**Echocardiographic examinations**
A transthoracic echocardiographic examination was performed within 1 week of the VEST study in all patients. Standard transthoracic M-mode and two-dimensional echocardiographic studies were performed to identify and quantify morphologic features of the LV, using criteria established by the American Society of Echocardiography.[15] LV dimensions and the thicknesses of the interventricular septum (IVST) and LV posterior wall (PWT) were measured at the level of the tips of the mitral valve leaflets, and the IVST/PWT ratio was calculated. Fractional shortening was calculated as the difference in end-diastolic and end-systolic dimensions divided by the end-diastolic dimension.

**Genetic Studies**
Genomic DNA was purified from subjects' white blood cells, after which *in vitro* amplification was performed by polymerase chain reaction (PCR). Oligonucleotide primers were used to amplify exons of the 3 cardiac troponin genes (C, T and I) using standard protocols (primer sequences and conditions for PCR amplification available upon request). Single-strand conformational polymorphism analysis of amplified DNA was then performed. For abnormal single-strand conformational polymorphism patterns, the nucleotide sequences of the cloned PCR products were determined on both strands by the dye terminator cycle sequencing method with use of an automated fluorescent sequencer (ABI PRISM$^{TM}$ 310 Genetic Analyzer, PE Biosystems, Foster City, CA). The sequence variation was confirmed by restriction enzyme digestion.

Based on the mutational analysis, the patients were divided into 2 groups: group A had the cardiac troponin gene mutations, and group B had other genes or unknown genotype.

**Statistical analysis**
Values are expressed as mean (SD). Differences between values measured at baseline and peak exercise were analyzed by the Student paired t test. Differences between groups were analyzed by the Student unpaired t test. Categorical data were compared by chi-square analysis. A multiple regression analysis was performed to detect factors that influenced exercise-induced systolic dysfunction. A p value $< 0.05$ was considered statistically significant. StatView 5.0 (Abacus Concepts, Inc., Berkeley, CA) was used for data analysis.

## RESULTS

**Study patients**
In the 52 study patients, cardiac troponin genes mutations were identified in 10 patients. Of the 10 patients, 4 had TnT mutations (Arg92Trp, n = 3; Phe110Ile, n = 1) and 6 had TnI mutations (Lys183Del, n = 6). Mutations in the cardiac troponin C gene were not

identified in this cohort. All mutations have been previously identified and described elsewhere.[7, 8, 16]

**Baseline characteristics**

The clinical and echocardiographic features of the study groups are summarised in table 1.

Table 1 Baseline characteristics

| | Group A (With troponin gene mutation) | Group B (Without troponin gene mutation) | p Value |
|---|---|---|---|
| Number of cases | 10 | 42 | |
| Male | 5 (50%) | 36 (85.7%) | 0.013 |
| Age (years) | 43.6 (16.1), 19-63 | 53.4 (12.6), 25-72 | 0.0416 |
| Family history of HCM | 10 (100%) | 10 (23.8%) | <0.0001 |
| Family history of SCD | 10 (100%) | 7 (16.7%) | <0.0001 |
| History of angina | 4 (40%) | 21 (50%) | 0.57 |
| History of syncope | 1 (10%) | 5 (11.9%) | 0.87 |
| NYHA functional class | | | |
| I | 7 (70%) | 32 (76.2%) | 0.12 |
| II | 2 (20%) | 10 (23.8%) | |
| III | 1 (10%) | 0 | |
| Echocardiogram | | | |
| maxWT (mm) | 19.0 (4.3), 13-26 | 17.9 (3.4), 13-25 | 0.36 |
| IVST (mm) | 17.5 (5.1), 11-23 | 17.4 (3.8), 10-25 | 0.92 |
| PWT (mm) | 12.4 (2.9), 6-17 | 12.1 (2.2), 9-22 | 0.74 |
| IVST/PWT | 1.41 (0.27), 1.00-1.83 | 1.45 (0.33), 0.84-2.20 | 0.67 |
| LAD (mm) | 38.6 (7.0), 29-54 | 36.8 (6.7), 22-47 | 0.46 |
| LVDd (mm) | 46.9 (6.3), 40-55 | 45.3 (6.3), 34-53 | 0.46 |
| LVDs (mm) | 30.3 (6.2), 24-41 | 27.0 (4.9), 19-40 | 0.08 |
| FS (%) | 36.1 (5.4), 27-43 | 40.7 (6.8), 26-56 | 0.06 |
| Radionuclear | | | |
| Exercise duration (min) | 7.3 (2.9), 4-12 | 8.2 (2.0), 5-12.4 | 0.22 |
| Peak exercise (W) | 100.0 (37.3), 50-150 | 104.2 (21.3), 75-150 | 0.64 |

Data presented are mean value (SD), range, or a number (%) of patients.
FS, fractional shortening; IVST, interventricular septal thickness; LAD, left atrial dimension; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; maxWT, maximum LV wall thickness; NYHA, New York Heart Association; PWT, left ventricular posterior wall thickness; SCD, sudden cardiac death.

Age was significantly younger in group A than in group B. The proportion of women, family history of HCM, and family history of sudden cardiac death were higher in group A than in group B. Echocardiographic parameters did not differ significantly between the 2 groups.

**Haemodynamic changes during supine ergometer exercise**

Exercise was terminated because of chest pain in one patient and dyspnoea and/or leg fatigue in the other patients. No patients terminated exercise because of hypotension or malignant arrhythmia. All examinations were performed without serious complications.

Exercise time and peak exercise load did not differ between the 2 groups (table 1). Haemodynamic responses and changes in haemodynamic parameters during ergometer exercise are summarised in table 2 and figure 1.

Table 2 Haemodynamic responses during ergometer exercise

| | Baseline | Peak Exercise | Difference | p Value |
|---|---|---|---|---|
| Heart rate (beats/min) | | | | |
| Group A | 62.0 (10.1), 49-87 | 132.2 (19.7), 96-164* | 70.2 (16.8) | 0.4 |
| Group B | 60.2 (6.1), 49-75 | 125.0 (20.1), 83-183* | 64.8 (18.2) | |
| Blood pressure (mm Hg) | | | | |
| Systolic BP | | | | |
| Group A | 118.9 (14.9), 104-176 | 157.9 (28.6), 104-204† | 39.0 (19.6) | 0.0098 |
| Group B | 128.3 (16.9), 92-142 | 197.7 (35.0), 109-256* | 69.4 (34.2) | |
| Diastolic BP | | | | |
| Group A | 68.1 (14.8), 43-93 | 86.5 (25.8), 65-149‡ | 18.4 (17.0) | 0.51 |
| Group B | 73.7 (12.0), 48-109 | 96.9 (23.9), 45-134* | 23.7 (23.6) | |
| Radionuclear | | | | |
| Relative EDV (%) | | | | |
| Group A | 100 | 109.7 (4.4), 92.7-150.9* | 10.7 (7.5) | 0.67 |
| Group B | 100 | 110.7 (7.5), 93.0-137.0* | 9.7 (4.4) | |
| Relative ESV (%) | | | | |
| Group A | 33.4 (10.8), 35.1-57.0 | 51.1 (18.9), 28.3-41.4‡ | 17.7 (12.7) | 0.0031 |
| Group B | 33.2 (7.2), 35.2-50.9 | 36.6 (12.0), 26.3-37.6 | 3.4 (13.2) | |
| LVEF (%) | | | | |
| Group A | 67.1 (10.8), 52.7-87.8 | 53.0 (17.5), 54.2-86.4‡ | -14.1 (11.1) | 0.0025 |
| Group B | 67.1 (6.3), 53.3-77.5 | 66.3 (11.0), 51.3-87.4 | -1.2 (11.7) | |

Data presented are mean value (SD), range.
EDV, end-diastolic volume; ESV, end-systolic volume; LVEF, ejection fraction.
p values concern the comparison of the differences from baseline to peak exercise between the group A and the group B by unpaired t-test.
*p <0.0001, †p <0.001, ‡p <0.05 compared with baseline within-group by paired t-test.

During exercise heart rate, systolic blood pressure, and diastolic blood pressure increased in the 2 groups. However the differences from baseline to peak exercise of systolic blood pressure in group A increased significantly less than in group B, and the changes in systolic blood pressure increased significantly less in group A than in group B. The differences from baseline to peak exercise of the LVEDV did not differ between the 2 groups, and the LVEDV increased similarly in the 2 groups. In contrast, the differences from baseline to peak exercise of the LVESV in group A increased significantly in contrast with group B, and the changes in LVESV increased significantly more in group A than in group B. Consequently, the differences from baseline to peak exercise of the LVEF in group A decreased significantly in contrast with group B, as shown in table 2 and figure 2. Additionally, the changes in LVEF decreased significantly more in group A than in group B and the changes in stroke volume decreased in group A, whereas they increased in group B. The changes in systemic vascular resistance decreased similarly in group A and group B.

