

METABOLISM

### 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Inhibitors Improve Myocardial High-Energy Phosphate Metabolism in Men

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#### ABSTRACT

*Purpose.* We intended to prove that 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or statins have a beneficial impact on the human myocardial, high-energy, phosphate metabolism. *Methods.* The present study included 18 male patients (mean age  $49.8 \pm 10.3$ ) with statin-treated, familiar hypercholesterolemia (FH) and 13 male patients with untreated FH (mean age  $44.6 \pm 9.5$ ). Twenty-six healthy male volunteers served as controls (mean age  $44.2 \pm 12.1$ ). Phosphorus-31, two-dimensional chemical shift imaging (<sup>31</sup>P 2D CSI) of the heart was performed in all subjects using a 1.5 Tesla whole-body magnetic resonance (MR) scanner. The ratios between phosphocreatine (PCr) and  $\beta$ -adenosine-triphosphate ( $\beta$ -ATP) were calculated for the left ventricular myocardium. Furthermore, echocardiographic evaluation and stress tests were performed in all individuals. *Results.* The untreated patients with FH exhibited a significant decrease in left ventricular PCr to  $\beta$ -ATP ratios ( $1.78 \pm 0.34$ ) compared with statin-treated FH patients ( $2.15 \pm 0.26$ ,  $p < 0.001$ ) and healthy controls ( $2.04 \pm 0.26$ ,  $p = 0.009$ ). The left ventricular PCr-to- $\beta$ -ATP ratios of the treated FH patients were in the range of the healthy controls. *Conclusions.* Our study shows for the first time an improvement of the high-energy, phosphate metabolism in the left ventricular myocardium of patients with statin-treated FH compared with untreated FH patients.

*Key Words:* Phosphorus-31 two-dimensional chemical shift imaging; Hypercholesterolemia; Statins; Myocardium.

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## INTRODUCTION

A high concentration of circulating, low density lipoproteins (LDL) is believed to be a major risk factor for atherosclerosis, which is the underlying disorder in the majority of patients with cardiovascular disease.

Several large clinical trials have demonstrated that 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or statins are powerful inhibitors of the cholesterol biosynthesis and decrease the incidence of coronary heart disease (The Scandinavian Simvastatin Survival Study). Since high cholesterol levels correlate closely with coronary heart disease, the cholesterol reduction by statins was generally assumed to be the main pathway for their beneficial impact on the reduction of cardiovascular events. However, the overall clinical benefits observed under statin therapy appear to exceed the effects, which might be expected from changes in lipid profile alone. This notion was strengthened by several subgroup analyses of large clinical trials, such as the Cholesterol and Recurrent Events (CARE) and West of Scotland Coronary Prevention (WOSCOP) studies. These analyses indicate that statin treated persons have a significantly lower risk of coronary heart disease than placebo-treated persons, while serum cholesterol levels were comparable for the statin-treated and placebo groups in these studies (Sacks et al., 1996; Shepherd et al., 1995). Furthermore, individuals treated with other cholesterol-lowering agents have shown a higher risk of myocardial infarction than statin-treated individuals, whereas comparable reductions in serum cholesterol levels were detected in both study groups. These findings suggest that statins may have beneficial impacts on the cardiovascular system that go beyond pure cholesterol reduction (Lefer et al., 2001; Takemoto and Liao, 2001).

We hypothesized that statins may modulate myocardial, high-energy phosphate metabolism. This assumption is based on the known cardiovascular

alterations caused by hypercholesterolemia, as well as on the recently published, beneficial cardiovascular effects of statins that are not related to cholesterol lowering (Lefer et al., 2001; Takemoto and Liao, 2001; Tsao and Cooke, 1998). Therefore, we used phosphorus-31, two-dimensional, chemical shift imaging ( $^{31}\text{P}$  2D CSI) to prove that the left ventricular high energy phosphate metabolism, as measured by the PCr-to- $\beta$ -ATP ratios, is improved in statin-treated individuals with familiar hypercholesterolemia (FH) compared with untreated FH patients. Previous  $^{31}\text{P}$  MR spectroscopy ( $^{31}\text{P}$  MRS) studies have shown that different cardiovascular risk factors such as aging and diabetes mellitus modulate left ventricular, high-energy, phosphate metabolism (Metzler et al., 2002; Schocke et al., 2003). Therefore, we believe that cardiac  $^{31}\text{P}$  MRS is a well-suited method for testing our hypothesis.

