Impact of Hypertriglyceridemia on Endothelial Dysfunction During Statin ± Ezetimibe Therapy in Patients With Coronary Heart Disease

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Despite the use of statin therapy and achieving the target for low-density lipoprotein cholesterol, a substantial number of coronary events are not prevented, and residual risk factors remain unsettled. Recently, ezetimibe has been shown to reduce not only low-density lipoprotein cholesterol but also triglyceride (TG) levels. The aim of this study was to investigate the associations of residual risk factors, mainly hypertriglyceridemia, with endothelial function during statin therapy in patients with coronary heart disease and examine the effect of ezetimibe add-on therapy. A total of 109 consecutive patients with coronary heart disease during statin therapy were enrolled. Lipid profile was measured and endothelial function was assessed by flow-mediated dilation (FMD) of the brachial artery in a fasting state. Next, 32 patients with high TG levels (≥150 mg/dl) were prospectively assigned to the ezetimibe add-on group or the no-ezetimibe group, and endothelial function was assessed after 3 months. Multivariate linear regression analysis demonstrated that serum TG and high-density lipoprotein cholesterol levels were independent determinants of percentage FMD ($\beta = -0.210$ and 0.208, respectively, $p < 0.05$). In patients with high TG levels, ezetimibe add-on therapy significantly improved percentage FMD (from 3.3 ± 1.1% to 4.0 ± 1.1%, $p < 0.005$), whereas no significant change was observed in the no-ezetimibe group. Moreover, the improvement in percentage FMD was significantly associated with reduction in serum TG levels ($\beta = -0.387$, $p < 0.05$) independent of the change in serum low-density lipoprotein cholesterol levels. In conclusion, hypertriglyceridemia is independently associated with endothelial dysfunction in patients with coronary heart disease during statin therapy. Ezetimibe add-on therapy improves endothelial function in these high-risk populations. © 2011 Elsevier Inc. All rights reserved. **(Am J Cardiol 2011;108:333–339)**

The aim of the present study was to investigate the associations between residual risk factors, mainly serum triglyceride (TG) levels, and endothelial function during statin therapy in patients with coronary heart disease (CHD). We also investigated the effect of ezetimibe add-on therapy on endothelial function in patients with hypertriglyceridemia.

Methods

The study included 109 consecutive outpatients with stable CHD who had undergone percutaneous coronary intervention or coronary bypass surgery and who visited Okayama University Hospital regularly from January 2009 to May 2010. All enrolled subjects had been receiving statins for dyslipidemia and secondary prevention of CHD for >3 months (atorvastatin 42%, rosuvastatin 28%, pitavastatin 13%, and pravastatin 17%). Patients were excluded from the study if they had acute coronary syndromes, stroke, or heart failure or had undergone major surgery <3 months before enrollment or if they had variant angina, concomitant inflammatory diseases, or malignant tumors. Hypertension was diagnosed as defined by the 1999 World Health Organization and International Society of Hypertension guidelines[1] or concurrent treatment with antihypertensive medication. Diabetes was defined as a fasting blood glucose level ≥126 mg/dl and glycosylated hemoglobin ≥6.1% or requiring antidiabetic medication. Dyslipidemia was diagnosed according to the 2007 Japan Atherosclerotic Society guidelines[2] or concurrent treatment with cholesterol-lowering medication. Metabolic syndrome was defined as waist circumference ≥85 cm for men and ≥90 cm for women as an essential component combined with ≥2 of the following components according to the 2005 definition and diagnostic criteria of metabolic syndrome in Japanese patients: TG ≥150 mg/dl and/or high-density lipoprotein (HDL) cholesterol <40 mg/dl, systolic blood pressure ≥130 mm Hg and/or diastolic blood pressure ≥85 mm Hg, and fasting blood glucose ≥110 mg/dl. All studies were

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approved by the ethics committee of Okayama University Hospital, and written informed consent was obtained from all patients before the procedure.

Venous blood samples from all 109 patients were obtained when they visited the outpatient clinic after overnight fasting for ≥8 to 12 hours. Lipid profiles and other markers were measured at SRL Company Ltd. (Tokyo, Japan). Serum levels of TG and uric acid were measured using the enzymatic method. Serum levels of low-density lipoprotein (LDL) cholesterol and HDL cholesterol were measured by direct method, and malondialdehyde-modified (MDA) LDL levels were measured using an enzyme-linked immunosorbent assay method. Concentrations of fasting plasma glucose were measured using the ultraviolet hexokinase method, and glycosylated hemoglobin levels were measured using the latex agglutination photometric immunoassay method. Concentrations of C-reactive protein and adiponectin were measured by the latex turbidimetric immunoassay method, and brain natriuretic peptide levels were measured using a chemiluminescent enzyme immunoassay method. Endothelium-dependent and endothelium-independent vascular function, as determined using flow-mediated dilation (FMD) and nitroglycerin-mediated dilation of the brachial artery, were measured by 1 skilled technician at the same time. Then, the factors associated with percentage FMD were evaluated.

Of the 109 patients, 32 patients with high serum TG levels (≥150 mg/dl) were assigned to either the ezetimibe group (statin, conventional diet and exercise, and ezetimibe; n = 16) or the no-ezetimibe group (statin and conventional diet and exercise; n = 16). Lipid profile and endothelial function were assessed at baseline and after 3 months in all patients.

Endothelium-dependent and endothelium-independent dilation was assessed as a parameter of vasodilation according to the guidelines for ultrasound assessment of FMD of the brachial artery.[3] Using a 10-MHz linear-array transducer probe (Unex Company Ltd., Nagoya, Japan), longitudinal images of the brachial artery at baseline were recorded with a stereotactic arm, and measurements of artery diameter were made after supine rest for ≥5 minutes. The diameter of the artery was measured from clear anterior (media-adventitia) and posterior (intima-media) interfaces, which were manually determined. Then, suprasystolic compression (50 mm Hg higher than systolic blood pressure) was performed at the right forearm for 5 minutes, and measurements of artery diameter were made continuously from 30 seconds before to ≥2 minutes after cuff release. After ≥10 minutes of rest from FMD measurement, artery diameter at baseline and for 5 minutes after administration of sublingual nitroglycerin 0.3 mg was also measured. Maximum vasodilation was then evaluated from the change in artery diameter after release of occlusion (percentage FMD) and after the administration of nitroglycerin (percentage nitroglycerin-mediated dilation).

Results are expressed as mean ± SD or mean ± SE. Categorical variables were compared using the chi-square test or Fisher's exact test. The Mann-Whitney U test was used to compare 2 groups. Differences in lipid profile and endothelial function between baseline and after 3 months in the 2 groups were compared using the paired Student's *t* test or Wilcoxon's signed-rank test when the variance was heterogenous. Factors independently associated with percentage FMD or change in percentage FMD (ΔFMD) were assessed using linear regression analyses, and transformed values in logarithm were used as variables when the continuous variables did not have a normal distribution. A p value <0.05 was considered statistically significant. Before assigning to either the ezetimibe or no-ezetimibe group, we performed a power calculation to determine the appropriate sample size. On the basis of the estimated FMD reported in other recent studies,[4] we assumed that the mean improvement in percent FMD was 1.4 ± 1.1%. To use a 2-sided test for differences, a minimal sample size of 11 patients was required in each group to detect statistical differences in percent FMD with 80% power an α-type error of 5% in statistical analysis.

Table 1
Univariate and multivariate linear regression analyses for predicting percent flow-mediated dilation (n = 109)

Variable	Univariate Analysis		Multivariate Analysis	
	r	p Value	β	p Value
Age (years)	−0.114	0.24		
Men	−0.222	0.02	−0.023	0.80
Body mass index	0.239	0.01	−0.021	0.82
Systolic blood pressure	−0.019	0.84		
Diastolic blood pressure	−0.021	0.83		
Baseline brachial artery diameter*	−0.368	<0.0001	−0.249	0.009
TG*	−0.410	<0.0001	−0.210	0.034
LDL cholesterol	−0.044	0.65		
HDL cholesterol*	0.379	<0.0001	0.208	0.031
MDA LDL cholesterol	−0.186	0.053		
Uric acid*	−0.215	0.02	−0.148	0.09
Fasting blood glucose*	−0.013	0.89		
Glycosylated hemoglobin*	−0.155	0.16		
C-reactive protein*	−0.059	0.54		
Estimated glomerular filtration rate	0.151	0.12		
Brain natriuretic peptide*	−0.004	0.97		
Adiponectin*	0.270	0.005	0.100	0.28

Multivariate analysis included significant factors (p <0.05) of univariate analysis.
* Logarithmically transformed.

Results

Results of univariate and multivariate linear regression analyses of factors associated with percentage FMD are listed in Table 1. In univariate analysis, male gender, body mass index, baseline brachial artery diameter, and serum levels of TG, HDL cholesterol, uric acid, and adiponectin were significantly associated with percentage FMD in patients with CHD during statin therapy. Moreover, multivariate linear regression analysis demonstrated that serum TG and HDL cholesterol levels, in addition to baseline brachial artery diameter, were independent determinants of percent

Table 2
Baseline clinical characteristics and endothelial function in patients with normal and high triglyceride levels

