Adiponectin acts as a positive indicator of left ventricular diastolic dysfunction in patients with hypertrophic cardiomyopathy

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Adiponectin acts as a positive indicator of left ventricular diastolic dysfunction in patients with hypertrophic cardiomyopathy

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ABSTRACT

Background Adiponectin is an adipose-derived plasma protein that exhibits beneficial actions on the heart. Recently, it was shown that adiponectin levels were elevated in patients with systolic heart failure.

Objective To investigate the association between adiponectin levels and left ventricular (LV) diastolic function in patients with hypertrophic cardiomyopathy (HCM), characterised by diastolic dysfunction.

Methods Twenty-six patients with HCM showing LV ejection fraction of >60% were enrolled. LV pressure half-time ($T_{1/2}$) was measured as an index of myocardial relaxation. Patients were divided into two groups on the basis of baseline $T_{1/2}$ (group A: $T_{1/2}$< 35 ms, group B: $T_{1/2}\geq$ 35 ms). Blood samples were simultaneously collected from the coronary sinus (CS) and aortic root (Ao) as well as the peripheral vein (PV) for measurement of plasma adiponectin levels.

Results Plasma adiponectin levels were significantly higher in group B than in group A. Adiponectin levels in the PV were positively correlated with the baseline $T_{1/2}$ in patients with HCM. The transcardiac gradient of adiponectin as calculated by the Ao−CS difference was significantly higher in group A than in group B. The transcardiac gradient of adiponectin also inversely correlated with the baseline $T_{1/2}$ and adiponectin levels in PV in patients with HCM. The expression of AdipoR1 but not AdipoR2 in the heart decreased in group B. The baseline $T_{1/2}$ was negatively associated with AdipoR1 expression in patients with HCM.

Conclusions These data document that adiponectin is an indicator of LV diastolic dysfunction in patients with HCM.

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is characterised by left ventricular (LV) hypertrophy and diastolic dysfunction.[1] It has been reported that abnormal cardiac energy metabolisms contribute to the development of HCM.[2] Particularly, it has been noted that myocardial oxidative metabolism and free fatty acid metabolism are increased and inversely related to hypertrophic response in patients with a genetically identical cause for HCM.[3] Thus, it is clinically valuable to clarify the relationship between cardiac energy metabolisms and the property of HCM.

Adiponectin is an adipose tissue-derived hormone whose concentration is downregulated in subjects with obesity-related diseases.[4] Adiponectin acts as a biologically relevant modulator of a variety of metabolic and cardiovascular disorders, including insulin resistance, atherosclerosis and cardiac injury.[5] Importantly, adiponectin stimulates fatty acid oxidation in the heart.[6] It has been shown that adiponectin-deficient (APN-KO) mice exhibit delayed free fatty acid clearance after injection of fat emulsion.[7] This indicates that adiponectin may have a crucial role in regulation of energy metabolism in various pathological heart conditions such as HCM.

Several clinical studies have investigated the interaction of adiponectin levels with cardiac hypertrophy and diastolic dysfunction. Low adiponectin levels are associated with the progression of LV hypertrophy, which is accompanied by diastolic dysfunction.[8] In non-complicated obese subjects, adiponectin levels are negatively correlated with LV mass index.[9] Plasma adiponectin levels are also inversely associated with LV hypertrophy by electrocardiographic and echocardiographic analysis in healthy subjects.[10 11] Consistent with these clinical observations, a number of experimental studies show that APN-KO mice exhibit enhanced concentric cardiac hypertrophy after pressure overload, and impaired LV systolic dysfunction after permanent coronary ischaemia.[12 13] Conversely, supplementation of adiponectin attenuates pathological cardiac hypertrophy and cardiac dysfunction under ischaemic conditions.[12 13] These data suggested that adiponectin directly affects signalling in the heart and has beneficial effects on several pathological heart conditions, including cardiac hypertrophy.

