among the groups. The infarct tissues consist of thin layer of fibrotic tissue with few myocytes and occupy only little area in the total myocardium, explaining unexpectedly small values of infarct areas in comparison with the values of infarct lengths.

Left ventricle/body weight ratio (LVW/BW, Fig. 3) and MCSA (Fig. 4) were not different between sham-operated *Agtr2*+ mice and *Agtr2*− mice, indicating that the lack of AT2R did not affect normal myocardial growth. In vehicle-treated *Agtr2*+ and *Agtr2*− mice, LVW/BW ($n$=11 and 9, respectively) and MCSA ($n$=6 and 8, respectively) were significantly increased 14 days after surgery to similar extents, suggesting that AT2R does not play a major role in the development of myocardial hypertrophy without pharmacological intervention.

Treatment with valsartan significantly decreased both LVW/BW ($n$=10) and MCSA ($n$=7) in *Agtr2*+ mice. In *Agtr2*− mice, valsartan reduced LVW/BW ($n$=8) and MCSA ($n$=8) to a lesser extents than those in *Agtr2*+ mice, where only the reduction in MCSA was statistically significant. These results indicate that the anti-hypertrophic effect of valsartan is mediated by stimulation of AT2R and partly by blockade of AT1R.

As shown in Fig. 5, lung/body weight ratio (Lung W/BW), an index for pulmonary congestion, was significantly larger in vehicle-treated *Agtr2*− mice ($n$=9) than in vehicle-treated *Agtr2*+ mice ($n$=11). In these vehicle-treated groups, parameters of LV morphology and function were not different (Figs. 1, 3 and 4)), suggesting that factors other than LV function are responsible for the deterioration of pulmonary congestion in *Agtr2*− mice. In other words, the lack of AT2R in the kidney and vasculature may be involved in the mechanism of the pulmonary congestion of *Agtr2*− mice. Treatment with valsartan significantly reduced LungW/BW similarly in the two strains of mice 14 days after surgery, suggesting that this effect of valsartan was mediated by

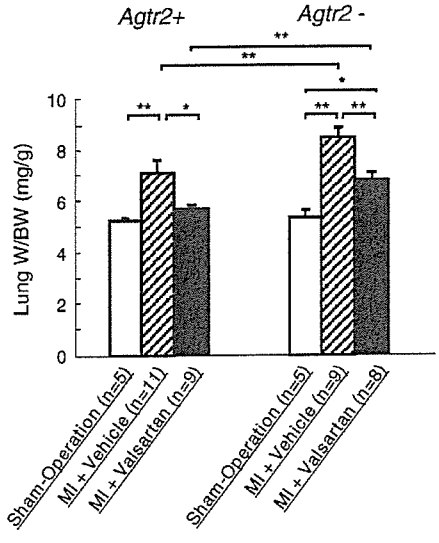


Fig. 5. Lung weight/body weight ratio (Lung W/BW) on day 14 was significantly increased both in *Agtr2*+ and *Agtr2*− mice treated with a vehicle. Valsartan significantly decreased Lung W/BW in both strains of mice. However, Lung W/BW was not restored to the level of sham-operated group in *Agtr2*− mice. Each data point is the mean of pooled data from 5–11 mice. MI; myocardial infarction, $^{**}P<0.001$, $^{*}p<0.05$.

AT1Rs in the cardiac- and extra-cardiac tissues. Taken together, the results suggest that deletion of AT2R leads to deterioration of post-infarction congestion and valsartan attenuates the LV remodeling and heart failure through stimulation of AT2R and blockade of AT1R.

MI increased IFI in both *Agtr2*− and *Agtr2*+ mice, but valsartan treatment did not affect IFI during the study period (data not shown).

## Discussion

LV remodeling after MI is a complex process consisting of acute phase infarct expansion and subsequent myocyte hypertrophy and interstitial fibrosis in the residual myocardium, all of which lead to LV dysfunction. In the present study, effects of valsartan on LV remodeling during the first 2 weeks after MI were examined in *Agtr2*+ and *Agtr2*− mice. Valsartan effectively inhibited cardiomyocyte hypertrophy, LV dilatation, and pulmonary congestion, thereby improving survival rate. On the other hand, in *Agtr2*− mice, valsartan had no effect on LV dilatation and had only a limited effect on cardiomyocyte hypertrophy, suggesting that effects of valsartan on post-infarction remodeling were largely mediated by AT2R.

Consistent with our observations, Xu et al. (2002) previously reported that AT2R activation during AT1R blockade plays an important role in the therapeutic effect of ARB in post-MI LV remodeling. Those authors treated AT2R-deficient mice with valsartan from the 4th week after MI for a period of 20 weeks and then demonstrated its benefits on interstitial fibrosis, cardiomyocyte hypertrophy and LV dysfunction, which are mainly mediated by AT2R. Similarly, Liu et al. (2004) demonstrated the role of kinins as a partial mediator of AT2R signaling in a rat post-MI heart failure model using a similar protocol focusing on the late phase of remodeling. Voros et al. (2006) provided support for these observations by results obtained by using a different experimental model, transgenic mice overexpressing AT2R in the heart. In those mice, AT2R overexpression and treatment with an AT1R antagonist had equivalent beneficial effects on the LV remodeling. All of those studies have established the benefit of an AT1R antagonist and the importance of AT2R as a mediator of its effects on late phase post-MI remodeling. On the other hand, relatively little is known about the roles of Ang II and the benefit of AT1R antagonist during the early period after MI, when the risks of LV dysfunction, arrhythmia, and cardiac rupture are high (Pfeffer and Braunwald, 1990). In such a time period, administration of an AT1R antagonist could be harmful because of hypotension (Pourdjabbar et al., 2005) or other causes. In the present study, we demonstrated that valsartan is effective for inhibiting acute phase LV dilatation and dysfunction and that most of its effects are dependent on AT2R. Consistent with our observation, the VALIANT trial (Pfeffer et al., 2003) demonstrated safety of starting valsartan as early as day 1 post-MI. Taken together, valsartan is effective to inhibit both of the early and late phases of post-MI LV remodeling and its effects are mediated by AT2R.

In our previous study (Oishi et al., 2003), we demonstrated that the survival rate of *Agtr2*+ mice during a period of 14 days

after MI was significantly higher than that of *Agtr2*− mice. In the present study, the survival rate of vehicle-treated *Agtr2*+ mice during a period of 14 days was also higher than that of vehicle-treated *Agtr2*− mice (41% vs. 33%). Valsartan improved the survival rates in both strains of mice to the same extents (41% to 61% in *Agtr2*+ mice and 33% to 52% in *Agtr2*− mice). However, neither the difference between the mortality rates in the two strains nor the improvement in survival rate by valsartan did reached statistical significance. In the present study, we induced larger MI than that in our previous study (Oishi et al., 2003) in order to accomplish earlier progression of LV dilatation and heart failure after MI. The infarct length in the present study was 65%, which is larger than the values (30–55%) reported in previous studies (Fuchs et al., 2003; Matsushima et al., 2006). The mortality rate of wild-type mice in this study was approximately 2-fold higher than that previously reported in this model (Harada et al., 1999; Liu et al., 1997; Xu et al., 2002). Such a severe MI may have overwhelmed the beneficial effects of AT2R and/or valsartan treatment, explaining, at least in part, the failure to detect statistical difference in the survival rates between the two mouse strains and valsartan's effects on it. Similarly, this large MI caused extremely low LV contraction (% FS, Fig. 1c) in both strains of mice, which may have been too severe to be restored by valsartan.

In the present study, the beneficial effect of valsartan on LV remodeling was abolished in *Agtr2*− mice (Figs. 1 and 4), suggesting that the effect of valsartan on LV remodeling was fully mediated by AT2R in the heart. However, valsartan treatment also improved the pulmonary congestion (Fig. 5) and the survival of *Agtr2*− mice, suggesting that (1) the pulmonary congestion and the survival rate were not determined solely by the extent of LV remodeling and that (2) some of the beneficial effects of valsartan in *Agtr2*− mice were mediated in part by AT1R *in the heart as well as* in the extra-cardiac tissues such as the vasculature and the kidney. It is plausible that the dominant receptor subtype that mediates the Ang II action may be AT1R in the vasculature and the kidney, which may explain the similar improvement in heart failure by valsartan in *Agtr2*+ and *Agtr2*− mice.

However, it should be noted that the extent of pulmonary congestion (Fig. 5) was more severe in *Agtr2*− mice than in *Agtr2*+ mice. This difference may be attributed to the absence of AT2R. Since AT2Rs in the kidney and systemic vasculature are involved in the mechanisms of natriuresis (Carey et al., 2000) and vasorelaxation (Akishita et al., 1999; Carey et al., 2000), respectively, a deletion of AT2R might cause volume overload and increase in systemic vascular resistance, leading to the deterioration of systemic hemodynamics after MI.

The effects of ARBs may change depending on the tissue content of Ang II and the expression levels of AT1R and AT2R. In the failing heart, tissue level of Ang II is increased and the increase in Ang II level is further facilitated by ARB administration (Spinale et al., 1997). MI results in upregulation of AT1R (Matsubara, 1998) and AT2R (Adachi et al., 2003; Matsubara, 1998). It has been also reported that ARBs upregulated AT2R (Jugdutt and Menon, 2004). These findings may explain the effectiveness of ARB in post-MI remodeling, particularly through its action on AT2R.

An unexpected result was that IFI was not improved by valsartan. There is a consensus that administration of ARBs inhibits interstitial fibrosis after MI both in rodents (Liu et al., 2004, 1997; Voros et al., 2006; Xu et al., 2002) and humans (Di Pasquale et al., 1999). However, previous studies (Harada et al., 1999; Liu et al., 1997; Voros et al., 2006) suggest that it takes approximately four weeks until significant fibrosis develops after MI in rodents. In the present study, focusing on the early phase of post-MI LV remodeling, we observed LV dilatation during a period of two weeks after MI, a period that was not sufficient to study post-MI interstitial fibrosis. Furthermore, in the present study, the infarct size was large, resulting in a delay in the healing process involving fibrosis formation.

A growing body of evidence (Dickstein and Kjekshus, 2002; Pfeffer et al., 2003) suggests that ARBs are important agents for treating heart failure. However, the advantage of this class of drugs over angiotensin converting enzyme inhibitors is not well understood. Likewise, possible differences in the pharmacological actions and clinical benefit among ARBs are not well elucidated. In the present study, we demonstrated an important action of valsartan to stimulate AT2R, which is associated with additional cardioprotection to a simple blockade of AT1R, particularly during early days after MI. It is of note that the cardioprotective role of AT2R was undetectable until valsartan was administered, suggesting that AT2R need to be "stimulated" by valartan to function as a cardioprotective mechanism. Precise studies on the effect of valsartan and other ARBs may be helpful for improving treatment of cardiovascular diseases.

