# High density lipoprotein downregulates angiotensin II type 1 receptor and inhibits angiotensin II-induced cardiac hypertrophy

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## ARTICLE INFO

## ABSTRACT

Angiotensin II (AngII) and its type receptor (AT1-R) play important roles in the development of cardiac hypertrophy. Low level of high density lipoprotein (HDL) is also an independent risk factor for cardiac hypertrophy. We therefore investigated in the present study whether HDL inhibits cardiac hypertrophy relatively to inhibition of AngII and AT1-R in both *in vitro* and *in vivo* experiments. Stimulation of cultured cardiomyocytes of neonatal rats with AngII for 24 h and infusion of AngII in mice for 2 weeks resulted in marked cardiac hypertrophic responses including increased protein synthesis, enlarged sizes of cardiomyocytes and hearts, upregulated phosphorylation levels of protein kinases and reprogrammed expression of specific genes, all of which were significantly attenuated by the treatment with HDL. Furthermore, AngII-treatment induced upregulation of AT-R expression either in cultured cardiomyocytes or in hearts of mice and HDL significantly suppressed the upregulation of AT1-R. Our results suggest that HDL may abrogate AngII-induced cardiac hypertrophy through downregulation of AT1-R expression.

## 1. Introduction

Cardiac hypertrophy is not only an adaptive state before cardiac failure, but also an independent risk factor for major cardiac events [1]. It is thus very important to prevent and/or to suppress the development of cardiac hypertrophy during various pathological stimulations. It has been previously shown that rennin–angiotensin systems (RAS) including angiotensin II (AngII) and its type 1 receptor (AT1-R) play a crucial role in the development of cardiac hypertrophy [2–4]. Studies using primary cultured neonatal cardiomyocytes have demonstrated that AngII directly induces cell enlargement and fetal gene expression [5,6]. When administered systemically at suppressor doses, AngII induces myocardial growth [7,8]. Continuous infusion of AngII in rats results in an increase in left ventricular (LV) mass independently of its pressure-elevating effects. Cardiac-specific overexpression of AngII in a transgenic murine model leads to cardiac hypertrophy in the absence of hypertension [9]. These results clearly indicate that AngII and AT1-R are critical to the development of cardiac hypertrophy. Clinically, inhibitors for RAS such as AngII converting enzyme inhibitors (ACEI) and AT1-R blockers (ARB) have been generally and effectively used in the treatment of not only hypertension but also cardiac hypertrophy. However, ACEI and ARB have severe side effect in some patients. It is therefore requited to explore whether there are other inhibitory factors for RAS.

High density lipoprotein (HDL) particles play a critical role in preventing atherosclerosis through the function of reverse cholesterol transport [10,11]. HDL cholesterol may also exert antioxidant and anti-inflammatory effects in patients with heart failure [12,13]. There has been study demonstrated that HDL is superior to B-type natriuretic peptide as a marker of systolic cardiac dysfunction in an elderly general population [14]. Recently, it has been indicated clinically that the low HDL levels are independent risk factors for LV hypertrophy [15–17]. Furthermore, it has been suggested that there is a cross-talk between RAS and clusters of lipoproteins [18–20]. HDL may reduce the hyperglycemia-induced upregulation of the AT1-R in human aortic endothelial cells [21]. These data collectively suggest that HDL might play a role in inhibition of RAS and thereafter in suppression of cardiac hypertrophy.

## 2. Methods

### 2.1. Animal models

C57BL/6 mice were purchased from Shanghai Animal Administration Center (Shanghai, China). AngII (200 ng/kg/min, Sigma–Aldrich), and HDL (Calbiochem US and Canada, 400 ng/kg/min) were continuously administered by Alzet osmotic minipumps (DURECT,

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Cupertino, California) implanted subcutaneously into the mice. All protocols were approved by the Animal Care and Use Committee of Fudan University and in compliance with "Guidelines for the Care and Use of Laboratory Animals" published by the National Academy Press (NIH Publication No. 85-23, Revised 1996).

## 2.2. Echocardiography and haemodynamic measurements

Transthoratic echocardiography was performed using 30 MHz high frequency scanhead (VisualSonics Vevo770, VisualSonics Inc. Toronto, Canada) [22]. All measurements were averaged for five consecutive cardiac cycles and were carried out by three experienced technicians who were unaware of the identities of the respective experimental groups. Blood pressure (BP) was evaluated as previously described [23,24]. Briefly, a micronanometer catheter (Millar 1.4F, SPR 835, Millar Instruments, Inc. Houston, TX) was inserted into the right common carotid artery. The transducer was connected to Power Laboratory System (AD Instruments, Castle Hill, Australia) and BP was recorded.