Variable	TG Level		p Value
	Normal (n = 77)	High (n = 32)	
Age (years)	69 ± 9	67 ± 8	0.22
Men	57 (74%)	28 (88%)	0.27
Body mass index (kg/m^2)	23.7 ± 3.8	25.3 ± 3.3	0.047
Hypertension*	56 (73%)	26 (81%)	0.48
Diabetes mellitus	37 (48%)	18 (56%)	0.50
Dyslipidemia†	77 (100%)	32 (100%)	>0.99
Current or previous smoker	51 (66%)	27 (84%)	0.14
Metabolic syndrome	29 (38%)	22 (69%)	0.01
Angina pectoris/healed myocardial infarction	62/15 (81/19%)	21/11 (34/66%)	0.22
Multivessel coronary disease	36 (47%)	21 (66%)	0.12
Coronary artery bypass grafting	3 (4%)	3 (9%)	0.65
Cerebral vascular disease	13 (17%)	4 (13%)	0.72
Peripheral artery disease	6 (8%)	3 (9%)	0.90
Medications			
Antiplatelet agents	77 (100%)	32 (100%)	>0.99
Angiotensin-converting enzyme inhibitors or angiotensin II type 1 receptor blockers	60 (78%)	25 (78%)	0.99
β blockers	43 (56%)	16 (50%)	0.63
Statins	77 (100%)	32 (100%)	>0.99
Strong statin use‡	62 (81%)	31 (97%)	0.18
High dose/normal dose§	22/55 (29/71%)	7/25 (22/78%)	0.58
Calcium antagonists	33 (43%)	14 (44%)	0.94
Nitrates	10 (13%)	4 (13%)	0.97
Eicosapentaenoic acid	10 (13%)	6 (19%)	0.64
Sulfonylurea	12 (16%)	3 (9%)	0.61
Pioglitazone	21 (27%)	7 (22%)	0.66
Insulin	7 (9%)	6 (19%)	0.43
Laboratory data			
TG (mg/dl)	97 ± 28	206 ± 50	<0.0001
LDL cholesterol (mg/dl)	83 ± 24	92 ± 22	0.06
HDL cholesterol (mg/dl)	46 ± 12	38 ± 7	0.0002
MDA LDL cholesterol (U/L)	89 ± 34	106 ± 32	0.0096
Uric acid (mg/dl)	6.0 ± 1.6	6.5 ± 1.9	0.10
Fasting blood glucose (mg/dl)	122 ± 44	141 ± 60	0.038
Glycosylated hemoglobin (%)	5.9 ± 0.7	6.4 ± 1.4	0.27
C-reactive protein (mg/dl)	0.34 ± 0.66	0.19 ± 0.25	0.90
Brain natriuretic peptide (pg/ml)	83 ± 84	101 ± 100	0.50
Adiponectin (μg/ml)	12.0 ± 5.7	8.8 ± 5.1	0.005
Estimated glomerular filtration rate (ml/min/1.73 m^2)	62.3 ± 21.6	59.6 ± 21.5	0.61
Endothelial function			
Baseline brachial artery diameter (mm)	4.01 ± 0.52	4.12 ± 0.46	0.41
Maximum diameter after hyperemia (mm)	4.18 ± 0.53	4.23 ± 0.45	0.99
Maximum diameter after nitroglycerin (mm)	4.70 ± 0.63	4.80 ± 0.47	0.45
FMD (%)	4.4 ± 1.6	3.0 ± 1.1	<0.0001
Nitroglycerin-mediated dilation (%)	16.4 ± 5.2	16.0 ± 3.8	0.71

Data are expressed as mean ± SD or as number (percentage).

* Systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, as defined by the 1999 World Health Organization and International Society of Hypertension guidelines or concurrent treatment with antihypertensive medication.

† LDL cholesterol ≥140 mg/dl or HDL cholesterol <40 mg/dl or TG ≥150 mg/dl, as defined by the 2007 Japan Atherosclerotic Society guidelines or concurrent treatment with cholesterol-lowering medication.

‡ Atorvastatin, rosuvastatin, and pitavastatin.

§ High dose indicates the maximum dose of statin approved in Japan (atorvastatin 20 mg, rosuvastatin 20 mg, pitavastatin 4 mg, pravastatin 20 mg).

FMD (TG: $\beta = -0.210$, $p < 0.05$; HDL cholesterol: $\beta = 0.208$, $p < 0.05$).

Of the 109 patients, 83 (76%) achieved target LDL cholesterol levels (<100 mg/dl) recommended by the 2006 American Heart Association and American College of Cardiology guidelines for secondary prevention.[5] In contrast, 32 patients (29%) had high serum TG levels (≥150 mg/dl). The patients were divided into 2 groups according to serum TG level: normal (<150 mg/dl; n = 77) and high (≥150 mg/dl; n = 32) TG. Baseline patient characteristics, laboratory data, and endothelial function are listed in Table 2. Obesity and metabolic syndrome were more prevalent in the

Table 3
Baseline clinical characteristics in the ezetimibe and no-ezetimibe groups

Variable	Ezetimibe (n = 15)	No-Ezetimibe (n = 15)	p Value
Age (years)	66 ± 6	69 ± 10	0.21
Men	13 (87%)	13 (87%)	>0.99
Body mass index (kg/m^2)	25.2 ± 2.4	24.9 ± 3.8	0.37
Hypertension	13 (87%)	13 (87%)	>0.99
Diabetes mellitus	9 (60%)	7 (47%)	0.53
Current or previous smoker	14 (93%)	12 (80%)	0.53
Metabolic syndrome	11 (73%)	9 (60%)	0.53
Angina pectoris/healed myocardial infarction	11/4 (73/27%)	9/6 (60/40%)	0.53
Multivessel coronary disease	11 (73%)	8 (53%)	0.35
Medications			
Antiplatelet agents	15 (100%)	15 (100%)	>0.99
Angiotensin-converting enzyme inhibitors or angiotensin II type 1 receptor blockers	14 (93%)	10 (67%)	0.21
β blockers	7 (47%)	8 (53%)	0.76
Statin	15 (100%)	15 (100%)	>0.99
Strong statin use	14 (93%)	15 (100%)	0.76
High dose/normal dose	2/13 (13/87%)	4/11 (27/73%)	0.53
Calcium antagonists	6 (40%)	7 (47%)	0.76
Nitrates	3 (20%)	1 (7%)	0.53
Eicosapentaenoic acid	3 (20%)	3 (20%)	>0.99
Sulfonylurea	3 (20%)	0 (0%)	0.35
Pioglitazone	6 (40%)	1 (7%)	0.12
Insulin	3 (20%)	2 (13%)	0.76

Data are expressed as mean ± SD or as number (percentage).

high-TG group (body mass index p <0.05, metabolic syndrome p <0.05). Regarding the laboratory data, mean serum TG, MDA LDL, and plasma blood glucose levels were significantly higher and mean serum HDL cholesterol and adiponectin levels were significantly lower in the high-TG group than in the normal-TG group. In addition, endothelial function assessed by percentage FMD was severely impaired in the high-TG group compared to that in the normal-TG group (p <0.0001). In contrast, percentage nitroglycerin-mediated dilation, endothelium-independent vasodilation reflecting vascular smooth muscle function, was not significantly different between the 2 groups.

Two of the 32 patients with high serum TG levels, including 1 patient in the ezetimibe group who stopped taking ezetimibe without permission and 1 patient in the no-ezetimibe group who started taking an antihypertensive medication, were withdrawn. Thus, a total of 30 patients (15 in each group) were available for analysis. No adverse events occurred during the study. At baseline, patient characteristics did not differ between the 2 groups (Table 3). Table 4 lists the laboratory profiles and endothelial function at baseline and after 3 months in the 2 groups. There were no significant differences between baseline laboratory data for the ezetimibe and no-ezetimibe groups. The lipid profile was not changed after 3 months in the no-ezetimibe group, but serum TG, LDL cholesterol, and MDA LDL levels decreased significantly after 3 months in the ezetimibe add-on group. As shown in Figure 1, percentage changes in serum TG, LDL cholesterol, and MDA LDL levels in the ezetimibe add-on group were significantly greater than those in the no-ezetimibe group (TG p <0.05, LDL cholesterol p <0.001, MDA LDL p <0.0001). Regarding endothelial function, percentage FMD increased significantly in the ezetimibe add-on group (from 3.3 ± 1.1% to 4.0 ± 1.1%, p <0.005), whereas no significant change was observed in the no-ezetimibe group (from 2.8 ± 1.0% to 2.9 ± 0.9%, p = 0.36) (Figure 1). Table 5 lists the results of univariate and multivariate linear regression analyses of factors associated with ΔFMD. In the ezetimibe and no-ezetimibe groups, ΔFMD was significantly associated with changes in serum TG ($\beta = -0.387$, p <0.05) and MDA LDL ($\beta = -0.363$, p <0.05) independent of change in serum LDL cholesterol and other profiles in multivariate analysis.

Discussion

The present study first demonstrated that serum TG and HDL cholesterol levels were independently associated with endothelial dysfunction in patients with CHD during statin therapy. We also showed that coadministration of ezetimibe improved endothelial function, which was significantly associated with TG reduction independent of serum LDL cholesterol levels, in CHD patients with hypertriglyceridemia.

Some previous studies of patients without CHD have shown that hypertriglyceridemia is closely associated with brachial artery endothelial dysfunction,[6,7] whereas other studies have failed to show a relation.[8] Schnell et al[8] demonstrated the negative finding that modest elevations of TG do not affect brachial artery endothelium-dependent vasodilation. However, that study was based on subjects free from other cardiac risk factors, indicating that synergism with several risk factors may be required to impair endothelial function. In the present study, patients with high TG levels had a high prevalence of obesity and metabolic syndrome, suggesting that these metabolic conditions characterized by glucose intolerance and atherogenic dyslipidemia may strongly affect endothelial function. The underlying mechanism on the adverse effects of hypertriglyceridemia may be explained several ways. Hypertriglyceridemia is associated with leukocyte activation, which involves the adhesion of monocytes to the endothelium with subsequent transmigration into the vascular wall.[9] Moreover, TG-rich lipoproteins have been shown to upregulate the expression of proinflammatory cytokines on the endothelium[10] and contribute to endothelial dysfunction via the generation of reactive oxygen species.[11] The results of these studies support our finding of serum TG level being an independent determinant of FMD and indicate enhanced therapeutic potential for reducing serum TG levels even when LDL cholesterol target levels have been achieved.

New or high-dose statin therapy can often reduce serum LDL cholesterol and TG levels but is sometimes still inadequate. Indeed, in our study, about 30% of the patients still had high serum TG levels even after the continuous administration of a statin for >3 months. Moreover, subjects with elevated TG had lower HDL cholesterol and higher oxidized LDL cholesterol levels, and the same results were

Table 4
Laboratory data and endothelial function at baseline and after 3 months in the ezetimibe and no-ezetimibe groups

Variable	Ezetimibe Group			No-Ezetimibe Group		
	Baseline	3 Months	p Value	Baseline	3 Months	p Value
Laboratory data						
TG (mg/dl)	213 ± 43	175 ± 43	0.0022	199 ± 55	191 ± 52	0.29
LDL cholesterol (mg/dl)	91 ± 12	77 ± 11	0.0007	90 ± 29	92 ± 34	0.75
HDL cholesterol (mg/dl)	39 ± 9	40 ± 8	0.21	37 ± 5	38 ± 7	0.41
MDA LDL cholesterol (U/L)	102 ± 29	86 ± 22	0.0007	103 ± 30	104 ± 28	0.78
Uric acid (mg/dl)	6.2 ± 2.1	5.9 ± 1.9	0.09	6.5 ± 1.5	6.4 ± 0.9	0.85
Fasting blood glucose (mg/dl)	141 ± 36	133 ± 30	0.13	139 ± 79	131 ± 64	0.26
Glycosylated hemoglobin (%)	6.7 ± 1.7	6.5 ± 1.6	0.23	5.9 ± 0.8	6.0 ± 0.8	0.55
C-reactive protein (mg/dl)	0.17 ± 0.17	0.12 ± 0.10	0.08	0.15 ± 0.14	0.20 ± 0.22	0.66
Adiponectin (μg/ml)	8.7 ± 5.7	8.8 ± 5.4	0.26	8.9 ± 4.9	9.0 ± 4.7	0.73
Endothelial function						
Baseline brachial artery diameter (mm)	4.03 ± 0.51	4.06 ± 0.49	0.28	4.16 ± 0.42	4.13 ± 0.41	0.20
FMD (%)	3.3 ± 1.1	4.0 ± 1.1	0.0038	2.8 ± 1.0	2.9 ± 0.9*	0.36
Nitroglycerin-mediated dilation (%)	16.1 ± 3.8	16.8 ± 3.5	0.08	16.1 ± 3.8	16.3 ± 3.3	0.69

Data are expressed as mean ± SD.
* p <0.005 comparison to the 2 groups after 3 months.

