13**: 1857–1862.
62 Tsuruta M, Adachi H, Hirai Y, Fujiura Y, Imaizumi T. Association between alcohol intake and development of hypertension in Japanese normotensive men: 12-year follow-up study. *Am J Hypertens* 2000; **13**: 482–487.
63 Fuchs FD, Chambless LE, Whelton PK, Nieto J, Heiss G. Alcohol consumption and the incidence of hypertension: the Atherosclerosis Risk in Communities study. *Hypertension* 2001; **37**: 1242–1250.
64 Nakanishi N, Yoshida H, Nakamura K, Suzuki K, Tatara K. Alcohol consumption and the risk for hypertension in middle-aged Japanese men. *J Hypertens* 2001; **19**: 851–855.
65 Moreira MB, Fuchs FB, Moraes RS, Bnedemeier M, Duncan BB. Alcohol intake and blood pressure: the importance of time elapsed since last drink. *J Hypertens* 1998; **16**: 175–180.
66 Kawabe H, Saito I, Saruta T. Effects of nighttime alcohol intake on evening and next morning blood pressure in Japanese normotensives. *Clin Exp Hypertens* 2007; **29**: 43–49.
67 Howes LG, Reid JL. Changes in blood pressure and autonomic reflexes following regular, moderate alcohol consumption. *J Hypertens* 1986; **4**: 421–425.
68 Potter JT, Beevers DG. Pressor effect of alcohol in hypertension. *Lancet* 1984; **i**: 119–122.
69 Puddey IB, Beilin LJ, Vandongen R, Rouse IL, Rogers P. Evidence for a direct effect of alcohol consumption on blood pressure in normotensive men: a randomized controlled trial. *Hypertension* 1985; **7**: 707–713.
70 Puddey IB, Beilin LJ, Vandongen R. Regular alcohol use raises blood pressure in treated hypertensive subjects: a randomized, controlled trial. *Lancet* 1987; **i**: 647–651.
71 Ueshima H, Ogihara T, Baba S, Tabuchi Y, Mikawa K, Hashizume K, Mandai T, Ozawa H, Kumahara Y, Asakura S, Hisanari M. The effect of reduced alcohol consumption on blood pressure: a randomized, controlled, single blind study. *J Hum Hypertens* 1987; **1**: 113–119.
72 Xin X, He J, Frontini MG, Ogden LG, Motsamai OI, Whelton PK. Effects of alcohol reduction on blood pressure: a meta-analysis of randomized controlled trials. *Hypertension* 2001; **38**: 1112–1117.
73 Abe H, Kawano Y, Kojima S, Ashida T, Kuramochi M, Matsuoka H, Omae T. Biphasic effects of alcohol intake on 24-h blood pressure in hypertensive patients. *Circulation* 1994; **89**: 2626–2633.
74 Kawano Y, Abe H, Takishita S, Omae T. Effects of alcohol restriction on 24-h ambulatory blood pressure in Japanese men with hypertension. *Am J Med* 1998; **105**: 307–311.
75 Minami J, Yoshii M, Todoroki M, Nishikimi T, Ishimitsu T, Fukunaga T, Matsuoka H. Effects of alcohol restriction on ambulatory blood pressure, heart rate, and heart rate variability in Japanese men. *Am J Hypertens* 2002; **15**: 125–129.
76 Howes LG, Krum H, Phillips PA. Effects of regular alcohol consumption on 24 h ambulatory blood pressure recordings. *Clin Exp Pharmacol Physiol* 1990; **17**: 247–250.
77 Rakic V, Puddey IB, Burke V, Dimmitt SB, Beilin LJ. Influence of pattern of alcohol intake on blood pressure in regular drinkers: a controlled trial. *J Hypertens* 1998; **16**: 165–174.

