Prevention of Cardiac Hypertrophy by Atorvastatin in a Transgenic Rabbit Model of Human Hypertrophic Cardiomyopathy


Abstract—Cardiac hypertrophy, a major determinant of morbidity and mortality in hypertrophic cardiomyopathy (HCM), is considered a secondary phenotype and potentially preventable. To test this hypothesis, we screened 30 5- to 6-month-old β-myosin heavy chain Q403 transgenic rabbits by echocardiography and selected 26 without cardiac hypertrophy. We randomized the transgenic rabbits to treatment with atorvastatin (2.5 mg/Kg/d), known to block hypertrophic signaling or a placebo. We included 15 nontransgenic rabbits as controls. Cardiac phenotype was analyzed serially before, 6 and 12 months after randomization. Serum total cholesterol levels were reduced by 49% with atorvastatin administration. Left-ventricular mass, wall thickness; myocyte size, myocardial levels of molecular markers of hypertrophy, lipid peroxides, and oxidized mitochondrial DNA; and the number of terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL)-positive myocytes were increased significantly in the placebo but not in the atorvastatin group. Myocardium catalase mRNA levels were decreased by 5-fold in the placebo but were normal in the atorvastatin group. Catalase protein level and activity were not significantly changed. Levels of membrane-bound Ras and phospho-p44/42 mitogen-activated-protein kinase (MAPK) were increased in the placebo group (~2.5 fold) but were reduced in the atorvastatin group. Levels of GTP- and membrane-bound RhoA and Rac1, phospho-p38, and phospho-c-Jun NH2-terminal kinases were unchanged. Thus, atorvastatin prevented development of cardiac hypertrophy; determined at organ, cellular, and molecular levels, partly through reducing active Ras and p44/42 MAPK. The results indicate potential beneficial effects of atorvastatin in prevention of cardiac hypertrophy, a major determinant of morbidity in all forms of cardiovascular diseases, and beckon clinical studies in humans with HCM. (Circ Res. 2005;97:0-0.)

Key Words: hypertrophy ■ genetics ■ prevention ■ gene expression ■ statins

Familial hypertrophic cardiomyopathy (HCM), the most common cause of sudden cardiac death in the young and a major cause of morbidity in the elderly, is a genetic disease caused by mutations in sarcomeric proteins (reviewed in Maron and Marian1,2). The molecular genetic basis of HCM is all but elucidated and several hundred mutations in multiple genes have been identified.2 However, the molecular links between the causal sarcomeric mutations and the phenotype of hypertrophic cardiomyopathy are largely unknown. The results of molecular genetic and experimental studies indicate the initial phenotypes, imparted by the causal mutations, are diverse and primarily functional.3 Accordingly, cardiac hypertrophy is considered a secondary or a distant phenotype mediated by the intermediary molecular phenotype expressed in response to the functional defects. Thus, we postulate cardiac hypertrophy could be prevented, attenuated or reversed by blocking the intermediary signaling molecules essential for the cardiac hypertrophic response.

We have generated a transgenic-rabbit model by cardiac-restricted expression of β-myosin heavy chain (MyHC)-Q403 that fully recapitulate the phenotype of human HCM.4,5 The β-MyHC-Q403 rabbits exhibit cardiac hypertrophy, interstitial fibrosis, myocyte disarray, and cardiac dysfunction.4,5 Using the β-MyHC-Q403 rabbits, we have shown that treatment with a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor reversed established cardiac hypertrophy and interstitial fibrosis and improved cardiac function.6 The beneficial effects of HMG-CoA reductase inhibitors in attenuating cardiac hypertrophy also extends to acquired forms, such as pressure-overload induced cardiac hypertrophy and cardiac remodeling.7,8 The antihypertrophic effects of HMG-CoA reductase inhibitors have been largely...
attributed to the inhibition of isoprenylation of RhoA and Rac1, essential molecules in hypertrophic signaling (reviewed in Marian\textsuperscript{3}). Accordingly, there has been considerable interest in the potential clinical utility of these drugs in preventing cardiac phenotype in HCM. We tested this hypothesis in a randomized place-control study in the \( \beta \)-MyHC-Q403 transgenic rabbits.