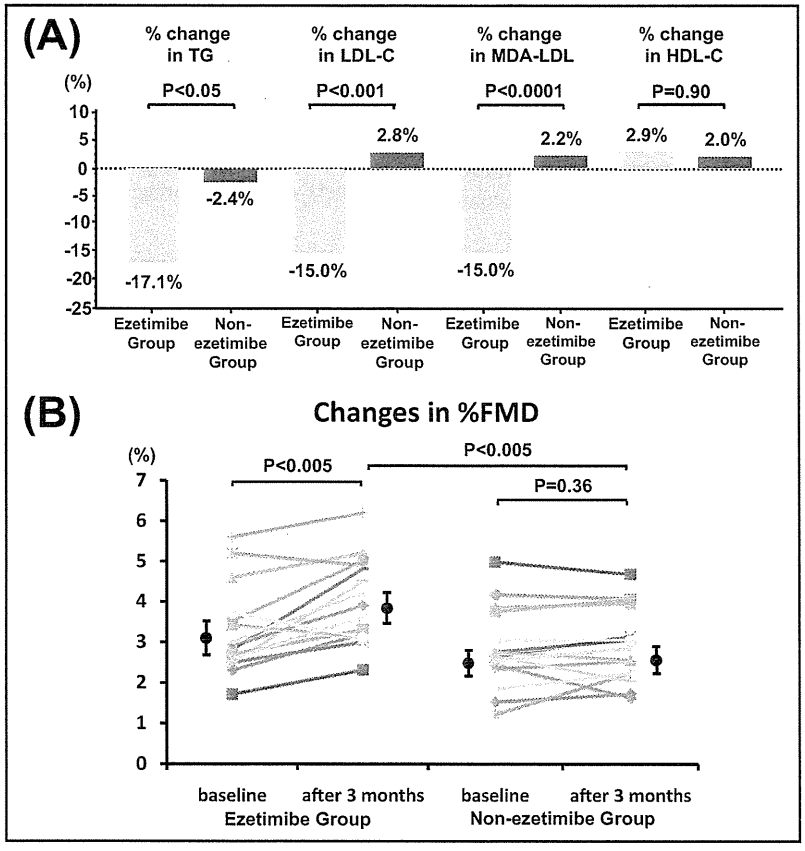

Figure 1. *(A)* Percentage changes in lipid profile in the ezetimibe and no-ezetimibe groups. *(B)* Changes in endothelial function assessed by FMD of the brachial artery between baseline and after 3 months in the ezetimibe and no-ezetimibe groups. Data are expressed as mean ± SE.

obtained in a recent study.[12] Ezetimibe is 1 of the potent agents currently available for residual risk factors. A recent clinical trial by Masuda et al[13] showed that ezetimibe reduces fasting and postprandial hypertriglyceridemia in patients with type IIb hyperlipemia. Regarding endothelial function, ezetimibe monotherapy or combination therapy with a statin has a beneficial effect on fasting or postprandial endothelial function in patients with metabolic syndrome or dysglycemia.[14–17] In the present study, we demonstrated that ezetimibe add-on therapy resulted in significantly greater reduction in TG than that in previous studies in which subjects with not only high TG concentrations but also normal TG concentrations were enrolled.[18,19] Moreover, reduction of serum TG level was independently

Table 5
Univariate and multivariate linear regression analyses for predicting change in flow-mediated dilation (n = 30)

Variable	Univariate Analysis		Multivariate Analysis	
	r	p Value	β	p Value
Change in body mass index	0.046	0.81		
Change in TG	−0.520	0.0032	−0.387	0.0243
Change in LDL cholesterol	−0.212	0.26		
Change in HDL cholesterol	0.023	0.91		
Change in MDA LDL cholesterol	−0.505	0.0045	−0.363	0.0339
Change in uric acid	−0.194	0.30		
Change in fasting blood glucose	0.016	0.93		
Change in glycosylated hemoglobin	−0.357	0.0531		
Change in C-reactive protein	−0.120	0.53		
Change in adiponectin	0.251	0.18		

Multivariate analysis included significant factors ($p < 0.05$) in univariate analysis.

associated with improvement in endothelial function. These findings suggest that ezetimibe has a beneficial effect mostly in patients with high baseline TG levels, many of whom have postprandial hyperlipemia and glucose intolerance. Thus, combination therapy with ezetimibe and a statin could be a potent therapy in these high-risk patients.

Recently, the Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction (PROVE-IT–TIMI 22) trial showed that on-treatment TG level <150 mg/dl was independently associated with a lower risk for recurrent CHD events after acute coronary syndromes.[20] In that study, each 10 mg/dl decrease in on-treatment TG reduced the risk for adverse cardiovascular events by 1.6%. Moreover, a previous study demonstrated that improvement in FMD after 6 months of optimized therapy was associated with adverse outcomes in CHD patients.[21] FMD values are affected by numerous factors, and our study was a short-term follow-up. Therefore, further large-scale, long-term clinical trials are needed to assess the relation between ezetimibe therapy and clinical outcomes in high-risk populations.

Unexpectedly, the administration of ezetimibe did not increase HDL cholesterol levels in the present study; however, previous clinical trials have shown that ezetimibe therapy increases HDL cholesterol levels significantly.[18,19,22] In addition, several studies have indicated possible mechanisms that may contribute to HDL-induced improvement of endothelial function[23,24] and have shown that HDL-increasing pharmacologic therapy improves endothelial function, especially in patients with low baseline HDL cholesterol levels.[25] These data suggest that improvement of HDL cholesterol might also have a beneficial effect on FMD. Moreover, in our study, improvement of FMD after ezetimibe coadministration was paralleled by a reduction of MDA LDL levels. Ezetimibe has been shown to prolong the lag time of LDL cholesterol oxidation when used as monotherapy and in combination with a statin[26] and to reduce the uptake of oxidized LDL cholesterol by human monocyte-derived macrophages.[27] These findings suggest that the improvement of endothelial function by ezetimibe coadministration was mediated, at least in part, by suppression of an oxidant mechanism. Further studies are needed to clarify this point.

There were several important limitations of our study. First, this study was an open-label, nonrandomized, small-scale study. Therefore, a degree of patient selection bias might have occurred. If the 2 patients excluded from the study could have been included, the results of our study would be more persuasive. However, these potential limitations do not alter the conclusion. Second, recent studies have shown that nonfasting TG levels are better for predicting future cardiovascular events than are fasting TG levels.[28] Atherosclerosis may be a postprandial phenomenon in which remnant lipoproteins play a dominant role; however, fasting TG measurements may be more reliable because of controlled conditions. Third, exercise and diet therapy as well as several drugs often affect FMD, but we did not assess exercise habit and lifestyle in this study. This was due to difficulties associated with making an accurate evaluation for each outpatient. Therefore, the possibility of an influence of these factors on FMD cannot be ruled out. However, patients continued optimal management for >3 months before the study and tried not to change their lifestyles and not to receive other drugs during the study to minimize the effects of factors other than ezetimibe.

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ORIGINAL ARTICLE
Heart Failure

The Role of Echocardiography in Predicting Responders to Cardiac Resynchronization Therapy

– Results From the Japan Cardiac Resynchronization Therapy Registry Trial (J-CRT) –

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Background: This multicenter prospective cohort study aimed to identify both ability of echocardiographic parameters to detect cardiac resynchronization therapy (CRT) volume responders and relation of these parameters with clinical outcomes.

Methods and Results: CRT responder was defined as ≥15% reduction of left ventricular (LV) end-systolic volume at 6 months. Seven echocardiographic dyssynchrony parameters were evaluated. The clinical endpoint comprised time to death from any cause or unplanned hospitalization for a major cardiovascular event. Of the 217 patients enrolled, 63 percent were classified as volume responders, in whom significantly fewer events occurred than in non-responders (log rank, P<0.001). No single echocardiographic criterion had significant power to detect volume responders, but a combining measurement of dyssynchrony between septum and LV free wall with M-mode and tissue Doppler imaging was independently associated with volume responders. In addition, this combined parameter was associated with the endpoint (hazard ratio, 0.66, 95% confidence interval 0.30–0.98, P=0.04). In contrast, left bundle branch block was identified as an independent predictor of volume responders and more strongly associated with the endpoint (hazard ratio, 0.38, 95% confidence interval 0.20–0.72, P=0.003).

Conclusions: Echocardiographic parameters did not show significant power to detect CRT responders independently. (*Circ J* 2011; **75:** 1156–1163)

Key Words: Cardiac resynchronization therapy; Echocardiography; Heart failure

Cardiac resynchronization therapy (CRT) by means of bi-ventricular pacing has been established by large clinical trials as a non-pharmacologic therapy for patients with drug-refractory heart failure and dyssynchrony.[1–3] These studies showed that CRT significantly improves heart failure symptoms, quality of life, exercise tolerance, and left ventricular (LV) systolic performance. The indication of CRT has been extended even in pediatrics.[4] However, approximately 30% of patients do not respond to bi-ventricular pacing, and inadequate selection criteria for identifying potential responders based on QRS duration result in a high rate of non-responders.[5,6] Alternatively, numerous Doppler echocardiographic parameters have been proposed to improve prediction of CRT responders, and echocardiography has had a central role in this prediction.[7–11] However, some limitations of echocardiography to predict CRT responders are causes of concern. First, echocardiographic parameters have been reported from single-center studies. Recently, the results of the

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Participating investigators and study centers of J-CRT are listed in the Appendix.