78 Aguilera MT, de la Sierra A, Coca A, Estruch R, Fernandez-Sola J, Urbano-Marquez A. Effect of alcohol abstinence on blood pressure: assessment by 24-h ambulatory blood pressure monitoring. *Hypertension* 1999; **33**: 653–657.
79 McFadden CB, Brensinger CM, Berlin JA, Townsend RR. Systematic review of the effect of daily alcohol intake on blood pressure. *Am J Hypertens* 2005; **18**: 276–286.
80 Kawano Y, Pontes CS, Abe H, Takishita S, Omae T. Effects of alcohol consumption and restriction on home blood pressure in hypertensive patients: serial changes in the morning and evening records. *Clin Exp Hypertens* 2002; **24**: 33–39.
81 Strickland JA, Wooles WR. Blood ethanol level and physiologic measurements during ethanol-induced hypertension. *Alcohol* 1989; **6**: 109–114.
82 Vasdev S, Sampson CA, Prabhakaran VM. Platelet free calcium and vascular calcium uptake in ethanol-induced hypertensive rats. *Hypertension* 1991; **18**: 116–122.
83 Puddey IB, Burke V, Croft K, Beilin LJ. Increased blood pressure and changes in membrane lipids associated with chronic ethanol treatment of rats. *Clin Exp Pharmacol Physiol* 1995; **22**: 655–657.
84 Harada H, Kitazaki K, Tsujino T, Watari Y, Iwata S, Nonaka H, Hayashi T, Takeshita T, Morimoto K, Yokoyama M. Oral taurine supplementation prevents the development of ethanol-induced hypertension in rats. *Hypertens Res* 2000; **23**: 277–284.
85 Abdel-Rahman AA. Alcohol abolishes the hypotensive effect of clonidine in spontaneously hypertensive rats. *Hypertension* 1994; **24**: 802–807.
86 Howe PRC, Rogers PF, Smith RM. Antihypertensive effect of alcohol in spontaneously hypertensive rats. *Hypertension* 1989; **13**: 607–611.
87 Hatton DC, Bukoski RD, Edgar S, McCarron DA. Chronic alcohol consumption lowers blood pressure but enhances vascular contractility in Wistar rats. *J Hypertens* 1992; **10**: 529–537.
88 Beilin LJ, Hoffmann P, Nilsson H, Skarphedinsson J, Folkow B. Effect of chronic ethanol consumption upon cardiovascular reactivity, heart rate and blood pressure in spontaneously hypertensive and Wistar-Kyoto rats. *J Hypertens* 1992; **10**: 645–650.
89 El-Mas MM, Abdel-Rahman AA. Ovariectomy alters the chronic hemodynamic and sympathetic effects of ethanol in radiotelemetered female rats. *Clin Exp Hypertens* 2000; **22**: 109–126.
90 Crandall DL, Ferraro GD, Lozito RJ, Cervoni P, Clark LT. Cardiovascular effects of intermittent drinking: assessment of a new animal model of human alcoholism. *J Hypertens* 1989; **7**: 683–687.
91 Capasso JM, Li P, Guideri G, Anversa P. Left ventricular dysfunction induced by chronic alcohol ingestion in rats. *Am J Physiol* 1991; **261**: H212–H219.
92 Schlicht I, Falk S, Krossin A, Mohnhaupt A. Lifetime prolongation in voluntary alcohol-consuming rats (SHR) treated with clofibrate. *Alcohol* 1992; **9**: 139–148.
93 Ishikawa J, Kario K, Eguchi K, Morinari M, Hoshide S, Ishikawa S, Shimada K. Regular alcohol drinking is a determinant of masked morning hypertension detected by home blood pressure monitoring inmmedicated hypertensive patients with well controlled clinic blood pressure: The Jichi Morning Hypertension Research (J-MORE) study. *Hypertens Res* 2006; **29**: 679–686.
94 Kawano Y, Abe H, Kojima S, Takishita S, Omae T. Interaction of alcohol and an $á_1$ blocker on ambulatory blood pressure in patients with essential hypertension. *Am J Hypertens* 2000; **13**: 307–312.
95 Bailey DG, Spence JD, Edgar B, Bailiff CD, Arnold JMO. Ethanol enhances the hemodynamic effects of felodipine. *Clin Invest Med* 1989; **12**: 357–362.
96 Kawano Y, Abe H, Kojima S, Yoshimi H, Sanai T, Kimura G, Matsuoka H, Takishita S, Omae T. Different effects of alcohol and salt on 24-h blood pressure and heart rate in hypertensive patients. *Hypertens Res* 1996; **19**: 255–261.
97 Kawano Y, Okuda N, Minami J, Takishita S, Omae T. Effects of a low energy diet and an insulin-sensitizing agent on ambulatory blood pressure in overweight hypertensive patients. *J Hypertens* 2000; **18**: 1451–1455.