Recently, several prospective studies have shown an association between adiponectin levels and LV systolic dysfunction. High adiponectin levels are associated with increased mortality and severity in patients with advanced systolic heart failure, including dilated cardiomyopathy.[14] Similar results were obtained by George *et al*,[15] who speculated that high adiponectin levels might represent an expression of high energy expenditure. In contrast, adiponectin levels are not predictive of heart failure in asymptomatic men.[16] To the best of our knowledge, nothing is known about the relationship of adiponectin with HCM. Here, we examined the association between adiponectin levels and diastolic function in patients with HCM.

PATIENTS AND METHODS

Patient selection

A total of 26 patients (mean age 61.2±2.2 years) with non-obstructive HCM with an LV ejection fraction (EF) of ≥ 60% were enrolled in this study. All the patients were diagnosed on the basis of clinical, electrocardiographic, echocardiographic and endomyocardial biopsy findings according to previously proposed criteria.[17] We excluded patients with coronary artery disease, primary valvular heart disease, congestive heart failure, primary or secondary hypertension, chronic atrial fibrillation and diabetes mellitus. Secondary HCMs such as Fabry's disease or mitochondrial encephalomyopathy were excluded by clinical and endomyocardial biopsy findings. All cardiovascular agents were discontinued at least 4 days before the catheter study and blood sampling. This protocol was approved by the ethics committee of the Nagoya University School of Medicine, Japan, and all subjects enrolled in this study provided written informed consent.

Biventricular cardiac catheterisation

Biventricular catheterisation was performed to measure LV end-diastolic pressure and the pressure half-time ($T_{1/2}$) as an index of LV isovolaemic relaxation as described previously.[18] A Swan–Ganz thermodilution catheter was positioned in the right pulmonary artery to measure pulmonary artery wedge pressure and cardiac index.

Biomarker analysis

Blood samples were obtained at the time of biventricular catheterisation to determine plasma adiponectin levels. After 20 min with the subjects resting in the supine position, vital signs were recorded and blood was simultaneously collected from the aortic root (Ao) and coronary sinus (CS) as well as the peripheral vein (PV). The catheter positions were confirmed by injection of contrast medium before sampling. Plasma adiponectin levels were determined with adiponectin ELISA kits (Otsuka Pharmaceutical Co, Tokyo, Japan). Blood samples were also collected from the PV for measurement of fasting glucose, HbA1c, homoeostasis model assessment of insulin resistance (HOMA-IR), low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, triglyceride, creatinine clearance, brain natriuretic peptide, norepinephrine and aldosterone.

Echocardiography analysis

Two-dimensional echocardiography was performed by the same experienced sonographer using a Sonos 2500 ultrasound system (Hewlett-Packard, Andover, Massachusetts, USA) with a 2.5 MHz phased-array transducer. LV wall thickness, LV end-diastolic dimension, LV end-systolic dimension and left atrial dimension were measured from standard M-mode measurements as recommended by the American Society of Echocardiography.[19] The LV mass was calculated by standard cube formula. Early diastolic annular velocity (Ea) and LV outflow tract pressure gradient were obtained by placing a tissue Doppler sample volume, and the E (early diastolic transmitral flow velocity)/Ea ratio was calculated.

Real-time reverse transcriptase-polymerase chain reaction analysis

Total RNA from frozen ventricular biopsy specimens was isolated with the use of an RNeasy Fibrous Tissue Mini-Kit (Qiagen, Valencia, California, USA). The RNA was subjected to reverse transcription with an RNA PCR Core kit (Applied Biosystems, Foster, California, USA), and the resulting cDNA was subjected to quantitative polymerase chain reaction (PCR) analysis with the Stratagene Mx 3000 Real-Time PCR System. Primers were as follows: 5′-AACCTCAGATAAGCCCGTCG-3′ and 5′-ATGGCAGAGAGGAGGTTGAC-3′ for human AdipoR1; 5′-GCATCCACTTCCCAACCA-3′ and 5′-CTTCCTCATCTTCATCGTCAT-3′ for human AdipoR2; and 5′-GGACTTCGAGCAGGAGATGG-3′ and 5′-GCACCGTGTTGGCGTAGAGG-3′ for human glyceraldehyde-3-phosphate dehydrogenase.