## MATERIALS AND METHODS

### Patients and Volunteers

The present study included 18 male patients with statin-treated hypercholesterolemia and 13 untreated hypercholesterolemic male patients, whose demographic and clinical data are listed in Table 1. Twenty-six healthy male volunteers served as controls. None of these patients had a history of coronary heart disease or any other cardiovascular disease. Apart from statins, the treated patients did not take any other continuous medication. Both patient groups did not show any substantial differences in cigarette smoking habits, alcohol consumption, or aspirin use. All individuals who had risk factors for the MR examination, such as cardiac pace makers or intracranial or intraocular metal devices, were excluded from this study. All subjects gave

**Table 1.** Clinical and demographic data of the FH patients and healthy controls.

	Patients with FH		Healthy controls
	Treated	Untreated	
N	18	13	26
Age (years)	49.8 $\pm$ 10.3	44.6 $\pm$ 9.5	44.2 $\pm$ 12.1
PCr-to- $\beta$ -ATP	2.15 $\pm$ 0.26	1.78 $\pm$ 0.34*	2.04 $\pm$ 0.26
BMI (kg/m <sup>2</sup> )	25.4 $\pm$ 2.5	25.2 $\pm$ 2.7	24.4 $\pm$ 3.2
Cholesterol (mg/dl)	227 $\pm$ 31.1	260.1 $\pm$ 24*	187.3 $\pm$ 25.9
LDL (mg/dl)	145.7 $\pm$ 27	168.3 $\pm$ 27.1*	105.4 $\pm$ 26.9
Glucose (mg/dl)	93.6 $\pm$ 7.8	91.8 $\pm$ 7.6	92.2 $\pm$ 24.4

\*Significant difference between the groups ( $p < 0.001$ ).

informed consent to the study, which was approved by the ethical committee of the medical faculty of the University of Innsbruck.

### Magnetic Resonance Protocol

The  $^{31}\text{P}$  MRS method used in the present experiments was recently published by Metzler et al. (2002) and Schocke et al. (2003). Both cardiac  $^{31}\text{P}$  MRS and cine MR imaging were performed in all subjects by using a 1.5 Tesla whole-body MR scanner (Magnetom Vision, Siemens Erlangen, Germany), and a circular, polarized, double resonator surface coil permitting the transmission and receipt of  $^1\text{H}$  resonances at 63.5 MHz and  $^{31}\text{P}$  resonances at 25.8 MHz.

Cine MRI was based on a flash sequence in bright blood preparation with a repetition time (TR) of 60 ms, an echo time (TE) of 6.8 ms, a slice thickness of 8 mm, a field of view (FoV), and a matrix of  $256 \times 128$ . The cine MR images were used for accurate superimposition of the spectroscopic grid.

The spectroscopic measurements were carried out by using a cardiac-gated, transversal, two-dimensional, chemical shift imaging sequence ( $8 \times 8$  phase encoding steps; slab thickness, 4 cm; flip angle,  $90^\circ$ ; echotime, 3 ms). For further evaluation the spectroscopic data were postprocessed with the help of Fourier interpolation (matrix  $32 \times 32$ ) and corrected for partial saturation effects as well as nucleus Overhauser enhancement, as recently published (Metzler et al., 2002; Schocke et al., 2003).

The PCr-to- $\beta$ -ATP ratios were formed in order to quantitate high-energy, phosphate metabolism. Mean left ventricular PCr-to- $\beta$ -ATP values were determined for each subject by averaging eight zero-filled voxels that were derived from the original voxel covering the left ventricle.

### Echocardiographic Examination

Each patient and control subject underwent echocardiography using an Acuson ultrasound imaging system (Acuson, Sequoia C256, Siemens, Erlangen, Germany) equipped with a 3.5 MHz-transducer (harmonic imaging). Parasternal long- and short-axis, as well as apical two-, four-, and long-axis chamber views, were obtained. Left ventricular (LV) volumes and ejection fractions (EF) were measured by the area-length-method (modified Simpsons method). The thickness of the left ventricular posterior wall and interventricular septum were used to calculate the left ventricular muscle mass