**Materials and Methods**

**\( \beta \)-MyHC-Q403 Transgenic Rabbits**

Generation and phenotypic characterization of the mutant \( \beta \)-MyHC-Q403 transgenic rabbits are as published.\textsuperscript{4,5}

**Design of the Placebo-Controlled Study and Administration of Atorvastatin**

We performed M mode and 2D echocardiography in 30 \( \beta \)-MyHC-Q403 transgenic rabbits and 15 nontransgenic rabbits between the ages of 5 to 6 months, which is considered the prehypertrophic stage.\textsuperscript{5} We determined the mean and the 95% confidence interval (CI) of the left ventricular mass indexed to body weight (LVMI) in the nontransgenic rabbit. We used the upper limit of the 95% CI (LVMI of \( >1.80 \) g/Kg) as the evidence for cardiac hypertrophy and excluded 4 \( \beta \)-MyHC-Q403 rabbits. The remainder 26 transgenic rabbits were randomized to either a soybean-based diet alone, as a placebo (Purina Test diet) or 2.5 mg/Kg/d of atorvastatin mixed in a soybean-based diet. M-mode, 2D and Doppler echocardiography were performed in all rabbits at the baseline, 6 and 12 months after randomization. The primary end point was a change in the LVMI over a 1-year study period. In addition, we analyzed changes in interventricular septal thickness, posterior wall thickness, left-ventricular fractional shortening, myocardocyte cross-sectional area (CSA), collagen volume fraction (CVF), and the extent of myocyte disarray. We also determined expression levels of molecular markers of cardiac hypertrophy, fibrosis, and oxidative stress-responsive genes as well as levels of selected hypertrophic signaling molecules, myocardial lipid peroxides, and oxidized nuclear and mitochondrial DNA.

**Serum Cholesterol Levels**

Serum cholesterol levels were measured by an enzymatic assay, per recommendation of the manufacturer (ThermoTrace). The assay is based on spectrophotometric (500 nm) measurement of quinone-imine dye generated in a chemical reaction catalyzed by peroxidase in the presence of hydrogen peroxide, a product of oxidation of cholesterol; hydroxybenzoic acid and 4-aminoantipyrine. The assay has linearity between 0 to 20 mmol/L (0 to 774 mg/dL) and sensitivity of 60 µA per mmol/L.

**Echocardiographic Studies**

Echocardiographic images were obtained and analyzed without knowledge of the group assignment as published.\textsuperscript{5}

**Histological Studies**

Myocyte CSA, CVF, and myocyte disarray were detected and analyzed by an examiner who had no knowledge of the group assignment, as published.\textsuperscript{8}

**Expression Levels of mRNAs of Molecular Markers of Cardiac Hypertrophy and Fibrosis**

Expression levels of mRNAs for A-type natriuretic peptide (NPPA), \( \alpha \)-MyHC (MYH6), skeletal \( \alpha \)-actin (ACTA1) and sarcoplasmic reticulum calcium ATPase 2 (SERCA2 or ATP2A2), procollagens COL1a1 (COL1A1), and gyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined by real-time quantitative RT-PCR (qRT-PCR) in an 7900HT SDS unit (Applied Biosystems, Inc), as published (\( N=4 \) to 6 animals per group).\textsuperscript{10} The sequence of the primers and assays are shown in online Table I available at http://circres.ahajournals.org. We could not develop a reliable qRT-PCR assay to detect expression levels of B-type natriuretic peptide (NPPB) in rabbits.

**Expression Levels of mRNAs of Oxidative Stress Response Genes**

Expression levels of mRNA for relative mRNA levels of catalse (CAT), glutathione peroxidase 1 (GPX1), and heme oxygenase 1 (HMOX1), encoding catalase, glutathione peroxidase 1, and heme oxygenase 1, respectively, were detected and quantified by qRT-PCR in triplicates in at least 4 rabbits per group (online Table I).

