Clinical Investigations

# Impact of Systolic Dysfunction in Genotyped Hypertrophic Cardiomyopathy

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ABSTRACT

*Background:* Hypertrophic cardiomyopathy (HCM) is a disease of the sarcomere, and approximately 5% of cases of HCM show systolic dysfunction with poor prognosis. Few data exist regarding the systolic dysfunction in a large population of genotyped HCM subjects.

*Hypothesis:* The aim of this study was to assess the systolic dysfunction and prognosis in sarcomere gene mutation carriers.

*Methods:* The study included 157 sarcomere gene mutation carriers from 69 unrelated HCM families (87 males; mean age, 46.5 ± 20.5 years). After exclusions for systolic dysfunction at baseline, 107 subjects underwent serial echocardiograms.

*Results:* At a mean follow-up of 7.0 years, 12 subjects experienced systolic dysfunction. In multivariate Cox analysis, systolic dysfunction was related to age and ejection fraction at initial evaluation ($P < 0.001$ and $P = 0.020$, respectively), and was associated with the absence of mutations in the cardiac myosin-binding protein C gene (*MYBPC3*) ($P = 0.042$). When the subjects were divided into *MYBPC3* and non-*MYBPC3* mutation carriers, and time from birth to development of systolic dysfunction was compared, the rate of systolic dysfunction was higher in the non-*MYBPC3* group than in *MYBPC3* group (Kaplan-Meier, log-rank test, $P = 0.010$). After the onset of systolic dysfunction, 11 of 12 subjects died during a mean follow-up of 8.3 years.

*Conclusions:* Non-*MYBPC3* mutation carriers developed left ventricular systolic dysfunction more frequently than *MYBPC3* mutation carriers, and the majority of sarcomere gene mutation carriers with systolic dysfunction had fatal outcomes during follow-up. This suggests that subjects with mutations in sarcomeric genes require careful management for systolic dysfunction.

## Introduction

Hypertrophic cardiomyopathy (HCM) is a primary disorder of the myocardium that causes distinctive anatomic and histologic features,[1] and it is the most frequent cause of sudden cardiac death in young athletes.[2] Epidemiologic data indicate that 2 in 1000 young adults have unexplained hypertrophy.[3] Mutations in genes that encode sarcomere proteins including cardiac myosin-binding protein C gene (*MYBPC3*), cardiac troponin T gene (*TNNT2*), cardiac troponin I gene (*TNNI3*), and cardiac β-myosin heavy chain gene (*MYH7*) are well-established causes of the disease.[1,4] Studies in populations with familial HCM often reported that mutations in sarcomere proteins could be detected in around 50% of study subjects.[4–6] Left ventricular systolic dysfunction has been regarded as a relatively common disease complication of HCM.[7–10] However, the characterization of left ventricular systolic dysfunction has been hindered by the small number of subjects with the disease (single cases or small groups of subjects).[11–13] We have reported that subjects with HCM caused by mutations in *TNNT2* and *TNNI3* start to develop left ventricular systolic dysfunction at around 40 years of age.[14–17] In addition, we and others have reported that subjects with HCM caused by mutations in *MYBPC3* also progress to left ventricular systolic dysfunction,[18,19] although the clinical features of HCM associated with mutations in *MYBPC3* have late onset and a favorable clinical course.[20] These data may provide useful information on genetic counseling strategies of affected subjects with sarcomere gene mutations. However, these previous studies have been based largely on HCM subjects with a single gene mutation.[14–19]

The primary aim of the present study was to compare the clinical course of left ventricular systolic dysfunction

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in subjects with HCM in a relatively large population with mutations in various sarcomeric genes. The secondary aim was to assess the prognosis of the HCM subjects who developed left ventricular systolic dysfunction.

## Methods

This study included 69 unrelated probands with HCM exhibiting disease-causing mutations in genes such as *MYBPC3, TNNT2, TNNI3,* and *MYH7.* In addition, their family members were evaluated clinically and genetically, and 88 carriers with the same etiological sarcomere gene mutation as each proband were identified. Thus, a total 157 genetically affected subjects (87 males; mean age, 46.5 ± 20.5 years) comprised the study population. All subjects were identified at Kanazawa University Hospital or its affiliated hospitals (from primary to tertiary care centers) between 1998 and 2009. The diagnosis of HCM was based on the echocardiographic demonstration of left ventricular hypertrophy (LVH) (maximal left ventricular wall thickness ≥13 mm) in the absence of other cardiac or systemic causes for the left ventricular hypertrophy. These subjects also met the definition and classification proposed by the 1995 World Health Organization/International Society and Federation of Cardiology Task Force.[21] Subjects with systolic dysfunction were also included in this study. To compare the differences in the clinical course between several disease-causing genes, carriers with multiple mutations were not included in this study. Written informed consent was obtained from all subjects or from the parents of minors participating in the study in accordance with the guidelines of the Bioethical Committee on Medical Research, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan.

DNA was isolated from peripheral white blood cells of all subjects by use of a DNA extractor (ABI 341 Genepure Nucleic Acid Purification System; Applied Biosystems, Carlsbad, CA). In vitro amplification of genomic DNA was performed via polymerase chain reaction (PCR). Oligonucleotide primers were used to amplify all exons and exon-intron boundaries of 4 sarcomere genes, namely *MYBPC3, TNNT2, TNNI3,* and *MYH7* using standard protocols as previously described.[14–18] Single-strand conformational polymorphism analysis of amplified DNA was then performed as previously described,[14–18] with a slight modification. We also screened for mutations in sarcomere genes using high-resolution melt analysis as previously described.[22] For abnormal single-strand conformational polymorphism patterns or abnormal melt profiles, the nucleotide sequences of the cloned PCR products were determined on both strands (bidirectional sequencing) by the dye terminator cycle sequencing method using an automated fluorescent sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems). The sequence variation was confirmed by restriction enzyme digestion. Samples from 400 chromosomes of 200 ethnicity-matched normal individuals were also analyzed.

Standard transthoracic M-mode and 2-dimensional echocardiographic studies were performed to identify and quantify the morphological features of the left atrium and left ventricle. The left atrial dimension was measured at end-systole. Maximal wall thickness (MWT) of the left ventricle was defined as the greatest thickness in any single segment. Left ventricular end-diastolic dimension (LVDd), left ventricular end-systolic dimension (LVDs), interventricular septal thicknesses (IVST), and posterior wall thickness (PWT) were measured at the level of the tips of the mitral valve leaflets. Ejection fraction (EF) was calculated by Teicholz's method and by modified Simpson's method when left ventricular dilatation or regional decrease of left ventricular wall motion occurred. Systolic dysfunction was defined as EF <50% according to a previous report.[23] The echocardiographic parameters and the course of development of systolic dysfunction were compared among the genotyped HCM subjects. Differences between values measured at baseline and follow-up were analyzed by the Student paired *t* test. Differences between groups were analyzed by the Student unpaired *t* test. Categorical variables were compared by the $\chi^2$ test for independent variables. A multivariate Cox analysis was performed to find predictor factors of systolic dysfunction. The age that subjects developed systolic dysfunction for the first time was estimated according to the Kaplan-Meier method, and comparison of the 2 groups was performed by means of the log-rank test. A $P$ value <0.05 was considered statistically significant in all analyses. Statistical analyses were carried out with the computer software SPSS version 17.0 (IBM SPSS, Armonk, NY).

## Results

The clinical and genetic characteristics of the study population are presented in Table 1; 26 different mutations were identified in 157 subjects. Left ventricular outflow tract obstruction (pressure gradient at rest >30 mm Hg) was detected only in 4 subjects (hypertrophic obstructive cardiomyopathy), and none of them underwent percutaneous septal ablation. Most of these mutations have been identified and described elsewhere.[14–19,24–27] Three nonsense mutations, p.Gln541ter, p.Tyr816ter, and p.Gln827ter in *MYBPC3* and 3 missense mutations, p.Ala200Thr, p.Ala321Val, and p.Ser866Pro in *MYH7* were novel and presumed to be pathogenic by standard criteria of the absence of the mutation in large numbers of normal controls, alteration of evolutionarily conserved residues, and/or predicted impact on protein structure. Two missense mutations p.Val85Leu in *TNNT2* and the missense mutation p.Met822Leu in *MYH7* were due to alteration in the nucleotide sequences, GTG to TTG, ATG to CTG, respectively; this the first report of these nucleotide alterations in these genes.

At the initial evaluation, 9 subjects showed systolic dysfunction. Of the 9 subjects, 3 were *MYBPC3* mutation carriers (2 of p.Arg820Gln from 2 families and 1 of p.Gln998Glu), 2 were *TNNT2* mutation carriers (both p.Arg92Trp from 1 family), 2 were *TNNI3* mutation carriers (both p.Lys183del from 2 families), and 2 were *MYH7* mutation carriers (1 of p.Gly733Glu and 1 of p.Met822Leu). As for the frequencies of subjects with systolic dysfunction, 5.4% (3/56) were *MYBPC3* mutation carriers, 8.7% (2/23) were *TNNT2* mutation carriers, 3.6% (2/56) were *TNNI3* mutation carriers, and 9.1% (2/22) were *MYH7* mutation carriers. There were no differences among the carriers


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Table 1. Clinical and Genetic Characteristics of the Study Population

| | |
|---|---|
| No. | 157 |
| Age (y) | 46.5 ± 20.5 |
| Male (%) | 87 (55.4) |
| Echocardiography | |
| LAD (mm) | 37.6 ± 7.5 |
| IVST (mm) | 14.7 ± 5.4 |
| PWT (mm) | 10.5 ± 2.3 |
| MWT (mm) | 15.3 ± 5.6 |
| LVDd (mm) | 44.5 ± 6.3 |
| LVDs (mm) | 28.0 ± 7.0 |
| EF (%) | 67.1 ± 10.9 |
| HOCM | 4 (2.5%) |
| Disease-causing gene (%) | |
| *MYBPC3* | 56 (35.7) |
| *TNNT2* | 23 (14.6) |
| *TNNI3* | 56 (35.7) |
| *MYH7* | 22 (14.0) |
| Medications (%) | |
| Calcium channel blocker | 27 (17.2) |
| β-Blocker | 24 (15.3) |
| ACE-I or ARB blocker | 29 (18.5) |

Abbreviations: ACE-I, angiotensin-converting enzyme-inhibitor; ARB, angiotensin receptor blocker; EF, ejection fraction; HOCM, hypertrophic obstructive cardiomyopathy; IVST, interventricular septal thickness; LAD, left atrial dimension; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; MWT, maximal wall thickness; *MYBPC3*, cardiac myosin-binding protein C gene; *MYH7*, cardiac β-myosin heavy chain gene; PWT, posterior wall thickness; *TNNI3*, cardiac troponin I gene; *TNNT2*, cardiac troponin T gene. Values are mean ± SD unless otherwise shown.

of the 4 genes with respect to the frequency of systolic dysfunction ($P = 0.72$). To study the clinical course of the development of systolic dysfunction, 9 subjects who had systolic dysfunction at the initial evaluation were excluded, and 107 of the 157 subjects underwent serial echocardiography at least a month apart. The data from 107 subjects (54 males; mean age, 44.7 ± 20.9 years; mean follow-up, 7.0 ± 4.9 years) were analyzed. During the mean follow-up period of 7 years, 12 subjects out of 107 experienced systolic dysfunction (1.60 cases per 100 person-years). In multivariate Cox analysis, systolic dysfunction was closely related to the age and ejection fraction at initial evaluation ($P < 0.001$ and $P = 0.020$, respectively), and was closely related to the absence of mutation in *MYBPC3* as a disease-causing gene ($P = 0.042$). Therefore, we divided the 107 subjects into 2 groups, those with *MYBPC3* mutations and those with other sarcomere gene mutations (non-*MYBPC3*), and compared the age to the development of systolic dysfunction.

