Natriuretic peptide receptor C contributes to disproportionate right ventricular hypertrophy in a rodent model of obesity-induced heart failure with preserved ejection fraction with pulmonary hypertension

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Abstract
Heart failure with preserved ejection fraction (HFpEF) currently has no therapies that improve mortality. Right ventricular dysfunction and pulmonary hypertension are common in HFpEF, and thought to be driven by obesity and metabolic syndrome. Thus, we hypothesized that an animal model of obesity-induced HFpEF with pulmonary hypertension would provide insight into the pathogenesis of right ventricular failure in HFpEF. Two strains of mice, one susceptible (AKR) and one resistant (C3H) to obesity-induced HFpEF, were fed high fat (60% fat) or control diet for 0, 2, or 20 weeks and evaluated by cardiac catheterization and echocardiography for development of right ventricular dysfunction, pulmonary hypertension, and HFpEF. AKR, but not C3H, mice developed right ventricular dysfunction, pulmonary hypertension, and HFpEF. NPRC, which antagonizes beneficial natriuretic peptide signaling, was found in RNA sequencing to be the most differentially upregulated gene in the right ventricle, but not left ventricle or lung, of AKR mice that developed pulmonary hypertension and HFpEF. Overexpression of NPRC in H9C2 cells increased basal cell size and increased expression of hypertrophic genes, MYH7 and NPPA. In conclusion, we have shown that NPRC contributes to right ventricular modeling in obesity-induced pulmonary hypertension-HFpEF by increasing cardiomyocyte hypertrophy. NPRC may represent a promising therapeutic target for right ventricular dysfunction in pulmonary hypertension-HFpEF.

Keywords
pulmonary hypertension, congestive heart failure, right ventricle function and dysfunction, obesity and metabolic syndrome, diabetes and dyslipidemias, natriuretic peptides

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Introduction
Pulmonary hypertension due to left heart disease (PH-LHD) is the most common form of pulmonary hypertension (PH). Among the various etiologies of PH-LHD, heart failure with preserved ejection fraction (HFpEF) represents a growing subset of these patients.1 Notably, 80% of patients with HFpEF have concomitant PH, and the presence of PH in HFpEF (PH-HFpEF) independently increases morbidity and mortality.2,3 Recent studies suggest a major cause of morbidity and mortality in PH-HFpEF is right ventricular (RV) failure, occurring independent of the severity of PH.4,5 Therapies targeting pathways known to affect pulmonary vascular and left ventricular remodeling have largely been...
neutral or harmful in PH-HFpEF, but no therapies to date have targeted adverse RV remodeling directly in PH-HFpEF. This is, in part, due to poor understanding of the pathophysiology of RV failure in HFpEF.

Understanding the mechanisms contributing to adverse RV remodeling in HFpEF has been limited by the relative lack of pre-clinical animal models of RV failure in PH-HFpEF. Human studies have shown that co-existing obesity and metabolic syndrome independently contribute to worsening RV function as well as development of HFpEF with pulmonary vascular remodeling. Thus, to better understand the mechanisms underlying maladaptive RV remodeling in PH-HFpEF, we used a validated animal model of obesity-induced PH-HFpEF to study early pathways that contribute to RV remodeling. Using a second rodent strain resistant to obesity-induced PH-HFpEF, we aimed to identify unique signals that contribute to the development of obesity-induced PH-HFpEF.

Methods

All animal studies were approved by the Vanderbilt University Medical Center IACUC (protocol M1800073).

Animal studies

Male mice, approximately eight weeks of age, were purchased from Jackson Laboratories. Equal numbers of AKR and C3H mice (n=8 for each time point and group) were fed either a high fat diet chow consisting of 60% lard content (BioServ F3832, San Diego, CA) or a nutrient matched control diet (BioServ F4031, San Diego, CA). At 0, 2, and 20 weeks after starting diets, mice underwent echocardiography and cardiac catheterization prior to sacrifice. A subset of mice (n=5 in each group) underwent echocardiography and cardiac catheterization, while another subset (n=3 in each group) underwent cardiac catheterization for histologic analysis of tissue.