**Myocardial Lipid Peroxide Levels**

Myocardial levels of malondialdehyde (MDA) and 4-hydroxyalkenals (HAE), the end products derived from peroxidation of polyunsaturated fatty acids and related esters, were measured using a commercially available kit, per instructions of the manufacturer (Calbiochem, Inc.). The assay is based on the spectrophotometric measurement of a chromophore, at 586 nm, generated by condensation of either MDA or HAE with 2 molecules of N-methyl-2-phenylindole, as the chromogenic reagent. The reactions were performed in triplicates and in 8 animals per group.

**Isolation of mtDNA and Detection of Oxidized DNA Levels**

Nuclear DNA and mtDNA were extracted from myocardial tissues per a published method with some modifications.\textsuperscript{11} Aliquots of myocardial tissue (25 to 30 mg) were homogenized in ice-cold Tris-HCl buffer pH 7.4 and centrifuged at 1000g for 10 minutes at 4°C. The pellets were used for extraction of nuclear DNA and the supernatants were centrifuged at 10 000g for 15 minutes at 4°C to pellet the mitochondria fraction. Nuclear DNA and mtDNA were extracted using a commercial kit based on silica-gel-membrane technique (Qiagen) without using phenol, chloroform, or ethanol precipitation. The concentration of DNA was quantified by measuring the absorbance at 260 nm. To determine the purity of isolated mtDNA, it was digested with BanHI, which results in 2 fragments of \( ~8 \) and 9 Kbp in size.

Immuno-slot blots to detect DNA oxidation were performed according to a published protocol with several changes.\textsuperscript{12} Briefly, 2 µg aliquots of nuclear DNA and mtDNA were dissolved in 100 µL with 2X SSC buffer, heat denatured at 95°C for 5 minutes and cooled on ice for 10 minutes. Ethidium bromide (1.2X dilution) was added to the DNA samples in a ratio 1:2 and samples were immobilized on nitrocellulose membranes (Bio-Rad) using a Mini-fold II, 72 well Slot Blot microfiltration apparatus. The membranes, following UV cross-linked of DNA, were blocked for nonspecific binding for 1 hour with 5% nonfat milk powder in PBS-Tween 20 (0.1%) and incubated with anti 7,8-dihydro-8-oxo-guanine (1:500) in PBS-T containing 5% non fat milk powder, overnight at 4°C. Following a couple of washes with PBS-T for 10 minutes, the membranes were incubated with goat anti-mouse IgG–HRP conjugate (1:4000) for 1 hour. Subsequently, the membranes were washed 3 times with PBS-T for 10 minutes and the immuno-slot blots were developed with ECL chemiluminescence (Amersham Bioscience). The intensity of the signal was quantified by densitometry and compared among the groups (\( N=6 \) per group).

**Signaling Kinases and Molecules**

Expression levels and activation of selected signaling molecules, namely: cytosolic, membrane, total and GTP bound Rac1 and RhoA; total and Ras-GTP; as well as total and phosphorylated p44/42 mitogen-activated-protein kinase (MAPK), p38, and c-Jun NH2-terminal kinase (JNK) were detected, as described.\textsuperscript{5,10} Protein expression level of catalse was detected by immunoblotting using a mouse monoclonal antibody in 4 animals per group (Sigma, St. Louis, Mo).
Measurement of Catalase Activity
Catalase activity in heart homogenates was determined using a commercially available kit (Cayman Chemicals) per manufacturer’s instructions. The assay is based on the peroxidatic function of catalase, which reacts with methanol in the presence of an optimal concentration of hydrogen peroxide to form formaldehyde, which is then measured spectrophotometrically at 540 nm in a plate reader with 4-aminoazobenzene-5-mercapto-1,2,4-triazole (purpald) as the chromogen. Catalase activity was determined in heart homogenates in duplicates and in 4 animals per group. Catalase activity is expressed in terms of nanomol of formaldehyde formed per minute per mg of protein at 25°C.

Detection of Myocyte Apoptosis
Myocyte apoptosis was detected by 3 complimentary techniques of agarose gel electrophoresis to detect DNA laddering, terminal deoxynucleotidyl transferase (TdT)-mediated nick end-labeling (TUNEL) assay, and immunoblotting to detect expression of truncated caspase 3.