**Multiple regression analysis**

Because the patient groups differed with regard to age, sex, family history of sudden cardiac death, and family history of HCM, multiple regression analysis was performed to evaluate the relationship between these and other factors *v* exercise-induced systolic dysfunction. Multiple regression analysis between group A and B, including troponin gene mutations, age, sex, family history of sudden cardiac death, and family history of HCM as covariates showed that troponin gene mutation was independently associated with the difference from baseline to peak exercise of the LVEF ($p = 0.0089$), but age, sex, family history of sudden cardiac death, and family history of HCM were not independently associated with this defect.

## DISCUSSION

The major finding of this study was that HCM patients with cardiac troponin gene mutations displayed exercise-induced systolic dysfunction of the LV more frequently than the other HCM patients.

The contraction and relaxation of cardiac muscle are regulated by the troponin complex, which acts as a $Ca^{2+}$ sensor. Hence, mutations in the troponin subunits that can alter these interactions are likely to lead to functionally important effects, and their impact on *in vivo* and *in vitro* contractility has been studied.[17, 18] Based on these findings we speculate that mutations in both TnT and TnI genes may cause an increase in the $Ca^{2+}$ sensitivity of force development that would result in increased force at submaximal $Ca^{2+}$ concentrations. An increase in $Ca^{2+}$ sensitivity of force development in TnT and TnI genes mutations may account for well-preserved systolic function in patients with troponin gene mutations at baseline.

In contrast, patients with cardiac troponin gene mutations showed exercise-induced LV systolic dysfunction in this study, and a significant difference was found in the change in LVEF and stroke volume between the 2 groups. Precise mechanisms of the difference in the frequency of LV systolic dysfunction between the patients with and without cardiac troponin gene mutations are not known, but the following possibility may account for these observations. Wall thickening and lumen narrowing of intramural coronary arteries has been reported in hearts with cardiac troponin T gene mutations.[19] If these histopathological changes occur more frequently in the hearts with cardiac troponin gene mutations, myocardial ischaemia might be induced more frequently during exercise and result in LV systolic dysfunction. In addition, myocardial ischaemia may induce cardiac troponin I degradation,[20] and degraded cardiac troponin I may change $Ca^{2+}$ sensitizing effects on force generation in cardiac muscle. On the other hand, an alternate mechanism for the exercise-induced LV systolic dysfunction in patients with troponin mutations may relate an abnormality of cardiac energy metabolism. It is well known that post-exercise systolic dysfunction due to the stunning phenomenon occurs in ischaemic heart disease. Since the stunning phenomenon may be caused by hypoxia and energy deficiency, this would fit well with the concept of HCM as a disease of energy deficiency.[21] Based on this hypothesis, it is possible that troponin mutations, which are regarded as particularly profligate in terms of energy dissipation, may cause both "stunning" and systolic dysfunction. Additional studies will be needed to clarify these mechanisms in the future.

Our study demonstrated that the LVEF during exercise in group A decreased significantly in contrast to group B. Moreover, in group A, the change in LVEF decreased in 9 of 10 patients, however, it increased in only one patient with a Phe110Ile mutation in TnT, which is associated with a favorable prognosis (fig 2).[16] Yanaga *et al* suggested that Arg92Gln mutant TnT enhanced $Ca^{2+}$ sensitivity of myofibrillar ATPase activity without affecting the maximum level of the ATPase activity, whereas the Phe110Ile TnT mutant enhanced a maximum level of ATPase activity without affecting $Ca^{2+}$ sensitivity in the functional analysis.[22, 23] These findings indicate that HCM-linked troponin mutations have at least two different effects on the $Ca^{2+}$-sensitive ATPase activity, i.e. $Ca^{2+}$-sensitization, and potentiation of the maximum level of the ATPase activity. The difference between these effects of HCM-linked TnT mutations may be one of the factors influencing exercised-induced LV systolic dysfunction.

Previous studies have suggested that an abnormal blood pressure response during exercise is related to the risk of sudden death and a high prevalence of cardiac events.[11-13] Moreover, an abnormal blood pressure response is associated with exercise-induced LV systolic dysfunction.[13, 24] In addition, previous reports have suggested that peak exercise induced systolic dysfunction in HCM represents a feature of poor prognosis.[25] In the present study, the difference from baseline to peak exercise of systolic blood pressure was significantly less in group A than in group B. Although the difference from baseline to peak exercise of exercise duration and the changes in heart rate and systemic vascular resistance did not differ between the 2 groups, the changes in stroke volume differed significantly. From these findings, the difference of systolic blood pressure response during exercise between the 2 groups

appears to result from lack of an appropriate increase in stroke volume, which in turn may be influenced by troponin gene mutations. This condition may relate to a specific clinical phenotype characterised by sudden cardiac death and progression to DCM. On the other hand, it is recognized that the cause of blood pressure alterations in exercise in HCM may be due to paradoxical vasodilation caused by aberrant mechano/baro receptor responses.[26] In contrast, Ciampi et al [24] showed that a similar decrease in systemic vascular resistance was present during exercise in patients with HCM that demonstrated both an abnormal and normal blood pressure response, and systemic vascular resistance did not differ between the 2 groups. It appears, therefore, that the mechanism of abnormal blood pressure response on exercise in HCM remains controversial. In our study 4 patients in group A (40%) and 3 patients in group B (7.1%) had an abnormal blood pressure response to exercise, and despite the small numbers the striking difference in the rate of abnormal blood pressure responses in the two groups was associated with a statistically significant difference between them.

**Limitations**

There are several potential limitations. First, the major limitation of the study is the disparity in the size of the two groups. The small number of patients, particularly in group A, may have special bearing on the outcomes. In this group we observed 1 troponin deletion and 2 distinct troponin T point mutations, and also noted that the distribution of troponin mutations was unusual (6 and 4, respectively) compared with previous reports. With such small numbers it is difficult to know if these findings would be representative of those in a larger group. There could also be some heterogeneity in Group B patients due to alterations in unknown genotypes, and this might also be reflected in the finding of considerable heterogeneity in LVEF (Figure 2). This variability makes it difficult to recommend that exercise testing be used routinely as a tool to evaluate this group of patients. However, our results do serve to improve understanding of pathophysiology of HCM patients with troponin gene mutations. Additional studies with a larger number of patients are needed in order to confirm and clarify our results.

Second, we may have experienced some referral bias, based on the fact that group A had a significantly increased family history of HCM and SCD compared to Group B. The question arises whether identification of exercise-induced systolic dysfunction in group A may be related to the manner in which patients with troponin mutations were referred to this clinic rather than representing a systematic feature of troponin mutations in general. The patients in this study, however, were referred to the Kanazawa University Hospital or its related hospitals (from primary to tertiary care centres). Multiple regression analysis revealed that family history of sudden cardiac death and family history of HCM were not independently associated with exercise-induced systolic dysfunction. Furthermore, no statistically significant differences were detected in characteristics of patients referred directly to our molecular cardiology clinic vs. those referred via our associated hospitals and care centres (data not shown). These findings mitigate against the likelihood of serious underlying referral bias in our study groups.

Finally, there are differences in cardiovascular haemodynamics during upright and spine exercise. The magnitude of exercise-induced subaortic pressure gradients may be affected by the exercise position, and pressure gradients may be difficult to induce during supine exercise because of an increase in venous return. Although we did not

evaluate the haemodynamic responses to upright exercise in the present study because of logistical concerns, we excluded patients with obstructive HCM diagnosed clinically and patients with latent obstruction detected by dobutamine stress study in the supine position. Furthermore, although subaortic pressure gradients with doppler echocardiography increase during upright bicycle exercise, there is no increase in the pressure gradient during supine exercise in patients with HCM.[10] Therefore we believe that during supine exercise, the patients in this study did not develop outflow tract gradients that might have influenced the study results.

**Conclusions**

The relation between the genotype and the haemodynamic phenotype in patients with HCM has not been established. In this study, we assessed specific haemodynamic changes during exercise with VEST between HCM patients with mutations in the cardiac troponin genes and those without them. HCM patients with cardiac troponin gene mutations may display exercise-induced LV systolic dysfunction more frequently than HCM patients without this abnormality.

**Figure legends**

**Figure 1.** Exercised-induced haemodynamic responses expressed as percent changes from baseline for group A and group B. BP, blood pressure; EDV, end-diastolic volume; ESV, end-systolic volume; LVEF, ejection fraction; SVR, systemic vascular resistance. Vertical bars indicate SD. *P < 0.05 versus group A.

**Figure 2.** Left ventricular ejection fraction under resting (baseline) conditions and peak exercise in the 10 patients with troponin genes mutations and the 42 patients without these mutations. Vertical bars indicate mean (SD) values for each group.

