Published in final edited form as:

J Cardiovasc Pharmacol. 2004 March ; 43(3): 454–461.

Simvastatin Preserves Cardiac Function in Genetically Determined Cardiomyopathy

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Abstract

Endothelial dysfunction characterizes heart failure (HF). Simvastatin (Sim) increases endothelial nitric oxide (NO) independent of lipid-lowering. We evaluated the effect of Sim on cardiac function and remodeling, apoptosis and NO availability in HF.

Methods—Five-month old cardiomyopathic (CM) hamsters were divided into - Sim (20 mg/kg, 6 weeks, n=6), and Untreated groups (n=6). Age-matched normal hamsters served as controls (n=6). Serial echocardiograms were performed to measure LV function and myocardial apoptosis and myocardial capillary density were measured at 6 weeks.

Results—CM hamsters had lower LV shortening fraction (SF) compared to controls (17±3% vs 59±2%), higher LV end-diastolic volume (30±3 vs 6±2 ml/m²) and lower LV mass/volume ratio (0.5±0.04 vs 0.72±0.02 mg/ml, p<0.001). During follow-up, SF decreased (9±2%) and LV volume increased (38±1 ml/m²) in untreated hamsters (p<0.05 from baseline) but did not change significantly in Sim group (p<0.05 vs untreated). Myocardial caspase-3 activity was higher and apoptotic nuclear density was lower in Sim compared to untreated CM hamsters (0.072±0.02% vs 0.107±0.03%, p<0.01). Myocardial capillary density was highest in Sim group (p<0.05).

Conclusions—Sim retards the progression of HF in CM hamsters. This may be related to an increase in coronary microvasculature with increase in NO availability and decreased apoptosis.

Keywords

Statins; remodeling; heart failure; angiogenesis; nitric oxide

INTRODUCTION

Hydroxy-methyl-glutaryl Coenzyme A reductase inhibitors or statins are drugs designed to inhibit the rate-limiting enzyme of cholesterol synthesis in the liver, thereby decreasing the hepatic production of LDL and lowering the circulating levels of LDL. In normocholesterolemic animals, statin therapy has been shown to protect against stroke¹, ischemia-reperfusion injury of the heart² and vascular inflammatory responses^{3,4} through mechanisms that may be mediated by an increase in endothelium-derived nitric oxide (NO) production. Heart failure is characterized by down regulation of NO production and endothelial NO synthase (eNOS) gene expression^{5–8}. The lack of NO contributes to myocardial apoptosis

or programmed cell death, a critical event in cardiac remodeling and progression of heart failure⁹. Our previous studies have shown that NO mediated regulation of apoptotic enzyme activity is reduced in failing hearts¹⁰. Statins upregulate endothelial NO production independent of their lipid lowering action and recent experimental studies have demonstrated their angiogenic potential^{11–13}. Although statins have been studied in heart failure following myocardial infarction^{14,15}, their effect on cardiac function in a non-ischemic, genetic model of heart failure has not been well studied.

The cardiomyopathic (CM) Syrian hamster (BIOTO2) is a widely studied model of heart failure. The disease in this strain of hamsters has phenotypic features of dilated CM in humans. The cardiac phenotype is characterized by early hypertrophy at 2 months of age that progresses to a dilated CM by 5–6 months with progression to end-stage heart failure by 9 months of age. The disease is caused by an inherited autosomal recessive mutation in the gene coding for delta-sarcoglycan, a component of the dystrophin complex^{16,17}. These animals die prematurely from progressive myocardial necrosis and heart failure. A recent study reported early onset of vascular dysfunction that preceded the development of decompensated heart failure¹⁸.

We have previously demonstrated that progression from compensated heart failure in 4 month old hamsters to decompensated heart failure in 9 month old hamsters using serial echocardiography is associated with increased caspase 3 activity which is a common downstream effector enzyme of apoptosis¹⁰. For this study, we followed 6 month old hamsters for the pathophysiologic events that contribute to the transition from compensated to decompensated heart failure and the ability of a statin drug to prevent this transition. Our study was designed to assess the effect of Sim on LV function and remodeling in CM hamster hearts, and the role of increased nitric oxide and reduced apoptosis in mediating these effects of Sim.