Table 2. Serial Changes of Echocardiographic Parameters During Follow-up

| | Baseline | *P* Value | Follow-up | *P* Value |
|---|---|---|---|---|
| Cases | | | | |
| *MYBPC3* | 36 | | | |
| Non-*MYBPC3* | 71 | | | |
| Male (%) | | | | |
| *MYBPC3* | 24 (66.7) | 0.017 | | |
| Non-*MYBPC3* | 30 (42.3) | | | |
| Age, y | | | | |
| *MYBPC3* | 50.1 ± 19.9 | 0.055 | | |
| Non-*MYBPC3* | 41.9 ± 21.0 | | | |
| HOCM (%) | | | | |
| *MYBPC3* | 2 (5.6) | 0.480 | | |
| Non-*MYBPC3* | 2 (2.8) | | | |
| Medications (%) | | | | |
| Calcium channel blocker | | | | |
| *MYBPC3* | 8 (22.2) | 0.721 | | |
| Non-*MYBPC3* | 18 (25.4) | | | |
| β-Blocker | | | | |
| *MYBPC3* | 5 (13.9) | 0.287 | | |
| Non-*MYBPC3* | 16 (22.5) | | | |
| ACE-I or ARB | | | | |
| *MYBPC3* | 7 (19.4) | 0.598 | | |
| Non-*MYBPC3* | 17 (23.9) | | | |
| IVST | | | | |
| *MYBPC3* | 15.8 ± 5.6 | 0.565 | 17.2 ± 6.0 | 0.118 |
| Non-*MYBPC3* | 15.1 ± 5.6 | | 15.4 ± 5.5 | |
| PWT | | | | |
| *MYBPC3* | 11.1 ± 2.3 | 0.178 | 10.4 ± 1.8 | 0.529 |
| Non-*MYBPC3* | 10.4 ± 2.6 | | 10.2 ± 2.5 | |
| MWT | | | | |
| *MYBPC3* | 16.6 ± 5.9 | 0.456 | 17.5 ± 6.0 | 0.194 |
| Non-*MYBPC3* | 15.7 ± 5.8 | | 15.9 ± 5.9 | |
| LVDd | | | | |
| *MYBPC3* | 43.8 ± 4.9 | 0.754 | 44.9 ± 5.8 | 0.717 |
| Non-*MYBPC3* | 43.5 ± 5.5 | | 44.4 ± 7.0 | |

There were 36 subjects in *MYBPC3* and 71 subjects in non-*MYBPC3* groups. At the time of initial evaluation,

Table 2. *Continued*

| | Baseline | *P* Value | Follow-up | *P* Value |
|---|---|---|---|---|
| LVDs | | | | |
| *MYBPC3* | 26.5 ± 3.8 | 0.829 | 27.7 ± 5.5 | 0.344 |
| Non-*MYBPC3* | 26.7 ± 5.1 | | 29.1 ± 7.9[a] | |

Abbreviations: ACE-I, angiotensin-converting enzyme-inhibitor; ARB, angiotensin receptor blocker; HOCM, hypertrophic obstructive cardiomyopathy; IVST, interventricular septal thickness; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; MWT, maximal wall thickness; PWT, posterior wall thickness. Values are mean ± standard deviation unless otherwise shown. *P* values concern the comparison of the values at baseline and follow-up between *MYBPC3* and non-*MYBPC3* (12 of *TNNT2*, 46 of *TNNI3*, and 13 of *MYH7*). [a]P < 0.05 compared with baseline within group by paired *t* test.

no significant differences were found between *MYBPC3* and non-*MYBPC3* groups, respectively, for the following variables: IVST, PWT, MWT, LVDd, and LVDs (Table 2). There was no significant difference in EF between the 2 groups at baseline. However, the percent decrease in EF was significantly greater in the non-*MYBPC3* group between baseline and last evaluation or the time of development of systolic dysfunction (69.0 ± 8.4% vs 63.7 ± 13.0%; $P < 0.001$) (Figure 1), whereas there was no significant change in the *MYBPC3* group in this time interval. Furthermore, Kaplan-Meier analysis comparing the age at which systolic dysfunction developed showed a significant difference between *MYBPC3* and non-*MYBPC3* groups (log-rank test, $P = 0.010$) (Figure 2). In the *MYBPC3* group, 1 subject out of 36 subjects developed to systolic dysfunction at the age of 71 in 6.3 years (0.44 cases per 100 person-years). On the other hand, in the non-*MYBPC3* group, 11 subjects out of 71 subjects developed to systolic dysfunction in 7.3 years (2.12 cases per 100 person-years, $P = 0.093$).

Next, we investigated the prognosis of the 12 subjects who developed systolic dysfunction (Table 3). Systolic dysfunction had a wide range of age for onset (ie, 41 to 74 years; mean, 58.9 ± 11.9 years). Three subjects (25.0%) were 41 to 50 years old, 4 subjects (33.3%) were 51 to 60 years old, 1 subject (8.3 %) had onset age of 62, and 4 subjects (33.3 %) were over 70 years old. Four subjects (33.3%) were male, and the mean age at death or the most recent evaluation was 67.3 ± 11.5 years. All 12 subjects were admitted to hospital for heart failure, and 11 out of 12 subjects (91.7%) died within the follow-up period (8.3 ± 4.0 years). Four out of 11 subjects (36.4%) died of refractory heart failure, 4 subjects (36.4%) died suddenly from causes related to heart failure, 2 subjects (18.2%) died of interstitial pneumonia after heart failure, and 1 subject (9.1%) died of cerebral infarction with atrial fibrillation with use of an appropriate dose of warfarin.

## Discussion

In this longitudinal study, 107 of the 157 genotyped HCM subjects underwent serial echocardiography, and during the mean follow-up period of 7 years, 12 subjects out of 107 experienced systolic dysfunction (1.60 cases per 100 person-years). The major finding was, that in a relatively large population of genotyped HCM subjects, EF decreased significantly in the non-*MYBPC3* group (69.0 ± 8.0% to 63.7 ± 13.0%, $P < 0.001$) (Figure 1), and freedom from systolic dysfunction was lower in subjects in the non-*MYBPC3* group than those in *MYBPC3* group (Kaplan-Meier, log-rank test, $P = 0.010$) (Figure 2).

The clinical features of HCM are particularly heterogeneous.[7,8] A patient subset characterized by clinical progression of left ventricular systolic dysfunction has been described.[9–13] Most of the prior literature about systolic dysfunction is, however, limited to isolated or small groups of subjects.[9–13] Recently, Harris et al reported on a cohort of HCM subjects with systolic dysfunction in a multicenter study that included the largest group of subjects with systolic dysfunction reported to date.[23] In that study, incidence of systolic dysfunction was 1.12 cases per 100 person-years. In our study, incidence of systolic dysfunction was 12 cases per 749 person-years of follow-up (1.60 cases per 100 person-years); the rates in these 2 studies were similar, which suggests that the cohort of 157 genotyped HCM subjects in this study was comparable to the cohort of 1259 study subjects in the former study in terms of systolic dysfunction.

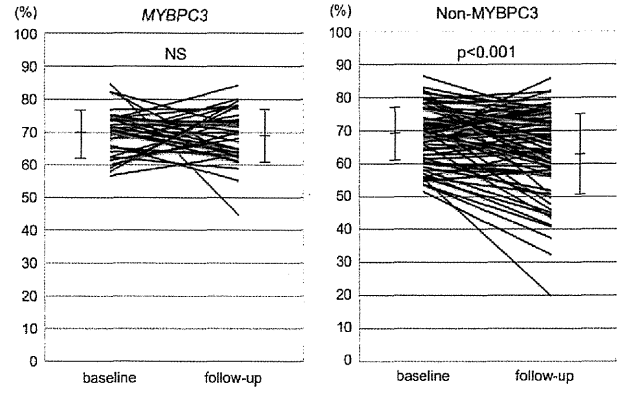


Figure 1. The percent decrease in ejection fraction in *MYBPC3* and non-*MYBPC3* groups. *MYBPC3*, myosin-binding protein C gene; NS, not significant.

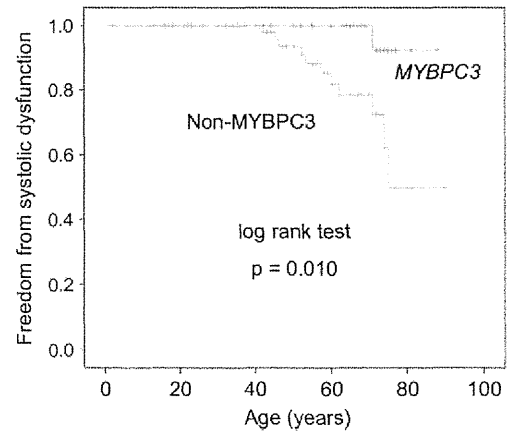


Figure 2. Kaplan-Meier analysis comparing the age at which systolic dysfunction developed in subjects in the myosin-binding protein C (*MYBPC3*) gene and non-*MYBPC3* groups.


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Recent advances in molecular genetics have demonstrated that HCM can be considered as a disease of the sarcomere. Some studies have investigated the relationship between specific sarcomere gene mutation and systolic dysfunction; however, these were limited to subjects with mutations in a single gene only.[14–19] In the multicenter cohort of over 1200 HCM subjects,[23] 44 subjects demonstrated systolic dysfunction, and 3 probands were genotyped to HCM-causing sarcomere protein mutations: p.Gly716Arg in *MYH7* in 2 and G-791 in *MYBPC3* in 1. This number of subjects (6.8%, 3 of 44) is very limited. On the other hand, we studied a relatively large population of HCM subjects with several sarcomere gene mutations and analyzed the relationship between disease-causing genes and systolic dysfunction.

We found that freedom from systolic dysfunction was lower in subjects in the non-*MYBPC3* group than those in *MYBPC3* group in this longitudinal study (Kaplan-Meier, log-rank test, $P = 0.010$) (Figure 2). This could be explained by several findings in the recent study. Sakata et al investigated left ventricular function reserve in HCM patients with and without mutations in cardiac troponin genes (*TNNT2* and *TNNI3*) before transition to systolic dysfunction.[28] Interestingly, the group of subjects with troponin gene mutations showed a significant increase in left ventricular end-systolic volume during an exercise test. This suggests that subjects with troponin gene mutations display exercise-induced left ventricular systolic dysfunction more frequently than HCM subjects without troponin gene mutations. The authors speculated that the mechanism of the systolic dysfunction was myocardial ischemia due to lumen narrowing of intramural coronary arteries. It was reported that the use of calcium channel blockers in advance of established clinical disease could prevent HCM caused by sarcomere protein gene mutations in a mouse model,[29] therefore the use of calcium channel blockers to ameliorate ischemia in advance of established systolic dysfunction may be useful to prevent the progression to systolic dysfunction in HCM.

Next, we investigated the prognosis of the 12 subjects who developed systolic dysfunction during follow-up (Table 3). At the time of presentation of systolic dysfunction, the mean age of the 12 subjects was 58.9 years (range, 41–74 years) and 67.3 years (range, 51–84 years) at death or the most recent evaluation. All of the 12 subjects were admitted to the hospital for heart failure, and 11 out of 12 subjects (91.7%) died within the follow-up period, which means the prognosis of genotyped subjects with HCM demonstrating systolic dysfunction is poor. The present study demonstrated that HCM subjects with mutations in sarcomere-related genes other than *MYBPC3* develop left ventricular systolic dysfunction more frequently than those with *MYBPC3* mutations, and most of those who developed systolic dysfunction died within mean period of 8.3 years. These observations may provide important prognostic information in the clinical practice of HCM, which suggests that subjects with mutations in sarcomeric genes require careful management for systolic dysfunction from the point of view of prognosis.

There remain several limitations of the present study. First, in recent studies, multiple gene mutations have been reported, which may further contribute to the disease heterogeneity.[30–35] We did not include carriers with multiple mutations in this study to compare the differences in the clinical course between multiple disease-causing genes; however, we should also assess the influence of multiple mutations on HCM phenotype as the next step. Second, heart transplantation should have been considered for many subjects who showed systolic dysfunction in our study; however, heart transplantation is not common in Japan. It

Table 3. Clinical Data From 12 Subjects Who Showed Systolic Dysfunction During Follow-up

| No. | Gene | Gender | A, y | Last Evaluation | B, y | A to B, y | Etiology of Death |
|---|---|---|---|---|---|---|---|
| 1 | *MYBPC3* | Male | 71 | Death | 76 | 5 | Interstitial pneumonia after heart failure |
| 2 | *MYH7* | Male | 74 | Death | 78 | 4 | Sudden death during heart failure |
| 3 | *TNNT2* | Female | 60 | Death | 69 | 9 | Refractory heart failure |
| 4 | *TNNT2* | Female | 53 | Death | 62 | 9 | Refractory heart failure |
| 5 | *TNNI3* | Female | 46 | Death | 52 | 6 | Sudden death during chronic heart failure |
| 6 | *TNNI3* | Female | 52 | Death | 59 | 7 | Sudden death during heart failure |
| 7 | *TNNI3* | Female | 45 | Death | 51 | 6 | Refractory heart failure |
| 8 | *TNNI3* | Female | 57 | Death | 74 | 17 | Cerebral infarction with atrial fibrillation |
| 9 | *TNNI3* | Female | 71 | Death | 84 | 13 | Refractory heart failure |
| 10 | *TNNI3* | Male | 62 | Death | 70 | 8 | Interstitial pneumonia after hear failure |
| 11 | *TNNI3* | Male | 75 | Death | 79 | 4 | Sudden death during chronic heart failure |
| 12 | *TNNI3* | Female | 41 | Alive | 53 | 12 | Not available |
| | | | 58.9 ± 11.9 | | 67.3 ± 11.5 | 8.3 ± 4.0 | |

Abbreviations: A, age at systolic dysfunction; A to B, interval from A to B; B, age at death or last evaluation.

