

UC Irvine

UC Irvine Previously Published Works

Title

LCZ696 (Sacubitril/Valsartan), an Angiotensin-Receptor Neprilysin Inhibitor, Attenuates Cardiac Hypertrophy, Fibrosis, and Vasculopathy in a Rat Model of Chronic Kidney Disease

Permalink

<https://escholarship.org/uc/item/9xt45515>

Journal

Journal of Cardiac Failure, 24(4)

ISSN

1071-9164

Authors

Suematsu, Yasunori
Jing, Wanghui
Nunes, Ane
[et al.](#)

Publication Date

2018-04-01

DOI

10.1016/j.cardfail.2017.12.010

Peer reviewed

Accepted Manuscript

Title: LCZ696 (Sacubitril/valsartan), an Angiotensin-Receptor Neprilysin Inhibitor, Attenuates Cardiac Hypertrophy, Fibrosis and Vasculopathy in a Rat Model of Chronic Kidney Disease

Author: Yasunori Suematsu, Wanghui Jing, Ane Nunes, Moti L. Kashyap, Mahyar Khazaeli, Nosratola D. Vaziri, Hamid Moradi

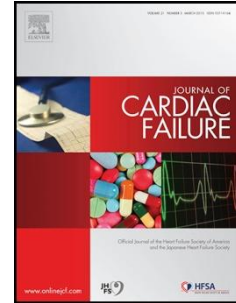
PII: S1071-9164(18)30002-2
DOI: <https://doi.org/10.1016/j.cardfail.2017.12.010>
Reference: YJCAF 4083

To appear in: *Journal of Cardiac Failure*

Received date: 2-9-2017
Revised date: 11-12-2017
Accepted date: 21-12-2017

Please cite this article as: Yasunori Suematsu, Wanghui Jing, Ane Nunes, Moti L. Kashyap, Mahyar Khazaeli, Nosratola D. Vaziri, Hamid Moradi, LCZ696 (Sacubitril/valsartan), an Angiotensin-Receptor Neprilysin Inhibitor, Attenuates Cardiac Hypertrophy, Fibrosis and Vasculopathy in a Rat Model of Chronic Kidney Disease, *Journal of Cardiac Failure* (2018), <https://doi.org/10.1016/j.cardfail.2017.12.010>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Title

LCZ696 (Sacubitril/valsartan), an Angiotensin-Receptor Neprilysin Inhibitor, Attenuates Cardiac Hypertrophy, Fibrosis and Vasculopathy in a Rat Model of Chronic Kidney Disease

Short Title: LCZ696 Attenuates Hypertrophy and Fibrosis

Yasunori Suematsu, MD, PhD^{*1,2}, Wanghui Jing, PhD^{*1,3}, Ane Nunes, PhD¹, Moti L. Kashyap⁴,

Mahyar Khazaeli, MD¹, Nosratola D. Vaziri, MD¹, Hamid Moradi, MD^{1,2}

¹Division of Nephrology and Hypertension, Department of Medicine, University of California, Irvine, CA, USA

³Nephrology Section, Long Beach VA Healthcare System, CA, USA

³School of Pharmacy, Xi'an Jiaotong University, Xi'an, PR China

⁴Cardiology Section, Long Beach VA Healthcare System, CA, USA

* Authors contributed equally to this manuscript

E-mail address of each author: YS is gjsnh423@yahoo.co.jp, WJ is wanghuij@uci.edu, AN is nunes.acf@gmail.com, MK is kashyapmoti@gmail.com, MK is mahyar.khazaeli@gmail.com, NV is ndvaziri@uci.edu, and HM is hmoradi@uci.edu

Address correspondence to:

Hamid Moradi, MD

Department of Medicine, Nephrology Section, Long Beach VA Healthcare System

5901 E. 7th street, Long Beach, CA 90822

Tel: +1-714-456-5142, Fax: +1-714-456-6034, Email: hamid.moradi@va.gov

N.D. Vaziri MD

Division of Nephrology and Hypertension, University of California, Irvine

333 City Blvd West, Suite 400, Orange, CA 92868

Tel: +1-714-456-8338, Fax: +1-714-456-6034, Email: ndvaziri@uci.edu

Highlights

- The effect of LCZ696 (Sacubitril/valsartan) against cardiorenal syndrome is proposed.
- LCZ696 more improved CKD related heart failure than valsartan therapy alone.
- LCZ696 attenuated CKD related cardiac hypertrophy and fibrosis and aortic fibrosis.
- Effects of LCZ696 for heart are mediated by anti-inflammation and oxidative stress.
- LCZ696 also improved indicators of mitochondrial mass/function.

Abstract

Background: Chronic kidney disease (CKD) is associated with cardiac hypertrophy, fibrosis and increased risk of cardiovascular mortality. LCZ696 (Sacubitril/valsartan) is a promising agent which has shown significant potential in treatment of heart failure. We hypothesized that LCZ696 is more effective than valsartan alone in treatment of cardiovascular abnormalities associated with experimental CKD.

Methods and Results: Male Sprague Dawley rats underwent 5/6 nephrectomy and were subsequently randomized to no treatment (CKD), 30 mg/kg valsartan (VAL), or 60 mg/kg LCZ696 (LCZ). After 8 weeks, cardiovascular parameters including markers of inflammation, oxidative stress, mitochondrial abundance/function, hypertrophy and fibrosis were measured. Treatment with LCZ resulted in significant improvements in the heart/body weight ratio and serum concentrations of N-terminal pro B-type natriuretic peptides (NT-pro BNP) and fibroblast growth factor-23 (FGF-23) along with improvement of kidney function. In addition, LCZ ameliorated aortic fibrosis and cardiac hypertrophy and fibrosis, reduced markers of cardiac oxidative stress and inflammation and improved indicators of mitochondrial mass/function. While VAL-therapy also improved some of these indices, treatment with LCZ was more effective than VAL therapy alone.

Conclusions: CKD-associated cardiovascular abnormalities including myocardial hypertrophy, fibrosis, inflammation, oxidative stress, and mitochondrial depletion/dysfunction were more effectively attenuated by LCZ treatment than VAL therapy alone.

Keywords: LCZ696, Chronic kidney disease, Fibrosis, Hypertrophy

Accepted Manuscript

Introduction

Chronic kidney disease (CKD) is an irreversible condition marked by progressive kidney damage culminating in end stage renal disease (ESRD). Due to the pervasive nature of the underlying conditions that cause CKD such as diabetes and hypertension, the prevalence of CKD has continued to increase in the United States (US) and worldwide.^{1,2} However, it has also been noted that while approximately 9% of the US population have CKD translating into more than 20 million adults, only a small fraction reach ESRD.¹ This discrepancy is partly attributed to the significantly increased risk of cardiovascular disease (CVD) and mortality in this patient population^{1,3} and it is understood that in a majority of patients with CKD, premature death from CVD is a more prominent risk than progression to ESRD.⁴ In addition, regardless of the stage of CKD, CVD and mortality are major contributors to the exceedingly poor outcomes in this patient population.

The pathogenesis of cardiac and vascular dysfunction in CKD is complex and multifactorial. While traditional risk factors, including hypertension and diabetes, make a significant contribution to pathogenesis of CVD, there are numerous nontraditional risk factors such as systemic inflammation, oxidative stress, mitochondrial dysfunction, reduced Klotho and elevated FGF-23 levels, which also play central roles in this process. As a result of the interplay between these factors, CKD is associated with ischemic heart disease, left ventricular (LV) hypertrophy and fibrosis and increased risk of sudden cardiac death. Therefore, therapeutic strategies which address the mechanisms responsible for these pathophysiologic processes hold significant potential in treatment of CKD-related CVD and mortality.

One novel therapeutic approach which has shown a promise in the treatment of chronic heart failure (HF) is enhancement of vasoactive peptides such as natriuretic peptides (NPs). Modulation of NPs (including atrial, B-type and C-type natriuretic peptide) can address many of the underlying pathologic processes involved in CKD-related cardiomyopathy including inflammation, cardiac hypertrophy and fibrosis. The level of endogenous vasoactive peptides can be enhanced via inhibition of neprilysin which is the key enzyme involved in the degradation of these proteins.⁵ In fact pharmacologic inhibition of neprilysin has been shown to be effective in treatment of many different

forms of HF and cardiomyopathy.⁶⁻⁸ While previous formulations of neprilysin inhibitors, which were combined with angiotensin converting enzyme inhibitors (ACEI), increased bradykinin production and risk of angioedema, a more recent drug combines an angiotensin receptor blocker (valsartan) and a neprilysin inhibitor prodrug (sacubitril). This new drug, which is called LCZ696, has been shown to provide the same beneficial effects with less risk of angioedema.⁹ Furthermore, it has been demonstrated that LCZ696 is more effective in the treatment of HF with reduced ejection fraction (HFrEF) than standard monotherapy with ACEIs. In the PARADIGM trial, LCZ696 therapy resulted in a significant reduction of serum N-terminal pro B-type natriuretic peptides (NT-proBNP), HF hospitalizations and risk of cardiovascular death when compared with enalapril.⁹

In light of the above observations, we hypothesized that administration of LCZ696 in an animal model of CKD will ameliorate CKD-associated cardiac and vascular hypertrophy and fibrosis beyond ARB therapy alone. We used renal mass reduction via surgical 5/6 nephrectomy. In addition, we included a group of CKD animals treated with valsartan alone to further delineate any potential benefit of LCZ696 beyond that conferred by renin-angiotensin-aldosterone system (RAAS) blockade alone.