## 2.3. Morphology and histological analyses

Excised hearts were weighed, perfused with PBS followed by 4% polyformaldehyde for global morphometry and fixed in 10% formalin for histological analysis. Paraffin embedded hearts were sectioned at 4-μm thickness and stained with hematoxylin and eosin (H–E). Cardiomyocytes were chosen from each section at a high magnification, and cross sectional area (CSA) of cardiomyocytes was measured by a video camera (Leica Qwin 3) attached to a micrometer in 20 different randomly chosen points from each cross section of LV free wall.

## 2.4. Cell culture and treatment

Neonatal rat cardiomyocytes were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) for 24 h and then in serum-free DMEM for 24 h. After pretreated with HDL (100 μg/ml) for 60 min, AngII (1 μmol/l) or vehicle was added to the cells. After 24 h incubation, cardiomyocytes were collected and lysed for the further real-time RT-PCR analysis.

## 2.5. [$^3$H] leucine incorporation

Cultured cardiomyocytes were incubated with [$^3$H] leucine (1 Ci/ml) in the presence of vehicle (Control), AngII (1 μmol/l), HDL (100 μg/ml) or HDL plus AngII 48 h. Cells were then treated with 5% trichloroacetic acid, the protein precipitates were dissolved in 1 ml of 100 mmol/L NaOH, and radioactivities were determined with a liquid scintillation counter.

## 2.6. Real-time RT-PCR

Total RNA was isolated from the LV tissues or cultured cells using TRIZol® reagent according to the manufacturer's instructions. After purification RNA was subjected to the real-time RT-PCR analysis for expression of *atrial natriuretic peptide* (*ANP*) and *skeletal α-actin* (*SAA*) on a BIO-RAD IQ5 multicolor detection system. Melting curves and quantization were analyzed using Light Cycler and Rel Quant software, respectively. A comparative CT method was used to determine relative quantification of RNA expression. All PCR reactions were performed at least triplicate.

## 2.7. Western blot analyses

Total proteins isolated from LV tissues or culture cells were subjected to Western blot analysis for phosphorylation of extracellular signal-regulated protein kinases (ERKs). The amounts of AT1-R were examined after dividing the membrane fraction and the cytosolic fraction. Briefly, cells were lysed and first centrifuged at 200g to remove nuclei. The supernatant was centrifuged at 15,000g for 30 min to pellet cell membrane. The total proteins or pelleted membranes were size-fractionated by SDS–PAGE and transferred to Immobilon-P membranes (Millipore). The blotted membranes were incubated with antibodies against phosphorylated ERKs (Cell Signaling Technology, 9101L) and AT1-R (Santa Cruz Biotechnology, sc-1173G), and subjected to an ECL Detection system (GE Healthcare, RPN2106).

## 2.8. Immunofluorescence

Cardiomyocytes cultured on glass cover slides in the presence of vehicle (Control), AngII (1 μmol/l), HDL (100 μg/ml) or HDL plus AngII 48 h were incubated with an anti-α-myosin heavy chain (MHC) (Upstate, catalogue 05-716, USA) antibody and then with the secondary antibody conjugated with FITC (Invitrogen, A21206) according to the manufacturer's directions. The surface area (SA) of cardiomyocytes stained by anti-α-MHC antibody was determined with image analysis software (Leica Qwin 3) and calculated as the mean of 100–120 cells from randomly selected fields.

## 2.9. Statistical analysis

Data are shown as means ± S.E.M. Comparison was performed by one-way or two-way analysis of variance followed by Newman–Keuls test for post hoc analysis to determine the difference among groups.

# 3. Results

## 3.1. HDL inhibited hypertrophic responses induced by AngII in cultured cardiomyocytes

After incubated with [$^3$H] leucine (1 Ci/ml) in the presence of AngII, HDL, HDL plus AngII or vehicle (Control) for 48 h, the cultured neonatal rat cardiomyocytes were subjected to test the rate of the cell protein synthesis. AngII significantly increased the protein synthesis of cardiomyocytes (Fig. 1A). We then measured the SA of cardiomyocytes by immunostaining using antibody against α-MHC. Similarly, treatment with AngII increased the SA of cardiomyocytes by approximately 78% (Fig. 1B). The upregulation of phosphorylation of ERKs was thought to be an important hypertrophic response. We thus examined the phosphorylation levels of ERKs in AngII-treated cardiomyocytes. The phosphorylation levels were significantly increased by AngII (Fig. 1C). Similar results were observed in detection of expression of fetal type genes, *ANP* and *SAA*, both of which were upregulated in AngII-treated cardiomyocytes (Fig. 1D).