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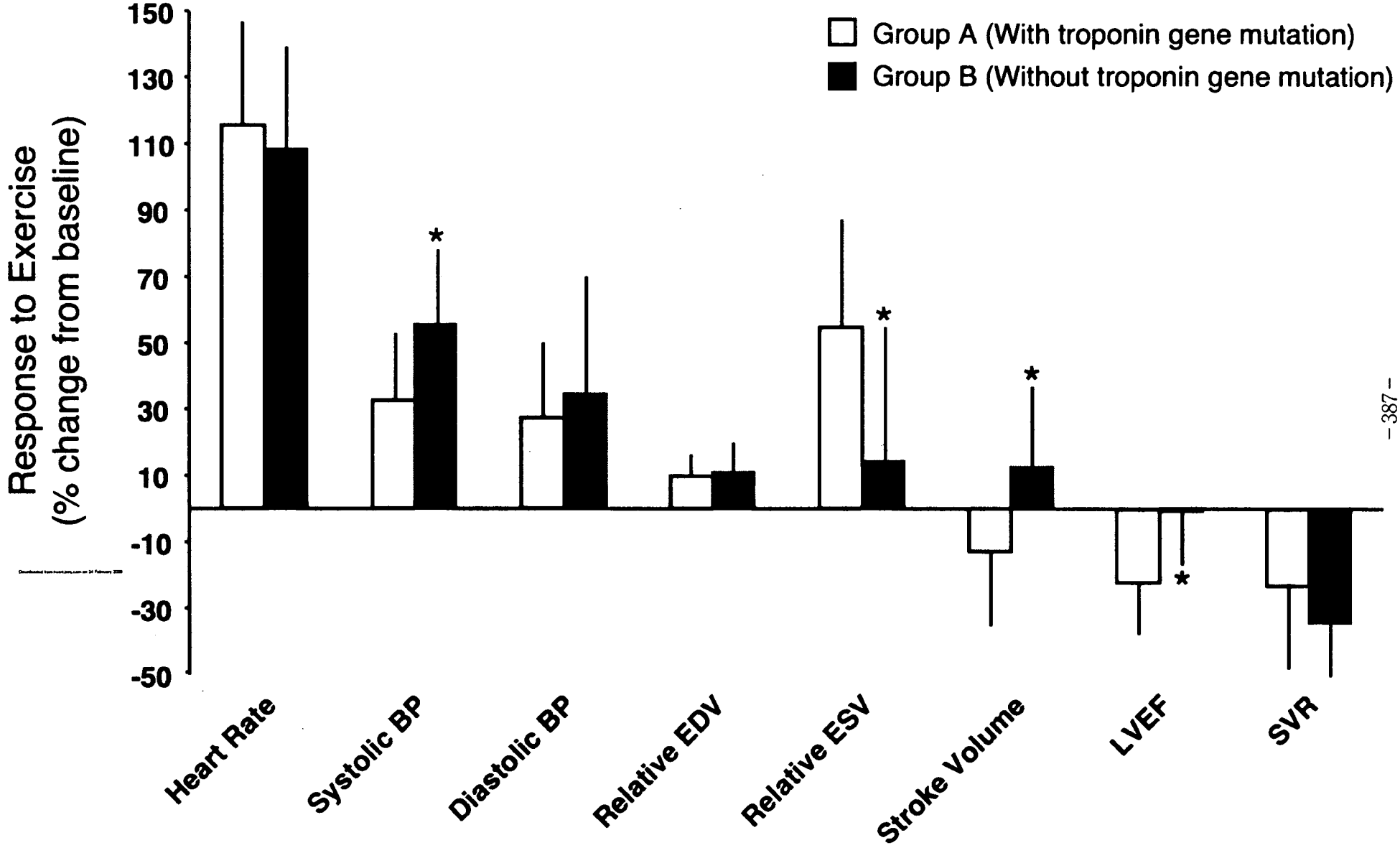
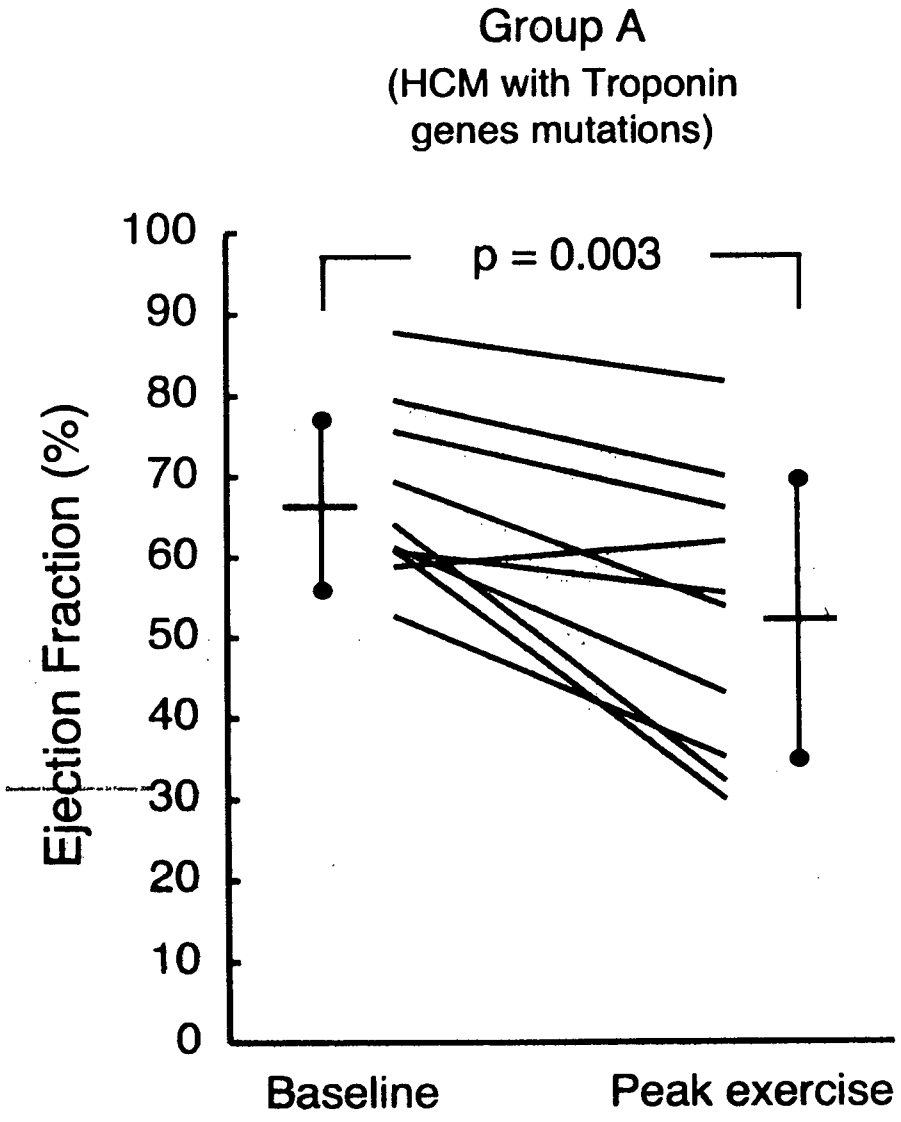
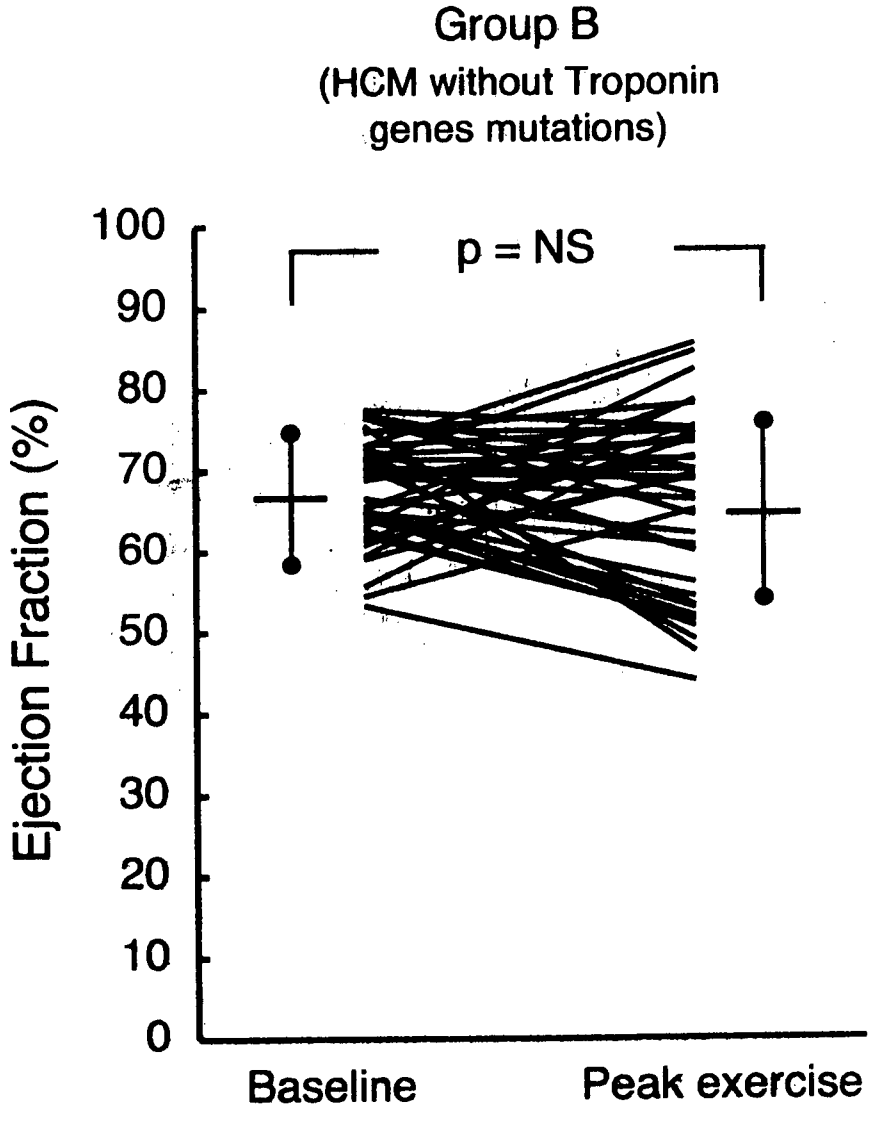