98 Kawano Y. Role of blood pressure monitoring in non-pharmacological management of hypertension. *Blood Press Monit* 2002; **7**: 51–54.
99 Walsh CR, Larson MG, Evans JC, Djousse L, Ellison C, vasan RS, Levy D. Alcohol consumption and risk of congestive heart failure in the Framingham Heart Study. *Ann Intern Med* 2002; **136**: 181–191.
100 Bryson CL, Mukamal KJ, Mittleman MA, Fried LP, Hirsch CH, Kitzman DW, Siscovick DS. The association of alcohol consumption and incident heart failure: the Cardiovascular Health Study. *J Am Coll Cardiol* 2006; **48**: 305–311.
101 Klatsky AL, Chartier D, Udaltsova N, Gronningen S, Brar S, Friedman GD, Landstrom RJ. Alcohol drinking and risk of hospitalization for heart failure with and without associated coronary artery disease. *Am J Cardiol* 2005; **96**: 346–351.
102 Cohen EJ, Klatsky AL, Armstrong A. Alcohol use and supraventricular arrhythmia. *Am J Cardiol* 1988; **62**: 971–973.
103 Frost L, Vestergaard P. Alcohol and the risk of atrial fibrillation or flutter. *Arch Intern Med* 2004; **164**: 1993–1998.
104 Conen D, Tedrow UB, Cook NR, Moorthy MV, Buring JE, Albert CM. Alcohol consumption and risk of incident atrial fibrillation in women. *JAMA* 2008; **300**: 2489–2496.
105 Boffetta P, Garfinkel L. Alcohol drinking and mortality among men enrolled in an American Cancer Society prospective study. *Epidemiology* 1990; **1**: 342–348.
106 Rimm EB, Giovanucci EL, Willett WC, Colditz DA, Ascherio A, Rosner B, Stampfer MJ. Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet* 1991; **338**: 464–468.
107 Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. *Br Med J* 1999; **319**: 1523–1528.
108 Beulens JWJ, Rimm EB, Ascherio A, Spiegelman D, Hendriks HFJ, Mukamal KJ. Alcohol consumption and risk for coronary heart disease among men with hypertension. *Ann Intern Med* 2007; **146**: 10–19.
109 Vliegenhart R, Oei HS, van den Elzen APM, van Rooij FJA, Hofman A, Oudkerk M, Witteman JCM. Alcohol consumption and coronary calcification in a general population. *Arch Intern Med* 2004; **164**: 2355–2360.
110 Ikehara S, Iso H, Toyoshima H, Date C, Yamamoto A, Kikuchi S, Kondo T, Watanabe Y, Koizumi A, Wada Y, Inaba Y, Tamakoshi A. Japan Collabrative Cohort Study Group: alcohol consumption and mortality from stroke and coronary heart disease among Japanese men and women: the Japan Collaborative Cohort Study. *Stroke* 2008; **39**: 2936–2942.
111 Langer RD, Criqui MH, Reed DM. Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. *Circulation* 1992; **85**: 910–915.
112 Albert MA, Glynn RJ, Ridker PM. Alcohol consumption and plasma concentration of C-reactive protein. *Circulation* 2003; **107**: 443–447.
113 Renaud SC, Gueguen R, Siest G, Salamon R. Wine, beer, and mortality in middle-aged men from eastern France. *Arch Intern Med* 1999; **159**: 1865–1870.
114 Di Castelnuovo A, Rotondo S, Iacoviello L, Donati MB, de Gaetano G. Meta-analysis of wine and beer consumption in relation to vascular risk. *Circulation* 2002; **105**: 2836–2844.
115 Rimm EB, Klatsky A, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits? *Br Med J* 1996; **312**: 731–736.
116 Camargo Jr CA. Moderate alcohol consumption and stroke: the epidemiologic evidence. *Stroke* 1989; **20**: 1611–1626.
117 Iso H, Baba S, Mannami T, Sasaki S, Okada K, Konishi M, Tsugane S. Alcohol consumption and risk of stroke among middle-aged men: the JPHC study Cohort I. *Stroke* 2004; **35**: 1124–1129.
118 Elkand MSV, Sciacca R, Baden-Albela B, Rundek T, Paik MC, Sacco RF. Moderate alcohol consumption reduces risk of ischemic stroke: the Northern Manhattan Study. *Stroke* 2006; **37**: 13–19.
119 Djousse L, Ellison C, Beiser A, Scaramucci A, D'Agostino RB, Wolf PA. Alcohol consumption and risk of ischemic stroke: the Framingham Study. *Stroke* 2002; **33**: 907–912.