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# Statins Protect Human Aortic Smooth Muscle Cells From Inorganic Phosphate-Induced Calcification by Restoring Gas6-Axl Survival Pathway

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*Abstract*—Vascular calcification is clinically important in the development of cardiovascular disease. It is reported that hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors (statins) inhibited vascular calcification in several clinical trials. However, the mechanism is poorly understood. Recently, it has been suggested that apoptosis is one of the important processes regulating vascular smooth muscle cell (VSMC) calcification. In this study, we investigated the effect of statins on VSMC calcification by testing their effect on apoptosis, focusing in particular on regulation of the survival pathway mediated by growth arrest-specific gene 6 (Gas6), a member of the vitamin K–dependent protein family, and its receptor, Axl. In human aortic smooth muscle cells (HASMC), statins significantly inhibited inorganic phosphate (Pi)-induced calcification in a concentration-dependent manner (reduced by 49% at 0.1 μmol/L atorvastatin). The inhibitory effect of statins was mediated by preventing apoptosis, which was increased by Pi in a concentration-dependent manner, and not by inhibiting sodium-dependent phosphate cotransporter (NPC) activity, another mechanism regulating HASMC calcification. Furthermore, the antiapoptotic effect of statins was dependent on restoration of Gas6, whose expression was downregulated by Pi. Restoration of Gas6 mRNA by statins was mediated by mRNA stabilization, and not by an increase in transcriptional activity. Suppression of Gas6 using small interfering RNA and the Axl-extracellular domain abolished the preventive effect of statins on Pi-induced apoptosis and calcification. These data demonstrate that statins protected HASMC from Pi-induced calcification by inhibiting apoptosis via restoration of the Gas6-Axl pathway. (*Circ Res.* 2006;98:1024-1031.)

**Key Words:** calcification ■ statins ■ apoptosis ■ Gas6 ■ Axl

Vascular calcification, such as coronary and aortic calcification, is a significant feature of vascular pathology, because this lesion is associated with cardiovascular disease.[1,2] It has been recognized that statins exhibit various protective effects against atherosclerosis, including modification of endothelial function,[3] decreased inflammation,[4] and inhibition of vascular smooth muscle cell (VSMC) proliferation and migration,[5] all of which cannot be accounted for by lipid reduction. One of the interesting pleiotropic effects of statins is the inhibition of vascular calcification. Results from clinical trials suggest an association of statin use with slowed progression of calcific aortic stenosis[6–8] and coronary artery calcification.[9] Statins also inhibited calcification of atherosclerotic plaques in experimental hyperlipidemic animals.[10,11] On the other hand, some recent clinical trials were not able to find such an inhibitory effect.[12,13] To clarify these discrepancies, it is important to identify the detailed regulatory mechanism of vascular calcification and the target of effect of statins.

Based on clinical findings,[14] inorganic phosphate (Pi) has been shown to be an important inducer of VSMC calcification, which is morphologically similar to that observed in calcified human heart valves and the aortic media. Transport of Pi into VSMC has been suggested to play an important role in the initiation of extracellular matrix calcification.[15] Recently, it has been shown that similar structures to matrix vesicles, derived from apoptotic VSMC, have been identified in human calcified arteries.[16] These vesicles have the capacity to concentrate and crystallize Ca, initiating calcification. Pi has been shown to induce apoptosis of hypertrophic chondrocytes, which is associated with cell maturation and extracellular matrix mineralization.[17] However, it is not clear whether or not apoptosis plays a regulatory role in the occurrence of VSMC calcification induced by Pi.

Recently, it was shown that growth arrest-specific gene 6 (Gas6), a member of the vitamin K–dependent protein family, and its receptor, Axl, a membrane receptor tyrosine kinase, are decreased on calcification of vascular pericytes.[18]

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Gas6 is a secreted protein that harbors a γ-carboxylglutamic acid–rich domain and 4 epidermal growth factor–like repeats.[19] Gas6-Axl interaction has been shown to be implicated in the regulation of multiple cellular functions, including growth, survival, adhesion, and chemotaxis.[20–23] In particular, they are known to protect a range of cell types from apoptotic death. However, there is no evidence that Gas6-Axl interaction is involved in Pi-induced apoptosis and calcification of VSMC.

In the present study, we found that statins inhibited Pi-induced calcification by preventing apoptosis in human aortic smooth muscle cells (HASMC). The effect of statins was dependent on restoration of the Gas6-Axl pathway. Furthermore, this beneficial effect was mediated by Gas6 mRNA stabilization, and not by increasing the transcription rate. Our results reveal a novel pathway by which statins regulate Pi-induced calcification in HASMC.

## Materials and Methods

### Materials

Pravastatin, atorvastatin, and fluvastatin were supplied by Sankyo Co Ltd, Pfizer Inc (New York), and Tanabe Seiyaku Co Ltd, respectively. Recombinant human Gas6 (rhGas6) and Axl-ECD were prepared as described previously.[22,24] All other reagents were of analytical grade.

### Cell Culture

HASMC were obtained from Clonetics. They were cultured in DMEM supplemented with 20% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in a humidified atmosphere with 5% $CO_2$. HASMC were used up to passage 8 for the experiments.

### Induction and Quantification of Calcification

For Pi-induced calcification, Pi (a mixed solution of $Na_2HPO_4$ and $NaH_2PO_4$ whose pH was adjusted to 7.4) was added to serum-supplemented DMEM to final concentrations of 2.0, 2.6, and 3.2 mmol/L ("calcification medium"). After the indicated incubation period, cells were decalcified with 0.6 mol/L HCl, and Ca content in the supernatant was determined by the *o*-cresolphthalein complexone method (C-Test, WAKO). The remaining cells were solubilized in 0.1 mol/L NaOH/0.1% SDS, and cell protein content was measured by Bio-Rad protein assay. Calcification was visualized by von Kossa's method. Briefly, the cells were fixed with 4% formaldehyde and exposed to 5% aqueous $AgNO_3$.

### Induction of Apoptosis

Two different time courses were tested to investigate Pi-induced apoptosis and examine the effect of statins. (1) Short-term condition: Pi was added at final concentrations of 2.0, 2.6, and 3.2 mmol/L for 24 hours at confluence, after the cells were incubated with serum-free DMEM for 48 hours. To test the effect of statins on apoptosis, they were added 24 hours after incubating the cells with serum-free DMEM (12 hours before adding Pi). (2) Long-term condition: at confluence, the medium was switched to calcification medium and cells were cultured for up to 10 days. The medium was changed every 2 days. To test the effect of statins, each was added simultaneously when the medium was switched to the calcification medium.

### RNA Extraction, Northern Blot, and mRNA Stability Analysis

The 304-bp product of the Gas6 cDNA probe (forward, 5′-GCGTGGCCAAGAGTGTGAAGT-3′; reverse, 5′-CGCCACTCC-TCAACAGAGAT-3′) was amplified by RT-PCR. For Northern blot analysis, harvested RNA (≈5 to 10 μg) was fractionated on 1.4% formaldehyde-agarose gel and transferred to a nylon filter. The filter was hybridized at 68°C for 2 hours with $^{32}P$-labeled Gas6 cDNA and 18S probe in QuickHyb solution (Stratagene) and autoradiographed. To examine Gas6 mRNA stability, serum-starved HASMC were incubated with actinomycin D (Act D, 5 μg/mL) in the presence of 2.6 mmol/L Pi after 12 hours of atorvastatin (0.1 μmol/L) treatment. Total RNA was harvested at 0, 1, 3, and 6 hours for Northern blot analysis. Signal density of the Gas6 mRNA was normalized to that

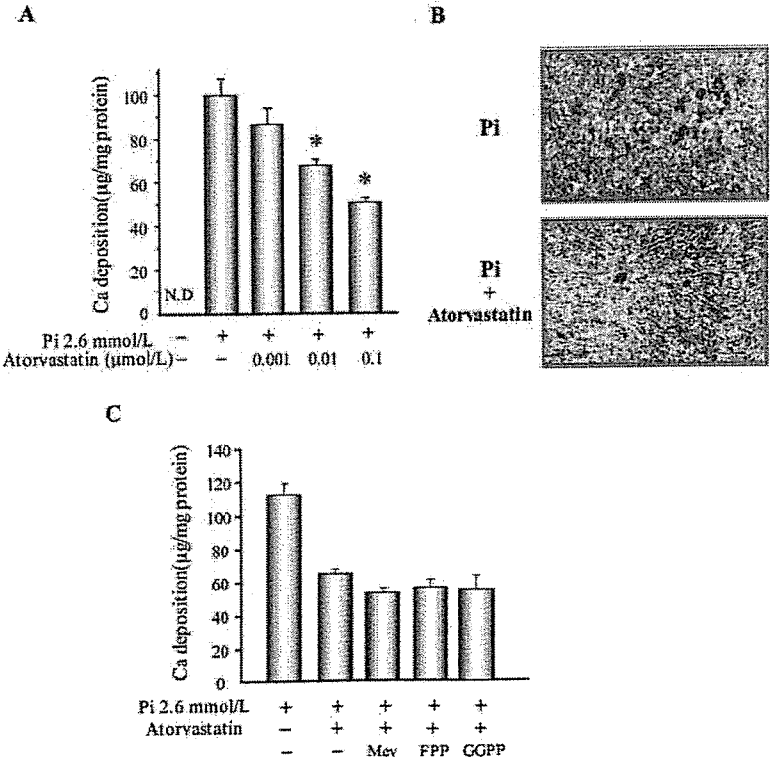


**Figure 1.** Statins prevent HASMC calcification. A, HASMC were cultured with the indicated concentrations of atorvastatin in the presence of 2.6 mmol/L Pi for 6 days. Ca deposition was measured by *o*-cresolphthalein complexone method and normalized by cell protein content. All values are presented as mean±SEM (n=6). *$P<0.05$ vs statin (-) by Fisher's test. N.D. indicates not detected. B, On day 6, the inhibitory effect of atorvastatin (0.1 μmol/L) on 2.6 mmol/L Pi-induced Ca deposition was evaluated at the light microscopic level with von Kossa's staining. The arrow points to an area of Ca deposition. C, HASMC were cultured with mevalonate (100 μmol/L), farnesylpyrophosphate (1 μmol/L), or geranylgeranylpyrophosphate (1 μmol/L) in the presence of atorvastatin (0.1 μmol/L) and 2.6 mmol/L Pi for 6 days. All values are presented as mean±SEM (n=6).