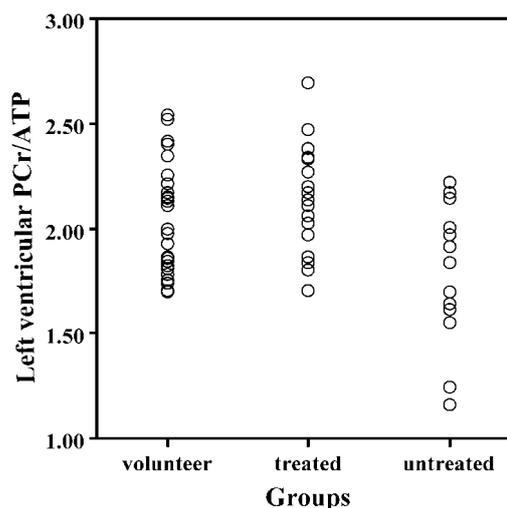
(Penn formula). Left ventricular diastolic filling was evaluated by pulsed Doppler echocardiography by the determination of the early (E)-to-atrial (A) peak ratio.

### Exercise Protocol

A modified Bruce protocol was used on an electrically-braked bicycle ergometer (Sheffield, 1988). Both standard blood pressure and 12-lead electrocardiographic (ECG) monitoring were performed (Marquette Electronics Inc., Solingen, Germany). Patients were encouraged to exercise until exhausted under physician supervision.

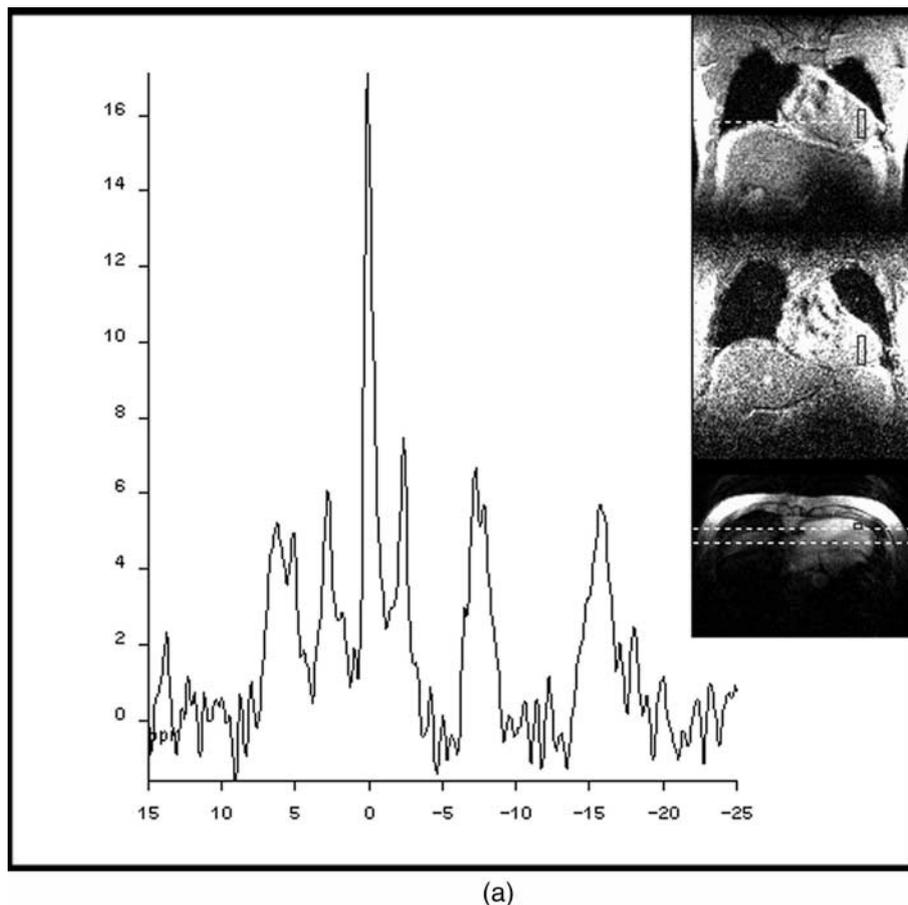
### Statistical Analysis

The statistical evaluation was performed by using SPSS 10.0 for windows (SPSS Inc., USA). Since the Kolgomorov-Smirnov test revealed normal distribution for most of our data, the univariate ANOVA with Bonverent correction was used for further evaluation. The significance level was set at  $p < 0.05$ .



**Figure 1.** A phosphorus-31, two-dimensional, chemical shift imaging of the heart was performed in all patients with untreated or statin-treated FH and in healthy controls. The PCr-to- $\beta$ -ATP ratios from the left ventricle of untreated hypercholesterolemic patients ( $1.78 \pm 0.34$ ) were significantly decreased compared with statin-treated FH patients ( $2.15 \pm 0.26$ ,  $p < 0.001$ ) and healthy controls ( $2.04 \pm 0.26$ ,  $p = 0.009$ ). There was no significant difference between the statin-treated FH patients and the healthy controls.