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Table 1. Dyssynchrony Parameters and Cut-Off Values to Predict CRT Responders

Dyssynchrony location	Parameter	Cutoff value
Intraventricular	SPWMD: the shortest interval between the maximal posterior displacement of the septum and the maximal displacement of the left posterior wall. Anatomical M-mode imaging should not be used to measure SPWMD.	>130 ms
Intraventricular	12Ts-SD: standard deviation of time from QRS to the largest peak systolic velocity in ejection phase for 12 LV segments.	>34.4 ms
Intraventricular	Ts (lateral-septal): delay between time to the largest peak systolic velocity in ejection phase at basal septal and lateral segments.	>65 ms
Interventricular	LV-PEP defined as the duration from onset of QRS to onset of pulsed Doppler LV out flow.	>140 ms
Interventricular	IMD defined as the difference between LV-PEP and RV-PEP. IMD=LV-PEP−RV-PEP	>40 ms
Intra- and interventricular	Sum of asynchrony: sum of LV asynchrony and LV-RV asynchrony measured by the time from QRS to regional onset of contraction (EMCT) with tissue pulsed Doppler. LV asynchrony; the difference between the maximum and the minimum EMCT in LV basal lateral, septal, and posterior wall. LV-RV asynchrony; the difference between the EMCT in the RV free wall and the maximum EMCT in LV basal lateral, septal, and posterior wall.	>102 ms
Atrioventricular	DFT/RR: LV diastolic filling time measured by Doppler transmitral flow to cardiac cycle length.	<40%

CRT, cardiac resynchronization therapy; SPWMD, septal-to-posterior wall motion delay; LV, left ventricular; PEP, pre-ejection period; IMD, interventricular mechanical delay; RV, right ventricular; EMCT, electromechanical coupling time.

Predictors of Response to CRT (PROSPECT) trial, a multicenter prospective cohort study to evaluate selected, predefined baseline echocardiographic parameters for their ability to predict clinical and echocardiographic response to CRT, were published.[1,2] The PROSPECT study showed that no single echocardiographic measure of dyssynchrony had the ability to predict CRT responders. However, the PROSPECT trial has been the only such multicenter trial to date. Therefore, the predictive ability of echocardiographic parameters requires verification by further studies. In addition, an appropriate method to evaluate the effects of CRT has not been standardized. Because LV reverse remodeling using LV end-systolic volume reduction has been used as a surrogate of CRT responders, identification of predictors of volume responders has been a focal issue. However, the role of volume responders as a surrogate of clinical outcomes has not been confirmed in multiple cohort studies. Finally, although clinical outcomes after CRT have been used as endpoints in large trials and associated factors unrelated to echocardiographic parameters have been identified, the relation between predefined baseline echocardiographic parameters and clinical outcomes has not been determined.[1–3,5] Considering these unresolved issues, a multicenter prospective cohort study was conducted in Japan to assess the following: (1) the ability of predefined baseline echocardiographic dyssynchrony parameters to predict volume responders after CRT, (2) implications of volume responders as a surrogate of clinical outcomes, and (3) the ability of baseline echocardiographic dyssynchrony parameters to predict clinical outcomes after CRT.

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Methods

The Japan Cardiac Resynchronization Therapy Registry Trial (J-CRT) was a multicenter prospective cohort study of patients undergoing CRT in Japan. The study was approved by the local ethics committee of each participating institution. All patients provided their written informed consent. A 2-day workshop to acquaint participating echocardiologists and electrophysiologists with the study protocol and echocardiographic recording was performed by the J-CRT Committee in August 2005, in Tokyo.

Study Population

Patient inclusion criteria included presence of congestive heart failure refractory to optimal medical therapy and QRS duration ≥120 ms, New York Heart Association (NYHA) class III or IV, and LV ejection fraction (LVEF) ≤35%. Patients were excluded if they were expected to die within 1 year because of non-cardiac disease, if they were scheduled for catheter intervention or cardiac surgery including cardiotomy or coronary bypass, or if they were expected to be lost to follow-up during the first year after CRT. Patients were scheduled to undergo Doppler echocardiographic studies with NYHA functional class assessment and measurements before, and at 1 week, 6 months, and 12 months after CRT. Patients were followed up for at least 6 months.

CRT Responders and Clinical Endpoints

A volume responder to CRT was defined as a patient with ≥15% reduction of LV end-systolic volume at 6 months after CRT.[12] Clinical outcomes were assessed with the following endpoint: a composite of death from any cause or an unplanned hospitalization for a major cardiovascular event.

Doppler Echocardiography and Dyssynchrony Parameters

The echocardiographic measurements were performed by cardiologists or well trained sonographers in each individual center in a prospective way, not knowing the patients' outcome. In Doppler echocardiographic examinations, LV volumes, LV dimensions, LV wall thickness, left atrial (LA) dimension, Doppler-derived parameters of LV diastolic function, mitral regurgitation severity as the ratio of color Doppler mitral regurgitant jet area to LA area, and pressure gradients derived from tricuspid regurgitant flow were assessed by standard methods. In this study, 7 dyssynchrony parameters,

Table 2. Baseline Patient Characteristics

Characteristic	All (n=217)	Responder (n=130)	Non-responder (n=77)	P value*
Age, years	65±12	67±12	63±12	0.01
Male, n (%)	149 (69)	90 (69)	53 (69)	0.94
Ischemic/non-ischemic, n (%)	63 (29)/154 (71)	39 (30)/91 (70)	22 (29)/55 (71)	0.83
NYHA class III/IV, n (%)	194 (91)/20 (9)	119 (92)/11 (8)	71 (92)/6 (8)	0.87
Basic rhythm, n (%)				0.81
Sinus	143 (67)	85 (65)	50 (65)	
Atrial fibrillation	27 (13)	15 (12)	11 (14)	
Pacemaker	39 (18)	26 (20)	15 (20)	
Other	5 (2)	4 (3)	1 (1)	
PQ interval, ms, n=156	192±47	186±44 (n=90)	199±51 (n=57)	0.13
QRS duration, ms	160±31	159±29	165±33	0.21
Heart rate, beats/min	73±15	73±16	71±13	0.36
Systolic blood pressure, mmHg	109±19	110±16	107±21	0.18
Diastolic blood pressure, mmHg	64±11	64±12	63±10	0.49
Conduction disorder, n (%)				0.002
LBBB	133 (61)	92 (71)	37 (48)	
RBBB	23 (11)	8 (6)	14 (18)	
Non-specific	61 (28)	30 (23)	26 (34)	
Primary ventricular tachyarrhythmia, n (%)				0.67
Sustained VT	30 (14)	19 (15)	8 (10)	
Non-sustained VT	73 (34)	44 (34)	27 (35)	
Ventricular fibrillation	11 (5)	6 (5)	4 (5)	
Other arrhythmia, n (%)				0.46
Paroxysmal atrial fibrillation	30 (14)	15 (12)	15 (20)	
Chronic atrial fibrillation	25 (12)	15 (12)	8 (11)	
Atrial flutter	8 (4)	3 (2)	4 (5)	
BNP, pg/ml	693±766	670±757	612±505	0.56
Medication, n (%)				
Intravenous inotropes	20 (9)	3 (2)	14 (18)	<0.001
β-blocker	164 (76)	87 (67)	69 (90)	<0.001
ACE-I or ARB	177 (82)	106 (82)	64 (83)	0.77
Loop diuretics	175 (81)	105 (81)	63 (82)	0.85
Spironolactone	125 (58)	70 (53)	50 (65)	0.11
Other diuretics	18 (8)	9 (6)	9 (12)	0.23
Oral inotropes	22 (10)	8 (6)	12 (16)	0.02
Digitalis	49 (23)	31 (23)	16 (20)	0.61
Statins	56 (26)	38 (29)	14 (18)	0.07

*Responder vs. non-responder.
NYHA, New York Heart Association; LBBB, left bundle branch block; RBBB, right bundle branch block; VT, ventricular tachycardia; BNP, B-type natriuretic peptide; ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blocker.

which are summarized in Table 1, were assessed for their ability to identify CRT responders.[7–11]

Statistical Analysis

Data are presented as mean±standard deviation or percentages. Comparisons between groups were performed with unpaired Student's t-test used for continuous variables and χ^2 tests used for categorical variables. We assessed the performance of each dyssynchrony parameter to predict volume responders and ≥1 class improvement of NYHA class at 6 months after CRT using the area under the curve of the receiver operating characteristic curve (AUC). Independent determinants of the volume responders were assessed by logistic regression analysis. The risk of clinical endpoints was determined with Cox proportional hazard models. The univariate factors with a value of P<0.05 and age and male sex were entered into the multivariable model to assess the impact of the parameters on the endpoint. Kaplan–Meier analysis was done to determine the influence of volume responders on the endpoint. A P-value <0.05 was considered to indicate statistical significance. Analyses were performed with SPSS version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Baseline Characteristics

The J-CRT trial enrolled 225 patients who were initially implanted with CRT devices in 18 Japanese centers. Enrollment began in April 2006 and ended in July 2008, and 6-month follow-up was completed in March 2009. In the entire cohort,

Table 3. Baseline Echocardiographic Parameters

Parameter	All (n=217)	Responder (n=130)	Non-responder (n=77)	P value*
LVESV (ml)	160±80	162±83	160±75*	0.96
LVEDV (ml)	218±111	214±95	211±84*	0.84
LVEF (%)	26±9	26±10	25±10	0.53
LVDs (mm)	58±11	58±11	60±11	0.16
LVDd (mm)	67±11	66±11	68±11	0.24
IVSth (mm)	9.1±2.5	9.4±2.5	8.7±2.6	0.06
PWth (mm)	9.7±2.1	9.8±1.8	9.7±2.4	0.41
LA dimension (mm)	44±8	44±8	45±8	0.45
E (cm/s)	74±30 (n=203)	76±32 (n=125)	73±28 (n=69)	0.42
A (cm/s)	67±33 (n=155)	72±33 (n=93)	61±30 (n=54)	0.05
E/A	1.5±1.4 (n=152)	1.4±1.5 (n=92)	1.6±1.4 (n=52)	0.59
DT (ms)	196±81 (n=196)	203±88 (n=120)	185±70 (n=68)	0.22
E' (cm/s)	4.4±2.0 (n=189)	4.5±2.0 (n=116)	4.4±1.9 (n=65)	0.75
E/E'	19±12 (n=191)	20±13 (n=115)	18±8 (n=67)	0.37
MR index (%)	24±17 (n=156)	24±19 (n=98)	22±13 (n=52)	0.63
TR (mmHg)	29±12 (n=156)	27±10 (n=91)	32±13 (n=58)	0.03

*Responder vs. non-responder.
LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVDs, left ventricular dimension at end systole; LVDd, left ventricular dimension at end diastole; IVSth, intraventricular septal thickness; PWth, posterior wall thickness; LA, left atrial; E, early diastolic peak velocity of Doppler transmitral flow; A, peak velocity at atrial contraction of Doppler transmitral flow; E/A, ratio of E to A; DT, deceleration time of E; E', early diastolic mitral annular velocity; E/E', ratio of E to E'; MR, mitral regurgitation; TR, tricuspid regurgitation.