120 Mukamal KJ, Ascherio A, Mittelman MA, Conigrave KM, Camargo Jr CA, Kawachi I, Stampfer MJ, Willett WC, Rimm EB. Alcohol and risk for ischemic stroke in men: the role of drinking patterns and usual beverage. *Ann Intern Med* 2005; **142**: 11–19.
121 Mukamal KJ, Longstreth Jr WT, Mittelman MA, Crum RM, Siscovick DS. Alcohol consumption and subclinical findings on magnetic resonance imaging of the brain in older adults: the Cardiovascular Health Study. *Stroke* 2001; **32**: 1939–1946.
122 Ding J, Eigenbrodt ML, Mosley Jr TH, Hutchinson RG, Folsom AR, Harris TB, Nieto FJ. Alcohol intake and cerebral abnormalities on magnetic resonance imaging in a community-based population of middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Stroke* 2004; **35**: 16–21.
123 Jepson RG, Fowkes FG, Donnan PT, Housley E. Alcohol intake as a risk factor for peripheral artery disease in the general population in the Edinburgh Artery Study. *Eur J Epidemiol* 1995; **11**: 9–14.
124 Camargo Jr CA, Stampfer MJ, Glynn RJ, Gaziano M, Manson JE, Goldhaber SZ, Hennekens CH. Prospective study of moderate alcohol consumption and risk of peripheral artery disease in US male physicians. *Circulation* 1997; **95**: 577–580.
125 Djousse L, Levy D, Murabito JM, Cupples LA, Ellison PC. Alcohol consumption and risk of intermittent claudication in the Framingham Heart Study. *Circulation* 2000; **102**: 3092–3097.
126 Vliegenthart R, Gleijinse JM, Hofman A, Meijer WT, van Rooij FJA, Grobbee DE, Witteman JCM. Alcohol consumption and risk of peripheral arterial disease. *Am J Epidemiol* 2002; **155**: 332–338.
127 Bagousslavsky J, Van Melle G, Despland PA, Regli F. Alcohol consumption and carotid atherosclerosis in the Lausanne Stroke Registry. *Stroke* 1990; **21**: 715–720.
128 Demirovic J, Nabulsi A, Folsom AR, Carpenter MA, Szklo M, Sorlie PD, Barnes RW. Alcohol consumption and ultrasonographycally assessed carotid artery wall thickness and distensibility. *Circulation* 1993; **88**: 2787–2793.
129 Kiechl S, Willeit J, Egger G, Oberhollenzer M, Aichner F. Alcohol consumption and carotid atherosclerosis: evidence of dose-dependent atherogenic and antiatherogenic effects: results from the Bruneck Study. *Stroke* 1994; **25**: 1593–1598.
130 Schminke U, Luedemann J, Berger K, Alie D, Mitusch R, Wood WG, Jachinski A, Barnow S, John U, Kessler C. Association between alcohol consumption and subclinical carotid atherosclerosis: the Study of Health in Pomerania. *Stroke* 2005; **36**: 1746–1752.
131 Sierksma A, Muller M, van der Schouw YT, Grobbee DE, Hendriks HFJ, Bots ML. Alcohol consumption and arterial stiffness in men. *J Hypertens* 2004; **22**: 357–362.
132 van den Elzen AP, Sierksma A, Oren A, Vos LE, Witteman JC, Grobbee DE, Handriks HF, Uitarwaal CS, Bots ML. Alcohol intake and aortic stiffness in young men and women. *J Hypertens* 2005; **23**: 731–735.
133 Kurihara T, Tomiyama H, Hashimoto H, Yamamoto Y, Yano E, Yamashina A. Excessive alcohol intake increases the risk of arterial stiffening in men with normal blood pressure. *Hypertens Res* 2004; **27**: 669–673.
134 Stampfer MJ, Hu FB, Manson JE, Rimm EB, Willett WC. Primary prevention of coronary heart disease in women through diet and lifestyle. *N Engl J Med* 2000; **343**: 16–22.
135 Franco OH, Bonneux L, de Laet C, Peeters A, Steyerberg EW, Mackenbach JP. The polymeal: a more natural, safer, and probably tastier (than polypill) strategy to reduce cardiovascular disease by more than 75%. *Br Med J* 2004; **329**: 1147–1150.
136 Gronbeck M, Becker U, Johansen D, Cottschau A, Schmahr P, Hein HO, Jensen G, Sorenses TIA. Type of alcohol consumed and mortality from all causes, coronary heart disease, and cancer. *Ann Intern Med* 2000; **133**: 411–419.
137 Rehm J, Greenfield TK, Rogers JD. Average volume of alcohol consumption, patterns of drinking, and all-cause mortality: results from the US National Alcohol Survey. *Am J Epidemiol* 2001; **153**: 64–71.