**Figure 2.** Comparison of selected  $^{31}\text{P}$  two-dimensional CSI spectra from the myocardium of a representative experiment from the left ventricle of an untreated FH patient (2a), a statin-treated FH patient (2b), and a healthy control person (2c). PCr was taken as 0 ppm, the peak of  $\beta$ -ATP is about  $-15$  ppm. The PCr-to- $\beta$ -ATP ratios were measured from the integrated areas of the PCr and  $\beta$ -ATP peaks.

## RESULTS

### $^{31}\text{P}$ 2D CSI

#### Laboratory Findings and Clinical Characteristics

The clinical data of the FH patients (treated and untreated) and the control subjects are shown in Table 1. The untreated FH patients exhibited significantly increased cholesterol ( $260.2 \pm 24$  mg/dl) and LDL ( $168.4 \pm 27.1$  mg/dL) levels compared to the treated group (cholesterol,  $227 \pm 31.1$  mg/dl,  $p = 0.001$ ; LDL,  $145.7 \pm 27$ ,  $p < 0.001$ ) and healthy controls (cholesterol,  $187.3 \pm 25.9$  mg/dl,  $p < 0.001$ ; LDL,  $105.4 \pm 26.9$ ,  $p < 0.001$ ). The differences in age, body mass index (BMI), and fasting serum glucose were not significant.

Figure 1 shows the PCr-to- $\beta$ -ATP ratios from the left ventricle of all groups. The left ventricular PCr to  $\beta$ -ATP ratios of the untreated FH patients ( $1.78 \pm 0.34$ ; Fig. 2a) were significantly decreased compared to the statin-treated FH patients ( $2.15 \pm 0.26$ ,  $p < 0.001$ ; Fig. 2b) and the healthy controls ( $2.04 \pm 0.26$ ,  $p = 0.009$ ; Fig. 2c). There was no significant difference between the latter two.

#### Echocardiographic Examination and Exercise Capacity

We found no significant differences among the groups concerning with EF, the E/A ratio, and the LV mass (data not shown).

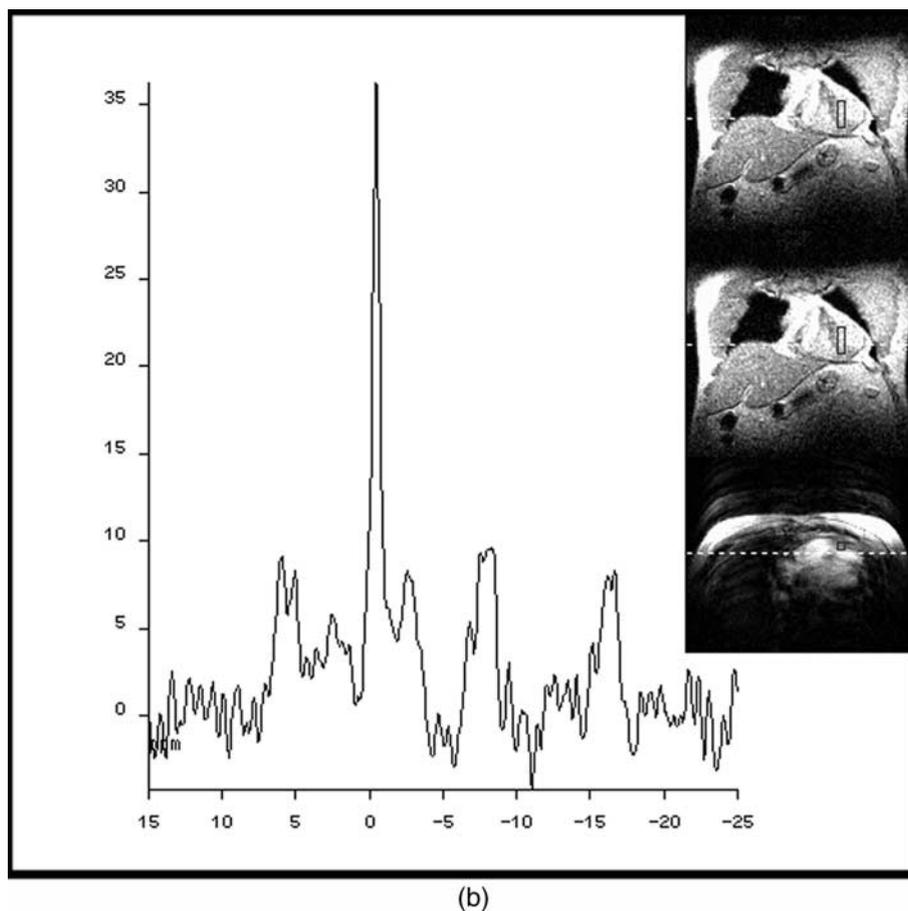


Figure 2. Continued.