For gel electrophoresis, DNA was extracted from cardiac tissues using a commercial kit (Qiagen DNeasy Tissue kit). Aliquots of 20 μg of genomic DNA were loaded onto a 1.6% agarose gel, subjected to electrophoresis and photographed following staining with ethidium bromide.

TUNEL assay was performed using a commercially available kit (In Situ Cell Death Detection Fluorescein Kit, Roche Diagnostics Corporation). In brief, thin myocardial sections were deparaffinized, hydrated, incubated with “Tunel” mixture containing terminal deoxynucleotidyl transferase and fluroscein-dUTP at 37°C for 1 hour to label DNA strand breaks. Nuclei were stained by incubating the slides in a 10 μl g/mL solution of DAPI for 30 seconds. Slides were also costained with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (purpald) as the chromogen. Catalase activity was determined in heart homogenates in duplicates and in 4 animals per group. Catalase activity is expressed in terms of nanomol of formaldehyde formed per minute per mg of protein at 25°C.

Results
Baseline Characteristics
There were no significant differences in the mean age, male/female ratio, body weight and heart rate at the baseline among the 3 experimental groups (online Table I).

Serum Levels of Total Cholesterol
Mean serum total cholesterol level was reduced by 47% in the atorvastatin group as compared with baseline (1.78±0.57 mmol/L at the baseline versus 0.94±0.19 mmol/L at the follow up, T=5.2, P<0.001), providing a direct evidence for the effective delivery of atorvastatin. There were no significant time-interval changes in the serum total cholesterol levels in the nontransgenic (1.65±0.44 versus 1.60±0.38 mmol/L) or in the transgenic rabbits in the placebo group (1.72±0.50 versus 1.63±0.31 mmol/L).

Cardiac Hypertrophy and Function
The LVMI, the primary end point of the study, was increased by 15% in the nontransgenic rabbits and by 4% in the atorvastatin group at the end of 1-year study (Figure 1). In contrast, LVMI was increased by 90% in the transgenic rabbits in the placebo group. Echocardiographic indices of cardiac hypertrophy and function at the baseline, 6 and 12 months following randomization are shown in online Table II. The mean interventricular septal thickness, posterior wall thickness, and LV Mass were similar in nontransgenic and atorvastatin groups, although they were increased by ~30% to 70% in the β-MyHC-Q403 transgenic rabbits in the placebo group.

Myocyte CSA and CVF
Myocyte CSA (~6000 myocytes per rabbit) was larger by 23% in the placebo group as compared with nontransgenic rabbits. In contrast, myocyte CSA was not significantly different between the atorvastatin and the nontransgenic rabbits (Figure 2A and 2B). The mean number of myocytes per high-power magnification field (X600, 120 field per rabbit) was smaller in the placebo group but similar between the atorvastatin and nontransgenic groups (Figure 2C).

CVF was increased by 41% in the placebo group as compared with nontransgenic rabbits (Figure 3A and 3B). It was reduced by 17% in the atorvastatin group, as compared with the placebo group. Similarly, expression levels of COL1A1 mRNA were higher in the placebo group as compared with nontransgenic and were modestly reduced in the atorvastatin group (Figure 3C). The differences in CVF and COL1A1 mRNA levels between the placebo and atorvastatin groups were not statistically significant.

Expression Levels of Markers of Cardiac Hypertrophy
The results are summarized in Figure 4. In brief, expression levels of MYH6 and ATP2A2 mRNAs were decreased by 4- to 5-fold in the β-MyHC-Q403 transgenic rabbits in the placebo group, as compared with the corresponding levels in the nontransgenic rabbits (P<0.001). In contrast, mRNA levels
for the above markers were normal in the atorvastatin group (Figure 4A and 4B). Expression levels of NPPA was increased by 2-fold in the placebo group and was near normal in the atorvastatin group (Figure 4C). Expression levels of ACTA1 mRNA were not significantly changed among the 3 groups.

