Annual Report Department of Physiology

2017 2018

Physiology VUmc Amsterdam

Content

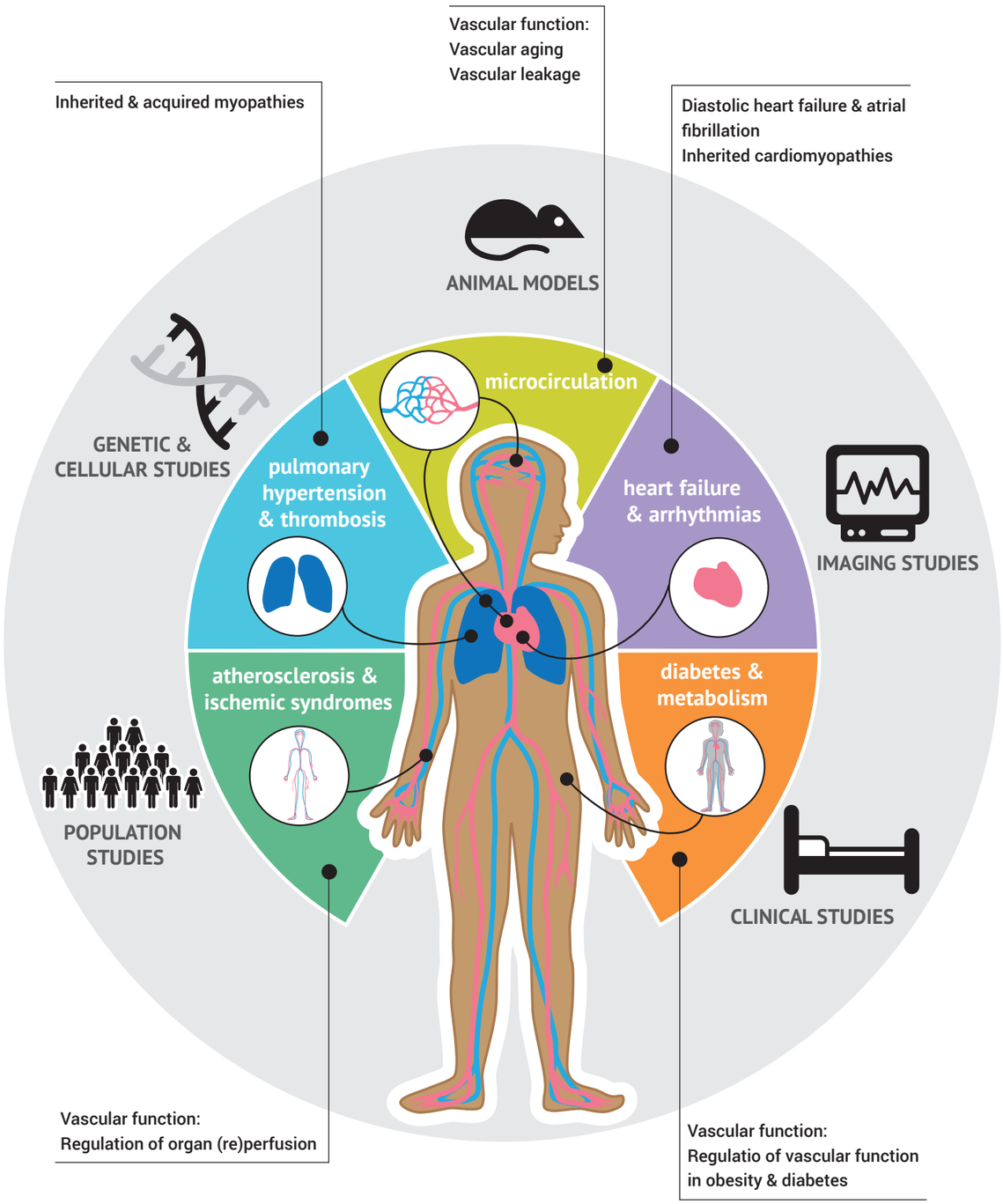
Preface

This report provides an overview of the highlights in 2017-2018, and the organization of our research lines led by principal investigators of our department. The term principal investigator, in short PI, is relatively new to the people working at the VU location of Amsterdam UMC. As part of our alliance with the AMC, we now adopt the term PI and link it to the research themes of the Amsterdam Cardiovascular Sciences institute. The alliance between AMC and VUmc means that we bring together two 'different' cultures, and aim to pick the best of both. The PI system of AMC is used to identify the investigators who are in charge of the main research themes and to bring focus and external funding to Amsterdam UMC which is required to maintain our high level of research. This PI system increases competition between individuals, which is needed to be (inter)nationally competitive. On the other hand, research is not an individual effort, but highly dependent on team efforts, which has been a strong asset at the VUmc for many years as research is organized in research institutes which aim to connect scientists from different disciplines and stimulates collaboration. We need both competition, which is enforced by the PI system, and collaboration, which is facilitated by the research institutes.

We are not only actively involved in research, but also bring the science and knowledge on the cardiovascular system to the future working force by our teaching program, which runs from bachelor courses to the cardiovascular research master. Dr. Pieter Koolwijk and Dr. Dop Simonides have been extremely active in optimizing our Physiology courses and lectures. Overall, our teaching is well rated by students, and Dr. Alice Muller has been selected as runner up for best lecturer of the bachelor program of Medicine.

I can only conclude that the years 2017 and 2018 have been a success both in research and teaching. Let's try to keep quality of our work at a high level by working as a solid team!

Jolanda van der Velden,
Head of the department



Overview Research

The Department of Physiology at VU University Medical Center studies (patho)mechanisms of the cardiovascular system. Our research projects are organized in 3 major research lines: (1) vascular (dys)function, (2) diastolic heart failure and atrial fibrillation and (3) inherited (cardio)myopathies. Our research aims to improve cardiac muscle function and tissue reperfusion and prevent vascular dysfunction in various clinical pathologies (e.g. diabetes). The majority of our research projects is performed in collaboration with investigators from other (clinical) departments. Most studies are translational going from bench to the bedside and back. Finally, our translational research lines are well-related to major research themes of the Amsterdam Cardiovascular Sciences institute as illustrated below.

Heart failure and cardiomyopathies

Prof. Jolanda van der Velden
Prof. Walter Paulus
Dr. Diederik Kuster (UD)
Dr. Willem van der Laarse (UD)
Dr. Warner Simonides (UHD) & Dr. Alice Muller (UD)

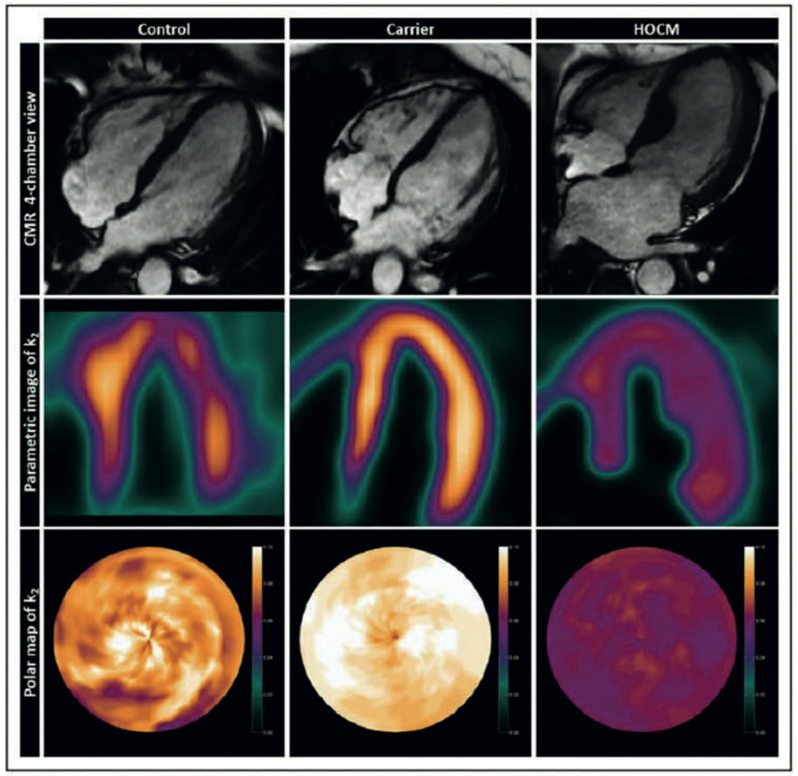
Sarcomeric proteins in cardiac performance

The main research interest of the van der Velden group is to study the role of sarcomeric proteins in cardiac performance for which specific protein analyses and functional assays have been designed. As mutations in sarcomeric proteins are a frequent cause of heart disease at young age, research on this topic was initiated with funding from the European Union and prestigious national grants (VICI). The national CVON-consortium (DOSIS) funded by the Netherlands Heart Foundation, aims to study genetic and environmental effects in cardiomyopathy development. Studies on the genetic heart disease hypertrophic cardiomyopathy include basic cell and tissue analyses, which are combined with in vivo cardiovascular imaging in mouse models and human patients.

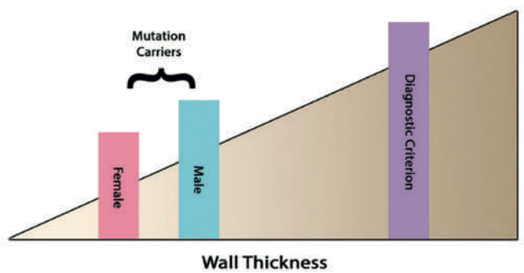
Recent studies revealed that 1) mutations resulting in altered sarcomere proteins cause inefficient contraction of the heart and 2) this cardiac inefficiency is already present in mutation carriers before onset of cardiomyopathy. In 2017, Ahmet Guclu defended his thesis entitled ‘Myocardial O2 utilization and energetics of the left ventricle in hypertrophic cardiomyopathy” in which he described these results. The translational research projects helped to build sufficient proof to initiate a clinical trial sponsored by ZonMW and the Netherlands Heart Foundation.

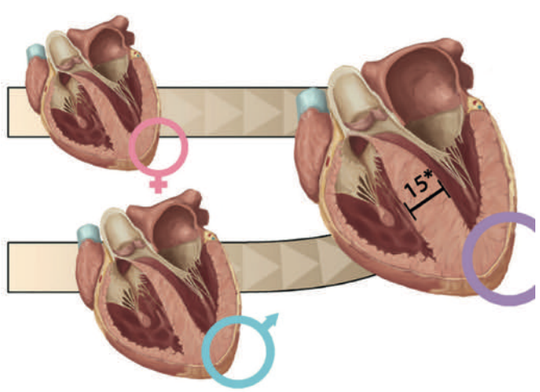
In the ENERGY trial, PhD student Beau van Driel studies the effect of a metabolic drug, Trimetazidine, on cardiac efficiency in asymptomatic mutation carriers. The project is performed in collaboration with the Cardiology departments of Amsterdam UMC (location VU) and Erasmus MC, and Nuclear Medicine of Amsterdam UMC (location VU). Ilse Bollen initiated studies in pediatric and peripartum dilated cardiomyopathy, and defended her thesis entitled: Cardiac remodeling and genotype-specific pathogenic effects in dilated cardiomyopathy (2017). Studies in pediatric cardiomyopathy are continued by Maike Schuldt (PhD student) who will perform a proteomics study in collaboration with the group of Jennifer van Eyk sponsored by the patient organization Hartedroom. In addition, Aref Najafi defended his thesis in 2018 on the interaction between calcium, titin isoform composition and beta-adrenergic receptor stimulation. Louise Nijenkamp published an important study on sex-differences in HCM in Circulation – Heart Failure for which she received the best presentation award during the annual cardiac surgery meeting in 2018. She observed that women have more severe diastolic dysfunction than men at the time of surgery, which may indicate that women already have a more advanced disease stage (more cardiac remodeling). Several of these studies provided the basis for the VICI project awarded to van der Velden in 2018: “Sarcomere inefficiency at the heart of hypertrophic cardiomyopathy.”

