

**International Conference on Heart Failure with Preserved  
Ejection Fraction: *Basic mechanisms***

**Scientific programme, abstracts of poster  
presentations**

Organized by the **Committee on HFPEF of the Heart Failure Association**

Budapest, Hungary

September 23 – September 25, 2011

**Friday 23<sup>rd</sup> September**

<b>12.00-18.00</b>	Arrival, registration
<b>18.00-18.20</b>	Welcome address: <b>Zoltán Papp</b> (HU), <b>Burkert Pieske</b> (AT), <b>Piotr Ponikowski</b> (PL)
<b>18.20-19.40</b>	<b>Session I</b> (L1-L4) <u>Ventriculo-Arterial coupling</u>  <u>Chairs:</u> <b>Piotr Ponikowski</b> (PL), <b>Paolo N. Marino</b> (IT), <b>Stefan D. Anker</b> (DE)
18.20-18.40	<b>Daniel Burckhoff</b> (US): Ventricular properties and ventriculo-vascular coupling in an animal model of HFPEF and similarities/dys-similarities to observations in humans
18.40-19.00	<b>Vojtech Melenovsky</b> (CZ): The role of arterial stiffness and ventriculo-vascular coupling in HFPEF
19.00-19.20	<b>Alan G. Fraser</b> (UK): Asymmetry and dyssynchrony of ventriculo-arterial coupling - a target for treatment?
19.20-19.40	<b>Jens-Uwe Voigt</b> (DE): Myocardial mechanics in diastole, and wall-motion - blood-flow interaction  <u>Discussants:</u> <b>Gilles De Keulenaer</b> (BE), <b>Erwan Donal</b> (FR)
<b>20.00</b>	Dinner
<b>21.30</b>	Social Program (Welcome at the Buda castle)

**08.00-10.00** **Session II** (L5-L10) Myocardial function, cardiomyocytes and myofilaments

Chairs: **Burkert Pieske** (AT), **Ger J.M. Stienen** (NL)

8.00-8.20 **Thomas Eschenhagen** (DE): Hypertrophic Cardiomyopathy - a model for HFPEF

8.20-8.40 **Karin Sipido** (BE): Heterogeneity of cellular remodeling in ischemic cardiomyopathy

8.40-9.00 **Stefan Janssens** (BE): Molecular mechanisms governing functional and structural changes in the pressure-overloaded human heart

9.00-9.20 **Attila Borbély** (HU): Cardiomyocyte dysfunction in HFPEF: the role of myofilament protein alterations

9.20-9.40 **Wolfgang Linke** (DE): Phosphorylation of titin as an important determinant of myocardial passive stiffness

9.40-10.00 **Otto Smiseth** (NO): Mechanisms of LV lengthening velocity - is it only about diastolic function?

Discussants: **Frank Rademakers** (BE), **Zoltán Papp** (HU)

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**10.20-10.40** **Break**

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**10.40-13.00** **Session III** (L11-16) Neurohumoral signaling

Chairs: **Andrew Coats** (UK), **Heikki Ruskoaho** (FI)

10.40-11.00 **Adelino Leite-Moreira** (PT): Acute neurohumoral modulation of diastolic function

11.00-11.20 **István Szokodi** (HU): Paracrine regulators in diastolic dysfunction

11.20-11.40 **Ajay Shah** (UK): Cell-specific role of NADPH oxidases in diastolic dysfunction

11.40-12.00 **Cristoph Maack** (DE): Mitochondrial redox regulation and myocardial remodelling

12.00-12.20 **Walter Paulus** (BE): Low myocardial protein kinase G activity in HFPEF: causes and consequences

12.20-12.40 **Leon De Windt** (NL): Pro-hypertrophic signaling and diastolic dysfunction

Discussants: **Rodolphe Fischmeister** (FR), **Michael Fu** (SE)

**13.00-14.00** Lunch

**14.00-15.40** **Session IV** (L17-20) Exercise testing and clinical data

Chairs: **Dirk Brutsaert** (BE), **Alan G. Fraser** (UK)

14.00-14.20 **Barry Borlaug** (USA): Cardiovascular Reserve Function with Exercise in HFPEF

14.20-14.40 **David Kaye** (AU): The role of invasive exercise hemodynamics in the diagnosis of HFPEF

14.40-15.00 **Dirk J. Duncker** (NL): Effects of exercise training on cardiac remodeling and dysfunction: Importance of the underlying pathology

15.00-15.20 **Aldo Maggioni** (IT): HFPEF in the Heart Failure Pilot Survey (ESC-HF Pilot)

Discussants: **Walter Paulus** (BE), **Franck Flachskampf** (BE)

**15.40-16.00**

**Break**

**16.00-18.00** **Session V** (L21-25) Extracellular matrix

Chairs: **Javier Díez** (SP), **Gerasimos Filippatos** (GR)

16.00-16.20 **Carsten Tschöpe** (DE): Inflammation and matrix regulation in HFPEF

16.00-16.40 **Faiez Zannad** (FR): Biomarkers of extracellular matrix turnover in LVH and HFPEF

16.40-17.00 **Kenneth McDonald** (IR): Collagen and new biomarkers

17.00-17.20 **Stephane Heymans** (NL): The matricellular proteins osteoglycin and osteonectin regulate matrix maturation and cardiac function

17.20-17.40 **Javier Díez** (SP): Looking for new therapeutic targets of myocardial fibrosis in HFPEF. From the bench to the bedside

Discussants: **Adelino Leite-Moreira** (PT), **Dirk J. Duncker** (NL)

**18.00-20.00** **"MEDIA" Discussion / Posters**

**20.30** Dinner Cruise on the Danube

**08.00-10.00**    **Session VI** (L26-30) New directions

Chairs: **Carsten Tschöpe** (DE), **Ajay Shah** (UK)

- 8.00-8.20                    **Petar Seferovic** (RS): Diastolic dysfunction in diabetes mellitus: The dawn of cardiometabolic continuum
- 8.20-8.40                    **Gilles De Keulenaer** (BE) Salvaging the diabetic heart with endothelial factors
- 8.40-9.00                    **Marco Guazzi** (IT): Pulmonary hypertension in HFPEF
- 9.00-9.20                    **Stefan Chlopicki** (PL): Revealing diastolic dysfunction in mice, a lesson from MRI in vivo
- 9.20-9.40                    **Michael Frenneaux** (UK) Exercise-induced diastolic and systolic dysfunction in HFPEF – mechanisms and implications

Discussants: **Thomas Eschenhagen** (DE), **Ludwig Neyses** (UK)

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**10.00-10.10**

**Break**

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**10.10-12.40**    **Session VII** (L31-36) Therapy

Chairs: **Walter Paulus** (BE), **Faiez Zannad** (FR)

- 10.10-10.30                    **Andrew Coats** (UK): Beta blockers in HFPEF
- 10.30-10.50                    **Frida Grynspan** (IS): HFPEF and stem cells
- 10.50-11.10                    **Stephane Heymans** (NL): Cell therapy for the failing heart: from gene to protein
- 11.10-11.30                    **Burkert Pieske** (AT): Exercise as therapeutic intervention in HFPEF
- 11.30-11.50                    **Thomas H. Marwick** (US): Meta-analysis on medical therapy
- 11.50-12.10                    **Scott Solomon** (US): Why have HFPEF clinical trials failed so far?

Discussants: **Stefan Chlopicki** (PL) and **Burkert Pieske** (AT)

- 12.30-12.40    Summary and Conclusions: **Burkert Pieske** (AT), **Zoltán Papp** (HU)