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appears that without the option of heart transplantation, the natural course of systolic dysfunction related to sarcomere genes mutations often follows an adverse course with fatal outcome. Last, 46 out of the 71 subjects in the non-*MYBPC3* group were *TNNI3* mutation carriers (Table 2), which might reflect a founder effect. However, we also included subjects with mutations in *MYBPC3* that were reported to be associated with systolic dysfunction.[18,19]

## Conclusion

Non-*MYBPC3* mutation carriers developed left ventricular systolic dysfunction more frequently than *MYBPC3* mutation carriers, and the majority of sarcomere gene mutation carriers with systolic dysfunction had fatal outcomes during follow-up. This suggests that subjects with mutations in sarcomeric genes require careful management for systolic dysfunction.

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Original article

# Constitutive SIRT1 overexpression impairs mitochondria and reduces cardiac function in mice

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ABSTRACT

Heart failure is associated with a change in cardiac energy metabolism. SIRT1 is a $NAD^+$-dependent protein deacetylase, and important in the regulation of cellular energy metabolism. To examine the role of SIRT1 in cardiac energy metabolism, we created transgenic mice overexpressing SIRT1 in a cardiac-specific manner, and investigated cardiac functional reserve, energy reserve, substrate uptake, and markers of mitochondrial function. High overexpression of SIRT1 caused dilated cardiomyopathy. Moderate overexpression of SIRT1 impaired cardiac diastolic function, but did not cause heart failure. Fatty acid uptake was decreased and the number of degenerated mitochondria was increased dependent on SIRT1 gene dosage. Markers of reactive oxygen species were decreased. Changes in morphology and reactive oxygen species were associated with the reduced expression of genes related to mitochondrial function and autophagy. In addition, the respiration of isolated mitochondria was decreased. Cardiac function was normal in transgenic mice expressing a low level of SIRT1 at baseline, but the mice developed cardiac dysfunction upon pressure overload. In summary, the constitutive overexpression of SIRT1 reduced cardiac function associated with impaired mitochondria in mice.



## 1. Introduction

Congestive heart failure (CHF) is associated with a significant change in the energy metabolism of the heart, and the altered energetics is hypothesized to play an important role in the progression of CHF [1]. The alteration includes changes in substrate utilization, mitochondrial function, and energy transfer by the creatine shuttle system. Despite previous intense efforts, the mechanism by which these changes are induced, and the roles of the changes in the progression of CHF are still not clear.

Silent mating type information regulation 2 homolog 1 (SIRT1) is a nicotinamide adenine dinucleotide ($NAD^+$)-dependent protein deacetylase and a homolog of the yeast silencing information regulator 2 (sir2) [2]. Sir2 plays a critical role in lifespan extension caused by caloric restriction [2]. As expected from its important role in caloric restriction, SIRT1 has been shown to be important in the energy metabolism of cells [3]. SIRT1 deacetylates peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and increases mitochondrial function in adipocytes [4]. Transient transfection of SIRT1 also increased fatty acid oxidation in a skeletal myocyte cell line [5]. However, cellular respiration was decreased in skeletal muscle cells stably transfected with SIRT1 [6], and SIRT1 overexpression in

*Abbreviations:* ANF, atrial natriuretic factor; Atg, autophagy-related protein; Bmal1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like1; β-MHC, β-myosin heavy chain; BNP, brain natriuretic peptide; CD36/FAT, CD36/fatty acid translocase; CHF, congestive heart failure; Clock, circadian locomotor output cycles kaput; COX, cytochrome c oxidase; CPT-1b, carnitine palmitoyltransferase-1b; Cyt, cytochrome; ERRα, estrogen-related receptor α; FOXO, forkhead box O; Gabarapl1, γ-aminobutyric acid [GABA] receptor-associated protein-related protein-like 1; GLUT, glucose transporter; IDH3α, isocitrate dehydrogenase 3α; LC, microtubule-associated protein1 light chain; MCAD, medium-chain acyl-coenzyme A dehydrogenase; $NAD^+$, nicotinamide adenine dinucleotide; NAM, nicotinamide; NAMPT, nicotinamide phosphoribosyltransferase; ND4, NADH dehydrogenase 4; NMN, nicotinamide mononucleotide; NMNAT, nicotinamide mononucleotide adenylyltransferase; NRF-1, nuclear respiratory factor-1; NTg, non-transgenic; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; PPARα, peroxisome proliferator-activated receptor α; ROS, reactive oxygen species; Sir2, silencing information regulator 2; SIRT1, silent mating type information regulation 2 homolog 1; SOD, superoxide dismutase; TAC, transverse aortic constriction; Vps, vacuolar protein sorting.

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skeletal muscle tissue decreased mitochondrial function in animals [7]. These results indicate the role of SIRT1 in energy metabolism to be dependent on the tissue or duration of the activation.

SIRT1 has been implicated in the development of cardiac hypertrophy and failure [8–12]. The amount of SIRT1 protein was increased in hypertrophied or failing hearts of animals [8,10]. SIRT1 attenuated hypertrophy and cell death in cultured cardiac myocytes [8]. In addition, mild (2.5-fold) and moderate (7.5-fold) overexpression of SIRT1 in a heart-specific manner attenuated some of the age-associated changes of the heart, whereas marked (12.5-fold) overexpression caused dilated cardiomyopathy in mice and moderate overexpression protected the heart against oxidative stress [10]. Moderate overexpression of SIRT1 also protects heart tissue against ischemia reperfusion injury [12]. However, the role of SIRT1 in cardiac energy metabolism is not well characterized [8–11]. Here, we created transgenic mice overexpressing SIRT1 in a cardiac-specific manner, and examined cardiac functional reserve, energy reserve, substrate uptake, and markers of mitochondrial function.

## 2. Methods

See the online-only Data Supplement for additional details.

### 2.1. Animals

Transgenic lines overexpressing mouse SIRT1 in a heart-specific manner were generated on a C57BL6 background using the α-myosin heavy chain promoter [13]. SIRT1 mice and non-transgenic (NTg) littermates were sacrificed at 3 months of age after overnight fasting and analyzed. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All animal experiments and methods were approved by the Animal Care and Use Committees of Kyoto University Graduate School of Medicine.

### 2.2. Transverse aortic constriction (TAC)

Twelve-week-old male mice were subjected to transverse aortic constriction (TAC) [14]. Briefly, the animals were intubated and ventilated with a small-animal respirator (model SN-480-7-10; Shinano Seisakusyo, Tokyo, Japan), at a rate of 110 cycles/min and a tidal volume of 1 ml/100 g body weight. Aortic constriction was performed by tying a 7-0 silk string ligature around a 26-gauge needle and then removing the needle. The chest was then closed and the mice were extubated and allowed to recover.

### 2.3. Statistical analysis

All data were expressed as the mean ± SEM. Differences between the groups were compared using a one-way ANOVA followed by Fisher's protected least significant difference for multiple comparisons. Survival was analyzed by the standard Kaplan–Meier method with a log-rank test. For the analysis of hemodynamic data, a two-way repeated-measure ANOVA was used to test the differences between groups in the response to dobutamine. In all tests, a value of $p<0.05$ was considered significant.

## 3. Results

### 3.1. Generation of transgenic mice overexpressing SIRT1 in the heart

Transgenic mice overexpressing murine SIRT1 in a heart-specific manner were generated using the αMyHC promoter on a C57BL6 background. Twenty independently derived founders were produced from 110 screened mice. Ten of the founders expressed the transgene product as determined by a Western blot analysis. We analyzed 3 lines of SIRT1 mice expressing different levels of the SIRT1 protein in the heart (low: 3.2-fold, moderate: 6.8-fold, high: 20-fold compared with levels in non-transgenic (NTg) mice) (Fig. 1A). Hereafter, the three transgenic lines are designated as SIRT1 (L, low), SIRT1 (M, moderate), and SIRT1 (H, high) mice, according to the level of SIRT1 protein expression. Over a follow-up period of 1 year, SIRT1 (L) and SIRT1 (M) mice survived normally. SIRT1 (H) mice died of heart failure at a mean age of 3–4 months ($p<0.0001$, by log-rank test, Fig. 1B).

### 3.2. Cardiac function was preserved in SIRT1 (L) mice, and reduced in SIRT1 (M) and SIRT1 (H) mice

Echocardiographic analysis revealed left ventricular diastolic diameter (LVDd), fractional shortening (FS), and diastolic posterior wall thickness (PWTd) to be normal in SIRT1 (L) and SIRT1 (M) mice. However, in SIRT1 (H) mice, LVDd was increased and FS and PWTd were decreased (Table 1). Thus, the systolic function of SIRT1 (H) mice at rest was reduced. Next, we measured cardiac function using cardiac catheterization in mice infused with dobutamine. Cardiac function was normal in SIRT1 (L) mice. In SIRT1 (M) mice, left ventricular pressure (LVP) and maximum dP/dT were preserved, but minimum dP/dT was impaired, suggesting diastolic dysfunction in response to dobutamine. In SIRT1 (H) mice, LVP, maximum dP/dT, and minimum dP/dT were markedly impaired, indicating that both systolic and diastolic functions were affected. Thus, SIRT1 expression impaired cardiac function in a transgene dosage-dependent manner (Fig. 1C).

### 3.3. Myocardial energy reserve measured by in situ $^{31}P$ magnetic resonance spectroscopy was preserved in SIRT1 (L) and SIRT1 (M) mice

We measured the energy reserve of the heart by measuring high-energy phosphates using in situ $^{31}P$ magnetic resonance spectroscopy (MRS). The phosphocreatine (PCr)/ATP ratio was identified as a marker of myocardial energy reserve, and reported to be a prognostic indicator of CHF [15]. Representative cardiac $^{31}P$ MR spectra from mice are shown in Fig. 2B. The mean cardiac PCr/ATP ratio of NTg mice was 2.0, consistent with a previous report [16]. We analyzed SIRT1 (L) and SIRT1 (M) mice at rest, since it was difficult to do stress tests in the MRS apparatus. The cardiac PCr/ATP ratio was preserved in SIRT1 (L) and SIRT1 (M) mice (Fig. 2C). We could not measure the PCr/ATP ratio of SIRT1 (H) mice since they did not tolerate the anesthesia for the 30 min required for the analysis.

### 3.4. Fatty acid uptake decreased and glucose uptake increased in SIRT1 (M) and SIRT1 (H) mice

To examine the change in substrate utilization in the SIRT1 mouse heart, we examined the myocardial uptake of fatty acids and glucose using $^{125}$I-labeled 15-(*p*-iodophenyl)-9-*R,S*-methylpentadecanoic acid ($^{125}$I-9MPA) and $^{18}$fluorodeoxyglucose ($^{18}$FDG), respectively [17]. $^{125}$I-9MPA uptake was decreased by 25% in SIRT1 (M) mice and by 55% in SIRT1 (H) mice compared with the level in NTg control mice (Fig. 2D). $^{18}$FDG uptake was increased 3.1-fold in SIRT1 (M) mice and 5.3-fold in SIRT1 (H) mice. The gene expression of glucose transporter (GLUT) 1 and GLUT4 was not increased in SIRT1 (M) mice, and the expression of GLUT4 was decreased and that of GLUT1 was increased in SIRT1 (H) mice (Fig. 2E). Thus, a shift in substrate utilization from fatty acids to glucose, commonly observed in animal models of CHF [18], occurred in a manner that was dependent on the SIRT1 transgene dosage.