Material and Methods

Study groups

Male Sprague-Dawley rats were purchased from Charles River Labs, Raleigh, NC, USA and used in this study. All experiments were approved by the University of California Irvine Institutional Committee for the Use and Care of Experimental Animals. All surgeries were performed as previously described and based on well-established protocols. For details please see supplement section. Animals were randomly assigned and underwent sham surgery (CTL, n=6) or 5/6 nephrectomy and after two weeks the animals with nephron mass reduction were randomized into three groups: no treatment (CKD, n=10), valsartan 30 mg/kg (VAL, n=12) or LCZ696 60 mg/kg (LCZ, n=12).¹⁰ Animals were euthanized after 8 weeks of treatment and the tissue of interest were either fixed in 10% buffered formalin or snap frozen in liquid nitrogen and stored at -80°C for protein

analysis. Serum creatinine was measured by George M O'Brian Kidney Research Core at University of Texas Southwestern Medical Center. Urinary protein, serum blood urea nitrogen (BUN), NT-proBNP and fibroblast growth factor 23 (FGF-23) and heart protein carbonyl content were measured using commercial kits according to manufacturer protocols (see methods supplement).

Histologic Analysis

The heart and aorta were removed immediately after sacrifice and prepared for histological and Western blot analyses. The sections taken for histological analysis were fixed in 10% buffered formalin. The cross-sections of the heart and aorta were embedded in paraffin and cut at two micron sections. Subsequently, these sections were stained with hematoxylin & eosin (H&E) or Masson's trichrome and analyzed using established protocols (see supplement section for details).^{11, 12}

Western blot analyses

Cytoplasmic and nuclear protein extracts from the LV tissue were prepared and the proteins of interest were measured by Western blot analysis as previously described¹³, using the appropriate antibodies (for details please see the material supplement section). The expression level was determined by measurement of the corresponding band intensities using Quantity one 1-D Analysis Software version 4.5.2 (Bio-Rad Laboratories, Richmond, CA, USA). Protein levels were expressed relative to housekeeping protein levels and the basal expression relative value in CTL group was considered to be 1.0.

Statistical analysis

The statistical analysis was performed using GraphPad Prism, version 6.01 (GraphPad Software, Inc. San Diego, CA, USA). The values are expressed as the mean \pm standard error. Group differences were analyzed by one-way analysis of variance with post hoc comparison. Tukey's post-test was used to determine differences between the groups. Statistical significance was defined as a p-value of less than 0.05.

Results

General Data

We analyzed the data 8-week post-randomization. As expected, BUN, serum creatinine and urine protein excretion were significantly higher in CKD animals compared with CTL animals (Table 1). In addition, there was a significantly higher in the ratio of heart to body weight and a modest but statistically significantly higher in systolic arterial pressure (SBP) in CKD animals when compared to the CTL group. Serum concentration of NT-pro BNP and FGF-23 were significantly higher in the CKD group when compared to CTL animals. Both valsartan and LCZ administration resulted in a significant improvement of SBP and urinary protein excretion while LCZ therapy also resulted in a significant improvement in BUN and serum creatinine concentration. While the ratio of heart to body weight and hypertrophy were significantly improved in both VAL and LCZ treated animals, the amelioration of these abnormalities was substantially greater with LCZ therapy when compared to VAL therapy alone.

Impact of CKD and treatment modalities on cardiac hypertrophy

Representative photomicrographs of H&E stained LV cardiomyocytes are shown in Figure 1 and Supplemental Table 1. The LV tissue in the CKD animals exhibited significant hypertrophy and large size of cardiomyocytes. In addition, cardiac markers of hypertrophy including LV protein abundance of myosin heavy chain 7B (MyH7B), tropomyosin and myocardin were significantly higher in CKD animals when compared to the CTL group. Both VAL and LCZ therapies resulted in reduction in LV cardiomyocyte size and protein abundance of MyH7B and myocardin. However, the degree of improvement in these indices was significantly greater with LCZ treatment when compared with VAL therapy alone.

Impact of CKD and treatment modalities on cardiac and aortic fibrosis

Representative photomicrographs of Masson's trichrome stained LV tissue are shown in Figure 2 and Supplemental Table 1. The ratio of interstitial and perivascular fibrosis were significantly higher in LV of animals with CKD when compared with the CTL group. In addition, the molecular markers of cardiac fibrosis including the LV protein abundance of transforming growth factor- β (TGF- β) and α smooth muscle (α -SM) actin were significantly higher in the CKD group when compared to the CTL

animals. Valsartan and LCZ therapy significantly attenuated LV fibrosis and reduced the abundance of the protein markers of fibrosis. However, the degree of improvement in perivascular fibrosis and TGF- β content was significantly greater with LCZ treatment compared with VAL therapy alone. We also evaluated the ratio of wall to lumen and collagen content in an area of the aorta (Figure 3 and Supplemental Table1). The CKD animals showed a significantly higher aorta wall to lumen ratio and collagen content when compared to CTL group. While both LCZ and VAL were effective in improving the wall to lumen ratio, only LCZ therapy significantly reduced the collagen content in the aorta.

Impact of CKD and treatment modalities on cardiac inflammatory and oxidative pathways

Data are shown in Figures 4 and 5. The nuclear translocation of the p65 subunit of nuclear factor-kappa B (NF- κ B) indicating its activation was significantly higher in untreated CKD animals when compared to CTL group. This was accompanied by upregulation of pro-inflammatory and reactive oxygen, nitrogen and halogen species-generating proteins including monocyte chemotactic protein 1 (MCP-1), cyclooxygenase 2 (COX-2), gp91^{Phox}, nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX-4), myeloperoxidase (MPO) and higher cardiac protein carbonyl content. While both VAL and LCZ therapy reversed or markedly attenuated many of these abnormalities, treatment with LCZ was more effective than VAL therapy alone.

Impact of CKD and treatment modalities on cardiac Nrf2 pathway

Data are shown in Figure 6. The LV tissue from the untreated CKD animals showed marked reduction in nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) and down-regulation of its key target gene products including copper-zinc superoxide dismutase (Cu/Zn SOD), catalase, glutathione peroxidase (GPX), heme oxygenase 1 (HO-1), and endothelial nitric oxide synthase (eNOS). These findings point to impairment of the Nrf2 pathway which contributes to the prevailing oxidative stress, inflammation and fibrosis of the cardiac tissue in CKD. While both VAL and LCZ therapy were effective in significantly improving the protein abundance of many of these enzymes, only LCZ treatment was associated with a significantly higher nuclear translocation of Nrf2 leading to the activation of this important antioxidant pathway.

Impact of CKD and treatment modalities on cardiac mitochondrial proteins

Data are shown in Figure 7. The protein abundance of adenosine triphosphate (ATP) synthase components beta/porin-1 was significantly decreased in LV cardiac tissue from untreated CKD animals when compared with controls. In addition, porin-1/GAPDH ratio determined in the whole heart tissue homogenate was markedly reduced in the CKD animals indicating a significant reduction in cardiac mitochondrial mass. LCZ therapy ameliorated these abnormalities more effectively than VAL therapy alone.

Discussion

As expected, CKD resulted in significant cardiac and aortic fibrosis. Furthermore, serum indicators of cardiovascular hypertrophy and fibrosis, mainly FGF-23 and NT-pro BNP, were significantly elevated in animals with CKD when compared to controls. These findings were accompanied by upregulation of markers of hypertrophy and fibrosis as well as activation of inflammatory and oxidative stress pathways in the cardiac tissue. Furthermore, we found impairment of cardiac antioxidant machinery caused by a disruption of the Nrf2 pathway. These findings were consistent with previously reported impairment of Nrf2 pathway and upregulation of oxidative, inflammatory and fibrosis pathways in the kidney and vascular tissue of CKD rats^{13, 14} and impaired Nrf2 activity in the circulating leukocytes of ESRD patients.¹⁵ Additionally, animals with CKD had reduced left ventricular mitochondrial mass as well as decreased abundance of proteins involved in mitochondrial function. Treatment with LCZ resulted in a substantial improvement of these indices, beyond that achieved with valsartan therapy alone. To our knowledge, this is the first study which comprehensively evaluates the impact of LCZ therapy on markers of cardiac and vascular pathology in a well-established rat model of CKD.