Van der Velden organized the ISHR-ES meeting in 2018 in Amsterdam, and currently represents the Talent program within the Dutch Cardiovascular Alliance (https://dcvalliance.nl/; official start in September 2018). Kuster together with Boon and Brundel organized the Dutch-German Joint Meeting of the Molecular Cardiology Working Groups in Amsterdam in 2018.



Cardia imaging of a control, genotype positive/phenotype negative (G+/Ph–), and patient with hypertrophic obstructive cardiomyopathy (HOCM). Cardiovascular magnetic resonance (CMR)-derived cardiac 4-chamber view and parametric images of [^{11}C]-acetate positron emission tomographic-derived average [^{11}C]-acetateclearance rate constant (k_2)with corresponing polar maps. As can be seen clearly, left ventricularremodeling occured in patients with HOCM, evident from an increase in left ventricle (LV) end-diastolic volume, LV mass, and left atrial volume. Myocardial oxygen metabolism was lower in patients with HOCM, whereas an increase in oxygen metabolism was observed in G+/Ph– compared with controls.





Louise Nijenkamp showed that female HCM patients have more severe diastolic dysfunction and cardiac remodelling at the time of myectomy indicating that the criterion for diagnosis may have to be adjusted, based on body size. Currently, the same diagnostic criterion (i.e. maximal wall thickness of 15 mm) is used for all patients, uncorrected for body size. Nijenkamp's study indicates that the threshold may have to be lower, or at least corrected for body size, in females. Currently, the smaller female heart has to 'travel a longer route' to reach the threshold of 15 mm maximal wall thickness, as depicted in the figure. (Van Driel et al. Current opinions in Cardiology 2019 (based on studies from Louise Nijenkamp)

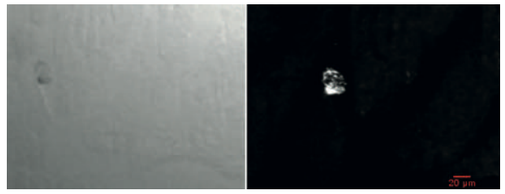
KEY FINDINGS

- Cardiac inefficiency is already present in mutation carriers before onset of cardiomyopathy → ENERGY trial with metabolic therapy.
- More severe diastolic dysfunction in female than male patients with hypertrophic cardiomyopathy → Studies initiated to define if diagnosis criterion of 15 mm wall thickness should be adjusted for body surface area.

Muscle energetics in heart failure

We study the energetics of heart and skeletal muscle with the aim to discover why overloaded muscle fails, as occurs for instance in the heart due to chronically increased blood pressure. In addition, we study ways to optimise muscle function in athletes.

By manipulating energy fluxes pharmacologically using blebbistatin, we have discovered that overloaded hearts fail because the available energy is not used for contraction but for processes related to activation and mitochondrial defects, whereas the contractile machinery functions normally. Mitochondrial efficiency decreases in chronic heart failure partly due to increased permeability of the mitochondrial inner membrane (which is related to cardiolipin metabolism and cytochrome c release). We are investigating a possible cause for this: hydrogen peroxide production by monoamine oxidase located on the outer mitochondrial membrane. It is a possibility that the catecholamines that are oxidised by monoamine oxidase are produced by intrinsic cardiac adrenergic cells (see Figure). The number of these cells (about 150/mm3 in rat heart) may increase with age, and their activity may increase with the load on the myocytes.



An Intrinsic Cardiac Adrenergic (ICA) cell in an unstained 20 µm thick cryostat section in bright-field (left) and the same cell in dark-field. Rat ventricular myocardium.

KEY FINDINGS

- Increased oxidative capacity in muscle fibres of athletes requires matched increased capillary density.
- In hypertrophied hearts capillary density decreases, whereas oxidative capacity increases.
- Mitochondrial function is affected in hypertrophied hearts - probably due to hypoxia.
- Mitochondrial efficiency can be determined in cryostat sections of the heart, allowing for diagnostic tests.
- Papillary muscles (used for in vitro studies) contain a variable number of intrinsic cardiac adrenergic cells.

Thyroid hormone metabolism in chronic heart failure

We and others have found complete impairment of cardiomyocyte-specific thyroid hormone (TH) signaling in various models of ventricular failure induced by chronic hemodynamic overload. This is associated with the induction in cardiomyocytes of the TH-degrading enzyme deiodinase type 3 (Dio3), which appears to be part of the re-activation of an otherwise ineffective cardiac growth program. The Dio3 activity results in low tissue TH levels and associated effects on the expression of key cardiac genes. In 2017-2018 we validated a conditional, cardiac-specific Dio3 knock-out mouse model for use in heart-failure models to test the hypothesized causal role of Dio3 expression in the development of chronic heart failure. This mouse will next be used in a model of cardiac hypertrophy and failure induced by chronic agonist infusion.

The long-standing collaboration with the group of Prof. Larsen and dr. Zavacki at the Brigham and Women's Hospital (Boston) resulted in 2018 in the elucidation of an unexpected role of the TH-activating enzyme deiodinase type 2 (Dio2) in the development of slow skeletal muscle in the mouse.

KEY FINDINGS

- Efficient Cre-mediated excision of the floxed Dio3 active locus in the conditional Dio3-KO mouse is not attained until 3 weeks after administration of Tamoxifen.
- Developmental activation of TH signaling by Dio2 in soleus muscle is required for the full development of slow skeletal muscle fiber-type composition and associated slow contractile properties and fatigue resistance.

PEOPLE

Post-docs:
Paul Wijnker

PhD students:
Edgar Nollet
Beau van Driel
Roy Huurman
Maike Schuldt
Larissa Dorsch
Tim Eijgenraam
Rahana Parbhudayal
Louise Nijenkamp

Technicians:
Max Goebel
Ruud Zaremba

HIGHLIGHTS AND FUNDING

- In 2017 van der Velden received the Outstanding Investigator Award of the International Society for Heart Research.
- NWO VICI grant – Innovational Research 2018. Sarcomere inefficiency at the heart of hypertrophic cardiomyopathy. €1,500,000 (van der Velden).
- Amsterdam Cardiovascular Sciences Out of the Box grant 2018. Targeting genetic heart disease with diabetes medication. Collaboration van der Velden with Coert Zuurbier (AMC). €25,000.
- Amsterdam Cardiovascular Sciences Out of the Box grant 2017. Keeping cardiomyocytes dynamic and exciting: a novel approach to prevent mechanical and electrical dysfunction in cardiomyopathies. Collaboration Kuster with Carol Ann Remme (AMC). €25,000.
- Amsterdam Cardiovascular Sciences equipment grant: Fluorescent Western blot Imager. Collaboraton Kuster with Charissa van den Brom. €28,000.
- Top-grant (ZonMW) 2018: Identification of molecular regulatory pathways for specification and maturation of human cardiac subtypes. Main applicant: Robert Passier. €675,000. Van der Velden Co-PI.
- Cardiovasculair Onderzoek Nederland (CVON) research grant 2017 – Prime: Towards Personalised Medicine in the Clinic: Novel RNA Therapies aimed at heritable forms of treatment-resistant Heart Failure. Principal investigator. National collaboration: coordinators: Prof dr. Y Pinto & Prof dr. L de Windt. €3 million (VUmc part: €100,000).
- Program translational research (ZonMW-Hartstichting) grant 2017. Extra energy. for hearts with a genetic defect: ENERGY trial. Coordinator: Jolanda van der Velden; collaboration with Michelle Michels (Erasmus MC). €400,000.

GRANTS

Crowd-funding Netherlands Heart Foundation (€70,000). Paul Wijnker. 2018.
Amsterdam Cardiovascular Sciences Post-doc grant. A disturbed redox-balance triggers cardiac disease in inherited cardiomyopathy (€75,000). Paul Wijnker. 2018.
First Contact Initiative Grant from the ESC Council on Basic Cardiovascular Science – travel grant. Charlotte Farah visit to Physiology VUmc Amsterdam (group Jean-Luc Balligand) (€2,500). 2017.
Mobility grant. Dorien Deluyker visit to Physiology VUmc Amsterdam (group Virginie Bito). 2017.
ISHR-Servier award (€30,000) to Vasco Sequeira. 2017.

KEY PUBLICATIONS

1. Nijenkamp LLAM, Bollen IAE, van Velzen HG, Regan JA, van Slegtenhorst M, Niessen HWM, Schinkel AFL, Krüger M, Poggesi C, Ho CY, **Kuster DWD**, Michels M, van der Velden J. *Sex-differences at the time of myectomy in hypertrophic cardiomyopathy.* Circulation Heart Failure 2018; 11(6):e004133.
2. **van der Velden J,** Tocchetti CG, Varricchi G, Bianco A, Sequeira V, Hilfiker-Kleiner D, Hamdani N, Leite-Moreira A, Mayr M, Falcão-Pires I, Thum T, Dawson DK, Balligand JL, Heymans S. *Metabolic changes in hypertrophic cardiomyopathies*. Cardiovascular Research 2018;114:1273-1280.
3. van Deel ED, Najafi A, Fontoura D, Valent E, Goebel M, Kardux K, Falcão-Pires I, **van der Velden J.** *In vitro model to study the effects of matrix stiffening on Ca2+ handling and myofilament function in isolated adult rat cardiomyocytes.* Journal of Physiology 2017;595:4597-4610. *With journal club paper by Callaghan NI, Lee XA. Extracellular matrix stiffness affects contractility in adult rat cardiomyocytes: implications for dynamic nitric oxide signalling and calcium handling.*
4. Bollen IAE, Schuldt M, Harakalova M, Vink A, Asselbergs FW, Pinto JR, Krüger M, **Kuster DWD, van der Velden J.** *Genotype-specific pathogenic effects in human dilated cardiomyopathy.* Journal of Physiology 2017;595:4677–4693. doi: 10.1113/JP274145
5. Güçlü A, Knaapen P, Harms HJ, Parbhudayal RY, Michels M, Lammertsma AA , van Rossum AC, Germans T, **van der Velden J.** *Disease stage-dependent changes in cardiac contractile performance and oxygen utilization underlie reduced myocardial efficiency in human inherited hypertrophic cardiomyopathy.* Circulation Cardiovascular Imaging 2017; 10(5). With editorial by Lu and Abraham. A good heart is hard to find even early in hypertrophic cardiomyopathy.
6. Parbhudayal RY, Garra AR, Götte MJW, Michels M, Pei J, Harakalova M, Asselbergs FW, van Rossum AC, **van der Velden J,** Kuster DWD. V*ariable cardiac myosin binding protein-C expression in the myofilaments due to MYBPC3 mutations in hypertrophic cardiomyopathy.* Journal of Molecular and Cellular Cardiology 2018 123:59-63
7. Janssen R, Muller A, **Simonides WS.** *Cardiac Thyroid Hormone Metabolism and Heart Failure* Eur Thyroid J. 6: 130-137, 2017.
8. Janssen R, Zuidwijk MJ, **Muller A**, van Mil A, Dirkx E, Oudejans CB, Paulus WJ, **Simonides WS.** *MicroRNA 214 Is a Potential Regulator of Thyroid Hormone Levels in the Mouse Heart Following Myocardial Infarction, by Targeting the Thyroid-Hormone-Inactivating Enzyme Deiodinase Type III.* Front Endocrinol. doi: 10.3389/fendo.2016.00022, 2016.
9. Janssen R, Zuidwijk MJ, **Kuster DW,** Muller A, **Simonides WS**. T*hyroid Hormone-Regulated Cardiac microRNAs are Predicted to Suppress Pathological Hypertrophic Signaling.* Front Endocrinol. doi: 10.3389/fendo.2014.00171, 2014.
10. Salvatore D, Simonides WS, Dentice M, et al. *Thyroid hormones and skeletal muscle-new insights and potential implications.* Nature Rev Endocrinol 10: 206-214, 2014.