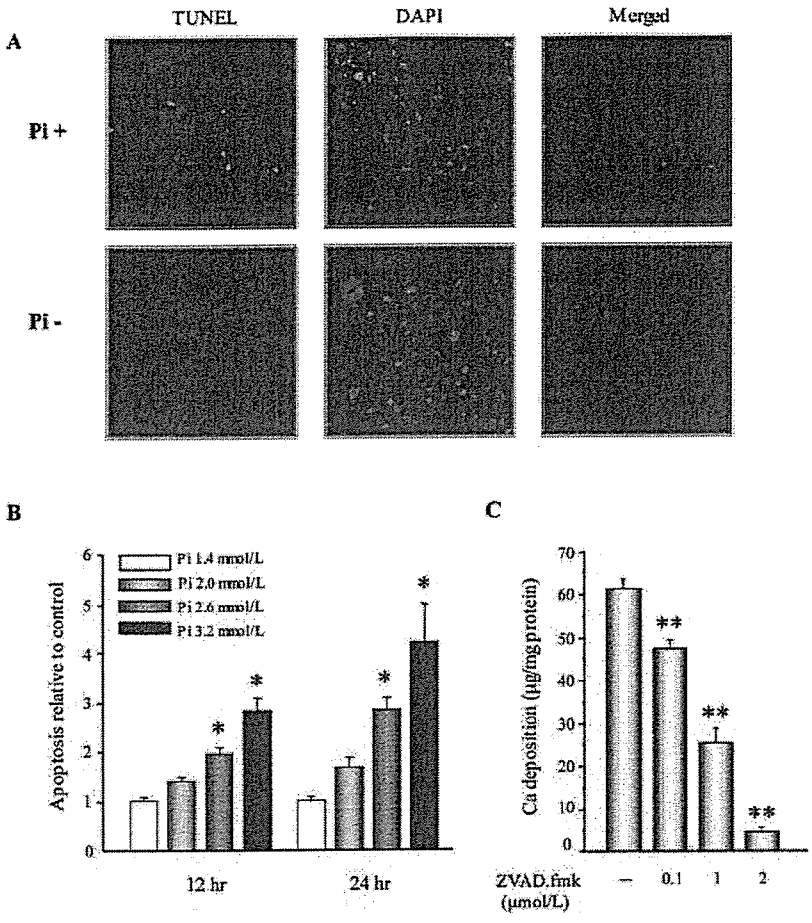


**Figure 2.** Pi induces apoptosis, and ZVAD.fmk inhibits Pi-induced calcification. A, After incubation with 1.4 (Pi-) and 3.2 mmol/L (Pi+) Pi for 10 days, apoptotic cells were identified by TUNEL staining (green). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). B, Serum-starved HASMC were cultured with the indicated concentration of Pi for 24 hours. A quantitative index of apoptosis, determined by ELISA, is presented as the relative value to that with 1.4 mmol/L Pi. All values are presented as mean±SEM (n=3). *$P$<0.05 vs 1.4 mmol/L Pi by Fisher's test. C, HASMC were incubated with the indicated concentration of ZVAD.fmk in the presence of 2.6 mmol/L Pi for 6 days. Ca content was measured and normalized by cell protein content. All values are presented as mean±SEM (n=6). **$P$<0.01 vs 2.6 mmol/L Pi, ZVAD.fmk(-) by Fisher's test. Experiments were performed with at least 3 different cell populations.

of the 18S RNA at each time point, and the half-life was calculated by linear extrapolation.

### Preparation of Small Interfering RNA Targeting Gas6 and Transfection

Two small interfering RNAs (siRNAs) were designed to target human Gas6 (accession no. NM_000820) using siRNA design software (Dharmacon). The sequences for Gas6 were 5'-GGACCTGCCAAGACATAGA-3' and 5'-ACCTCGTGCAGCCT-ATAAA-3'. Nonspecific control siRNA was synthesized using standard templates (Dharmacon). Twenty-four hours after HASMC seeding onto 12-well plates, cells were cultured in serum-free medium for an additional 24 hours, then transfected with Gas6 (100 nmol/L) and control siRNA using transfection reagent (Upstate). To evaluate the effect of Gas6 siRNA on Ca deposition, siRNA was transfected when HASMC had reached 80% to 90% confluence and then transfected every time the medium was changed (every 2 days) up to 6 days. The loss of Gas6 by transfection of siRNA was validated by immunoblotting for Gas6 protein in the cell lysates 48 hours and 6 days after siRNA transfection.

### Statistical Analysis

All results are presented as mean±SEM. Statistical comparisons were made by ANOVA, unless otherwise stated. A value of $P$<0.05 was considered to be significant.

An expanded Materials and Methods section can be found in the online data supplement available at http://circres.ahajournals.org.

## Results

### Statins Inhibit Pi-Induced HASMC Calcification

To induce HASMC calcification, cells were incubated with calcification medium for 10 days. We confirmed that high phosphate (≥2.6 mmol/L) induced Ca deposition in a concentration- and time-dependent manner, whereas 1.4 mmol/L Pi, equivalent to the human physiological serum phosphate level, was not able to induce Ca deposition up to 10 days. To investigate the effect of statins on Pi-induced calcification, HASMC were incubated with atorvastatin in the presence of 2.6 mmol/L Pi. On day 6, Ca deposition was significantly suppressed by atorvastatin in a concentration-dependent manner (51.1±1.9% of control at 0.1 μmol/L) (Figure 1A). An inhibitory effect of the statins on Ca deposition was also found by von Kossa's staining (Figure 1B). Atorvastatin was able to be added at as high a concentration as 0.1 μmol/L without cell damage. The inhibitory effect was also observed with fluvastatin (0.001 to 0.1 μmol/L) and pravastatin (0.01 to 50 μmol/L) (data not shown). The inhibitory effect of statins was not blocked by mevalonate (100 μmol/L), farnesylpyrophosphate (1 μmol/L), or geranylgeranylpyrophosphate (1 μmol/L), suggesting that the effect is not dependent on the mevalonate pathway (Figure 1C).

### Inhibitory Effect of Statins on Calcification Is Caused by Preventing Apoptosis, Not by Inhibiting Sodium-Dependent Phosphate Cotransporter Activity

Two different time courses were tested to examine the effect of Pi on HASMC apoptosis: short-term (up to 24 hours) and long-term (up to 10 days; practical time course of calcifica-

tion process). During calcification, Pi increased the rate of apoptotic cell death detected by terminal deoxyribonucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling (TUNEL) assay (Figure 2A). Furthermore, cytoplasmic histone-associated DNA fragments determined by ELISA, as a quantitative index of apoptosis, were also increased by Pi in a concentration- and time-dependent manner in both short-term (Figure 2B) and long-term conditions (supplemental Figure I). In addition, caspase 3 activation, detected by immunoblotting, by 2.6 mmol/L Pi was observed in short-term and long-term conditions (data not shown). To investigate the relationship between apoptosis and calcification, we used ZVAD.fmk, a general caspase inhibitor. We found that ZVAD.fmk significantly inhibited Pi-induced apoptosis as well as calcification in a concentration-dependent manner (Figure 2C).

It has been reported that sodium-dependent phosphate cotransporter (NPC) activity is an important pathway regulating Pi-induced HASMC calcification.[25] We confirmed that type III NPC (Pit-1) was expressed in the HASMC that we used, and its activity was enhanced by Pi treatment. Furthermore, a specific inhibitor of NPC, phosphonoformic acid (PFA), inhibited Ca deposition (reduced by 90.4% at 0.1 μmol/L), indicating that NPC-mediated Pi uptake is also essential for HASMC calcification.

To investigate the mechanisms of these statins, we examined the effect of atorvastatin on apoptosis and NPC activity. Atorvastatin, at concentrations exerting inhibition of calcification, reduced apoptosis in a concentration-dependent manner (Figure 3A). A beneficial effect of statins was also observed in the long-term condition (supplemental Figure II). On the other hand, statins did not inhibit NPC activity induced by Pi treatment (Figure 3B).

## Downregulation of Gas6-Axl Interaction Is Associated With Pi-Induced Apoptosis

Immunoblot analysis showed that the expression of Gas6 and Axl was markedly downregulated by 2.6 mmol/L Pi in both short-term (Figure 4A) and long-term (supplemental Figure III) conditions. To further examine whether Pi affects the secretion of Gas6 by HASMC, conditioned medium was collected after Pi treatment. Gas6 production in the medium was reduced by 2.6 mmol/L Pi, along with a reduction in its intracellular expression (Figure 4B). Gas6 production was also reduced in an immunoprecipitation-immunoblotting study on day 10 (Figure 4C). Next, to investigate the role of Gas6-Axl interaction in the process of apoptosis and calcification, rhGas6 and Axl-ECD were supplemented in Pi-treated HASMC. The addition of rhGas6 significantly inhibited both Pi-induced apoptosis and calcification. Addition of Axl-ECD to block the binding of Gas6 to Axl clearly abrogated the inhibitory effect of rhGas6 (Figure 4D and 4E). These results indicate that Pi-induced apoptosis and calcification are associated with downregulation of the Gas6-Axl interaction.

## Statin-Mediated Induction of Gas6 Expression Is Dependent on mRNA Stabilization, Not on Transcription

To investigate whether the antiapoptotic effect of statins is dependent on restoration of the Gas6-Axl interaction, we first assessed the effect of statins on Gas6 expression. As shown in Figure 5A, atorvastatin increased Gas6 expression, which was downregulated by Pi at both the mRNA and protein levels. Upregulation of Gas6 expression was also observed in the long-term condition (supplemental Figure IV). Furthermore, to elucidate the mechanism of statins on restoration of Gas6 mRNA, a promoter study was undertaken. Reporter assay using the −1.9 kb Gas6-luciferase DNA construct revealed that atorvastatin did not have a significant effect on Gas6 promoter activity (supplemental Figure V), as well as mRNA expression under the condition in which it was significantly inhibited by PDGF-BB (data not shown). Next, we investigated the effect of atorvastatin on mRNA stabilization using an RNA polymerase inhibitor, actinomycin D (ActD). As shown in Figure 5B, Gas6 mRNA expression was more stable in the presence of atorvastatin than in its absence under Pi and ActD treatment. The half-life was 15.9 hours with atorvastatin and 5 hours without atorvastatin, suggesting the capacity of statins to improve Gas6 mRNA stabilization (Figure 5C). Taken together, these findings suggest that the restoration of Gas6 mRNA by statins appears to be mediated by decreasing the mRNA degradation rate, and not by stimulating transcriptional activity.