### 3.5. Pathological examination of SIRT1 mice

The heart weight/body weight ratio and lung weight/body weight ratio were increased in SIRT1 (H) mice (Table 2). There was also a

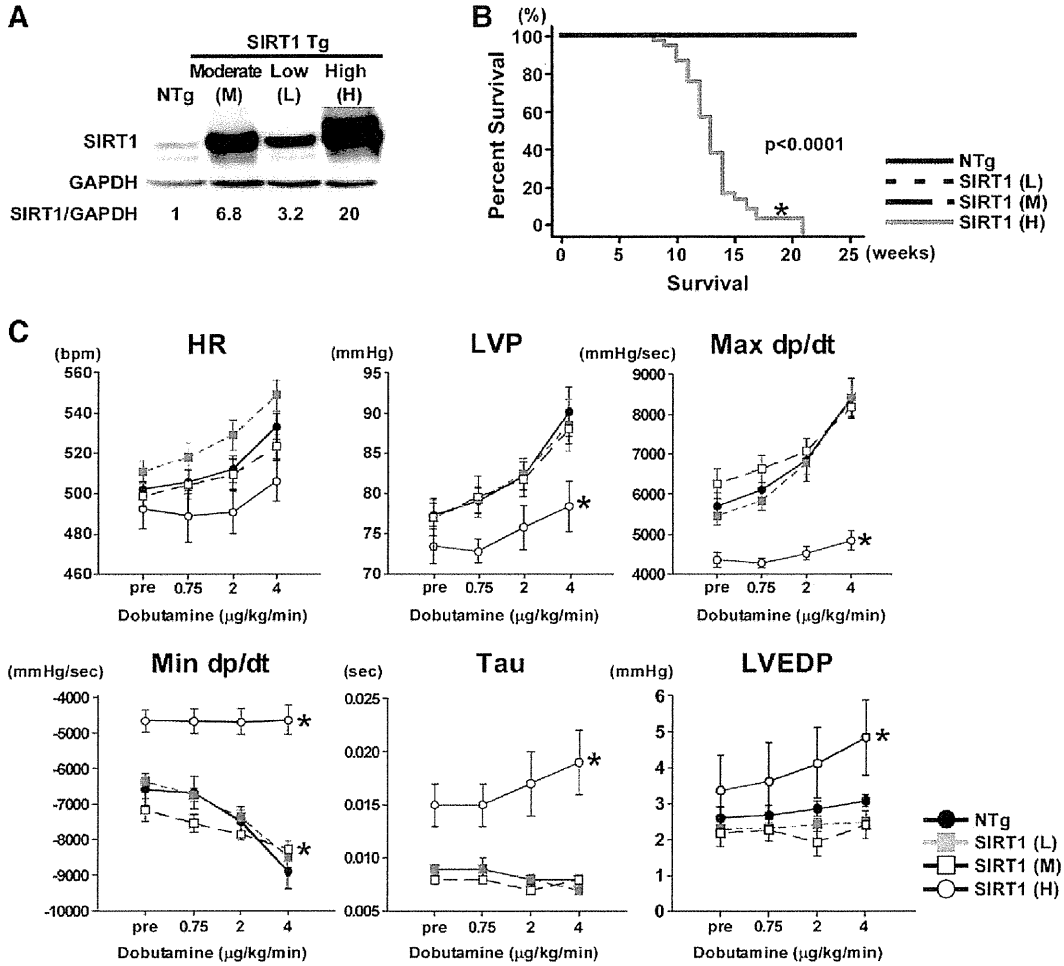


**Fig. 1.** Generation of transgenic mice overexpressing SIRT1 in heart. (A) Transgenic mice overexpressing SIRT1 in a heart-specific manner were generated using the α-MHC promoter on a C57BL6 background. The three transgenic lines were designated as SIRT1 (L, low), SIRT1 (M, moderate), and SIRT1 (H, high) mice, according to the level of SIRT1 protein expression. (B) Survival of SIRT1 transgenic lines. SIRT1 (H) mice died of heart failure at 3–4 months of age. (C) Cardiac function of SIRT1 transgenic mice. Cardiac function was measured using cardiac catheterization under dobutamine infusion. Diastolic function was impaired in SIRT1 (M) mice. Systolic and diastolic functional reserves were impaired in SIRT1 (H) mice. HR: heart rate, LVP: left ventricular pressure, LVEDP: left ventricular end diastolic pressure. A two-way repeated-measure ANOVA was used to test the differences between groups in response to dobutamine infusion. Values are the mean ± SEM. *$p<0.05$, interaction of dobutamine doses with differences between NTg mice and each Tg line ($n=8$ for each group).

marked increase in the size of the ventricular cavity and a thinning of the ventricular wall (Supplementary Fig. 1A). Thus, high overexpression of SIRT1 caused dilated cardiomyopathy, compatible with a previous report [10]. Cardiac gross morphology was normal in SIRT1 (L) and SIRT1 (M) mice. We examined the fibrosis and apoptosis of SIRT1 transgenic mice. A mild increase in myocardial fibrosis was observed in SIRT1 (H) mice (Supplementary Fig. 1B). Histological results were normal in SIRT1 (L) and SIRT1 (M) mice. A few TdT-mediated dUTP-biotin nick end-labeling (TUNEL)-positive nonmyocytes were observed in SIRT1 (H) mice, but the number of TUNEL-positive myocytes was not increased (Supplementary Fig. 1C). The levels of brain natriuretic peptide (BNP) and β-myosin heavy chain (β-MHC) mRNA were increased in SIRT1 (H) mice. The levels of atrial natriuretic factor (ANF) and α-skeletal actin mRNA did not increase in SIRT1 (H) mice despite severe heart failure (Supplementary Fig. 1D).

**Table 1**
Echocardiographic analysis of SIRT1 transgenic mice.

| | NTg | SIRT1 (L) | SIRT1 (M) | SIRT1 (H) |
|---|---|---|---|---|
| Number of animals | 6 | 6 | 6 | 6 |
| Heart rate (bpm) | 510 ± 6 | 517 ± 5 | 502 ± 5 | 508 ± 4 |
| LV diastolic diameter (mm) | 4.10 ± 0.07 | 3.93 ± 0.06 | 4.04 ± 0.06 | 4.41 ± 0.08* |
| LV systolic diameter (mm) | 2.49 ± 0.08 | 2.40 ± 0.06 | 2.33 ± 0.06 | 3.21 ± 0.03* |
| FS (%) | 39.4 ± 1.0 | 38.9 ± 1.0 | 42.1 ± 1.2 | 27.2 ± 0.8* |
| Diastolic posterior wall thickness (mm) | 0.73 ± 0.04 | 0.63 ± 0.03 | 0.73 ± 0.03 | 0.58 ± 0.03* |

Values are expressed as the mean ± SEM. NTg: non-transgenic mice; SIRT1: SIRT1 transgenic mice; bpm: beats per minute; LV: left ventricular; FS: fractional shortening.
* $p<0.05$ versus NTg mice.

### 3.6. Abnormal morphology of mitochondria in SIRT1 mice

Impaired cardiac function and the shift of substrate uptake from fatty acids to glucose suggest that cardiac energy metabolism is compromised in SIRT1 (M) and SIRT1 (H) mice. Thus, we examined the mitochondrial DNA content and morphology of mitochondria in SIRT1 mice. Mitochondrial DNA content assessed by quantitative PCR did not differ between the SIRT1 transgenic lines and NTg mice (Fig. 3C). Electron-microscopic analysis showed that the size and number of mitochondria did not differ among SIRT1 mice (Figs. 3A, C). However, degenerated or collapsed mitochondria were observed and their number increased dependent on the gene dosage of SIRT1 (Figs. 3B, C).

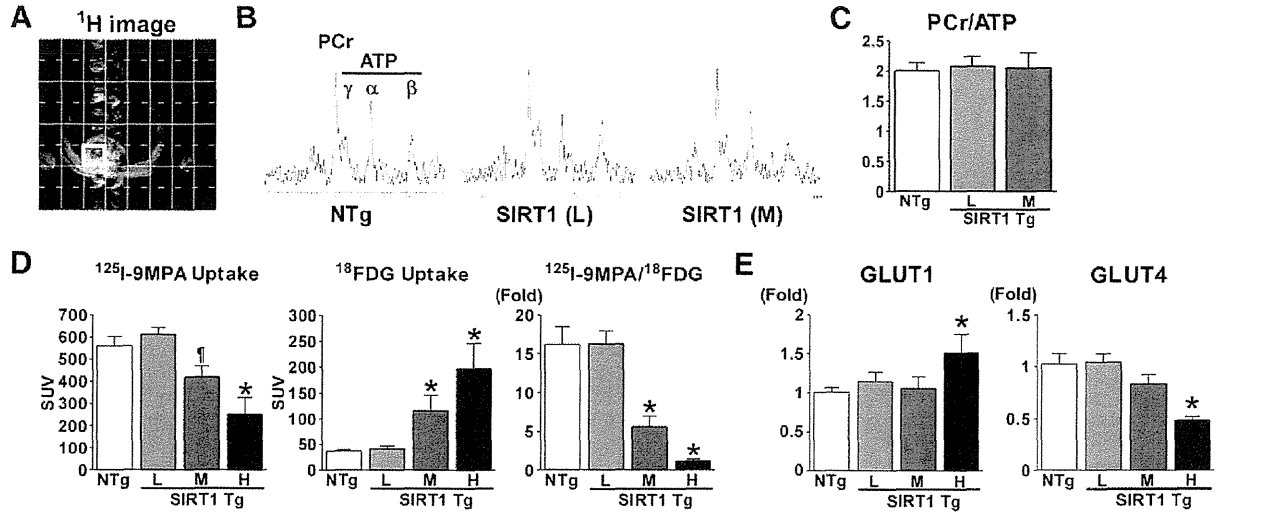


**Fig. 2.** Myocardial energy reserve measured by in situ $^{31}$P magnetic resonance spectroscopy was preserved in SIRT1 (L) and SIRT1 (M) mice. Fatty acid uptake decreased and glucose uptake increased in SIRT1 (M) and SIRT1 (H) mice. (A) A $^{1}$H magnetic resonance (MR) image to define the region of interest to measure a $^{31}$P MR spectrum of the LV anterior wall. (B) In vivo cardiac $^{31}$P MR spectra from NTg (left), SIRT1 (L) (middle), and SIRT1 (M) (right) mice. ppm, parts per million. (C) The PCr/ATP ratio was preserved in SIRT1 (L) and SIRT1 (M) mice compared with that in NTg mice (n = 10 for each group). (D) $^{125}$I-9MPA uptake decreased and $^{18}$FDG uptake increased in SIRT1 (M) and SIRT1 (H) mice. The $^{125}$I-9MPA/$^{18}$FDG ratio decreased in SIRT1 (M) and SIRT1 (H) mice. Values are the mean ± SEM. ¶p = 0.0533 versus NTg mice. *p<0.05 versus NTg mice (n = 7–9 for each group). SUV: standard uptake value. SUV = tissue concentration (MBq/g)/injected dose (MBq)/body weight (g). (E) Gene expression of glucose transporters (GLUT1 and GLUT4) was analyzed using qRT-PCR. The expression of GLUT4 was decreased and that of GLUT1 was increased in SIRT1 (H) mice. Values are the mean ± SEM. *p<0.05 versus NTg mice (n = 6 for each group).

### 3.7. SIRT1 modified expression of genes related to cardiac energy metabolism

Next, we examined the expression of genes involved in fatty acid oxidation. PGC-1α is a master regulator of mitochondrial biogenesis, and its level of mRNA did not change. Peroxisome proliferator-activated receptor α (PPARα), estrogen-related receptor α (ERRα), and nuclear respiratory factor-1 (NRF-1) are downstream of PGC-1α and regulate fatty acid metabolism. The level of PPARα mRNA increased in SIRT1 (L) mice, but the increase was not associated with downstream genes such as the CD36/fatty acid translocase (CD36/FAT), carnitine palmitoyltransferase-1b (CPT-1b), and medium-chain acyl coenzyme A dehydrogenase (MCAD) genes. NRF-1 mRNA decreased in a manner dependent on transgene dosage (Fig. 4A). Among the genes involved in fatty acid transport or breakdown, the CD36/FAT, CPT-1b, MCAD, and isocitrate dehydrogenase 3α (IDH3α) genes showed decreased mRNA levels in SIRT1 (M) and SIRT1 (H) mice (Fig. 4B). Several genes of mitochondrial respiratory chain complexes decreased in a manner that was dependent on SIRT1 gene dosage (Fig. 4C).

### 3.8. Reactive oxygen species and activity of mitochondrial electron transport chain complexes were decreased in SIRT1 mice

Since the generation of reactive oxygen species (ROS) is related to mitochondrial function, we measured markers of ROS. Thiobarbituric acid reactive substances (TBARS) are a marker of lipid peroxidation. TBARS decreased in a transgene dosage-dependent manner (Fig. 5A).

Mitochondria are known to be a major source of ROS. To measure mitochondrial ROS production, we measured $H_2O_2$ levels using isolated mitochondria. Mitochondrial ROS generation tended to be decreased in SIRT1 mice compared with NTg mice (¶p = 0.07, NTg versus SIRT1 (L) + (M) mice) (Fig. 5B). The levels of mRNA for Cu/Zn-superoxide dismutase (Cu/Zn-SOD), Mn-superoxide dismutase (Mn-SOD), and catalase decreased in SIRT1 (M) and SIRT1 (H) mice (Fig. 5C). Indeed, the gene expression of Cu/Zn-SOD, Mn-SOD, and catalase is known to increase in response to ROS [19–22]. Thus, the generation of ROS appeared to be attenuated in SIRT1 mice.

Next, we isolated mitochondria from heart tissue and measured the mitochondrial respiratory chain in SIRT1 (M) mice (Fig. 5D). To estimate the activities of mitochondrial electron transport complexes, we measured oxygen consumption of isolated mitochondria by adding substrates and inhibitors specific to each complex (Fig. 5D). Total oxygen consumption of isolated mitochondria was slightly decreased in SIRT1 (M) mice compared with that in NTg mice (p = 0.08, NTg versus SIRT1 (M) mice) (Fig. 5E). The respiration stimulated by *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) and ascorbate reflects the reserve capacity of complex IV supported respiration. TMPD/ascorbate stimulated oxygen consumption was significantly decreased in SIRT1 (M) mice (Fig. 5E).