Protein quality control in the heart

Prof. Bianca Brundel

The incidence and prevalence of cardiac diseases, which are the main cause of death worldwide, are likely to increase because of population ageing. Prevailing theories about the mechanisms of ageing feature the gradual derailment of cellular protein homeostasis (proteostasis) and loss of protein quality control as central factors. In the heart, loss of protein patency, owing to flaws in genetically-determined design or because of environmentally-induced 'wear and tear', can overwhelm protein quality control, thereby triggering derailment of proteostasis and contributing to cardiac ageing. Failure of protein quality control involves impairment of chaperones, ubiquitin-proteosomal systems, autophagy, and loss of sarcomeric and cytoskeletal proteins, all of which relate to induction of cardiomyocyte senescence.

The role of protein quality control systems is studied in the most common cardiac arrhythmia atrial fibrillation. By utilizing tachypaced atrial cardiomyocytes and Drosophila, the protein quality control is 1) genetically targeted to identify key modulators involved in AF onset and progression 2) pharmacologically targeted to test novel therapeutic strategies to be ultimately tested in patients with AF. Maintenance of cardiac proteostasis offers a novel therapeutic strategy to promote cardiac health and combat cardiac disease, including AF. Marketed drugs are available to explore this concept in the clinical setting. Currently, the first clinical trial in AF patients with an HSP-inducing compound is conducted. In addition, key modulators of proteostasis are measured in atrial tissue and serum samples of patients in various stages of AF to identify novel biomarkers to early predict the onset and progression of clinical AF.

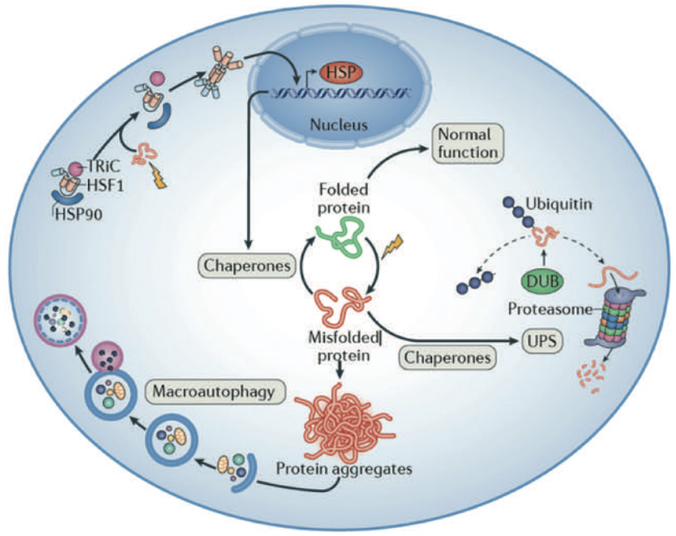


Figure 1: Overview of the protein quality control system. Stress causes misfolding of nascent proteins or damage to functional proteins, often resulting in the exposure of hydrophobic surfaces. Subsequent binding by chaperones either initiates protein refolding or prevents interaction and subsequent aggregation of misfolded or damaged proteins. Under nonstressful conditions, heat shock factor protein 1 (HSF1) monomers are associated with a chaperone complex that includes heat-shock protein (HSP) 90 and T complex protein 1 ring complex (TRiC). During protein misfolding, the chaperones dissociate from the complex and bind to unfolded proteins. Dissociation of the complex frees HSF1 monomers, which then translocate to the nucleus as HSF1 trimers and stimulate HSP expression. When (re)folding is not an option, chaperones keep the misfolded protein in a soluble state, so that protein-degradation systems can dispose of it. Ubiquitin–proteasome system (UPS)-mediated proteolysis is the primary degradation system that can remove soluble (chaperone-presented) misfolded, oxidized, mutant, or otherwise-damaged proteins. The UPS also degrades normal proteins that are no longer needed, providing temporal regulation of protein activity. If the proteins cannot be maintained in a soluble state, autophagy is the primary route to clear the aggregated proteins. DUB, deubiquitinating enzymes.

KEY FINDINGS

- Key modulators of proteostasis have been identified with prominent role in AF progression and recovery.
- Drugs directed at key modulators prevent AF and also aid in the recovery from AF-induced cardiomyocyte damage.
- Several key modulators represent biomarkers to predict stage and recurrence of AF after treatment.
- First clinical trial with HSP-inducing compound in symptomatic AF patients is started early 2018.
- Role proteostasis derailment is expanded to other cardiac diseases.

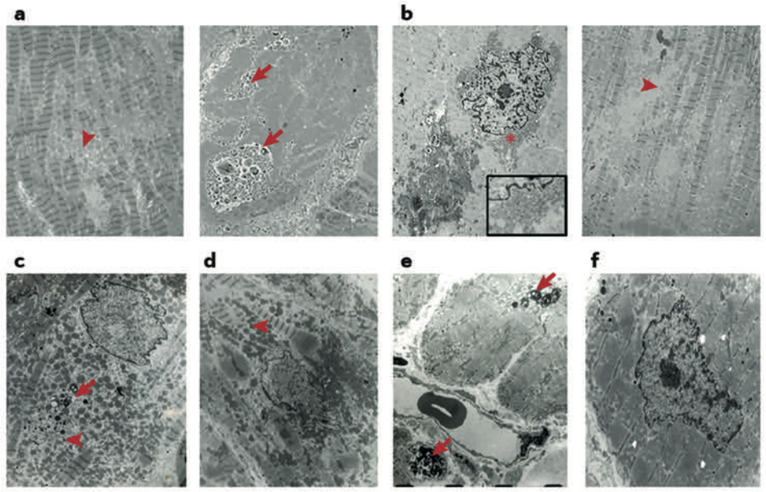


Figure 2: Loss of protein quality control in the myocardium of patients with cardiac disease. Electron microscopy pictures showing characteristics of protein degradation and activation of protein quality control in ventricular (top row) and atrial (bottom row) myocardium. a | A patient with hypertrophic cardiomyopathy resulting from a mutation in MYBPC3, showing loss of sarcomeric structure (myolysis; arrowhead in left panel) and autophagy (arrows in right panel). b | A patient with hypertrophic cardiomyopathy who was negative for sarcomere mutations, showing perinuclear aggregates (asterisk and inset magnification in left panel) and area with myolysis (arrowhead in right panel). c | A patient with >1 year of persistent atrial fibrillation. d | A patient after myocardial infarction. e | A patient with mitral valve disease. All cardiac diseases show significant loss of sarcomeric structure (myolysis; arrowheads), and autophagy is also observed (arrows). f | An atrial cardiomyocyte from a patient undergoing CABG surgery shows normal sarcomeres with no signs of derailment of protein quality control.

KEY PUBLICATIONS

1. Den Hoed M, Eijgelsheim M, Esko T, Brundel BJJM et al. Heart rate-associated loci and their effects on cardiac conduction and rhythm disorders- results from a GWAS in up to 181.171 individuals. Nature Genetics 2013; 45:621-31.
2. Zhang D, .., Brundel BJJM. Activation of Histone Deacetylase-6 (HDAC6) induces contractile dysfunction through derailment of α-tubulin proteostasis in experimental and human Atrial Fibrillation. Circulation 2014; 129:346-58.
3. Wiersma M, Meijering RAM, Qi XY, Zhang D, Liu T, Hoogstra-Berends F, Sibon OCM, Henning RH, Nattel S, Brundel BJJM. Endoplasmic Reticulum Stress Is Associated With Autophagy and Cardiomyocyte Remodeling in Experimental and Human Atrial Fibrillation. J Am Heart Assoc. 2017 Oct 24;6(10).
4. Henning RH, Brundel BJJM. Proteostasis in cardiac health and disease. Nat Rev Cardiol. 2017 Nov;14(11):637-653. doi: 10.1038/nrcardio.2017.89. Personal invitation
5. Luso A*, Wiersma M*, … Brundel BJJM, Haas D, Sibon OCM, Anikster Y. Mutations in phosphopantothenoylcystein synthetase (PPCS) cause dilated cardiomyopathy. American Journal of Human Genetics. 2018

PATENT

Mitochondrial DNA as biomarker in AF.

GRANTS

- **Deli Zhang:** Dr.Dekker junior postdoc grant Dutch Heart Foundation
- **Marit Wiersma:** ACS post doc grant
- AFIP foundation: Bayer sponsorship 100kE + other 50kE
- Medical Delta: 380 kE
- Amsterdam cardiovascular Sciences - Out of the Box: 3x 25kE

PEOPLE

PhD-students:
Xu Hu, Denise v Marion, Larissa Dorsch

Post-docs:
Marit Wiersma, Deli Zhang

Research assistant:
Lucienne Baks

Myofilaments in muscle contraction

Prof. Coen Ottenheijm

The unifying theme of our research concerns the regulatory and pathogenic role of myofilament proteins in muscle contraction, with special focus on the diaphragm.
Using unique diaphragm biopsies of critically ill patients, we have established that dysfunctional myofilaments are important contributors to inspiratory muscle weakness in critically ill patients. The function of these myofilaments can be improved with novel compounds, whose efficacy we test in our laboratory. Furthermore, we have identified the ubiquitin-proteasome pathway as a therapeutic target to combat diaphragm weakness in critically ill patients, and we are evaluating options to inhibit the activity of this pathway in muscle.
We established that myofilament dysfunction not only contributes to acquired (diaphragm) muscle weakness but also to weakness in patients with inherited forms of muscle disease, such as nemaline myopathy. Using biopsies of more than fifty patients with nemaline myopathy, as well as muscle of genetically engineered mouse models, we established that thin filament length dysregulation contributes only to specific forms of nemaline myopathy.