Statistical analysis

Data are presented as mean±SEM. Variables were compared between two groups of patients with HCM using the Student t test. Relations between continuous data were analysed by linear regression analysis. A p value of <0.05 was considered statistically significant.

RESULTS

Study population

A total of 26 consecutive patients with non-obstructive HCM who underwent cardiac catheterisation were enrolled in this

Table 1 Patient characteristics according to the baseline $T_{1/2}$

Characteristics	Group A ($T_{1/2}$ <35 ms, n=12)	Group B ($T_{1/2}$ ≥35 ms, n=14)
Age (years)	58.7±3.6	63.2±2.9
Female/male	2/10	4/10
BMI (kg/m^2)	24.3±0.7	21.0±2.4
Smoking	6/12	4/14
Echocardiography		
Maximal wall thickness (mm)	15.1±1.5	16.0±0.8
LVDd (mm)	46.4±1.0	48.2±1.2
LVDs (mm)	26.6±0.7	28.4±2.4
LAD (mm)	37.5±1.5	40.1±1.4
EF (%)	73.1±1.3	75.0±1.9
LVOT-PG (mm Hg)	5.8±2.2	8.4±4.9
E/Ea	11.0±0.48	14.9±1.0*
LV mass index (g/m^2)	124.8±6.6	153.2±7.9*
Cardiac catheterisation		
EDVI (ml/m^2)	65.1±4.3	70.5±5.6
ESVI (ml/m^2)	19.4±1.9	23.9±4.7
EDP (mm Hg)	11.4±1.7	16.2±1.3*
PCWP (mm Hg)	11.0±1.7	16.3±1.4*
CI (l/min/m^2)	3.2±0.2	2.9±0.3
Neurohormonal factors		
BNP (pg/ml)	70.9±23.9	221.9±53.2*
Norepinephrine (pg/ml)	701.5±152.1	568.1±60.7
Aldosterone (ng/l)	99.7±13.1	81.1±13.5
Biochemical data		
Fasting glucose (mg/dl)	103.4±3.2	101.9±5.0
HbA1c (%)	5.6±0.14	5.3±0.13
HOMA-IR	1.7±0.5	1.4±0.26
LDL-cholesterol (mg/dl)	122.8±6.2	134.1±8.0
HDL-choresterol (mg/dl)	46.8±2.4	57.0±6.2
Triglyceride (mg/dl)	172.0±23	134.0±20
Ccr (ml/min)	91.4±8.9	81.7±8.4
Adiponectin (μg/ml)	5.23±0.9	9.62±1.0*

Data are presented as means±SEM.
*$p<0.05$ versus group A.BMI, body mass index; BNP, B-type natriuretic peptide; Ccr, creatinine clearance; CI, cardiac index; E/Ea, early diastolic transmitral flow velocity/early diastolic annular velocity; EDP, end-diastolic pressure; EDVI, end-diastric volume index; EF, ejection fraction; ESVI, end-systolic volume index; HDL, high-density lipoprotein; HOMA-IR, homoeostasis model assessment of insulin resitance; LAD, left atrial dimension; LDL, low-density lipoprotein; LV, left ventricular; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVOT-PG, left ventricular outflow tract pressure gradient; PCWP, pulmonary capillary wedge pressure.

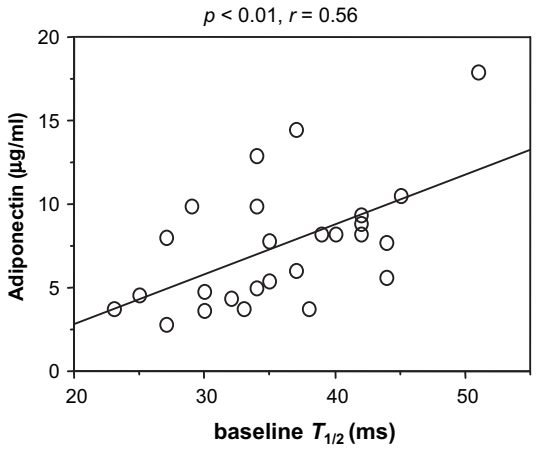

Figure 1 Scatterplots of the association between adiponectin levels and the $T_{1/2}$ values.