**12.40-14.00**    **Lunch**

14.00                    Departure

## Poster Presentations

- No 1. **Ágnes Balogh:** Myofilament protein alterations contribute to post-infarction contractile dysfunction
- No 2. **John Baugh:** Getting to the Heart of Cardiac Remodelling: Using Combined Confocal and Atomic Force Microscopy to Assess Collagen Type 1 and 3 Biomechanics
- No 3. **Daniel Czuriga:** Diastolic Stiffness Alters Sarcomeric Structure of Failing Human Cardiomyocytes
- No 4. **Tamas Erdei:** The rationale of a simple handheld echo protocol to exclude heart failure with normal ejection fraction
- No 5. **Nadezhda Glezeva:** Phenotypic analysis of monocyte subsets in Hypertension and Heart Failure with Preserved Ejection Fraction (HFpEF). Evidence of increased monocyte to M2 macrophage skewing in HFpEF
- No 6. **Nazha Hamdani:** Cyclic GMP Enhancing Therapy Promotes Titin Phosphorylation and Corrects High Cardiomyocyte Passive Stiffness in Diastolic Heart Failure
- No 7. **Judit Kalász:** Oxidative myocardial protein alterations contribute to increased passive stiffness of left ventricular human cardiomyocytes
- No 8. **Lea Lak:** Device-Based Treatment for Diastolic Heart Failure – Two Years Interim Clinical Study Results
- No 9. **Jan-Christian Reil :** Ivabradine improves vascular stiffness as well as left ventricular systolic and diastolic function in mice with type 2 diabetes
- No 10. **Michael Schwarzl:** DOCA/salt-induced hypertension + high-cholesterol/high-lipid diet: A large animal model of concentric LV hypertrophy with increased LV stiffness
- No 11. **Michael Schwarzl:** Experimental Coronary Microembolisation, a Model of No-Reflow-Infarction, causes Acute and Progressive Diastolic Heart Failure in Pigs
- No 12. **Marie-France Seronde:** Echocardiography finding in emergency room in patient with acute dyspnea
- No 13. **Yu Ting Tan:** Dyssynchronous three plane motion in patients with heart failure and normal ejection fraction
- No 14. **Elza van Deel:** eNOS-mediated oxidative stress exacerbates LV hypertrophy and dysfunction and masks beneficial effects of eNOS overexpression in pressure-overload
- No 15. **Loek van Heerebeek:** Statins Favourably Affect Cardiomyocyte Hypertrophy and Myocardial Protein Kinase G Activity in Heart Failure with Preserved Ejection Fraction
- No 16. **Loek van Heerebeek:** Increased Nitrosative/Oxidative Stress lowers Myocardial Protein Kinase G activity in Heart Failure with Preserved Ejection Fraction.
- No 17. **Chris Watson:** Serial changes in LRG, a novel biomarker of ventricular dysfunction and heart failure, reflects progressive cardiac remodeling

## Abstracts of Poster Presentations

### No 1. Myofilament protein alterations contribute to post-infarction contractile dysfunction

Ágnes Balogh\*, Enikő T. Pásztor, David Santer, Dániel Czuriga, Tünde Rácz, László Nagy, Szilvia Koncz, Bruno Podesser, Zoltán Papp

\*Division of Clinical Physiology, Institute of Cardiology, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

As a consequence of myocardial infarction (MI) contractile dysfunction of cardiomyocytes may occur together with replacement fibrosis. Both factors may increase left ventricular stiffness and impair diastolic function.

In the present study MI-associated alterations in the cardiomyocyte contractile performance were assessed. We studied whether cardiomyocytes contribute to the increased left ventricular (LV) stiffness and whether there are any regional differences in cardiomyocyte contractile function within the infarcted LV.

Cardiomyocytes were obtained from the LV of untreated (Control) and infarcted (MI) mice from two different areas: the anterior wall (Ant) and a remote area (Inf). In skinned cardiomyocytes,  $\text{Ca}^{2+}$ -independent passive force ( $F_{\text{passive}}$ ) and the  $\text{Ca}^{2+}$ -sensitivity of force production ( $\text{pCa}_{50}$ ) were determined. In parallel, the levels of kinase-specific phosphorylations of troponin I (TnI), markers of SH-oxidation and carbonylation of contractile proteins were assessed by Western immunoblotting and by the OxyBlot assay.

$\text{pCa}_{50}$  was significantly lower at the MI Ant site than in Control hearts [1.9  $\mu\text{m}$  sarcomere length (SL): Control (n=28):  $5.8 \pm 0.02$ ; MI Ant (n=30):  $5.7 \pm 0.03$ ; MI Inf (n=10):  $5.8 \pm 0.02$ ; mean  $\pm$  SEM].  $F_{\text{passive}}$  values were significantly higher of the MI Ant and Inf cardiomyocytes at 1.9  $\mu\text{m}$  SL compared to Controls (Control:  $0.3 \pm 0.1$  kN/m<sup>2</sup>; MI Ant:  $0.8 \pm 0.1$  kN/m<sup>2</sup>; MI Inf:  $0.6 \pm 0.1$  kN/m<sup>2</sup>). The increases in  $\text{pCa}_{50}$  and in active and passive forces by stretching the cardiomyocytes suggested the preservation of the Frank-Starling mechanism in MI. A decreased level of PKA-dependent TnI-phosphorylation could be detected selectively at the MI Ant site (MI Ant:  $59.9 \pm 7.6\%$  vs. Control:  $100 \pm 12.8\%$ ), whereas the levels of PKC-dependent TnI-phosphorylation were similar in every group. SH-oxidation of actin and carbonylation levels of actin and myosin heavy chain of the MI Ant cardiomyocytes were enhanced compared to Control. *In vitro* protein carbonylation resulted in a significant decrease in the calcium sensitivity of force production which could not be reverted by antioxidant treatment, however this type of oxidation had no effect on  $F_{\text{passive}}$ .

In conclusion, post-infarction remodeling resulted in a decrease of  $\text{Ca}^{2+}$ -sensitivity of force production and in an increase of passive tension at the Ant site. Higher passive stiffness of the cardiomyocytes may contribute to the increased wall stress of the infarcted hearts. Different types of posttranslational protein modifications, SH-oxidation, carbonylation may be responsible for each experienced post-MI alterations.

## **No 2. Getting to the Heart of Cardiac Remodelling: Using Combined Confocal and Atomic Force Microscopy to Assess Collagen Type 1 and 3 Biomechanics**

John Baugh\*, Patrick Collier, Christopher Watson, Maarten Van Es, Catherine McGorrian, Michael Tolan, Mark Ledwidge, Kenneth McDonald, John Baugh

*\*The Conway Institute, University College Dublin, Dublin, Ireland*

### **Background:**

Targeting cardiac interstitial abnormalities is a major focus of future preventative strategies for the management of HFpEF. Recent biomarker studies have attached particular pathological importance to accumulation of myocardial collagen subtype 3. For example, in a cross-sectional study we have shown increased serum evidence of collagen3 synthesis in patients with HFpEF (P3NP,  $p < 0.001$ ) with no increase in evidence of collagen1 synthesis (P1CP,  $p = \text{NS}$ ; P1NP,  $p = \text{NS}$ ) despite evidence of heightened collagen1 degradation (C1TP,  $p < 0.001$ ). These data are at odds with conventional wisdom which states that collagen subtypes 1&3 are inextricably linked within supra-molecular structures. Current knowledge regarding the component structures of myocardial collagen networks (often derived from non-human or even non-cardiac tissue) is limited, further delineation of which will require application of more innovative technologies.

### **Methods:**

Using ex-vivo right atrial tissue from patients undergoing coronary bypass, methodology involved immuno-histochemical and immuno-fluorescent staining in addition to combined confocal laser scanning and atomic force microscopy.

### **Results:**

We show for the first time, that collagen fibres within the human heart are subtype specific, with disparate anatomical locations and differential biomechanical properties. During single fibre deformation, overall median values of stiffness recorded in collagen3 were  $37 \pm 16\%$  lower than in collagen1 [ $p < 0.001$ ]. On fibre retraction, collagen1 exhibited greater degrees of elastic recoil [ $7 \pm 3\%$ ;  $p < 0.001$ ] and less energy dissipation than collagen3 [ $p < 0.001$ ]. In atrial biopsies taken from patients in permanent atrial fibrillation ( $n=5$ ) versus sinus rhythm ( $n=5$ ), stiffness of both collagen fibre subtypes was augmented ( $p < 0.008$ ).

### **Conclusions:**

We demonstrate that the two major collagen subtypes within the human heart form discrete fibres, have preferential anatomical locations and exhibit significantly different biomechanical properties. These data help explain observations that early increases in collagen3, as described in animal models of pressure overload, are associated with reduced chamber stiffness. We also describe altered collagen quality in chronic atrial fibrillation and highlight the importance of collagen cross-linking. A greater understanding of the post-translational modifications that contribute to altered collagen fibre biomechanics will be essential for our understanding of the role of collagen turnover and the relative importance of collagen “quality vs quantity” in HFpEF.



### No 3. Diastolic Stiffness Alters Sarcomeric Structure of Failing Human Cardiomyocytes

Daniel Czuriga\*, René Musters, Attila Borbély, Ines Falcao-Pires, Sylvia Bogaards, István Édes, Adelino Leite-Moreira, Jean Bronzwaer, Zoltán Papp, Jolanda van der Velden, Ger Stienen, Loek van Heerebeek, Walter Paulus

\*Division of Clinical Physiology, Institute of Cardiology, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

**Background:** cardiomyocytes isolated from failing human hearts have elevated diastolic stiffness, which is mainly attributed to the giant sarcomeric protein titin. Titin-based stiffness of failing cardiomyocytes can be partially reverted by *in vitro* protein kinase (PK)A, PKG treatment or by the reducing agent dithiotreitol (DTT). Gelsolin-induced thin filament extraction also modulates high stiffness through disruption of titin-actin interactions. In the present study we investigated whether the high cardiomyocyte stiffness has any impact on sarcomeric structure.