Furthermore, to determine whether Gas6 is required for statin-mediated effects, we tried to knock down the action of

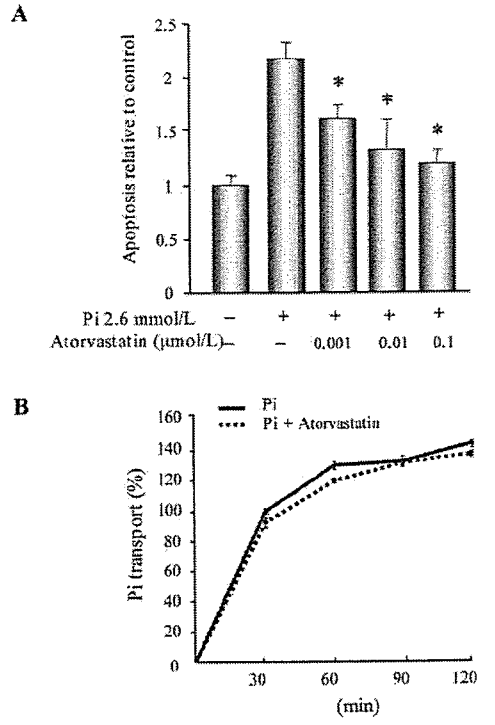


**Figure 3.** Effect of atorvastatin on Pi-induced apoptosis and NPC activity. A, HASMC were cultured with the indicated concentration of atorvastatin for 12 hours and then incubated with 2.6 mmol/L Pi for an additional 24 hours. All values are presented as mean±SEM (n=3). *$P<0.05$ vs 2.6 mmol/L Pi, statin (-) by Fisher's test. B, HASMC were treated with (dotted line) or without (solid line) 0.1 μmol/L atorvastatin in the presence of 2.6 mmol/L Pi. On day 6, NPC activity was determined in Earl's balanced salt solution containing 0.1 mmol/L $H_3{}^{32}PO_4$ (1 μCi/mL) with 143 mmol/L sodium chloride for the indicated period. All values are presented as mean±SEM (n=6).

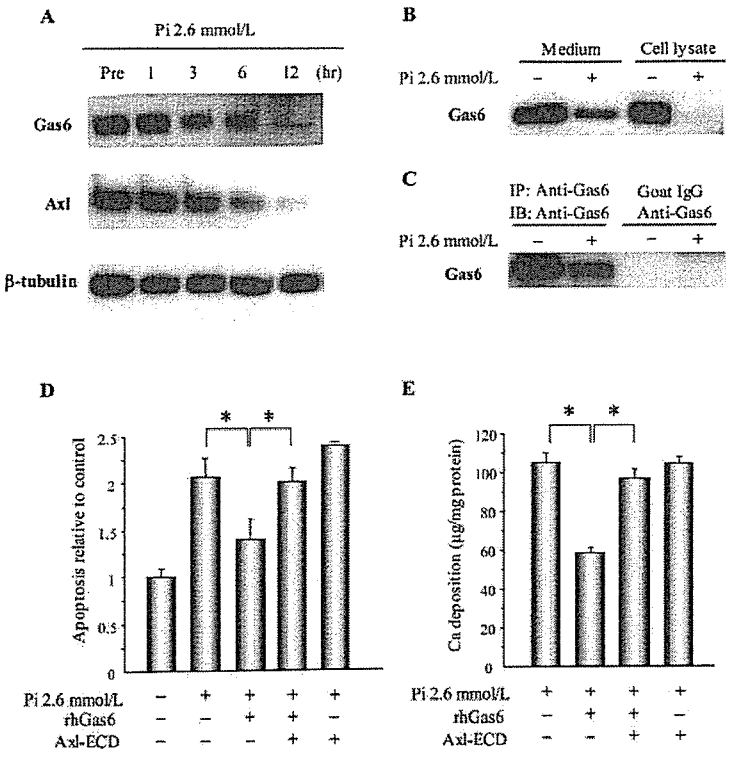


**Figure 4.** Pi reduces production of Gas6 and Axl, and rhGas6 inhibits Pi-induced apoptosis and calcification via Axl. A, HASMC were cultured in the presence of 2.6 mmol/L Pi for 12 hours. Cell lysates were subjected to SDS-PAGE followed by immunoblotting with antibodies to Gas6, Axl, or β-tubulin. B, Conditioned medium of HASMC in the absence (lane 1) or presence (lane 2) of 2.6 mmol/L Pi at 12 hours was concentrated and separated by SDS-PAGE along with cell lysates. C, Conditioned medium of HASMC on day 10 in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 2.6 mmol/L Pi was subjected to immunoprecipitation with anti-Gas6 antibody (lanes 1 and 2) or control goat IgG (lanes 3 and 4). Precipitates were immunoblotted with anti-Gas6 antibody. D, After pretreatment with rhGas6 (400 ng/mL) with or without Axl-ECD (1 μg/mL), apoptosis was induced by 2.6 mmol/L Pi. All values are presented as mean±SEM (n=3). *$P<0.05$ by Fisher's test. E, For measurement of Ca deposition, HASMC were cultured with rhGas6 (400 ng/mL) with or without Axl-ECD (1 μg/mL) in the presence of 2.6 mmol/L Pi for 6 days. All values are presented as mean±SEM (n=6). *$P<0.05$ by Fisher's test. Experiments were performed with at least 3 different cell populations.

Gas6 and examined the effect of atorvastatin on Pi-induced apoptosis and calcification. Transfection of Gas6 siRNA markedly decreased Gas6 expression in the short-term and long-term conditions (Figure 6A). The inhibitory effect of atorvastatin on Pi-induced apoptosis and calcification was reversed by Gas6 siRNA (Figure 6B and 6C). Similarly, the beneficial effect of atorvastatin was also abolished by blocking the binding of Gas6 to Axl using Axl-ECD (Figure 6D and 6E). These data support a critical role of Gas6 in the preventive effect of statins on apoptosis and calcification.

## Discussion

The present study demonstrated that statins protected HASMC from Pi-induced calcification. The clinical effect of statins on vascular calcification is controversial. Many retrospective clinical studies[6,7,9] and a prospective study[8] have shown beneficial effects, whereas recent prospective studies were unable to show such effects.[12,13] The reason is not yet clear, and the time window of statin use has been raised as an important matter. The discrepancy may also derive from the complex in vivo effects of statins. In this regard, it is important to analyze the detailed regulatory mechanism of statins in a simple model.

In Pi-induced calcification, HASMC undergo apoptosis. A causal link between apoptosis and calcification was evident from the finding that both apoptosis and calcification were inhibited by the general caspase inhibitor, ZVAD.fmk. As reported previously,[25] we confirmed that NPC-mediated Pi uptake is another essential mechanism for HASMC calcification. Given that apoptosis does not always lead to calcification, Pi-induced HASMC calcification is presumably dependent on both an NPC-mediated phenotypic transition from SMC to an osteoblastic phenotype and apoptotic cell death. With respect to the mechanism of action of statins, they clearly inhibited Pi-induced apoptosis, although they did not have an effect on Pi-induced NPC activity or osteoblastic differentiation; Pi-induced upregulation of matrix Gla protein (MGP) mRNA was not inhibited by atorvastatin (supplemental Figure VI). These results suggest that apoptosis is the target of statins in inhibiting HASMC calcification.

Another important signal in Pi-induced calcification is an increase in intracellular Ca ($[Ca^{2+}]_i$). Statins have been shown to inhibit VSMC proliferation[5] and reduce the acute increase of $[Ca^{2+}]_i$ in a mevalonate and isoprenoid pathway–independent manner.[26] On the other hand, $[Ca^{2+}]_i$ is reported to modulate Pi-induced apoptosis of terminally differentiated chondrocytes.[27] Therefore, modulation of $[Ca^{2+}]_i$ is another possible mechanism of the inhibition of apoptosis by statins. In this study, we investigated the association of proliferation with Pi-induced apoptosis and calcification. We found that Pi did not affect proliferation, measured by the incorporation of 5-bromo-2′-deoxyuridine (BrdU) during calcification (data not shown). We also found that the inhibitory effect of statins on calcification was not affected by an inhibitor of Rho kinase (Y-27632), an important modulator of the mevalonate and isoprenoid pathway affecting proliferation and apoptosis (supplemental Figure VII). These results suggest that proliferation is not associated with Pi-induced calcification. The inhibitory effect of statins on calcification was not blocked by mevalonate, farnesylpyrophosphate, geranylgeranylpyrophosphate, or Rho kinase inhibitor, suggesting that the effect of statins is not dependent on the mevalonate and isoprenoid pathways. Indeed, a mevalonate pathway–independent effect of statins has been reported previously,[26,28–30] although the precise mechanism has not been shown. The pleiotropism of statins is of continuing interest.

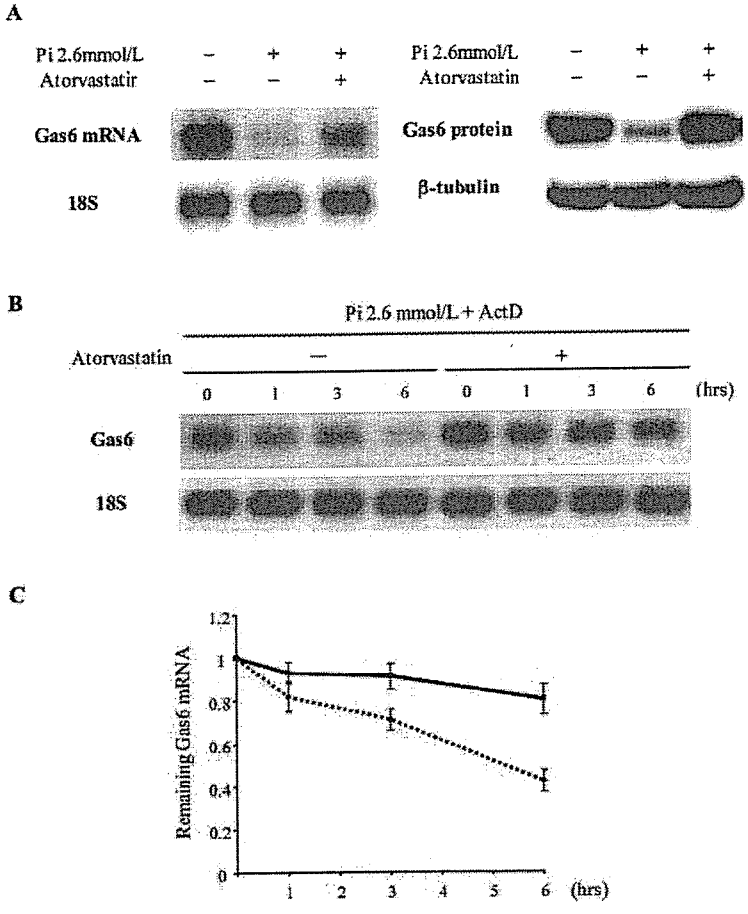


Figure 5. Atorvastatin enhances Gas6 mRNA stabilization, but not transcription. A, After pretreatment with atorvastatin (0.1 μmol/L) for 12 hours, apoptosis was induced by 2.6 mmol/L Pi. At 12 hours, mRNA was isolated and Northern blot analysis for Gas6 and 18S was performed. Simultaneously, cell lysates were collected and subjected to SDS-PAGE followed by immunoblotting with antibodies to Gas6 and β-tubulin. B, Serum-starved HASMC were incubated with actinomycin D (Act D) (5 μg/mL) in the presence of 2.6 mmol/L Pi after 12 hours of atorvastatin (0.1 μmol/L) treatment. Total RNA was harvested at 0, 1, 3, and 6 hours for Northern blot analysis. C, Signal density of Gas6 mRNA with (solid line) or without (dotted line) atorvastatin (0.1 μmol/L) in the presence of 2.6 mmol/L Pi and Act D (5 μg/mL) was normalized to that of 18S RNA at each time point. Gas6 mRNA level at time 0 was given the value 1. Each experiment was performed in triplicate for each condition.