Group A (With troponin gene mutation)
Group B (Without troponin gene mutation)
Response to Exercise
(% change from baseline)
150
130
110
90
70
50
30
10
-10
-30
-50
*
*
*
*
Heart Rate
Systolic BP
Diastolic BP
Relative EDV
Relative ESV
Stroke Volume
LVEF
SVR


Group A
(HCM with Troponin genes mutations)
p = 0.003
Ejection Fraction (%)
100
90
80
70
60
50
40
30
20
10
0
Baseline
Peak exercise


Group B
(HCM without Troponin genes mutations)
p = NS
Ejection Fraction (%)
100
90
80
70
60
50
40
30
20
10
0
Baseline
Peak exercise

# Comparison of Effects of Pitavastatin and Atorvastatin on Plasma Coenzyme Q10 in Heterozygous Familial Hypercholesterolemia: Results From a Crossover Study

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**An open, randomized, four-phased crossover study using 4 mg of pitavastatin or 20 mg of atorvastatin was performed to compare their efficacy and safety, especially regarding plasma levels of coenzyme Q10 (CoQ10) in 19 Japanese patients with heterozygous familial hypercholesterolemia. Pitavastatin and atorvastatin caused significant and almost comparable reductions in serum levels of total cholesterol (−35.4 vs. −33.8%), low-density lipoprotein cholesterol (−42.8 vs. −40.7%), and triglyceride (−26.1 vs. −29.4%), and significantly increased serum levels of high-density lipoprotein cholesterol (12.1 vs. 11.4%). Under these conditions, plasma levels of CoQ10 were reduced by atorvastatin (−26.1%, $P = 0.0007$) but not by pitavastatin (−7.7%, $P = 0.39$), although no adverse events or abnormalities of liver and muscle enzyme were observed after either statin treatment. It remains to be seen whether the observed changes in CoQ10 levels are related to the long-term safety of this drug.**

A line of clinical trials has shown the efficacy of cholesterol-lowering therapy using 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, statins, for primary and secondary prevention of coronary artery disease.[1–5] Recently, several clinical trials have demonstrated that aggressive cholesterol-lowering therapy using a high dose of statins appeared to be more effective than a standard dose of statins in reducing cardiovascular events.[6–8] Statins efficiently reduce serum low-density lipoprotein (LDL) cholesterol by inhibiting the synthesis of mevalonate, an intermediate in the cholesterol biosynthetic pathway, and increase the induction of LDL receptors mainly in hepatocytes[9] (**Figure 1**).

Inhibition of HMG-CoA reductase results in decreased synthesis of not only cholesterol but also of other products downstream of mevalonate such as coenzyme Q10 (CoQ10), which is an essential cofactor in the mitochondrial electron transport chain and exists in almost all human tissues. Ubiquinol-10, the reduced form of CoQ10, is a potent lipophilic antioxidant, and the ratio between ubiquinol-10 and ubiquinone-10 is considered to be a good marker of oxidative stress. More than 50% of plasma CoQ10 is considered to be endogenous.[10] Previously, we reported that 10 mg/day of atorvastatin reduced plasma CoQ10 levels by 43% and that the percent reduction of ubiquinol-10 and of total cholesterol showed a significant positive correlation in patients with primary hypercholesterolemia.[11] Mitochondrial myopathy, encephalopathy, lactoacidosis, and stroke-like episode (MELAS) is a congenital syndrome due to impaired mitochondrial function, and it is also known to be associated with diabetes mellitus.[12] Thus, it is possible to hypothesize that the intracellular depletion of CoQ10 due to statins, at least partially, may cause myopathy and worsening of diabetes mellitus. Pitavastatin is a new totally synthetic HMG-CoA reductase inhibitor, which is hardly metabolized via the cytochrome *P*-450-mediated pathway[13] and induces LDL receptors more effectively than the other statins *in vitro*.[14] It is thus expected to show fewer adverse effects.

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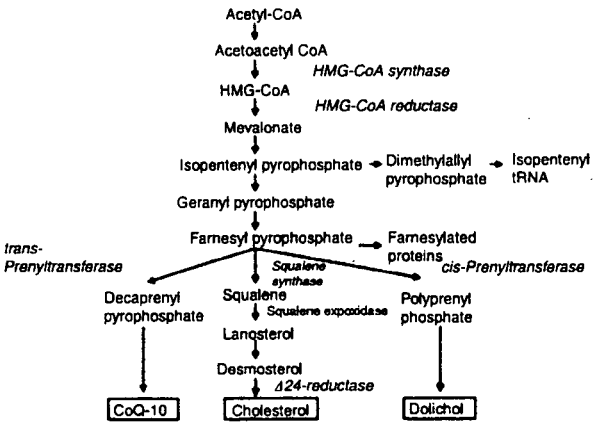


**Figure 1** The mevalonate pathway. Inhibition of HMG-CoA reductase results in decreased mevalonate metabolites as well as cholesterol, which is partially associated with "pleiotropic effects" of statins.

In this study, we compared the efficacy and safety of pitavastatin and atorvastatin in high doses, especially as they affected the plasma levels of CoQ10, in patients with heterozygous familial hypercholesterolemia (FH).

**Table 1 Baseline characteristics of the study subjects**

| | Group I ($n$=10) | Group II ($n$=9) |
|---|---|---|
| Age (years) | 51.6 ± 11.8 | 64.9 ± 11.7 |
| Sex (M/F) | 5/5 | 2/7 |
| BMI (kg/m$^2$) | 22.6 ± 1.7 | 22.9 ± 1.8 |
| *Risk factors* | | |
| Hypertension, $n$ (%) | 4 (40) | 4 (44) |
| Diabetes mellitus, $n$ (%) | 1 (10) | 1 (11) |
| Current smoking, $n$ (%) | 2 (20) | 0 (0) |
| Coronary artery disease, $n$ (%) | 1 (10) | 1 (11) |
| *Medications at baseline* | | |
| Atorvastatin, $n$ (%) | 9 (90) | 9 (100) |
| Colestimide, $n$ (%) | 3 (30) | 2 (22) |
| None, $n$ (%) | 1 (10) | 0 (0) |
| *Concomitant drugs* | | |
| Calcium channel blockers, $n$ (%) | 1 (10) | 3 (33) |
| Angiotensin receptor blockers, $n$ (%) | 2 (20) | 2 (22) |
| Beta blockers, $n$ (%) | 0 (0) | 1 (11) |
| Diuretics, $n$ (%) | 1 (10) | 2 (22) |

BMI, body mass index; F, female; M, male. Values are shown as mean ± SD.

## RESULTS

### Characteristics of study subjects

Nineteen subjects with Japanese heterozygous FH were enrolled in this study. Group I consisted of 10 patients, five men and five women aged 51.6 ± 11.8 years, and group II consisted of nine patients, two men and seven women aged 64.9 ± 11.7 years. The mean and SD of body mass index was 22.8 ± 2.4 kg/m$^2$. Coronary artery disease was already documented in two patients (11%), but none of the subjects had cerebral atherosclerotic vascular disease. Two patients (11%), who were under diet therapy, had diabetes mellitus with glycohemoglobin levels < 6.5% at the baseline. Eighteen patients were given atorvastatin, and 5 of 18 patients were administered colestimide before entering this study, whereas the remaining patient had never been treated with cholesterol-lowering agents. Colestimide was the only non-statin lipid-lowering medication that was given before entering this study. Seven patients were administered certain medications, such as antihypertensive drugs, before entering this study; however, the dosages of these coadministrated drugs were kept constant during the whole study period (**Table 1**).