study. Patients were 61.2 ±2.2 years of age (range 35–77), with six (23.1%) of the patients female. Patients had mild shortness of breath on effort or atypical chest pain and were classified as New York Heart Association functional class I or II. Echocardiography confirmed that the patients in this study had non-obstructive LV hypertrophy with a mean LVEF of 74.1 ±1.5%. Thus, all patients had asymptomatic or mildly symptomatic HCM.

Patient characteristics according to the baseline $T_{1/2}$ value

The baseline $T_{1/2}$ is considered to be highly sensitive as an index for diastolic dysfunction in patients with HCM. According to previous reports, healthy subjects show a baseline $T_{1/2}$ value of <35 ms.[18] In addition, the median baseline $T_{1/2}$ value in our study was 35 ms. Therefore, we divided the patients with HCM into two groups on the basis of baseline $T_{1/2}$ (group A: $T_{1/2}$< 35 ms, group B: $T_{1/2}\geq$ 35 ms). The LV end-diastolic pressure values in group B were significantly increased as compared with those in group A ($p<0.05$). Thus, patients in group B exhibited diastolic dysfunction as compared with patients in group A.

Clinical characteristics of each group are shown in table 1. Vital signs, echocardiography data and biomarkers are also shown. There were no significant differences between groups A and B in mean age, percentage of men, mean body mass index or smoking history. In echocardiographic data, no significant differences were seen in the levels of maximal wall thickness, left atrial dimension, LVEF and LV outflow tract pressure gradient for the two groups. E/Ea and LV mass index were significantly higher in group B as compared with those in group A ($p<0.05$). LV end-diastolic dimension, LV end-systolic dimension, and cardiac index derived from cardiac catheterisation were similar, whereas pulmonary artery wedge pressure was significantly higher in group B. Levels of brain natriuretic peptide but not norepinephrine and aldosterone were significantly higher in group B than those in group A. There were no differences between the two groups in the levels of blood glucose, HOMA-IR, creatinine clearance or lipid profiles. Plasma adiponectin levels were significantly higher ($p<0.05$) in group B (9.62±1.0 μg/ml) as compared with those in group A (5.23±0.9 μg/ml).

Correlation between adiponectin and baseline $T_{1/2}$ in patients with HCM

We examined the association between plasma adiponectin levels in the PV and the baseline $T_{1/2}$ values in patients with HCM, as circulating adiponectin levels in group B were markedly higher than those in group A. Plasma adiponectin levels in the PV were

