Differential Effects of Myocardial Afadin on Pressure Overload-Induced Compensated Cardiac Hypertrophy

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Background: Pressure overload induces cardiac hypertrophy, which often ends in heart failure. Afadin is an adaptor protein that is ubiquitously expressed and, in the heart, it localizes at intercalated disks. The current study aimed to examine the afadin-mediated cardiac phenotype in mice exposed to different types of pressure overload: transverse aortic constriction (TAC) burden and angiotensin II (Ang II) stimulation.

Methods and Results: Conditional knockout mice with selective deletion of afadin (afadin cKO) in cardiomyocytes were generated. TAC-operated and Ang II-infused mice at 4 weeks had a similar degree of pressure overload and cardiac hypertrophy in the heart. In afadin cKO mice, TAC operation caused progressive left ventricular dysfunction and heart failure, while Ang II infusion did not deteriorate cardiac function. Furthermore, TAC operation produced more fibrosis and apoptosis in the heart than Ang II infusion, and the expression of growth differentiation factor 15, which can promote apoptosis, in the afadin cKO heart was higher in TAC-operated mice than Ang II-infused ones.

Conclusions: In the 2 pressure overload models, myocardial afadin is involved in mechanical stress-induced, but not pharmacological Ang II-related, compensated cardiac hypertrophy.

Key Words: Angiotensin; Cardiac hypertrophy; Heart failure; Mechanical stress
with transforming growth factor-β signaling.

In the current investigation, we clarify the effects of afadin in another model of pressure overload in mice using Ang II delivery by micro-osmotic pump. Unexpectedly, Ang II infusion did not deteriorate cardiac function in afadin cKO mice, although similar progression of LV hypertrophy was observed between TAC and Ang II models. Our results suggest that afadin predominantly opposes cardiac dysfunction initiated by a TAC-induced mechanical burden, but not by Ang II-stimulated overload.

Methods

Conditional Knockout Mice

C57BL/6 mice with homozygous floxed exon 2 of the afadin gene were crossed with mice expressing Cre recombinase under the control of the α-myosin heavy chain gene promoter (Myh6-Cre; Jackson Laboratory, Bar Harbor, ME, USA). This was performed to create afadin cKO with selective afadin deletion in cardiomyocytes.18-19 Mice harboring afadin-floxed alleles alone were used as controls.

Chronic Pressure Overload Models in Mice

Treatment of the animals was approved and followed the guidelines of the Animal Ethics Committee of the Shiga University of Medical Science, Japan.

Ang II-Infusion Model

Mice were anesthetized by an intraperitoneal injection of 30 mg/kg pentobarbital sodium and implanted with subcutaneous micro-osmotic pumps (ALZET model 1004; Durect Corp., Cupertino, CA, USA) to deliver Ang II for 4 weeks at a constant flow of 400 ng kg⁻¹ min⁻¹ or saline, as previously reported.20

TAC-Operation Model

Mice were anesthetized, as described above, and ventilated through an intratracheal cannula (rodent ventilator model 28025; Ugo Basile, Varese, Italy). The tidal volume was set to 0.1–0.2 mL and the rate of respiration to 120/min. The aortic arch was approached through the left second intercostal space. Silk (7-0) surgical thread was tightened around a 27-gauge needle immediately distal to the innominate artery to create a fixed mechanical obstruction with a diameter of approximately 0.4 mm. Sham mice underwent a similar procedure to that of the TAC operation, except for ligation of the ascending aorta. Ang II/saline-infused and TAC/sham mice were sacrificed after 4 weeks of in vivo observation.

Measurement of Blood Pressure (BP)

Arterial BP was measured non-invasively in conscious mice by the plethysmographic tail-cuff method (model BP-98-AL; Softtron, Tokyo, Japan). Mice were warmed for at least 5 min at 37°C in the cylindrical thermostat of the BP-98-AL machine before and during the course of BP measurement. BP was measured in 2-min intervals and the mean of 5 steady state measurements was accepted as the true BP in each mouse.