### Changes in serum lipids and apolipoproteins

The changes in lipids and apolipoproteins in each study group are presented in **Table 2**. Because all parameters of serum lipids and apolipoproteins before the administration of each statin did not significantly differ between groups I and II, the data from both groups were combined (**Figure 2**). Both 4 mg of pitavastatin and 20 mg of atorvastatin significantly decreased the serum levels of total cholesterol (4 mg pitavastatin, $P<0.0001$; 20 mg atorvastatin, $P<0.0001$), LDL cholesterol ($P<0.0001$, $P<0.0001$), and triglyceride ($P<0.0001$, $P=0.0004$), and significantly increased the serum levels of high-density lipoprotein (HDL) cholesterol ($P=0.0001$, $P=0.002$) (**Figure 2**). Similarly, serum levels of apolipoprotein A-I and A-II were significantly increased, and those of apolipoprotein B, C-II, C-III, and E were significantly decreased by 4 mg of pitavastatin and 20 mg of atorvastatin (**Figure 2**). However, there was no significant difference in the changes in serum lipids and apolipoproteins between pitavastatin and atorvastatin treatment.

### Changes of lipoprotein lipid distributions

The changes in lipid distribution in each lipoprotein fraction were determined based on their density and size, namely ultracentrifugation and newly developed high-performance liquid chromatography (HPLC).[15] Because the pre-treatment levels of lipids in each lipoprotein fraction were not significantly different between groups I and II, the data were combined to compare the effects of pitavastatin and atorvastatin, as was performed in the other crossover studies.

**Table 3** shows the changes in cholesterol and triglyceride by pitavastatin and atorvastatin in each lipoprotein fractionated by ultracentrifugation. Both statins significantly decreased serum very LDL, intermediate-density lipoprotein, LDL1 cholesterol, and triglyceride and increased HDL cholesterol mainly in the HDL2 fraction. Although 20 mg of atorvastatin significantly decreased serum HDL3 cholesterol levels, there were no significant differences in the lipoprotein lipid levels after two statin treatments.

**Table 4** shows the pre- and post-treatment of lipid content in detailed fractioning lipoprotein using HPLC. Both

**Table 2 Serum lipid and apolipoprotein levels before and after treatment of each study group**

| | Pitavastatin | | | Atorvastatin | | |
|---|---|---|---|---|---|---|
| | Pre-treatment | Post-treatment | Mean % change | Pre-treatment | Post-treatment | Mean % change |
| *Group I (n=10)* | | | | | | |
| Total cholesterol (mg/dl) | 356.0 ± 34.2 | 227.1 ± 10.3 | −35.4*** | 355.2 ± 39.5 | 232.0 ± 25.4 | −34.1*** |
| Triglycerides (mg/dl) | 114.7 ± 44.0 | 84.5 ± 30.0 | −19.0 | 123.6 ± 51.5 | 74.0 ± 27.4 | −19.0* |
| LDL cholesterol (mg/dl) | 258.5 ± 25.9 | 144.9 ± 13.1 | −43.6*** | 263.3 ± 29.3 | 153.6 ± 23.3 | −41.3*** |
| HDL cholesterol (mg/dl) | 55.9 ± 9.7 | 60.6 ± 9.6 | 8.9*** | 55.1 ± 10.3 | 59.2 ± 7.8 | 8.7* |
| Apolipoprotein (mg/dl) | | | | | | |
| A-I | 134.7 ± 19.2 | 144.0 ± 14.6 | 7.9*** | 136.9 ± 19.1 | 141.6 ± 14.1 | 4.2 |
| A-II | 28.1 ± 3.2 | 28.9 ± 1.8 | 3.9 | 28.9 ± 2.4 | 29.1 ± 2.0 | 0.9 |
| B | 168.2 ± 20.4 | 110.4 ± 7.0 | −33.3*** | 174.8 ± 26.2 | 112.0 ± 14.0 | −35.3*** |
| C-II | 4.5 ± 1.7 | 3.8 ± 0.9 | −8.5 | 4.7 ± 1.4 | 3.4 ± 1.0 | −28.7** |
| C-III | 10.5 ± 2.7 | 8.8 ± 2.0 | −11.8 | 10.7 ± 2.3 | 8.5 ± 2.0 | −20.2*** |
| E | 6.0 ± 1.1 | 4.7 ± 0.9 | −20.9** | 6.3 ± 1.2 | 4.3 ± 0.9 | −31.2*** |
| *Group II (n=9)* | | | | | | |
| Total cholesterol (mg/dl) | 339.1 ± 42.8 | 226.3 ± 44.6 | −33.6*** | 366.1 ± 63.9 | 236.8 ± 47.7 | −35.4*** |
| Triglycerides (mg/dl) | 154.8 ± 82.1 | 107.2 ± 56.1 | −25.7* | 156.0 ± 61.8 | 98.3 ± 30.7 | −33.9*** |
| LDL cholesterol (mg/dl) | 234.7 ± 46.4 | 140.6 ± 38.5 | −40.0*** | 254.4 ± 58.6 | 149.1 ± 40.6 | −40.9*** |
| HDL cholesterol (mg/dl) | 50.0 ± 13.1 | 56.6 ± 14.0 | 14.4* | 53.0 ± 13.8 | 59.7 ± 13.0 | 15.5* |
| Apolipoprotein (mg/dl) | | | | | | |
| A-I | 125.9 ± 16.6 | 137.3 ± 14.6 | 10.3* | 132.2 ± 20.9 | 148.0 ± 14.0 | 13.8* |
| A-II | 26.1 ± 2.4 | 27.5 ± 2.9 | 6.0 | 27.7 ± 3.2 | 29.9 ± 2.9 | 8.8* |
| B | 162.8 ± 13.3 | 110.9 ± 17.4 | −31.7*** | 181.4 ± 25.8 | 113.6 ± 16.0 | −37.1*** |
| C-II | 5.8 ± 1.5 | 4.2 ± 0.9 | −24.3** | 6.2 ± 1.3 | 4.7 ± 1.0 | −21.4** |
| C-III | 11.2 ± 2.1 | 9.8 ± 1.6 | −10.0 | 12.2 ± 1.9 | 10.2 ± 1.2 | −14.0* |
| E | 7.2 ± 1.8 | 4.9 ± 1.4 | −30.4*** | 7.9 ± 2.0 | 5.3 ± 1.2 | −32.5*** |

HDL, high-density lipoprotein; LDL, low-density lipoprotein. Values are shown as mean ± SD. *$P<0.05$, **$P<0.01$, ***$P<0.001$; data compared the effects of two different statins.

statins significantly decreased cholesterol and triglyceride from segment no. 3 to no. 13, corresponding to very low-, low-, and intermediate-density lipoprotein, and significantly increased cholesterol from segment no. 16 to no. 17, corresponding to large to medium-size HDL. However, there was no significant difference in lipoprotein lipid distribution between pitavastatin and atorvastatin treatment.

**Adverse events**

Both 4 mg of pitavastatin and 20 mg of atorvastatin were well tolerated, and all 19 patients completed all study protocols. None of the patients suffered any severe adverse events that caused the discontinuation of either pitavastatin or atorvastatin, and no abnormalities in the laboratory findings were observed, including elevations in hepatic enzyme (aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, and alkaline phosphatase) or in creatine kinase more than three times the upper normal limits. Neither pitavastatin nor atorvastatin caused any impairment in glucose metabolism, as assessed using glycoalbumin (**Table 5**).

**Changes in plasma CoQ10 levels**

Because the plasma levels of total CoQ10, ubiquinol-10, and ubiquinone-10 before starting each statin between groups I and II were not significantly different, the values of plasma CoQ10 from both groups were combined and crossover-designed statistics were conducted (**Figures 3** and **4**).

Administration of pitavastatin did not change plasma levels of total CoQ10 significantly (838.6 to 737.3 nmol/l (−7.7%, $P=0.39$)), whereas atorvastatin significantly reduced plasma levels of total CoQ10 (864.6 to 599.9 nmol/l (−26.1%, $P=0.0007$)) (**Figure 3**). The reduction rate of plasma CoQ10 by atorvastatin treatment was significantly greater than that by pitavastatin treatment ($P<0.03$). Plasma levels of the reduced form of CoQ10, ubiquinol-10, showed similar changes as total CoQ10 after pitavastatin and atorvastatin treatment: from 659.9 to 572.2 nmol/l (−7.4%,

$P=0.14$) by pitavastatin and from 692.9 to 467.2 nmol/l (−23.0%, $P=0.006$) by atorvastatin. Interestingly, the reduction rate of plasma levels of the oxidized form of CoQ10, ubiquinone-10, by pitavastatin (−15.4%) was not significantly different from that by atorvastatin (−8.3%) ($P=0.4$).