Interestingly, addition of HDL to AngII-stimulated cardiomyocytes significantly abrogated all AngII-induced hypertrophic responses, such as increased cell protein synthesis, enlarged cell surface area, upregulated *p*-ERK and reprogrammed fetal type genes expression (Fig. 1A–D), clearly indicating an *in vitro* anti-hypertrophic effect of HDL against AngII.

## 3.2. HDL inhibited AngII-induced cardiac hypertrophy in vivo

To investigate whether HDL has an anti-hypertrophic effect *in vivo*, we treated the mice with low doses of AngII (200 ng/kg/min), which was insufficient to elevate the BP of mice, HDL (400 ng/kg/min) or both of them. Two weeks later, although BP was not different among all groups of mice (Fig. 2A),
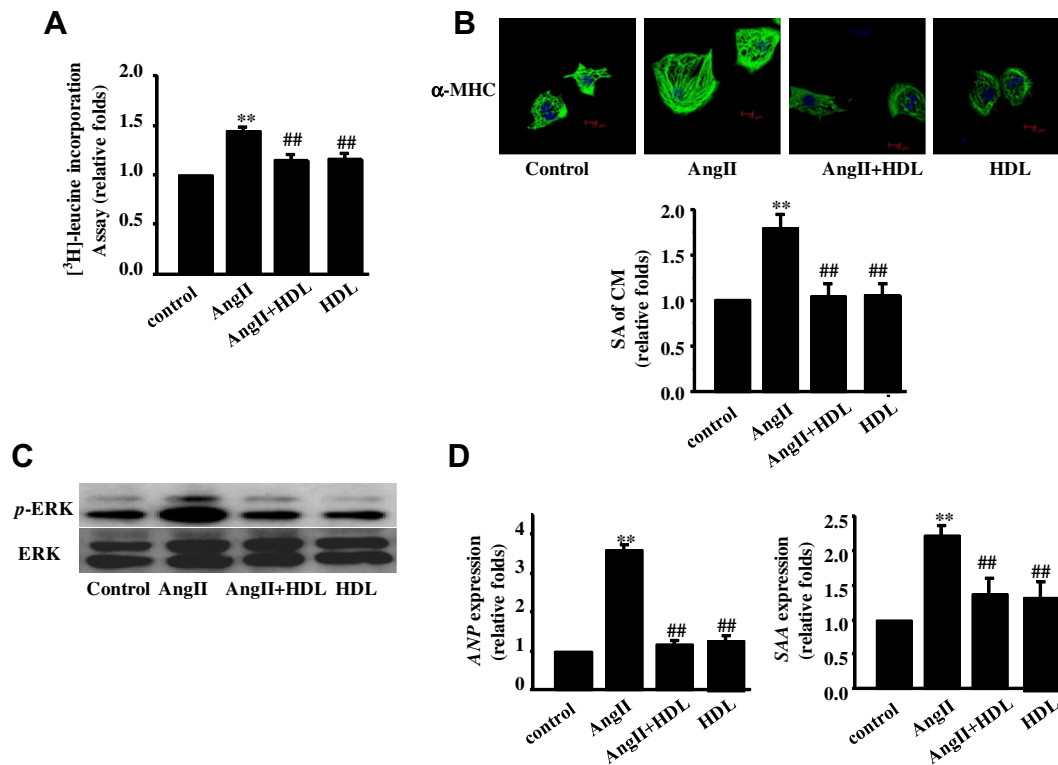
**Fig. 1.** Effects of HDL on AngII-induced cardiomyocyte hypertrophy in cultured cardiomyocytes. Cultured cardiomyocytes of neonatal rats were added by vehicle (Control), AngII (1 μmol/l), HDL (100 μg/ml) or HDL plus AngII for 48 h. (A) [³H]-leucine incorporation in cardiomyocytes. (B) Cardiomyocytes morphology and size. Cardiomyocytes were subjected to the immunofluorescent staining for α-MHC (green) and DAPI staining. Representative photographs from three independent experiments are shown (scale bar: 10 μm). Surface area (SA) of cardiomyocytes was evaluated by measuring 100 cardiomyocytes in one dish. (C) Cultured cardiomyocytes of neonatal rats were added by vehicle (Control), AngII (1 μmol/l), HDL (100 μg/ml) or HDL plus AngII for 8 min. Phosphorylation of ERKs. ERKs were examined by Western blotting using an anti-phosphor-ERKs antibody. Total ERKs were used as the loading control. Representative photograms from three independent experiments are shown. (D) Cultured cardiomyocytes of neonatal rats were added by vehicle (Control), AngII (1 μmol/l), HDL (100 μg/ml) or HDL plus AngII for 24 h. Expression of *ANP and SAA* genes evaluated by the real time RT-PCR. β-actin was served as internal control. All data are expressed as mean ± S.E.M from three independent experiments. ∗∗$p < 0.01$ vs. cardiomyocytes in Control; ##$p < 0.01$ vs. cardiomyocytes with AngII. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Echocardiographic measurement showed that AngII-infused mice developed a significant cardiac hypertrophy including the increased LVAWd (left ventricular anterior wall at end diastole) and LVPWd (left ventricular posterior wall at end diastole), decreased LVIDd (left ventricular internal dimension at end diastole) and preserved LVFS (left ventricular Fractional shortening) (Fig. 2B). Gross heart size and heart weight to body weight radio (HW/BW) were also increased by treatment with AngII (Fig. 2C). Measurement of CSA of cardiomyocytes in H–E stained LV sections revealed that AngII significantly enlarged the cardiomyocytes size (Fig. 2D). Similar to the results from *in vitro* experiments, the upregulation of phosphorylation of ERKs and expression of *SAA* and *ANP* were also observed in the hearts of AngII-treated mice (Fig. 2E and F).