KEY FINDINGS

- Muscle fiber weakness contributes to diaphragm weakness in critically ill patients, and can be restored by calcium sensitizers.
- Mechanical ventilaton of critically ill patients induces ‘longitudinal’ atrophy of diaphragm fibers, which may severely hamper weaning from ventilatory support.
- The giant protein titin is an important mechanosensor protein in the diaphragm that regulates muscle trophicity.
- Sarcomere dysfunction, in particular thin filament length dysregulation, contributes to muscle weakness in patients with nemaline myopathy due to nebulin mutations, a devastating myopathy for which no cure exists.
- Experiments on sarcomeres from patients revealed that impaired contractility of sarcomeres is an importnat contributor to muscle weakness in patients with actin-related nemaline myopathy.

KEY PUBLICATIONS

1. J. Lindqvist,…, **C.A.C. Ottenheijm**. Am J Respir Crit Care Med. 2018. *Positive end-expiratory pressure ventilation induces longitudinal atrophy in diaphragm fibers.* Am J Respir Crit Care Med. 2018; 198(4):472-485
2. Heunks L, **Ottenheijm C**. *Diaphragm-Protective Mechanical Ventilation to Improve Outcomes in ICU Patients?* Am J Respir Crit Care Med. 2018;197:150-152. (Editorial).
3. B. Joureau,…, **C.A.C. Ottenheijm.** *Dysfunctional sarcomere contractility contributes to muscle weakness in ACTA1-related nemaline myopathy (NEM3).* Annals of Neurol. 2018; 83:269-282.
4. van der Pijl R, Strom J, Conijn S, Lindqvist J, Labeit S, Granzier H, **Ottenheijm C**. *Titin-based mechanosensing modulates muscle hypertrophy*. J Cachexia Sarcopenia Muscle. 2018 Oct;9(5):947-961.
5. van den Berg M,..., **Ottenheijm C.A.C.** *Diaphragm Atrophy and Weakness in the Absence of Mitochondrial Dysfunction in the Critically Ill*. Am J Respir Crit Care Med. 2017;196:1544-1558. (Received Editorial).

GRANTS

Research grant; Prinsess Beatrix Muscle Foundation; COORDINATOR OF CONSORTIUM
Research grant; Foundation for Building Strength for Nemaline Myopathies (USA)
University Research Chair (VU/VUmc); (https://www.vu.nl/en/research/topresearchers-at-vu/university-research-chair/ottenheijm/index.aspx)

PEOPLE

Postdocs:
Michaela Yuen
Josine de Winter

PhD students:
Marloes van den Berg
Martijn van de Locht
Barbara Joureau
Robbert van der Pijl
Saskia
Lassche

Technicians:
Stefan Conijn
Sylvia Bogaards
Shengyi Shen

Endothelial integrity, vascular disease and aging

Prof. Peter Hordijk
Dr. Ed Eringa (UHD)
Prof. Reinier Boon
Dr. Pieter Koolwijk (UD)
Prof. Victor van Hinsbergh

Endothelial integrity

The control of endothelial integrity is one of the key research lines in the group. Already for > 15 years, we focus on the role of the small GTPases of the Rho family and their function in human endothelial cells. Rho GTPases such as RhoA, Rac1 and Cdc42 are all involved in various aspects of cytoskeletal dynamics and, consequently, control endothelial cell-cell adhesion and thereby vascular integrity.

In 2017/2018 we finalized a large set of data obtained by a focused siRNA screen aimed at all RhoGTPases and their regulators. This work has led to a series of publications by Amado-Azevedo et al., identifying novel molecular regulators of endothelial cell-cell contact. Notably, this was the Rac1 GAP HMHA1; a remarkable finding, since this Human Minor Histocompatibility Protein was originally suggested to be only expressed in hematopoietic cells. However, our data show that it plays an important role in the endothelium as a negative regulator of barrier function. Additional studies identified the GTPase Cdc42 as a main positive regulator of cell-cell contact in endothelium, in conjunction with specific regulators such as the GAP protein SYDE1 and the GEFs FARP1 and Tiam2.

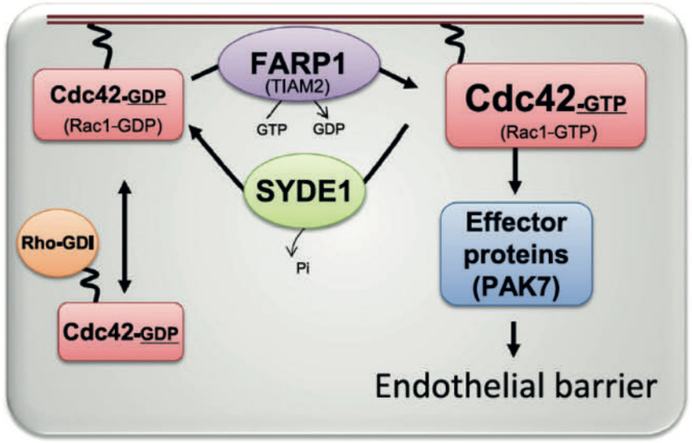


Figure 1: Model depicting the proteins that are part of a Cdc42-centered signalling unti regulating endothelial barrier function in human endothelium (Amado-Azevedo et al., 2017).

A significant body of work was dedicated to the ubiquitination and lysosomal degradation of endothelial RhoB. This work was published in the Journal of Cell Biology in 2018. In collaboration with Japanese colleagues, we identified KCTD10, in complex with CUL3 and Rbx1, as the ubiquitin ligase for RhoB. This is important, since constitutive degradation of RhoB maintains endothelial integrity. Conversely, loss of KCTD10 leads to elevated levels of RhoB, which is always active, and a consequent loss of endothelial barrier function.

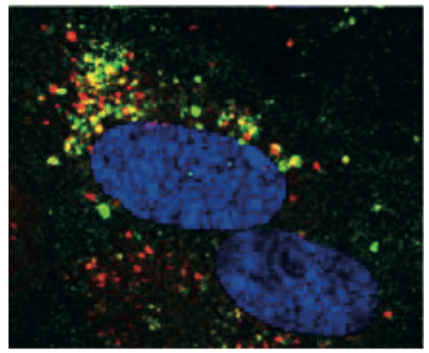


Figure 2: Endogenous RhoB (green) colocalizes with lysosomes (red) in TNFalfa-activated human endothelial cells (Kovacevic et al., 2018)

Finally, several translational studies were published, in part dedicated to the positive role of vitamin D on endothelial integrity, and in part focused on the link between hypertension and vascular inflammation and permeability. These studies were performed in close collaboration with clinical partners from the departments of nephrology and gynaecology.

KEY FINDINGS

- We have performed an extensive analysis comparing three highly related GTPases RhoA, RhoB and RhoC and found that they regulate cell-cell contact differently in resting vs. activated endothelial cells, with RhoB as the major, negative, regulator of endothelial integrity.
- Conversely, we showed that Cdc42 is a dominant positive regulator of endothelial integrity.
- We identified the ubiquitin ligase of the GTPase RhoB: this is a complex of CUL3-Rbx1-KCTD10.
- We also showed that the KCTD10 ligase promotes lysosomal degradation of RhoB.
- The minor histocompatibility antigen 1 (HMHA1)/ArhGAP45, which is a RacGAP was unexpectedly identified as a novel regulator of endothelial integrity.

Microvascular dysfunction in obesity and diabetes

In obesity and type 2 diabetes, impaired perfusion and microvascular inflammation contribute to insulin resistance and organ failure in these conditions. Specifically, we aim to elucidate the role of perivascular adipose tissue (PVAT) in (dys)regulation of blood flow. In the earliest pathogenesis of type 2 diabetes, this balance shifts towards vasoconstrictor adipokines. We have found that insulin controls perfusion of skeletal and cardiac muscle, and that this effect is progressively impaired during the pathogenesis of type 2 diabetes and the metabolic syndrome. Insulin enhances blood flow in healthy subjects, this effect is reduced after ~4 weeks of a hypercaloric diet, is abolished in obesity and worsens to an insulin-induced reduction of blood flow in type 2 diabetes. This microvascular insulin resistance precedes metabolic insulin resistance and hyperglycemia.

In addition to this insulin-specific vascular defect, we have shown that kidney failure, a common complication of obesity and diabetes, independently impairs organ blood flow through the endogenous NOS inhibitor asymmetric dimethyl arginine.

In collaboration with the department of Cardiology, we have translated these insights in microvascular dysfunction in skeletal muscle to the myocardial microcirculation during the past years with the initiation of the MICORDIS cohort of patients with non-obstructive coronary artery disease and age- and sex-matched controls. In this cohort, we are developing new diagnostic tools to identify specific impairments of blood flow in NOCAD patients and provide clinicians with individualized, specific targets for treatment in this heterogeneous group of patients.

KEY FINDINGS

- Perivascular adipose tissue in muscle selectively controls muscle blood flow during hyperinsulinemia
- Perivascular adipose tissue controls glucose uptake and protein expression in muscle
- Blood flow is specifically impaired during hyperinsulinemia in skeletal muscle and the myocardium in patients with type 2 diabetes (figure 1)
- Impairment of insulin-dependent vasodilatation in resistance arteries is impaired in the earliest development of obesity and type 2 diabetes, with identical findings in human volunteers and mice
- In a key complication of type 2 diabetes, kidney failure, FGF23 impairs NO-dependent vasodilatation (figure 2)

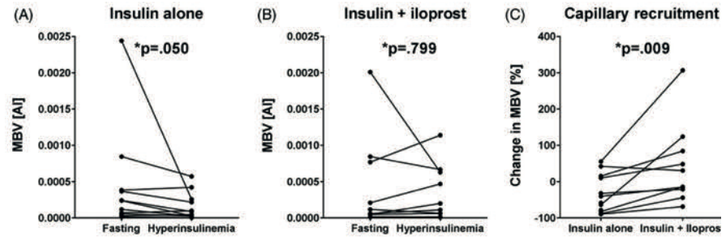


Figure 1: Insulin reduces muscle blood volume in patients with type 2 diabetes, and this impairment can be restored by the stable prostacyclin analogue Iloprost. A, Skeletal muscle microvascular blood volume (MBV) with insulin alone, fasting compared with hyperinsulinaemia; B, skeletal muscle microvascular blood volume (MBV) during insulin combined with iloprost, fasting compared with hyperinsulinaemia; C, percentage change from baseline in MBV (ie, capillary (de)recruitment), insulin alone compared with insulin combined with iloprost. †Wilcoxon-rank test. Abbreviation: AU, arbitrary units. Emanuel, Eringa et al., Diabetes Obes Metab. 2018 Nov;20(11):2523-2531.