**Table 2**
Heart weight and lung weight of SIRT1 transgenic mice.

| | NTg | SIRT1 (L) | SIRT1 (M) | SIRT1 (H) |
|---|---|---|---|---|
| Number of animals | 8 | 8 | 9 | 10 |
| Body weight (BW, g) | 21.5 ± 1.1 | 21.6 ± 1.1 | 21.6 ± 1.0 | 20.0 ± 0.8 |
| Tibial length (TL, mm) | 16.4 ± 0.1 | 16.7 ± 0.2 | 16.7 ± 0.1 | 16.4 ± 0.1 |
| Heart weight (HW, mg) | 94.5 ± 3.7 | 95.5 ± 4.5 | 91.0 ± 3.3 | 110.0 ± 4.9* |
| Lung weight (LW, mg) | 128.6 ± 2.8 | 131.8 ± 3.6 | 128.2 ± 3.9 | 144.9 ± 8.0* |
| HW/BW (mg/g) | 4.42 ± 0.07 | 4.42 ± 0.05 | 4.23 ± 0.07 | 5.52 ± 0.22* |
| LW/BW (mg/g) | 6.07 ± 0.25 | 6.16 ± 0.23 | 5.97 ± 0.15 | 7.25 ± 0.43* |
| HW/TL (mg/mm) | 5.77 ± 0.21 | 5.70 ± 0.23 | 5.46 ± 0.17 | 6.76 ± 0.28* |
| LW/TL (mg/mm) | 7.85 ± 0.15 | 7.88 ± 0.17 | 7.69 ± 0.19 | 8.86 ± 0.48* |

Values are expressed as the mean ± SEM. NTg: non-transgenic mice; SIRT1: SIRT1 transgenic mice.
* p<0.05 versus NTg mice.

### 3.9. Expression of autophagy related genes

Autophagy is important in maintaining mitochondrial homeostasis [23], and also implicated in cardiac physiology and pathology [24]. Thus, we examined the expression of genes involved in autophagy. The expression of LC3b, Gabarapl1, Vps34, and Atg4b decreased dependent on the SIRT1 dosage (Fig. 6A). The amount of LC3-II protein also decreased (Fig. 6B).

### 3.10. Nampt gene expression and $NAD^+$ were decreased in a SIRT1 gene dosage-dependent manner

$NAD^+$ is essential for the Sir2 deacetylase reaction. The pathway of $NAD^+$ biosynthesis from nicotinamide (NAM) in mammals is

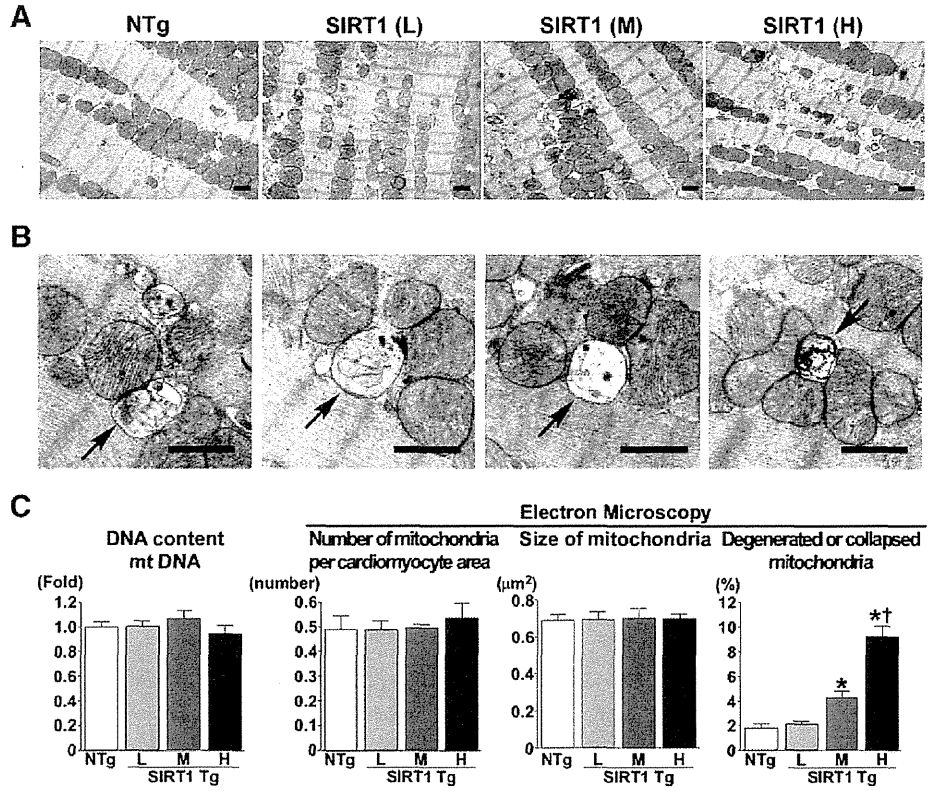


**Fig. 3.** Abnormal mitochondrial ultrastructure in SIRT1 transgenic mouse heart. (A) Low-power electron micrograph of histological sections prepared from 8-week-old SIRT1 transgenic mouse ventricle and NTg control mice. Bar represents 1 μm. (B) High-power electron micrograph of histological sections prepared from 8-week-old SIRT1 transgenic mice. Arrows represent degenerated and collapsed mitochondria with various morphologies. Bar represents 1 μm. (C) The number of mtDNA was analyzed using quantitative PCR. The number of mtDNA is represented using the ratio of the tRNA-Tyr/mt-Co1 gene to gapdh gene. The density of mitochondria (number per 100 μm²), the size of mitochondria (μm²), and the proportion of degenerated or collapsed mitochondria (%) within a cardiomyocyte were observed by electron microscopy. Values are the mean ± SEM. *$p<0.05$ versus NTg mice, †$p<0.05$ versus SIRT1 (M) mice.

shown in Fig. 7A. NAM is converted into nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT). NMN is directly converted into $NAD^+$ by nicotinamide mononucleotide adenylyltransferase (NMNAT1-3). The pathway is controlled by NAMPT. An increased dosage of NAMPT increased the total cellular $NAD^+$ level and enhanced the catalytic domain of Sir2α in mouse fibroblasts [25]. Thus, we analyzed the gene expression of Nampt and Nmnat1-3 in the heart using qRT-PCR. Nampt and Nmnat3 expression decreased in a SIRT1 gene dosage-dependent manner (Fig. 7B). We also analyzed the gene expression of Clock and Bmal1 in the heart since SIRT1 directly depresses Nampt gene expression by modifying Clock and Bmal1 [26]. Clock and Bmal1 expression tended to decrease in a SIRT1 gene dosage-dependent manner (Fig. 7C). Next, we examined the levels of $NAD^+$ in heart. $NAD^+$ decreased dependent on the SIRT1 dosage (Fig. 7D). Thus, constitutive SIRT1 overexpression was associated with decreased gene expression of the $NAD^+$ salvage pathway and cellular $NAD^+$ levels.

### *3.11. SIRT1 (L) mice developed LV systolic dysfunction in response to acute pressure overload*

SIRT1 is known to increase the stress resistance of cells [27]. Indeed, overexpression of SIRT1 reduced myocardial damage induced by ischemia reperfusion [12]. To examine the effect of SIRT1 overexpression on biomechanical stress, SIRT1 (L) mice were subjected to TAC. Fourteen days after TAC, the heart weights of NTg mice and SIRT1 (L) mice increased significantly. The normalized heart weight/tibial length ratio of SIRT1 (L) mice was not different to that of NTg mice after TAC (Table 4). In response to TAC, the left ventricular (LV) fractional shortening (FS) was significantly reduced, and the LV systolic diameter was significantly increased, in SIRT1 (L) mice compared with NTg controls (Fig. 8A, Table 3). The levels of BNP and β-MHC mRNA were significantly increased in SIRT1 (L) mice (Fig. 8B). Collectively, mild constitutive overexpression of SIRT1 caused LV systolic dysfunction upon pressure overload.

## 4. Discussion

Several papers have indicated that SIRT1 plays an important role in cardiac physiology and pathology [8–12]. In this study, we have added new information about the role of SIRT1 in cardiac biology by showing that the constitutive overexpression modified cardiac energy metabolism.

Alcendor et al. showed that SIRT1 protein increased by 8.8-fold in heart tissue after pressure overload, and a 7.5-fold overexpression in transgenic mice attenuated age-related changes of the heart and protected mice from paraquat-induced myocardial damage [10]. Thus, we believe that the finding in SIRT1 (M) mice (6.8-fold overexpression) is physiologically relevant. In the SIRT1 (M) mice, cardiac function examined by echocardiography was normal, although cardiac diastolic function in response to dobutamine was impaired. Gross and histological morphology were normal. Markers of cardiac hypertrophy, such as ANF and α-skeletal actin, were not increased. Although the SIRT1 (M) mice did not develop heart failure, fatty acid uptake was decreased, the number of degenerated mitochondria was increased, and the gene expression related to mitochondrial function was decreased. In addition, the respiration of isolated mitochondria and markers of oxidative stress were decreased in the SIRT1 (M) mice. Thus, the change observed in cardiac energy metabolism in SIRT1 (M) mice is not likely to be a secondary effect of heart

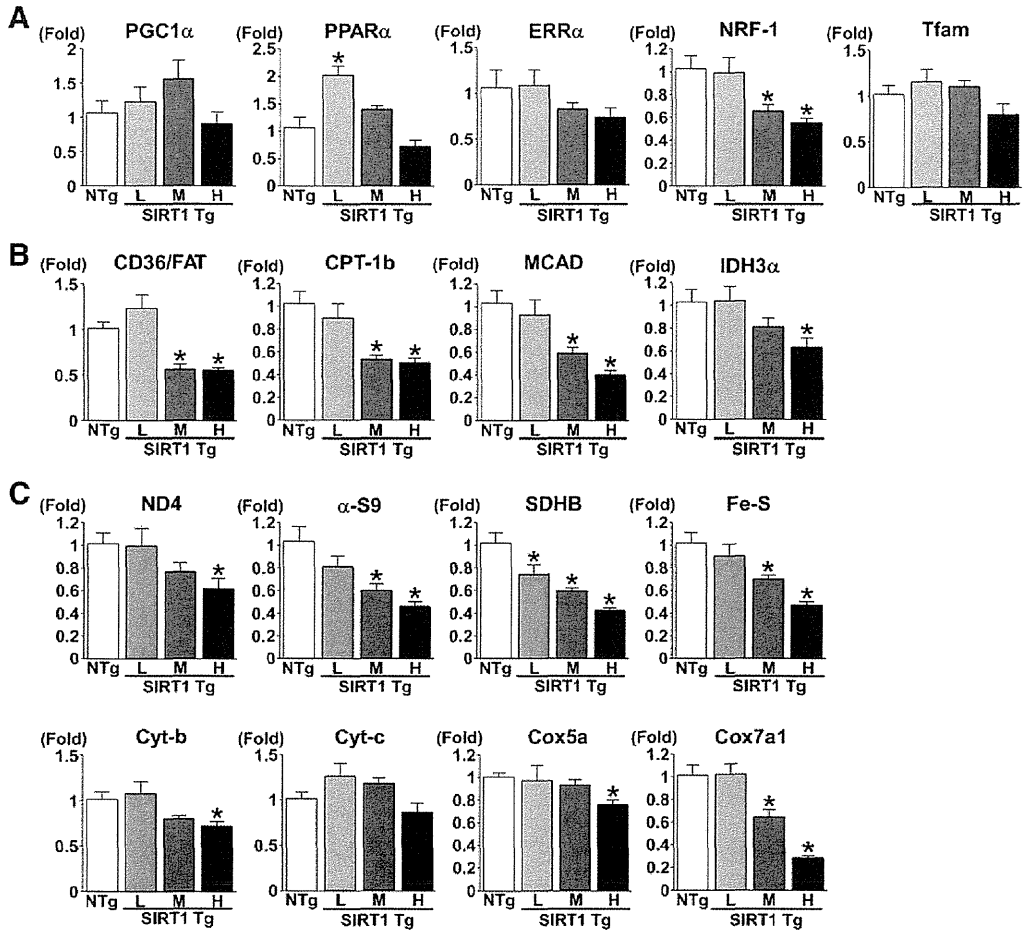


**Fig. 4.** Expression of genes related to fatty acid utilization. (A) Gene expression of transcriptional regulators of fatty acid utilization was analyzed using qRT-PCR. The level of peroxisome proliferator-activated receptor α (PPARα) was increased in SIRT1 (L) mice. The level of nuclear respiratory factor-1 (NRF-1) was decreased in SIRT1 (M) and SIRT1 (H) mice. (B) Gene expression for fatty acid transport or breakdown was analyzed using qRT-PCR. The levels of CD36/fatty acid translocase (CD36/FAT), medium-chain acyl coenzyme A dehydrogenase (MCAD), and carnitine palmitoyltransferase-1b (CPT-1b) decreased in SIRT1 (M) and SIRT1 (H) mice. (C) Gene expression of mitochondrial proteins was analyzed using qRT-PCR. The levels of NADH dehydrogenase 1 alpha subcomplex 9 (α-s9), succinate dehydrogenase complex subunit B (SDHB), complex III subunit Fe-S core protein (Fe-S), and cytochrome c oxidase VIIa1 (Cox7a1) were decreased in a manner that was dependent on transgene dosage. Values are the mean ± SEM. *$p<0.05$ versus NTg mice ($n=6$ for each group).

failure or a non-specific toxic effect of the transgene, but a direct effect of the constitutive overexpression of SIRT1.