An antiapoptotic effect of statins has been shown in various cell types.[31–34] In cardiomyocytes, apoptosis induced by hypoxia or protein kinase C (PKC) inhibitors was inhibited by 10 μmol/L pravastatin or 0.1 μg/mL atorvastatin, respectively.[31,32] Simvastatin (1 μmol/L) promoted endothelial cell survival.[33] In VSMC, 7-ketocholesterol–induced apoptosis was inhibited by 10 μmol/L pravastatin.[34] However, in contrast to the results of the present and other studies, a proapoptotic effect of statins has also been reported in VSMC,[35] endothelial cells,[36] and cardiac myocytes.[37] Although the precise mechanism is not understood, it can be postulated that statins have biphasic effects on cell survival (an antiapoptotic effect at low concentrations and a proapoptotic effect at high concentrations) depending on the type of cell, statins, and apoptotic stimulus. Indeed, Weis et al showed dose-dependent biphasic effects of statins on apoptotic activity in microvascular endothelial cells.[30] Consistent with these data, we found that 3 different statins displayed an antiapoptotic effect at low concentrations and a proapoptotic effect at high concentrations (>1 μmol/L for atorvastatin and fluvastatin; >100 μmol/L for pravastatin) (data not shown).

During Pi-induced apoptosis, we have shown that Pi downregulates the Gas6-Axl interaction, resulting in blockade of a survival signal, thereby promoting apoptosis and calcification. We previously proposed that Gas6 may allow Axl-expressing phagocytic cells, eg, macrophages and VSMC, to recognize cells exposing phosphatidylserine (PS) on the outer cell membrane, the initial step of the apoptotic process.[38] Proudfoot et al also showed that in vascular calcification, several PS-exposing cells are observed within and on the periphery of the nodules.[16] PS exposure by apoptotic bodies generates a potential Ca-binding site and membrane surface suitable for hydroxyapatite deposition.[39,40] Based on these observations, Gas6-Axl downregulation is presumably involved in decreased cell survival and clearance, both directing cells to apoptosis-mediated mineral deposition.

With regard to the molecular pathway of the restoration of Gas6 by statins, we have shown that statins retarded degradation of Gas6 mRNA, not increasing the transcriptional rate. Indeed, it was reported that statins improve mRNA stability as well as transcription.[41,42] In addition, the result that suppression of the action of Gas6 by siRNA and Axl-ECD abrogated the inhibitory effect of statins on apoptosis and inhibition clearly indicates a pivotal role of Gas6 in the effect of statins.

We conclude that statins inhibit Pi-induced HASMC calcification by preventing apoptosis via restoration of the Gas6-Axl pathway. The regulation of Gas6 by statins occurs at the posttranscriptional level. The present study provides evidence of a preventive role of statins in vascular calcification and further indicates the pleiotropic effects of statins, which could potentially contribute to the treatment of cardiovascular disease.

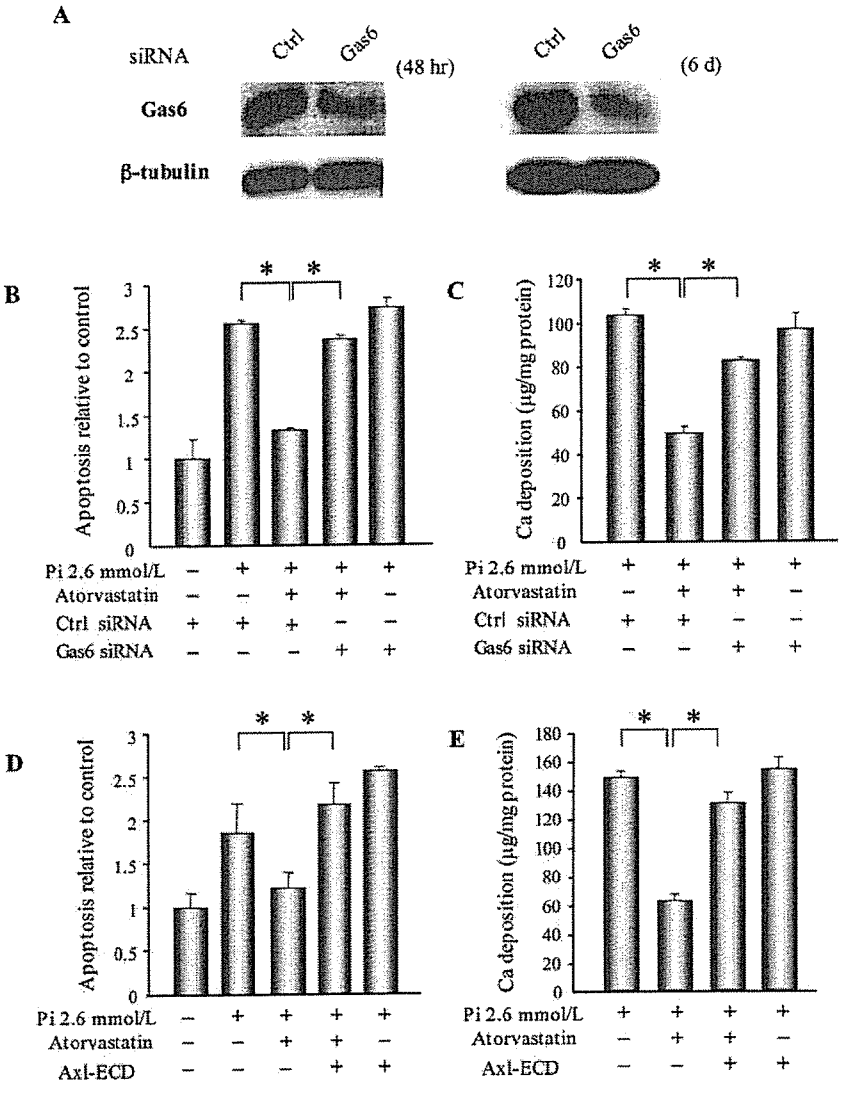


**Figure 6.** Gas6 knockdown abolishes inhibition of Pi-induced apoptosis and calcification by atorvastatin. A, Gas6-specific siRNA (100 nmol/L) and nonspecific siRNA (Ctrl siRNA) were transfected into HASMC, and immunoblotting was performed at 48 hours and 6 days after transfection. B, Serum-starved HASMC were transfected with 100 nmol/L Gas6 siRNA and control (Ctrl) siRNA. After transfection, cells were treated with atorvastatin (0.1 μmol/L) for 12 hours, then with 2.6 mmol/L Pi for an additional 24 hours before measurement of apoptosis (n=3). C, For measurement of Ca deposition, HASMC were transfected with 100 nmol/L Gas6 siRNA and control siRNA and incubated with atorvastatin (0.1 μmol/L) and 2.6 mmol/L Pi for 6 days (n=3). D, In the case of Axl-ECD, HASMC were pretreated with atorvastatin (0.1 μmol/L) and Axl-ECD (1 μg/mL) for 12 hours, then incubated with 2.6 mmol/L Pi for an additional 24 hours. Thereafter, a quantitative index of apoptosis was determined by ELISA (n=3). E, HASMC were cultured with atorvastatin (0.1 μmol/L) and Axl-ECD (1 μg/mL) in the presence of 2.6 mmol/L Pi for 6 days. Ca content was measured and normalized by cell protein content. All values are presented as mean±SEM (n=6). *$P<0.05$ by Fisher's test. Each panel shows a representative example of 3 independent experiments.

## Acknowledgments

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特集 アンドロロジー——課題と展望

# 診療内科医からみたアンドロゲン*

秋下雅弘**

**Key Words**：testosterone, DHEA, atherosclerosis, metabolic syndrome

## はじめに

アンドロゲンは男性のみならず女性にも存在し，ライフサイクルの中で変動を示す．アンドロゲンの血中濃度は成人以降加齢とともに低下するが，それが顕著になる中高年は，生活・社会環境の変化とも相まってそれまで健康であった者にも各種疾患が発症してくる時期でもある．そのような疾患のうちアンドロゲン低下に関連する病態をlate-onset hypogonadism(LOH)/partial androgen deficiency in the aging male(PADAM)と呼び，内科疾患も多く含まれる．アンドロゲンの経年的低下は徐々に起きるため，疾患との関係は年齢という交絡因子を加味して評価することが必要である．しかし，ホルモン濃度の加齢変化を考慮しても，アンドロゲンの低下が骨粗鬆症，動脈硬化やその原因となるメタボリックシンドローム，認知症といった疾患の発症と関係することが最近わかってきた．本稿では，アンドロゲンの低下がどのような内科疾患と関係するかについて男性を中心に解説する．

## アンドロゲンと骨粗鬆症

テストステロンと骨粗鬆症との関係は古くから指摘されているが，内因性ホルモンと骨量との関係については，テストステロンそのものよりも，テストステロンからアロマターゼにより変換されるエストラジオールの方が骨量に関係するという意見が強い．ただし，アンドロゲン受容体欠損マウスでは高代謝回転型の骨量減少をきたすことが報告されており[1]，アンドロゲンにも骨量を維持する作用はあると考えられる．

テストステロン補充療法の効果についても賛否あるが，Snyderらのプラセボ対照二重盲検比較試験[2]では，若年成人平均より1SD以上の低下を示した65歳以上の高齢男性に貼付剤によるテストステロン補充療法を行ったところ，群全体ではプラセボに比べて有意な骨量増加を認めなかったが，治療前のテストステロン濃度が低いほど骨量が増加した(図1)．

## アンドロゲンと動脈硬化

いくつかの症例対照比較試験で，テストステロンや副腎由来アンドロゲンであるdehydroepiandrosterone(DHEA)の低値が高齢男性の脳梗塞や心筋梗塞の発症，冠動脈狭窄度と関係することが報告されている．また，40～70歳の男性1,709名を対象としたMassachusetts Male Aging Studyという疫学研究[3]では，遊離テストステロン低値およびDHEAのsulfate型(DHEA-S)の低値は独立した心疾患の危険因子であった(図2)．

* The significance of androgen in internal medicine.