Echocardiography

Echocardiography was completed as previously described. Briefly, mice were anesthetized with 2–3% isoflurane. Depilatory cream was applied to the thorax. Mice were then positioned supine, ventral side up on a heated operating table. A vertical incision over the abdomen was made and cautery was used to cut the diaphragm and expose the heart. A 1.4 French Mikro-tip catheter was directly inserted into the left ventricle for measurement of pressure and volume within the left ventricle. Afterwards, the catheter was removed and directly inserted into the right ventricle. Hemostasis of the left ventricle was ensured prior to catheterization of the right ventricle to ensure no effect of volume loss on findings. Hemodynamics were continuously recorded with a Millar MPVS-300 unit coupled to a Powerlab 8-SP analog-to-digital converter acquired at 1000 Hz and captured to a Macintosh G4 (Millar Instruments, Houston, TX). Mice were then sacrificed with cervical dislocation and tissues (left and right ventricle) were measured and flash-frozen in liquid nitrogen. Whole hearts from a subset of mice in each group were placed in 10% neutral buffered formalin for histologic sectioning and analysis. Transpulmonary gradient was calculated by subtracting the left ventricular end-diastolic pressure from the mean pulmonary arterial pressure, which was estimated as 60% of the RV systolic pressure. Pulmonary vascular resistance was calculated in Wood units as the ratio of transpulmonary gradient to cardiac output (measured by echo as the product of velocity time integral and aortic cross-sectional area).

Cardiac catheterization

At 0, 2, and 20 weeks after starting diets, mice were anesthetized with 2–3% isoflurane and orotracheally intubated with 22 g catheter. Animals were mechanically ventilated at 18 cc/kg with vaporized isoflurane general anesthesia. Mice were weighted after 30 min and 1 h to ensure tissue was entirely dry.

Histologic analysis

Hearts were harvested from mice and immediately either placed in 10% neutral buffered formalin or Optimal Cutting Temperature medium for processing, sectioning, and staining with hematoxylin/eosin (H&E) and Masson’s Trichrome stains at three different levels. Only slides with complete short axis views of the right and left ventricle were used for analysis. Fibrosis was quantified using ImageJ to measure area of fibrosis on Masson’s Trichrome stains using the Colour Deconvolution plugin. Oil Red O stain was completed as previously described on frozen sections, and percentage of tissue with lipid deposition was quantified using ImageJ and the Colour Deconvolution plugin. Lung tissue were first perfused through the trachea with agaroise and then fixed in formalin for sectioning and staining with H&E.

Immunofluorescence

Slides were deparaffinized and underwent heat antigen retrieval for 20 min in Tris EDTA pH 9 antigen retrieval buffer.
Samples were blocked for 2 h in 10% goat serum and 1% bovine serum albumin (BSA) prior to incubation in primary antibody overnight in 0.5% BSA. They were then incubated in secondary antibody diluted in phosphate buffered saline prior to mounting with Vectashield 4',6-diamidino-2-phenylindole DAPI (Vector Laboratories, Burlingame, CA). Images were then taken on the Nikon Eclipse Ti confocal microscope (Nikon USA, Melville, NY). Cell staining was conducted on chamber slides. Cells were washed in ice cold phosphate buffered saline PBS and fixed in 4% paraformaldehyde for 10 min prior to permeabilizing with 0.1% Triton X in PBS for 10 min. Cells were blocked for 30 min in blocking buffer as above and incubated in primary antibody in 0.5% BSA for 1 h, followed by secondary antibody for 1 h. Cell slides were mounted in Vectashield DAPI and imaged as above. Antibodies used included: alpha smooth muscle actin (Abcam, ab5694) 1:100, natriuretic peptide clearance receptor C (NPRC) (Novus Biologics, 31365) 1:50, troponin-T (Abcam, ab10214) 1:100, and Alexa Fluor 488 or 594 conjugated secondary 1:500 goat anti-rabbit IgG (Thermo Fisher Scientific).

**Western blot**

NPRC antibody (Novus Biologics, 31365) was used at a concentration of 1:1000 diluted in 5% milk, Tris buffered saline TBS, and 0.1% Tween 20. Secondary antibody was then used at 1:5000 dilution. Bands were detected using the SuperSignal Western Pico Chemiluminescent Substrate (Thermo Scientific, Waltham, MA) and imaged using the BioRad ChemiDoc Touch System (Bio Rad, Hercules, CA).