Table 4. Comparisons of Dyssynchrony Parameter Values and Numbers of Evaluable Cases

Dyssynchrony parameters	Mean±SD (range), evaluable cases (%)			P value*
	All (n=217)	Responder (n=130)	Non-responder (n=77)	
SPWMD (ms)	162±104 (0–437), 203 (94)	188±109 (0–437), 123 (95)	132±89 (0–383), 70 (91)	<0.001, 0.24
LV-PEP (ms)	146±46 (4–283), 200 (92)	146±45 (40–256), 118 (91)	138±47 (4–283), 73 (95)	0.22, 0.29
IMD (ms)	45±28 (–92–133), 199 (92)	48±29 (0–133), 120 (92)	32±30 (–92–96), 72 (94)	0.001, 0.47
DFT/RR (%)	44±11 (13–72), 193 (89)	44±11 (13–70), 116 (89)	44±11 (13–72), 68 (88)	0.50, 0.88
12Ts-SD (ms)	47±23 (10–169), 170 (78)	48±24 (10–169), 102 (78)	44±21 (10–121), 61 (79)	0.27, 0.86
Ts (lateral-septal) (ms)	69±55 (0–289), 200 (92)	77±61 (0–289), 119 (92)	57±44 (0–208), 71 (92)	0.02, 0.87
Sum of asynchrony (ms)	128±97 (8–608), 135 (62)	129±99 (12–608), 79 (61)	127±86 (25–394), 49 (64)	0.93, 0.68

*Responder vs. non-responder.
Abbreviations as in Table 1.

8 patients (3.6%) were excluded because of CRT discontinuation immediately after implantation (n=3), withdrawal of consent (n=1), and incomplete data (n=4). Ultimately, 217 patients formed the final study group of the present report (Table 2). Almost all patients were NYHA class III, and the majority of the patients had non-ischemic heart disease, a history of non-sustained ventricular tachycardia, and left bundle branch block (LBBB).

Clinical Outcomes

The mean duration of follow-up was 288±177 days (range, 13 to 608 days). By the end of the study, 51 patients had reached the endpoint of a composite of death from any cause or unplanned hospitalization for a major cardiovascular event. Deaths occurred in 19 patients (9%): 11 patients died of heart failure, 6 died of non-cardiac death, and only 2 patients died from sudden cardiac death. Unplanned hospitalizations occurred in 23 patients for heart failure and in 9 patients for other cardiovascular events.

Volume Responder Study

LV volume measurements at 6 months were completed in 207 of 217 patients. Six-month data in the remaining 10 patients could not be assessed due to death in 9 patients (from heart failure in 4, sudden cardiac death in 1, and non-cardiac death in 4 and to loss to follow-up in 1 patient with cerebral infarction.

Table 5. Ability of Dyssynchrony Parameters to Predict Volume Responders

Dyssynchrony parameters	Sensitivity	Specificity	Positive predictive value	AUC
SPWMD >130 ms	0.65	0.51	0.71	0.65
LV-PEP >140 ms	0.57	0.50	0.64	0.56
IMD >40 ms	0.60	0.56	0.69	0.64
DFT/RR <40%	0.29	0.66	0.59	0.53
12Ts-SD >34.4 ms	0.70	0.37	0.66	0.55
Ts (lateral-septal) >65 ms	0.50	0.59	0.67	0.59
Sum of asynchrony >102 ms	0.50	0.47	0.60	0.50

AUC, area under the curve. Other abbreviations as in Table 1.

Table 6. Ability of Dyssynchrony Parameters to Predict NYHA Class Improvements

Dyssynchrony parameters	Sensitivity	Specificity	Positive predictive value	AUC
SPWMD >130 ms	0.61	0.66	0.64	0.68
LV-PEP >140 ms	0.58	0.64	0.62	0.59
IMD >40 ms	0.50	0.62	0.61	0.55
DFT/RR <40%	0.70	0.46	0.56	0.60
12Ts-SD >34.4 ms	0.68	0.41	0.54	0.62
Ts (lateral-septal) >65 ms	0.49	0.66	0.59	0.60
Sum of asynchrony >102 ms	0.54	0.62	0.59	0.60

Abbreviations as in Tables 1,2,5.

Table 7. Logistic Regression Analysis for Predefined Predictors of Volume Responders

Characteristic	Univariate		Multivariate model 1		Multivariate model 2	
	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value
Age, years	1.02 (0.99–1.04)	0.05	1.01 (0.89–1.05)	0.26	1.02 (0.99–1.05)	0.16
Sex, male	0.98 (0.53–1.81)	0.94				
Baseline NYHA class IV	0.81 (0.32–2.04)	0.66				
Ischemic cardiomyopathy	1.12 (0.64–2.12)	0.61				
QRS duration >150 ms	1.23 (0.74–2.26)	0.42				
PQ interval, ms	0.99 (0.99–1.02)	0.14				
LBBB	2.72 (1.51–4.89)	<0.001	2.91 (1.47–5.79)	0.02	2.58 (1.34–4.98)	0.004
Baseline BNP >350 pg/ml	1.34 (0.76–2.34)	0.31				
Intravenous inotropes	0.11 (0.03–0.38)	<0.001	0.07 (0.01–0.30)	<0.001	0.11 (0.03–0.43)	<0.001
β-blocker	0.24 (0.10–0.53)	<0.001	0.21 (0.08–0.57)	0.002	0.20 (0.09–0.53)	0.001
LVEDV, ml	1.00 (0.98–1.01)	0.76				
LVEF, %	1.01 (0.98–1.04)	0.52				
E/E'	1.01 (0.98–1.03)	0.46				
SP WMD >130 ms	2.12 (1.16–3.83)	0.01	1.71 (0.86–3.38)	0.12		
PEP >140 ms	1.38 (0.76–2.52)	0.29				
IMD >40 ms	1.91 (0.98–3.64)	0.06				
DFT/RR <40%	0.79 (0.41–1.53)	0.49				
12Ts-SD >34.4 ms	1.41 (0.71–2.77)	0.32				
Ts (lateral-septal) >65 ms	1.65 (0.93–2.90)	0.08				
Sum of asynchrony >102 ms	0.91 (0.44–1.85)	0.73				
Combined SPWMD and Ts (lateral-septal)	2.53 (1.35–4.79)	0.004			2.19 (1.05–4.56)	0.03

HR, hazard ratio; CI, confidence interval; Combined SPWMD and Ts (lateral-septal), a combined dyssynchrony criterion that requires at least one of SPWMD >130 ms or Ts (lateral-septal) >65 ms.
Other abbreviations as in Tables 1,2.

Of the 207 patients, 130 (63%) were identified as volume responders to CRT. Comparisons of baseline patient characteristics between the responder group and non-responder group are summarized in Table 2. Patients in the responder group were older and showed greater prevalence of LBBB compared to those in the non-responder group. The prevalence of patients being administered intravenous or oral inotropes was higher in the non-responder group, whereas the prevalence of patients being administered a β-blocker was less in the responder group.

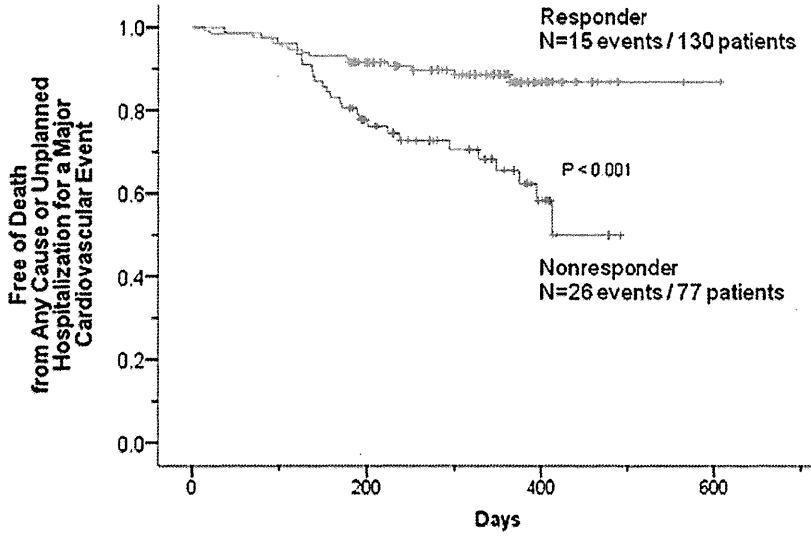

Figure. Kaplan–Meier estimates of the time to the endpoint of composite of death from any cause or unplanned hospitalization for a major cardiovascular event.

Table 8. Univariate and Multivariate Predictors of Death From Any Cause or Unplanned Hospitalization for a Major Cardiovascular Event

Predictor	Death from any cause or unplanned hospitalization for a major cardiovascular event							
	Univariate model		Multivariate model 1		Multivariate model 2		Multivariate model 3	
	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value
Age, years	0.99 (0.97–1.01)	0.38	1.01 (0.98–1.03)	0.50	1.00 (0.98–1.03)	0.71	1.01 (0.98–1.03)	0.53
Sex, male	0.89 (0.50–1.596)	0.68	0.73 (0.38–1.42)	0.34	0.66 (0.36–1.21)	0.18	0.78 (0.43–1.41)	0.40
QRS duration >150 ms	0.38 (0.22–0.67)	<0.001	1.23 (0.74–2.26)	0.42	0.62 (0.33–1.14)	0.12	0.63 (0.34–1.16)	0.14
Intravenous inotropes	2.74 (1.35–5.77)	0.005	2.38 (1.04–5.43)	0.04	1.93 (0.91–4.08)	0.09	1.90 (0.89–4.05)	0.10
LBBB	0.27 (0.15–0.49)	<0.001	0.47 (0.24–0.91)	0.03	0.37 (0.20–0.69)	0.002	0.38 (0.20–0.72)	0.003
SPWMD >130 ms	0.46 (0.26–0.84)	0.02	0.51 (0.28–0.95)	0.03				
Ts (lateral-septal) >65 ms	0.49 (0.27–0.89)	0.02			0.62 (0.33–1.15)	0.13		
Combined SPWMD and Ts (lateral-septal)	0.42 (0.24–0.74)	0.002					0.66 (0.30–0.98)	0.04

Abbreviations as in Tables 1,2,7.

Baseline echocardiographic parameters are compared in Table 3. Only transtricuspid pressure gradient was significantly higher in the non-responder group than in the responder group. Baseline dyssynchrony parameters are summarized in Table 4. The baseline evaluable rate of each parameter did not differ between the groups. Of the 7 dyssynchrony parameters, only septal-to-posterior wall motion delay (SPWMD), interventricular mechanical delay (IMD), and delay between time to peak systolic velocity in ejection phase at basal septal and lateral segments (Ts (lateral-septal)) were significantly higher in the responder group than in the non-responder group. The AUC to predict volume responders for each dyssynchrony parameter are listed in Table 5. Of all dyssynchrony parameters, SPWMD showed the highest AUC value (0.65), followed by that of IMD at 0.64. In addition, the AUC to predict ≥1 class improvement of NYHA class at 6 months after CRT are listed in Table 6. SPWMD showed the highest AUC value (0.68).