(continued)

The results of the exercise test also did not reveal any significant differences among the three groups regarding maximal exercise capacity, resting heart rate, and the resting blood pressure (systolic and diastolic). All patients and healthy controls did not show any significant ST-segment changes or angina pectoris during exercise (data not shown).

## DISCUSSION

Hitherto, we are the first to show a beneficial effect in vivo for the human myocardial, high-energy, phosphate metabolism following treatment with statins. Previous cardiac  $^{31}\text{P}$  MRS studies have shown a decreased PCr-to- $\beta$ -ATP ratio in patients with cardiomyopathy and coronary artery disease, suggesting an altered metabolism in the myocardial tissue. Furthermore, two recent  $^{31}\text{P}$

MRS studies demonstrated the impact of two major cardiovascular risk factors, diabetes mellitus and age, on cardiac, high-energy phosphate metabolism. Therefore, the improved left ventricular PCr-to- $\beta$ -ATP ratios in statin-treated FH patients, compared with the nontreated FH patients, indicate the high impact of statin treatment on the cardiovascular system and cell metabolism (Hardy et al., 1991; Neubauer et al., 1992; Weiss et al., 1990). It is known that the cholesterol-lowering effect of statins can improve per se the endothelial dysfunction (Tamai, 1997). Furthermore, the statins have other multiple beneficial effects on the cardiovascular system, such as stimulation and upregulation of the endothelial nitric oxide synthase (eNOS), inhibition of the adhesion molecules and endothelin-1 expression, and inhibition of the smooth muscle cell migration and proliferation (Lefer et al., 2001; Takemoto and Liao, 2001; Tsao and Cooke, 1998). The crucial "pleiotropic" effect, however, seems to be the