Consistent with the anti-hypertrophic effect of HDL against the stimulation with AngII in cultured cardiomyocytes, HDL significantly blunted the *in vivo* cardiac hypertrophy induced by AngII (Fig. 2A–F), as demonstrated by the thinner ventricular wall, approximately normal ventricular chamber and preserved systolic function without affecting the BP of mice. The HW/BW ratio and CSA of myocardiocytes were significantly abrogated by HDL. Moreover, treatment with HDL significantly inhibited the upregulation of phosphorylated ERK and SAA and ANP expression induced by AngII.

### 3.3. HDL inhibited the expression of AT1 receptor in AngII-treated myocardiocytes

It had been believed that AT1-R plays a critical role in the AngII-induced cardiac hypertrophy. Hence we tested whether HDL attenuated the AngII-induced cardiac hypertrophy through inhibition of AT1-R. We detected the expression of AT1-R in cultured cardiomyocytes and hearts after AngII treatment by the Western blotting. AngII treatment significantly induced upregulation of AT1-R expression both in cultured cardiacmyocytes and hearts and HDL significantly restrained the increased AT1-R expression, suggesting that downregulation of AT1-R maybe contribute to the anti-hypertrophic effects of HDL (Fig. 3).

## 4. Discussion

Previous studies indicated that HDL as an emerging player in the field of cardiovascular protection was largely attributed to it's key role in reverse cholesterol transport [25]. However, a growing body of evidence has revealed that HDL possesses several other potent biological functions, such as anti-inflammatory, anti-oxidative, anti-apoptotic and nitric oxide (NO)-generating properties independently of reverse cholesterol transport. Such properties could be of direct benefit for cardiac events [26–30]. Recent studies have shown that the underlying mechanism for HDL effects may be to inhibit inflammatory neutrophil recruitment and cardiomyocyte apoptosis [31]. The present study indicates that administration with HDL inhibits both *in vitro* and *in vivo* cardiomyocyte hypertrophy induced by AngII, and our results also suggest that the effect of HDL on cardiomyocyte hypertrophy is, at least in part, due to inhibition of AT1-R.