Cardiac Ultrasound Imaging

LV dimensions and pump function were monitored by transthoracic ultrasonography on the Vevo 2100 system (VisualSonics Inc., Toronto, Canada). During the procedure, mice were anesthetized on a 37°C heating table with 1.0–1.5% isoflurane. Two-dimensional (B mode)- and Doppler images were recorded in the para-sternal short-axis position at the level of the papillary muscles and used for evaluation of LV parameters. LV mass was calculated by using the following formula: LV mass (mg) = 1.055×[(LVdd+PWd+IVSd)³−LVdd³], where LVdd indicates LV diastolic diameter (mm), and PWd and IVSd indicate diastolic thickness of LV posterior wall and interventricular septum (mm), respectively.21,22 The aortic arch and TAC stenosis were visualized in the B mode and color Doppler mode. Peak blood velocity (V, m/s) through the stenotic site was assessed by pulsed-wave Doppler mode. The pressure drop/gradient was calculated using the simplified Bernoulli equation as follows: pressure gradient=4V² (mmHg).23,24 Addition of the value of the stenotic pressure gradient to systemic systolic BP provides prestenotic systolic BP in the ascending aorta.

Histological Staining of Heart Sections and Immunohistochemistry

Mice hearts were frozen within water-soluble medium (Surplus FSC 22; Leica Biosystems, Wetzlar, Germany) or fixed with 4% paraformaldehyde, and subsequently embedded in paraffin blocks. Cryosections, 10-μm thick, were fixed by 4% paraformaldehyde on the top of poly-L-lysine-coated slides immediately after cutting the sections in a cryostat (Leica Biosystems). The sections were then permeabilized with 0.1% Triton X-100 and blocked with 1% bovine serum albumin. Primary antibody (Ab) was applied in bovine serum albumin-containing blocking solution overnight followed by a 1-h incubation with fluorescent dye-labeled secondary Ab. Confocal images were taken by using CLiS Laser Scanning Microscope (Nikon, Tokyo, Japan). Paraffin sections, 4-μm thick, were stained with Masson’s trichrome using standard techniques. Images for analysis of myocardial histology were captured by using a color CCD camera (MicroPublisher 5.0 RTV; Qimaging, Surrey, BC, Canada) under the control of Image-Pro Plus software (Media Cybernetics, Rockville, MD, USA) mounted on a Nikon FXA light microscope. Interstitial fibrotic content in cardiac sections was quantified as a ratio of the collagen-positive area/whole area of visible myocardium×100 (%) using Fiji software.25

Apoptosis Assays

Activated apoptotic signaling in cardiomyocytes was assessed by the immunostaining of frozen cardiac sections for cleaved caspase 3, and the fractional area of positive staining was compared among all groups. To detect fragmented DNA in apoptotic nuclei, the terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling (TUNEL) reaction was performed on sections of frozen hearts (DeadEnd™ Fluorometric TUNEL System; Promega, Madison, WI, USA) and fluorescence of stained nuclei was visualized by using a confocal microscopy. For each studied group of mice, the percentage of TUNEL-positive nuclei was estimated after evaluation of 1000 nuclei in every cardiac section.