### Correlation between percent changes of LDL cholesterol and plasma CoQ10

Correlations between percent changes in serum LDL cholesterol and plasma CoQ10 by both statins are shown in **Figure 4**. Percent changes in serum LDL cholesterol tended to correlate with those in plasma CoQ10 after atorvastatin treatment ($r^2=0.17$, $P=0.08$), whereas there was no correlation between percent changes in serum LDL cholesterol and those in plasma CoQ10 after pitavastatin treatment ($r^2=0.09$, $P=0.21$).

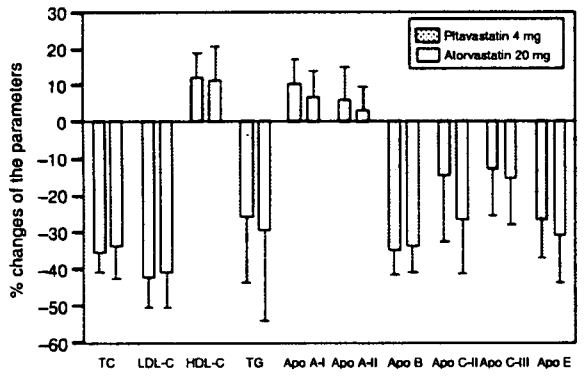


**Figure 2** The percent changes in serum lipids and apolipoproteins after treatment with pitavastatin and atorvastatin. Both pitavastatin (4 mg) and atorvastatin (20 mg) significantly decreased serum total cholesterol (−35.4 vs. −33.8%), LDL-cholesterol (−42.8 vs. −40.7%), and triglyceride levels (−26.1 vs. −29.4%) and significantly increased HDL-cholesterol (12.1 vs. 11.4%) levels. Serum levels of apolipoproteins also changed significantly. However, there were no significant differences between pitavastatin and atorvastatin in all these parameters. APO, apolipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

## DISCUSSION

Recently, several large clinical trials have demonstrated that lower levels of serum LDL cholesterol were more effective for the secondary prevention of coronary artery disease.[6–8,16] Thus, high doses of statins are frequently used in a clinical setting. Pitavastatin is a totally synthetic HMG-CoA reductase inhibitor that is available only in Japan and Korea so far. To test the efficacy and safety of this drug, comparison with the most common drug in the same category using a high dose would be reasonable. Thus, we chose patients with heterozygous FH as the study subjects to test the efficacy and safety of pitavastatin and atorvastatin, because they should be treated with a high dose of strong statins even if they do not have coronary artery disease (primary prevention).

The key findings of this study were that pitavastatin did not significantly reduce plasma CoQ10, whereas atorvastatin did, despite the fact that changes in serum lipid and apolipoprotein parameters, including detailed lipoprotein lipid distribution analysis and the short-term safety after both statin treatments, were almost comparable.

As shown in **Figure 2**, 4 mg of pitavastatin reduced serum total and LDL-cholesterol levels (−35.4 and −42.8%, respectively) to the same degree as 20 mg of atorvastatin (−33.8 and −40.7%, respectively), which is one of the most effective cholesterol-lowering drugs. Moreover, all of the following parameters were significantly and favorably changed by pitavastatin and atorvastatin, respectively: serum HDL

**Table 3 Serum levels of each lipoprotein cholesterol and triglyceride before and after treatment of each study group**

| | Pitavastatin | | | Atorvastatin | | | Pitavastatin vs. atorvastatin |
|---|---|---|---|---|---|---|---|
| Lipoprotein fraction | Pre-treatment | Post-treatment | Mean % change | Pre-treatment | Post-treatment | Mean % change | *P*-value |
| VLDL cholesterol (mg/dl) | 49.9 ± 14.9 | 29.0 ± 7.9 | −38.6*** | 50.3 ± 14.7 | 29.2 ± 8.8 | −39.8*** | 0.76 |
| triglyceride (mg/dl) | 73.2 ± 41.4 | 46.5 ± 23.9 | −25.5** | 78.1 ± 53.5 | 47.0 ± 34.1 | −25.3** | 0.76 |
| IDL cholesterol (mg/dl) | 30.1 ± 11.2 | 14.3 ± 5.3 | −44.5*** | 29.2 ± 13.7 | 13.2 ± 5.6 | −43.8*** | 0.88 |
| triglyceride (mg/dl) | 12.4 ± 4.3 | 8.7 ± 2.7 | −24.2** | 11.1 ± 4.6 | 8.1 ± 2.6 | −16.8** | 0.96 |
| LDL1 cholesterol (mg/dl) | 205.1 ± 38.0 | 118.1 ± 25.6 | −41.9*** | 199.4 ± 32.9 | 117.3 ± 25.0 | −41.0*** | 0.71 |
| triglyceride (mg/dl) | 30.1 ± 11.7 | 17.7 ± 5.7 | −34.8*** | 30.1 ± 9.4 | 16.8 ± 5.4 | −42.3*** | 0.43 |
| LDL2 cholesterol (mg/dl) | 29.4 ± 15.0 | 21.8 ± 5.7 | 1.9 | 22.8 ± 8.1 | 20.3 ± 4.1 | 4.8 | 0.94 |
| triglyceride (mg/dl) | 5.1 ± 2.4 | 4.5 ± 1.4 | 19.1 | 4.2 ± 2.3 | 4.6 ± 1.8 | 56.5 | 0.53 |
| HDL cholesterol (mg/dl) | 45.8 ± 10.0 | 50.7 ± 9.7 | 12.6** | 45.1 ± 10.5 | 49.6 ± 8.4 | 12.3** | 0.74 |
| triglyceride (mg/dl) | 9.3 ± 2.6 | 9.4 ± 2.1 | 6.9 | 9.7 ± 2.6 | 9.1 ± 2.8 | −4.0 | 0.36 |
| HDL2 cholesterol (mg/dl) | 28.0 ± 7.8 | 34.0 ± 8.5 | 24.5** | 26.9 ± 7.6 | 33.5 ± 7.7 | 28.2** | 0.79 |
| HDL3 cholesterol (mg/dl) | 17.8 ± 3.1 | 16.7 ± 2.0 | −3.9 | 18.2 ± 3.0 | 16.1 ± 1.8 | −8.7* | 0.25 |

HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein. Values are shown as mean ± SD. *$P<0.05$, **$P<0.01$, ***$P<0.001$; data compared the effects of two different statins.

**Table 4 Percent changes of the levels of cholesterol and triglyceride in each lipoprotein after treatment with pitavastatin and atorvastatin**