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Additive Interaction of Metabolic Syndrome and Chronic Kidney Disease on Cardiac Hypertrophy, and Risk of Cardiovascular Disease in Hypertension

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BACKGROUND
Recent epidemiologic analyses have demonstrated a link between the metabolic syndrome (MetS) and chronic kidney disease (CKD). We examined the association between MetS, CKD, and left ventricular hypertrophy (LVH), and prospectively investigated the predictive value of the combination of MetS and CKD for cardiovascular disease (CVD) in essential hypertension.

METHODS
A total of 1,160 essential hypertensive patients (mean age 63 years, 53% male) underwent clinical evaluation, laboratory testing, and Doppler echocardiography, and were monitored for a mean follow-up of 4.8 years.

RESULTS
At baseline, total subjects were divided into four groups according to the presence/absence of MetS and/or CKD, and, compared to the group without MetS and CKD ($MetS^-/CKD^-$); those with MetS and CKD ($MetS^+/CKD^+$) had a multivariate-adjusted odds ratio of 2.40 (95% confidence interval (CI) 1.66–3.48) for LVH. During the follow-up period, 172 subjects developed CVD. Multiple Cox regression analysis including LV mass index (LVMI) showed that the presence of MetS as well as that of CKD were each independent predictors of CVD (hazard ratio 1.90 for MetS, 1.82 for CKD). We then divided the total subjects into four groups, and found that, compared to the $MetS^-/CKD^-$ group, multivariate-adjusted HR for the $MetS^+/CKD^+$ group was 3.58 (95% CI 2.14–5.95).

CONCLUSIONS
Our findings suggest that, in essential hypertension, the combination of MetS and CKD is a strong risk for LVH as well as a strong and independent predictor of subsequent CVD. These findings highlight the clinical importance of the concomitance of MetS and CKD in essential hypertension.