Baseline predictors of a volume responder are shown in Table 7. In a multivariate logistic regression analysis, presence of LBBB, administration of intravenous inotropes, and non-administration of β-blockers were identified as the independent predictors of a volume responder. For the presence of LV dyssynchrony, univariate logistic regression analysis showed SPWMD >130 ms to be associated with volume responders, but it was not an independent predictor in multivariate model 1. In contrast, a combined dyssynchrony criterion that required at least one of SPWMD >130 ms or Ts (lateral-septal) >65 ms was independently associated with volume responders in multivariate model 2.

Outcomes and Volume Responders

Kaplan–Meier estimates of the time to the endpoint is shown in Figure. There were significantly fewer events in the volume responder group than in the non-responder group (log rank, P<0.001). Of the 15 events in the responder group, heart failure was the cause of death in 2 patients and of unplanned hospitalizations in 6 patients, and other cardiovascular events were the cause of unplanned hospitalizations in 7 patients. Of the 26 events in the non-responder group, heart failure was the cause of death in 5 patients and of unplanned hospitalizations in 17 patients. Sudden cardiac death occurred in 1 patient, and non-cardiac death occurred in 2 patients. Other cardiovascular events were the cause of unplanned hospitalization in 1 patient.

Baseline Parameters and Clinical Outcomes

Univariate Cox proportional hazard analyses adjusted for age and sex revealed the relations of 6 predefined parameters with a composite of death from any cause or an unplanned hospitalization for a major cardiovascular event (Table 8). Because 3 dyssynchrony parameters were associated with the endpoint, 3 multivariate analysis models combined with each dyssynchrony parameter were assessed, and SPWMD >130 ms or a combined dyssynchrony criterion that required at least one of SPWMD >130 ms or Ts (lateral-septal) >65 ms was associated with the endpoint independently of the presence of LBBB. In contrast, presence of a wide QRS of >150 ms was not independently associated with the endpoint.

Discussion

The present study showed the following major findings regarding the relation between Doppler echocardiographic parameters and CRT effect. Although the volume responders showed better outcomes compared to the volume non-responders, no dyssynchrony parameter could independently predict CRT volume responders. In addition, single dyssynchrony parameters could not predict NYHA class improvements after CRT. In contrast, a combined dyssynchrony criterion of parameters to detect dyssynchrony between the septum and LV free wall was associated with volume responders and the clinical outcome independently of QRS duration and presence of LBBB.

Intraventricular Dyssynchrony and Cardiac Remodeling After CRT

A dyssynchrony criterion combining SPWMD and Ts (lateral-septal) was independently associated with volume responders but each criterion alone did not appear to independently have significant power to detect volume responders, similar to the findings of the PROSPECT study.[12] As a rationale for the use of CRT with bi-ventricular pacing, LV mechanical dyssynchrony between the septum and free wall is a primary target for resynchronization by LV free-wall pacing.[13,14] Then, the present results are reasonable because the detection of typical mechanical dyssynchrony between the septum and free wall, which can be detected by SPWMD and Ts (lateral-septal), is a key concept for the use of Doppler echocardiography to predict CRT responders. Although multiple factors including NYHA class at baseline and etiology of heart failure are associated with LV reverse remodeling after CRT, as is also shown in a sub-analysis of the PROSPECT study, at the least, the presence of mechanical dyssynchrony between the septum and free wall at baseline should be a key indicator of LV reverse remodeling after CRT.[15]

In contrast, the presence of LBBB was the strongest predictor of CRT benefit. The finding suggests that the presence of LBBB can predict the presence of mechanical dyssynchrony between the septum and free wall more accurately than present echocardiographic parameters.

Issues With Cardiac Dyssynchrony Parameters

The modest power of SPWMD and Ts (lateral-septal) to predict volume responders raises some issues in the quantification of important dyssynchrony patterns by the Doppler echocardiographic parameters. In the present study, about one half (49%) of the patients who had mechanical dyssynchrony as identified by either the SPWMD or Ts (lateral-septal) definition had mechanical dyssynchrony identified by both the SPWMD and Ts (lateral-septal) parameters. This disagreement convincingly illustrates the issues of parameter measurements, despite the differences in motion analysis modalities and direction in longitudinal or radial motions. On the basis of the original definition of SPWMD, the surrogate points depend on the magnitude of displacement and the shortest interval between the maximum displacement peaks between septum and posterior wall, independent of the timing of the peaks.[7] In addition to M-mode images without peaks associated with reduced wall motion, the determinant of a surrogate peak in the multiple samples of the septum on an M-mode image is also of great concern as instances in which measurements are impossible.[16,17] For Doppler-derived Ts (lateral-septal) measurements, the limited measurement phase during the LV ejection period might be an issue. Recently, septal flash motion has been receiving attention as a useful marker of CRT responders and is commonly observed in early systole including the pre-ejection period.[14,18,19] Thus, phase-restricted measurements cannot detect early septal contractions.[20] In addition, an atypical dyssynchrony pattern, in which a lateral peak occurs earlier than a septal peak, might be observed even in patients with typical LBBB.[21] In such cases, the time difference does not associate with the typical mechanical dyssynchrony sequence.

Mechanical Dyssynchrony and CRT Effect

The present study shows that mortality and morbidity rates of volume responders were better than those of non-responders. Therefore, the volume responder is a reliable surrogate of CRT effects, and patients who can be predicted to undergo reverse remodeling should be selected as the first candidates for CRT.[15,21,22] In contrast, 10 patients in the present study were excluded due to the occurrence of fatal events within 6 months after CRT, which suggests the limitation of volume responder criteria as a method of evaluating CRT effects. More importantly, the J-CRT trial is the first multicenter study to reveal the relation between predefined baseline echocardiographic dyssynchrony parameters and clinical outcome because earlier large trials did not use echocardiographic parameters to assess clinical outcomes in patients with CRT, except in a part of the Cardiac Resynchronization-Heart Failure (CARE-HF) Study results.[3] Dyssynchrony between the septum and LV free wall was associated with clinical outcome as well as volume responders. In the PROSPECT study,[12] a heart failure clinical composite score (CCS) that combined all-cause mortality, heart failure hospitalization, NYHA class, and patient global assessment into an outcome measure was used to assess the relation between dyssynchrony parameters and clinical outcomes. Although the CCS was evaluated as an outcome at 6 months after CRT, there was a discrepancy in relation to dyssynchrony parameters between the CCS and volume responders. Possible explanations for this finding are that the CCS includes relatively subjective data such as NYHA class and patient global assessment, and the duration of follow-up observation might be too short to assess clinical outcomes in patients with CRT. As a novel proposal, more accurate means of echocardiographic detection of LV dyssynchrony should be developed that focus on the electrical and mechanical dyssynchrony sequence that can be treated with CRT. If this is done, echocardiography might occupy a more critical role in the selection of CRT candidates and can be a more accurate predictor of the beneficial effects of CRT. In addition, the comparison between echocardiography and other imaging modalities including electrocardiogram-gated single-photon emission computed tomography myocardial perfusion imaging and magnetic resonance imaging might be

useful in identifying the need for mechanical dyssynchrony imaging.[23]

Study Limitations

The reproducibility of echocardiographic parameter measurements was not assessed in the present study. Although an echocardiographic recording workshop was provided to participating echocardiologists and electrophysiologists by the J-CRT Committee, data variability among the institutions participating in the J-CRT study might have affected the results as poor reproducibility was also shown in the PROSPECT study.[12]

Conclusions

Current echocardiographic parameters do not have significant power to detect clinical improvements and LV reverse remodeling after CRT, which has been identified as a reliable surrogate of clinical outcomes in patients with CRT. However, intraventricular dyssynchrony measured between the septum and LV free wall might be associated not only with CRT volume responders but also with the clinical outcome. These relations suggest that intraventricular mechanical dyssynchrony between the septum and LV free wall might be a key to the prediction of CRT benefit. Therefore, further studies are needed to identify the parameter that most accurately detects the mechanical dyssynchrony sequence.

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Appendix 1

The following study centers and investigators participated in the J-CRT Registry Trial: *Aichi Cardiovascular and Respiratory Center, Aichi:* Yoshimasa Murakami; *Gunma Prefectural Cardiovascular Center, Maebashi:* Yasuaki Tanaka; *Kobe University, Kobe:* Akihiro Yoshida; *Kokura Memorial Hospital, Fukuoka:* Takeshi Arita; *Koseikai Takeda Hospital, Kyoto:* Eiwa Zen; *Nagoya Daini Red Cross Hospital, Nagoya:* Yukihiko Yoshida; *Nagoya University, Nagoya:* Yasuya Inden; *National Cardiovascular Center, Suita:* Shiro Kamakura, Takashi Noda, Hideaki Kanzaki; *Nihon University Itabashi Hospital, Tokyo:* Toshiko Nakai, Ichiro Watanabe; *Nippon Medical School, Tokyo:* Takao Kato, Yasushi Miyauchi; *Saitama Medical University International Medical Center, Saitama:* Kazuo Matsumoto, Ritsushi Kato; *Sakurabashi Watanabe Hospital, Osaka:* Koichi Inoue, Ryusuke Kimura, Motoko Uehara; *Tohoku University, Sendai:* Yuji Wakayama; *Tokyo Women's Medical University, Tokyo:* Azusa Furugen; *Toyohashi Heart Center, Toyohashi:* Kohei Yamashiro; *Tsuchiya General Hospital, Hiroshima:* Yoshiko Masaoka; *Tsukuba Medical Center Hospital, Tsukuba:* Yuichi Noguchi, Miyako Igarashi; *University of Tsukuba, Tsukuba:* Yukio Sekiguchi, Tomoko Ishizu.

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ORIGINAL PAPER

Intermittent arm ischemia induces vasodilatation of the contralateral upper limb

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Abstract Intermittent arm ischemia before percutaneous coronary intervention induces remote ischemic preconditioning (RIPC) and attenuates myocardial injury in patients with myocardial infarction. Several studies have shown that intermittent arm ischemia increases coronary flow and is related to autonomic nerve system. The aim of this study was to determine whether intermittent arm ischemia induces vasodilatation of other arteries and to assess changes in the autonomic nerve system during intermittent arm ischemia in humans. We measured change in the right brachial artery diameter during intermittent left arm ischemia through three cycles of 5-min inflation (200 mmHg) and 5-min deflation of a blood-pressure cuff using a 10-MHz linear array transducer probe in 20 healthy volunteers. We simultaneously performed power spectral analysis of heart rate. Ischemia-reperfusion of the left arm significantly dilated the right brachial artery time-dependently, resulting in a 3.2 ± 0.4% increase after the 3rd cycle. In the power spectral analysis of heart rate, the high-frequency domain (HF), which is a marker of parasympathetic activity, was significantly higher after the 3rd cycle of ischemia-reperfusion than baseline HF ($P = 0.02$). Intermittent arm ischemia was accompanied by vasodilatation of another artery and enhancement of parasympathetic activity. Those effects may play an important role in the mechanism of RIPC.