Dilutions/Working Concentrations of Abs and Fluorescent Dyes

We used anti-cleaved caspase 3 (1:400) rabbit monoclonal Ab (Cell Signaling Technology, Danvers, MA, USA), anti-GDF15 (1:100) mouse monoclonal Ab (Santa Cruz Biotechnology, Dallas, TX, USA), Alexa Fluor 488 (1:1,000) goat anti-rabbit Ab, wheat germ agglutinin tetramethylrhodamin conjugate (5 μg/mL), propidium iodide (1 μg/mL) (Invitrogen, Carlsbad, CA, USA), and 4′,6-diamidino-
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Master Mix, Toyobo, Osaka, Japan) with a random primer. Samples for qPCR were prepared by using Light Cycler 480 SYBR Green I Master (Roche, Basel, Germany) and amplified in a Roche Light Cycler 480 with the following primers: natriuretic peptide A forward 5´-GAGGAGAAGATGCCGGTAGAAGA-3´ and reverse 5´-GAGCACTGCCGTCTCTCAGA-3´ (product length 148 bp); natriuretic peptide B forward 5´-AGCTGCTGGAGCTGATAAGAGAA-3´ and reverse 5´-TGCCCAAAGCAGCTTGAGATATG-3´ (product length 178 bp); connective tissue growth factor forward 5´-CTCCACCCGAGTTACCAATGAC-3´ and reverse 5´-GTGCAGCCAGAAAGCTCATTAC-3´ (product length 171 bp); tumor necrosis factor-α forward 5´-ATGAGCACAGAAAGCATGATC-3´ and reverse 5´-TACGGCTGTCTGACTGAATT-3´ (product length 276 bp); interleukin-6 forward 5´-GACAAAGCCAGAGTCCTTCAGA-3´ and reverse 5´-CTTCAGGGCCCTAGTAGATCTC-3´ (product length 170 bp); and growth differentiation factor 15 forward 5´-GAGACTCGAACTCAGAACCAAG-3´ and reverse 5´-CTTCAGGGCCCTAGTAGATCTC-3´ (product length 204 bp). The quantity of mRNA that was measured by the crossing points in qPCR was normalized to the value of β-actin in the same samples and the ratios were used for comparison.

2-phenylindole (DAPI) (1 µg/mL) (Dojindo, Kumamoto, Japan).

Langendorff’s Perfusion Protocols

Control and afadin cKO mice were anesthetized with pentobarbital sodium (30 mg/kg intraperitoneally). After thoracotomy, the heart was excised and immersed in the ice-cold Krebs-Henseleit bicarbonate buffer (composition in mmol/L: NaCl 118.5, NaHCO3 25, KCl 4.7, MgSO4 1.2, KH2PO4 1.2, glucose 11, and CaCl2 1.2), as described previously.26 The ascending aorta was cannulated with metal cannula (19 gauge). Then, the heart was mounted on the Langendorff’s apparatus (ADInstruments Pty Ltd, Bella Vista, NSW, Australia), and perfused with 95% oxygen/5% carbon dioxide bubbled, 37°C warmed Krebs-Henseleit buffer at the rate of 6 mL/min to apply high mechanical stress to LV cardiomyocytes. Heart perfusion was maintained for 20 min in order to avoid significant consequences of ischemia and edema on the myocardium. Flow rate and pressure are recorded by LabChart software (ADInstruments Pty Ltd).

Quantification of mRNA in Mouse Cardiomyocytes

Total RNA was extracted by using RNeasy Fibrous Tissue Kit (Qiagen, Hilden, Germany). cDNA was synthesized by reverse transcription PCR (ReverTra Ace™ qPCR RT Master Mix, Toyobo, Osaka, Japan) with a random primer. Samples for qPCR were prepared by using Light Cycler 480 SYBR Green I Master (Roche, Basel, Germany) and amplified in a Roche Light Cycler 480 with the following primers: natriuretic peptide A forward 5´-GAGGAGAAGATGCCGGTAGAAGA-3´ and reverse 5´-GAGCACTGCCGTCTCTCAGA-3´ (product length 148 bp); natriuretic peptide B forward 5´-AGCTGCTGGAGCTGATAAGAGAA-3´ and reverse 5´-TGCCCAAAGCAGCTTGAGATATG-3´ (product length 178 bp); connective tissue growth factor forward 5´-CTCCACCCGAGTTACCAATGAC-3´ and reverse 5´-GTGCAGCCAGAAAGCTCATTAC-3´ (product length 171 bp); tumor necrosis factor-α forward 5´-ATGAGCACAGAAAGCATGATC-3´ and reverse 5´-TACGGCTGTCTGACTGAATT-3´ (product length 276 bp); interleukin-6 forward 5´-GACAAAGCCAGAGTCCTTCAGA-3´ and reverse 5´-CTTCAGGGCCCTAGTAGATCTC-3´ (product length 170 bp); and growth differentiation factor 15 forward 5´-GAGACTCGAACTCAGAACCAAG-3´ and reverse 5´-CTTCAGGGCCCTAGTAGATCTC-3´ (product length 204 bp). The quantity of mRNA that was measured by the crossing points in qPCR was normalized to the value of β-actin in the same samples and the ratios were used for comparison.