The phenotype of SIRT1 (H) mice replicated the cardiomyopathic phenotype already reported by Alcendor et al. [10]. We suggest that impaired cardiac energy metabolism rather than non-specific toxicity of the transgene overexpression would explain the cardiomyopathic phenotype in SIRT1 (H) mice for the following reasons. In SIRT1 (L) mice, in which cardiac function was preserved, ROS generation was decreased and the mice developed cardiac dysfunction upon biomechanical stress. In SIRT1 (M) mice, in which cardiac diastolic function in response to dobutamine was impaired but systolic function was preserved and heart failure did not develop, decreased expression of genes related to mitochondrial function, decreased mitochondrial respiration and ROS generation, and abnormal mitochondrial morphology were observed. Collectively, these results indicate that the constitutive overexpression of SIRT1 perturbed mitochondria in a gene dosage-dependent manner and the mitochondrial abnormalities were not a secondary effect of heart failure but due to the constitutive overexpression of SIRT1. Thus, it is plausible that higher SIRT1 overexpression caused significant mitochondrial abnormalities, as supported by the abnormal morphology of mitochondria and the gene expression related to mitochondria in SIRT1 (H) mice, and caused heart failure.

The development of cardiac dysfunction in response to TAC in SIRT1 (L) mice is not necessarily inconsistent with reports that SIRT1 overexpression protected the heart against aging [10] or ischemia reperfusion [12]. We and others have reported that resveratrol, which activates SIRT1, attenuates CHF in animal models [28,29]. The apparent paradox may be explained by the alteration of mitochondria. The lower ROS levels protect the heart against aging or ischemic injury, but lower mitochondrial respiration might reduce cardiac energy supply and lead to the development of cardiac dysfunction upon pressure overload.

Cardiac substrate preference is tightly regulated at multiple levels. One of the most important mechanisms is the Randle reaction [30]. The Randle reaction implies that the metabolites of fatty acid oxidation suppress glucose metabolism. In this experiment, the expression of genes involved in fatty acid oxidation was decreased in SIRT1 mice. The morphology, respiration and ROS-generating capability of mitochondria were perturbed. In SIRT1 (M) mice, in which glucose uptake increased and mitochondrial respiration was decreased, the expression of GLUT1 and GLUT4 was not changed. Therefore, it is likely that mitochondrial dysfunction increased glucose metabolism via the Randle reaction.

The mechanism by which SIRT1 overexpression changed the expression of genes related to mitochondrial function is not clear.

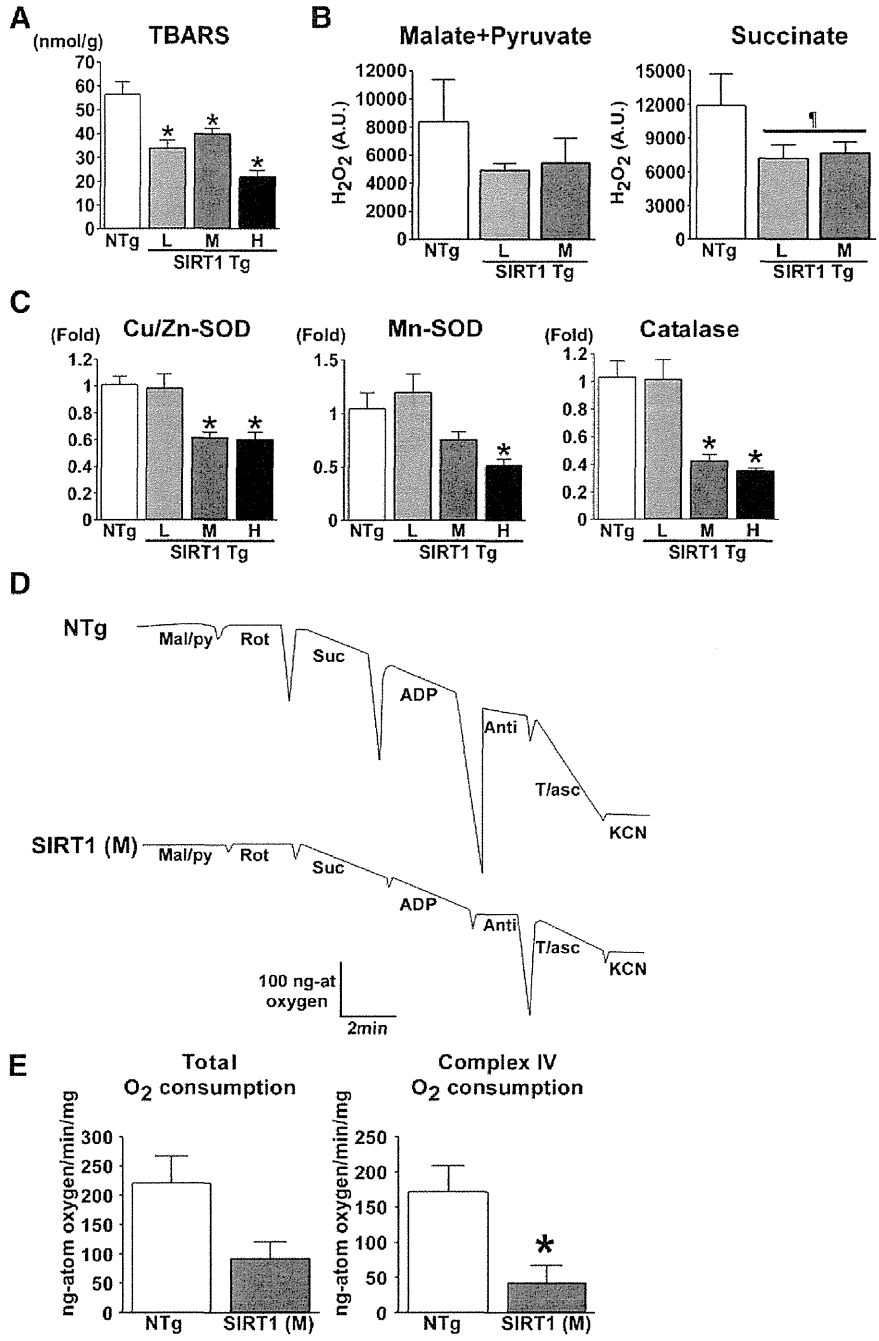


**Fig. 5.** Markers of reactive oxygen species in SIRT1 transgenic mouse heart. (A) Levels of myocardial thiobarbituric acid reactive substances (TBARS). Values are the mean ± SEM. *$p<0.05$ versus NTg mice ($n=6$ for each group). (B) Generation of $H_2O_2$ using isolated mitochondria was measured. Malate and pyruvate, or succinate, were used as substrates. Values are the mean ± SEM. ($n=3$ to 4 for each group.) Generation of $H_2O_2$ tended to be decreased in SIRT1 mice (succinate: ¶$p=0.07$ NTg versus SIRT1 (L)+(M) mice). (C) Gene expression of Cu/Zn-superoxide dismutase (Cu/Zn-SOD), Mn-superoxide dismutase (Mn-SOD), and catalase in heart was analyzed using qRT-PCR. Values are the mean ± SEM. *$p<0.05$ versus NTg mice ($n=6$ for each group). (D) Oxygen consumption of isolated mitochondria was measured using an oxygen electrode cuvette. Transient downward deflections indicate the points at which the substrates and inhibitors specific for various segments of the electron transport path were added to the samples in the oxygen electrodes at the final concentrations indicated (Mal/py, 5 mM malate + 5 mM pyruvate; Rot, 100 nM rotenone; Suc, 5 mM succinate; ADP, 1 mM adenosine diphosphate; Anti, 50 nM antimycin A; T/asc, 0.4 mM *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine + 1 mM ascorbate; KCN, 5 mM potassium cyanide). The downward slope indicates that oxygen was consumed. (E) Total oxygen consumption tended to be decreased in SIRT1 (M) mice ($p=0.08$). Oxygen consumption supported by complex IV was significantly decreased in SIRT1 (M) mice. Values are expressed as ng-atoms oxygen/min/mg. Values are the mean ± SEM. *$p<0.05$ versus NTg mice (NTg: $n=4$, SIRT1 (M) mice: $n=3$).

SIRT1 modifies the function of several proteins that play key roles in the energy metabolism of cells, such as PGC-1α [31], forkhead box O (FOXO) [27,32], and p53 [33]. PGC-1α is a master regulator of mitochondrial biogenesis and function [34]. Transient transfection of SIRT1 deacetylated PGC-1α and increased fatty acid oxidation in a skeletal myocyte cell line [5]. However, cellular respiration was decreased in skeletal muscle cells stably transfected with SIRT1 [6]. In addition, SIRT1 overexpression in skeletal muscle tissue decreased mitochondrial function in animals [7].

Another potential effector of SIRT1 mediating its metabolic effect is FOXO, an important downstream transcription factor of insulin signaling, because SIRT1 overexpression modified the expression of

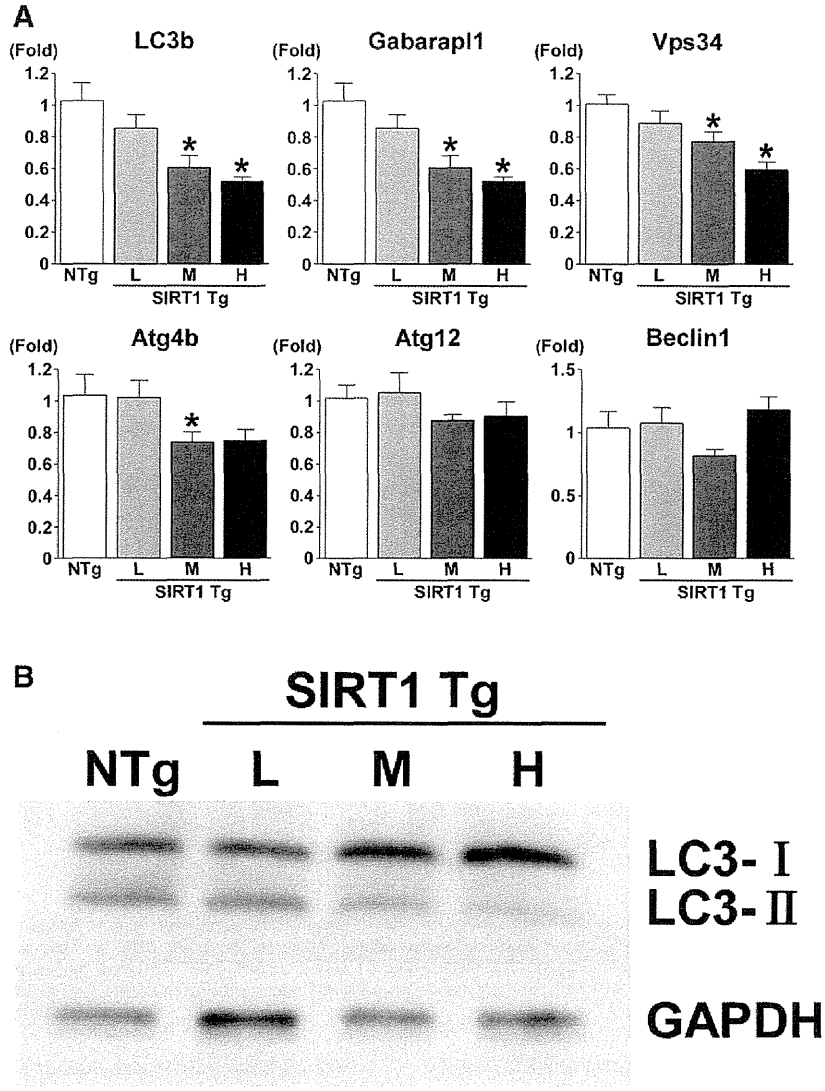


**Fig. 6.** Expression of autophagy-related genes. (A) mRNA expression of autophagy-related genes was measured using qRT-PCR. Values are the mean ± SEM. *$p<0.05$ versus NTg mice ($n=6$ for each group). (B) The protein levels of LC3 and GAPDH (loading control) were examined by Western blotting. A mixture of proteins from five hearts was used for each group.

autophagy-related genes and these genes are known targets of FOXOs [35]. The FOXO family contains four members in mammals: FOXO1, FOXO3, FOXO4, and FOXO6. FOXOs play critical roles in a wide variety of cellular processes, including proliferation, apoptosis, autophagy, metabolism, inflammation, differentiation, and stress resistance. Indeed, SIRT1 is reported to induce autophagy by deacetylating FOXO1 in cultured myocytes [36]. FOXOs can function as transcriptional activators and repressors, probably depending on the range of associated cofactors that they recruit upon binding DNA [37]. Particularly, FOXOs are involved in energy metabolism in liver [38,39] or pancreatic beta-cells [40,41]. In addition, it is becoming clear that insulin signaling is important in regulating mitochondrial homeostasis [42]. For example, FOXO1 increases the expression of heme oxygenase 1 which may cause defects in the mitochondrial electron transport complexes [43].