Keywords: blood pressure; cardiovascular disease; chronic kidney disease; hypertension; left ventricular hypertrophy; metabolic syndrome; risk factor

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Hypertension is a common 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. In the past few years, there has been growing attention to a condition known as the metabolic syndrome (MetS),[1] which is characterized by a cluster of atherosclerotic risk factors, including obesity, hypertension, insulin resistance, and dyslipidemia, as well as chronic kidney disease (CKD).[2] Individuals with MetS or CKD are at increased risk of CVD as well as death from CVD and all causes.[3–8] Furthermore, recent epidemiologic analyses have demonstrated a link between MetS and CKD.[9–11] However, whether the concomitance of MetS and CKD contributes to the development of CVD is unknown.

Echocardiography is a well-established procedure to diagnose increased left ventricular (LV) mass, and its presence is thought to increase CVD risk through a series of unfavorable metabolic, functional, and structural cardiac changes.[12–14] The assessment of LV geometry in addition to LV hypertrophy (LVH) is important for evaluation of the peculiar hemodynamic pattern such as a combination of pressure and volume stimuli, contractile efficiency, and prognosis.[15] Insulin resistance, oxidative stress, and inflammation have been implicated in the pathogenesis of MetS and CKD, which also have been shown to be associated with LVH. Increased LV mass has been shown to be associated with MetS and CKD;[16–20] however, we could not find any previous studies examining the hypothesis that the combination of MetS and CKD may be a strong risk for LVH.

The influence of increased LV mass on the association of MetS and/or CKD with CVD is also unknown. The

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association between MetS or CKD and increased CVD could be mediated through increased LV mass, and this may be one of the pathways linking MetS and CKD to CVD. Therefore, in this study, we investigated the potential interrelationship between MetS, CKD, and the risk of LVH in essential hypertensive subjects. Furthermore, we also examined prospectively whether MetS and CKD interact to substantially increase the risk of CVD in hypertension. Moreover, we additionally examined whether this association would be independent of LV mass.

METHODS

Study subjects. This study enrolled essential hypertensive patients in normal sinus rhythm, who had good-quality echocardiographic recordings, and monitored them for a mean follow-up of 4.8 ± 2.7 years. In our laboratory (the National Cardiovascular Center in Osaka, Japan), all hypertensive patients attended the echocardiography laboratory, and echocardiographic data were routinely collected consecutively. From 1,263 patients at the time of the baseline examination, we excluded patients with missing data of MetS or CKD components (n = 77) and patients receiving regular hemodialysis therapy (n = 26), leaving 1,160 patients (545 women) for this analysis. Exclusion criteria included acute coronary syndrome, congestive heart failure (CHF) (New York Heart Association class II or greater), secondary hypertension, moderate or severe aortic or mitral regurgitation, heart rate ≥100 bpm, and low ejection fraction (<45%). All procedures in this study were carried out in accordance with institutional and national ethical guidelines for human studies. All participants enrolled in this study were Japanese, and all gave informed consent to participate.

Baseline clinical characteristics. Hypertension was defined as systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg on repeated measurements, or receiving antihypertensive treatment. Diabetes mellitus was defined according to the American Diabetes Association criteria.[21] Smoking status was determined by interview, and defined as never-smoker, past-smoker (those with a history of habitual smoking but had quit), and current-smoker. Previous CVD was defined as a history of myocardial infarction, CHF, or stroke.

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 and urine sampling from all subjects was performed. Height and body weight were measured, and body mass index (BMI) was calculated. The following parameters were also determined: triglycerides, high-density lipoprotein cholesterol, C-reactive protein (CRP), and creatinine.

Definition of MetS and CKD. MetS was defined according to the guidelines of the National Cholesterol Education Program Third Adult Treatment Panel with modification for body size.[1]

In this study, all patients were hypertensive and thus, participants had MetS if they fulfilled two or more of the following.