AngII plays a critical role in cardiac growth, resulting in increases in cardiac fibrosis, remodeling and dysfunction [32–34]. The present study provides an evidence for HDL to have protective
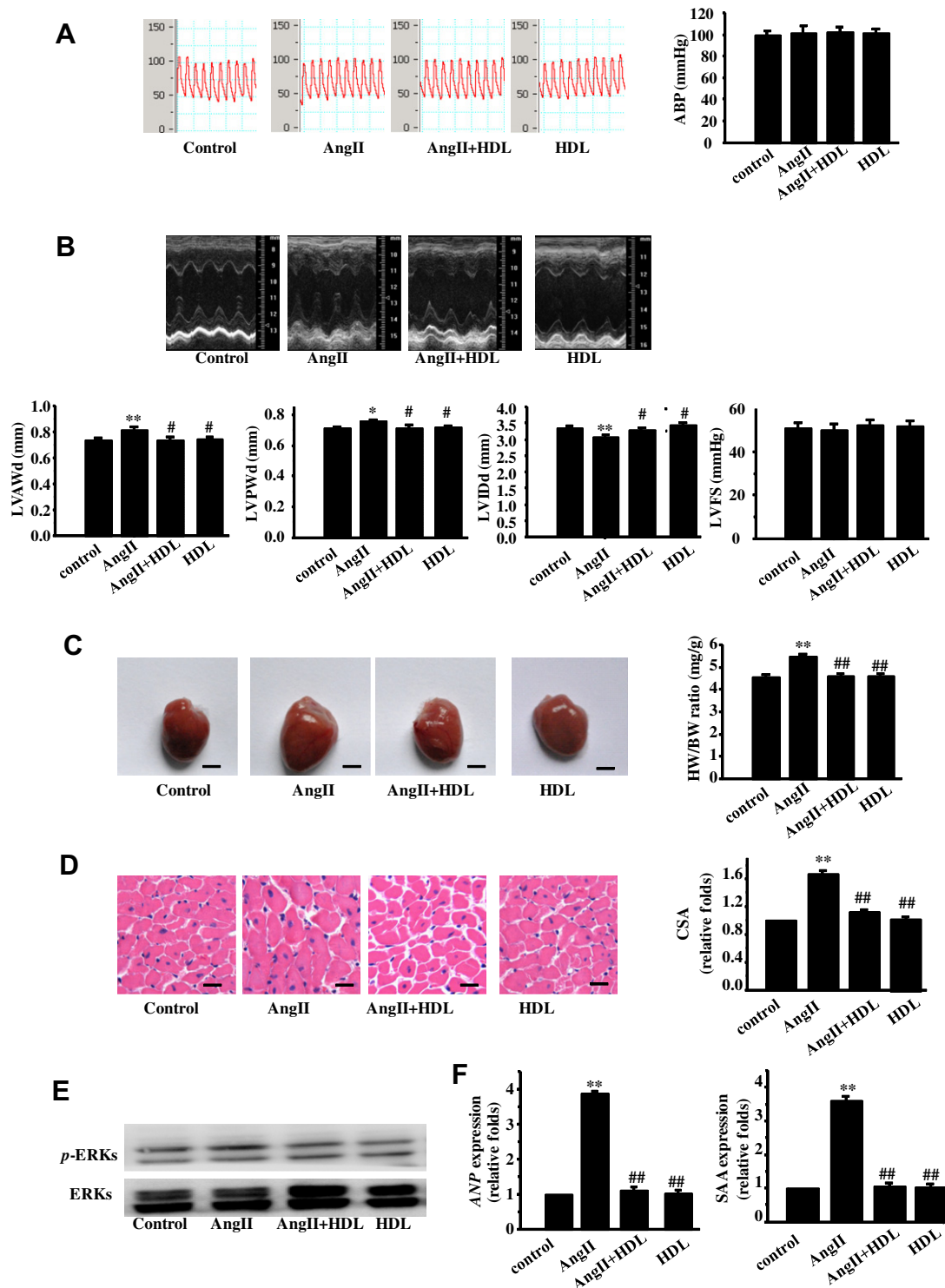
**Fig. 2.** Inhibition of AngII-induced cardiac hypertrophy by HDL in mice. C57B/L6 mice were infused with vehicle (Control), AngII (200 ng/kg/min), HDL (400 ng/kg/min) or AngII plus HDL for 2 weeks. (A) BP recordings. Representative recordings from five mice are shown. (B) Echocardiographic analysis. Representative M-mode tracings from five mice are shown. All echocardiographic measurements are shown as mean ± S.E.M from five mice. LVAWd, LV anterior wall thickness at end-diastole; LVPWd, LV posterior wall thickness at end-diastole; LVIDd, LV internal dimension at end-diastole; LVFS, LV fraction shortening. (C) Heart morphology and weight. Representative global heart photographs of five mice are shown (scale bar: 2 mm). Heart weigh to body weight radio (HW/BW) was measured from five mice. (D) H–E stained LV sections of mice. Scale bar: 20 μm. Cross sectional area (CSA) of cardiomyocyte was measured from five sections for one heart and five hearts were examined. (E) Phosphorylation of ERKs. ERKs were examined by Western blotting using an anti-phosphor-ERKs antibody. Total ERKs were served as the loading control. Representative photograms from five hearts are shown. (F) Expression of *ANP and SAA* genes evaluated by the real time RT- PCR. β-actin was used as internal control. All values are expressed as mean ± S.E.M. of five mice in all groups. $*p < 0.05$, $**p < 0.01$ vs. Control; $#p < 0.05$, $##p < 0.01$ vs. AngII-treated mice.