**Methods:** single cardiomyocytes were isolated from left ventricular myocardial samples of 16 heart failure (HF) patients and 5 donor hearts (Con). Resting tension (RT) of individual cardiomyocytes (HF: n=59; Con: n=30) was measured to determine diastolic cardiomyocyte stiffness. Reversibility of high cardiomyocyte stiffness was tested by *in vitro* treatment with PKA, PKG, DTT and gelsolin. To determine slack sarcomere length (SL) and Z-disc width, single cardiomyocytes (HF: n=50; Con: n=32) were labeled with antibody against alpha-actinin. High quality, 3D stacks of optical sections of cardiomyocytes were obtained with digital imaging fluorescence microscopy. Actin extraction with gelsolin was used to test the effects of titin-actin interaction on Z-disc structure and SL.

**Results:** RT in HF ( $6.2 \pm 0.9$  kN/m<sup>2</sup>) was higher than in Con ( $2.5 \pm 0.2$  kN/m<sup>2</sup>;  $p=0.001$ ), and slack sarcomere length in HF was shorter than in Con ( $p=0.031$ ). Although PKA, PKG or DTT lowered RT in HF to  $4.4 \pm 0.6$ ,  $4.2 \pm 0.7$  and  $4.0 \pm 0.4$  kN/m<sup>2</sup> respectively, it still exceeded ( $p<0.05$ ) the RT of Con. Subsequent gelsolin treatment caused further reduction of RT ( $p<0.05$ ) and shortening of slack sarcomere length ( $p=0.048$ ) in HF. Z-disc width was larger in HF than in Con ( $p=0.032$ ), and it could be normalized with gelsolin treatment. Positive correlation was found between cardiomyocyte stiffness and Z-disc width ( $r=0.72$ ;  $p=0.03$ ).

**Conclusions:** High diastolic stiffness of failing cardiomyocytes creates an intrasarcomeric tension, which shortens slack SL and opens up adjacent Z-discs. *In vitro* phosphorylation, antioxidant treatment and thin filament removal can reduce high cardiomyocyte stiffness. Sarcomeric alterations of failing cardiomyocytes are relevant to the pronounced diastolic stiffness and LV dysfunction in HF patients.

#### **No 4. The rationale of a simple handheld echo protocol to exclude heart failure with normal ejection fraction**

Tamas Erdei\*, Alan G Fraser

*\*Wales Heart Research Institute, Cardiff University, Cardiff, UK*

**Background:** Accurate diagnosis of heart failure with normal ejection fraction (HFNEF) is important but difficult because detailed echocardiographic assessment of diastolic function is technically challenging and operator-dependent and because natriuretic peptides are less useful than for diagnosing heart failure with reduced ejection fraction (HFREF). All diagnostic variables are continuously distributed, and multiple diagnostic criteria with single cut-points give high sensitivity but low specificity for the current ESC recommendations for diagnosing HFNEF. The echocardiographic parameter E/e' correlates with mean left ventricular (LV) filling pressure but has been little validated in HFNEF and does not discriminate between impaired (early diastolic) relaxation and reduced (end-diastolic) compliance. The ESC recommendations included a separate algorithm for excluding HFNEF which has not yet been validated in an unselected population.

**Hypothesis and rationale:** Our hypothesis is that HFNEF can be excluded with high negative predictive value by using echocardiography *without* needing expert measurement of diastolic indices. Applying recent pathophysiological findings, we propose that older patients with breathlessness at rest or on exertion will not have HFNEF if there is no left ventricular hypertrophy and if LV ejection fraction, end-systolic volume, long-axis systolic velocity and annular displacement, and LA volume, are *all* normal.

**Study design:** Patients aged  $\geq 60$  years with unexplained breathlessness on exertion will be excluded if they have evidence of respiratory disease (by spirometry), ischaemic heart disease (ECG changes or regional wall motion abnormalities), HFREF, or other valvular and structural heart disease (by echocardiography). In  $\geq 100$  patients with possible HFNEF, a handheld echocardiographic study performed by the general practitioner, using the simple protocol, will be compared with a detailed examination including diastolic indices by a sonographer using a portable scanner. The definitive diagnosis will be established by a cardiologist after diastolic stress testing using a semi-supine bicycle and a high-end echocardiographic system, if there are significant abnormalities of diastolic function at rest or during exercise, and compared with NTproBNP assays. Age- and sex-matched healthy subjects will serve as controls. This study will compare three echo diagnostic strategies for excluding and diagnosing HFNEF; a simple but reliable protocol for excluding HFNEF using handheld echocardiography could be highly cost-effective.

*This study has been funded by a Research Fellowship from the HFA of the ESC; if you would like to test the same protocol in your own patients, please contact Dr. Tamas Erdei [[dr.tamas.erdei@gmail.com](mailto:dr.tamas.erdei@gmail.com)].*

## **No 5. Phenotypic analysis of monocyte subsets in Hypertension and Heart Failure with Preserved Ejection Fraction (HFpEF). Evidence of increased monocyte to M2 macrophage skewing in HFpEF**

Nadezhda Glezeva\*, Victor Voon, Kenneth McDonald, John Baugh

\**University College Dublin, Conway Institute, Dublin, Ireland*

*Background.* Inflammation is critically involved and contributes to the pathophysiological myocardial remodelling in the early stages of heart disease. The existence of a repetitive and progressive state of immune-inflammatory activation and fibrosis, with increased monocyte activation and infiltration into the cardiac tissue have been described and associated with the aetiology and progression of ventricular diastolic dysfunction (DD) and Congestive Heart Failure. Circulating monocytes differentiate into macrophages following exposure to appropriate stimuli to become classical (pro-inflammatory) type 1 macrophages (M1) or alternative (anti-inflammatory) type 2 macrophages (M2). M2 have been implicated in the pathogenesis of different inflammatory and neoplastic diseases including atherosclerosis, myocarditis, systemic sclerosis, pulmonary fibrosis and cancer. It has been suggested that macrophage polarization to M1 or M2 phenotype depends on the expression pattern of monocyte markers and cytokine mediators. Our aim was to analyse monocytes from patients at different stages of heart disease and associate peripheral blood markers to disease pathogenesis.

*Methods.* Peripheral blood was collected from 15 asymptomatic hypertensive (aHTN), 15 asymptomatic left ventricular DD (aLVDD), and 15 HFpEF patients. Inflammatory cytokines in patient plasma were identified by ELISA. PBMC and monocytes were purified and stained for CD14, CD163, and CD206 monocyte/macrophage-specific surface markers.

*Results.* An active state of inflammation in the HFpEF group as compared to the aHTN group was defined by the significantly elevated levels of classical inflammatory cytokines: TNF $\alpha$  (2.2 pg/ml vs 2.9 pg/ml), IL12 (68 pg/ml vs 113 pg/ml), IL6 (2.2 pg/ml vs 4.9 pg/ml), IL8 (2.7 pg/ml vs 3.2 pg/ml), MCP1 (206 pg/ml vs 230 pg/ml), and CXCL10 (99 pg/ml vs 244 pg/ml). Plasma levels of the M2-associated cytokine TARC/CCL17 were also significantly increased in HFpEF (94 pg/ml vs 57 pg/ml in aHTN). In addition, the percentage of CD14+ monocytes was increased in HFpEF (83.7% - aHTN vs 87.7% - HFpEF). Interestingly, the percentage of CD14+ cells expressing the M2 marker CD206 was increased in HFpEF as compared to aHTN (85.1% vs 80.7%,  $p < 0.05$ ).

*Conclusion.* We identified increased numbers of CD14+CD206+ monocytes and elevated levels of the M2-associated cytokine TARC/CCL17 in the peripheral blood of patients with HFpEF. A M2 monocyte profile was not characteristic of aHTN suggesting that a skewing of monocytes towards a regulatory, pro-fibrotic M2 phenotype occurs during the early stages of heart disease and these changes are associated with progression to HF.

## No 6. Cyclic GMP Enhancing Therapy Promotes Titin Phosphorylation and Corrects High Cardiomyocyte Passive Stiffness in Diastolic Heart Failure

Nazha Hamdani\*, Kalkidan G Bishu, Jolanda van der Velden, Ger JM Stienen, Margaret M Redfield, Wolfgang A Linke

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**Background:** Myofiber passive stiffness is lowered by phosphorylation of the giant sarcomeric protein titin, with beneficial effects on diastolic function. Titin can be phosphorylated by cGMP-activated protein kinase (PK)G, a pathway stimulated by B-type natriuretic peptide (BNP) or PDE-5A inhibitor (sildenafil). Whether titin phosphorylation and stiffness are affected by PKG activation in vivo had not been studied. Here we examined how dogs with experimental hypertension and diastolic dysfunction, induced by renal wrapping, respond to treatment with  $\beta$ -blockers, sildenafil, and BNP, in terms of altered titin phosphorylation and passive stiffness.