Heart mitochondria exist as two populations, namely subsarcolemmal mitochondria (SSM) which are situated beneath the plasma membrane, and interfibrillar mitochondria (IFM) which are located among the myofibrils [44]. In this study, subsarcolemmal mitochondria were used to measure mitochondrial respiration and ROS generation. The two heart mitochondrial populations have similarities and differences in morphology, biochemistry and function, under physiological and pathological conditions [45]. The two populations were affected differently in models of genetic cardiomyopathy [46] and aging [47,48]. In contrast, other studies report that both populations are equally affected in heart failure caused by hypertension or myocardial infarction [49,50].

Previous experiments suggest that complexes I and III are major sources of ROS from mitochondria [51,52], and that the loss of respiratory chain activity causes a loss of mitochondrial membrane potential leading to the generation of ROS [53]. Indeed, multiple mechanisms are likely to regulate the formation of ROS in mitochondria [53]. Cytochrome c oxidase (COX) is a large protein complex and functions as complex IV. A component of COX positively regulates ROS production in yeast [54] and mice [55]. Since TMPD/ascorbate stimulated respiration decreased and the expression of genes of complex IV significantly decreased in SIRT1 mice, it is possible that a defective COX reduced the level of ROS in this experiment.

It is possible that compensatory mechanisms act to reduce the potentially harmful effects of constitutive SIRT1 overexpression. Indeed, constitutive overexpression of PGC-1α did cause dilated cardiomyopathy in mice [56]. The decrease in the expression of genes in the

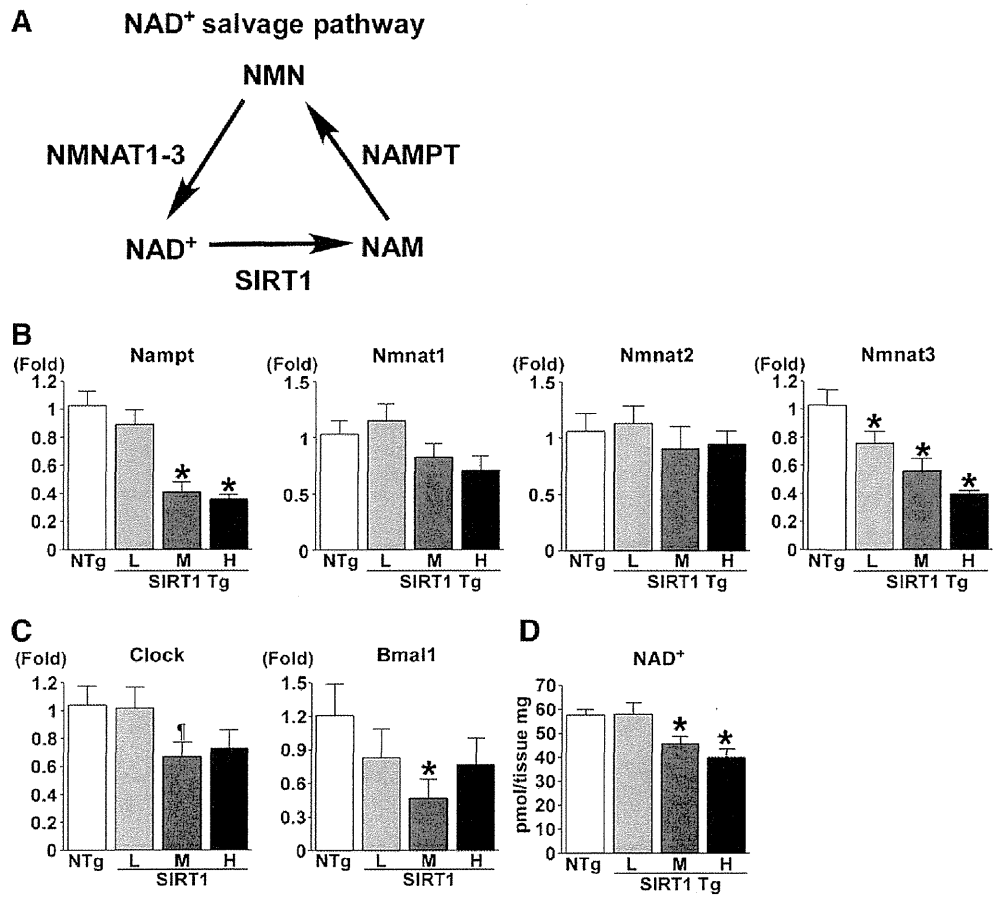


**Fig. 7.** SIRT1 controls Nampt gene expression. (A) Scheme of the $NAD^+$ salvage pathway in mammals. (B) Gene expression of Nampt and Nmnat1-3 in heart was analyzed using qRT-PCR. Nampt and Nmnat3 were decreased in a SIRT1 gene dosage-dependent manner. Values are the mean ± SEM. *p<0.05 versus NTg mice (n = 6 for each group). (C) Gene expression of Clock and Bmal1 in heart was analyzed using qRT-PCR. Clock and Bmal1 were decreased in SIRT1 (M) mice. Values are the mean ± SEM. ¶p = 0.0533 versus NTg mice. *p<0.05 versus NTg mice (n = 6 for each group). (D) $NAD^+$ in heart tissue was analyzed. Values are the mean ± SEM. *p<0.05 versus NTg mice (n = 4 for each group).

$NAD^+$ salvage pathway, which could limit a cofactor of SIRT1, may be one of the mechanisms. SIRT1 directly depresses Nampt gene expression by modifying Clock and Bmal1 [26]. It is not clear whether the decreased levels of markers of autophagy were an adaptive response to SIRT1 overexpression. However, decreased autophagy may cause impaired mitochondrial morphology and function in this experiment, since autophagy of mitochondria plays a critical role in maintaining mitochondrial homeostasis [57].

Sir2 modulates aging and extends lifespan in yeast, *Caenorhabditis elegans*, and *Drosophila* [2]. Resveratrol improved the health and survival of mice on a high-calorie diet [58]. SIRT1 and resveratrol protected mice from neurodegenerative diseases [59]. However, the inhibition of SIRT1 also protected neurons [60]. Systemic SIRT1 overexpression by knocking-in SIRT1 into the β-actin locus caused embryonic lethality [61], and SIRT1 overexpression did not protect neurons but caused a reference memory deficit [62]. The role of SIRT1 in the formation of tumors is also controversial [63]. These results indicate that SIRT1 does not always play a protective role under physiological or pathological

**Table 3**
Echocardiographic analysis of SIRT1 transgenic mice after 14 days of TAC.

| | Sham | | TAC | |
|---|---|---|---|---|
| | NTg | SIRT1 (L) | NTg | SIRT1 (L) |
| Number of animals | 5 | 4 | 12 | 6 |
| Heart rate (bpm) | 507 ± 6 | 508 ± 8 | 517 ± 5 | 507 ± 6 |
| LV diastolic diameter (mm) | 3.95 ± 0.05 | 3.90 ± 0.04 | 3.99 ± 0.07 | 4.19 ± 0.11* |
| LV systolic diameter (mm) | 2.52 ± 0.05 | 2.47 ± 0.03 | 2.63 ± 0.05 | 2.94 ± 0.09*,** |
| FS (%) | 36.4 ± 0.5 | 36.8 ± 0.6 | 34.1 ± 0.8 | 29.9 ± 1.2*,** |
| Diastolic posterior wall thickness (mm) | 0.62 ± 0.03 | 0.60 ± 0.03 | 0.94 ± 0.02* | 0.86 ± 0.07* |

Values are expressed as the mean ± SEM. TAC: transverse aortic constriction; NTg: non-transgenic mice; SIRT1: SIRT1 transgenic mice; bpm: beats per minute; LV: left ventricular; FS: fractional shortening.
* p<0.05 versus sham-operated mice.
** p<0.05 versus NTg with TAC.

**Table 4**
Heart weight and lung weight of SIRT1 transgenic mice after 14 days of TAC.

| | Sham | | TAC | |
|---|---|---|---|---|
| | NTg | SIRT1 (L) | NTg | SIRT1 (L) |
| Number of animals | 5 | 4 | 8 | 9 |
| Body weight (BW, g) | 24.9 ± 1.3 | 25.0 ± 1.3 | 26.4 ± 0.6 | 24.3 ± 0.9 |
| Tibial length (TL, mm) | 16.7 ± 0.1 | 17.2 ± 0.1 | 17.1 ± 0.1 | 17.1 ± 0.2 |
| Heart weight (HW, mg) | 111.8 ± 5.3 | 112.8 ± 4.5 | 176.1 ± 8.9* | 187.6 ± 15.0* |
| Lung weight (LW, mg) | 149.2 ± 6.2 | 140.0 ± 2.5 | 226.6 ± 3.9 | 265.8 ± 49.4* |
| HW/BW (mg/g) | 4.49 ± 0.11 | 4.52 ± 0.19 | 6.65 ± 0.25* | 7.90 ± 0.87* |
| LW/BW (mg/g) | 6.00 ± 0.13 | 5.62 ± 0.14 | 8.59 ± 1.10 | 11.47 ± 2.46 |
| HW/TL (mg/mm) | 6.68 ± 0.31 | 6.57 ± 0.29 | 10.30 ± 0.47* | 11.00 ± 0.90* |
| LW/TL (mg/mm) | 8.91 ± 0.36 | 8.17 ± 0.17 | 13.22 ± 1.64 | 15.53 ± 2.83* |

Values are expressed as the mean ± SEM. TAC: transverse aortic constriction; NTg: non-transgenic mice; SIRT1: SIRT1 transgenic mice.
* p<0.05 versus sham-operated mice.

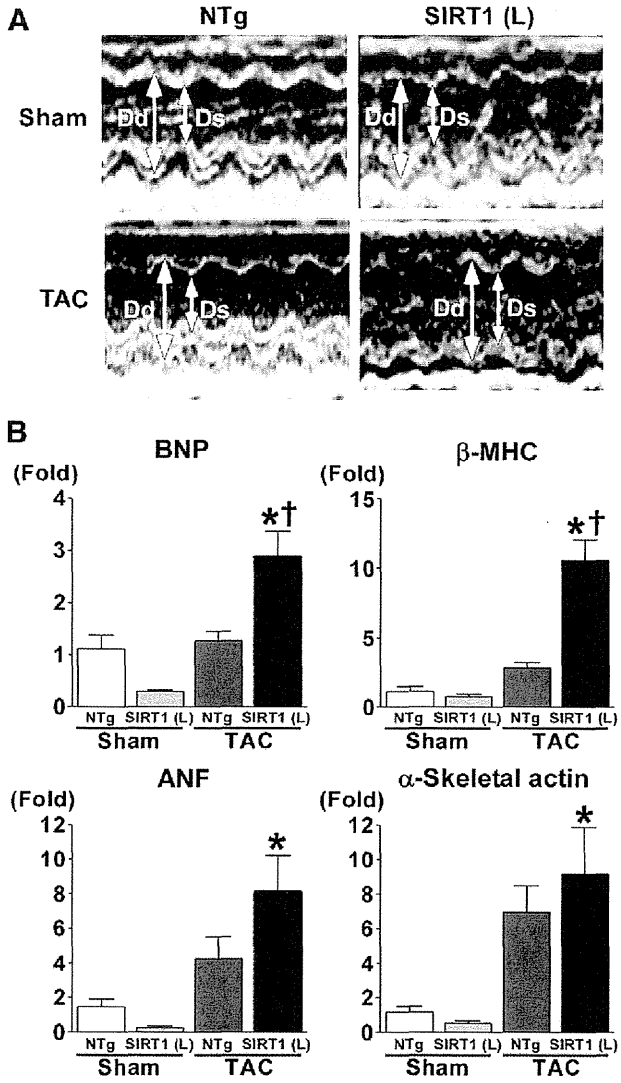


**Fig. 8.** SIRT1 (L) mice developed LV systolic dysfunction in response to acute pressure overload. (A) Echocardiographic analysis of NTg and SIRT1 (L) mice after 14 days of acute pressure overload. SIRT1 (L) mice developed LV systolic dysfunction in response to transverse aortic constriction (TAC). (B) 14 days after TAC, the expression of cardiac fetal genes was analyzed using qRT-PCR. Levels of BNP and β-myosin heavy chain (β-MHC) mRNAs were increased in SIRT1 (L) mice with TAC compared to NTg mice with TAC. Values are the mean ± SEM. *$p<0.05$ versus sham-operated mice, †$p<0.05$ versus NTg mice with TAC (sham-operated NTg: n = 4, sham-operated SIRT1 (L): n = 4, NTg with TAC: n = 7, SIRT1 (L) with TAC: n = 9).

conditions, and the effect of SIRT1's modulation is likely to depend on where the experiments are performed.