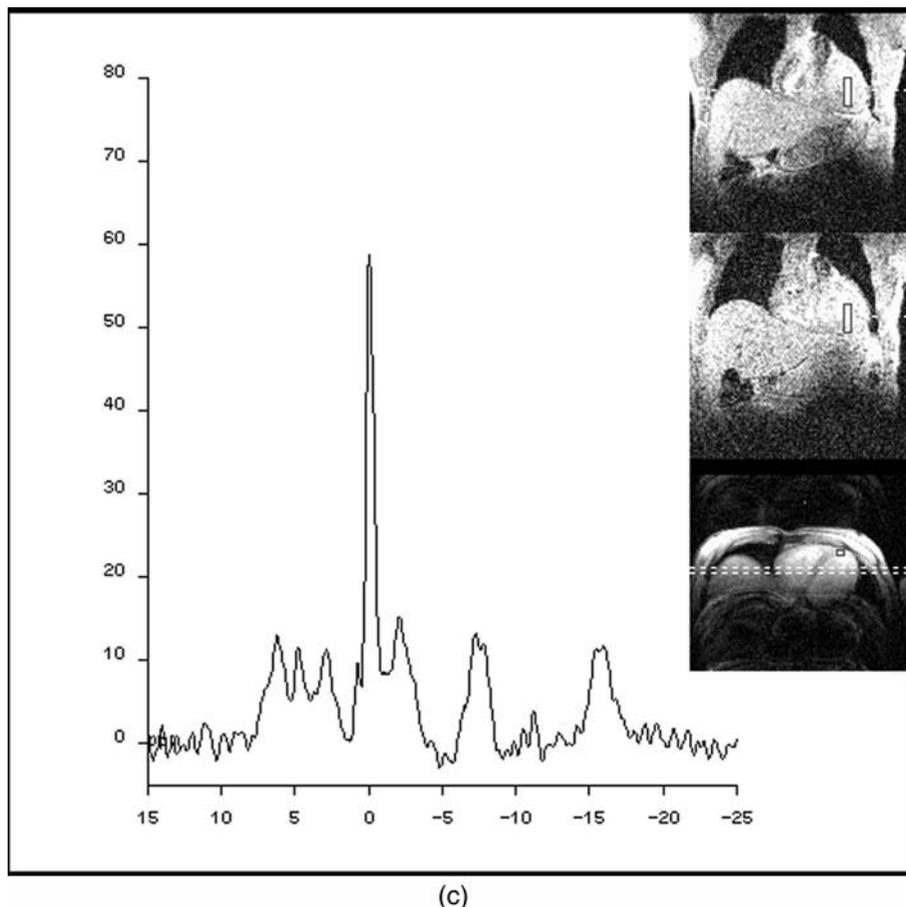


Figure 2. Continued.

enhanced NO biosynthesis, which is accomplished by increasing the stability for the mRNA for eNOS and by enhancing NO release from endothelial cells. These effects preserve the vascular reactivity and result in a better blood supply of the myocardium. Thus, NO acts as a paracrine signal released from the vascular endothelium for the regulation of the adjacent cellular respiration and the improvement of the myocardial, metabolic efficiency (Shen et al., 1995). The inhibition of NOS by nitro-L-arginine (NLA) has been shown to accomplish a shift from a prevalent utilization of free fatty acids to a carbohydrate utilization in the myocardium (Recchia et al., 1998). Furthermore, statin treatment leads also to an increase in inducible nitric oxide synthase (iNOS) expression in cardiac myocytes (Ikeda et al., 2001).

Another potential mechanism for improvement of the endothelial function is given by the antioxidant effects of statins. Statins have been shown to enhance endothelial-dependent relaxation by inhibiting the production of reactive oxygen species (ROS) such as

superoxide and hydroxyl radicals in the aortas of cholesterol-fed rabbits (Rikitake et al., 2001).

Our data are in accordance with the recently published in vitro data from Kawabata et al. (2001) showing a significant inhibition of ATP-depletion in statin-treated, ischemic rabbit hearts compared to nontreated animals. These results suggest that statins improve the myocardial, high-energy, phosphate metabolism, which is provided by K(ATP) channels and NO. Cardiac K(ATP) channels, mediated by NO, appear to be an important cardioprotective mechanism (Kawabata et al., 2001).

Another, interesting protective mechanism is the statin-activated protein kinase Akt. The protein kinase Akt serves as a multifunctional regulator of cell survival, growth, and metabolism (Datta et al., 1999). The mechanism of Akt activation by statins is not well known, but it seems that PI 3-kinase signaling is involved in this process. Kureishi et al. (2000) have provided a very elegant demonstration for statin-induced activation

of Akt signaling, protecting cardiomyocytes from apoptosis after ischemia-reperfusion in vivo. In addition to this cytoprotective role, Akt plays an important role in the activation of eNOS production and thereby controls the vasomotor activity (Kureishi et al., 2000). The statin-activated protein kinase Akt also promotes angiogenesis comparable to the vascular endothelial growth factor (VEGF).

Furthermore, statins also inhibit the isoprenoid synthesis, which is required for the posttranslational modification of the Rho GTPases. The Rho GTPases are supposed to play an important role in the mediation of cardiovascular diseases (Goldstein and Brown, 1990; Laufs and Liao, 2000). Activators of Rho include growth factors, cytokines, integrins, and G protein-coupled, receptor ligands of hormones such as bradykinin or lysophosphatidic acid (Hall, 1998). Therefore, the inhibition of Rho may account for some of the cholesterol-independent pleiotropic effects of statins.

In conclusion, our study shows improved left ventricular PCr-to- $\beta$ -ATP ratios in statin-treated FH patients compared with nontreated FH patients. Our results indicate the beneficial impact of statins on the cardiac, high-energy, phosphate metabolism. Although the use of cardiac  $^{31}\text{P}$  MRS permits only limited mechanistic insights in the human setting, we believe that our data may help to further elucidate the link between the recently published signaling and molecular effects of statins and their appreciated clinical benefits. Several important pieces of the puzzle, which will bridge the enhanced myocardial, high-energy, phosphate metabolism with the clinical benefits of statins, are still missing. However, increasing evidence emerges that the statin-induced, increased nitric oxide biosynthesis seems to play a pivotal role in that context. This notion is strengthened by several, elegantly conducted, experimental data as discussed above. Our findings may challenge investigators in this research field to clarify the molecular basis of this observation and thus provide new insights into the cardioprotective role of statins.

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