1. Elevated BMI (in lieu of waist measurement, which was not available in our database). The frequency of BMI ≥30 kg/m^2 is 2–3% in Japan and 20–30% in Western countries.[22–24] Because of the differences in BMI between Japanese and Western populations, values ≥25 kg/m^2 were considered elevated (in contrast to ≥30 kg/m^2 in Western populations) according to the criteria of the Japan Society for the Study of Obesity.[22,25]
2. Elevated triglycerides (≥150 mg/dl).
3. Low high-density lipoprotein cholesterol (<40 mg/dl in men, <50 mg/dl in women).
4. Impaired fasting glucose (fasting plasma glucose ≥110 mg/dl and/or a history of diabetes).

The estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease formula in ml/min. CKD and its stages were defined according to the guidelines of the National Kidney Foundation classification of CKD,[2] which defines from eGFR of <60 ml/min/1.73 m^2 or dipstick proteinuria (≥1+) as follows: eGFR ≥90 ml/min/1.73 m^2 without proteinuria (high BP), eGFR 60–89 ml/min/1.73 m^2 without proteinuria (high BP with reduced GFR), eGFR ≥90 ml/min/1.73 m^2 with proteinuria (stage 1), eGFR 60–89 ml/min/1.73 m^2 with proteinuria (stage 2), and stages 3–5 were classified according to the level of kidney function (eGFR 30–59, 15–29, and <15 ml/min/1.73 m^2, respectively), regardless of the presence of other markers of kidney damage.[2] Subjects were diagnosed with CKD if they were classified as CKD stage 1–5.

Echocardiographic methods and calculation of derived variables. Phased-array echocardiography with M-mode, two-dimensional, pulsed, and color-flow Doppler capabilities was performed in all study participants, as previously described.[26,27] Estimates of LV mass were calculated according to the American Society of Echocardiography criteria[28] applied to the formula of Devereux *et al.*[29] LV mass index (LVMI) was calculated by dividing LV mass by body surface area. LVH was defined as LVMI >125 g/m^2 for men and >110 g/m^2 for women.[30] Relative wall thickness (RWT) was calculated as (interventricular septal + posterior wall thickness)/LV internal diameter.[31] Concentric remodeling was defined as normal LVMI with RWT >0.45 (ref. 31). Eccentric hypertrophy was defined as LVH with RWT ≤0.45. Concentric hypertrophy was defined as LVH with RWT >0.45 (ref. 32). LV filling was assessed by recording mitral flow by a standard pulsed Doppler technique, and the following parameters were considered: the ratio of peak early-to-late diastolic filling velocity (*E*/*A* ratio) and the deceleration time of early diastolic LV filling.

Clinical end points. For survival analysis, observation began on the date of echocardiography, with verified dates updated through October 2007. All of the subjects were followed at

the National Cardiovascular Center in Osaka and treated by implementation of standard lifestyle and pharmacological measures. CVD events of interest in this study were acute myocardial infarction, stroke, aortic dissection, CHF requiring hospitalization, and cardiovascular death. The occurrence of myocardial infarction was confirmed if symptoms met the criteria of the World Health Organization and if the event was associated with abnormal levels of cardiac enzymes and diagnostic electrocardiographic criteria. Stroke was confirmed if the participant had a new neurologic deficit that persisted for >24 h. Computed tomographic scans or magnetic resonance images were available for all the events and were used to distinguish hemorrhagic from ischemic events. Aortic dissection was defined as any nontraumatic dissection when a participant was admitted to hospital with a dissection that required intervention, and diagnosis was based on confirmatory imaging, intraoperative visualization, or autopsy. CHF was defined by the Framingham Heart Study criteria,[33] which require the simultaneous presence of at least two major criteria, or one major criterion in conjunction with two minor criteria, and requiring treatment with a diuretic, vasodilator, or antihypertensive drug. The cause of death was classified as CVD if there was sudden death from CVD. All CVD events were determined 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 evidence of pneumonia or uremia were excluded. For patients who experienced multiple nonfatal episodes of CVD, the analysis included only the first event.