## 5. Conclusion

The current study demonstrates that the constitutive overexpression of SIRT1 reduced cardiac function associated with impaired mitochondria. SIRT1 may be involved in altered cardiac energy metabolism in CHF since SIRT1 levels are reported to be increased in CHF. However, the precise mechanism by which the constitutive SIRT1 overexpression impaired mitochondria needs to be determined.

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## Conflict of interest

None to declare.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.yjmcc.2011.09.013.

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# 心臓移植および補助人工心臓の経験

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## はじめに

心筋障害が高度な末期心不全に対する治療手段として，人工心臓と心臓移植があり，心臓移植は欧米を中心にすでに10万例以上に行われている[1]．またわが国では，左心補助人工心臓(LVAS)として，従来体外設置型が用いられてきたが，最近では植込み型LVASが心臓移植へのブリッジ(BTT)として認可され，その施行数も増加している．さらに米国での市販後調査(Interagency Registry for Mechanically Assisted Circulatory Support：INTERMACS)においては，年間1,000例以上が報告されるようになり，HeartMate Ⅱ(Thoratec社，Pleasanton)では総施行数が1万例を超えたと報告されている．

わが国においては，1997年に施行された臓器移植法により1999年から心臓移植が開始された．その後施行数は徐々に増加したが，年間施行数は10例程度であった[2,3]．2010年7月から改正臓器移植法が施行され，施行数は改正前の69例から改正後の2年4ヵ月で74例と，著明な増加を認めている(図1)．また2011年4月には，わが国で開発された2種の遠心ポンプによる植込み型LVASがBTTとして保険償還されるようになり，植込み型LVASによる在宅での心臓移植待機患者数も増加している．当センターでは，1999年5月に第1例目の心臓移植を施行してから，2012年10月までに50例の心臓移植を実施してきた．またBTTとして，従来国循型ニプロ-東洋紡LVAS(ニプロ社，大阪)を用いてきたが，最近では植込み型LVASを第一選択として適応している．このような末期心不全に対する心臓移植とLVASの現状について，当センターにおける経験を報告する．

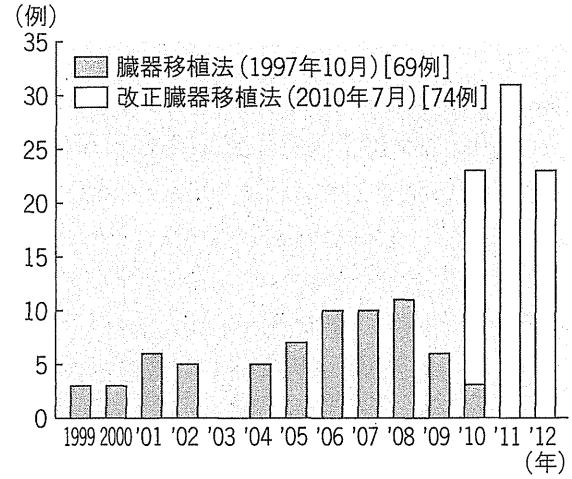


**図1．わが国における心臓移植**(2012年10月31日，計143例)

## Ⅰ．当センターにおける心臓移植

1999年5月～2012年10月に，当センターに

キーワード：心臓移植，補助人工心臓，心臓移植へのブリッジ

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表1. 当センターにおける心臓移植(1)

| | |
|---|---|
| 症　例(例) | 50 |
| 移植時年齢(歳) | 14～61(平均37) |
| 性　別(男/女)[例] | 39/11 |
| 原　病 | |
| 拡張型心筋症 | 32 |
| 拡張相肥大型心筋症 | 8 |
| 虚血性心筋疾患 | 2 |
| その他 | 8 |

表2. 当センターにおける心臓移植(2)

| | |
|---|---|
| 待機状況(例) | status 1 50<br>[うち LVAS 45(90%)] |
| LVAS(例) | |
| 体外設置型 | |
| ニプロ-東洋紡 | 39 |
| 植込み型 | |
| HeartMate VE | 2 |
| Novacor | 1 |
| Evaheart | 1 |
| Jarvik 2000 | 1 |
| HeartMate Ⅱ | 1 |
| 待機期間(日) | 29～3,838(平均1,049) |
| status 1 | 29～1,476(平均812) |
| LVAS補助期間(日) | 39～1,703(平均886) |

LVAS：左心補助人口心臓

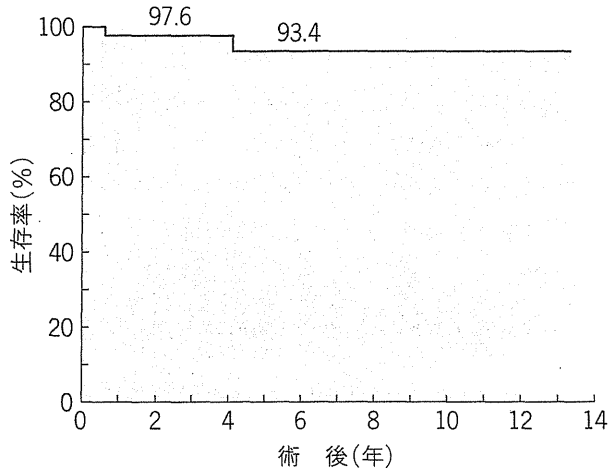


図2. 当センターにおける心臓移植の生存率
(13年以上2例)

おいて50例の心臓移植を施行した（表1）．移植時年齢は平均37歳で，体外設置型LVAS装着で待機していた60歳以上の3例が含まれる．原病は64%が拡張型心筋症で，うち1例は右胸心を伴っていた．待機状況は全例status 1で，90%は各種LVASによるブリッジ例であった（表2）．用いたLVASは，87%が体外設置型のニプロ-東洋紡LVASで，ほかは植込み型の拍動流および連続流ポンプであった．待機期間は平均1,049日で，status 1での待機期間は平均812日，最長1,476日に及んだ．またLVAS補助期間は平均886日で，最長は体外設置型による1,703日であった．最近施行した1例は入院リハビリテーション中であるが，47例は外来経過観察中で，これまでに2例が死亡した．心臓移植後の生存率を図2に示す．2例が感染症で死亡（移植後8ヵ月および4年2ヵ月後）したが，13年以上経過したものが2例であった．また10年生存率は93.4%と，国際心肺移植学会レジストリーより良好な成績を示している[4]．

これまでの年間施行数は，臓器移植法が改正されるまでは年間5例以内であったが，改正臓器移植法施行後は2ヵ月で5例や，4週連続した施行などもみられるようになり，2011年には年間9例，2012年は9月までに9例となってきた．また2004年2月には同時に発生したドナー情報に対応し，2例同時に施行することも経験している．連続するドナー情報に対応できる体制であることが必要である．

ドナー情報への対応においては，ドナー心およびレシピエントの状況，搬送経路など総合的に検討して心臓移植を実施する必要がある．当センターの36例の移植例においては，78%がマージナルドナーであり，移植後7例においてprimary graft dysfunctionを認めた．しかし移植後早期死亡はなく，4週後において心エコー上有意な差を認めなかった[5]．

移植手術においては，Lower-Shumway法，bi-caval法をそれぞれ1例施行したが，第3例目以降はmodified bi-caval法を用いている[2]．心筋保護は，St.Thomas液を当初用いたが，7例目からはCelsior液を用いており，心筋保護効果は良好である[5]．免疫抑制療法は，カルシニューリン

阻害薬，mycophenolate mofetil（MMF）およびステロイドによる三者併用療法を用いている[3,7]．カルシニューリン阻害薬は，当初はciclosporin（CYA）を用いたが，その後CYAあるいはtacrolimus（Tac）を用い，最近ではTacを第一選択としている．腎機能障害例などに対してはinduction therapyを行うこととしているが，当初はmuromonab-CD3を，最近ではbasiliximab（Simulect）を使用している[4]．また最近では，腎機能障害例や移植後冠状動脈病変例などに対し，新しい免疫抑制薬であるeverolimusを積極的に用いている[8]．

またわが国の心臓移植例は，その大半がブリッジ例で大量輸血を経験しており，抗体関連型拒絶反応に対する配慮が重要である．当センターでは初期から，病理検査のみならずpanel reactive antibody（PRA）検査などを実施し，必要に応じてガンマグロブリン投与や血漿交換により対応している[9]．

臓器移植法の改正により18歳未満からの臓器提供が可能となり，心臓移植では18歳未満からの提供において，登録時年齢18歳未満の症例を優先することとなった．当センターにおいては，10歳代男児に対し18歳未満からの臓器提供に基づく心臓移植を施行した．本例はLVAS補助施行中（補助期間764日）で，status 1にて341日間待機中であった．移植後退院し，就業している．

現在生存中の48例において，入院加療中およびリハビリテーション中が23%であるが，ほかは復職，再就職，復学，主婦などの社会復帰を行っている．

心臓移植施行数は増加したものの，待機者数も増加傾向にある．このため当センターにおいても，図3で示すように改正前に施行した27例と改正後の23例で，待機日数（status 1）および補助期間を検討すると，改正後実施例において著明に増加していた．

## Ⅱ．当センターにおけるLVAS

1999年以降心臓移植対象患者139例に対し，LVASを適応してきた．当初は体外設置型のニプロ-東洋紡LVASを用いた．2001年以降は，拍動流植込み型として，治験としてHeartMate

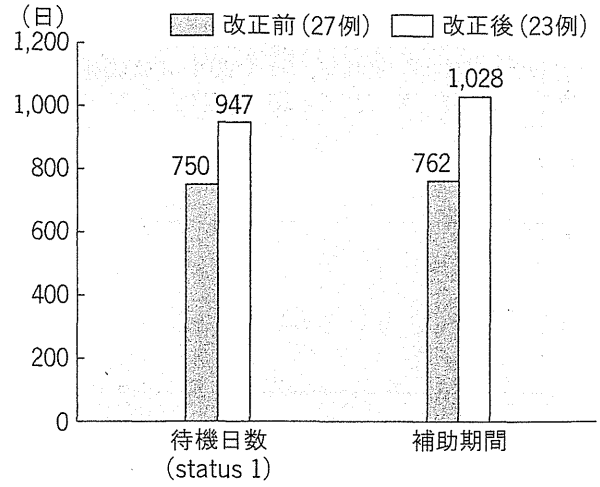


**図3．当センターにおける心臓移植待機日数および補助期間（平均）**

Ⅰ（HM-1：Thoratec社，Pleasanton）を，心臓移植へのブリッジとして保険償還されたNovacor（World Heart社，Salt Lake City）を用いた．しかしHM-1は承認がなされず，Novacorはバッテリーの供給ができないことより使用できなくなった．2005年からはわが国で開発された遠心ポンプを用いたEvaheart（サンメディカル社，長野），DuraHeart（テルモ社，東京）の治験に参加し，その後米国で開発された軸流ポンプを用いた植込み型LVASであるHeartMate Ⅱおよび Jarvik（Jarvik Heart社，New York）の治験に参加した．2011年4月にはEvaheart，DuraHeartが心臓移植へのブリッジとして保険償還され，以後心臓移植へのブリッジ例に対してEvaheartあるいはDuraHeartを第一選択とする方針で臨んできた．これまでに体外設置型111例，各種の植込み型28例の装着を行ってきた．また最近では，年間15例以上の装着を行っている．前項で述べたように，国内移植で45例のブリッジ例を経験している．

LVAS例の管理における問題点としては，感染症および脳血管障害がある．感染症に対しては，送血・脱血管あるいはドライブラインの皮膚貫通部の処置に配慮するとともに，必要に応じて抗生物質投与を行う．またLVASの機種に応じた抗凝固および抗血小板療法が必要であり，脳梗塞や脳出血に配慮した管理が必要である．脳出血発症時には，乾燥ヒト血液凝固第Ⅸ因子複合体などを用いてプロトロンビン時間国際標準比（PT-