It is well known CKD is associated with significant cardiac and vascular abnormalities which result in increased risk of cardiovascular mortality. Many diverse mechanisms contribute to the development of CVD in CKD which manifest mainly as LV hypertrophy, cardiovascular fibrosis, arteriosclerosis and atherosclerosis.¹⁶ These mechanisms include oxidative stress, inflammation, fluid and electrolyte

disorders, retained uremic metabolites and mitochondrial dysfunction. In this regard, studies have documented oxidative stress and inflammation in the cardiac tissue of animals with experimental CKD.¹⁷⁻¹⁹ CKD also results in mitochondrial depletion and dysfunction in the myocardium and skeletal muscle.^{20,21} In fact, mitochondria from cardiac tissue of CKD animals were unable to retain calcium, had diminished cytochrome C levels and decreased respiratory function. Given the known vulnerability of mitochondria to calcium and oxidative stress, CKD-induced mitochondrial dysfunction may be, in part, related to the pervasive oxidative stress and altered calcium metabolism.²¹

Partly as a result of the above-mentioned mechanisms, CKD results in significant myocardial hypertrophy and activation of cellular apoptotic signals which trigger activation of extracellular matrix production pathways leading to fibrosis.^{16,22} In fact CKD induced by subtotal nephrectomy leads to increased cross-sectional area of cardiomyocytes and increased abundance of protein markers of hypertrophy such as α -SM actin.²³ In addition, cardiac tissue from CKD animals had increased matrix and TGF- β abundance and reduced matrix metalloproteinase-1.²⁴ It should also be noted that there are other CKD-related factors which play a role in the pathogenesis of uremic cardiomyopathy such as increased concentrations of FGF-23, Klotho deficiency and activation of the RAAS.^{25,26} The effectiveness of RAAS inhibition in treatment of HF has been demonstrated in numerous clinical trials using ACEI or ARB therapy.²⁷ Moreover, use of RAAS blockade in patients with CKD is advocated based on its renoprotective properties as well as the cardioprotective potential shown on the sub-analyses of data from cardiovascular trials.²⁸ However, despite optimum use of RAAS inhibition, patients with CKD continue to suffer from a significant residual risk for CVD and poor cardiovascular outcomes. Therefore, RAAS blockade only partially addresses the underlying mechanisms responsible for uremic CVD. In this regard, a new therapeutic target which has shown significant potential is augmentation of vasoactive peptides such as NPs. The latter consist of a family of peptides which have a wide range of cardiovascular effects including amelioration of volume overload and hypertension.²⁹ While the inhibitory effect of NPs on the RAAS pathway undoubtedly plays a major role in their cardioprotective characteristics, there is also evidence that NPs have important

cardioprotective antioxidant, anti-inflammatory and antifibrotic properties.^{29,30} For example, NPs have been shown to inhibit activation of the nuclear factor NF- κ B and reduce the production of inflammatory mediators in macrophages, endothelial cells and cardiomyocytes.^{31,32} In addition, NPs have been shown to have important protective effects on myocyte mitochondrial function and cardiac mitochondria by preventing aberrant calcium handling and reducing mitochondrial ROS production.³³ Furthermore, enhancement of NPs in CKD-related CVD would represent a logical therapeutic approach given that inflammation, oxidative stress and mitochondrial dysfunction are major contributors to uremic cardiomyopathy.

There are several limitations of the current study which need to be mentioned. Firstly, in the present study we did not assess functional markers using echocardiography or Millar conductance catheter system and future studies are needed to provide further details regarding vascular compliance and mitochondrial function. Secondly, in our study we did not check LV weight in isolation given concerns for introducing operator bias/variability. In addition, the contribution of the myocyte hypertrophy and collagen deposition to heart weight/LV weight changes cannot be estimated based on our findings. Therefore, future studies are needed to build on our findings and provide further histological details regarding the impact of LCZ on cardiomyocytes in CKD. Thirdly, additional mechanistic studies are needed to further elucidate the molecular pathways by which LCZ therapy may mediate the many different effects observed in this study. Furthermore, clinical utility and effectiveness of LCZ will need to be confirmed in randomized clinical trials in patients with CKD.³⁵ Finally, the model used in this study is that of advanced CKD due to renal mass reduction and the effectiveness of LCZ therapy in treatment of CVD in different models of CKD such as that of glomerulosclerosis due to heavy proteinuria should be determined in future investigations.

Conclusions

LCZ therapy in rats with uremic cardiomyopathy resulted in significant improvement of cardiac and aortic hypertrophy and fibrosis. These findings may be a result of a direct cardioprotective impact of LCZ and secondary beneficial effects of improved BP control and renal function. These improvements were associated with and likely mediated by a significant amelioration of myocardial

inflammation and oxidative stress, improvement of Nrf2-mediated antioxidant system, and restoration of mitochondrial abundance and activity. It should also be noted that LCZ therapy was more effective than valsartan treatment alone in ameliorating the markers of uremic cardiomyopathy in this experimental model.

Acknowledgements

We thank UT Southwestern O'Brien Kidney Center for measuring serum creatinine concentrations for this project.

Disclosure

This work was supported by Novartis (LCZ696BUSNC03T). HM is supported by a career development award from the Office of Research and Development of the Department of Veterans Affairs 1 IK CX 001043-01A2. YS is supported overseas research scholarship by Sumitomo Life Welfare and Culture Foundation and Fukuoka University, School of Medicine Alumni, Eboshikai. WHJ is partly supported by the International Postdoctoral Exchange Fellowship Program (No.20150050). HM has received grant funding from the NIH, VA ORD and Novartis.

References

1. US Renal Data System: Excerpts from the USRDS. Annual Data Report: Atlas of End-Stage Renal Disease in the United States. 2012.
2. Zhang L, Wang F, Wang L, Wang W, Liu B, Liu J, et al. Prevalence of chronic kidney disease in China: a cross-sectional survey. *Lancet*. 2012;379:815-22.
3. Chronic Kidney Disease Prognosis Consortium, Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet*. 2010;375:2073-81.
4. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, Jafar TH, Heerspink HJ, Mann JF, et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet*. 2013;382:339-52.
5. Wilkins MR, Redondo J, Brown LA. The natriuretic-peptide family. *Lancet*. 1997;349:1307-10.
6. Packer M, Califf RM, Konstam MA, Krum H, McMurray JJ, Rouleau JL, et al. Comparison of omapatrilat and enalapril in patients with chronic heart failure: the Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events (OVERTURE). *Circulation*. 2002;106:920-6.
7. Suematsu Y, Miura S, Goto M, Matsuo Y, Arimura T, Kuwano T, et al. LCZ696, an angiotensin receptor-neprilysin inhibitor, improves cardiac function with the attenuation of fibrosis in heart failure with reduced ejection fraction in streptozotocin-induced diabetic mice. *Eur J Heart Fail*. 2016;18:386-93.
8. Von Lueder TG, Wang BH, Kompa AR, Huang L, Webb R, Jordaan P, et al. Angiotensin receptor neprilysin inhibitor LCZ696 attenuates cardiac remodeling and dysfunction after myocardial infarction by reducing cardiac fibrosis and hypertrophy. *Circ Heart Fail*. 2015;8:71-8.
9. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med*. 2014;371:993-1004.
10. Vaziri ND, Ni Z, Oveisi F, Liang K, Pandian R. Enhanced nitric oxide inactivation and

- protein nitration by reactive oxygen species in renal insufficiency. *Hypertension*. 2002;39:135-41.
11. Zhao QD, Viswanadhapalli S, Williams P, Shi Q, Tan C, Yi X, et al. NADPH oxidase 4 induces cardiac fibrosis and hypertrophy through activating Akt/mTOR and NFkappaB signaling pathways. *Circulation*. 2015;131:643-55.
 12. Bhatta A, Yao L, Toque HA, Shatanawi A, Xu Z, Caldwell RB, et al. Correction: angiotensin II-induced arterial thickening, fibrosis and stiffening involves elevated arginase function. *PLoS One*. 2015;10:e0127110.
 13. Kim HJ, Vaziri ND. Contribution of impaired Nrf2-Keap1 pathway to oxidative stress and inflammation in chronic renal failure. *Am J Physiol Renal Physiol*. 2010;298:F662-71.
 14. Aminzadeh MA, Reisman SA, Vaziri ND, Shelkovnikov S, Farzaneh SH, Khazaeli M, et al. The synthetic triterpenoid RTA dh404 (CDDO-dhTFEA) restores endothelial function impaired by reduced Nrf2 activity in chronic kidney disease. *Redox Biol*. 2013;1:527-31.
 15. Pedruzzi LM, Cardozo LF, Daleprane JB, Stockler-Pinto MB, Monteiro EB, Leite M, Jr., et al. Systemic inflammation and oxidative stress in hemodialysis patients are associated with down-regulation of Nrf2. *J Nephrol*. 2015;28:495-501.
 16. Di Lullo L, Gorini A, Russo D, Santoboni A, Ronco C. Left Ventricular Hypertrophy in Chronic Kidney Disease Patients: From Pathophysiology to Treatment. *Cardiorenal Med*. 2015;5:254-66.
 17. Chen J, Kieswich JE, Chiazza F, Moyes AJ, Gobbetti T, Purvis GS, et al. IkappaB Kinase Inhibitor Attenuates Sepsis-Induced Cardiac Dysfunction in CKD. *J Am Soc Nephrol*. 2017;28:94-105.
 18. Hernandez-Resendiz S, Correa F, Garcia-Nino WR, Buelna-Chontal M, Roldan FJ, Ramirez-Camacho I, et al. Cardioprotection by curcumin post-treatment in rats with established chronic kidney disease. *Cardiovasc Drugs Ther*. 2015;29:111-20.
 19. Bai Y, Sigala W, Adams GR, Vaziri ND. Effect of exercise on cardiac tissue oxidative and inflammatory mediators in chronic kidney disease. *Am J Nephrol*. 2009;29:213-21.
 20. Yazdi PG, Moradi H, Yang JY, Wang PH, Vaziri ND. Skeletal muscle mitochondrial