Figure 1. Hemodynamic effects in mice exposed to transverse aortic constriction (TAC) burden or angiotensin II (Ang II) infusion for 4 weeks. (A) Visualization and measurement of the pressure gradient through the TAC stenosis (arrow). B mode and color Doppler mode images show morphology and blood flow through the normal aortic arch and stenosis caused by TAC. Numbers in the B mode/sham image indicate the following: 1, ascending aorta; 2, innominate artery; 3, left common carotid artery; and 4, left subclavian artery. Pulsed-wave (PW) Doppler images show enhanced blood flow velocity through the stenotic site. (B) Mean peak blood flow velocity and calculated pressure gradient in the ascending aorta in sham mice and across TAC stenosis. (C) Mean systemic systolic blood pressure (BP) in TAC-operated mice as measured from the tail artery, and calculated systolic BP in the ascending aorta proximal to the TAC stenosis. *P<0.05. (D) BP and heart rate (Inset) in mice that were infused with saline or Ang II. *P<0.05 vs. 0 week, n=8 per group.
Statistical Analysis
All numerical values are shown as mean±standard error of the mean. Differences between 2 groups were examined by using the Student’s t-test. Three or more grouped data were evaluated by one-way analysis of variance followed by Tukey’s post-hoc test.

Results

Hemodynamics in Mice Exposed to TAC vs. Ang II
We first compare hemodynamics between TAC-operated or Ang II-infused mice for 4 weeks. To estimate mechanical load on LV during systole in TAC-operated mice, we calculated prestenotic systolic BP in the ascending aorta by echocardiography. The aortic arch in mice after the 4-week sham or TAC procedure was depicted in B mode (Figure 1A). Blood flow velocity through the intact and stenotic aortic arches was visualized in color Doppler mode (Figures 1A; Movies S1, S2). Measurement of peak blood flow velocity was conducted in pulsed-wave Doppler mode (Figure 1A). Then, the mean peak blood flow velocity and the pressure gradient were calculated (Figure 1B). The values are in agreement with previous studies.23-27 Systolic BP proximal to TAC (ascending aorta) was estimated by addition of the pressure gradient to systemic systolic BP, as measured from the tail artery (Figure 1C). Ang II induced systolic and diastolic hypertension without heart rate alteration, and saline had no effect on BP and heart rate (Figure 1D). Taken together, systolic BP elevated by Ang II was similar to that proximal to TAC for 4 weeks, although the mode of increase of BP is different between the 2 models. TAC causes an immediate increase in mechanical tension, resulting in rapid BP elevation proximal to the stenosis,27 while Ang II infusion gradually raises systemic BP, reaching the maximal values within approximately 2 weeks.28

Comparison of Cardiac Function Between TAC- and Ang II-Challenged Mice
The LV dimensions and function were followed in vivo by cardiac echography before and every 2 weeks during the observational period. As presented in our previous report,18 TAC in afadin cKO mice evoked LV hypertrophy, with an increase in LV wall thickness and mass, and attenuated LV pump function evaluated by ejection fraction (n=8–9 per group). Control: afadin-floxed mice. *P<0.05 vs. 0 week.
Afadin cKO as well as control mice. Other echocardiographic parameters are shown in Table.

Morphometry of the hearts and lungs after 4 weeks of TAC and Ang II challenge confirmed the above findings. TAC operation and Ang II infusion similarly enlarged the heart, compared with sham operation (Figure 3A, B). A direct effect of LV pump failure is congestion in the pulmonary veins and lungs (Figure 3C).