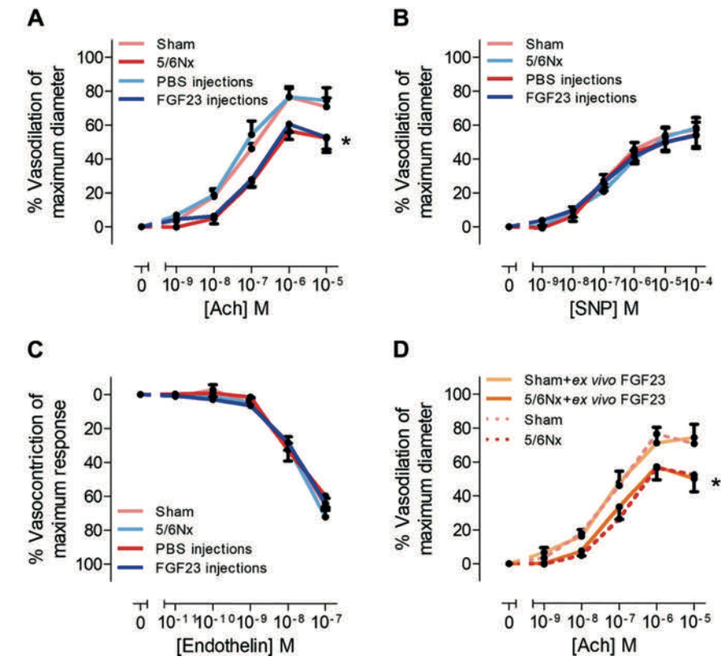


Figure 2: 5/6 Nephrectomy (5/6Nx) impairs endothelial but not vascular smooth muscle function, which is mimicked by chronically increasing circulating fibroblast growth factor 23 (FGF23) concentration. A: 5/6Nx attenuated endothelium-dependent vasodilator responses of the gracilis artery, and this effect was mimicked by increasing FGF23 intraperitoneal injections. The pink line shows sham surgery (n = 12), the light blue line shows PBS intraperitoneal injections (n = 8), the red line shows 5/6Nx surgery (n = 8), and the dark blue line shows FGF23 intraperitoneal injections (n = 7). B: 5/6Nx and increased FGF23 concentration in isolation did not impair vasodilator responses of gracilis resistance arteries to the endothelium-independent vasodilator sodium nitroprusside (SNP). The pink line shows sham surgery (n = 9), the light blue line shows PBS intraperitoneal injections (n = 8), the red line shows 5/6Nx surgery (n = 8), and the dark blue line shows FGF23 intraperitoneal injections (n = 7). C: 5/6Nx and high concentration of FGF23 in the absence of chronic kidney disease do not impair vasoconstrictor responses to endothelin. The pink line shows sham surgery (n = 6), the light blue line shows PBS intraperitoneal injections (n = 5), the red line shows 5/6Nx surgery (n = 5), and the dark blue line shows FGF23 intraperitoneal injections (n = 7). D: arteries from sham and 5/6Nx mice were incubated for 1 h with recombinant FGF23 and subsequently exposed to acetylcholine (ACh), but this did not change endothelial function compared with arteries without incubation with FGF23. The light orange line shows sham surgery and ex vivo FGF23 incubation (n = 7), the dark orange line shows 5/6Nx surgery and ex vivo FGF23 incubation (n = 7), the pink dotted line shows sham surgery (n = 12), and the red dotted line shows 5/6Nx surgery (n = 8). Data are means ± SE. *P ≤ 0.05 vs. sham or PBS, by linear mixed models. M Verkaik, M Vervloet, E Eringa et al., Am J Physiol Heart Circ Physiol. 2019 in press

Aging in cardiovascular disease

Aging is the main independent risk factor for cardiovascular disease. In recent years, it has become clear that the majority of RNA is not translated to protein. These so-called non-coding RNAs control various cellular functions by interacting with DNA, other RNAs and proteins. Our group focuses on long non-coding RNAs (lncRNAs) that are involved in aging of endothelial cells and cardiomyocytes. A third research line focuses on lncRNAs that play a role in biomechanical signaling in endothelial cells induced by shear stress.

We have identified several lncRNAs that control key processes in cardiovascular cells, including the endothelial lncRNAs Meg3, Meg8, H19, Lassie, linc-pint and Aerrie. Meg3 is induced by aging and regulates angiogenesis, whereas H19 is repressed during aging, which causes excessive Stat3 signaling and inflammatory activation. Lassie and Aerrie are highly shear stress sensitive and control endothelial barrier function. Meg8 and linc-pint are mainly regulated by aging and regulate barrier function and DNA damage signalling, respectively. Additionally, we rah, a cardiomyocyte-enriched aging-re- that controls cardiomyocyte survival via helix-mediated interaction with the proval genes.

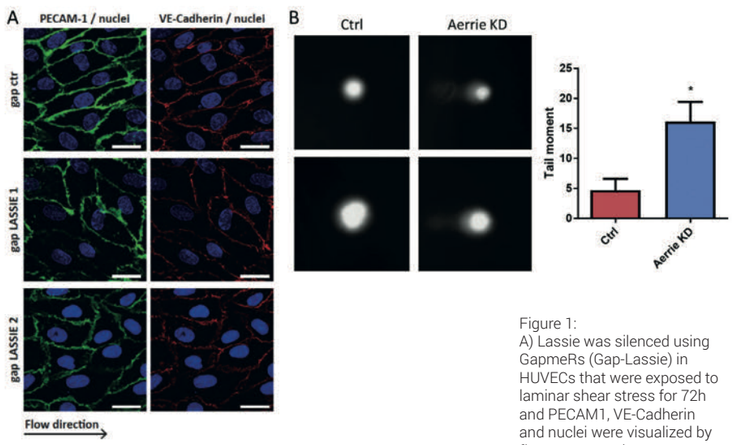


Figure 1:
A) Lassie was silenced using GapmeRs (Gap-Lassie) in HUVECs that were exposed to laminar shear stress for 72h and PECAM1, VE-Cadherin and nuclei were visualized by fluorescence microscopy.
B) Aerrie was silenced using GapmeRs (Aerrie KD) in HUVECs. DNA damage was analyzed with a comet assay.

KEY FINDINGS

- Non-coding RNAs are involved in aging of the cardiovascular system.
- The aging-regulated lncRNA Meg8 regulates endothelial barrier function, partly through control of neighbouring miRNAs.
- The cardiomyocyte-enriched, aging-regulated lncRNA Sarrah is a positive regulator of cardiomyocyte survival and contractile function via triple helix formation and transcription activation.
- The shear stress-regulated lncRNA Lassie and H19 regulate endothelial cell function.
- The aging- and shear stress-regulated lncRNA Aerrie regulates DNA damage signaling.

Endothelial Cardiomyocyt interactions in heart failure

In 2017 we started a project to study the mutual interaction between human cardiac microvascular endothelial cells and rat cardiomyocytes in particular in the context of heart failure. To that end a model was set up that enabled the co-culture of these cells and the subsequent multiple analysis of contraction and relaxation of paced rat cardiomyocytes. The project was part of and supported by the CVON RECONNECT program.

We showed that CMEC enforced both the contractile and relaxing forces of cardiomyocytes, a property that was lost after inflammatory activation of CMEC with TNFα or inteleukin-1β. The beneficial effect of the CMEC was largely dependent of the production of NO, a property that was lost due to elevated production of reactive oxygen species after TNFα. Interestingly, co-incubation of CMEC with TNFα and the SGLT-2 inhibitor empagliflozin and subsequent washing prevented the enhanced ROS production and the loss of NO production, and maintained the improved cardiomyocyte contractility induced by CMEC (published in 2019 in JACC: Basic to Translational Science). These data fit with the outcome of present trials showing a beneficial effect of SGLT-2 inhibitors on heart failure and can help to better understand the underlying mechanisms and future improvements of heart failure with preserved ejection fraction.

Hypoxia and tissue repair

The hypoxia-inducible factors, HIF-1α and HIF-2α play an important role in the endothelial adaptation to a low oxygen environment. We and other previously showed in human microvascular endothelial cells that while HIF-1α stimulates the formation of new sprouts, HIF-2α stabilizes neovessels and prevents sprout formation. By comparing differentially regulated genes in hypoxia (1% O2) with the genes that were differentially regulated upon silencing of HIF-2α in hypoxia we identified 51 genes, which were subsequently screened for their role in endothelial sprouting in hypoxia. From si-RNA inhibition of these genes individually, 4 new genes (ARRDC3, MME, PPARG and RALGPS2) were recognized that directly influenced endothelial sprouting during prolonged hypoxic culturing (Nauta et al, 2017).

Endothelial colony forming cells (ECFCs) are cells with an endothelial nature that can be retrieved and cultured from circulating blood cells and display a large growth potential. They can be used both as a source of endothelial cells from individual patients e.g. for patients with lung disease (Smits et al, 2018) and as a source of endothelial cells for potential revascularization of tissue-engineered (Tasev et al, 2016; Medina et al 2017).

Upon evaluation of the effect of hypoxia on ECFCs we observed that sprouting was affected in a similar way, but that also their proliferation was retarded in contrast to microvascular endothelial cells (Tasev et al, 2018). Gene array of hypoxia-altered gene expression revealed that 3 of the 4 HIF-2α induced candidate genes mentioned above were also induced in ECFCs, together with several known angiogenic factors. We suggest that ECFCs may perform best in neovascularization at the border between normoxic and hypoxic tissue.

PEOPLE

Post-docs:
Igor Kovacevic
Rio Juni
Noelia Lozano-Vidal
Diewertje Bink
Kim Sjoberg (University of Copenhagen)

PhD students:
Joana Amado-Azevedo
Manon Pronk
Marc Vila-Cuenca
Jisca Majolee
Vanessa Meyn
Dimitar Tasev
Laura Stanicek
Phat Pham
Veerle Kremer
Kelly ‘t Hart
Pien Spoor (i.c.w. dept. Cardiology),
Jayron Habibe (i.c.w. dept. Biochemistry),
Anna Emanuel (i.c.w. dept. Internal Medicine)

Technicians:
Jan van Bezu
Michiel van Wijhe
Zeineb Gam

GRANTS (RB)

- ACS PhD Student grant (VUmc&AMC), €240K
- ERA-CVD Consortium (INNOVATION), €1100K total, €240K Ams
- EU Horizon 2020 Consortium (Cardioregenix), €15M total, €1100K Ams
- ACS PhD Student grant (VUmc&AMC), €240K
- Rembrandt Research Award (Rembrandt Institute), €225K
- Vidi Grant from the Dutch Scientific Organization (NWO), €800K

GRANTS (EE)

- Netherlands Heart and Lung Institute, 100 k€
- VUMC Jubileum Foundation, 50 k€
- Netherlands Heart Foundation, innovation grant, 60 k€
- Novo Nordisk, 50 k€
- Amgen, 50 k€
- EU (Horizon 2020) – IMPROVE-PD: 3,2 M€ (with M Vervloet and E Lutgens)

AWARD (EE)