METHODS

Animals

Five month old male Syrian CM hamsters (BIO TO2 strain) (n=12) and age and sex-matched normal hamsters (BIO FIB strain) (n=6) were obtained from Bio Breeders Inc (Watertown, MA). All experimental protocols were approved by the Institutional Animal Care and Use Committee of Columbia University and conform to the “Guiding Principles for the Use and Care of Laboratory Animals” of the NIH and the American Physiological Society. CM hamsters were divided into two groups (i) Simvastatin (Sim) group (n=6) received 20mg/kg Sim (Merck, NJ) daily by oral gavage for six weeks (ii) Untreated group (n=6). All hamsters underwent echocardiography at baseline and serially for a 6 week period. After 6 weeks, control and CM hamsters were euthanized with 75 mg/kg sodium pentobarbital intraperitoneal injection, the hearts were removed, weighed, and LV myocardium was snap frozen in liquid nitrogen at –80° C. A piece of ventricular myocardium was also preserved in formalin for immuno-histochemistry.

Echocardiographic assessment of ventricular function

The hamsters were sedated with ketamine 100mg/kg intramuscular and serial 2-dimensional trans-thoracic echocardiograms were performed at baseline and 3 week intervals thereafter until the end of the study (i.e. 6 weeks) using an Acuson Sequoia with a 13-MHz linear transducer (15L8) in a phased-array format with real-time digital acquisition, storage, and review capabilities. LV contractile function and cardiac dimensions indexed to body surface area were compared between groups. The heart was imaged in the para-sternal short axis view at the level of the papillary muscles to obtain LV wall thickness and fractional shortening, and para-sternal long axis view to measure LV systolic and diastolic volumes and ejection fraction.

Three beats were averaged for each measurement. All calculations were derived using standard formulas¹⁹.

LV chamber dimensions and wall thickness—LV end-diastolic (LVEDD) and end-systolic (LVESD) diameters and wall thickness were measured from M-mode tracings.

LV Mass: was calculated according to uncorrected cube assumptions using the equation, $LV\ mass = 1.055[(IVST + LVEDD + PWT)^3 - (LVEDD)^3]$ where 1.055 is the specific gravity of the myocardium, IVST is the inter-ventricular septal thickness, and PWT is the posterior wall thickness.

LV shortening fraction: was calculated from the M-mode LV dimensions using the equation, $SF\ (\%) = [(LVEDD - LVESD) / LVEDD] \times 100$

Myocardial Caspase 3 activity assay

LV myocardium was homogenized in cell lysis buffer, centrifuged at 12,000 g, and supernatant was separated to obtain cell lysates. Caspase 3 enzyme cleaves the p-nitroaniline (pNA) labeled substrate Ac-DEVD-pNA to release pNA which was assayed colorimetrically at an absorbance wavelength of 405 nm in assay buffer. The reaction was started by adding 5 μ l of substrate to each well containing myocardial cell lysates and measuring absorbance at 10 minute intervals for an hour. Activity was expressed as slope of absorbance over time in pmol/20 μ g protein/min. Activity was also measured after pre-incubation of the cell lysates with nitro-L-arginine methyl ester (L-NAME, 0.1 mM/L), an inhibitor of endogenous NOS. The NO donor, S-nitroso-N-acetyl penicillamine (SNAP) was added to cell lysates to confirm the ability of exogenous NO to inhibit caspase 3 activity.

TUNEL POD assay for myocyte apoptosis

Paraffin-embedded sections of LV myocardium (5 μ m thick) were dewaxed, rehydrated and permeabilized using a Proteinase K working solution. Myocyte apoptosis was detected using an *in situ* cell death detection kit (Roche Diagnostics, Indianapolis, IN). DNA degradation is a key biochemical event of apoptosis, resulting in cleavage of nuclear DNA into oligonucleosome-sized fragments. Terminal deoxynucleotidyl transferase (TdT) mediated d-UTP nick-end labeling (TUNEL) was used to label DNA strand breaks as an early marker of apoptosis. The fluorescent label was converted into a colorimetric signal using the TUNEL-peroxidase (POD) as a secondary detection system. DNA fragmentation was seen as brown staining of nuclei. Tissue sections were examined microscopically at 40 X magnification and the apoptotic nuclei were expressed as a percentage of normal nuclei counted in a minimum of five low power fields. Tissue sections incubated with TUNEL Label only solution instead of the TUNEL reaction mixture served as the negative control while those incubated with DNase to induce DNA strand breaks just before adding the TUNEL reaction mixture served as the positive control.