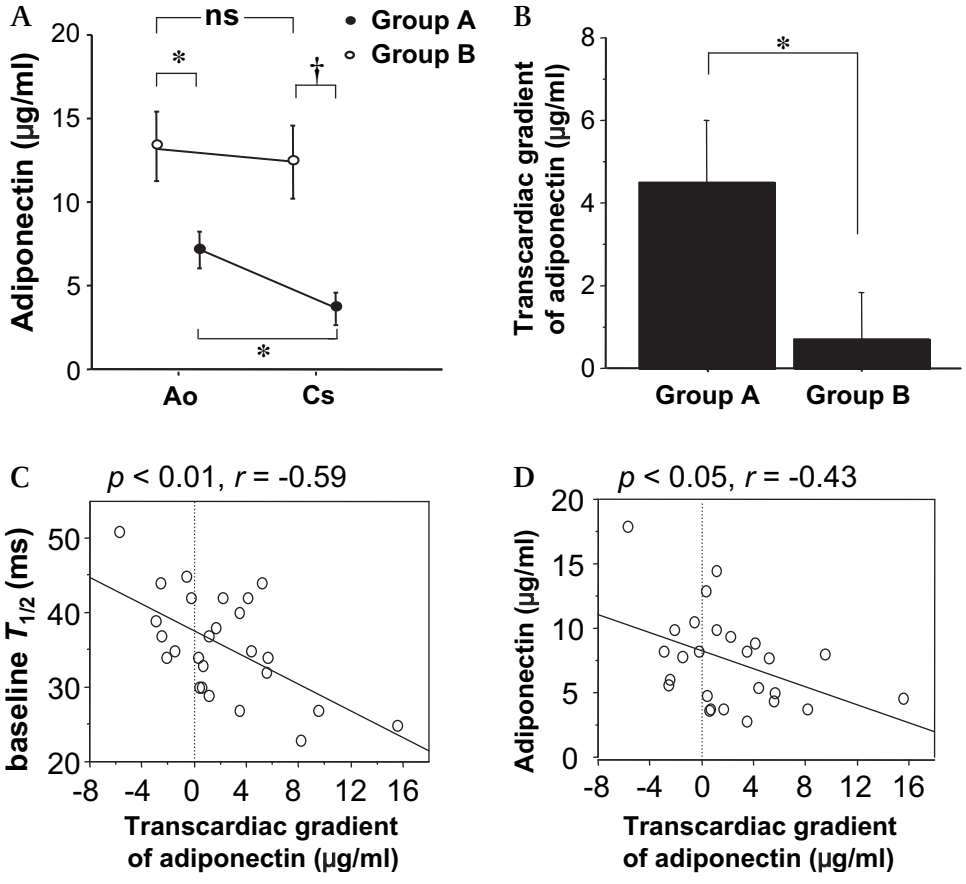

Figure 2 Transcardiac gradient of adiponectin in patients with hypertrophic cardiomyopathy. (A) Plasma adiponectin levels in the aortic root (Ao) and coronary sinus (CS) in group A (closed circle) and group B (open circle). (B) The transcardiac gradient of adiponectin as calculated by the Ao–CS difference in groups A and B. (C) Scatterplots of the association between $T_{1/2}$ values and the transcardiac gradient of adiponectin. (D) Scatterplots of the association between adiponectin levels in the peripheral vein and the transcardiac gradient of adiponectin. *$p<0.05$, †$p<0.01$ (group A: n=12, group B: n=14).

positively correlated with the baseline $T_{1/2}$ values ($p<0.01$, $r=0.56$) in patients with HCM (figure 1).

Transcardiac gradient of adiponectin levels in patients with HCM

To examine the transcardiac utilisation of adiponectin, we simultaneously collected blood from the Ao and CS as well as the PV. Adiponectin levels in both the Ao and CS were significantly higher in group B than in group A (figure 2A). In group A, adiponectin levels were significantly lower in the CS than in the Ao. In contrast, group B exhibited no significant differences in adiponectin levels in the Ao and the CS (figure 2A). The transcardiac gradient of adiponectin as calculated by the Ao−CS difference was significantly higher in group A than in group B (figure 2B). The transcardiac gradient of adiponectin also inversely correlated with the values of baseline $T_{1/2}$ and adiponectin levels in the PV in patients with HCM (figure 2C,D). Thus, cardiac uptake of adiponectin was negatively associated with LV diastolic dysfunction in patients with HCM.

Adiponectin receptors in patients with HCM

Lastly, we assessed the expression of the adiponectin receptors AdipoR1 and AdipoR2 in human hearts by reverse transcriptase-PCR analysis (n=8: each group). The expression of AdipoR1 in group B was significantly reduced in comparison with group A (figure 3A), whereas there were no significance differences in the expression of AdipoR2 between the two groups (figure 3B). Furthermore, the baseline $T_{1/2}$ values were negatively associated with the expression levels of AdipoR1 ($r=-0.57$, $p<0.05$) but not with the expression levels of AdipoR2 ($r=0.11$, $p=0.69$) in patients with HCM (figure 3C,D). The transcardiac gradient of adiponectin also positively correlated with the expression levels of AdipoR1 ($r=0.59$, $p<0.05$) but not with the expression levels of AdipoR2 ($r=0.24$, $p=0.59$) (figure 3E,F).