| Fraction number | Particle diameter (nm) | Lipid | Major and subclass name | Pitavastatin | | | Atorvastatin | | | Pitavastatin vs. atorvastatin |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pre-treatment | Post-treatment | Mean % change | Pre-treatment | Post-treatment | Mean % change | *P*-value |
| 1 | >90 | Cholesterol (mg/dl) | CM | 0.23 ± 0.27 | 0.06 ± 0.05 | −39.4** | 0.23 ± 0.24 | 0.18 ± 0.20 | −14.6 | 0.19 |
| | | Triglyceride (mg/dl) | | 0.81 ± 1.03 | 0.22 ± 0.24 | 4.6* | 0.95 ± 1.26 | 0.79 ± 1.21 | −38.8 | 0.85 |
| 2 | 75 | Cholesterol (mg/dl) | CM | 0.19 ± 0.21 | 0.05 ± 0.05 | −48.8** | 0.22 ± 0.23 | 0.11 ± 0.11 | −39.7** | 0.23 |
| | | Triglyceride (mg/dl) | | 0.60 ± 0.67 | 0.18 ± 0.21 | −12.7** | 0.81 ± 0.98 | 0.47 ± 0.64 | −47.2* | 0.89 |
| 3 | 64 | Cholesterol (mg/dl) | Large VLDL | 2.18 ± 1.77 | 0.86 ± 0.55 | −33.1 | 2.37 ± 1.68 | 0.89 ± 0.72 | −61.3*** | 0.13 |
| | | Triglyceride (mg/dl) | | 6.09 ± 5.27 | 2.78 ± 2.23 | −36.2** | 6.94 ± 5.86 | 3.42 ± 3.50 | −44.3** | 0.76 |
| 4 | 53.6 | Cholesterol (mg/dl) | Large VLDL | 9.11 ± 4.58 | 3.64 ± 2.02 | −57.2*** | 9.20 ± 4.07 | 3.55 ± 2.26 | −61.2*** | 0.53 |
| | | Triglyceride (mg/dl) | | 19.49 ± 12.22 | 11.34 ± 6.74 | −32.0*** | 20.71 ± 13.15 | 10.73 ± 8.28 | −38.7** | 0.58 |
| 5 | 44.5 | Cholesterol (mg/dl) | Large VLDL | 25.24 ± 7.57 | 12.25 ± 3.90 | −50.1*** | 24.98 ± 7.14 | 11.35 ± 4.25 | −55.2*** | 0.38 |
| | | Triglyceride (mg/dl) | | 30.90 ± 14.32 | 19.76 ± 9.93 | −29.5*** | 30.93 ± 16.49 | 17.15 ± 11.14 | −35.3** | 0.61 |
| 6 | 36.8 | Cholesterol (mg/dl) | Medium VLDL | 29.26 ± 7.42 | 15.59 ± 4.67 | −46.0*** | 29.97 ± 6.48 | 14.75 ± 4.80 | −51.2*** | 0.26 |
| | | Triglyceride (mg/dl) | | 23.90 ± 9.01 | 16.86 ± 7.28 | −25.5*** | 23.02 ± 9.76 | 14.19 ± 6.97 | −32.3** | 0.38 |
| 7 | 31.3 | Cholesterol (mg/dl) | Small VLDL | 21.14 ± 4.85 | 10.38 ± 2.67 | −50.3*** | 18.46 ± 4.23 | 9.74 ± 3.16 | −47.7*** | 0.58 |
| | | Triglyceride (mg/dl) | | 10.74 ± 3.27 | 8.06 ± 2.92 | −21.4*** | 9.83 ± 3.21 | 7.23 ± 2.53 | −21.9** | 0.61 |
| 8 | 28.6 | Cholesterol (mg/dl) | Large LDL | 57.12 ± 10.59 | 31.06 ± 6.34 | −45.2*** | 52.26 ± 10.61 | 30.36 ± 8.26 | −42.0*** | 0.20 |
| | | Triglyceride (mg/dl) | | 15.13 ± 4.61 | 10.73 ± 3.10 | −25.8*** | 14.30 ± 4.07 | 10.15 ± 2.92 | −27.0*** | 0.53 |
| 9 | 25.5 | Cholesterol (mg/dl) | Medium LDL | 81.52 ± 13.13 | 47.23 ± 8.23 | −41.6*** | 79.69 ± 12.26 | 48.37 ± 10.03 | −38.1*** | 0.13 |
| | | Triglyceride (mg/dl) | | 16.99 ± 5.92 | 11.64 ± 3.11 | −27.4*** | 16.72 ± 4.82 | 11.62 ± 3.04 | −28.6*** | 0.82 |
| 10 | 23.0 | Cholesterol (mg/dl) | Small LDL | 52.65 ± 10.58 | 31.53 ± 5.16 | −38.6*** | 52.51 ± 8.36 | 33.42 ± 5.40 | −35.4*** | 0.10 |
| | | Triglyceride (mg/dl) | | 10.61 ± 4.16 | 7.51 ± 1.86 | −22.0** | 10.83 ± 3.59 | 7.70 ± 1.98 | −25.3*** | 0.53 |
| 11 | 20.7 | Cholesterol (mg/dl) | Very small LDL | 18.43 ± 5.47 | 11.84 ± 2.42 | −31.5*** | 19.44 ± 4.74 | 12.79 ± 2.29 | −31.8*** | 0.55 |
| | | Triglyceride (mg/dl) | | 4.25 ± 1.91 | 3.16 ± 0.91 | −15.1* | 4.40 ± 1.66 | 3.34 ± 1.00 | −18.2** | 0.48 |
| 12 | 18.6 | Cholesterol (mg/dl) | Very small LDL | 6.20 ± 1.97 | 4.05 ± 1.06 | −32.9*** | 6.34 ± 1.67 | 4.34 ± 0.98 | −29.3*** | 0.26 |
| | | Triglyceride (mg/dl) | | 1.45 ± 0.78 | 1.09 ± 0.42 | −12.3* | 1.56 ± 0.68 | 1.16 ± 0.51 | −22.7** | 0.22 |
| 13 | 16.7 | Cholesterol (mg/dl) | Very small LDL | 1.94 ± 0.48 | 1.34 ± 0.34 | −29.8*** | 1.97 ± 0.38 | 1.37 ± 0.29 | −29.4*** | 0.67 |
| | | Triglyceride | | 0.54 ± 0.29 | 0.37 ± 0.17 | −13.3** | 0.55 ± 0.24 | 0.43 ± 0.16 | −9.9* | 0.53 |
| 14 | 15.0 | Cholesterol (mg/dl) | Very large HDL | 2.22 ± 0.60 | 2.02 ± 0.63 | −9.7* | 2.10 ± 0.34 | 1.94 ± 0.48 | −8.2 | 0.43 |
| | | Triglyceride (mg/dl) | | 0.57 ± 0.26 | 0.46 ± 0.14 | −3.5 | 0.59 ± 0.23 | 0.47 ± 0.19 | −4.7* | 0.26 |
| 15 | 13.5 | Cholesterol (mg/dl) | Very large HDL | 3.63 ± 1.91 | 4.15 ± 2.20 | 15.7* | 2.98 ± 1.01 | 3.53 ± 1.96 | 12.0 | 0.67 |
| | | Triglyceride (mg/dl) | | 0.88 ± 0.52 | 0.78 ± 0.43 | −7.9 | 0.84 ± 0.47 | 0.81 ± 0.64 | −8.4 | 0.91 |

Table 4 continued on following page

**Table 4 Continued**

| Fraction number | Particle diameter (nm) | Lipid | Major and subclass name | Pitavastatin | | | Atorvastatin | | | Pitavastatin vs. atorvastatin |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pre-treatment | Post-treatment | Mean % change | Pre-treatment | Post-treatment | Mean % change | *P*-value |
| 16 | 12.1 | Cholesterol (mg/dl) | Large HDL | 12.86 ± 5.92 | 16.41 ± 7.01 | 37.3*** | 12.15 ± 5.72 | 15.79 ± 5.94 | 42.1** | 0.40 |
| | | Triglyceride (mg/dl) | | 3.30 ± 1.40 | 3.56 ± 1.09 | 25.5 | 3.19 ± 1.54 | 3.69 ± 1.44 | 27.7 | 0.43 |
| 17 | 10.9 | Cholesterol (mg/dl) | Medium HDL | 15.70 ± 3.40 | 18.81 ± 2.63 | 26.7*** | 15.83 ± 4.42 | 18.06 ± 3.41 | 19.0** | 0.20 |
| | | Triglyceride (mg/dl) | | 3.88 ± 1.19 | 3.97 ± 1.03 | 10.7 | 4.04 ± 1.09 | 3.91 ± 1.15 | 2.2 | 0.55 |
| 18 | 9.8 | Cholesterol (mg/dl) | Small HDL | 13.90 ± 1.61 | 14.49 ± 2.16 | 5.3 | 14.25 ± 2.13 | 14.23 ± 2.03 | 0.5 | 0.22 |
| | | Triglyceride (mg/dl) | | 3.44 ± 1.10 | 3.18 ± 1.16 | −6.5 | 3.65 ± 1.16 | 3.12 ± 1.18 | −9.5 | 0.38 |
| 19 | 8.8 | Cholesterol (mg/dl) | Very small HDL | 4.93 ± 1.18 | 4.82 ± 1.13 | −1.1 | 5.30 ± 1.31 | 5.33 ± 1.43 | −0.2 | 0.62 |
| | | Triglyceride (mg/dl) | | 1.05 ± 0.56 | 0.81 ± 0.49 | −21.5** | 1.15 ± 0.51 | 0.93 ± 0.48 | −20.0* | 0.82 |
| 20 | 7.6 | Cholesterol (mg/dl) | Very small HDL | 2.70 ± 0.42 | 2.64 ± 0.41 | −1.9 | 2.84 ± 0.47 | 2.82 ± 0.47 | −0.7 | 0.55 |
| | | Triglyceride (mg/dl) | | 1.29 ± 0.35 | 1.18 ± 0.23 | 11.1* | 1.34 ± 0.31 | 1.23 ± 0.26 | −4.9 | 0.69 |

CM, chylomicron; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein. Values are shown as mean ± SD. *$P<0.05$, **$P<0.01$, ***$P<0.001$; data compared the effects of two different statins.

cholesterol (12.2 vs. 11.4%), triglycerides (−26.1 vs. −29.4%), apolipoproteins A-I (10.7 vs. 7.1%), A-II (6.2 vs. 3.3%), B (−35.1 vs. −33.6%), C-II (−14.6 vs. −26.6%), C-III (−12.9 vs. −15.4%), and E (−26.4 vs. −30.8%); however, there was no significant difference between those two treatments. Thus, we can conclude that 4 mg of pitavastatin has the same potential as 20 mg of atorvastatin to change lipid parameters in heterozygous FH.