**RNA isolation and Reverse Transcription PCR**

RNA was isolated using RNAeasy kit (Qiagen, Hilden, Germany). Reverse transcription was completed using Quantitect Reverse Transcriptase Kit (Qiagen, Hilden, Germany). Thereafter, quantitative PCR was completed using SYBR Green Master PCR Mix (Applied Biosystems, Foster City, CA) and primer pairs on the QuantStudio 3 PCR machine (Applied Biosystems). PCR primers used included (5'-3'): NPR3 Mus musculus F- AACGTCTGCCTA CAATTTCG, R- GCACACATGATCACCACCTCG; HPRT Mus musculus F-TGCTCGAGATGTACATGAAGGAG, R-TTGGATGCTGCAGATGCTCAAG; NPPA Rattus norvegicus F- ATCCCGTAAATCCACGAGTCCAGC; NPRC Rattus norvegicus F- ATCCCCGTATACAGGTGGT, R- CTCC TCCAGGTTGCTACTACA; MYH7 Rattus norvegicus F- TGGCCACCGAGTACAAATA, R- TACAAGTGCAA TGCTCGAC; NPR3 Rattus norvegicus F- GGAC CTGGAGACACATAGTGC; R- CCACGAGCCATCTCCGTAAG; HPRT Rattus norvegicus F-TGCTCGAGATGCTATGAAGGAG, R-TCCACACCTTCGAGAGGTCC.

**RNA sequencing**

RNA isolated from the right ventricle at 0 and 2 weeks from mice was submitted for RNA sequencing by the Vanderbilt University Medical Center. All samples with RNA integrity number RIN values greater than 6 were submitted for sequencing analysis. Experimental conditions included four or five replicates of a total of eight groups: two mouse strains (AKR vs C3H), two experimental groups (control diet vs high fat diet), and two time points (0 and 2 weeks). RNA sequencing was performed on an Illumina HiSeq system with a paired-end mRNA library prep, PE-150, with 30 million reads. Initial alignment and quantification of sequences was performed using the Partek Flow package. STAR 2.5.3a was used to align RNA-Seq reads, with quantification to Ensembl Transcripts Release 83 using Partek E/M. Reads were normalized to total count. Approximately 77% of all reads aligned to genes. A total of 22,232 genes were identified with at least one read in each sample. Genes with fewer than four reads per million were filtered out, yielding 1452 genes. To remove the effect of diet alone, genes with similar changes between diets in both mouse strains (AKR high fat vs control diet, C3H high fat vs control diet) were filtered. Additionally, genes were filtered for those that had a statistically significant (p < 0.05), >1.5 fold change difference between mouse strains in response to high fat diet (AKR vs C3H high fat diet), resulting in a total of 42 differentially regulated genes between AKR and C3H strains in response to high fat diet. Over-representation analysis was completed using WebGestalt (webgestalt.org) for the Mus musculus organism and gene ontology database, comparing against the genome-protein coding database. Multiple test adjustment was conducted using the Benjamini-Hochberg procedure and a false discovery rate threshold of 0.05 or lower was considered significant.

**H9C2 cell culture and plasmid transfection**

Cardiomyocyte-like H9C2 cells were cultured in Dulbecco’s Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS). Prior to studies, they were cultured in DMEM containing 1% FBS for 48 h. A flag-tagged human NPRC construct was generated and confirmed by sequencing. A subset of H9C2 cells were transduced using Lipofectamine 2000 following manufacturer’s recommended protocol with either plasmid containing NPRC or empty vector alone. Transduced cells underwent selection for one week with 1 mg/ml G418 (Sigma) followed by maintenance in 0.8 mg/ml G418 thereafter. Plasmid transfection was confirmed using PCR and Western blot for NPRC. Cells were cultured in DMEM supplemented with 10% FBS (Corning Biosciences) and G418.

**Cell hypertrophy assays**

H9C2 cells transduced with either empty vector or NPRC were differentiated into cardiomyocyte-like cells for 48 h. A subset of cells was fixed on chamber slides and permeabilized (as above) for immunofluorescent staining with...
phalloidin (Sigma, 1:200 in PBS for 30 min) or harvested for RT-PCR in RLT buffer (Qiagen, Hilden, Germany). Cell size was measured randomly on 50 cells for a given experiment, and repeated three times. Cell area was quantified using ImageJ with a reader blinded to the experimental group. To analyze the effect of the addition of an NPRC-specific ligand, ANP-4-23, on cell hypertrophy, transfected cells were treated with 200 nM of ANP-4-23 (Phoenix Pharmaceuticals, 005-26, Burlingame, CA) for 24 h prior to immunofluorescent staining and RT-PCR.

**Statistical analysis**

Statistical analysis was completed in the GraphPad Prism 5.0 package (GraphPad Software, La Jolla, CA) using one-way ANOVA with Tukey post-hoc test or two-way ANOVA (for muscularized artery analysis) with Bonferroni post-hoc correction. For pair-wise comparisons, students t-test was used assuming equal variances. A threshold of $p < 0.05$ was considered significant.