### Table. Echocardiographic Parameters

|         | TAC         | Ang II       |
|---------|-------------|--------------|
|         | Control     | Afadin cKO   | Control     | Afadin cKO   |
| IVSd (mm) |             |              |             |              |
| 0 weeks | 0.58±0.02   | 0.58±0.02    | 0.68±0.02   | 0.68±0.01    |
| 2 weeks | 0.80±0.03*  | 0.77±0.02*   | 0.75±0.03   | 0.78±0.03*   |
| 4 weeks | 0.80±0.03*  | 0.77±0.03*   | 0.87±0.04*  | 0.87±0.03*   |
| LVDd (mm) |             |              |             |              |
| 0 weeks | 3.97±0.08   | 3.85±0.10    | 3.87±0.08   | 3.87±0.03    |
| 2 weeks | 3.86±0.08   | 3.88±0.07    | 4.12±0.15   | 3.78±0.04    |
| 4 weeks | 3.93±0.11   | 4.06±0.10    | 3.98±0.15   | 3.91±0.04    |
| FS (%)   |             |              |             |              |
| 0 weeks | 34.41±2.61  | 34.17±1.39   | 37.00±1.45  | 37.20±1.89   |
| 2 weeks | 34.00±1.64  | 26.92±2.74   | 35.47±1.81  | 35.27±2.40   |
| 4 weeks | 35.56±3.20  | 22.73±2.30*  | 39.42±1.85  | 33.20±1.67   |

Ang II, angiotensin II; cKO, conditional knockout; FS, fractional shortening; IVSd, interventricular septal thickness at diastole; LVDd, left ventricular end-diastolic diameter; TAC, transverse aortic constriction. *P<0.05 vs. 0 week.

Figure 3. Morphometry of the hearts and lungs after a 4-week transverse aortic constriction (TAC) burden or angiotensin II (Ang II) infusion. (A) Typical images of hearts exposed to sham operation, TAC burden or Ang II infusion. Heart weight is indicated in each image. (B) Summary graphs of normalized heart weight (heart weight to tibia length ratio). (C) Summary graphs of normalized lung weight (lung weight to tibia length ratio). In (B) and (C), n=3–4 per group. Control: afadin-floxed mice. *P<0.05.

Figure 4. Effects of transverse aortic constriction (TAC) and angiotensin II (Ang II) on myocardial collagen production after 4-week treatment. (A) Masson’s staining of cardiac sections from mice of each group. The blue color indicates collagen deposition. (B) Summary graph of quantified fibrotic areas (stained in blue). Control: afadin-floxed mice. *P<0.05, n=6 per group.
pulmonary circulation and a resultant rise in lung mass.\(^\text{29}\) There was a small but significant increase in normalized lung weight in afadin cKO mice compared with control mice after TAC operation, but Ang II treatment or sham operation did not increase normalized lung weight (Figure 3C). These results suggest that the inhibitory role of afadin in the development of cardiac dysfunction and failure is dependent on the types of pressure overload.

**Different Cardiac Responses After TAC Operation and Ang II Infusion**

To explore the reason why afadin deletion in cardiomyocytes differentially responded to TAC operation and Ang II infusion, we examined the interstitial fibrosis, which affects cardiac function,\(^\text{30}\) by Masson’s staining that detects collagen deposition. Positively stained areas in the sections of TAC-operated afadin cKO hearts were more abundant than those in control hearts, while these areas were similar between Ang II-infused control and afadin cKO hearts (Figure 4). Moreover, the level of collagen accumulation in the heart of TAC-operated afadin cKO mice was higher than that of Ang II-infused afadin cKO mice. These findings suggest that deletion of afadin accelerates TAC-induced, but not Ang II-related, myocardial fibrosis, leading to cardiac dysfunction.