**Levels of Markers of Oxidative Stress**

Myocardial levels of MDA and 4-HAE were similar between the nontransgenic and atorvastatin groups, although they were increased by a 2-fold in the placebo group (Figure 5A). Expression levels of mRNAs for 3 oxidative stress genes, namely CAT, GPX1, and HMOX1 are depicted in Figure 5B, 5C, and 5D, respectively. The CAT mRNA levels were reduced by 4.5-fold in the placebo group, as compared with nontransgenic rabbits, although levels were normal in the atorvastatin group. Levels of catalase protein (data not shown) and enzymatic activity were not significantly different among the 3 groups (enzymatic activity: 137.6±19.6 versus 123.5±17.3 versus 136.8±14.9 nM/μg/min in nontransgenic, placebo, and atorvastatin groups, respectively, P=0.49).

**Levels of Oxidized Nuclear and mtDNA**

There was a modest 27% increase in the level of oxidized mtDNA in the placebo group as compared with nontransgenic rabbits, which was of borderline statistical significance (Figure 6A). In contrast, levels of oxidized mtDNA were normal in the atorvastatin group. Levels of oxidized nuclear DNA were not significantly different among the 3 groups (7B).

**Levels of Intracellular Signaling Kinases and Molecules**

Levels of phosphorylated p44/42 MAPK were not significantly different between the nontransgenic and atorvastatin groups, although they were increased by 2.5-fold in the placebo group (Figure 7). Expression levels of phosphorylated p38 and JNKs also followed the same pattern, ie, similar between the nontransgenic and atorvastatin groups and increased by 20% in the placebo group (data not shown). Levels of total p44/42 MAPK, p38, and JNKs were unchanged. Another notable finding was an 36% reduction in the levels of membrane-bound Ras in the atorvastatin group (Figure 7), whereas levels of total and GTP-bound Ras, Rac1, and RhoA were not significantly different (data not shown).

**Myocyte Apoptosis**

The mean numbers of TUNEL-positive cells were 1.33±0.82, 3.33±1.86, and 0.67±1.03 per 100 000 myocytes in the nontransgenic, placebo, and atorvastatin groups, respectively (Figure 8). There were no discernible fragmented DNA in any of the 3 groups and myocardial levels of the truncated 19 KDa fragment of caspase 3 were not significantly different among the experimental groups (Figure 8).

**Discussion**

We performed a randomized placebo-controlled study and showed administration of atorvastatin to the β-MyHC-Q403 transgenic rabbits, early and before expression of cardiac hypertrophy, prevented development of cardiac hypertrophy over a 1-year period of observation. Prevention of cardiac hypertrophy was demonstrated at organ, cell, and molecular levels. It was associated with reduced levels of active Ras, phosphorylated p44/42 MAPK, lipid peroxides, oxidized mtDNA, and the number of TUNEL-positive cells. The findings indicate the potential utility of atorvastatin in preventing evolving cardiac hypertrophy in HCM, the most common cause of sudden cardiac death in the young and a major cause of morbidity in the elderly.13

Atorvastatin was administered early and before development of cardiac hypertrophy, as determined by echocardiographic assessment of LVMI. There were no significant differences in the baseline demographic and echocardio-
graphic phenotypes between the nontransgenic, transgenic-placebo and transgenic-atorvastatin groups. To reduce potential biases, the data were acquired and interpreted without knowledge of the group assignment. The results are strengthened by serial and multi-level phenotypic characterization. Furthermore, the use of β-MyHC-Q403 transgenic rabbits, known to recapitulate the phenotype of human HCM, strengthens the potential application of the findings to human patients. Moreover, the dose of atorvastatin was chosen based on our previous data,6 and considering the relative potency of the 2 HMG CoA reductase inhibitors, at least, as it relates to their effects on plasma levels of low-density lipoprotein-cholesterol. We documented the effectiveness of the chosen dose in reducing plasma total cholesterol levels (≈50% reduction). Thus, we administered an effective biological dose, which is expected to inhibit generation of isoprenoid intermediates of cholesterol biosynthesis.9 Moreover, the results were concordant for strong beneficial effects on prevention of molecular, histological, and morphological phenotypes of HCM without asserting an adverse effect on cardiac function. Finally, the observed beneficial effects of atorvastatin in prevention of cardiac hypertrophy in our genetic rabbit model of human HCM are also in accord with the effects of HMG-Co A reductase inhibitors on prevention of acquired forms of cardiac and myocyte hypertrophy.7,14–16