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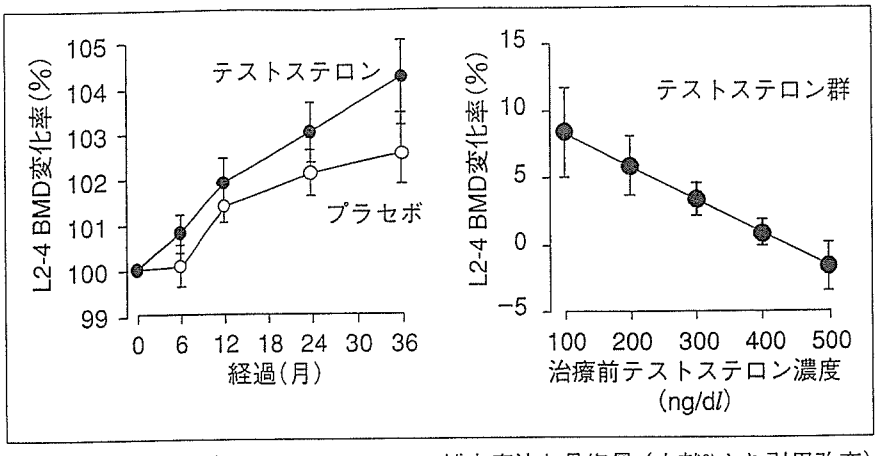


図1　高齢男性に対するテストステロン補充療法と骨塩量（文献[2]より引用改変）

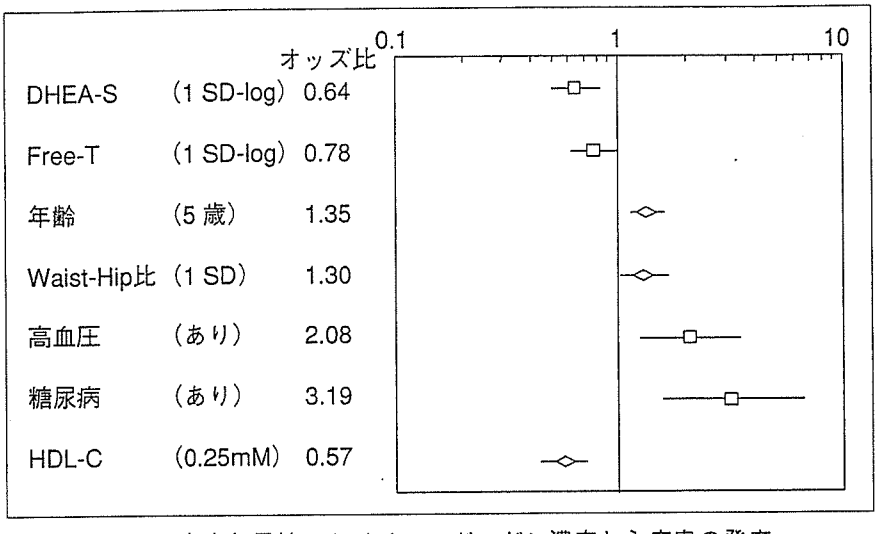


図2　中高年男性におけるアンドロゲン濃度と心疾患の発症
Massachusetts Male Aging Study, 40～70歳の男性1,709名の追跡研究．DHEA-S：Dehydroepiandrosterone-sulfate, Free-T：遊離テストステロン，HDL-C：HDLコレステロール
（文献[3]より引用改変）

血管内皮機能障害は動脈硬化の初期変化として重要な指標であり，主に一酸化窒素の利用障害に基づくと考えられる．著者の検討では，内皮依存性血管拡張反応である上腕動脈の血流依存性血管拡張能は血清テストステロン濃度と正相関した(図3)．両者の関連は年齢および高血圧，高脂血症，糖尿病，喫煙といった危険因子で補正してもかわらず，血流依存性血管拡張能はエストラジオール濃度とは関連しなかったことから，テストステロンが直接血管内皮に作用する可能性を示唆する．一方，テストステロンを急性投与すると，内皮依存性血管拡張反応が改善すること，冠動脈疾患患者の運動負荷に対する心虚血の出現が遅れることが報告されている．

このような研究結果はテストステロンが血管拡張作用を有することを意味し，基礎実験でもテストステロンはアンドロゲン受容体を介して血管内皮と平滑筋の双方に作用することが示されている．一方で，テストステロンはアロマターゼによりエストロゲンに変換されるため，テストステロンの作用の一部は変換されたエストロゲンによるものかもしれない．実際，LDL受容体欠損マウスに高コレステロール食を負荷したモデルでは，テストステロンによる粥状硬化の抑制はアロマターゼ阻害薬で消失した[4]．また，テストステロン，DHEAいずれにしても，アンドロゲン補充療法による長期効果，たとえば動脈硬化の進展や心筋梗塞，脳卒中を含む心血管イベントに対する効果などは不明である．

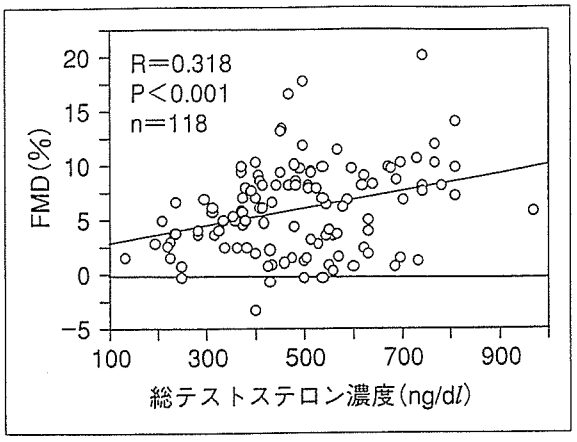


図3　中高年男性患者(年齢51±14歳)におけるテストステロン濃度と血管内皮機能
上腕動脈血流依存性血管拡張反応(FMD)と血清総テストステロン濃度との関連を散布図と回帰直線で示す．年齢および高血圧，高脂血症，糖尿病，喫煙の有無で補正しても関連に影響しなかった．

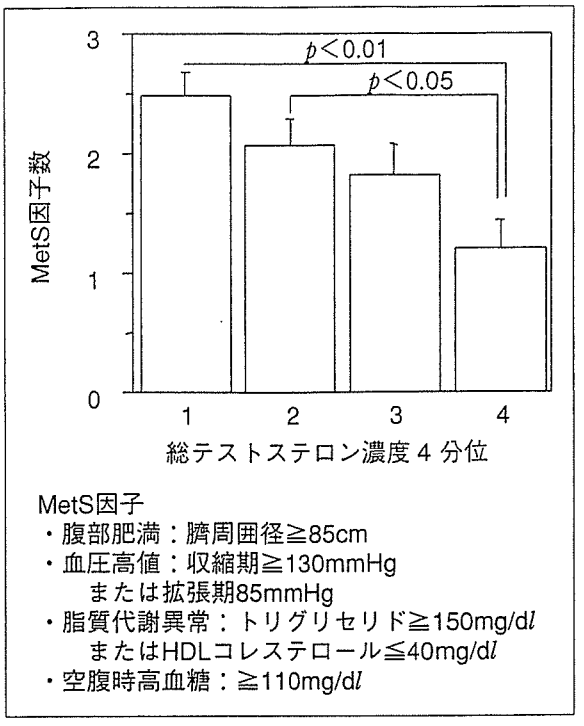


図4　中高年男性における総テストステロン濃度とメタボリックシンドローム(MetS)の関連

## アンドロゲンとメタボリックシンドローム

アンドロゲン低下に伴う代謝性変化として，HDLコレステロール低下，トリグリセリド増加といった脂質変化に加え，インスリン抵抗性，内臓脂肪蓄積が指摘されており，動脈硬化の進行にかかわると考えられる．上述したMassachusetts Male Aging Studyでは，テストステロン低値がその後の2型糖尿病発症につながることを報告している．さらに，男性ではテストステロン低値が高血圧と関連することを示唆する報告もある．

これらの結果を総合すると，内臓肥満を基盤として糖・脂質代謝異常，高血圧が蓄積した病態であるメタボリックシンドローム(MetS)にアンドロゲン低下がかかわっている可能性が考えられる．実際，欧米の複数の研究が，血清総テストステロン値あるいはDHEA-S値の低下がその後のMetS発症に関連したことを報告している．

著者も30～69歳の中高年男性症例118名を対象に検討した．8学会合同委員会による日本の診断基準に従い，MetSを診断し，また腹囲を含む各因子の保有数をカウントした．図4に示すように，総テストステロン濃度で4分位すると，低値ほどMetS因子の保有数が多く，年齢調整しても1階層の低下により，MetS診断に対する相対危険度は1.61(95%信頼区間，1.1～2.3)増加することがわかった．このような関係はDHEA-S濃度についてはみられなかった．次に，MetS各要素に関係する測定値がテストステロン濃度と関連するかどうかを年齢調整して検討したところ(表1)，総テストステロン濃度はHDLコレステロールを除くすべての項目に対して有意な決定変数であった(LDLコレステロールは本来MetSとは独立した動脈硬化危険因子である)．つまり，総テストステロン濃度の低い中高年男性は，腹部肥満があり，血圧が高く，トリグリセリドが高く，空腹時血糖が高いという結果であった．

それでは本当にアンドロゲン低下がMetSの原因となるのか，あるいは肥満やMetSの結果として性腺機能が低下するのかという疑問がある．フィンランドで行われた中年男性(平均51歳)の疫学研究では，11年間の追跡期間中に21%がMetSを，8%が糖尿病を発症したが，開始時のテストステロン濃度が下位1/4の群では，年齢調整後の発症率がMetS，糖尿病ともに2.3倍であった[5]．逆に，開始時にMetSの診断基準を満たした者は11年間にアンドロゲン低下症のリスクが2.6倍であった[6]．