Immunohistochemistry for eNOS staining

Paraffin-embedded sections of LV myocardium were immuno-stained with monoclonal anti-human eNOS antibody using a modification of the avidin-biotin-peroxidase method. Anti-eNOS, a murine monoclonal antibody to human eNOS (1:25 dilution, Transduction Laboratories, Lexington, KY) was used as the primary antibody and biotinylated goat anti-mouse serum (1:200 dilution) was used as the secondary antibody. Myocardial capillaries with more than 50% circumferential staining of the lumen for eNOS (excluding vessels with muscular layer) were identified in tissue sections using 400 X magnification. Myocardial capillary density was expressed as number of capillaries per 3 high power fields.

Western Blots for myocardial eNOS

Myocardial proteins were prepared and separated on SDS/polyacrylamide gel electrophoresis. Immuno-blotting was performed using a murine monoclonal antibody to human eNOS (1:2500 dilution, Transduction Laboratories, Lexington, KY). Immuno-detection was obtained using a sheep anti-mouse secondary antibody HRPO (1:2500 dilution). Autoradiography was performed at 23°C, and the blots were quantitated by densitometry.

Statistical Analysis

All data were expressed as mean $\pm$ SEM. Two-way ANOVA was used to compare serial echocardiographic data between groups and unpaired Student t test was used to compare caspase activity, apoptotic nuclear density, eNOS density and myocardial capillary density between groups. The Student-Newman-Keuls post hoc analysis was used to identify which means were different (Sigma Stat 2.03). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Cardiac function and remodeling

The results of echocardiography are shown in Table 1. Figures 1 and 2 show serial changes in LV function and size in individual CM hamsters. One of the six CM hamsters in each group did not have echocardiograms at every time point, therefore their data is not shown in the figures. At baseline study, CM hamsters had lower LV shortening fraction compared to controls, higher indexed LV end-diastolic volume, a higher indexed LV mass and wall thickness, and a lower LV mass/volume ratio ($p < 0.001$ vs controls). Serial echocardiograms demonstrated a progressive decline in LV shortening fraction from $17 \pm 1\%$ to $9 \pm 2\%$, $p < 0.05$ (Figure 1a) and an increase in LV end-diastolic volume from 28 ± 4 to 38 ± 1 ml/m² (Figure 2a). In the Sim-treated hamsters, there was no deterioration in LV function or increase in LV dilatation during serial follow-up (Figures 1b, 2b). LV mass and mass/volume ratio did not change significantly in treated and untreated CM hamsters (Fig 3). The control hamsters demonstrated normal LV function with no significant change in LV mass, volume or function during follow-up (see Table 1)

Myocardial Apoptosis

Myocardial caspase 3 activity—Caspase 3 activity was higher in the CM hamsters (2.1 ± 0.1 pmol/20 μ g/min) compared to controls (1.3 ± 0.7 pmol/20 μ g/min). In the CM hamsters, caspase 3 activity was lower in the Sim group (1.8 ± 0.3 pmol/20 μ g/min) compared to the untreated group ($p < 0.05$). NOS inhibitor, L-NAME, increased caspase 3 activity in the Sim group (2.7 ± 0.6 pmol/20 μ g/min, $p < 0.05$) but not in the untreated group (1.8 ± 0.3 pmol/20 μ g/min) (Figure 4). NO donor, SNAP, caused a rapid decay in caspase 3 activity in both groups ($p < 0.05$).

TUNEL assay—TUNEL staining revealed a significantly higher percentage of apoptotic nuclei in the untreated CM hamster hearts compared to controls ($0.107 \pm 0.03\%$ vs. $0.032 \pm 0.02\%$, $p < 0.001$). Sim treatment was associated with a lower percentage of apoptotic nuclei ($0.072 \pm 0.02\%$, $p < 0.01$) compared to untreated hamsters (Figure 5a). Representative microscopic images of TUNEL stained myocardial sections are shown in Figure 5b.

Myocardial eNOS Capillary Staining

Myocardial capillary density per three high power fields in untreated CM hamsters was 56 ± 11 . This was not significantly different from controls (47 ± 9). Sim treatment was associated with a higher myocardial capillary density (110 ± 12) compared to both controls and untreated CM

hamsters ($p<0.01$) (Figure 6a). Representative microscopic images of myocardial sections stained with eNOS are shown in Figure 6b. Myocardial total eNOS expression measured by Western blot was not different between the three groups (representative blots are shown in Figure 6c).