**Methods and Results:** Isolated permeabilized cardiomyocytes from left ventricular (LV) biopsies untreated (n=5), treated with  $\beta$ -blockers (n=7), followed by sildenafil (n=7) and BNP (n=7) were attached to a force transducer and the passive length tension relation ( $F_{\text{passive}}$ ) was measured between 1.8 and 2.4 $\mu\text{m}$  sarcomere length (SL) before and after PKA and PKG administration. We also assessed phosphorylation of myofibrillar proteins including titin, cMyBP-C, desmin, cTnT, cTnI and MLC2 by gel electrophoresis using SYPRO Ruby (total-protein) and ProQ Diamond (phospho-protein) stain and expression of titin PEVK, phospho-PEVK, cTnI, phospho-cTnI (at Ser 23/24), PKC $\alpha$  and phospho-PKC $\alpha$  by Western blotting. Compared to untreated cardiomyocytes,  $F_{\text{passive}}$  was unaltered with  $\beta$ -blocker therapy, but significantly reduced with sildenafil; with BNP  $F_{\text{passive}}$  did not change further. Ex-vivo administration of PKG lowered  $F_{\text{passive}}$  of untreated and  $\beta$ -blocker cardiomyocytes to the levels measured in sildenafil and BNP samples, and additional administration of PKA did not further change  $F_{\text{passive}}$ . Total titin phosphorylation was low in untreated samples and with  $\beta$ -blockade, but significantly increased with sildenafil and BNP. Total cTnI phosphorylation and phospho-cTnI at Ser 23/24 were low in untreated compared to treated samples but were similar between  $\beta$ -blocker, sildenafil and BNP groups. Phospho-PEVK and phospho-PKC $\alpha$  were high in untreated samples and with  $\beta$ -blockade, but reduced with sildenafil and BNP.

**Conclusions:** Acute cGMP enhancing therapy with sildenafil and BNP improves LV diastolic function through correction of a titin phosphorylation deficit, thereby reducing cardiomyocyte passive stiffness.

	Untreated	$\beta$ -blocker	Sildenafil	BNP
$F_{\text{passive}}$ (kN/m <sup>2</sup> ) (at SL 2.2 $\mu$ m)	6.6 $\pm$ 0.2	6.0 $\pm$ 2.0	3.7 $\pm$ 2.7 <sup>†</sup>	1.8 $\pm$ 1.0 <sup>†</sup>
Titin phosphorylation (% relative to total titin)	30 $\pm$ 2	37 $\pm$ 2	80 $\pm$ 3 <sup>†</sup>	85 $\pm$ 3 <sup>†</sup>
Phospho-PEVK N2BA (% relative to total titin)	55 $\pm$ 6	59 $\pm$ 6	25 $\pm$ 3 <sup>†</sup>	21 $\pm$ 1 <sup>†</sup>
Phospho-PEVK N2B (% relative to total titin)	57 $\pm$ 14	58 $\pm$ 10	29 $\pm$ 4 <sup>†</sup>	33 $\pm$ 5 <sup>†</sup>
Phospho-MyBPC (% relative to total MyBPC)	20 $\pm$ 1	16 $\pm$ 1	20 $\pm$ 0.6	80 $\pm$ 0.6 <sup>#†</sup>
Phospho-Tnl (% relative to total MyBPC)	30 $\pm$ 20	50 $\pm$ 10	80 $\pm$ 30	80 $\pm$ 40
Phospho-TnT (% relative to total MyBPC)	210 $\pm$ 50	120 $\pm$ 10	90 $\pm$ 30	120 $\pm$ 40
Phospho-MLC2 (% relative to total MyBPC)	210 $\pm$ 70	130 $\pm$ 10	100 $\pm$ 20	140 $\pm$ 60
Phospho-desmin (% relative to total MyBPC)	190 $\pm$ 20	190 $\pm$ 60	140 $\pm$ 60	110 $\pm$ 50
Phospho-cTnl (Ser23/24) (normalized to actin)	0.1 $\pm$ 0.02	0.1 $\pm$ 0.02	0.13 $\pm$ 0.02	0.11 $\pm$ 0.001
Phospho-PKC $\alpha$ (normalized to actin)	0.9 $\pm$ 0.02	0.9 $\pm$ 0.06	0.4 $\pm$ 0.1 <sup>†</sup>	0.4 $\pm$ 0.1 <sup>†</sup>
# p<0.05 sildenafil vs BNP ; <sup>†</sup> p<0.05 sildenafil/BNP vs untreated/ $\beta$ -blocker				

## No 7. Oxidative myocardial protein alterations contribute to increased passive stiffness of left ventricular human cardiomyocytes

Judit Kalász\*, Ágnes Balogh, Enikő Tóth Pásztorné, Miklós Fagyas, Sahar Pahlavan, Attila Tóth, István Édes, Zoltán Papp, Attila Borbély

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**Purpose:** Oxidative sarcomeric protein modifications have been implicated in cardiomyocyte diastolic dysfunction. Myeloperoxidase (MPO) generates several oxidants, such as hypochlorous acid from chloride ions and hydrogen peroxide ( $H_2O_2$ ) during ischaemia-reperfusion and myocardial inflammation. In the present study we aimed to characterize the effects of MPO and of its inhibitor on  $Ca^{2+}$ -activated and  $Ca^{2+}$ -independent forces ( $F_{active}$  and  $F_{passive}$ ) of left ventricular (LV) human cardiomyocytes.

**Methods:** Force measurements were performed in permeabilized human cardiomyocytes isolated from donor LV myocardium.  $F_{active}$  and  $F_{passive}$  and the calcium sensitivity of isometric force production ( $pCa_{50}$ ) were determined before and after sequential applications of  $H_2O_2$ , MPO in the presence or absence of an MPO-inhibitor (4-aminobenzhydrazide) and/or a reducing agent dithiotreitol (DTT) at a sarcomere length of 2.3  $\mu m$ . An Ellman's assay was used to quantify the extent of  $H_2O_2$  and/or MPO-induced sulfhydryl group (SH) oxidation of myofilament proteins.

**Results:** Application of  $H_2O_2$  (30  $\mu M$ ) significantly decreased  $F_{active}$  (from  $23.1 \pm 3.7$  kN/m<sup>2</sup> to  $16.0 \pm 2.8$  kN/m<sup>2</sup>, n=7, P<0.01) and increased  $F_{passive}$  (from  $3.5 \pm 0.9$  kN/m<sup>2</sup> to  $4.0 \pm 0.9$  kN/m<sup>2</sup>, n=7, P<0.01). A further reduction in  $F_{active}$  (from  $18.8 \pm 2.7$  kN/m<sup>2</sup> to  $10.6 \pm 1.8$  kN/m<sup>2</sup>, n=12, P<0.05) and an additional increase in  $F_{passive}$  (from  $1.7 \pm 0.1$  kN/m<sup>2</sup> to  $2.7 \pm 0.3$  kN/m<sup>2</sup>, n=12, P<0.01) were observed when  $H_2O_2$  and MPO (2.67 nM) were applied together. 4-aminobenzhydrazide (50  $\mu M$ ) could partially prevent these effects on active and passive force productions (from  $16.3 \pm 3.4$  kN/m<sup>2</sup> to  $11.1 \pm 1.6$  kN/m<sup>2</sup>; and from  $1.8 \pm 0.4$  kN/m<sup>2</sup> to  $2.3 \pm 0.5$  kN/m<sup>2</sup> (n=5), respectively). Combined application of  $H_2O_2$  and MPO (13 nM) significantly decreased the relative SH content of myofilament proteins (to  $87.04 \pm 1.2\%$  of the initial 100%, P<0.05), which effect was reversed by the reducing agent DTT (i.e. to  $114.9 \pm 9.5\%$ ). In addition, DTT also reversed the MPO induced increase in  $F_{passive}$  (from  $2.4 \pm 0.3$  kN/m<sup>2</sup> to  $1.4 \pm 0.2$  kN/m<sup>2</sup>, n=6).  $H_2O_2$  alone did not affect  $pCa_{50}$ , however, combined application of  $H_2O_2$  and MPO significantly reduced  $pCa_{50}$  ( $5.82 \pm 0.02$  vs.  $5.67 \pm 0.02$ , P<0.05, n=12). 4-aminobenzhydrazide prevented the changes in  $pCa_{50}$  in the presence of  $H_2O_2$  and MPO ( $pCa_{50}=5.84 \pm 0.03$  vs.  $5.87 \pm 0.07$ , n=5).

**Conclusions:** MPO derived oxidants may contribute to diastolic dysfunction via increasing cardiomyocyte passive stiffness in parallel with SH oxidation of myofilament proteins.

## **No 8. Device-Based Treatment for Diastolic Heart Failure – Two Years Interim Clinical Study Results**

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### **Background**

Diastolic heart failure (DHF) accounts for over 40% of HF cases. Evidence-based treatment is lacking. The ImCardia™ device operates by harnessing energy exerted by the left ventricle (LV) during systole and returning it to the ventricle during diastole, to enhance diastolic performance. This extra-ventricular device is composed of a series of elastic elements, interposed between spiral screws attached to the epimyocardium of the LV free-wall.

Device safety and indications of efficacy were examined in patients undergoing aortic valve replacement (AVR) for aortic stenosis.