MetS患者あるいは肥満者にアンドロゲン補充

表1　メタボリックシンドローム指標に対する総テストステロン濃度の寄与度

| | 従属変数 | 標準回帰係数 | $p$ 値 |
|---|---|---|---|
| 肥満 | Body mass index | −0.366 | <0.001 |
| | ウエスト周囲径 | −0.378 | <0.001 |
| | ウエスト／ヒップ比 | −0.383 | <0.001 |
| 血圧 | 収縮期血圧 | −0.315 | <0.001 |
| | 拡張期血圧 | −0.226 | 0.012 |
| 脂質 | 遊離脂肪酸 | −0.237 | 0.018 |
| | トリグリセリド | −0.207 | 0.026 |
| | HDLコレステロール | 0.065 | 0.490 |
| | LDLコレステロール | 0.001 | 0.992 |
| 糖代謝 | 空腹時血糖 | −0.231 | 0.011 |
| | ヘモグロビン$A_{1c}$ | −0.211 | 0.020 |
| | HOMA-IR | −0.305 | 0.002 |

総テストステロン濃度と年齢を独立変数とし，表のいずれかを従属変数とした重回帰分析．

療法を行うと改善するかどうかであるが，MetSの各指標をきちんと検討した研究はない．ただ，高齢男性に対するテストステロン補充療法により筋肉量の増加に対応した体脂肪の減少がみられることは，小規模ではあるがプラセボ対照研究[7]でも示されている(図5)．逆に，肥満者が運動療法により痩せるとアンドロゲンが増加することは数多く報告されているし，中年男性を食事療法により9週間で十数キログラム痩せさせたところ，遊離テストステロン濃度が増加したとの報告がある．

## アンドロゲンと認知症

Rancho Bernardo Studyという米国のコホート研究では，健常高齢者を対象に認知機能や気分についての評価を行い(表2)，男性ではbio-available testosterone濃度と認知機能評価尺度mini-mental state examination(MMSE)の点数とが正相関を示したのに対し[8]，DHEA-S濃度は関係なかった．著者の老人保健施設通所・入所男性における検討(54名，平均82歳)[9]では，総テストステロン濃度および遊離テストステロン濃度は，認知機能のみならずADLや意欲とも正相関した(図6)．とくに遊離テストステロンと認知機能，意欲との関係は強く，年齢や各種栄養の指標で補正しても有意であった．一方，DHEA(-S)濃度は認知機能とのみ相関した．さらにこの集団を追跡したところ，遊離テストステロン濃度5 pg/ml未満の男性は，5 pg/ml以上の男性に比べてその後3年間の死亡率が高かった．高齢男性に対するアンドロゲン補充療法により認知機能が改善するとの報告があるが，結果は必ずしも一致しない．

## 女性におけるアンドロゲンの低下と内科疾患

閉経後の女性では，エストロゲンの産生が著しく低下しているせいか，エストロゲン濃度よりもむしろアンドロゲン濃度の方が各種疾患に関係するとされ，男性と同様，低アンドロゲン血症が骨粗鬆症，肥満，高脂血症の発症と関連したことが報告されている．

閉経後女性を16年間追跡した研究[10]では，骨粗

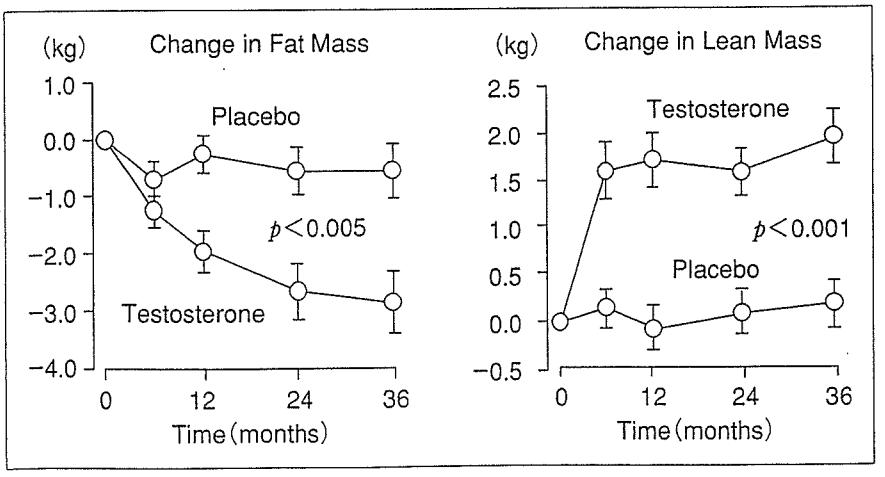


図5　高齢男性に対するテストステロン補充療法と体組成の変化
(文献[7]より引用改変)

表2　地域在住高齢者における性ホルモン濃度と認知機能，気分(米国Rancho Bernardo Studyの結果をまとめた)

| | | 認知機能 | うつスケール |
|---|---|---|---|
| 女　性 | Testosterone | ↑↑ | → |
| | Estradiol | → | → |
| | DHEA | ↑ | ↓ |
| 男　性 | Testosterone | ↑↑ | ↓ |
| | Estradiol | ↓ | → |
| | DHEA | → | 未検 |

↑↑：強い正の相関，↑：正の相関，↓：負の相関，→：相関なし．性ホルモンは，実際にはそれぞれbioavailable testosterone, bioavailable estradiol, DHEA sulfateとして測定したものについて有意な関係を認めた．

鬆症の圧迫骨折に由来すると思われる身長低下に，開始時のテストステロン濃度は関連したがエストラジオールやエストロンは関連しなかった．同様に，DHEA-S濃度が骨量と関連したことが複数報告されている．補充療法の効果については，エストロゲンにテストステロンを併用することで骨量がより増加したこと，DHEA補充療法で骨量が増加したことが報告されているが，エストロゲンへの変換を介した機序がどの程度関係するのかは不明である．

アンドロゲン低値は，男性と同様，閉経後女性における動脈硬化の進行にも関係しているようである．101名の更年期女性(平均47歳)を対象に超音波で頸動脈肥厚を調べたイタリアの研究[11]では，アンドロゲン濃度，とくにDHEA-Sが肥厚度と逆相関した．アンドロゲン補充による動脈硬化への影響は不明である．

多嚢胞性卵巣症候群の女性では高アンドロゲン血症と内臓肥満を呈する．自然閉経後の女性でもアンドロゲン高値が肥満と関連するという報告と，逆にアンドロゲン低値が体脂肪蓄積と関連するという報告がみられる．テストステロン補充により内臓脂肪が減少したという報告もあるが，脂肪分化とそれに伴う代謝異常には多くのホルモンがかかわるため，女性の肥満に対するアンドロゲンの作用はまだ明確でない．

上述したRancho Bernardo Studyでは，bioavailable testosteroneとMMSEの点数とは正相関したほか，DHEA-Sと記憶に関する一部の項目とが正相関した(表2)．

## おわりに

従来，女性におけるエストロゲンの骨粗鬆症や動脈硬化，代謝異常に対する保護作用と対比して，アンドロゲン，とくにテストステロンはこれらの疾患に悪い作用を及ぼすというイメージが強かった．しかし，近年の研究で，少なくとも男性においては，アンドロゲンの低下が動脈硬化をはじめとする内科疾患のリスクとなることが明らかとなってきた．まだ作用機序に不明な部分が多いことや日本では製剤の制限もあり，生活習慣病の治療や予防目的で積極的にアンドロゲン補充療法が行われることはない．ただ，加齢に伴う疾患の発症とアンドロゲンの加齢変化という点では，アンドロゲン補充療法は理にかなった予防・治療のようにも思われる．効果を確かめるための臨床研究と作用機序を明確にするための基礎研究の発展は必須の課題である．

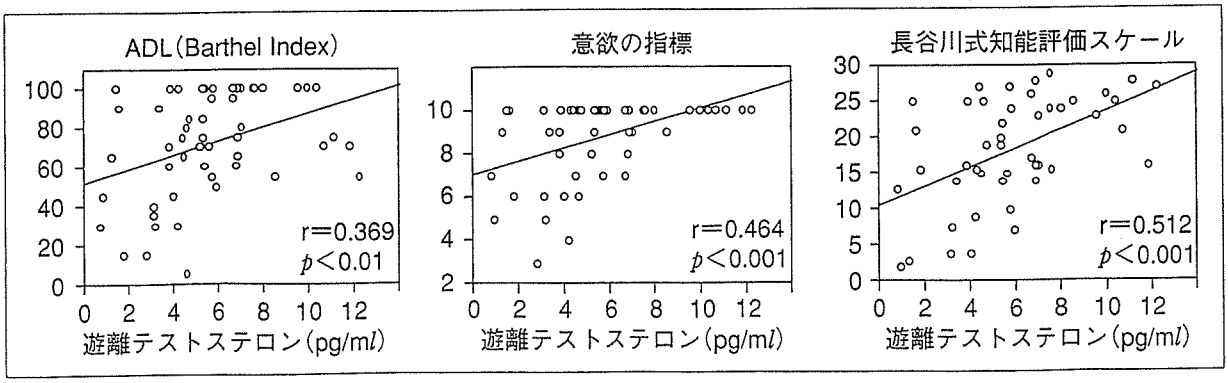


図6　虚弱高齢男性における血中遊離テストステロン濃度と日常生活機能の関係(文献[9]より引用改変)

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基礎病態である動脈硬化の危険因子

# 加齢・性

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[Summary]

虚血性心疾患およびその原因である動脈硬化には，明確な加齢変化と性差がみられる．動脈硬化の加齢による進行は，男性の方がより若年ではじまる．一方，若年女性の動脈硬化は非常に少なく，閉経を境にして進行が早くなり，高齢者では男性のレベルに近づく．このような動脈硬化の加齢変化と性差には，高血圧，高脂血症など他の動脈硬化危険因子の影響に加えて，血管自体の老化や性ホルモンの血管作用が関係していると考えられる．