Cardiomyocyte loss in the presence of accelerated apoptosis is a causative factor in the progression of cardiac dysfunction and heart failure.\(^\text{31}\) We investigated activation of apoptosis by using 2 methods: the main cellular effector of the apoptotic process, cleaved caspase 3,\(^\text{32}\) and the TUNEL reaction, which detects DNA fragments that are produced by digestion of nuclear DNA in the caspase 3-mediated apoptotic process.\(^\text{33}\) In TAC-operated mice, staining of cleaved caspase 3, an active form of caspase 3, by immunohistochemistry detected significant activation of apoptosis in afadin cKO hearts, compared with control hearts (Figure 5A, B). In addition, TAC-operated afadin cKO hearts had more accentuated staining than Ang II-infused hearts. In Ang II-infused mice, there was no difference in the staining between control and afadin cKO hearts. Corresponding to cleaved caspase 3 staining, TUNEL-positive nuclei showed a significantly higher density in TAC-operated afadin cKO hearts than in control hearts (Figure 5C, D). In the afadin cKO TAC group, the percentage of TUNEL-positive nuclei was higher than that in the afadin cKO Ang II group. Increased apoptosis in afadin cKO hearts exposed to TAC may be associated with the promotion of myocardial fibrosis, and crucially contribute to deterioration of cardiac function. To exclude the possibility that afadin-deleted cardiomyocytes have less contractility against mechanical stress than control cardiomyocytes, we conducted the Langendorff’s perfusion experiment using the hearts isolated from control and afadin cKO mice. When the hearts were perfused with high constant flow (6 mL/min) to induce excessive mechanical load to LV cardiomyocytes, both hearts generated equal LV systolic (~210 mmHg) and diastolic (~160 mmHg) pressure with identical LV beating rate (Figure S1), suggesting that the functional contraction of cardiomyocytes is not reduced by ablation of afadin in the pressure-overloaded conditions.

Finally, we aimed to understand the mechanism responsible for the difference in cardiac phenotype in the TAC and Ang II models. We quantified mRNA of several molecules that are related to cardiac injury or inflammation...
is dissimilar at the beginning of TAC and Ang II exposure. There is a slow increase in LV afterload in the Ang II model, and is an abrupt and steady mechanical stress to LV in TAC procedure. Afadin might be important for instantaneous resistance against the mechanical burden to LV. Slow development of pressure overload in the Ang II model may allow activation of protective mechanisms that maintain LV function even in the absence of afadin, although Ang II strongly stimulates various signaling pathways in cardiomyocytes, which modulates cardiac function, mainly through AT 1R, a G protein-coupled receptor (GPCR). Based on the fact that we did not identify an adverse cardiac phenotype in afadin cKO hearts of Ang II-infused mice, it appears that there is no interaction of afadin with AT 1R itself and/or its downstream signaling molecules activated by Ang II.

In addition, Ang II type 1 receptor (AT 1R) is one of the mechanosensors in the heart. It is demonstrated that even in the absence of Ang II, AT 1R and downstream signaling are activated and cardiac hypertrophy is developed by TAC-mediated mechanical stretch. It is also shown that the active conformation of AT 1R during mechanical stimulation is not identical to its active conformation after binding of Ang II. Particular conformation of activated GPCRs such as AT 1R is known to have selective affinity to specific G proteins. Therefore, the signaling triggered by different stimuli acting on the same GPCR depends on the activation of a specific G protein and its downstream molecules. Considering the above, the inconsistency in the cardiac phenotype between Ang II-treated and TAC-operated afadin cKO mice in our study is most probably a

**Discussion**

TAC burden and Ang II treatment produce chronic pressure overload, and in our experiments, a similar degree of cardiac hypertrophy was observed in both TAC- and Ang II-challenged hearts. In control mice, TAC operation or Ang II infusion did not affect LV pump function. In afadin cKO mice, however, responses to TAC and Ang II were different; Ang II infusion did not deteriorate cardiac function, while TAC operation induced LV dysfunction and heart failure. The discordant effect of TAC and Ang II on LV function in afadin cKO mice may be due to the differences in at least hemodynamics. Only considering the tension of LV, the time-course of LV hemodynamic load