DISCUSSION

The results of our study demonstrate that Sim administration preserves myocardial function, decreases ventricular remodeling and retards the progression of heart failure in cardiomyopathic hamsters. This may be related to an increase in myocardial capillary density in failing hearts resulting in increased endothelial nitric oxide availability and reduced myocardial apoptosis.

The CM hamsters develop progressive LV dilatation and heart failure secondary to a mutation in the delta-sarcoglycan protein^{16,17}. In the present study, the progression of CM was characterized by LV dilatation and progressive deterioration in cardiac function during a 6 week follow up. Administration of Sim attenuated the progressive LV remodeling, prevented LV wall thinning and dilatation and prevented deterioration of myocardial function compared to untreated CM hamsters. While other studies have shown the ability of statins to improve LV function and decrease LV hypertrophy, dilatation and fibrosis following myocardial infarction^{14,15}, our study is the first to show that a statin can retard the progression of heart failure in a genetic model of non-ischemic CM. Previous studies by Takemoto and colleagues²⁰ and Nahrendorf and colleagues²¹ demonstrated an anti-hypertrophic effect of a statin drug in isolated cardiomyocytes and in rats after myocardial infarction respectively. In our study, Sim did not lower LV mass but prevented progressive LV thinning and dilatation thereby retarding disease progression (see Table 1).

The molecular mechanisms of the beneficial effects of statins in heart failure are not completely understood. Heart failure is associated with an impaired ability of the vascular endothelium to produce NO secondary to decreased eNOS expression⁵. Reduced NO contributes to increased apoptosis or programmed death of myocytes²². Apoptosis is mediated by a family of protease enzymes called caspases. NO S-nitrosylates the active cysteine site on the enzyme, in particular caspase 3, a common downstream effector enzyme of apoptosis, to decrease apoptosis²³. In a previous study, we demonstrated that myocardial caspase 3 activity is increased in CM hamsters secondary in part to reduced NO¹⁰. In the present study, baseline myocardial caspase 3 activity was higher in CM hamsters compared to controls. Sim inhibited caspase 3 activity by increasing NO bioavailability. The ability of exogenous NO to inhibit caspase 3 in both groups of CM hamsters suggests that the effect of Sim on caspase 3 was due to increase in NO availability rather than an increase in enzyme sensitivity to NO. The higher caspase activity in CM hamsters was associated with greater myocardial apoptosis. Treatment with Sim reduced myocardial apoptosis compared to untreated hamsters (Figure 5). Therefore, reduction in apoptosis may contribute to the beneficial effects of Sim on cardiac function and remodeling.

Despite a higher myocardial mass, myocardial capillary density in untreated CM hamsters was not different compared to normal hamsters, reflecting either a failure of new blood vessel formation in response to increased myocardial mass and oxygen demand or a loss of coronary microvasculature due to the disease process. The inability of the coronary microcirculation to compensate for the increased demands of a hypertrophied and dilated myocardium may explain in part the progressive myocyte loss in CM hamsters. Our findings are consistent with other investigators who have reported reduced microvessel density in CM hearts, both ischemic and non-ischemic²⁴. Sim-treated hamsters, on the other hand, demonstrated a significantly higher myocardial capillary density compared to both untreated hamsters and to normal hamsters (Figure 6). This may reflect a preservation of coronary microvasculature due to a direct vasculo-

protective effect of Sim. Moreover, recent studies have demonstrated a direct angiogenic effect of Sim with reports of neo-angiogenesis in ischemic limb vasculature following treatment with Sim¹³. More recently, Taniyama and colleagues reported increased blood flow and capillary density and reduced myocardial fibrosis in CM hamsters transfected with hepatocyte growth factor using a viral vector²⁵. Our findings of increased coronary microvascular density are consistent with a possible direct angiogenic effect of Sim on new capillary formation. These findings suggest that systemic administration of a statin can mimic the effects of local gene transfection into failing hearts on new blood vessel formation and provides a novel mechanism for the benefit of statins in heart failure.

To determine if increased NO in Sim treated hamsters was related to increased eNOS expression, we performed Western blots for eNOS on myocardial isolates. Total eNOS expression was not different between the groups. This result is not unexpected. Some investigators have demonstrated that Sim can upregulate vascular eNOS expression secondary to a post-transcriptional increase in eNOS mRNA stability due to changes in isoprenoid synthesis²⁶. Other papers report that Sim can also activate eNOS directly by a post-translational mechanism. Kureishi et al reported the ability of a statin drug to phosphorylate eNOS through increased activity of phospho-kinase B or Akt in human saphenous vein endothelial cells. The downstream effects of this activation included enhanced endothelial cell survival and angiogenesis¹³. Although we did not measure phosphorylated eNOS in the hamster hearts, the ability of Sim to increase NO without an increase in eNOS expression suggests that Sim likely increases eNOS phosphorylation through a post-translational mechanism.