- depletion and dysfunction in chronic kidney disease. *Int J Clin Exp Med*. 2013;6:532-9.
21. Taylor D, Bhandari S, Seymour AM. Mitochondrial dysfunction in uremic cardiomyopathy. *Am J Physiol Renal Physiol*. 2015;308:F579-87.
 22. Lekawanvijit S, Kompa AR, Wang BH, Kelly DJ, Krum H. Cardiorenal syndrome: the emerging role of protein-bound uremic toxins. *Circ Res*. 2012;111:1470-83.
 23. Lekawanvijit S, Kompa AR, Manabe M, Wang BH, Langham RG, Nishijima F, et al. Chronic kidney disease-induced cardiac fibrosis is ameliorated by reducing circulating levels of a non-dialysable uremic toxin, indoxyl sulfate. *PLoS One*. 2012;7:e41281.
 24. Yin J, Lu Z, Wang F, Jiang Z, Lu L, Miao N, et al. Renalase attenuates hypertension, renal injury and cardiac remodelling in rats with subtotal nephrectomy. *J Cell Mol Med*. 2016;20:1106-17.
 25. Hu MC, Shi M, Cho HJ, Adams-Huet B, Paek J, Hill K, et al. Klotho and phosphate are modulators of pathologic uremic cardiac remodeling. *J Am Soc Nephrol*. 2015;26:1290-302.
 26. Hoogwerf BJ. Renin-angiotensin system blockade and cardiovascular and renal protection. *Am J Cardiol*. 2010;105:30A-5A.
 27. Sayer G, Bhat G. The renin-angiotensin-aldosterone system and heart failure. *Cardiol Clin*. 2014;32:21-32, vii.
 28. Sarafidis PA, Ruilope LM. Aggressive blood pressure reduction and renin-angiotensin system blockade in chronic kidney disease: time for re-evaluation? *Kidney Int*. 2014;85:536-46.
 29. Kerkela R, Ulvila J, Magga J. Natriuretic Peptides in the Regulation of Cardiovascular Physiology and Metabolic Events. *J Am Heart Assoc*. 2015;4:e002423.
 30. Fujita S, Shimojo N, Terasaki F, Otsuka K, Hosotani N, Kohda Y, et al. Atrial natriuretic peptide exerts protective action against angiotensin II-induced cardiac remodeling by attenuating inflammation via endothelin-1/endothelin receptor A cascade. *Heart Vessels*. 2013;28:646-57.
 31. Della Penna SL, Roson MI, Toblli JE, Fernandez BE. Role of angiotensin II and oxidative stress in renal inflammation by hypernatremia: benefits of atrial natriuretic peptide, losartan, and tempol. *Free Radic Res*. 2015;49:383-96.
 32. Kapoun AM, Liang F, O'Young G, Damm DL, Quon D, White RT, et al. B-type natriuretic

peptide exerts broad functional opposition to transforming growth factor-beta in primary human cardiac fibroblasts: fibrosis, myofibroblast conversion, proliferation, and inflammation. *Circ Res.* 2004;94:453-61.

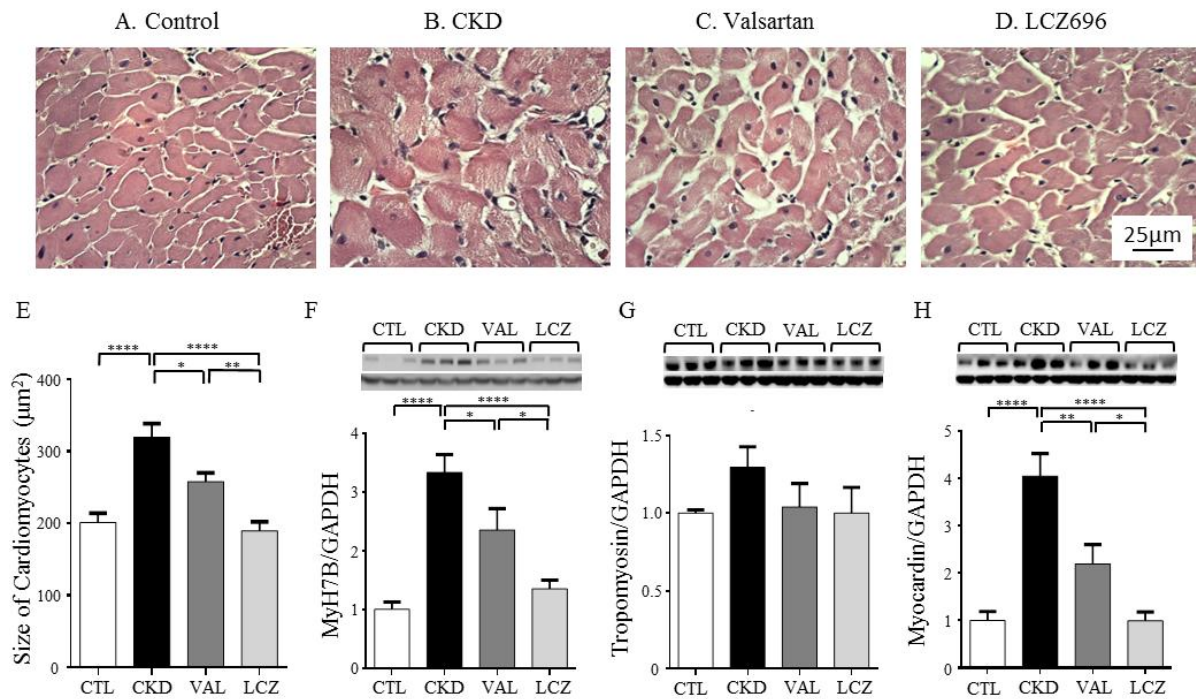
33. Grois L, Hupf J, Reinders J, Schroder J, Dietl A, Schmid PM, et al. Combined Inhibition of the Renin-Angiotensin System and Neprilysin Positively Influences Complex Mitochondrial Adaptations in Progressive Experimental Heart Failure. *PLoS One.* 2017;12:e0169743.

34. Murakami W, Kobayashi S, Susa T, Nanno T, Ishiguchi H, Myoren T, et al. Recombinant Atrial Natriuretic Peptide Prevents Aberrant Ca²⁺ Leakage through the Ryanodine Receptor by Suppressing Mitochondrial Reactive Oxygen Species Production Induced by Isoproterenol in Failing Cardiomyocytes. *PLoS One.* 2016;11:e0163250.