**Results**

**AKR, but not C3H, mice develop HfEF**

After 20 weeks of high fat diet, AKR mice developed biventricular hypertrophy and increased mass when compared to C3H mice (Fig. 1a–c). By echocardiography, all groups of mice demonstrated preserved ejection fraction (Fig. 1d), but only AKR mice fed high fat diet showed evidence of concentric hypertrophy of the LV and E to A velocity reversal on transmitral Doppler signals, consistent with HfEF (Fig. 1b). By direct cardiac catheterization, AKR mice fed high fat diet, but not control diet, showed eccentric and concentric remodeling of the RV and LV (Fig. 1c). End-diastolic pressure was also selectively elevated in both the

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**Fig. 1.** Cardiac changes after 20 weeks of high fat diet. (a) H&E stain of cross-section of hearts from AKR and C3H mice fed high fat diet confirms that AKR, but not C3H, mice develop biventricular cardiac hypertrophy. (b) M-mode and transmitral Doppler echocardiography confirms ventricular hypertrophy and E to A reversal in AKR mice. (c) Representative pressure volume loops from AKR mice show biventricular concentric and eccentric remodeling consistent with HfEF. (d) Preserved ejection fraction by echo in AKR and C3H mice. (e) and (f) AKR mice fed high fat diet selectively demonstrate elevated right and left ventricular end-diastolic pressures. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to other groups. Data presented as mean ± standard deviation.

HFD: high fat diet. LVEF: left ventricular ejection fraction; LVEDP: left ventricular end-diastolic pressure; RVEDP: right ventricular end-diastolic pressure.
RV and LV of AKR mice fed high fat diet (Fig. 1e–f), suggestive of both LV and RV congestion and confirming the HFP EF phenotype.

**AKR, but not C3H, mice develop PH and pulmonary vascular remodeling**

After 20 weeks, histologic analysis of perfusion fixed lungs suggested that AKR mice developed more perivascular remodeling than C3H mice or either group of mice fed control diet (Fig. 2a). By catheterization, AKR mice fed high fat diet showed an increased RV systolic pressure and transpulmonary gradient, consistent with PH and pulmonary vascular remodeling (Fig. 2b). AKR mice fed high fat diet also showed evidence of lung congestion based on an increased ratio of wet to dry lung weight compared to other groups (Fig. 2c). Immunostaining of the fixed lung sections also showed that there was a significant increase in the number of muscularized small vessels (0–25 μM) in AKR mice fed high fat diet, as well as a decrease in proportion of muscularized medium to large vessels (50–100 μM, and >100 μM) (Fig. 2d and e). Of note, no significant changes were noted in pulmonary vascular remodeling, hemodynamically or histologically, between any of the groups at two weeks after diet change.

![Pulmonary vascular changes after 20 weeks of high fat diet. (a) AKR mice fed a high fat diet demonstrate peri-vascular remodeling. (b and c) AKR mice fed a high fat diet demonstrated elevated right ventricular systolic pressures and transpulmonary gradients. (d) AKR mice fed a high fat diet demonstrate increased lung congestion as measured by wet-to-dry lung weight. (e and f) AKR mice fed high fat diet have a greater proportion of small and medium sized muscularized vessels. *p < 0.05, **p < 0.01, and ***p < 0.001 when compared to AKR mice fed control diet. Data presented as mean ± standard deviation. HFD: high fat diet; RV: right ventricle.](image)
**AKR mice develop early disproportionate RV remodeling**

To determine early changes that contribute to the pathogenesis of PH-HFpEF in AKR mice, mice were evaluated at an early time point (two weeks) post-initiation of diet. After two weeks of high fat diet, AKR mice fed a high fat diet had a greater free wall thickness by echocardiography and higher Fulton index compared to all other groups (Fig. 3a and c) without any significant change in RV systolic pressure or pulmonary vascular resistance (Fig. 3b), consistent with disproportionate RV hypertrophy. By histologic analysis, there was evidence of increased myocyte size, but no change in lipid accumulation or fibrosis (Fig. 3d–f).