- Annual Dutch Diabetes Research Meeting 2018, best abstract

KEY PUBLICATIONS

1. Kovačević I, Sakaue T, Majoleé J, Pronk MC, Maekawa M, Geerts D, Fernandez-Borja M, Higashiyama S, **Hordijk PL.** The Cullin-3-Rbx1-KCTD10 complex controls endothelial barrier function via K63 ubiquitination of RhoB. J Cell Biol. 2018 Mar 5;217(3):1015-1032.
2. Amado-Azevedo J, Reinhard NR, van Bezu J, van Nieuw Amerongen GP, **van Hinsbergh VWM, Hordijk PL.** *The minor histocompatibility antigen 1 (HMHA1)/ArhGAP45 is a RacGAP and a novel regulator of endothelial integrity.* Vascul Pharmacol. 2018 Feb;101:38-47.
3. Reinhard NR, Mastop M, Yin T, Wu Y, Bosma EK, Gadella TWJ Jr, Goedhart J, **Hordijk PL.** *The balance between Gα(i)-Cdc42/Rac and Gα(1)(2)/(1)(3)-RhoA pathways determines endothelial barrier regulation by sphingosine-1-phosphate.* Mol Biol Cell. 2017 Nov 7;28(23):3371-3382.
4. Verkaik M, Juni RP, van Loon EPM, van Poelgeest E, Kwekkeboom RFJ, Gam Z, Richards WG, Ter Wee PM, Hoenderop JGJ, Vervloet MG, and Eringa EC. *FGF23 impairs peripheral microvascular function in renal failure.* Am J Physiol Heart Circ Physiol. 2018 Nov 1;315(5):H1414-H1424.
5. Emanuel AL, de Clercq NC, Koopen AM, van Poelgeest E, Serlie MJM, van Raalte DH, Kramer MHH, Nieuwdorp M, Serne EH, and **Eringa EC**. *Iloprost infusion prevents the insulin-induced reduction in skeletal muscle microvascular blood volume but does not enhance peripheral glucose uptake in type 2 diabetic patients.* Diabetes Obes Metab Nov;20(11):2523-2531, 2018.
6. Emanuel AL, Meijer RI, Muskiet MH, van Raalte DH, Serné EH, and **Eringa EC.** *Role of Insulin-Stimulated Adipose Tissue Perfusion in the Development of Whole-Body Insulin Resistance.* Arterioscler Thromb Vasc Biol 37: 411-418, 2017.
7. Meijer RI, Serné EH, Yudkin JS, van Hinsbergh VW, Smulders YM, and **Eringa EC.** *Perivascular fat in human muscle.* Lancet Diabetes Endocrinol 4: 958, 2016.
8. Meijer RI, Serné EH, Korkmaz HI, van der Peet DL, de Boer MP, Niessen HW, **van Hinsbergh VW,** Yudkin JS, Smulders YM, and **Eringa EC.** *Insulin-induced changes in skeletal muscle microvascular perfusion are dependent upon perivascular adipose tissue in women.* Diabetologia 58: 1907-1915, 2015.

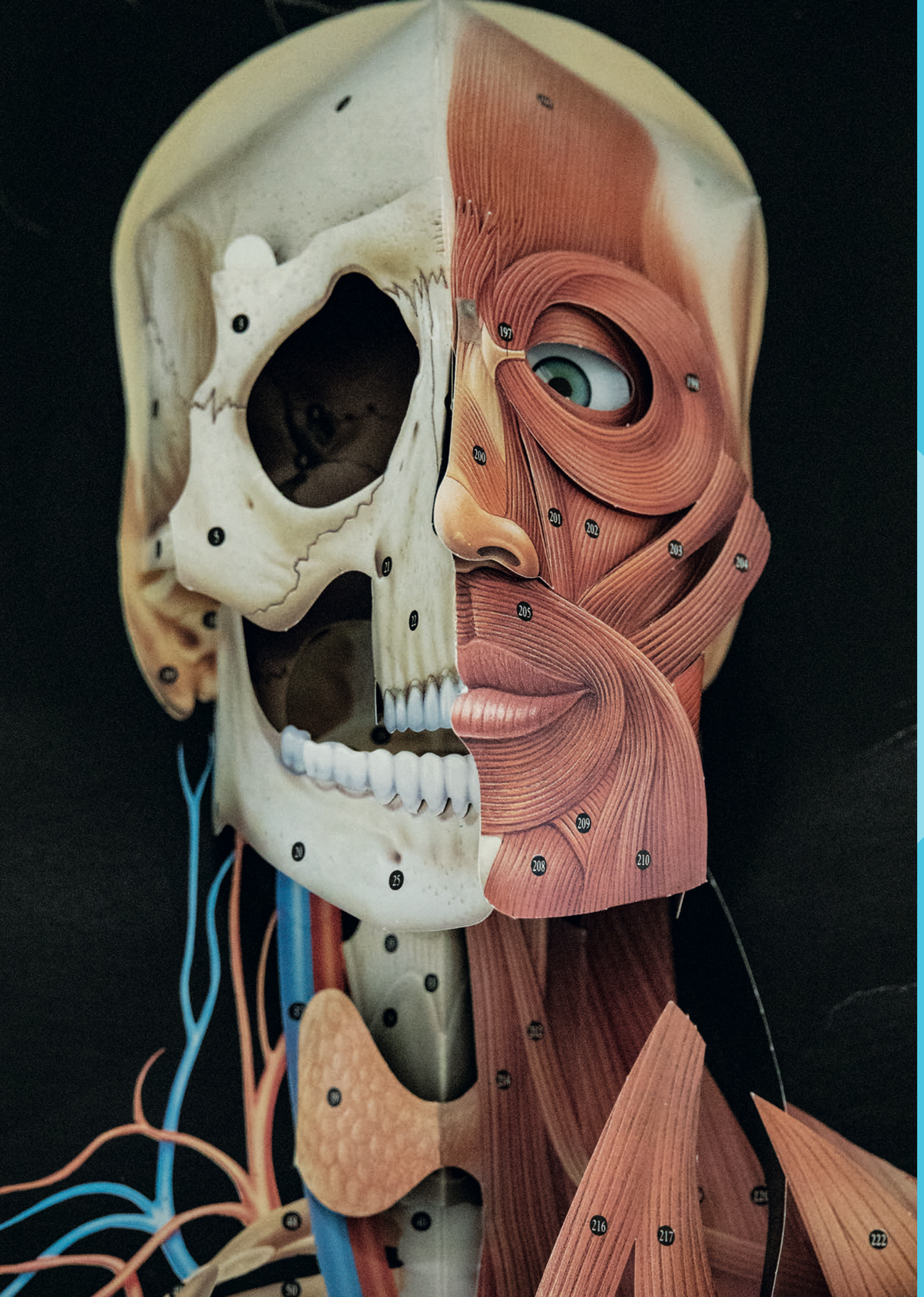
9. **Boon RA,*** Iekushi K,* Lechner S, Seeger T, Fischer A, Heydt S, Kaluza D, Treguer K, Carmona G, Bonauer A, Horrevoets AJ, Didier N, Girmatsion Z, Biliczki P, Ehrlich JR, Katus HA, Müller OJ, Potente M, Zeiher AM, Hermeking H, Dimmeler *S. MicroRNA-34a controls cardiac aging and function*. Nature 2013 495(7439):107-10.
10. **Boon RA,** Hofmann P, Michalik KM, Lozano-Vidal N, Berghäuser D, Fischer A, Knau A, Jaé N, Schürmann C, Dimmeler S. *Long Noncoding RNA Meg3 Controls Endothelial Cell Aging and Function: Implications for Regenerative Angiogenesis.* J Am Coll Cardiol. 2016 68(23):2589-2591.
11. Doddaballapur A, Michalik KM, Manavski Y, Lucas T, Houtkooper RH, You X, Chen W, Zeiher AM, Potente M, Dimmeler S, **Boon RA.** *Laminar Shear Stress Inhibits Endothelial Cell Metabolism via KLF2-Mediated Repression of PFKFB3.* Arterioscler Thromb Vasc Biol. 2015 35(1):137-45.
12. Michalik KM, You X, Manavski Y, Doddaballapur A, Zörnig M, Braun T, John D, Ponomareva Y, Chen W, Uchida S, **Boon RA,*** Dimmeler S.* The Long Noncoding RNA MALAT1 Regulates Endothelial Cell Function and Vessel Growth. Circ Res. 2014 114(9):1389-97.
13. Manavski Y, Lucas T, Glaser SF, Dorsheimer L, Günther S, Braun T, Rieger MA, Zeiher AM, Boon RA*, Dimmeler S*. *Clonal Expansion of Endothelial Cells Contributes to Ischemia-Induced Neovascularization.* Circ Res. 2018 122(5):670-677.
14. Juni R, Kuster DWD, Goebel M, Helmes M, Musters RJ, van der Velden J, **Koolwijk P,** Paulus WJ, van Hinsbergh VWM, *Cardiac microvascular endothelial enhancement of cardiomyocyte function is impaired by inflammation and restored by empagliflozin.* J Am Coll Cardiol Basic Trans Science, 2019; 4: 575-591.
15. Nauta TD, van den Broek M, Gibbs S, van der Pouw-Kraan TCTM, Oudejans CB, **van Hinsbergh VWM, Koolwijk P,** *Identification of HIF-2α –regulated genes that play a role in human microvascular endothelial sprouting during prolonged hypoxia in vitro.* Angiogenesis, 2017; 20:39-54.
16. Smits J, Tasev D, Andersen S, Szulcek R, Botros L, Ringgaard S, Andersen A, Vonk-Noordegraaf A, **Koolwijk P,** Bogaard HJ, B*lood Outgrowth and Proliferation of Endothelial Colony Forming Cells are Related to Markers of Disease Severity in Patients with Pulmonary Arterial Hypertension.* Int J Mol Sci. 2018; 19(12): E3763. doi: 10.3390/ijms19123763.
17. Tasev D, **Koolwijk P, van Hinsbergh VWM,** *Therapeutic potential of human-derived endothelial-colony forming cells in animal models.* Tissue Eng. Part B Rev 2016; 22:371-382.
18. Medina RJ, Barber CL, Sabatier F, Dignat-George F, Melero-Martin JM, Khosrotehrani K, Ohneda O, Randi AM, Chan JKY, Yamaguchi T, van Hinsbergh VWM, Yoder MC, Stitt AW, *Endothelial Progenitors: A consensus statement on Nomenclature.* Stem Cells Translational Medicine, 2017; 6: 1316-1320.
19. Tasev D, Dekker-Vroling L, van Wijhe M, Broxterman HJ, **Koolwijk P, van Hinsbergh VWM.** *Hypoxia Impairs Initial Outgrowth of Endothelial Colony Forming Cells and Reduces Their Proliferative and Sprouting Potential.* Front Med (Lausanne). 2018; 5:356. doi: 10.3389/fmed.2018.00356.

*These authors contributed equally

Overview Education

Within the VUmc School of Medical Sciences our main education tasks involve the bachelor study of Medicine and the two-year master program Cardiovascular Research (Director: Dr. Warner Simonides). At the VU campus we coordinate several cardiovascular and medical physiology courses for the studies Medical Natural Sciences, Biomedical Sciences, Health & Lifestyle, and Health Sciences of the Faculty of Sciences. In addition, our department provides (patho)physiology courses for students of the Amsterdam University College (AUC).

Practical courses play a central role in physiology education and are part of the training of the medical and biomedical students at the Amsterdam UMC and the VU. A team of 22 student-assistants and 12 PhDs or PhD-students, supervised by Dr. Pieter Koolwijk, take care of 51 practical courses in more than 230 sessions each year, teaching nearly 10.000 students. The logistics and the maintenance of the practical set-ups are tasks of the amanuensis Rob de Jong and the electrical engineers Duncan van Groen and Andreas de Haas.