Figure 4. Expression levels of molecular markers of cardiac hypertrophy. A, Relative mRNA levels of MYH6, encoding α-myosin heavy chain. B, mRNA levels of ATP2A2, encoding sarcoplasmic reticulum ATPase 2A. C, mRNA level of NPPB, encoding B-type natriuretic peptide. D, Expression level of ACTA1, encoding skeletal α-actin. The P values reflect differences among the 3 groups and *P<0.05 in pairwise comparisons.

Figure 5. Expression levels of markers of oxidative stress. A, Levels of myocardial lipid peroxides; B, CAT; C, GPX1; and D, HMOX1. *P<0.05 in Tukey pairwise comparisons.
The main antihypertrophic effects of HMG CoA reductase inhibitors have been attributed to inhibition of isoprenylation of Ras, RhoA, Rac1, and Cdc42 as well as Rac1-mediated generation of reactive oxygen species via NADH oxidase activity.7,17 We detected a significant reduction in levels of membrane-bound Ras and phosphorylated p44/42 MAPK but not in the levels of activated RhoA and Rac1. The lack of discernible differences in levels of activated RhoA and Rac1 could simply reflect the inadequate resolution of immuno-blotting in detecting relatively modest changes that are expected in a genetic animal model of cardiac hypertrophy, wherein the stimulus is chronic and of relatively low magnitude. This is in contrast to in vitro cell culture experiments, wherein the stimulus is acute, direct and potent and hence, the anticipated changes in levels of activated GTPases are greater, as shown previously.6 Thus, the data does not necessarily exclude involvement of RhoA and Rac1 GTPases or Rac1-mediated oxidative stress in mediating the antihypertrophic effects of atorvastatin. It is also noteworthy that expression levels of CAT mRNA were reduced significantly in the hearts of β-MyHC-Q403 transgenic rabbits in the placebo group but not in atorvastatin group. Decreased expression level of CAT mRNA has been observed in a porcine model with naturally occurring HCM.18 Catalase, which reacts very efficiently with H2O2 to form water and molecular oxygen, in conjunction with GPX1 are the primary responsible enzymes for the removal of H2O2. Although it is plausible that reduced expression level of CAT could provide for a mechanism for the excess levels of H2O2 in the hypertrophic conditions, we did not detect significant reduc-
tions in the levels of catalase protein and activity. Therefore, it is unlikely that the antihypertrophic effect of the atorvastatin was predominantly attributable to upregulation of expression of CAT in the heart, as has been noted previously. Other redox-regulating proteins, such as thioredoxin (TXN), GPX1, and Cu-Zn superoxide dismutase (SOD1) also have been implicated in regulating cardiac hypertrophy and failure. We detected no significant changes in the expression levels of GPX1 and HOMX1 in the β-MyHC-Q403 rabbits. We could not determine the expression levels of TXN and SOD in the heart because of lack of reliable assays in rabbits. Finally, the number of TUNEL-positive myocytes, which reflect DNA break points, was higher in the placebo and lower in the atorvastatin, as compared with the nontransgenic group. Although TUNEL-positive cells are generally considered apoptotic cells, in the absence of a discernible evidence of DNA fragmentation or increased expression of 19 kDa caspase 3, the findings could reflect enhanced myocyte DNA synthesis in the hypertrophic state in the placebo group and reduced DNA synthesis in the atorvastatin group.