**NPRC is selectively increased in the RV of AKR mice**

To determine genes and pathways differentially regulated in AKR mice fed a high fat diet, transcriptomic changes in RNA expression of all groups was assessed by RNA sequencing. After filtering out genes that showed concordant change in expression between C3H and AKR mice to account for the effect of high fat diet alone, there were 42 genes differentially expressed between C3H and AKR mice fed high fat diet (Fig. 4a). Among the genes, the most differentially expressed gene between C3H and AKR mice was npr3, encoding for a NPRC (Fig. 4b). Validation RT-PCR confirmed that the RV, but not LV or lung parenchyma, of AKR mice showed an increase in NPRC expression in

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Fig. 3. Cardiac changes occurring after two weeks of high fat diet. (a) After two weeks, AKR mice fed high fat diet showed an increase in RV mass. (B) No change was noted in pulmonary vascular resistance after two weeks. (C) The free wall thickness of the RV was increased in AKR mice fed high fat diet. (D) No changes in fibrosis were noted between groups. (E) An increase in myocyte area was noted in AKR mice fed high fat diet histologically. (F) No change in lipid deposition was noted by Oil Red O stain between groups. *p < 0.05 and **p < 0.01 compared to control. Data presented as mean ± standard deviation.

HFD: high fat diet; RV: right ventricle; LV: left ventricle; PVR: pulmonary vascular resistance.
response to high fat diet (Fig. 4c). Western blot also confirmed that the increase in RV NPRC expression occurred primarily in AKR mice fed high fat diet at 2 and 20 weeks (Fig. 4d). Immunofluorescent staining also showed disproportionate increase in NPRC expression in the RV, but not LV, of AKR mice fed high fat diet with greater expression in the epicardium of the RV (Fig. 4e).

As NPRC is considered thought to contribute to clearance of natriuretic peptides from circulation, circulating N-terminal pro B-natriuretic peptide NT-pro-BNP levels were measured in AKR and C3H mice fed high fat or control diets. From 0 to 20 weeks, only AKR mice fed high fat diet showed a decrease in circulating NT-pro-BNP levels, as expected with increased NPRC expression. Regardless of diet, C3H mice showed an increase in NT-pro-BNP after 20 weeks (Fig. 5a). Finally, to determine the extent to which other genes within the natriuretic peptide system were affected, RNA sequencing data demonstrated that

![Figure 4](image-url)

**Fig. 4.** (a and b) RNA sequencing of the right ventricle at two weeks post-diet identified differentially regulated genes between AKR and C3H mice, of which NPRC was the most differentially expressed. (c–e) RT-PCR, high fat blot, and immunostaining confirmed increased NPRC expression selectively in the right ventricle. *p < 0.05 and ***p < 0.001 compared to control. Data presented as mean ± SEM.

NPRC: natriuretic peptide clearance receptor C; HFD: high fat diet; RV: right ventricle; LV: left ventricle.
only one natriuretic peptide receptor, NPRC, and not NPRA or NPRB, was changed in expression in the RV of AKR mice fed high fat diet (Fig. 6b–d). Additionally, no change in expression of natriuretic peptide ANP (gene NPPA) was noted, but there was a decrease in BNP (NPPB) expression (Fig. 6e and f). Of note, expression of NPPC and neprilysin NEP) were not detected in sufficient quantities in the RV.

**NPRC increases pathologic cell hypertrophy of cardiomyocytes in vitro**

To determine the effect of NPRC upon cardiomyocyte hypertrophy, cardiomyocyte-like H9C2 cells were transduced with a plasmid containing either human NPRC or an empty vector. Transduced cells demonstrated an increase in in transcript expression of NPRC (Fig. 6a) and protein expression by both immunofluorescence and Western blot (Fig. 6b). H9C2 cells overexpressing NPRC overall demonstrated an increase in cell size compared to empty vector controls (Fig. 6d). They also showed an increase in β-cardiac myosin heavy chain (MYH7) and atrial natriuretic peptide (NPPA), both genes activated in pathologic cardiomyocyte hypertrophy (Fig. 6e and f). Upon treatment with a selective ligand for NPRC, ANP-4-23, H9C2 cells overexpressing NPRC displayed a decrease in cell size (Fig. 7a) and decrease in expression of MYH7 (Fig. 7b) but not NPPA (Fig. 7c).

**Discussion**

PH due to left heart disease, and specifically HFpEF, is a highly prevalent and morbid disease with no current therapeutic options to improve mortality. This is, in part, due to a lack of animal models that faithfully recapitulate features of HFpEF, and a lack of understanding of the pathophysiology of RV dysfunction in the context of PH-HFpEF.
Given that obesity and metabolic syndrome are central to the pathogenesis of PH, RV dysfunction, and HFpEF, our study validated and extended a relevant, obesity-induced model of PH and HFpEF to study the pathogenesis of RV dysfunction in HFpEF. Exploiting common genetic variation among available mouse strains to account for effects of diet alone, we identified genes uniquely contributing to the development of RV dysfunction in the context of obesity-induced PH-HFpEF. Of these genes, we identified a therapeutic target in natriuretic peptide receptor C, a clearance receptor for natriuretic peptide, and showed that it contributes to cardiomyocyte hypertrophy.