Awards 2017-2018

Walter Paulus
Life Time Achievement Award by the Heart Failure Association of the ESC, May 2018

Reinier Boon
Laura Stanicek
Young Investigator Award, Netherlands Vascular Biology Meeting, November 2017
Young Investigator Award, Joint Dutch-German Vascular Biology Conference, March 2018
Keystone Symposia Scholarship (Long Noncoding RNAs: From Molecular Mechanism to Functional Genetics), November 2018

Diederik Kuster
Maike Schuldt
3rd Poster Prize, 1st Cardiovascular translational research meeting, Utrecht, 2017 (shared with Larissa)
Best Poster Award, Annual ACS symposium, Amsterdam, 2017
Best Poster Award, Annual ACS symposium, Amsterdam, 2018
Young Investigator Award, International Society for Heart Research - European Section (ISHR-ES), Amsterdam, 2018

Paul Wijnker
International Society for Heart Research - European Section (ISHR-ES) Poster Prize Award, the 34th Meeting of the European Section of the ISHR-ES, Hamburg, Germany, July 2017 (€250)

Larissa Dorsch
3rd Poster Prize, 1st Cardiovascular translational research meeting, Utrecht, 2017 (shared with Maike)
3rd prize Winner Contest Communicating Science to People in 2018 Young@Heart and Netherlands Heart Institute

Ed Eringa
NVDO 2018, best abstract (shared with Alexander Turaihi).

Theses 2017 -2018

JM de Winter, 27-01-2017
Nemaline myopathy: Pathophysiology and Therapeutic targets

T Nauta, 15-02-2017
HIF-2a regulates in vitro neovascularization during hypoxia

CPM Franssen, 23-02-2017
Heart Failure with Preserved Ejection Fraction: The Media Message

RFJ Kwekkeboom, 11-04-2017
Ultrasound triggered microbubble destruction for targeted delivery of miRNA-therapeutics

A Güçlü, 18-05-2017
Myocardial O2 utilazation and energetics of the left ventricle in hypertrophic cardiomyopathy

NJ Koning, 23-06-2017
Protection of the microcirculation during cardiac surgery with cardiopulmonary bypass

D Tasev, 22-02-2018
Endothelial Colony Forming Cells (ECFCS) for tissue regeneration in vitro characterization

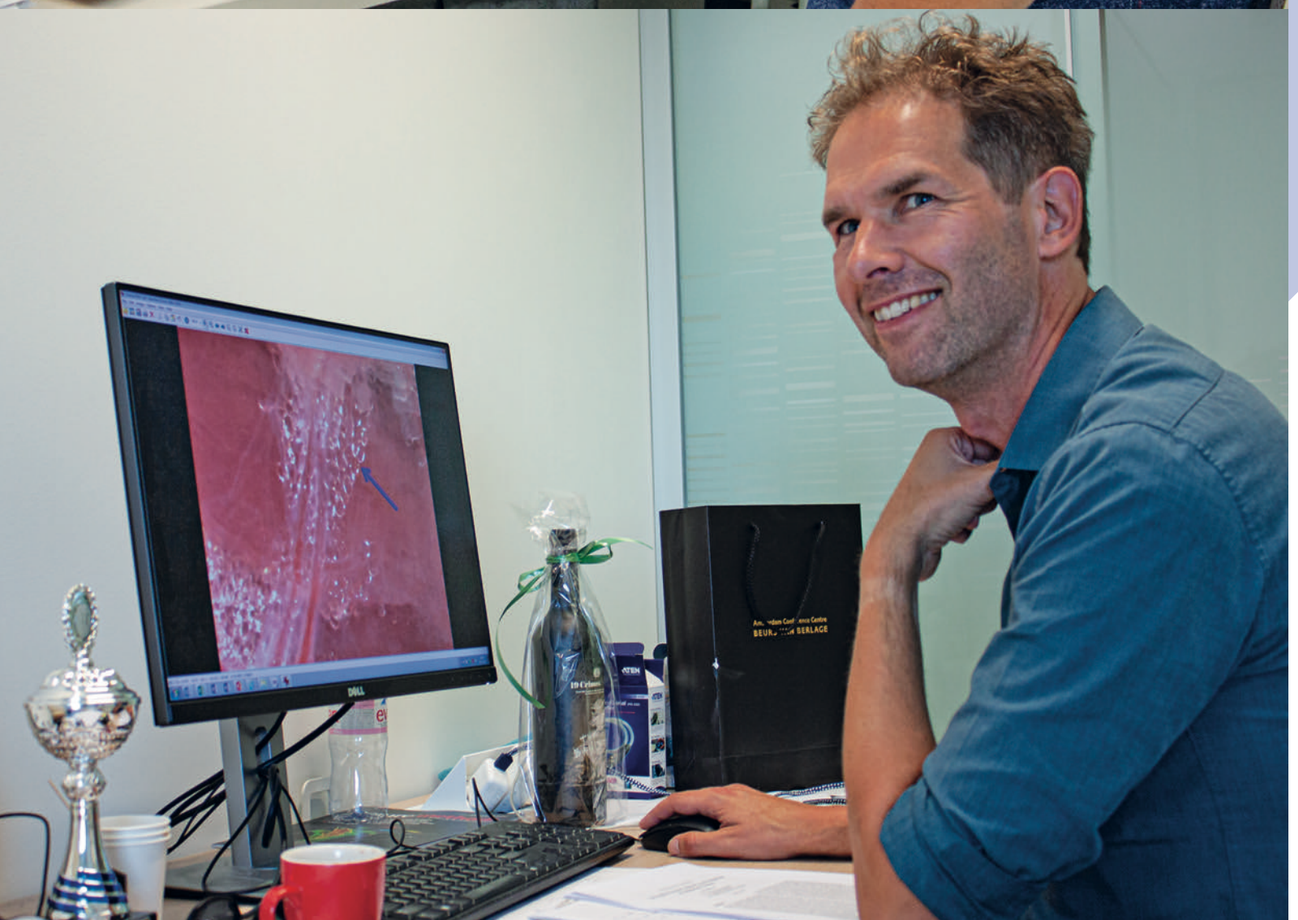
A.E. Bollen, 29-01-2018
Cardiac remodeling and genotype-specific pathogenic effects in dilated cardiomyopathy

A Turaihi, 05-10-2018
Effect of PVAT on muscle microcirculation in vivo: Life Style, Genetic and Surgical Approaches

MCA Pronk, 24-10-2018
RhoGTPases, post-translational modifications and tyrosine kinases in endothelial barrier regulation

A Najafi, 08-11-2018
Disease modifiers in hypertrophic cardiomyopathy

JM Coelho Amado de Azevedo, 17-12-2018
Regulation of vascular permeability by RhoGTPases: Fiat lux! (Let there be light!)



Publications 2017-2018

20. Alma, L.J., De Groot, C.J.M., De Menezes, R.X., Hermes, W., Hordijk, P.L., and Kovacevic, I. (2018). *Endothelial dysfunction as a long-term effect of late onset hypertensive pregnancy disorders: High BMI is key.* Eur J Obstet Gynecol Reprod Biol 225, 62-69.

21. Amado-Azevedo, J., de Menezes, R.X., van Nieuw Amerongen, G.P., van Hinsbergh, V.W.M., and Hordijk, P.L. (2018). *A functional siRNA screen identifies RhoGTPase-associated genes involved in thrombin-induced endothelial permeability.* PLoS One 13, e0201231.

22. Amado-Azevedo, J., Reinhard, N.R., van Bezu, J., de Menezes, R.X., van Beusechem, V.W., van Nieuw Amerongen, G.P., van Hinsbergh, V.W.M., and Hordijk, P.L. (2017). *A CDC42-centered signaling unit is a dominant positive regulator of endothelial integrity.* Sci Rep 7, 10132.

23. Amado-Azevedo, J., Reinhard, N.R., van Bezu, J., van Nieuw Amerongen, G.P., van Hinsbergh, V.W.M., and Hordijk, P.L. (2018). *The minor histocompatibility antigen 1 (HMHA1)/ArhGAP45 is a RacGAP and a novel regulator of endothelial integrity.* Vascul Pharmacol 101, 38-47.

24. Attaye, I., Smulders, Y.M., de Waard, M.C., Oudemans-van Straaten, H.M., Smit, B., Van Wijhe, M.H., Musters, R.J., Koolwijk, P., and Spoelstra-de Man, A.M.E. (2017). T*he effects of hyperoxia on microvascular endothelial cell proliferation and production of vaso-active substances.* Intensive Care Med Exp 5, 22.

25. Baniaamam, M., Paulus, W.J., Blanken, A.B., and Nurmohamed, M.T. (2018). *The effect of biological DMARDs on the risk of congestive heart failure in rheumatoid arthritis: a systematic review.* Expert Opin Biol Ther 18, 585-594.

26. Boa, B.C.S., Yudkin, J.S., van Hinsbergh, V.W.M., Bouskela, E., and Eringa, E.C. (2017). *Exercise effects on perivascular adipose tissue: endocrine and paracrine determinants of vascular function.* Br J Pharmacol 174, 3466-3481.

27. Boas, S.E.M., Carvalho, J., van den Broek, M., Weijers, E.M., Goumans, M.J., Koolwijk, P., and Merks, R.M.H. (2018). *A local uPAR-plasmin-TGFbeta1 positive feedback loop in a qualitative computational model of angiogenic sprouting explains the in vitro effect of fibrinogen variants.* PLoS Comput Biol 14, e1006239.

28. Bokslag, A., Franssen, C., Alma, L.J., Kovacevic, I., Kesteren, F.V., Teunissen, P.W., Kamp, O., Ganzevoort, W., Hordijk, P.L., Groot, C.J.M., et al. (2018). *Early-onset preeclampsia predisposes to preclinical diastolic left ventricular dysfunction in the fifth decade of life: An observational study.* PLoS One 13, e0198908.

29. Bokslag, A., Teunissen, P.W., Franssen, C., van Kesteren, F., Kamp, O., Ganzevoort, W., Paulus, W.J., and de Groot, C.J.M. (2017). *Effect of early-onset preeclampsia on cardiovascular risk in the fifth decade of life.* Am J Obstet Gynecol 216, 523 e521-523 e527.

30. Bollen, I.A.E., Ehler, E., Fleischanderl, K., Bouwman, F., Kempers, L., Ricke-Hoch, M., Hilfiker-Kleiner, D., Dos Remedios, C.G., Kruger, M., Vink, A., et al. (2017). *Myofilament Remodeling and Function Is More Impaired in Peripartum Cardiomyopathy Compared with Dilated Cardiomyopathy and Ischemic Heart Disease.* Am J Pathol 187, 2645-2658.

31. Bollen, I.A.E., Schuldt, M., Harakalova, M., Vink, A., Asselbergs, F.W., Pinto, J.R., Kruger, M., Kuster, D.W.D., and van der Velden, J. (2017). Genotype-specific pathogenic effects in human dilated cardiomyopathy. J Physiol 595, 4677-4693.

32. Bollen, I.A.E., van der Meulen, M., de Goede, K., Kuster, D.W.D., Dalinghaus, M., and van der Velden, J. (2017). *Cardiomyocyte Hypocontractility and Reduced Myofibril Density in End-Stage Pediatric Cardiomyopathy.* Front Physiol 8, 1103.

33. Bondue, A., Arbustini, E., Bianco, A., Ciccarelli, M., Dawson, D., De Rosa, M., Hamdani, N., Hilfiker-Kleiner, D., Meder, B., Leite-Moreira, A.F., et al. (2018). *Complex roads from genotype to phenotype in dilated cardiomyopathy: scientific update from the Working Group of Myocardial Function of the European Society of Cardiology.* Cardiovasc Res 114, 1287-1303.