Keywords Ischemic heart disease · Autonomic nervous system · Ischemia-reperfusion

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Introduction

Remote ischemic preconditioning (RIPC) is a powerful innate mechanism by which brief ischemia in one region or organ protects distant tissue or organs from a sustained event of ischemia [1]. In 1993, Przyklenk et al. [2] reported for the first time that brief circumflex artery occlusion could reduce the size of myocardial infarct induced by subsequent sustained occlusion of the left anterior descending artery in dogs. This intramyocardial protection was later extended to non-cardiac organs, with a report that myocardial infarct size could be actually reduced in the animal heart by inducing brief ischemia and reperfusion in either the kidney [3, 4] or the small bowel [5]. In clinical settings, several studies have shown that RIPC by intermittent arm ischemia is useful in protection from myocardial injury after percutaneous coronary intervention [6, 7] and is controversial in myocardial protection after coronary artery bypass graft [8, 9].

Transient limb ischemia reduced coronary resistance and increased coronary blood flow in a porcine model [10]. In human cases, coronary diastolic flow velocity was also increased by 3 cycles of remote intermittent ischemia-reperfusion [11]. However, it is unclear whether

intermittent arm ischemia accompanies vasodilatation of distant tissue in human cases. Therefore, we examined whether intermittent arm ischemia induces vasodilatation of another artery, an artery of the contralateral upper limb.

There are several potential mechanisms underlying RIPC in neural and hormonal pathways and systematic response [1, 12]. Loukogeorgakis et al. [13] reported that trimetaphan, an autonomic ganglion blocker, attenuated RIPC by intermittent arm ischemia in humans. The autonomic nerve system may be associated with RIPC by intermittent arm ischemia in humans. Thus, we hypothesized that RIPC by intermittent arm ischemia changes the autonomic balance and induces vasodilatation of the distant organ's artery. The purpose of this study was to determine the validity of this hypothesis. We evaluated changes in autonomic nerve balance during intermittent arm ischemia using power spectral analysis of the RR interval of the electrocardiography (ECG) and whether vasodilatation of the brachial artery occurred using 2D gray-scale echography.

Methods

Subjects

This study was performed on 20 healthy volunteers (17 men and 3 women; mean age 34 ± 5 years, age range 24–42 years; height 169 ± 2 cm; weight 62 ± 3 kg; right brachial artery diameter 3.78 ± 0.1 mm). The diameter of the brachial artery was measured 3–5 cm above the antecubital space. All of the studies were approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, and informed consent was obtained from all subjects before the procedure. The investigation conformed to the principles outlined in the Declaration of Helsinki.

Induction of intermittent ischemia

Studies were performed in a temperature-controlled laboratory (24–26°C). Intermittent ischemia was induced by inflating a 13.5-cm-wide blood pressure cuff placed around the upper part of the left arm. The cuff was inflated to 200 mmHg for 5 min (ischemia) followed by 5-min deflation. The inflation/deflation cycle was performed three times, as described previously [13].

Assessment of artery response

Artery response was continuously assessed by measuring change in the right brachial artery diameter through three cycles of 5-min inflation (200 mmHg) and 5-min deflation of the blood pressure cuff. Using high-resolution ultrasound with a 10-MHz linear array transducer, the right brachial artery diameter was measured as previously described [14]. Longitudinal images of the artery were recorded at baseline and continuously from the first to third cycles of ischemia-reperfusion. The diameter of the brachial artery was determined semi-automatically using an instrument equipped with software for monitoring brachial artery diameter (Unex Co., Ltd., Nagoya, Japan). Briefly, continuous recording of a 2D gray scale image and A-mode waves of the brachial artery in the longitudinal plane was conducted with a novel stereotactic probe-holding device. A segment with clear anterior (media-adventitia) and posterior (intima-media) interfaces was manually determined. Then these border interfaces were identified automatically on the A-mode waves. The diastolic per-beat diameter of the brachial artery was synchronized with the electrocardiographic R-wave and tracked automatically. The changes in diameter were continuously recorded. Then vasodilatation was examined as percent change in the diameter over the baseline value at maximal vasodilatation after every reperfusion. Both the baseline values and each maximum vasodilatation value came from a single beat. Three-millimeter-wide longitudinal images of the artery in A-mode were obtained, and the images were divided into 20 parts. Mean diameter of the 20 parts was calculated as the brachial artery's diameter.

Power spectral analysis

To evaluate indices of sympathetic activity and parasympathetic activity, we performed power spectral analysis. ECG was recorded using a two-channel recorder during the protocol (Fukuda Denshi: model Digital walk FM190, Fukuda Denshi Co., Ltd., Tokyo, Japan). The power spectra of the RR interval were calculated using the maximum entropy method with MemCalc software (Suwa Trust Co., Ltd., Tokyo, Japan) [15] at 15-s intervals for 30-s periods. We performed measurements at two time points, baseline and 3rd ischemia. The interpolation was performed by using the linear supplement method after cleaning noise. The interval of resampling is determined as the difference between the mean and minimum values of the RR interval. The high-frequency (HF 0.15–0.40 Hz) domain was used as a marker of parasympathetic activity, and the ratio of low-frequency (LF 0.04–0.15 Hz) domain to HF (LF/HF) was used as an indicator of sympathetic activity [16]. The averaged value during the 10-min period before RIPC was defined as baseline.

Study protocols

After 10-min bed rest in the supine position, the diameter of the right brachial artery was measured at the four times,

before and after each of three cycles of 5-min inflation (200 mmHg) and 5-min deflation of the blood pressure cuff in the left upper arm. We obtained the percent change in diameter over the baseline value at maximal vasodilatation in each period (Fig. 1a). Simultaneously, ECG monitoring with a Fukuda Denshi FM-190 (Fukuda Denshi) was performed. In the control study without ischemia-reperfusion of the left upper arm, the diameter of the right brachial artery was measured at four times, before and after each of 3 cycles of 10-min bed rest in the supine position (Fig. 1b).

Laboratory measurements

Fasting blood samples were drawn before and after 3 cycles of ischemia-reperfusion from 11 of the 20 volunteers who gave informed consent (Fig. 1a). We took both blood samples by needle insertion. To evaluate the relationship between contralateral artery dilatation and nitric oxide (NO), we measured cyclic GMP, a second messenger of NO. To evaluate the relationship between contralateral artery dilatation and sympathetic activity using parameters other than heart rate variability, we measured catecholamine. The plasma levels of cyclic GMP and catecholamine (adrenaline, noradrenalin and dopamine) were measured using the RIA DCC method and HPLC method (SRL, Tokyo, Japan)

Statistical analysis

All data are expressed as mean ± SE unless otherwise stated. Brachial artery diameter was measured in millimeters, and dilatation was expressed as percentage increase from baseline diameter. Data were compared using Student's paired *t* test or repeated measures analysis of variance (ANOVA), as appropriate. For assessment of the changes in the power spectral, hemodynamics and laboratory data, Student's paired *t* test was applied. For assessment of the changes in artery diameter, analysis of variance (ANOVA) was applied. *P* value by ANOVA was Bonferroni-adjusted. In all cases, $P < 0.05$ was considered statistically significant.

Results

Effect of ischemia-reperfusion of the left arm on the right brachial artery

All subjects tolerated the procedures without any complications and any pains. The ischemia-reperfusion protocol had no effect on hemodynamics except for heart rate (Table 1). Ischemia-reperfusion of the left arm gradually

Table 1 Changes in hemodynamics

	Pre	Post
Systolic BP (mmHg)	108 ± 8	106 ± 7
Diastolic BP (mmHg)	66 ± 6	66 ± 8
HR (/min)	64 ± 2	59 ± 2*

Data are mean ± SE of the mean

BP Blood pressure, *HR* heart rate, *Pre* before 3 cycles of intermittent arm ischemia-reperfusion, *Post* after 3 cycles of intermittent arm ischemia-reperfusion

* Significantly different from Pre, $P < 0.05$

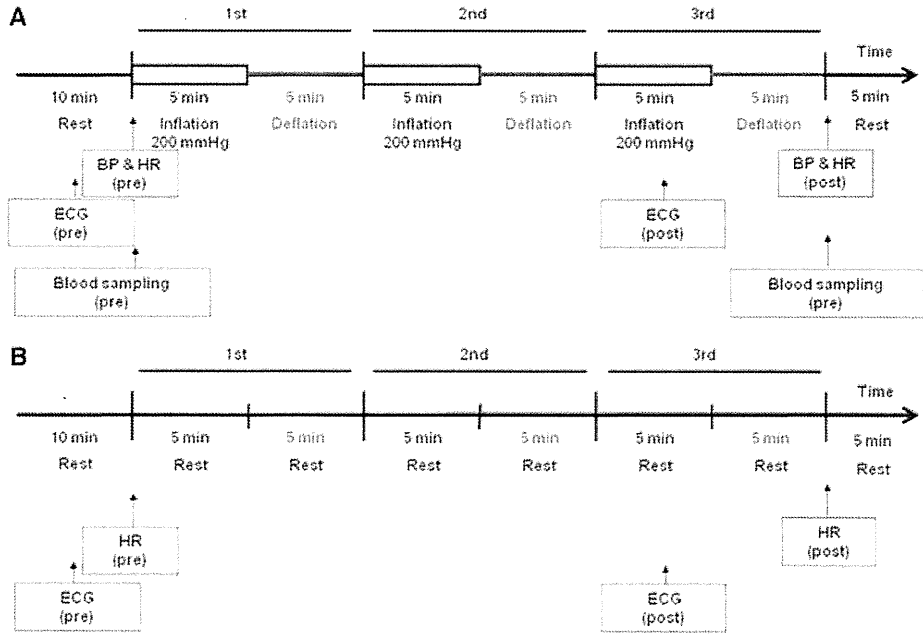

Fig. 1 Study protocols with and without ischemia-reperfusion. **a** Study protocol with ischemia-reperfusion. **b** Study protocol without ischemia-reperfusion. **a** After 10-min bed rest in the supine position, the diameter of the right brachial artery was measured four times, before and after each of three cycles of 5-min inflation (200 mmHg) and 5-min deflation of a blood pressure cuff in the left arm. **b**. In the control study without ischemia-reperfusion of the left arm, the diameter of the right brachial artery was measured four times, before and after 3 cycles of 10-min bed rest in the supine position. *BP* Blood pressure, *HR* heart rate, *ECG* electorcardiogram