effects against AngII-induced cardiomyocyte hypertrophy. As estimated by the heart weight/body weight ratio, cardiomyocyte area, echocardiographic measurements, and *ANP* and *SAA* mRNA expres-

sion, cardiac hypertrophy by AngII infusion for 2 weeks was significantly formed in mice independently of BP. Administration with HDL significantly attenuated all AngII-induced cardiac
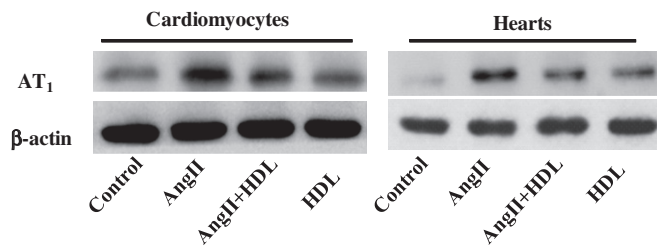
**Fig. 3.** Effects of HDL on expression of AT1-R proteins. Cultured cardiomyocytes and mice were treated with vehicle, AngII, HDL or AngII plus HDL. Membrane proteins were extracted from cardiomyocytes and LV of mice and subjected to Western blot analyses for expression of AT1-R proteins using an anti AT1-R antibody as described in (Section 2). β-actin in whole cell lysate was used as the loading control. Representative photograms from three independent experiments for cardiomyocytes or from five hearts of mice are shown.

hypertrophic responses. Taken together with our *in vitro* results that AngII–induced cardiomyocytes hypertrophy was also attenuated by HDL treatment, our present observations demonstrate that HDL prevents from AngII-induced cardiac hypertrophy.

Our study reveals a suppression of AngII-increased AT1-R expression by HDL, suggesting a possible mechanism for HDL actions. We postulated that the reduction of AT1-R expression by HDL is a significant mechanism for subsequent reduced cardiac hypertrophy during AngII stimulation. Under cardiac hypertrophy condition, the RAS is activated [3]. Given the known protective effects of HDL [13,35,36], we hypothesized that HDL could also regulate AT1-R expression under cardiac hypertrophy conditions. To investigate this hypothesis, we administered HDL via continuous infusion of HDL into mice and added HDL to cultured cardiomyocytes in the presence of AngII stimulation. Because the expression level of AT1-R defines the biological efficacy of AngII, HDL-induced downregulation of AT1-R reflects antagonism of HDL to AngII-mediated cardiac hypertrophy. Here, the HDL-caused downregulation of AT1-R in cardiomyocytes was in agreement with a decrease of cardiac hypertrophy. Our finding that HDL reduces AT1R expression and subsequent AngII–stimulated cardiac hypertrophy is consistent with the recent observation [37], who demonstrated that HDL inhibited the activation of Rac1, which is a downstream signal of AngII-stimulated AT1-R activation [38]. The exact mechanism by which HDL suppresses AngII-simulated AT1-R expression during the development of cardiac hypertrophy requires further fundamental studies.

In conclusion, we demonstrate that HDL protects against AngII-induced cardiac hypertrophy possibly through downregulation of AT1-R. Our results suggest a possible strategy for the treatment of cardiac hypertrophy through elevation of HDL levels.

## Acknowledgments

## References

[1] D. Levy, R.J. Garrison, D.D. Savage, W.B. Kannel, W.P. Castelli, Prognostic implications of echocardiographically determined left-ventricular mass in the framingham heart-study – reply, New England Journal of Medicine 323 (1990) 1706–1707.

[2] H. Yue, W. Li, R. Desnoyer, S.S. Karnik, Role of nuclear unphosphorylated STAT3 in angiotensin II type 1 receptor-induced cardiac hypertrophy, Cardiovascular Research 85 (2010) 90–99.

[3] J.F.X. Ainscough, M.J. Drinkhill, A. Sedo, N.A. Turner, D.A. Brooke, A.J. Balmforth, S.G. Ball, Angiotensin II type-1 receptor activation in the adult heart causes blood pressure-independent hypertrophy and cardiac dysfunction, Cardiovascular Research 81 (2009) 592–600.

[4] Q.H. Oin, L. Brako, L.H. Cheng, W. Lewis, D. Floyd, Q.L. Yang, Mice with cardiomyocyte-restricted smad4 deletion resist to angiotensin II induced cardiac hypertrophy, Circulation 116 (2007) 1223.

[5] D. Frank, C. Kuhn, M. Van Eickels, D. Gehring, H.A. Katus, N. Frey, Calsarcin-1 inhibits angiotensin-II induced cardiac hypertrophy *in vivo* and *in vitro*, European Journal of Heart Failure 6 (2007) 44–45.

[6] C.M. Hu, Y.H. Chen, M.T. Chiang, L.Y. Chau, Heme oxygenase-1 inhibits angiotensin II-induced cardiac hypertrophy *in vitro* and *in vivo*, Circulation 110 (2004) 309–316.