The molecular mechanisms that lead to activation of p44/42 MAPK in the β-MyHC-Q403 rabbits, a finding that is in accord with our previous data, were not determined specifically, but likely to involve multiple pathways including excess oxidative stress, which has been shown to affect post-translation oxidative modification of thiols on Ras. Nonetheless, the signaling pathways that could activate p44/42 MAPK are complex and interactive, encompassing Ras-dependent and –independent pathways, such as the activation of phospholipase C and protein kinase C pathways. Additional investigations would be required to delineate the molecular signaling involved in cardiac hypertrophic response in the β-MyHC-Q403 rabbits. Administration of atorvastatin had a modest but not statistically significant effect on increased myocardial CVF and expression level of COLIA1 mRNA in the β-MyHC-Q403 rabbits. The finding appears in discord with our previous data showing normalization of myocardial CVF with simvastatin therapy in the β-MyHC-Q403 rabbits and those of others, showing prevention of interstitial fibrosis post myocardial infarction in rats. The discrepancy could reflect potential differences in the biological effects of HMG-CoA reductase inhibitors and/or the experimental design of the studies including the drug dosages. Nonetheless, the findings suggest dissociation of the antihypertrophic and antifibrotic effects of atorvastatin, either because of the differential dose effects on hypertrophy and fibrosis and/or involvement of different molecular mechanisms by which HMG-CoA reductase inhibitors exert their antihypertrophic and antifibrotic effects.

We have proposed that myocardial dysfunction is the initial functional defect caused by the β-MyHC-Q403 mutation that provokes reactive cardiac hypertrophy in HCM. In the present as well as in the previous studies, indices of global left ventricular systolic function were normal in the β-MyHC-Q403. The finding of normal global left ventricular systolic function does not detract from the proposed hypothesis, because myocardial contraction and relaxation abnormalities, as detected by tissue Doppler imaging, were reduced despite preserved global left ventricular systolic function. Impaired myocardial contraction and relaxation velocities were initially observed in the β-MyHC-Q403 transgenic rabbits and were subsequently confirmed in humans with HCM mutations but no discernible cardiac hypertrophy. It is also noteworthy that the left-ventricular fractional shortening was higher in 6 month-old (baseline) as compared with 18 month-old rabbits (12-month follow up) in all 3 groups. The decline was slightly greater in and statistically significant in the nontransgenic group (NTG) group. The reason(s) for the age-dependent decline in the left ventricular fractional shortening is unclear. It could be the normal physiology or could reflect an increase in body weight with aging that occurred in all three groups. However, the increased load could have a greater impact on the load-dependent indices of left ventricular function, such as the fractional shortening, in the

![Figure 8. Detection of apoptosis. A. Results of TUNEL assay. The upper panels represent the NTG, the middle panels transgenic rabbits in the placebo group, and the bottom panels transgenic rabbits treated with atorvastatin. The first micrograph in each row shows immunofluorescent-stained thin myocardial sections with anti-cardiac troponin T antibody JLT12; the second, DAPI stained nuclei; the third, TUNEL-positive cells, and the last micrograph is the overlay of the 3. B. Quantitative data on the mean number of TUNEL-positive cells per 100 000 myocytes per groups. *P<0.05 in Tukey pairwise comparisons. C. Gel electrophoresis of nuclear DNA to detect apoptosis. No significant laddering was detected in any of the groups. D. Immunoblots using an antibody against full-length (35 kDa) and truncated (19 kDa) caspase 3 proteins (upper) and β tubulin (lower) in the experimental groups. No significant differences were detected in expression levels of truncated caspase 3 among the experimental groups.](http://circres.ahajournals.org/content/7/6/775/suppl/DC1)
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The results of the present study showing prevention of cardiac hypertrophic response in a transgenic rabbit model of human HCM have considerable clinical implications. The clinical significance of the findings is underscored by the lack of an effective pharmacological intervention to prevent cardiac hypertrophy in human patients with HCM. Moreover, current pharmacological therapy is largely empiric and none has been shown to prevent, attenuate or reverse cardiac hypertrophy in HCM. The results, once confirmed in humans, would raise the possibility for an early intervention, using a safe and well-established class of pharmacological agents, in HCM mutation carriers to prevent the development of cardiac phenotype. Furthermore, because hypertrophy is the common response of the heart to many forms of stress and a major determinant of mortality and morbidity, regardless of the cause, the findings could have broader implications in treatment and prevention of many forms of cardiovascular disease.