**Key Words:**

*年齢□高齢者□女性ホルモン□男性ホルモン*

## はじめに

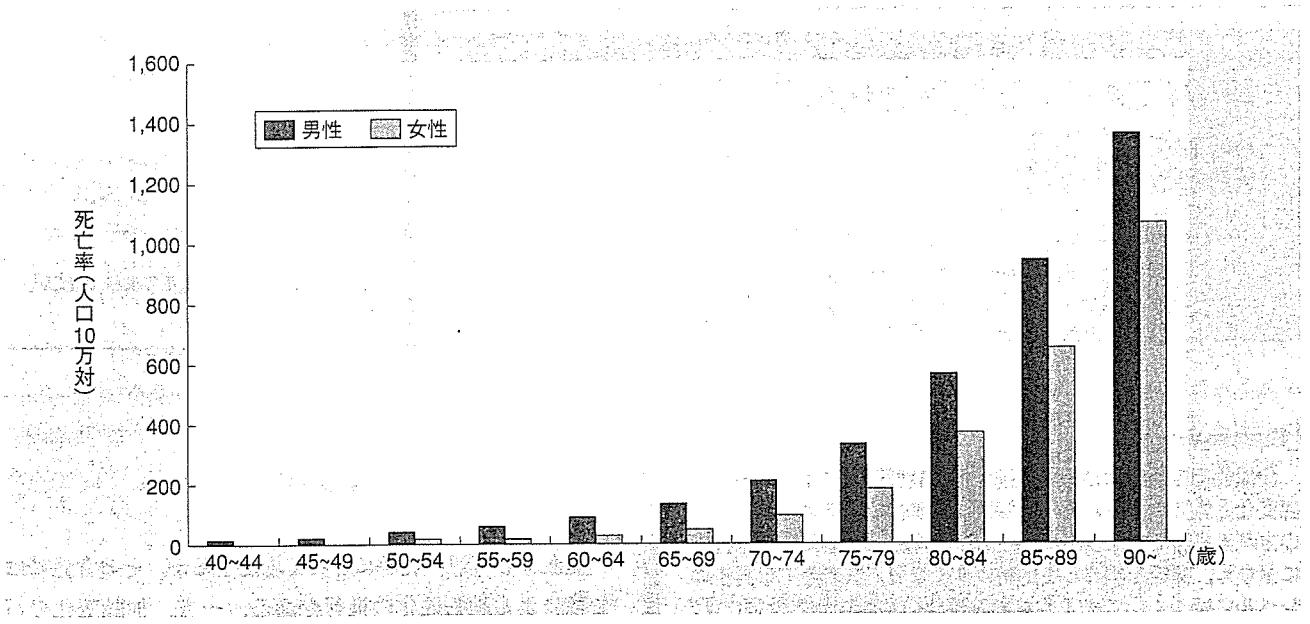
虚血性心疾患は加齢に伴って増加するが，その背景には加齢による動脈硬化の進行がある．一方，加齢変化のパターンは男女で異なり，男性では成人以降動脈硬化がほぼ直線的に進行するのに対し，女性の場合は閉経を境に緩やかな勾配から急勾配へとシフトする．動脈硬化の加齢変化と性差には，高血圧，高脂血症など他の動脈硬化危険因子の影響に加えて，血管自体の老化や性ホルモンが関係していると考えられ，本稿ではこれらの点について概説する．

## 加齢と動脈硬化

### 1．加齢による虚血性心疾患とその危険因子の増加

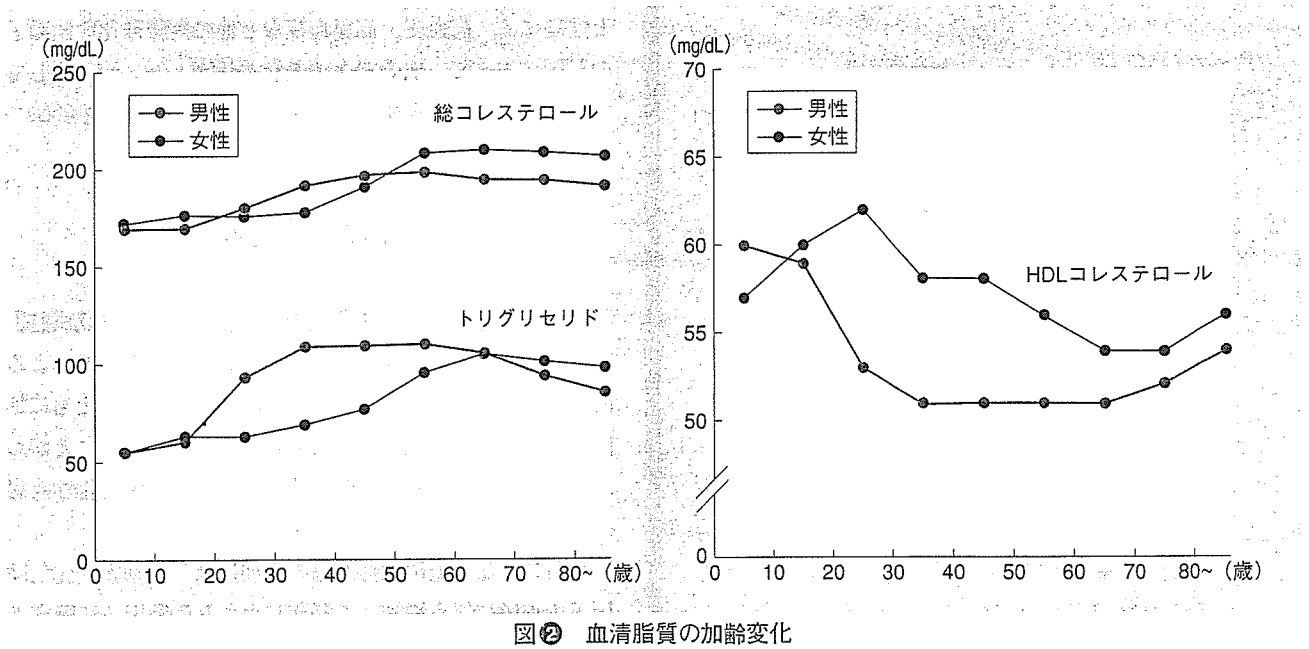
図❶は平成14年のわが国における虚血性心疾患による死亡率を性別・年代別に示したものであるが，男女ともに加齢により虚血性心疾患による死亡が増加していることがわかる．脳梗塞や閉塞性動脈硬化症など他の動脈硬化性疾患についても，同様な加齢変化を認める．

加齢による動脈硬化性疾患の増加には，①動脈硬化危険因子の加齢に伴う増加，②長年にわたり危険因子に曝露された結果，③血管自体の加齢変化などが関係すると考えられる．図❷に日本人における血清脂質の加齢変化を示す




図❶ 虚血性心疾患による性別・年齢別死亡率

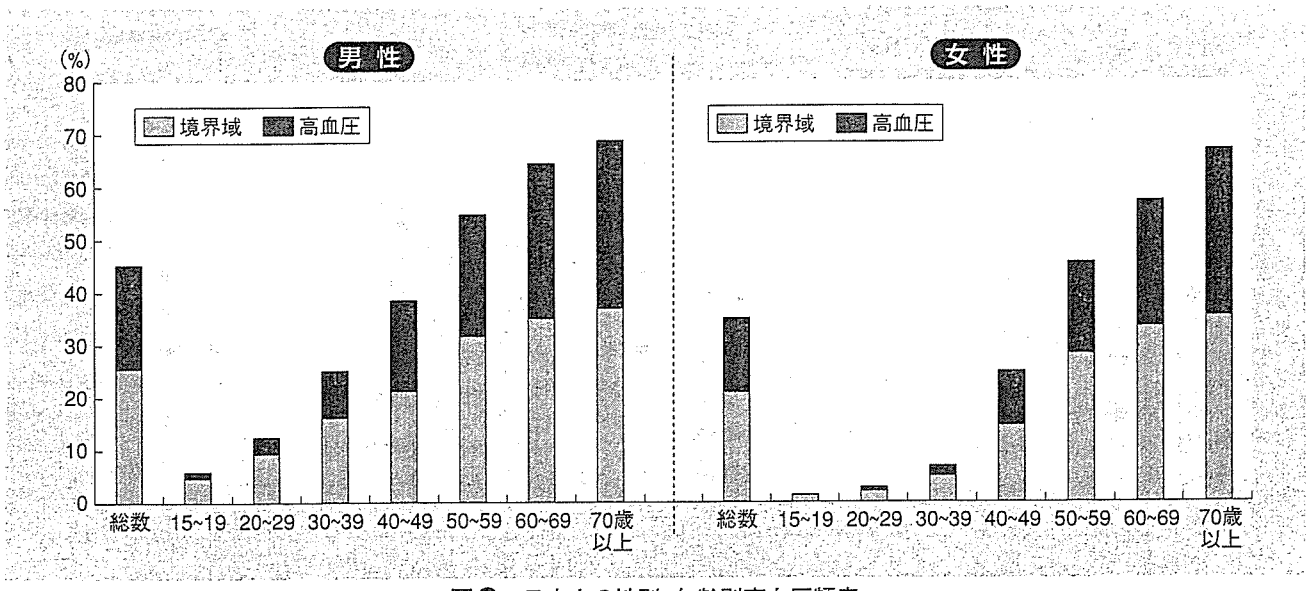
（厚生労働省 平成14年人口動態調査より）




図❷ 血清脂質の加齢変化

加齢とともに血清総コレステロール，トリグリセリドは上昇し，HDLコレステロールは低下する．また，明らかに男女差が存在する

（平成9年国民栄養調査より）




図❸　日本人の性別・年齢別高血圧頻度

（平成9年国民栄養調査より）

が，加齢とともに血清総コレステロール，トリグリセリドは上昇し，HDLコレステロールは低下する．その結果，高脂血症は加齢に伴って増加し，やはり危険因子である高血圧（図❸）や糖尿病も加齢により増加する．

このような危険因子の増加は，加齢そのものの動脈硬化への影響をわかりにくくしている．しかし，日本動脈硬化学会による動脈硬化性疾患診療ガイドライン2002において，男性45歳以上，女性55歳以上が危険因子として取り扱われているように，一般に加齢は動脈硬化の独立した危険因子と考えられている．各種危険因子による虚血性心疾患の発症に対する寄与度を調べたPooling Project Research Groupの成績によれば，高コレステロール血症，喫煙，肥満の相対危険度は加齢とともに低下する．裏を返せば，年を経るに従って加齢自体の相対危険度が増大することを意味している．また，最近の脂質低下薬による大規模介入試験をみると，脂質低下による虚血性心疾患の相対危険度低下は，若年者と高齢者で同等であるが，絶対危険度の低下は高齢者の方が大きくなる．これは，高齢者の発症頻度がより高いためであり，同等な脂質レベルの場合，高齢者の方が動脈硬化自体は進行していることを意味する．

## 2．血管の老化

加齢とともに動脈壁は厚くなり，弾性が失われる．病理学的には，びまん性内膜肥厚と呼ばれる比較的若年からみられる線維組織などの間質成分の増加と，高齢者に多くみられる石灰沈着に由来する．このような変化は，いわゆる粥状硬化による動脈硬化とは異なるもので，全身性に起こるが，最近は粥状硬化の好発部位としても認識されてきた．臨床的には，血管壁硬化として脈波伝播速度（PWV，図❹）[1]の増加やX線上の血管石灰化として観察され，その後の虚血性心疾患発症と関連することがわかっている．

動脈硬化の初期変化として重要な内皮細胞の機能低下も，血管老化の表現と考えられる．一酸化窒素（NO）分泌能は代表的な内皮機能であるが，それを血流依存性血管拡張反応として臨床的に評価すると，男性は40歳，女性は50歳から加齢による低下が明瞭にみられた（図❺）[2]．