### **Method**

The extra-ventricular device was implanted as an add-on to AVR (study group, n=10), and compared to AVR only (control group, N=9). The patients were followed-up for up to 24 months.

### **Results**

There were four non-device related operative deaths: due to right coronary artery osteal-obstruction by the prosthetic sewing-ring (1 study group patient) and insufficient myocardial protection (2 study group patients, 1 control group patient). During the follow-up period there were no device-related complications or adverse events; 2 device group patients died at 29 month and at 31 month after implantation from diverticulitis and GI bleeding + pneumonia respectively. Improvement in NYHA functional class, 6-minutes walk-test and quality of life was similar for both groups. LV mass regression after 24 months was  $-83.49 \text{ gr} \pm 78.62 \text{ gr}$  in the control group Vs  $-115.4 \text{ gr} \pm 46.67 \text{ gr}$  in the study group. Left atrial area, an important marker of diastolic dysfunction, increased slightly after 24 month in the control group ( $+0.57 \text{ cm}^2 \pm 3.21 \text{ cm}^2$ ) but decreased in the study group ( $-2.71 \text{ cm}^2 \pm 3.5 \text{ cm}^2$ ). BNP level regression after 24 month was  $-78.37 \text{ pg/ml} \pm 112.45 \text{ pg/ml}$  in the study group vs.  $+47.28 \text{ pg/ml} \pm 163.84 \text{ pg/ml}$  in the control group. None of the patients had mitral valve disease at baseline or at follow-up.

### **Conclusion**

A passive elastic device which transfers energy from systole to diastole, may improve diastolic performance as suggested by further decrease in left atrial size beyond that expected from AVR only; the device did not induce LV hypertrophy neither it limited mass regression.

This is a limited size study; a larger scale study is needed to verify the efficacy trends.

### **Next step**

An intra-ventricular device, working on the same mechanical principle, is under advanced R&D stages with preclinical follow-up of up to 9 months. Clinical study is to be initiated beginning of 2012.



## **No 9. Ivabradine improves vascular stiffness as well as left ventricular systolic and diastolic function in mice with type 2 diabetes**

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### **Background:**

Selective heart rate reduction by ivabradine improves morbidity and mortality in patients with systolic heart failure. It is unclear, however, whether heart rate reduction has beneficial effects in heart failure with preserved ejection fraction (HFPEF). Since HFPEF is associated with diabetes, but treatment options in patients with HFPEF have been disappointing so far, it was the aim of the present study to examine whether – and by which mechanisms - ivabradine improves LV function in mice with diabetes (db-) and features of HFPEF.

### **Methods:**

10 control mice ( $450 \pm 10$  bpm), 11 db- mice (HR  $580 \pm 15$  bpm) and 8 db- mice treated over 5 weeks with ivabradine (20 mg/kg body weight/d;  $430 \pm 20$  bpm) were investigated. Aortic distensibility was measured by MRI to determine vascular stiffness. Pressure-volume analysis was performed in isolated working hearts, followed by protein expression analyses and histological studies.

### **Results:**

In db- mice aortic distensibility was significantly reduced, which was prevented by ivabradine treatment. Ees, an index of systolic stiffness, was increased in db- mice compared to controls ( $5.9 \pm 1.5$  vs.  $3.3 \pm 0.8$  mmHg/ $\mu$ l,  $p < 0.05$ ), whereas  $dP/dt_{max}$  was reduced ( $p < 0.05$ ). Ivabradine treatment improved Ees ( $4.5 \pm 1.0$  mmHg/ml,  $p < 0.05$ ) and normalized contractility. Active relaxation was prolonged, while end-diastolic capacitance at a preload of 10 mmHg was significantly decreased in db- mice compared to controls ( $15.0 \pm 3.5$  vs.  $25.8 \pm 5.2$   $\mu$ l,  $p < 0.05$ ). Both parameters were largely normalized in ivabradine treated mice. Hemodynamic changes in db- mice were accompanied by increased titin N2B expression and reduced phosphorylation of phospholamban, both being reversed by ivabradine treatment. There were no signs of myocardial fibrosis or cardiac hypertrophy in db- mice.

### **Summary and conclusion:**

Diabetic mice have systolic and diastolic dysfunction, which is associated by increased vascular stiffness, altered myofilament composition and calcium handling, but not by increased myocardial fibrosis. Ivabradine decreases vascular and LV systolic stiffness and normalizes altered myofilament composition and alterations of calcium handling, thereby improving LV contractility and diastolic function in db- mice. Thus, heart rate reduction by ivabradine might be a new therapeutic tool for patients with HFPEF.



## No 10. DOCA/salt-induced hypertension + high-cholesterol/high-lipid diet: A large animal model of concentric LV hypertrophy with increased LV stiffness

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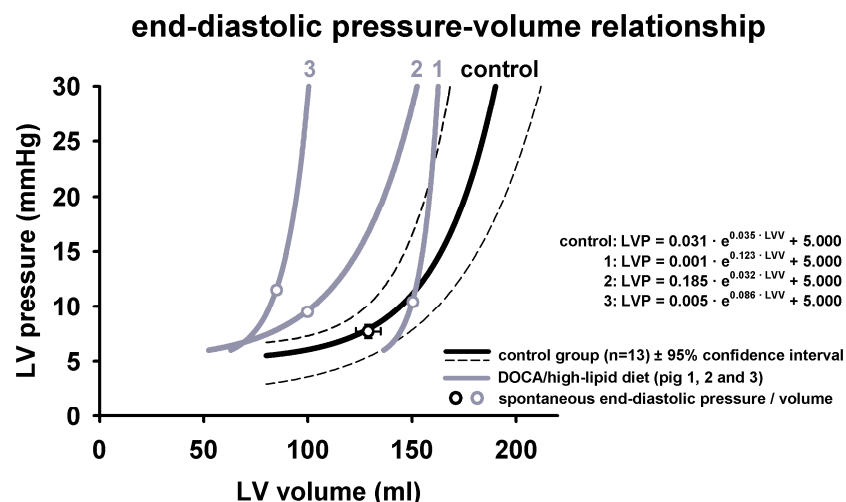
Background: Chronic heart failure with preserved ejection fraction (HFPEF) is of increasing importance in the aging population and associated with the accumulation of cardiovascular risk factors. However, the underlying pathophysiological mechanisms are poorly understood, and no clinical therapeutic strategies have been established, yet. This is in part related to the lack of animal models mimicking a HFPEF cardiac phenotype.

Aim: We aimed to study the impact of risk factors (hypertension, hyperlipidemia) on cardiac dimensions and function in a large animal.

Methods: In a preliminary study in 3 pigs, DOCA (deoxycorticosterone acetate) pellets were implanted subcutaneously, accompanied by a diet containing high amounts of salt, sugar, cholesterol and saturated fat, and followed for 12 weeks. Three further pigs on a regular diet served as a time control.

Results: DOCA/high lipid diet treated pigs developed persistent hypertension (tail-cuff systolic blood pressure after 12 weeks during light sedation:  $135 \pm 10$  vs.  $95 \pm 8$  mmHg) and had 7fold increased plasma cholesterol-levels. Left ventricular (LV) septal wall thickness was  $16 \pm 1$  vs.  $11 \pm 1$  mm, while LV ejection fraction was similar ( $68 \pm 5$  vs.  $64 \pm 6\%$ ). Compared to control pigs and weight-matched historical controls (total  $n=13$ ), the end-diastolic pressure-volume relationship (EDPVR, invasive pressure-volume analysis, aortic occlusion) was steeper and shifted leftwards in DOCA/high-lipid diet treated pigs (see graph).

Conclusion: DOCA/salt induced hypertension and a high lipid diet in pigs induce a shift of the LV EDPVR comparable to changes reported in patients with HFPEF. An upcoming larger number of experiments will allow for further in-vivo and in-vitro characterization of this model.



## No 11. Experimental Coronary Microembolisation, a Model of No-Reflow-Infarction, causes Acute and Progressive Diastolic Heart Failure in Pigs

Michael Schwarzl\*, Paul Steendijk, Stefan Huber, Heinrich Mächler, Martini Truschnig-Wilders, Burkert Pieske, Heiner Post

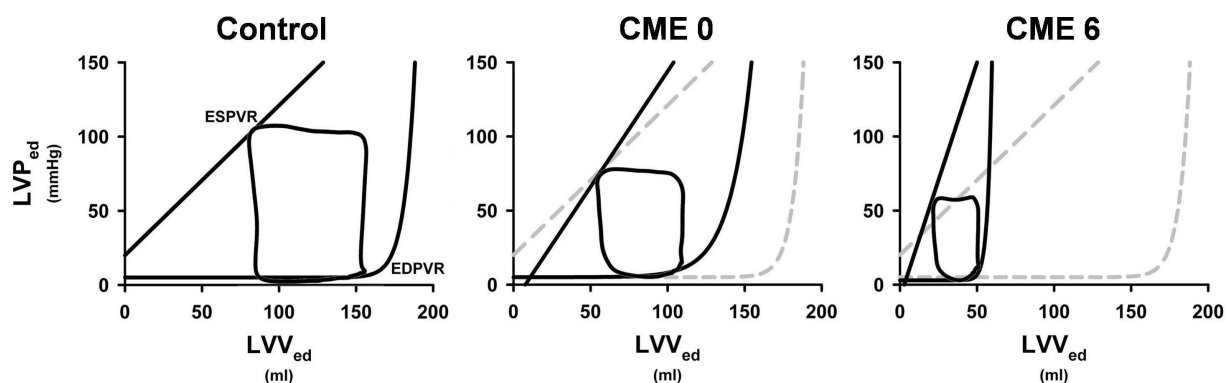
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Background: Coronary microembolisation (CME) from ruptured atherosclerotic plaques occurs spontaneously and often complicates percutaneous interventions. Experimental CME of the left circumflex coronary artery (LCX) causes a progressive reduction of regional myocardial wall thickening. We aimed to study the acute effects of CME on global LV function.