In summary, in a randomized placebo-controlled study, we have shown that administration of atorvastatin early and before development of cardiac hypertrophy, prevented evolution of cardiac hypertrophy at organ, cell and molecular levels and reduced levels of myocardial lipid peroxides and oxidized mtDNA as well as the number of TUNEL-positive myocytes. These findings, beckon the need for clinical studies in humans carriers of HCM mutations to determine the potential beneficial effects of HMG-CoA reductase inhibitors in prevention of cardiac hypertrophy.

Acknowledgments
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References
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### TABLE1: Sequence of Primers Used to Detect Expression of the Hypertrophic Markers and Antioxidant Genes by qRT-PCR

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</tr>
<tr>
<td>Baseline</td>
<td>3.53 ± 0.24</td>
<td>3.41 ± 0.14</td>
</tr>
<tr>
<td>6 Months</td>
<td>3.94 ± 0.26</td>
<td>3.95 ± 0.26</td>
</tr>
<tr>
<td>12 Months</td>
<td>4.02 ± 0.24</td>
<td>4.10 ± 0.22</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ST (mm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.00 ± 0.15</td>
<td>2.00 ± 0.22</td>
</tr>
<tr>
<td>6 Months</td>
<td>2.01 ± 0.12</td>
<td>2.32 ± 0.13</td>
</tr>
<tr>
<td>12 Months</td>
<td>2.11 ± 0.17</td>
<td>2.58 ± 0.15</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>0.083</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>PWT (mm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.08 ± 0.19</td>
<td>2.28 ± 0.26</td>
</tr>
<tr>
<td>6 Months</td>
<td>1.97 ± 0.20</td>
<td>2.18 ± 0.14</td>
</tr>
<tr>
<td>12 Months</td>
<td>2.03 ± 0.18</td>
<td>2.43 ± 0.17</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>0.498</td>
<td>0.024</td>
</tr>
<tr>
<td><strong>LVEDD (mm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>13.8 ± 0.97</td>
<td>13.3 ± 0.89</td>
</tr>
<tr>
<td>6 Months</td>
<td>14.8 ± 1.6</td>
<td>15.4 ± 1.8</td>
</tr>
<tr>
<td>12 Months</td>
<td>15.8 ± 1.0</td>
<td>16.4 ± 1.4</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>LVESD (mm)</strong></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>8.0 ± 0.7</td>
<td>8.1 ± 1.9</td>
</tr>
<tr>
<td>6 Months</td>
<td>9.6 ± 2.3</td>
<td>10.4 ± 1.6</td>
</tr>
<tr>
<td>12 Months</td>
<td>11.0 ± 1.4</td>
<td>11.5 ± 1.5</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>0.002*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>FS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.41 ± 0.08</td>
<td>0.39 ± 0.12</td>
</tr>
<tr>
<td>6 Months</td>
<td>0.35 ± 0.10</td>
<td>0.32 ± 0.06</td>
</tr>
<tr>
<td>12 Months</td>
<td>0.30 ± 0.08</td>
<td>0.30 ± 0.05</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>0.011</td>
<td>0.285*</td>
</tr>
<tr>
<td><strong>LVM (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.34 ± 1.11</td>
<td>5.05 ± 0.91</td>
</tr>
<tr>
<td>6 Months</td>
<td>5.62 ± 1.24</td>
<td>7.08 ± 1.74</td>
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<tr>
<td>12 Months</td>
<td>6.88 ± 1.07</td>
<td>8.69 ± 1.36</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>0.005</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NTG: Non-transgenic; MyHC: Myosin heavy chain; Q: Glutamine; M/F: Male/Female; bpm: beat per minute; IVST: Interventricular septal thickness; PWT: Posterior wall thickness; LVEDD: Left ventricular end diastolic dimension; LVESD: Left ventricular end systolic diameter; FS: Fractional shortening; LVM: left ventricular mass;
* denotes p values by Kruskall-Wallis test