Clinical Implications

Although caution should be exercised when extrapolating results obtained from animal studies to the clinical setting, our data suggests that statins may have therapeutic implications in heart failure. Earlier studies have demonstrated that Sim reduces the incidence of heart failure in patients with coronary artery disease^{27,28}. This may be in large part due to a reduction in recurrent coronary events and also a beneficial effect on LV remodeling. The findings of our study highlight the need for clinical trials in humans to prospectively evaluate the benefits of statins in heart failure. Reduced eNOS expression in heart failure limits the efficacy of drugs like ACE inhibitors whose effects are partly NO-mediated. The concurrent use of Sim may increase the efficacy of these drugs by increasing eNOS activation²⁹.

Limitations

(i) This study did not evaluate a survival effect of Sim in the CM hamsters. Additional prospective long-term studies are needed to determine if the beneficial effects of Sim on cardiac function and remodeling translate into improved survival in heart failure and to determine if the effects of Sim represent a class effect or are unique to Sim. This is important in the context of a recent study by Marz et al who showed that lovastatin and pravastatin did not alter survival in male CM hamsters. In fact, lovastatin reduced survival in female CM hamsters the mechanism of which was not clear³⁰. (ii) The statin dose used in this study is high compared with that used in the clinical setting. This dose was selected based on a study by Endres and colleagues who reported a dose-dependent increase in vascular eNOS expression and stroke protection in mice treated with 0.2, 2, and 20 mg/kg/day Sim with largest effect seen with the highest dose. The use of a high dose allowed us to see a biologic effect within a relatively short duration of follow-up. We and other investigators have used these doses of Sim in animal studies without any adverse effects^{1,12,29}. Although we did not measure liver enzymes or muscle enzymes, we found no clinical evidence of adverse or toxic clinical effects during Sim treatment. The hamsters continued to demonstrate adequate weight gain with good appetite and activity level throughout the study period. (iii) A cause-and-effect relationship showing

that the beneficial effects of Sim on remodeling were NO-related was not conclusively demonstrated in this study. The remodeling effects occurred in association with an increase in myocardial capillary eNOS staining. We plan to perform future studies to determine if administration of the NOS inhibitor, L-NAME, can prevent the effects of Sim to confirm the role of NO in this process. (iv) We did not perform studies using CD31 or bromo-deoxyuridine to determine if the increased microvascular density represented true neo-angiogenesis. However, the finding that microvessel density in Sim treated hamsters was higher than both untreated CM hamsters and controls supports the notion of new blood vessel formation.

CONCLUSION

In conclusion, simvastatin, an HMG-CoA reductase inhibitor, can attenuate cardiac remodeling in heart failure. By virtue of its ability to upregulate eNOS, simvastatin can reduce cardiomyocyte apoptosis and preserve myocardial function, thus retarding the progression of heart failure. Randomized clinical trials, specifically designed to assess the effects of statins on the morbidity and mortality in heart failure patients may prove statins to be an important drug in the armamentarium used to treat heart failure.

Acknowledgements

This work was presented in part at the Annual Meeting of the American College of Cardiology, Chicago, March 2003

ABBREVIATIONS

Sim	Simvastatin
NO	Nitric oxide
eNOS	Endothelial nitric oxide synthase
HF	Heart failure
CM	Cardiomyopathy
SF	Shortening fraction
EDV	End-diastolic volume
EDD	End-diastolic diameter
ESD	End-systolic diameter
TUNEL	Terminal deoxynucleotidyl transferase mediated d-UTP nick-end labeling
POD	Peroxidase

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Figure 1. shows serial changes in LV shortening fraction in (a) untreated, and (b) Simvastatin-treated cardiomyopathic (CM) hamsters. (a) LV shortening fraction decreased during a 6-week follow-up in untreated CM hamsters. (b) LV shortening fraction did not change significantly in the Sim-treated CM hamsters (n=5 in each group). * p<0.05 from baseline; + p<0.01 from heart failure
HF=Heart failure; Sim=Simvastatin; Ham=Hamster