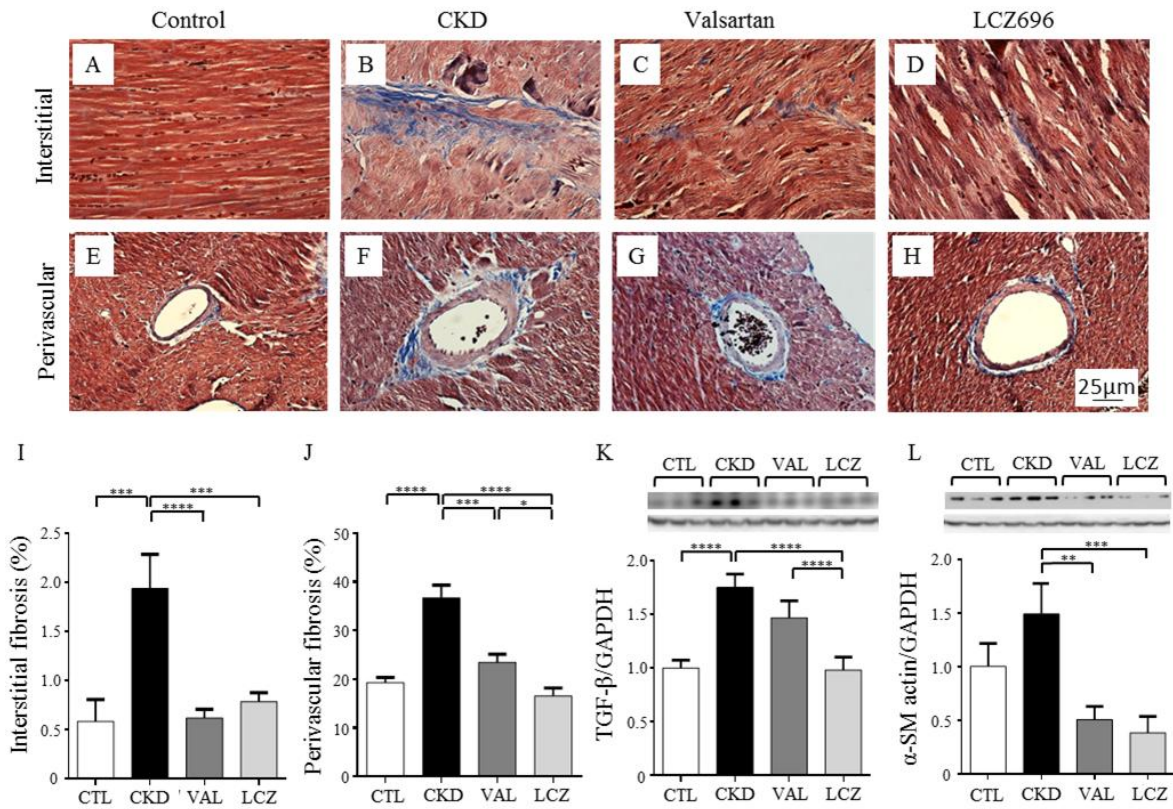
35. Group. UH-IC. Randomized multicentre pilot study of sacubitril/valsartan versus irbesartan in patients with chronic kidney disease: United Kingdom Heart and Renal Protection (HARP)- III- rationale, trial design and baseline data. *Nephrol Dial Transplant.* 2016.

Figures and figure legends

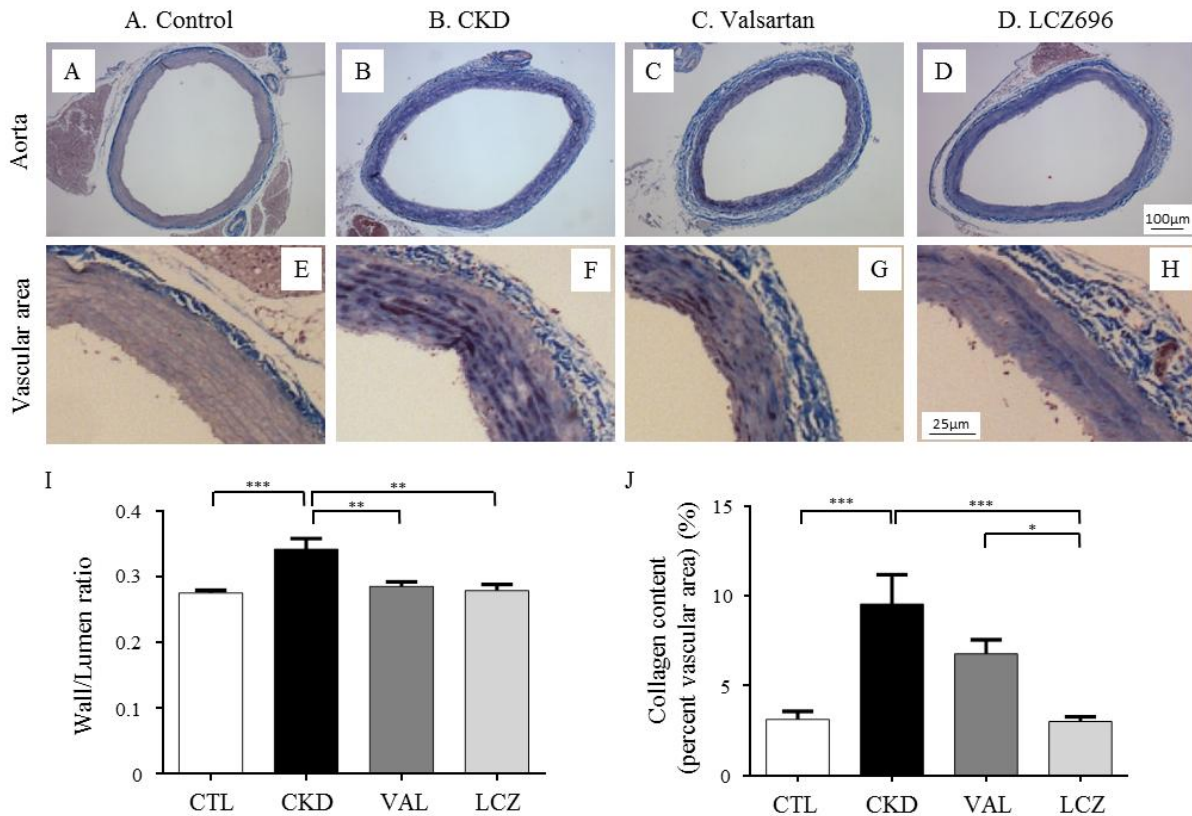
Figure 1. Impact of treatment on cardiac hypertrophy



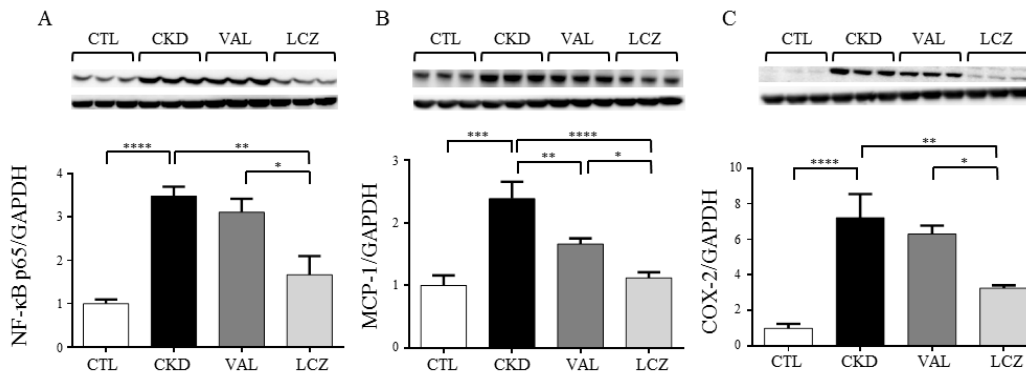
Representative microphotographs of LV tissue stained with hematoxylin & eosin in the following groups (A) CTL, (B) CKD, (C) VAL, and (D) LCZ. (E) Quantification analysis for mean cross sectional areas of cardiomyocytes in each group. (F through H) Representative Western blots and group data depicting the LV protein abundance of (F) MyH7B, (G) tropomyosin, and (H) myocardin 8-weeks post-randomization. Data are mean ± SEM. CTL (n=6), CKD (n=10), VAL (n=12), LCZ (n=12) were investigated. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Figure 2. Impact of treatment on cardiac fibrosis

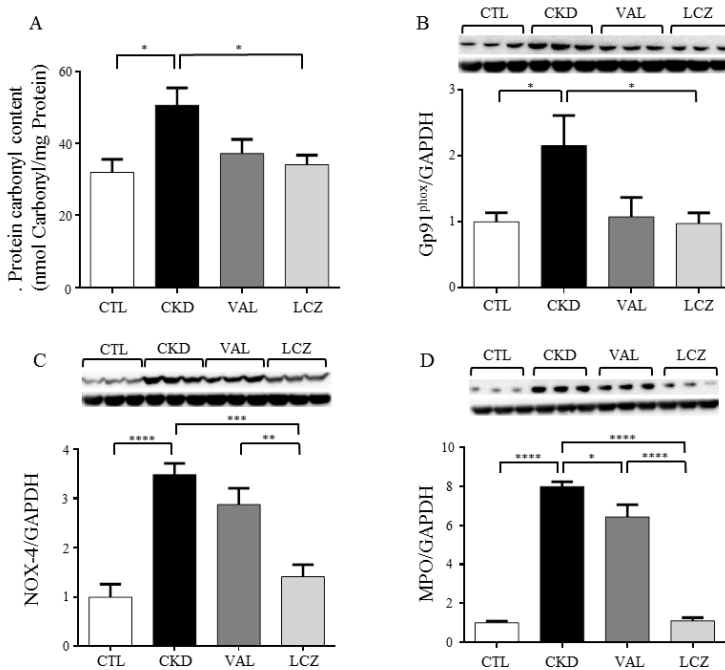
Representative photomicrographs of the Masson's trichrome stained LV interstitial and perivascular sections in the (A, E) CTL, (B, F) CKD, (C, G) VAL, and (D, H) LCZ. (I) Quantification analysis for the ratio of LV interstitial fibrotic area, (J) Quantification analysis for the ratio of LV perivascular fibrotic area, and representative Western blots and group data depicting the LV protein abundance of (K) TGF-β and (L) PAI-1 8-weeks post-randomization. Data are mean ± SEM. CTL (n=6), CKD (n=10), VAL (n=12), LCZ (n=12) were investigated. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Figure 3. Impact of treatment on aortic fibrosis