Statistical analysis. Statistical analyses were performed with SPSS, version 15.0 (SPSS, Chicago, IL). Data are presented as mean ± s.d. for continuous variables and as proportions for categorical variables. First, we divided the participants into four groups according to the presence/absence of MetS and/or CKD. Differences in baseline characteristics among the four groups were determined by one-way analysis of variance (ANOVA) with Dunnett's multiple comparison post-test for continuous variables, and χ^2 test for categorical variables. Because of the right skew in CRP distribution, levels of CRP were log-transformed to examine the significance of any difference between groups. The number of subjects in whom CRP was measured was small (n = 997) compared with the total number of study subjects. Therefore, CRP was not included in the following analysis.

We used logistic regression analysis to determine the odds ratio (OR) of LVH as a function of MetS or CKD. In multivariate models, we entered both MetS and CKD into the same model, and included variables that might confound the relation between LVH and MetS or CKD: age, sex, duration of hypertension, systolic BP, smoking status, previous CVD, and receiving antihypertensive medication. We next divided the subjects into four groups according to the presence/absence of MetS and/or CKD, and the relative ORs of LVH were assessed in crude, age- and sex-adjusted, and multivariate regression models (adjusting for the same variables as listed above). Relative ORs were calculated using the $MetS^-/CKD^-$ group as a reference for each.

Survival analysis was performed using cumulative event-free Kaplan–Meier curves according to the presence/absence of MetS or CKD, and the groups were compared by Mantel log-rank test. Cox proportional hazard analysis was used to examine the association between variables and the cumulative incidence of CVD in crude and multivariate models. In multivariate models, both MetS and CKD were entered into the same model after accounting for relevant variables, using a P value of <0.05 as the selection criterion. These effects were measured by the hazard ratio (HR) and 95% confidence interval (CI) based on Cox regression models.

We then evaluated the joint associations of MetS and CKD with incident CVD by dividing the subjects into four groups according to the presence/absence of MetS and/or CKD. Event-free survival analysis was performed using the Kaplan–Meier method to plot the cumulative incidence of CVD. The relative risk of CVD events in Cox proportional hazard analysis was assessed in crude and multivariate models, and the cumulative incidence of CVD was calculated using the $MetS^-/CKD^-$ group as a reference for each. In these analyses, HRs of CVD were calculated using the whole participants or excluding subjects with previous CVD and/or diabetes from the analysis. A P value <0.05 was considered to be statistically significant.

RESULTS

Characteristics of study subjects

The baseline clinical and biochemical characteristics of the study subjects are shown in Table 1. Their mean age was 63.3 ± 11.2 years, and 53.0% were men. Overall, 42.4% had MetS, and 50.6% had CKD. We first divided the subjects into four groups according to the presence/absence of MetS and/or CKD. As a result, the total subjects were divided into four groups as follows; no MetS and no CKD ($MetS^-/CKD^-$), MetS without CKD ($MetS^+/CKD^-$), CKD without MetS ($MetS^-/CKD^+$), and MetS and CKD ($MetS^+/CKD^+$). As shown in Table 1, compared with the $MetS^-/CKD^-$ group, the $MetS^+/CKD^+$ group showed an increased risk of cardiovascular morbidity, such as significantly longer duration of hypertension, higher prevalence of previous CVD, diabetes, and current-smoking, higher age, BMI, systolic BP, fasting glucose, and CRP, worse dyslipidemia, and lower eGFR. In addition, the $MetS^+/CKD^+$ group showed a significantly longer duration of hypertension, lower eGFR, and higher CRP than the $MetS^+/CKD^-$ group, and a significantly higher prevalence of diabetes, higher BMI, fasting glucose, and CRP, and worse dyslipidemia than the $MetS^-/CKD^+$ group.

Relations of MetS and CKD to LVH

The baseline echocardiographic characteristics of the study subjects are shown in Table 2. At baseline, 58.3% of the total subjects were found to have LVH. Univariate logistic