Methods: 8 anesthetized pigs (69±2 kg) were acutely instrumented (closed chest preparation) with a Swan-Ganz catheter, a LV pressure-volume catheter, a right atrial pacing probe and an intraaortic balloon catheter. Repetitive injections of 45 µm polystyrole microspheres into the LCX were administered until cardiac power output decreased by >40 %. Data are reported at control, immediately (CME 0), and at 6 h after the last embolisation (CME 6). \*: p<0.05 vs. control, #: p<0.05 vs. CME 0.

Results: Heart rate increased from 86±4 and 86±4 to 99±6\*,# bpm, cardiac output decreased from 6.2±0.3 to 4.2±0.2\* and 3.5±0.3\* l/min, LV peak pressure from 121±5 to 86±3\* and 68±4\*,# mmHg, LV dP/dtmax from 1838±98 to 1266±57\* and 1015±76\*,# mmHg/s, and central venous oxygen saturation from 67±1 to 51±2\* and 42±3\*,# %. Due to the concomitant fall of stroke volume (72±4, 49±2\* and 36±3\*,# ml) and LV end-diastolic volume (134±7, 95±4\* and 66±4\*,# ml), LV ejection fraction remained constant (52±2, 52±2 and 55±3 %). The end-diastolic pressure-volume relationship was progressively shifted leftwards (see original registration), while the end-systolic pressure-volume relationship did not reflect reduced contractility.

Conclusion: The major part of global LV pump failure in this model of no-reflow infarction is related to diastolic heart failure, as the LV is progressively unable to maintain an adequate preload. LV ejection fraction did not detect this critically reduced LV pump function. An acute diastolic heart failure may be underestimated in patients with acute myocardial ischemia.



## **No 12. Echocardiography finding in emergency room in patient with acute dyspnea**

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**Introduction:** The aim of the study was to evaluate the role of echocardiography performed during the first hours of hospitalization in patients with acute dyspnea.

**Methods:** patients with acute dyspnea presenting to the emergency department were prospectively enrolled within 4 hours after admission. Cardiac ultrasound study was performed by a trained cardiologist blinded for the BNP results. Each patient was classified into acute heart failure (AHF) and non AHF group. The diagnosis was adjudicated by two doctors without BNP and without echocardiography results. In the heart failure group, we analyzed the echocardiography and BNP in the subgroup of patient with preserved systolic function (with clinical signs of AHF and ejection fraction >50%).

**Results:** Among these patients, there were 18 patients in AHF group and 8 patients in non AHF group. Plasma BNP levels were higher into AHF group (1230±884 vs 77±99 pg/ml, p=0.0094). The acute heart failure patients had a LV diameter larger than non AHF patients (58±10 vs 45±6 mm, p=0.0048) and left ventricle ejection fraction (LVEF) was more altered in AHF (44±18 versus 63±13%, p=0.0095).

In patient with acute heart failure and preserved systolic function, we compare the BNP level at admission; it was increased in two groups. The echocardiography results and BNP level were resumed in the table n°1.

We observed 22% of the AHF patient had a combined origin of their dyspnea (pulmonary and cardiac), and these patient had preserved LVEF. So we compared this group with non AHF patient. The results were presented in the table n°2.

**Discussion:** The feasibility of echocardiography, in the Emergency Room, in patient with dyspnea is more complex than in patient in stable condition, in echocardiography lab. So a number of variables could not be collected in all patients (assessment of left ventricular volumes and LVEF by Simpson's method, and the systolic pulmonary pressure on the speed of the tricuspid regurgitation).

In the AHF group with preserved LVEF, the diastolic dysfunction parameters were the same that the dysfunction systolic group (tablen°1). But we observed that mean E/E' ratio was slightly increased than the results of other studies. These results could be explained by small effective and by a significative proportion of patients with combined dyspnea.

When we compared echocardiography results of AHF population with preserved LVEF and non AHF group we observed that the only significant differences were the left ventricle and left atrial size. The echocardiographic indicators of diastolic dysfunction were no significantly different. Although the AHF with preserved systolic function group had a trend for increase of E/E' and E/A ratio, and decrease DTE level. These results must be confirmed by a larger number of patients.

**Conclusions:** The number of patients is still low. However, it seems that echographic parameters of diastolic dysfunction may be inadequate in the population of our study. So it

would be interesting to combine echocardiography in dosage of natriuretic peptides as well as other biomarkers then the recruitment is also active.

Table n°1: echocardiography results and BNP level of the AHF group according to LVEF.

	AHF LVEF<50%, n=11	AHF LVEF ≥50%, n=7	p
LVED(mm)	60±12	55±8	0.36
LVES(mm)	48±13	40±12	0.24
LVEF (%)	31±8	62±10	<0.001
E/A	2.5±1.4	2.3±2.7	0.84
E/E' means	20±12	11±4	0.10
DTE (msec)	112±40	140±50	0.0155
VCI diameter (mm)	24±4.5	21.4±3	0.22
LA surface (cm <sup>2</sup> )	27±6	21±4	0.071
VTI Ao (m/sec)	11±4	18±8	0.0486
BNP (pg/ml)	1484±829	863±944	0.089

Table n°2: echocardiography results and BNP level of the AHF patients with preserved LVEF and non AHF patients.

	AHF LVEF>50%, n=7	No AHF LVEF >50, n=8	p
LVED (mm)	55±8	45±6	0.0152
LVES(mm)	40±12	28±7	0.0177
LVEF (%)	62±10	63±13	0.63
E/A	2.3±2.7	1.09±0.5	0.29
E/E' means	11±4	9.5±2	0.25
DT E (msec)	140±50	179±49	0.25
VCI diameter (mm)	21.4±3	17±5	0.34
LA surface (cm <sup>2</sup> )	21±4	15±3	0.0215
BNP (pg/ml)	863±944	77±99	0.039

### No 13. Dyssynchronous three plane motion in patients with heart failure and normal ejection fraction

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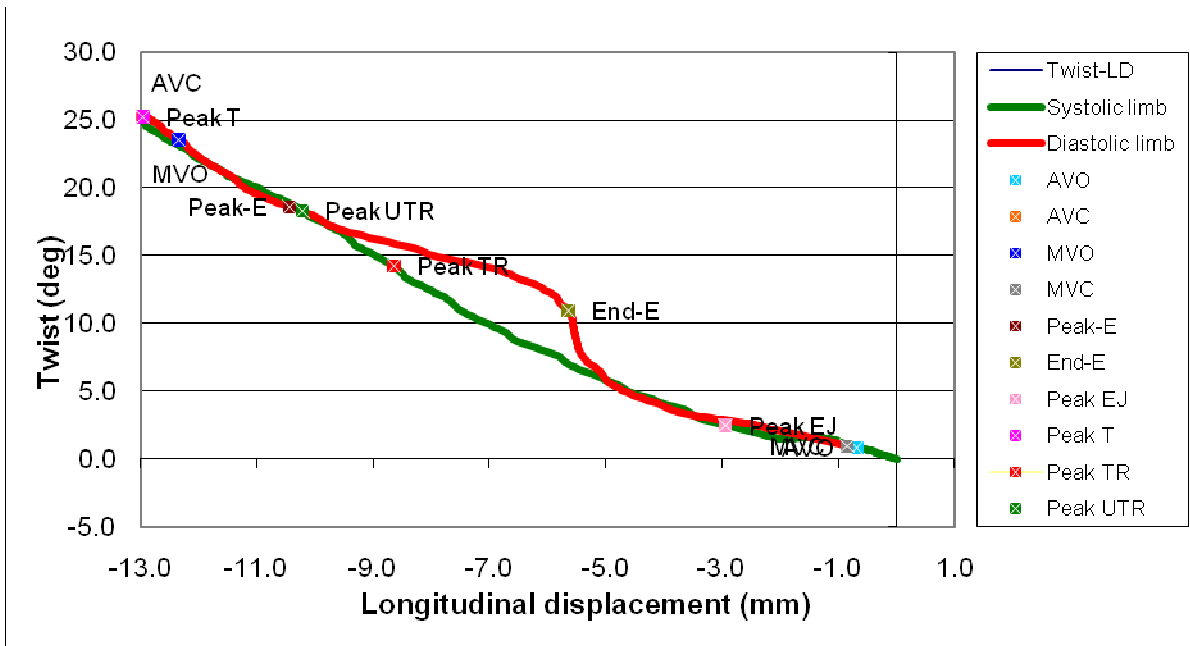
Background: Dyssynchrony is reportedly present in some patients with heart failure and normal ejection fraction (HFNEF) at rest. However, it is unknown if dyssynchrony is present on exercise and affects myocardial function when the patient is symptomatic.