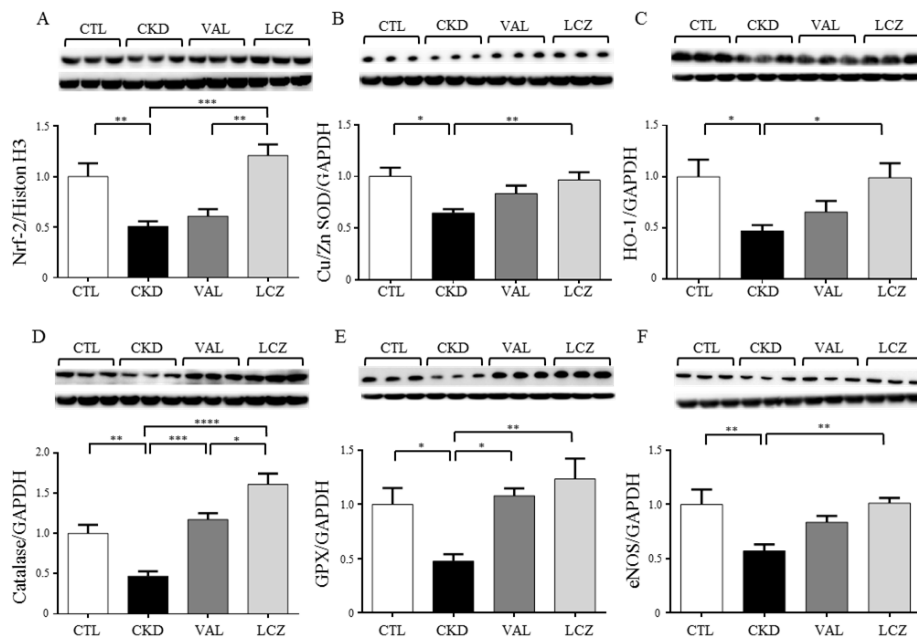
Representative photomicrographs of the Masson's trichrome stained aortic tissue in the (A, E) CTL, (B, F) CKD, (C, G) VAL, and (D, H) LCZ. (I) The ratio of wall to lumen, (J) the ratio of collagen content in vascular area was quantified for each group 8-weeks post-randomization. Data are mean \pm SEM CTL (n=6), CKD (n=10), VAL (n=12), LCZ (n=12) were investigated. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Figure 4. Impact of treatment on cardiac markers of inflammation

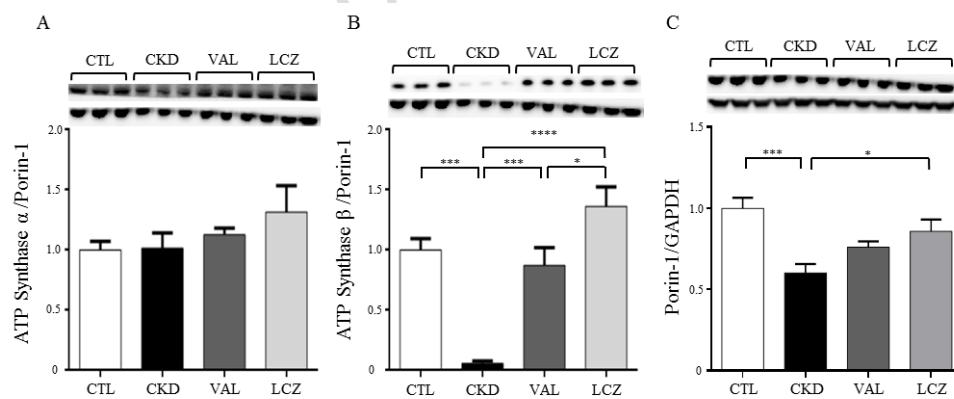
Representative Western blots and group data depicting the LV protein abundance of (A) NF-κB p65, (B) MCP-1, and (C) COX-2 8-weeks post-randomization. Data are mean \pm SEM. CTL (n=6), CKD (n=10), VAL (n=12), LCZ (n=12) were investigated. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Figure 5. Impact of treatment on cardiac markers of oxidative stress

Heart protein carbonyl content was shown (A). Representative Western blots and group data depicting the LV protein abundance of (B) Gp91^{phox}, (C) NOX-4, and (D) MPO 8-weeks post-randomization. Data are mean \pm SEM. CTL (n=6), CKD (n=10), VAL (n=12), LCZ (n=12) were investigated. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Figure 6. Impact of treatment on cardiac Nrf2 pathway

Representative Western blots and group data depicting the LV nuclear protein abundance of markers of cardiac Nrf-2 pathway including (A) nuclear protein abundance of Nrf-2, (B) Cu/Zn SOD, (C) HO-1, (D) catalase, (E) GPX, and (F) eNOS 8-weeks post-randomization. Data are mean \pm SEM. CTL (n=6), CKD (n=10), VAL (n=12), LCZ (n=12) were investigated. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Figure 7. Impact of treatment on cardiac mitochondrial proteins

Representative Western blots and group data depicting the LV nuclear protein abundance of (A) ATP synthase subunit α /porin-1, (B) ATP synthase subunit β /porin-1, and (C) porin-1/GAPDH 8-

weeks post-randomization. Data are mean \pm SEM. CTL (n=6), CKD (n=10), VAL (n=12), LCZ (n=12) were investigated. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Accepted Manuscript

Table1. General and laboratory data

	CTL (n=6)	CKD (n=10)	VAL (n=12)	LCZ (n=12)
Heart weight/body weight (mg/g)	2.6±0.05 [*]	3.3±0.12 ^{*,§,}	2.9±0.10 ^{§,#}	2.5±0.04 ^{,#}
Heart weight (mg)	1.18±0.02 ^{*,‡}	1.30±0.05 ^{*,§,}	1.14±0.03 ^{§,#}	0.99±0.03 ^{‡, ,#}
Body weight (mg)	430.3±10.3	394.5±12.9	394.4±10.3	397.8±7.5
Systolic blood pressure (mmHg)	92.5±2.8 ^{*,†,‡}	143.1±2.2 ^{*,§,}	119.7±2.6 ^{†,§}	117.7±2.2 ^{‡,}
Blood urea nitrogen (mg/dl)	8.7±0.3 ^{*,†,‡}	53.9±1.8 ^{*,}	53.7±2.2 ^{†,#}	44.6±1.4 ^{‡, ,#}
Serum creatinine (mg/dl)	0.33±0.03 ^{*,†,‡}	1.12±0.11 ^{*,}	1.09±0.05 ^{†,#}	0.86±0.04 ^{‡, ,#}
Urine protein (mg/24 h)	4.1±0.7 [*]	22.8±3.0 ^{*,§,}	8.4±1.1 [§]	8.9±0.6
FGF-23 (ng/ml)	1.8±0.2 [*]	4.2±0.6 ^{*,}	2.6±0.4	2.2±0.3
NT-pro BNP (ng/dl)	48±4 ^{*,†}	137±4 ^{*,§,}	105±10 ^{†,§,#}	39±10 ^{,#}

We analyzed the data 8-weeks post-randomization. Data are mean ± SEM. * p<0.05: CTL vs. CKD,

†p<0.05: CTL vs. VAL, ‡p<0.05: CTL vs. LCZ, §p<0.05: CKD vs. VAL, ||p<0.05: CKD vs. LCZ,

#p<0.05: VAL vs. LCZ.