Figure 2. shows serial changes in LV end-diastolic volume in (a) untreated, and (b) Simvastatin-treated cardiomyopathic (CM) hamsters. (a) LV end-diastolic volume increased during a 6-week follow-up in untreated CM hamsters. (b) LV end-diastolic volume did not change in the Sim-treated CM hamsters (n=5 in each group). * $p < 0.05$ from baseline; $\ddagger p < 0.01$ from heart failure HF=Heart failure; Sim=Simvastatin; Ham=Hamster

Figure 3.

shows serial changes in LV mass/volume ratio in the three groups of hamsters. LV mass/volume ratio was significantly lower in the untreated cardiomyopathic (HF, closed squares) hamsters and treated cardiomyopathic (HF/SIM, open triangles) compared to controls (closed diamonds). LV mass/volume did not change significantly in any group during follow-up. + p<0.001 from controls.

HF=Heart failure; Sim=Simvastatin

Figure 4.

shows LV myocardial caspase 3 activity at baseline (open bars), with L-NAME, inhibitor of nitric oxide synthase (hatched bars), and with NO donor, SNAP (gray bars) in untreated (HF) and treated (HF/Sim) hamsters. Baseline myocardial caspase 3 activity was lower in Sim-treated hamsters compared to untreated hamsters. L-NAME increased caspase activity in treated but not in untreated hamsters indicating increased NO in treated hamsters. SNAP decreased caspase 3 activity in both treated and untreated hamsters. * p<0.05 from baseline; ‡ p<0.05 from HF

HF=Heart failure; Sim=Simvastatin; L-NAME=Nitro-L-arginine methyl ester; SNAP=S-nitroso N-acetyl penicillamine.

Figure 5.

LV myocardial apoptotic nuclear density i.e the percentage of apoptotic nuclei to normal nuclei was significantly higher in the untreated cardiomyopathic hamsters (hatched bar) compared to controls (solid bar). Apoptotic nuclear density was lower in Simvastatin-treated hamsters (open bar) compared to untreated group. (n=6 in each group). * $p<0.001$ from controls; + $p<0.05$ from untreated HF hamsters. (b) Representative microscopic images of myocardial sections stained for DNA fragmentation in the 3 groups of hamsters are shown. The brown-stained nuclei represent apoptotic nuclei compared to the normal blue nuclei. A positive control is also shown.

HF=Heart failure; Sim=Simvastatin

eNOS Capillary Staining

Myocardial eNOS Western Blot

Figure 6.

(a) LV myocardial capillary density i.e the number of capillaries per three high-power fields was significantly higher in the Simvastatin-treated cardiomyopathic hamsters (open bar) compared to controls (solid bar) and untreated cardiomyopathic hamsters (hatched bar). (n=6 in each group). + p<0.01 from controls; + p<0.01 from untreated HF hamsters. (b) Representative microscopic images of myocardial sections stained for eNOS in the 3 groups of hamsters are shown. The brown-staining represents eNOS staining of capillaries. HF=Heart failure; Sim=Simvastatin; eNOS=endothelial nitric oxide synthase

Table 1

Serial echocardiographic data in control and cardiomyopathic hamsters

		Baseline	6 weeks
LV SF (%)	Control	59±2	51±3
	HF	17±1 [†]	9±2 ^{*†}
	HF/SIM	16±2 [†]	17±1 ^{†‡}
LV Mass (mg/m ²)	Control	4.1±0.8	8.7±0.8
	HF	14.8±2 [†]	18.3±1 [†]
	HF/SIM	14.9±2 [†]	15.6±1 [†]
LVEDV (ml/m ²)	Control	6±2	14±1
	HF	28±4 [†]	38±1 ^{*†}
	HF/SIM	31±2 [†]	30±1 ^{†‡}
Mass/Volume ratio	Control	0.72±0.02	0.63±0.04
	HF	0.53±0.05 [†]	0.49±0.03 [†]
	HF/SIM	0.48±0.04 [†]	0.51±0.02 ^{†‡}
LV free wall (mm/m ²)	Control	27±3	38±3
	HF	32±2	38±5
	HF/SIM	33±3	35±4
LVEDD (mm/m ²)	Control	135±7	183±12 [*]
	HF	305±17 [†]	314±15 [†]
	HF/SIM	297±14 [†]	288±15 ^{*†}

SF=Shortening fraction; EDV=End-diastolic volume; EDD=End-diastolic diameter;

*
p<0.05 from baseline;†
p<0.01 from controls;‡
p<0.01 for HF vs HF/Sim