While our model shares many features with other models of HFpEF, including the use of high fat diet in mice prone to development of metabolic syndrome, our study is unique in that it focused on RV dysfunction, a common cause of death in this patient population. Using a relevant model of obesity-induced HFpEF, we found RV hypertrophy to be an early manifestation of disease in response to high fat diet in this model. Our model builds upon a previous study by Meng et al. that identified the AKR mouse strain as susceptible to high fat diet-induced hemodynamic and structural changes consistent with HFpEF and PH. Our study shows very similar hemodynamic and structural changes in the heart and lung after 20 weeks of high fat diet compared to their study, including similar increases in RV systolic pressure measurement, left ventricular mass, RV mass, left ventricular end-diastolic pressure, and pulmonary vascular resistance. A key addition of our study is the finding of changes in RV mass as early as two weeks post-high fat diet.

**Fig. 6.** (a) Confirmation of increased NPRC expression by PCR and immunofluorescence in transfected H9C2 cells. (B) Transfected H9C2 cells retain their ability to differentiate based on troponin stain. (c and d) Overexpression of NPRC in H9C2 causes increased cell hypertrophy based on increased cell size. (e) Increased expression of hypertrophic markers, MYH7 and NPPA in NPRC overexpressing H9C2 cells. *p < 0.05, **p < 0.01, and ***p < 0.001 compared to control. Data presented as either mean ± standard deviation or Tukey box-and-whisker plot. NPRC: natriuretic peptide clearance receptor C.
may contribute to a unique phenotype within the population such as diabetes mellitus, obesity, and metabolic syndrome. Clinical studies have suggested that metabolic derangements responsible for the HFpEF phenotype in patients with metabolic syndrome. Since NPRC’s expression and function is integral to the development of metabolic syndrome, our findings also support the emerging hypothesis that metabolic derangements may be a driver of HFpEF in certain subsets of patients. Future studies are necessary to directly test the extent to which NPRC is responsible for the HFpEF phenotype in patients with metabolic syndrome, though.

A key finding of our study identifies natriuretic peptide receptor C as possible contributor to RV hypertrophy. Our study shows an association between RV hypertrophy and increased NPRC expression in vivo, and both an increase in cell size and activation of a hypertrophic gene program in vitro. The natriuretic peptides are central mediators of the body’s systemic response to increased ventricular strain, generally activating pathways that promote myocyte lusitropy, systemic vasorelaxation, and natriuresis to reduce strain upon the heart. Recent studies, however, also suggest that natriuretic peptides play a prominent role in regulating obesity and metabolic syndrome. Functioning through its two canonical receptors, NPRA and NPRB, natriuretic peptides have been found to increase mitochondrial biogenesis and prevent obesity in a guanosine monophosphate cGMP-dependent fashion. The third receptor, NPRC, is a transmembrane membrane receptor similar to NPRA and NPRB, but has a short cytoplasmic tail lacking a guanylyl cyclase catalytic domain. While thought to be a “clearance receptor” that internalizes natriuretic peptides, studies have shown that it can also directly alter signaling through inhibition of adenylyl cyclase and adenosine cyclic 3’5’-monophosphate cAMP production via pertussis-sensitive Gi protein. Additionally, genetic knockout of NPRC restores natriuretic responsiveness in adipose tissue to reduce insulin resistance and obesity. The finding that a receptor that regulates activity and function of circulating natriuretic peptides may be important in the pathogenesis of PH-HFpEF and RV dysfunction is consistent with the growing perception that both HFpEF and PH are organ-specific manifestations of systemic derangements.

This study has a number of limitations. The two mouse strains differ genetically in a number of ways, and thus there are many genetic differences between the two mouse strains that could have given rise to a differential response to PH-HFpEF and RV dysfunction. Even in the current study, a total of 42 genes were noted to be differentially regulated between AKR and C3H mice in the RV in response to high fat diet. Of them, NPRC was chosen for further study given its known connection to heart failure, PH, metabolic

![Figure 7](image_url)
Conflict of interest
The author(s) declare that there is no conflict of interest.

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