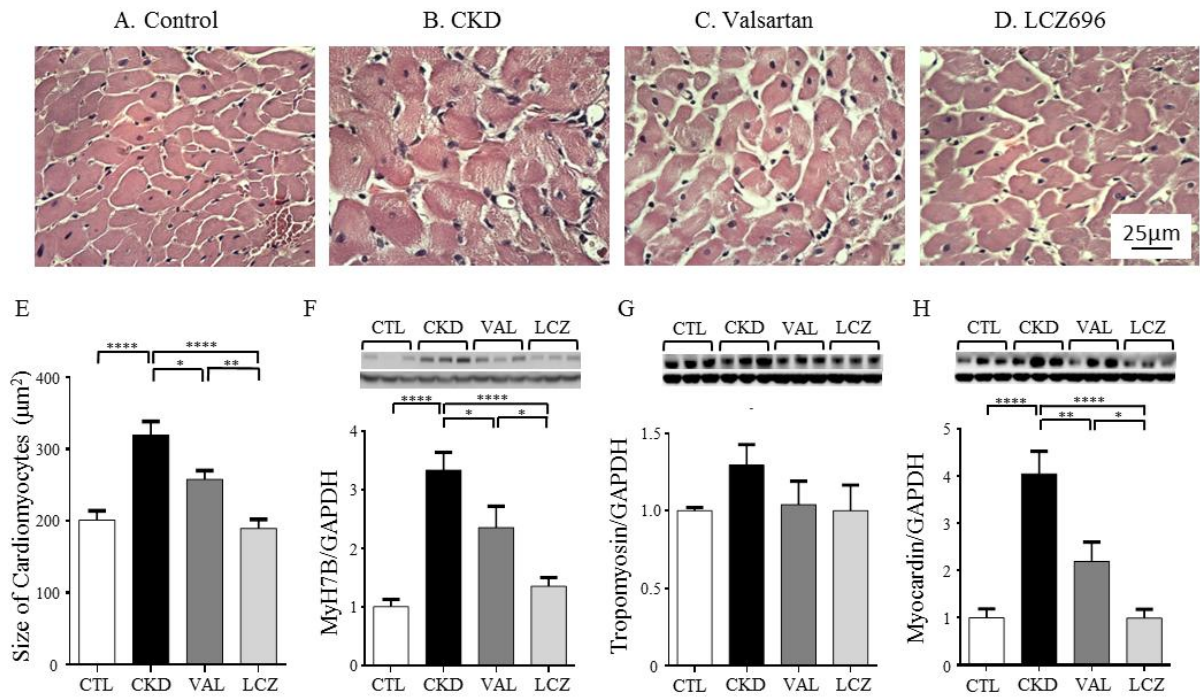


Figure 1_bestsetConverted.png

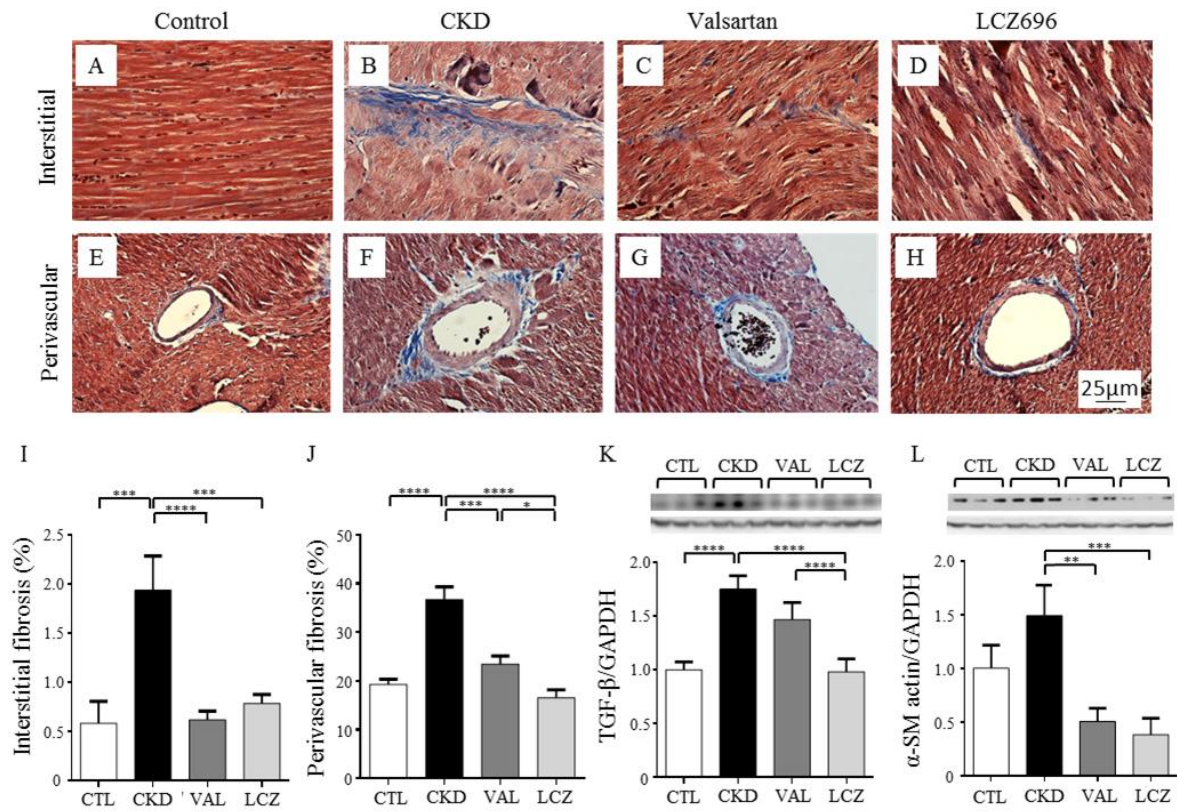


Figure 2_bestsetConverted.png

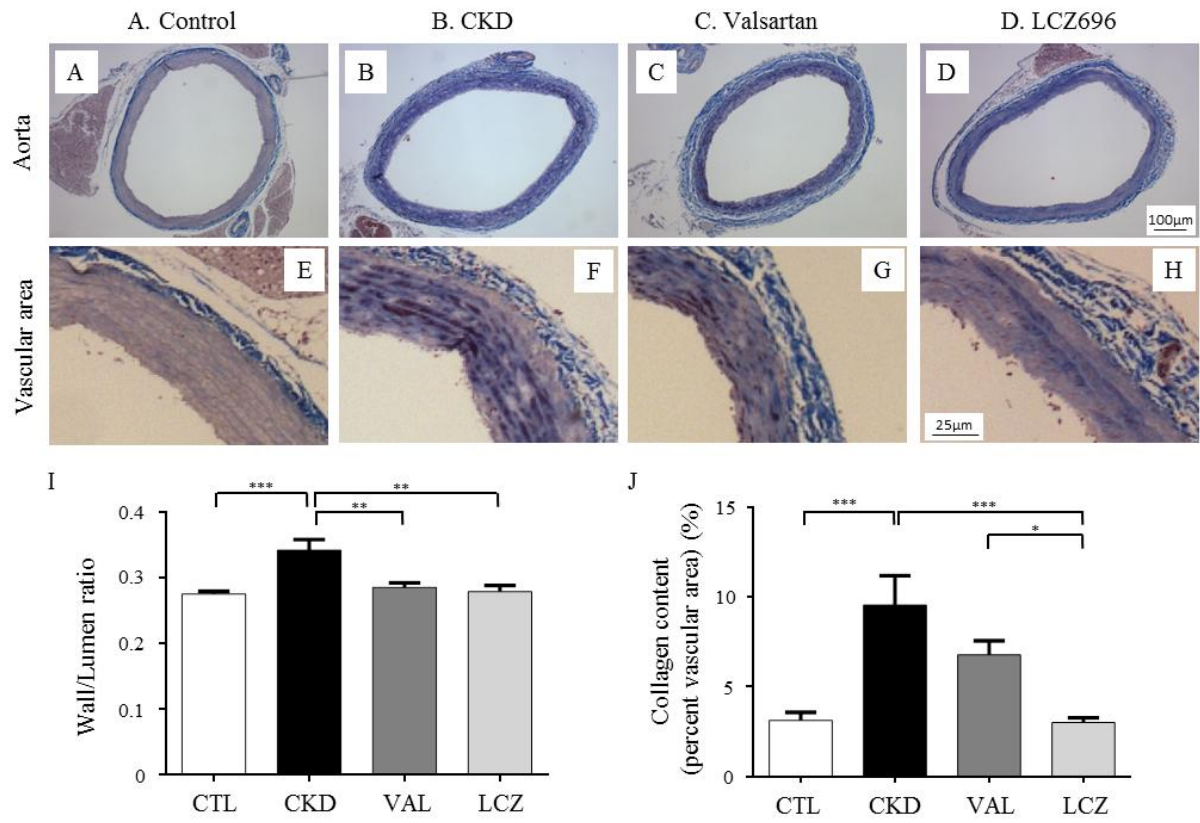


Figure 3_bestsetConverted.png

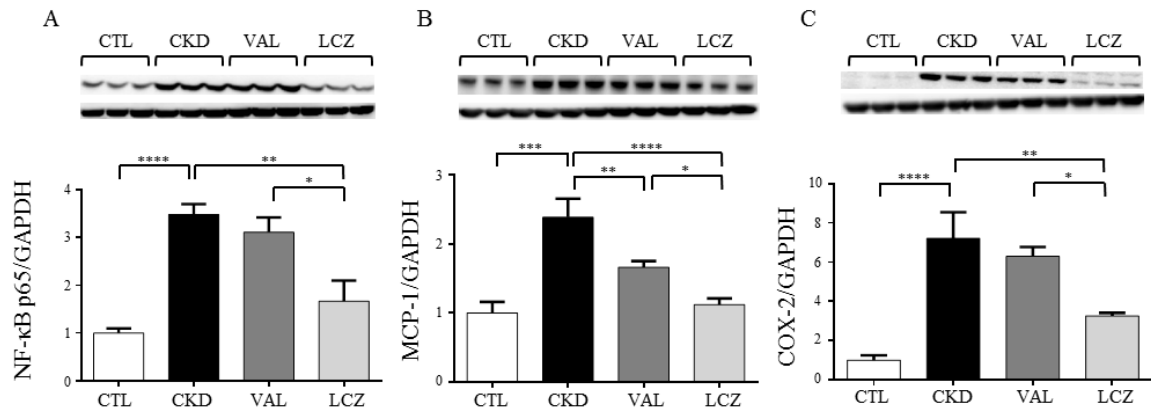