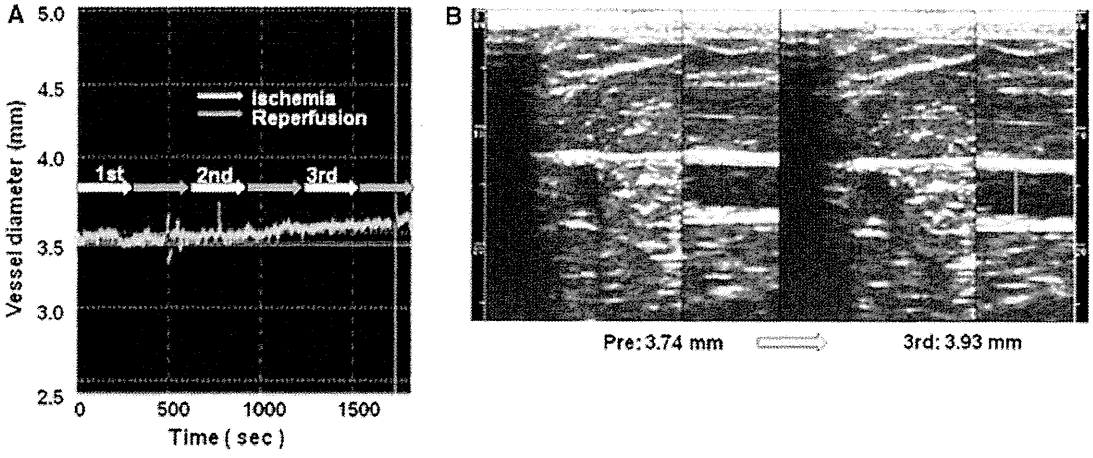

Fig. 2 Ischemia-reperfusion of the left arm gradually enhanced right brachial artery dilatation time-dependently. **a** Representative diameter of a contralateral artery, right brachial artery. **b** Representative ultrasonographical images of the right brachial artery before (*left*) and after (*right*) 3 cycles of intermittent arm ischemia

enhanced dilatation of the right brachial artery, an artery in the contralateral limb, and this effect was dependent on the number of trials (Figs. 2, 3a). Mean vessel diameters of all subjects were increased significantly [vessel diameter: 3.78 ± 0.1 mm before procedure (control), 3.83 ± 0.1 mm after the 1st cycle of ischemia-reperfusion, 3.86 ± 0.1 mm after the 2nd cycle and 3.89 ± 0.1 mm after the 3rd cycle] (control vs. 1st cycle, $P < 0.001$; control vs. 2nd cycle, $P < 0.001$; control vs. 3rd cycle, $P < 0.001$). Intermittent arm ischemia of the left upper arm increased % changes of vessel diameter in individual subjects. The percent changes were 1.5 ± 0.3, 2.4 ± 0.3 and 3.2 ± 0.4% of the baseline diameter, respectively (control vs. after 1st cycle, $P < 0.001$; control vs. after 2nd cycle, $P < 0.001$; control vs. after 3rd cycle, $P < 0.001$) (Fig. 3a).

We examined whether the vessel diameter was changed during bed rest without intermittent arm ischemia for the control study in 9 subjects out of the same 20 volunteers with intermittent arm ischemia. Post mean heart rate was significantly lower than pre mean heart rate (pre mean heart rate 65 ± 3/min, post mean heart rate 60 ± 3/min; pre mean heart rate vs. post mean heart rate, $P < 0.05$). Mean vessel diameters of all subjects were not increased significantly. The vessel diameters were 3.81 ± 0.32, 3.81 ± 0.32, 3.81 ± 0.33 and 3.82 ± 0.33 mm, respectively (control vs. 1st cycle, $P = 1.000$; control vs. 2nd cycle, $P = 1.000$; control vs. 3rd cycle, $P = 1.000$). Bed rest without intermittent arm ischemia did not increase % changes of vessel diameter in individual subjects. The percent changes were 0.1 ± 0.07, 0.2 ± 0.14 and 0.2 ± 0.14%, respectively (control vs. after 1st cycle, $P = 1.000$; control vs. after 2nd cycle, $P = 1.000$; control vs. after 3rd cycle, $P = 1.000$) (Fig. 3b).

Effect of ischemia-reperfusion of the left arm on the autonomic nerve system

The high-frequency domain, a marker of parasympathetic activity, after the 3rd reperfusion was significantly higher than the baseline mean HF domain (425 ± 69 ms^2/Hz at baseline vs. 617 ± 108 ms^2/Hz after 3rd ischemia, $P < 0.05$) (Fig. 4a). On the other hand, bed rest without intermittent arm ischemia did not increase the HF domain (370 ± 79 ms^2/Hz at baseline vs. 357 ± 59 ms^2/Hz after 3rd cycle, $P = 0.81$) (Fig. 4b).

The ratio of LF to HF, an indicator of sympathetic activity, after the 3rd ischemia was lower than baseline, but not significantly (2.6 ± 0.5 at baseline vs. 2.0 ± 0.3 after the 3rd cycle, $P = 0.07$). Bed rest without intermittent arm ischemia did not change LF/HF (2.6 ± 0.5 baseline vs. 2.3 ± 0.3 after the 3rd cycle, $P = 0.30$).

Plasma noradrenalin levels after the 3rd cycle of ischemia-reperfusion were significantly lower than those before ischemia-reperfusion. Cyclic GMP, adrenaline and dopamine levels after the 3rd cycle of ischemia-reperfusion tended to be lower than those before ischemia-reperfusion (Table 2).

Discussion

This study showed for the first time in humans that intermittent ischemia in an upper limb was accompanied by vasodilatation in the contralateral limb and enhancement of parasympathetic nerve activity.

There are several possible mechanisms by which vasodilatation in the contralateral limb is induced by intermittent

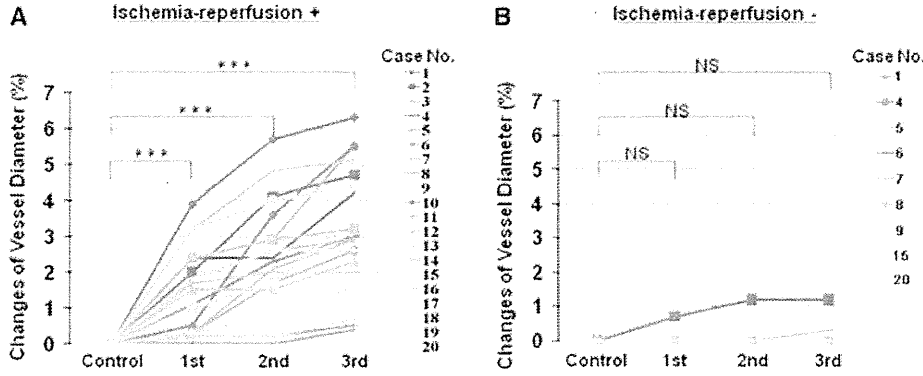

Fig. 3 % changes of vessel diameter in individual subjects with or without ischemia-reperfusion. **a** Effect of ischemia-reperfusion of the left arm on the right brachial artery. ***$P < 0.001$. **b** Effect of bed rest without ischemia-reperfusion of the left arm on the right brachial artery

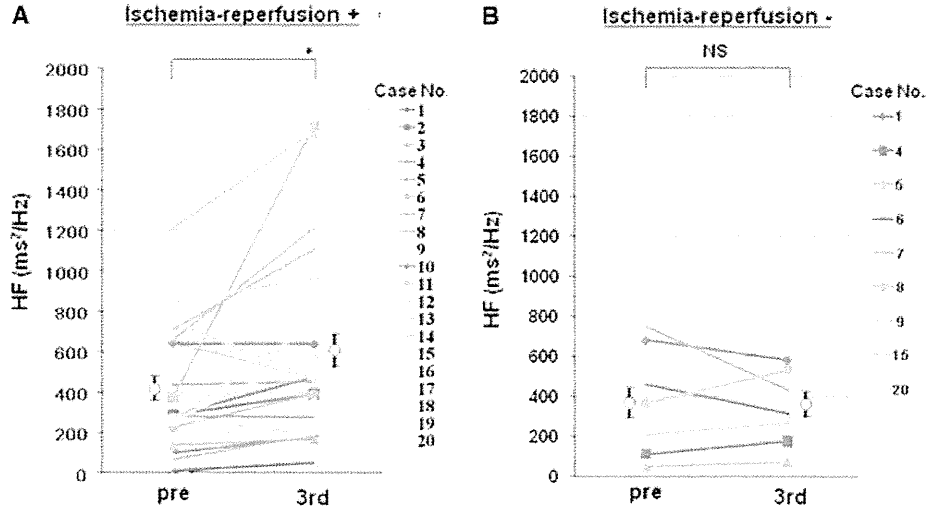

Fig. 4 Changes in the high frequency (HF) domain with or without ischemia-reperfusion. **a** Effect of ischemia-reperfusion of the left arm on HF domain. *$P < 0.05$. **b** Effect of bed rest without ischemia-reperfusion of the left arm on HF domain

Table 2 Peripheral blood chemical analysis

	Pre	Post
Cyclic GMP (pmol/ml)	3.6 ± 0.5	3.1 ± 0.4
Adrenaline (pg/ml)	31.3 ± 5.7	25.5 ± 4.8
Noradrenaline (pg/ml)	302.4 ± 31.3	230.0 ± 31.7*
Dopamine (pg/ml)	8.2 ± 1.2	6.6 ± 0.8

Data are mean ± SE of the mean

GMP Guanosine monophosphate, *Pre* before 3 cycles of intermittent ischemia-reperfusion, *Post* after 3 cycles of intermittent ischemia-reperfusion

* Significantly different from Pre, $P < 0.05$

upper limb ischemia. First, several studies have indicated that RIPC is associated with hormonal factors such as adenosine and bradykinin, and other factors such as nitric oxide [12]. Accordingly, contralateral upper limb vasodilatation might be induced by these vasoactive factors. Our results showed that cyclic GMP was not significantly changed, indicating that nitric oxide does not participate in the vasodilatation. Second, in humans, the neural pathway is involved in the RIPC mechanism of the early (short) phase [13] and late phase [17–19], but the afferent-efferent pathway has not been elucidated. Recently, Gourine et al. reported that RIPC might be associated with the nerve reflex, which is the sensory afferent pathway from the peripheral organ (limbs) and parasympathetic vagal efferent outflow to the heart in rats [20]. Previous studies showed that there is evidence of parasympathetic vasodilator in the masseter muscle [21], lower lip [22] and brain vessels [23] in animals. Those findings indicated that vasodilatation is a result of reflex via increasing parasympathetic activity.

It is well known that increased sympathetic activity and reduced parasympathetic activity are induced by cardiovascular diseases. Previous studies showed that physical training could significantly improve exercise capacity and ameliorate the autonomic derangement in chronic heart failure by increasing parasympathetic activity [24–26].