![Figure 6](image-url). Comparison of expression levels of regulatory molecules related to cardiac function and inflammation after 4-week of sham operation, transverse aortic constriction (TAC) burden or angiotensin II (Ang II) infusion in afadin conditional knockout (cKO) mice. (A) mRNA levels of each molecule quantified by quantitative polymerase chain reaction (qPCR). NppA, natriuretic peptide A; NppB, natriuretic peptide B; CTGF, connective tissue growth factor; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; GDF15, growth differentiation factor 15. (B) Western blotting for GDF15. The value of sham-operated hearts is set as 1.0. *P<0.05, n=3–4 per group.
result of different intracellular processes, as suggested by quantification of GDF15 mRNA and protein levels (Figure 6). Afadin appears to participate in the signaling inside cardiomyocytes during mechanical overload, but not in Ang II-stimulated signaling. In fact, similar distinction of cardiac phenotype is reported about NADPH oxidase-induced cardiac hypertrophy. In gp91phox knockout hearts, NADPH oxidase activation and hypertrophic response are inhibited in Ang II-infused mice, but persist in the TAC-operated ones due to the stimulation of an alternative signaling mechanism.42

Systolic heart failure is known to be accompanied by ventricular cavity expansion due to the decline of the ejection fraction. Lack of increase of LV dimensions in the afadin cKO TAC hearts in our data looks unusual, but in fact, after 4 weeks of TAC procedure, LV of afadin cKO mice gradually expands, as can be seen in our previous study.43 It appears that during the first 4 weeks after TAC operation, LV can resist the expansion of the cavity because of relatively small systolic dysfunction and/or available collagen accumulation, which may counteract the increased intraventricular diastolic pressure that is created by the blood congestion in the left part of the circulation.

Accelerated fibrosis and apoptosis in the heart are the adverse cardiac responses, and are causative factors for transition to or progression of heart failure.30,41,44 We found significant upregulation of fibrosis and apoptosis in TAC-operated afadin cKO hearts compared with control hearts. Additionally, this upregulation was not observed in Ang II-treated hearts, and the apoptotic process and fibrogenesis in the heart were less activated by Ang II treatment than TAC operation in afadin cKO mice. This finding suggests that prevention of cardiomyocyte loss and fibrotic alteration appears to be a key factor to maintain cardiac function in Ang II-infused afadin cKO mice. In support of this observation, expression of GDF15, a promoter of apoptosis, was significantly reduced in the hearts of Ang II-infused afadin cKO mice compared with that of TAC-operated mice. This may also contribute to inhibition of the progress of LV dysfunction induced by Ang II-mediated pressure overload in the afadin-deleted heart.

In conclusion, afadin has different cardioprotective effects against stimuli that mediate a heavy cardiac burden. In afadin cKO mice, Ang II stimulation caused cardiac hypertrophy, but not dysfunction, whereas TAC burden induced cardiac hypertrophy and consequent dysfunction. Thus, afadin appears to interact with mechanical stress-induced signaling, but might not be associated with Ang II/AT1R-coupled pathways.

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Supplementary Files

Supplementary File 1

Figure S1. Langendorff’s perfusion of isolated control and afadin conditional knockout (cKO) hearts perfused with constant flow rate to induce strong mechanical burden on left ventricle (LV).

Supplementary File 2

Movie S1. Color Doppler mode echocardiographic recordings show aortic arch morphology and blood flow in the intact vessels (Movie S1) and after the transverse aortic constriction (TAC) operation (Movie S2). Anatomy of the aortic arch, and the origin of the innominate, common carotid and left subclavian arteries are clearly seen. The blue color indicates flow in the outward direction of the transducer, and the red color indicates flow toward the direction of the transducer. A turbulent jet in the stenotic location of the ascending aorta can be seen in colors of the yellow spectrum.

Supplementary File 3

Movie S2.

Please find supplementary file(s): http://dx.doi.org/10.1253/circj.CJ-17-0394