[7] H.R. De Smet, M.F. Menadue, J.R. Oliver, P.A. Phillips, Endothelin ETA receptor antagonism does not attenuate angiotensin II-induced cardiac hypertrophy *in vivo* in rats, Clinical and Experimental Pharmacology and Physiology 30 (2003) 278–283.

[8] C. Freund, R. Schmidt-Ullrich, A. Baurand, S. Dunger, W. Schneider, P. Loser, A. El-Jamali, R. Dietz, C. Scheidereit, M.W. Bergmann, Requirement of nuclear factor-kappa B in angiotensin II- and isoproterenol-induced cardiac hypertrophy *in vivo*, Circulation 111 (2005) 2319–2325.

[9] P. Paradis, N. Dali-Youcef, F. Paradis, G. Thibault, M. Nemer, Overexpression of angiotensin II type 1 receptor in cardiomyocytes induces cardiac hypertrophy and remodeling, Circulation 100 (1999) 43.

[10] C.J. Fielding, P.E. Fielding, Molecular physiology of reverse cholesterol transport, Journal of Lipid Research 36 (1995) 211–228.

[11] A.R. Tall, Plasma high-density lipoproteins – metabolism and relationship to atherogenesis, Journal of Clinical Investigation 86 (1990) 379–384.

[12] G. Clunn, A. Hughes, M. Schachter, Endotoxin-lipoprotein hypothesis, Lancet 356 (2000) 2097–2098.

[13] B.J. Ansell, K.E. Watson, A.M. Fogelman, M. Navab, G.C. Fonarow, High-density lipoprotein function – recent advances, Journal of the American College of Cardiology 46 (2005) 1792–1798.

[14] T. Kerola, T. Nieminen, S. Hartikainen, R. Sulkava, O. Vuolteenaho, R. Kettunen, High-density lipoprotein is superior to B-type natriuretic peptide as a marker of systolic dysfunction in an elderly general population, Scandinavian Journal of Clinical & Laboratory Investigation 69 (2009) 865–872.

[15] G. de Simone, M.H. Olsen, K. Wachtell, D.A. Hille, B. Dahlof, H. Ibsen, S.E. Kjeldsen, P.A. Lyle, R.B. Devereux, Clusters of metabolic risk factors predict cardiovascular events in hypertension with target-organ damage: the life study, Journal of Human Hypertension 21 (2007) 625–632.

[16] F. Anan, H. Yonemochi, T. Masaki, N. Takahashi, N. Fukunaga, Y. Teshima, T. Iwao, K. Kaneda, N. Eshima, T. Saikawa, H. Yoshimatsu, High-density lipoprotein cholesterol and insulin resistance are independent and additive markers of left ventricular hypertrophy in essential hypertension, Hypertension Research 30 (2007) 125–131.

[17] R. Ikee, Y. Hamasaki, M. Oka, K. Maesato, T. Mano, H. Moriya, T. Ohtake, S. Kobayashi, High-density lipoprotein cholesterol and left ventricular mass index in peritoneal dialysis, Peritoneal Dialysis International 28 (2008) 611–616.

[18] D.Y. Li, T. Saldeen, F. Romeo, J.L. Mehta, Oxidized LDL upregulates angiotensin II type 1 receptor expression in cultured human coronary artery endothelial cells – the potential role of transcription factor NF-kappa B, Circulation 102 (2000) 1970–1976.

[19] B.M. Singh, J.L. Mehta, Interactions between the renin–angiotensin system and dyslipidemia – relevance in the therapy of hypertension and coronary heart disease, Archives of Internal Medicine 163 (2003) 1296–1304.

[20] G. Nickenig, M. Bohm, Regulation of the angiotensin AT1 receptor expression by hypercholesterolemia, European Journal of Medical Research 2 (1997) 285–289.

[21] S. Van Linthout, F. Spillmann, M. Lorenz, M. Meloni, F. Jacobs, M. Egorova, V. Stangl, B. De Geest, H.P. Schultheiss, C. Tschope, Vascular-protective effects of high-density lipoprotein include the downregulation of the angiotensin II type 1 receptor, Hypertension 53 (2009) 682–687.

[22] M. Sano, T. Minamino, H. Toko, H. Miyauchi, M. Orimo, Y.J. Qin, H. Akazawa, K. Tateno, Y. Kayama, M. Harada, I. Shimizu, T. Asahara, H. Hamada, S. Tomita, J.D. Molkentin, Y.Z. Zou, I. Komuro, p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload, Nature 446 (2007) 444–448.

[23] O.F. Bueno, J.D. Molkentin, Involvement of extracellular signal-regulated kinases 1/2 in cardiac hypertrophy and cell death, Circulation Research 91 (2002) 776–781.