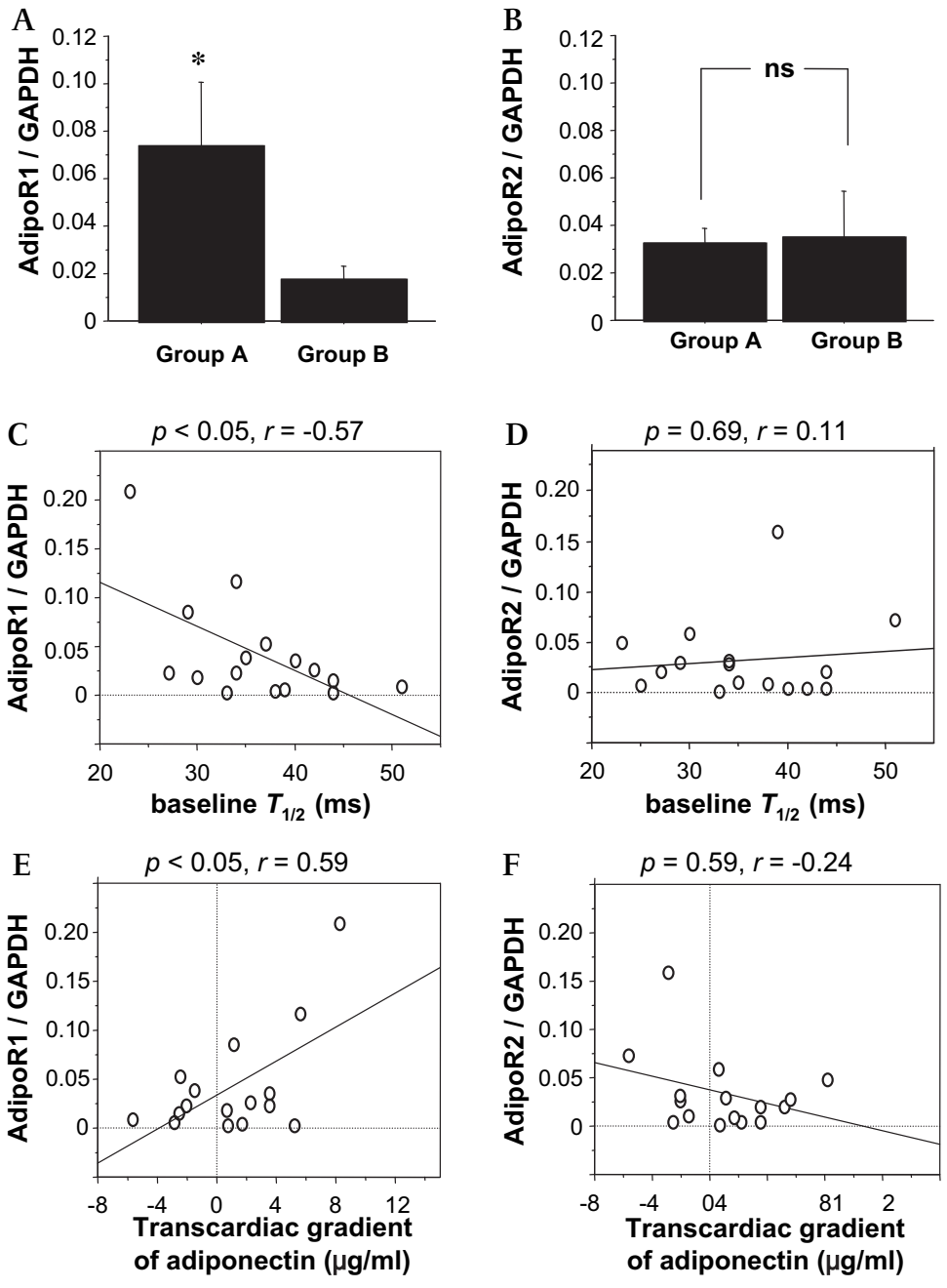

Figure 3 Expression of adiponectin receptors in heart tissues from patients with cardiomyopathy. AdipoR1 (A) and R2 (B) mRNA levels in heart tissues from group A and group B. (C) Scatterplots of the association between AdipoR1 levels and $T_{1/2}$ values. (D) Scatterplots of the association between AdipoR2 levels and $T_{1/2}$ values. (E) Scatterplots of the association between AdipoR1 levels and the transcardiac gradient of adiponectin. (F) Scatterplots of the association between AdipoR2 levels and the transcardiac gradient of adiponectin. Results describe expressed AdipoR1 and R2 mRNA levels relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels. *$p<0.05$ (n=8 in each group).

DISCUSSION

This study demonstrated that, in patients with HCM without systolic dysfunction, plasma adiponectin levels positively correlated with the $T_{1/2}$ values as an index of myocardial relaxation. These observations suggest that plasma adiponectin levels were associated with LV diastolic function in patients with HCM. Increased adiponectin levels could be an indicator of LV diastolic dysfunction in asymptomatic or mildly symptomatic patients with HCM. Our data appear to show that plasma adiponectin levels are low in the early stage of HCM (the group A: 5.23±0.9 μg/ml) in comparison with levels (6–8 μg/ml) in healthy subjects, as determined by the same assay method.[20 21] In this regard, cross-sectional studies demonstrate the inverse correlation between adiponectin levels and the presence of LV hypertrophy.[8–11] Future prospective studies are needed to clarify this relationship, because our results are obtained from observational study.

In this study, the transcardiac gradient of adiponectin is negatively correlated with the values of baseline $T_{1/2}$ and circulating adiponectin levels. Similarly, it was reported that a low transcardiac gradient of adiponectin in the coronary circulation was associated with extensive coronary artery disease in patients with type II diabetes.[22] Adiponectin protein also has been detected at the periphery of damaged myocytes in patients with myocardial infarction and dilated cardiomyopathy.[23 24] Recently, we demonstrated in experiments with mice that adiponectin protects the heart from injury by accumulating in tissues subjected to ischaemic damage through leakage from the vascular compartment.[25] Therefore, impairment of adiponectin utilisation in the heart is likely to contribute to diastolic dysfunction in our study populations.