As shown in **Figure 3**, 20 mg of atorvastatin significantly reduced plasma CoQ10 by 26.1% ($P=0.0007$). Because cholesterol and CoQ10 share a common biosynthetic pathway, it is expected that inhibition of HMG-CoA reductase by statins results in the reduction of both molecules (**Figure 1**).[11] Indeed, several studies have indicated that the depletion of plasma or tissue CoQ10 levels by lovastatin, pravastatin, simvastatin, and atorvastatin is almost the same degree as serum or plasma LDL-cholesterol reduction rate.[17,18] Moreover, CoQ10 is essentially insoluble in aqueous media, and thus CoQ10 is believed to be transported in the plasma by the lipoproteins.[19] Because LDL is a major lipoprotein in humans, especially in FH patients, the relative distribution of plasma CoQ10 in LDL is estimated to be higher in FH than in normal controls (75 vs. 60%).[19] It is also speculated that a reduction in LDL particles is the cause of depleted plasma CoQ10.

Ezetimibe, which is a selective cholesterol absorption inhibitor, did not change plasma CoQ10 levels despite significant −22.1% reduction of LDL cholesterol, possibly due to effective induction of CoQ10 in hepatocyte.[20] Cholestyramine, which is a bile acid-sequestering resin, also does not decrease plasma CoQ10 levels.[21] Thus, the depletion of plasma CoQ10 is not the direct result of serum lipid reduction. We have previously reported that percent reductions in serum ubiquinol-10 and those in total and LDL cholesterol showed a positive correlation ($r^2=0.39$, $P=0.0165$ and $r^2=0.28$, $P=0.0496$, respectively).[11] Similarly, in this study, the percent changes of plasma CoQ10 and those of serum LDL cholesterol by atorvastatin tended to be linearly correlated ($r^2=0.17$, $P=0.08$) (**Figure 4**). Interestingly, 4 mg of pitavastatin did not significantly reduce plasma CoQ10 levels despite the fact that its effects on serum lipoprotein metabolism, assessed by three independent methods (enzymatic lipid and apolipoprotein assay using whole serum, ultracentrifugation, and HPLC), were almost comparable with 20 mg of atorvastatin (**Figure 2**, **Tables 2**, **3**, and **4**). Thus, the mechanism by which LDL was lowered by pitavastatin does not appear to be as simple as that by atorvastatin, the latter lowering plasma LDL-cholesterol levels mainly through inhibition of HMG-CoA reductase. Indeed, Morikawa *et al.*[14] reported that the level of LDL receptor mRNA induced from HepG2 cells by pitavastatin was much higher than that by atorvastatin or simvastatin using a 200-fold excess of the inhibition concentration 50 ($IC_{50}$ concentrations (pitavastatin, 5.8 nM; atorvastatin, 33 nM; and simvastatin, 17 nM). Their findings combined with the results of this study suggest that pitavastatin inhibits HMG-CoA reductase and the production of CoQ10 much less than atorvastatin.

**Table 5 Alterations in liver enzyme, creatinine phosphokinase, myoglobine, and glycoalbumin during pitavastatin and atorvastatin therapy**

| | Pitavastatin | | | Atorvastatin | | | |
|---|---|---|---|---|---|---|---|
| | Pre-treatment | Post-treatment | Mean % change | Pre-treatment | Post-treatment | Mean % change | *P*-value |
| AST (IU/l) | 22.2 ± 6.3 | 26.9 ± 7.1* | 25.3 | 22.1 ± 6.1 | 25.3 ± 7.4* | 18.9 | 0.27 |
| ALT (IU/l) | 17.1 ± 4.9 | 22.0 ± 6.8* | 28.8 | 17.1 ± 5.9 | 22.5 ± 9.2* | 31.0 | 0.31 |
| γ-GTP (IU/l) | 23.9 ± 11.5 | 25.7 ± 13.6 | 7.4 | 24.6 ± 12.1 | 23.4 ± 10.4 | −0.4 | 0.42 |
| ALP (IU/l) | 210.1 ± 43.7 | 215.2 ± 52.1 | 2.8 | 211.6 ± 38.9 | 225.8 ± 46.7* | 7.0 | 0.19 |
| CK (IU/l) | 104.2 ± 32.8 | 148.1 ± 66.2* | 39.4 | 115.5 ± 44.7 | 124.9 ± 50.6 | 12.3 | 0.09 |
| Myoglobin (ng/ml) | 49.2 ± 15.1 | 62.1 ± 22.0* | 28.3 | 49.6 ± 16.5 | 54.4 ± 12.9 | 40.7 | 0.37 |
| Glycoalbumin (%) | 15.1 ± 0.9 | 15.0 ± 0.9 | −0.6 | 15.1 ± 1.0 | 15.0 ± 1.1 | −0.5 | 0.08 |

ALP, alkaline phosphatase; ALT, alanine aminotransferase, AST, aspartate aminotransferase; CK, creatine kinase; γ-GTP; gamma glutamyltransferase. Values are shown as mean ± SD. *$P<0.05$, **$P<0.01$, ***$P<0.001$; data compared the effects of two different statins.

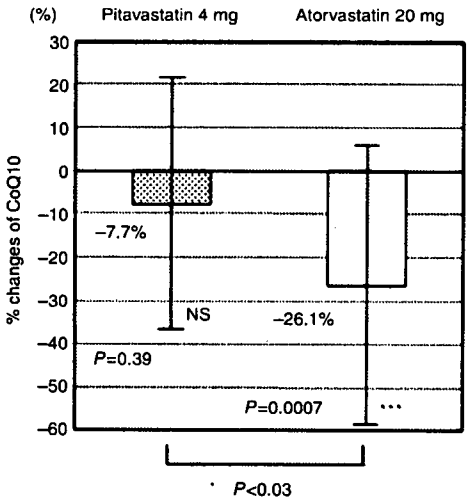


**Figure 3** The changes in plasma CoQ10 after treatments with pitavastatin and atorvastatin. Plasma levels of CoQ10 did not change by pitavastatin (4 mg) (−7.7%, $P = 0.39$) but did so by atorvastatin (20 mg) (−26.1%, $P = 0.0007$). The difference of the percent changes in plasma levels of CoQ10 was statistically significant ($P = 0.03$). NS, not significant.

CoQ10 is an essential cofactor in the mitochondrial electron transport chain, and 60% of plasma CoQ10 is endogenous. Although statins are generally well tolerated and safe, myopathy and an asymptomatic increase in hepatic enzymes are relatively frequent. The fatal rhabdomyolysis caused by cerivastatin led to its withdrawal from the market in 2001.[22] Cerivastatin has shown significant cytotoxicity in cultured human skeletal muscle cells and reduced ubiquinone levels in the rat heart compared with pitavastatin.[23] Administration of CoQ10 has been reported to ameliorate the increase in aspartate aminotransferase, creatinine phosphatase, and lactate dehydrogenase in the rat model of rhabdomyolysis induced by the combination of a high dose of simvastatin and gemfibrozil.[24] Thus, it can be speculated that depletion of tissue levels of CoQ10 may be at least a potential cause of myositis or liver toxicity in humans, although in this study we did not find any difference in the adverse symptoms or the abnormalities of laboratory data between these two statins (**Table 5**). We speculate that the period of this study might be too short to clarify the relationship between plasma CoQ10 levels and the adverse side effects of statin. Moreover, all study patients were administered pitavastatin for the first time, whereas 18 of the total 19 participants had been treated with 20 mg or a much higher dose of atorvastatin before entry into the study. Thus, it was expected that there might be fewer adverse effects against atorvastatin.

Our study has some limitations: 4 weeks of washout period, which might not be enough, was chosen because of ethical issues in treating high-risk patients. However, Chu *et al.*[25] have recently reported that serum CoQ10 levels decreased by 63% with 10 mg/day of atorvastatin, whereas it returned to baseline levels 3 days after withdrawal of atorvastatin. Thus, this relatively short washout period should be enough at least for plasma CoQ10 measurement. The other limitations are a relatively short treatment period, small sample size, and prior exposure to atorvastatin in terms of underestimation of adverse events or laboratory abnormalities of atorvastatin.

In conclusion, 4 mg of pitavastatin had the same effect on serum lipoprotein metabolism as 20 mg of atorvastatin, which is one of the most effective cholesterol-lowering drugs, without affecting plasma CoQ10 levels in heterozygous FH patients. It should be further established whether changes in CoQ10 levels after statin treatment are related to the long-term safety of this drug.

## METHODS

**Study patients.** This study population consisted of 19 patients (7 men and 12 women, aged 57 ± 13 (mean ± SD)) with heterozygous FH. All patients fulfilled the diagnostic criteria for FH:[26] primary hypercholesterolemia (> 230 mg/dl) with tendon xanthomas or first-degree relatives of previously diagnosed heterozygous FH patients showing primary hypercholesterolemia (> 230 mg/dl).