Figure 4_bestsetConverted.png

Accepted Manuscript

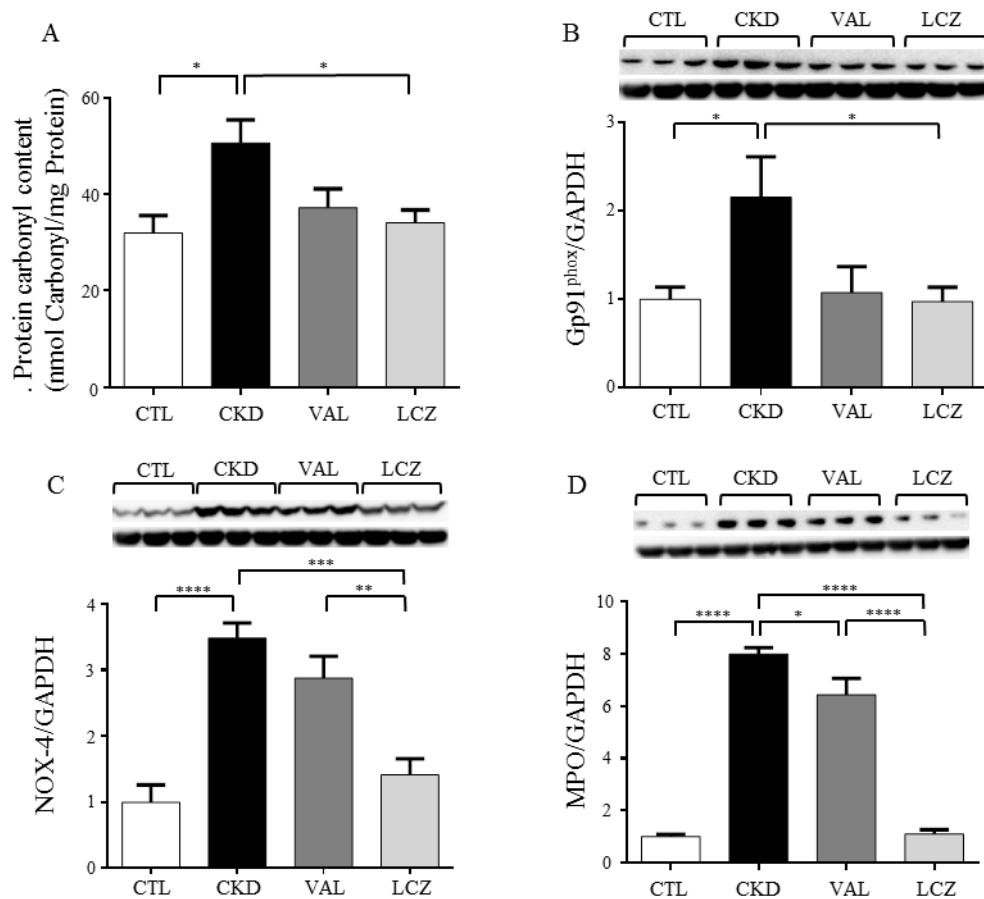


Figure 5_bestsetConverted.png

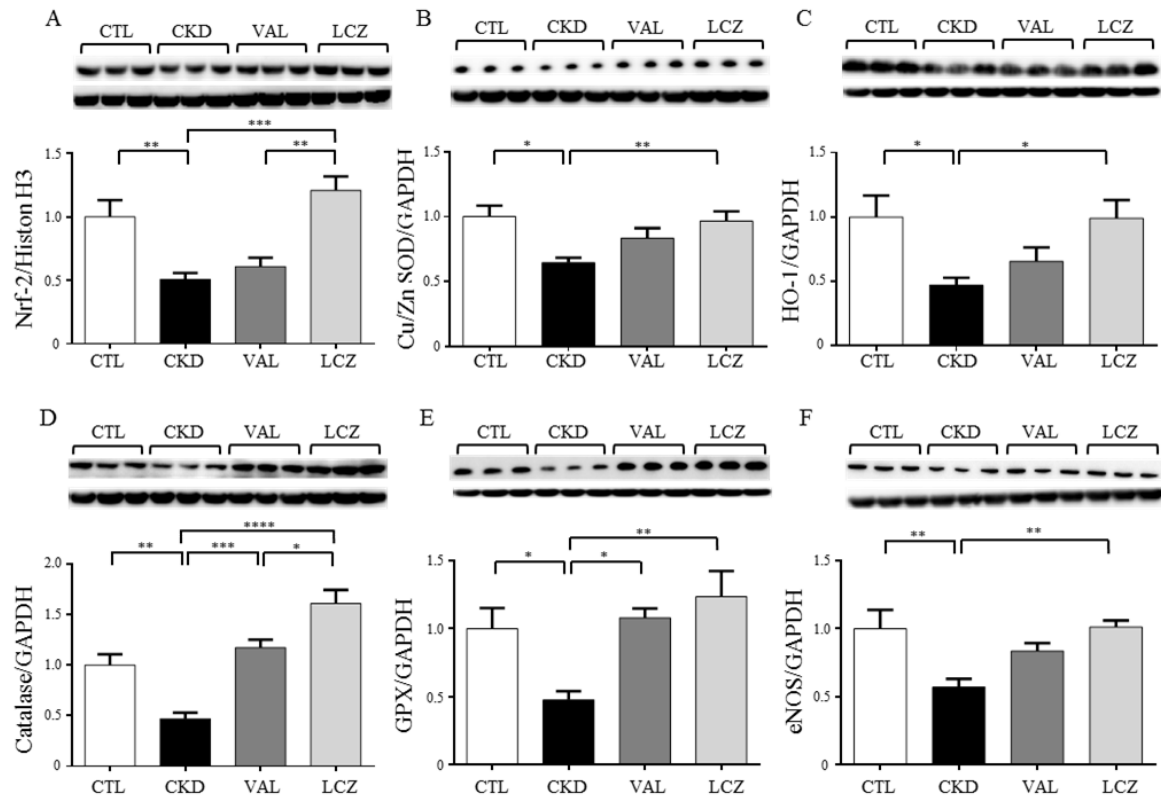


Figure 6_bestsetConverted.png

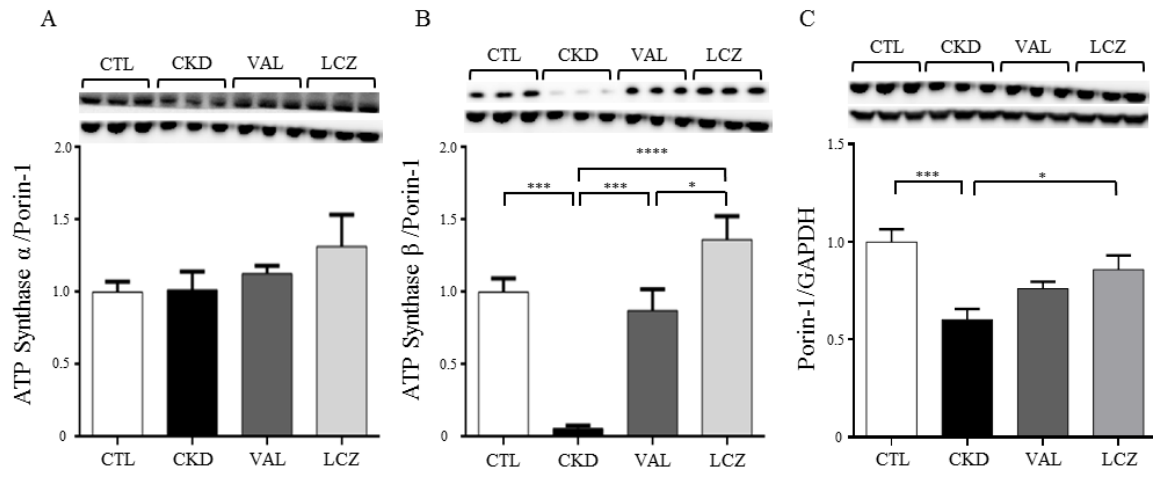


Figure 7_bestsetConverted.png