Adiponectin interactions with AdipoR1 and AdipoR2 are believed to mediate AMPK activation, leading to enhanced glucose uptake and fatty acid oxidation.[26] AdipoR1 and AdipoR2 are expressed in cardiac myocytes and heart tissues.[26 27] These receptors were found to mediate AMPK activation by adiponectin in cardiac myocytes in an in vitro model of hypertrophy.[27] AdipoR1 expressions were suppressed by the incubation of tumour necrosis factor α or norepinephrine in cultured cardiac myocytes.[28] The expression of AdipoR1, but not of AdipoR2, in myocardium was also reduced in a muse model of myocardial infarction.[27] Our data showed that the cardiac expression of AdipoR1 but not AdipoR2 decreased in patients with advanced diastolic dysfunction. It is possible that clearance of adiponectin by myocardium is impaired in patients with diastolic dysfunction, presumably owing to downregulation of adipoR1. Therefore, in light of the protective effects of adiponectin on the pathological cardiac remodelling, these disease conditions may be associated with a so-called 'adiponectin resistance'.[29 30] However, it is still unclear whether dysregulation of adiponectin is a cause or a result of diastolic cardiac dysfunction, and detailed biochemical and genetic studies are required to understand the underlying mechanism by which high adiponectin levels associate with diastolic dysfunction.

In patients with HCM, high adiponectin levels correlated with LV diastolic dysfunction, which may be associated with impaired utilisation of adiponectin in the heart. Therefore, continuous follow-up of adiponectin level might be helpful for assessment of the transition from hypertrophic status to diastolic heart failure in patients with HCM.

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Competing interests None.

Ethics approval This study was conducted with the approval of the ethics committee of the Nagoya University School of Medicine, Japan.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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