Method: 72 Patients (age  $73\pm 7$  years, 48 female) with exertional dyspnea and normal LV ejection fraction ( $60\pm 7\%$ ) and 38 age-matched healthy controls (age  $71\pm 7$  years, 29 female, EF  $63\pm 7\%$ ) underwent cardiopulmonary exercise testing and full Doppler 2D-echocardiography at rest and on supine exercise. Analysis of three plane motion was performed using speckle tracking imaging with customized interpolation software. Standard deviation (SD) of peak event timing and time delay of diastolic motions were calculated to assess synchrony.

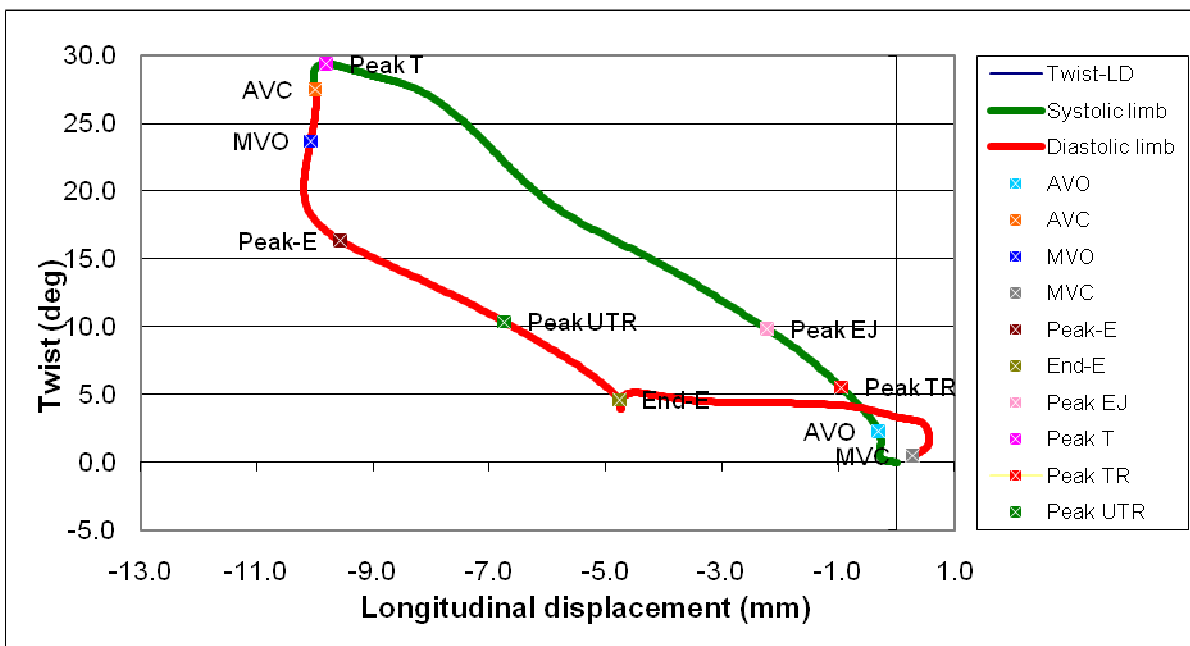
Results: The standard deviation of time to peak basal rotation, peak apical rotation, peak longitudinal and radial displacements was not different at rest ( $48.6\pm 32.9$  ms vs.  $43.1\pm 25.3$  ms,  $p=0.38$ ). On exercise healthy controls were able to synchronize all myocardial motions as shown by a significantly reduced SD of  $25.9\pm 15.5$  ms whereas patients showed almost no decrease ( $40.1\pm 27.2$  ms) suggesting the inability of the different muscle planes to contract cohesively at peak motion during exercise. Furthermore, the ratio of untwist and longitudinal extension during IVRT showed a significant steeper slope on exercise in patients indicating the presence of diastolic dyssynchrony.

Conclusion: Systolic and diastolic dyssynchrony in all three planes are present on exercise in HFNEF patients. This contributes to the overall deterioration of LV systolic function and impaired early diastolic filling causing reduced stroke volume and high filling pressures on exercise, the symptoms and exercise limitation.

	Patients rest	Controls rest	p value	Patients ex	Controls Ex	p value
SD Systolic Motions (ms)	$48.6\pm 32.9$	$43.1\pm 25.3$	0.38	$40.1\pm 27.2$	$25.9\pm 15.5$	0.01
Ratio Untwist /Extension in IVRT ( $^{\circ}/\text{mm}$ )	$25.3\pm 51.4$	$7.1\pm 10.7$	0.059	$9.6\pm 14.7$	$3.3\pm 3.8$	0.034



Twist-Longitudinal Displacement Curve of healthy control on exercise



Twist-Longitudinal Displacement Curve of HFNEF patient on exercise.

Red arrow, untwist only; Blue arrow, untwist and longitudinal extension; Green arrow, longitudinal extension only.

## No 14. eNOS-mediated oxidative stress exacerbates LV hypertrophy and dysfunction and masks beneficial effects of eNOS overexpression in pressure-overload

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Although initiated as a compensatory mechanism, pressure-overload induced left ventricular hypertrophy (LVH) increases the risk for heart failure. Previously, we showed endothelial Nitric Oxide synthase (eNOS) to be protective against myocardial infarction induced LV dysfunction. However, the role of eNOS in pressure overload induced ventricular remodeling remains controversial. Consequently, we studied the effect of eNOS expression levels on LVH and LV function 8 weeks after transverse aortic constriction (TAC, n=48) in eNOS knockout (Ko), wildtype (Wt) and eNOS overexpressing transgenic (Tg) mice. eNOS expression levels affected aortic pressure (Ko: 100±4 mmHg, Wt: 87±4 mmHg, Tg: 72±4 mmHg) but not influence LV weight and function in sham operated mice (n=41). TAC produced LVH and dilation, decreased LVdP/dtP40 and induced pulmonary congestion (lung fluid weight/tibia length) and interstitial fibrosis in Wt mice. Surprisingly, in spite of the beneficial effects of eNOS levels on afterload, the effects of TAC on LVH and LV function were less detrimental in eNOS-Ko and aggravated in eNOS-Tg mice (Table 1). Subsequently we investigated whether eNOS-uncoupling induced oxidative stress prevented protective effects of eNOS overexpression. TAC significantly increased uncoupling ratio three fold in eNOS-Tg mice. Moreover, scavenging of reactive oxygen species (ROS) with N-acetyl cysteine (NAC) had only mild effects in eNOS-Wt mice (P=0.08 for relative lung fluid weight and P>0.1 for all other parameters) but prevented LV dilation (3.7±0.1 mm), reduced relative LV weight (85±5 mg/cm) and lung weight (91±11 mg/cm) and improved fractional shortening (26±4 %) in eNOS-Tg mice. In conclusion, eNOS does not reduce but rather elevates pressure-overload induced ROS, thereby masking beneficial effects of NO. Supported by NHF grant 2007B024

Table 1. Effects of eNOS expression levels on TAC induced LVH and LV dysfunction

	eNOS-Ko	eNOS-Wt	eNOS-Tg
	n=17	n=17	n=14
LVSP (mmHg)	130±6*	122±4*	105±5*†‡
LV weight/TL (mg/cm)	87±4*	95±3*†	103±4*†‡
LV ED lumen (mm)	4.1±0.1*	4.4±0.1*†	4.5±0.1*†
Fractional Shortening (%)	19±2*	18±2*	16±2*
LV dP/dt <sub>P40</sub> (mmHg/s)	8030±730*	6080±350*	4660±330*†‡
Lung fluid weight/TL (mg/cm)	100±11*	115±11*	130±12*†
Interstitial fibrosis (%)	3.1±1.2*	10.3±2.1*†	12.0±2.4*†

\*P<0.05 vs corresponding sham, †P<0.05 vs corresponding Ko ‡ P<0.05 vs corresponding Wt

## **No 15. Statins Favourably Affect Cardiomyocyte Hypertrophy and Myocardial Protein Kinase G Activity in Heart Failure with Preserved Ejection Fraction**