[24] J. Sadoshima, S. Izumo, Molecular characterization of angiotensin-II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts – critical role of the At(1) receptor subtype, Circulation Research 73 (1993) 413–423.

[25] H.B. Rubins, S.J. Robins, D. Collins, C.L. Fye, J.W. Anderson, M.B. Elam, F.H. Faas, E. Linares, E.J. Schaefer, G. Schectman, T.J. Wilt, J. Wittes, C. Vet, Affairs high-density lipoprotein, gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol, New England Journal of Medicine 341 (1999) 410–418.

[26] R.P. Choudhury, J.X. Rong, E. Trogan, V.I. Elmalem, H.M. Dansky, J.L. Breslow, J.L. Witztum, J.T. Fallon, E.A. Fisher, High-density lipoproteins retard the progression of atherosclerosis and favorably remodel lesions without suppressing indices of inflammation or oxidation, Arteriosclerosis Thrombosis and Vascular Biology 24 (2004) 1904–1909.

[27] B. Idzior-Walus, M. Walus, A. Foltyn, High density lipoproteins and atherosclerosis, Przeglad Lekarski 61 (2004) 1433–1435.

[28] J. Lie, M. Moerland, T. van Gent, R. van Haperen, L. Scheek, F. Sadeghi-Niaraki, R. de Crom, A. van Tol, Sex differences in atherosclerosis in mice with elevated phospholipid transfer protein activity are related to decreased plasma high density lipoproteins and not to increased production of triglycerides, Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids 1761 (2006) 1070–1077.

[29] M. Gomaraschi, L. Calabresi, G. Rossoni, S. Iametti, G. Franceschini, J.A. Stonik, A.T. Remaley, Anti-inflammatory and cardioprotective activities of synthetic high-density lipoprotein containing apolipoprotein A-I mimetic peptides, Journal of Pharmacology and Experimental Therapeutics 324 (2008) 776–783.

[30] P. Keul, K. Sattler, B. Levkau, HDL and its sphingosine-1-phosphate content in cardioprotection, Heart Failure Reviews 12 (2007) 301–306.

[31] G. Theilmeier, C. Schmidt, J. Herrmann, P. Keul, M. Schafers, I. Herrgott, J. Mersmann, J. Larmann, S. Hermann, J. Stypmann, O. Schober, R. Hildebrand, R. Schulz, G. Heusch, M. Haude, K.V. Lipinski, C. Herzog, M. Schmitz, R. Erbel, J. Chun, B. Levkau, High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury *in vivo* via the S1P(3) lysophospholipid receptor, Circulation 114 (2006) 1403–1409.

[32] S. Grigoryan, A.A. Stepanyan, L.G. Hazarapetyan, Inhibitor of angiotensin II type I receptors eprosartan and antiodarone as a combined treatment of advanced heart failure and atrial fibrillation, European Journal of Heart Failure 6 (2007) 151.

[33] R. Yamamoto, H. Akazawa, K. Ito, H. Toko, M. Sano, N. Yasuda, Y. Qin, Y. Kudo, T. Sugaya, K.R. Chien, I. Komuro, Angiotensin II type 1a receptor signals are involved in the progression of heart failure in MLP-deficient mice, Circulation Journal 71 (2007) 1958–1964.

[34] K.M. Baker, M.I. Chernin, T. Schreiber, S. Sanghi, S. Haiderzaidi, G.W. Booz, D.E. Dostal, R. Kumar, Evidence of a novel intracrine mechanism in angiotensin II-induced cardiac hypertrophy, Regulatory Peptides 120 (2004) 5–13.

[35] S.D. Anker, R. Sharma, The syndrome of cardiac cachexia, International Journal of Cardiology 85 (2002) 51–66.

[36] R.S. Velagaleti, J. Massaro, R.S. Vasan, S.J. Robins, W.B. Kannel, D. Levy, Relations of lipid concentrations to heart failure incidence the framingham heart study, Circulation 120 (2009) 2345–2351.

[37] M. Tolle, A. Pawlak, M. Schuchardt, A. Kawamura, U.J. Tietge, S. Lorkowski, P. Keul, G. Assmann, J. Chun, B. Levkau, M. van der Giet, J.R. Nofer, HDL-associated lysosphingolipids inhibit NAD(P)H oxidase-dependent monocyte chemoattractant protein-1 production, Arteriosclerosis Thrombosis and Vascular Biology 28 (2008) 1542–1548.

[38] H. Ohtsu, H. Suzuki, H. Nakashima, S. Dhobale, G.D. Frank, E.D. Motley, S. Eguchi, Angiotensin II signal transduction through small GTP-binding proteins – mechanism and significance in vascular smooth muscle cells, Hypertension 48 (2006) 534–540.