Loek van Heerebeek\*, Nazha Hamdani, Ines Falcao-Pires, Adelino Leite-Moreira, Mark Begieneman, Jean Bronzwaer, Jolanda van der Velden, Ger Stienen, Gerrit Laarman, Aernout Somsen, Freek Verheugt, Hans Niessen, Walter Paulus

*\*Free University Medical Center, Amsterdam, the Netherlands*

Despite modern heart failure (HF) therapy, prognosis of HF with preserved ejection fraction (HFPEF) did not improve over the last decades. In experimental HF models, statin treatment reduces cardiomyocyte hypertrophy and lowers myocardial oxidative stress thereby enhancing nitric oxide bioavailability and protein kinase G (PKG)-dependent signaling. In addition, statin treatment was reported to be associated with improved survival in HFPEF patients (pts). The present study compared cardiomyocyte hypertrophy, myocardial nitrosative/oxidative stress and myocardial PKG activity in LV endomyocardial biopsy tissue from HFPEF pts treated with (HFPEF<sup>stat+</sup>)(n=15) or without statins (HFPEF<sup>stat-</sup>)(n=21). Moreover, as HFPEF cardiomyocytes have high diastolic stiffness evident from raised passive force ( $F_{\text{passive}}$ ), the present study also compared cardiomyocyte  $F_{\text{passive}}$  in both groups. All pts were free of coronary artery disease and biopsies demonstrated no evidence for infiltrative or inflammatory myocardial disease. All pts had LVEF>50%, LV end diastolic volume index <97 mL/m<sup>2</sup> and LV end diastolic pressure >16 mmHg. Cardiomyocyte diameter (MyD,  $\mu\text{m}$ ) was determined by histomorphometric analysis and  $F_{\text{passive}}$  was measured in single, mechanically isolated cardiomyocytes with sarcomere lengths fixed at 2.2  $\mu\text{m}$ . Myocardial nitrosative/oxidative stress was indirectly measured via detection of myocardial nitrotyrosine content. Myocardial PKG activity was immunohistochemically measured by ratio of vasodilatory stimulated phosphoprotein (VASP) phosphorylated at Ser<sup>239</sup> (PVASP) to total VASP (PVASP/VASP) ratio. Myocardial PKG activity was higher in HFPEF<sup>stat+</sup> than in HFPEF<sup>stat-</sup> ( $0.80\pm 0.03$  vs  $0.62\pm 0.04$ ;  $p=0.002$ ) pts. MyD ( $26.3\pm 0.2$   $\mu\text{m}$  vs  $29.3\pm 0.3$   $\mu\text{m}$ ;  $p<0.001$ ), cardiomyocyte  $F_{\text{passive}}$  ( $5.2\pm 0.2$  kN/m<sup>2</sup> vs  $7.9\pm 0.3$  kN/m<sup>2</sup>;  $p<0.001$ ) and myocardial nitrotyrosine content ( $2.84\pm 0.17\%$  vs  $4.49\pm 0.24\%$ ;  $p<0.001$ ), were lower in HFPEF<sup>stat+</sup> than in HFPEF<sup>stat-</sup> pts.

**Conclusion:** In HFPEF, statin treatment lowered both cardiomyocyte hypertrophy and stiffness, probably because of raised myocardial PKG activity in the presence of reduced myocardial nitrosative/oxidative stress. These findings suggest statin treatment to be of therapeutic value in HFPEF.



## **No 16. Increased Nitrosative/Oxidative Stress lowers Myocardial Protein Kinase G activity in Heart Failure with Preserved Ejection Fraction**

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Elevated cardiomyocyte passive force ( $F_{\text{passive}}$ ) contributes to raised diastolic left ventricular (LV) stiffness in patients (pts) with heart failure and preserved ejection fraction (HFPEF).  $F_{\text{passive}}$  in HFPEF is higher than in HF with reduced ejection fraction (HFREF) or in aortic stenosis (AS). High  $F_{\text{passive}}$  of HFPEF cardiomyocytes is also acutely lowered in-vitro following administration of protein kinase G (PKG). The present study therefore assessed myocardial PKG activity, cardiomyocyte  $F_{\text{passive}}$  and myocardial nitrosative/oxidative stress, which can lower PKG activity, in LV biopsies of pts with HFPEF (n=36), HFREF (n=43) and severe AS (n=67). All pts were free of coronary artery disease and biopsies demonstrated no evidence for infiltrative or inflammatory myocardial disease. HFPEF pts had LVEF>50%, LV end diastolic volume index <97 mL/m<sup>2</sup> and LV end diastolic pressure >16 mmHg. HFREF pts had a LVEF <45%. LV myocardium was procured by transvascular biopsy in HFPEF and HFREF and by peroperative biopsy in AS. Myocardial PKG activity was immunohistochemically measured by ratio of vasodilatory stimulated phosphoprotein (VASP) phosphorylated at Ser<sup>239</sup> (PVASP) to total VASP (PVASP/VASP) ratio. Myocardial nitrosative/oxidative stress was indirectly measured via detection of myocardial nitrotyrosine content.  $F_{\text{passive}}$  was measured in single, mechanically isolated cardiomyocytes with sarcomere lengths fixed at 2.2  $\mu\text{m}$ . Myocardial PKG activity was lower in HFPEF ( $0.70\pm 0.03$ ) than in HFREF ( $0.85\pm 0.03$ ;  $p<0.001$ ) and in AS ( $0.84\pm 0.02$ ;  $p<0.001$ ). Myocardial nitrotyrosine content was higher in HFPEF ( $3.94\pm 0.24\%$ ) than in HFREF ( $2.64\pm 0.24\%$ ;  $p<0.001$ ) and in AS ( $3.26\pm 0.13\%$ ;  $p=0.008$ ). Cardiomyocyte  $F_{\text{passive}}$  was also higher in HFPEF ( $7.6\pm 0.4$  kN/m<sup>2</sup>) than in HFREF ( $5.1\pm 0.2$  kN/m<sup>2</sup>;  $p<0.001$ ) and in AS ( $3.2\pm 0.2$  kN/m<sup>2</sup>;  $p<0.001$ ). More HFPEF pts were obese ( $p<0.05$ ) or had diabetes mellitus ( $p<0.05$ ).

**Conclusion:** Myocardial PKG activity is reduced in HFPEF because of high nitrosative/oxidative stress, possibly related to HFPEF pts having metabolic disorders. This low PKG activity raises cardiomyocyte stiffness and could become a valuable target for a specific HFPEF treatment strategy.

## **No 17. Serial changes in LRG, a novel biomarker of ventricular dysfunction and heart failure, reflects progressive cardiac remodeling**

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Heart failure with preserved ejection fraction (HFpEF) is commonly preceded by a prolonged asymptomatic phase during which progressive left ventricular diastolic dysfunction (LVDD) develops. HFpEF preventative strategies urgently require better biomarkers for identifying disease manifestations before the onset of symptoms and irreversible myocardial damage. In addition, biomarkers that predict the likelihood and rate of disease progression over time would help streamline and focus clinical efforts.

To help address this we adopted a proteomic screening approach (2D-DIGE and mass spectrometry) to dissect the coronary sinus serum proteome of asymptomatic hypertensive patients with low and high risk for future development of HFpEF. Risk was based on B-type natriuretic peptide (BNP) levels, a cardiac hormone that correlates with increased risk of cardiovascular events and is reflective of an active pathological process. We identified several differentially expressed disease-associated proteins, one of which was leucine-rich  $\alpha$ -glycoprotein (LRG). In various validation cohorts, LRG was found to be consistently over-expressed in the serum of patients who exhibit high BNP levels. Serum LRG levels correlated significantly with BNP in hypertensive, asymptomatic LVDD and HF patient groups ( $p < 0.05$ ) and were found to increase across the spectrum of disease. LRG levels were able to identify heart failure independent of BNP. Although a precise biological function for LRG is yet to be determined, LRG correlated with serum levels of TNF $\alpha$  ( $p < 0.01$ ) and IL-6 ( $p < 0.05$ ). Furthermore, LRG expression was detected in myocardial tissue and correlated with fibrotic genes ( $p < 0.01$ ).

To investigate the dynamics of LRG levels with progressive LVDD, we identified a cohort of 30 subjects from a population of over 500 asymptomatic hypertensive patients, whom following serial clinical and echocardiographic assessment exhibited evidence of progressive left ventricular diastology. Progression was based on changes in left atrial volume index (LAVI) which is a robust continuous echocardiographic measure of LVDD that has relative load independence. Progressors were identified as those having a change in  $\Delta\text{LAVI} \geq 3.5\text{mls/m}^2$  from an initial LAVI between 20 and 34mls/m<sup>2</sup>. A matched non-progressor cohort was selected and were similarly identified as those having  $\Delta\text{LAVI} < 3.5\text{mls/m}^2$ . Serum analysis revealed that, unlike BNP, LRG was able to predict changes in left ventricular diastology over time highlighting a potential role for identification of sub-clinical disease progression in pre-HFpEF syndromes.