34. Boon, R.A. (2018). *Non-coding RNAs in cardiovascular health and disease.* Noncoding RNA Res 3, 99.

35. Boon, R.A. (2018). *TUGging at heartstrings: control of aortic valve calcification by non-coding RNA.* Cardiovasc Res 114, 10-11.

36. Brynnel, A., Hernandez, Y., Kiss, B., Lindqvist, J., Adler, M., Kolb, J., van der Pijl, R., Gohlke, J., Strom, J., Smith, J., et al. (2018). *Downsizing the molecular spring of the giant protein titin reveals that skeletal muscle titin*

determines passive stiffness and drives longitudinal hypertrophy. Elife 7.

37. Buch, E.R., Santarnecchi, E., Antal, A., Born, J., Celnik, P.A., Classen, J., Gerloff, C., Hallett, M., Hummel, F.C., Nitsche, M.A., et al. (2017). *Effects of tDCS on motor learning and memory formation: A consensus and critical position paper.* Clin Neurophysiol 128, 589-603.

38. Carra, S., Alberti, S., Arrigo, P.A., Benesch, J.L., Benjamin, I.J., Boelens, W., Bartelt-Kirbach, B., Brundel, B., Buchner, J., Bukau, B., et al. (2017). *The growing world of small heat shock proteins: from structure to functions.* Cell Stress Chaperones 22, 601-611.

39. Chandrupatla, D., Molthoff, C.F.M., Ritsema, W., Vos, R., Elshof, E., Matsuyama, T., Low, P.S., Musters, R.J.P., Hammond, A., Windhorst, A.D., et al. (2018). *Prophylactic and therapeutic activity of alkaline phosphatase in arthritic rats: single-agent effects of alkaline phosphatase and synergistic effects in combination with methotrexate.* Transl Res 199, 24-38.

40. da Silva Goncalves Bos, D., Van Der Bruggen, C.E.E., Kurakula, K., Sun, X.Q., Casali, K.R., Casali, A.G., Rol, N., Szulcek, R., Dos Remedios, C., Guignabert, C., et al. (2018). *Contribution of Impaired Parasympathetic Activity to Right Ventricular Dysfunction and Pulmonary Vascular Remodeling in Pulmonary Arterial Hypertension.* Circulation 137, 910-924.

41. de Winter, J.M., and Ottenheijm, C.A. (2017). *A two-faced cysteine residue modulates skeletal muscle contraction. Focus on "S-nitrosylation and S-glutathionylation of Cys134 on troponin I have opposing competitive actions on Ca(2+) sensitivity in rat fast-twitch muscle fibers.* Am J Physiol Cell Physiol 312, C314-C315.

42. de Winter, J.M., and Ottenheijm, C.A.C. (2017). *Sarcomere Dysfunction in Nemaline Myopathy.* J Neuromuscul Dis 4, 99-113.

43. Deckx, S., Heggermont, W., Carai, P., Rienks, M., Dresselaers, T., Himmelreich, U., van Leeuwen, R., Lommen, W., van der Velden, J., Gonzalez, A., et al. (2018). *Osteoglycin prevents the development of age-related diastolic dysfunction during pressure overload by reducing cardiac fibrosis and inflammation.* Matrix Biol 66, 110-124.

44. Demolli, S., Doddaballapur, A., Devraj, K., Stark, K., Manavski, Y., Eckart, A., Zehendner, C.M., Lucas, T., Korff, T., Hecker, M., et al. (2017). *Shear stress-regulated miR-27b controls pericyte recruitment by repressing SEMA6A and SEMA6D.* Cardiovasc Res 113, 681-691.

45. Devaux, Y., Creemers, E.E., Boon, R.A., Werfel, S., Thum, T., Engelhardt, S., Dimmeler, S., Squire, I., and Cardiolinc, n. (2017). *Circular RNAs in heart failure.* Eur J Heart Fail 19, 701-709.

46. Dos Remedios, C.G., Lal, S.P., Li, A., McNamara, J., Keogh, A., Macdonald, P.S., Cooke, R., Ehler, E., Knoll, R., Marston, S.B., et al. (2018). *Correction to: The Sydney Heart Bank: improving translational research while eliminating or reducing the use of animal models of human heart disease.* Biophys Rev 10, 941.

47. Emanuel, A.L., de Clercq, N.C., Koopen, A.M., van Poelgeest, E., Serlie, M.J.M., van Raalte, D.H., Kramer, M.H.H., Nieuwdorp, M., Eringa, E.C., and Serne, E.H. (2018). *Iloprost infusion prevents the insulin-induced reduction in skeletal muscle microvascular blood volume but does not enhance peripheral glucose uptake in type 2 diabetic patients.* Diabetes Obes Metab 20, 2523-2531.

48. Emanuel, A.L., Meijer, R.I., Muskiet, M.H., van Raalte, D.H., Eringa, E.C., and Serne, E.H. (2017). *Role of Insulin-Stimulated Adipose Tissue Perfusion in the Development of Whole-Body Insulin Resistance.* Arterioscler Thromb Vasc Biol 37, 411-418.

49. Emanuel, A.L., Nieuwenhoff, M.D., Klaassen, E.S., Verma, A., Kramer, M.H., Strijers, R., Vrancken, A.F., Eringa, E., Groeneveld, G.J., and Serne, E.H. (2017). *Relationships Between Type 2 Diabetes, Neuropathy, and Microvascular Dysfunction: Evidence From Patients With Cryptogenic Axonal Polyneuropathy.* Diabetes Care 40, 583-590.

50. Esse, R., Teerlink, T., Koolwijk, P., Tavares de Almeida, I., Blom, H.J., and Castro, R. (2018). *Folinic Acid Increases Protein Arginine Methylation in Human Endothelial Cells.* Nutrients 10.

51. Franssen, C., Kole, J., Musters, R., Hamdani, N., and Paulus, W.J. (2017). *alpha-B Crystallin Reverses High Diastolic Stiffness of Failing Human Cardiomyocytes.* Circ Heart Fail 10, e003626.

52. Frantz, S., Falcao-Pires, I., Balligand, J.L., Bauersachs, J., Brutsaert, D., Ciccarelli, M., Dawson, D., de Windt, L.J., Giacca, M., Hamdani, N., et al. (2018). *The innate immune system in chronic cardiomyopathy: a European Society of Cardiology (ESC) scientific statement from the Working Group on Myocardial Function of the ESC.* Eur J Heart Fail 20, 445-459.

53. Gorter, T.M., van Veldhuisen, D.J., Bauersachs, J., Borlaug, B.A., Celutkiene, J., Coats, A.J.S., Crespo-Leiro, M.G., Guazzi, M., Harjola, V.P., Heymans, S., et al. (2018). *Right heart dysfunction and failure in heart failure with preserved ejection fraction: mechanisms and management. Position statement on behalf of the Heart Failure Association of the European Society of Cardiology.* Eur J Heart Fail 20, 16-37.

54. Greising, S.M., Ottenheijm, C.A.C., O'Halloran, K.D., and Barreiro, E. (2018). *Diaphragm plasticity in aging and disease: therapies for muscle weakness go from strength to strength.* J Appl Physiol (1985) 125, 243-253.

55. Groeneveld, M.E., Bogunovic, N., Musters, R.J.P., Tangelder, G.J., Pals, G., Wisselink, W., Micha, D., and Yeung, K.K. (2018). *Betaglycan (TGFBR3) up-regulation correlates with increased TGF-beta signaling in Marfan patient fibroblasts in vitro.* Cardiovasc Pathol 32, 44-49.

56. Groeneveld, M.E., van der Reijden, J.J., Tangelder, G.J., Westin, L.C., Renwarin, L., Musters, R.J.P., Wisselink, W., and Yeung, K.K. (2017). *Peroxynitrite Footprint in Circulating Neutrophils of Abdominal Aortic Aneurysm Patients is Lower in Statin than in Non-statin Users.* Eur J Vasc Endovasc Surg 54, 331-339.

57. Grote, P., and Boon, R.A. (2018). *LncRNAs Coming of Age.* Circ Res 123, 535-537.

58. Guazzi, M., van Heerebeek, L., and Paulus, W.J. (2017). *Phosphodiesterase-5 inhibition in heart failure with preserved ejection fraction: trading therapy for prevention.* Eur J Heart Fail 19, 337-339.

59. Heeren, A.M., van Dijk, I., Berry, D., Khelil, M., Ferns, D., Kole, J., Musters, R.J.P., Thijssen, V.L., Mom, C.H., Kenter, G.G., et al. (2018). *Indoleamine 2,3-Dioxygenase Expression Pattern in the Tumor Microenvironment Predicts Clinical Outcome in Early Stage Cervical Cancer.* Front Immunol 9, 1598.

60. Henning, R.H., and Brundel, B. (2017). *Proteostasis in cardiac health and disease.* Nat Rev Cardiol 14, 637-653.

61. Heunks, L., and Ottenheijm, C. (2018). *Diaphragm-Protective Mechanical Ventilation to Improve Outcomes in ICU Patients?* Am J Respir Crit Care Med 197, 150-152.

62. Horrevorts, S.K., Duinkerken, S., Bloem, K., Secades, P., Kalay, H., Musters, R.J., van Vliet, S.J., Garcia-Vallejo, J.J., and van Kooyk, Y. (2018). *Toll-Like Receptor 4 Triggering Promotes Cytosolic Routing of DC-SIGN-Targeted Antigens for Presentation on MHC Class I.* Front Immunol 9, 1231.

63. Hu, X., Van Marion, D.M.S., Wiersma, M., Zhang, D., and Brundel, B. (2017). *The protective role of small heat shock proteins in cardiac diseases: key role in atrial fibrillation.* Cell Stress Chaperones 22, 665-674.

64. Iuso, A., Wiersma, M., Schuller, H.J., Pode-Shakked, B., Marek-Yagel, D., Grigat, M., Schwarzmayr, T., Berutti, R., Alhaddad, B., Kanon, B., et al. (2018). *Mutations in PPCS, Encoding Phosphopantothenoylcysteine Synthetase, Cause Autosomal-Recessive Dilated Cardiomyopathy.* Am J Hum Genet 102, 1018-1030.

65. Janssen, R., Muller, A., and Simonides, W.S. (2017). *Cardiac Thyroid Hormone Metabolism and Heart Failure.* Eur Thyroid J 6, 130-137.

66. Joureau, B., de Winter, J.M., Conijn, S., Bogaards, S.J.P., Kovacevic, I., Kalganov, A., Persson, M., Lindqvist, J., Stienen, G.J.M., Irving, T.C., et al. (2018). Dysfunctional sarcomere contractility contributes to muscle weakness in ACTA1-related nemaline myopathy (NEM3). Ann Neurol 83, 269-282.

67. Joureau, B., de Winter, J.M., Stam, K., Granzier, H., and Ottenheijm, C.A. (2017). *Muscle weakness in respiratory and peripheral skeletal muscles in a mouse model for nebulin-based nemaline myopathy.* Neuromuscul Disord 27, 83-89.

68. Kovacevic, I., Sakaue, T., Majolee, J., Pronk, M.C., Maekawa, M., Geerts, D., Fernandez-Borja, M., Higashiyama, S., and Hordijk, P.L. (2018). *The Cullin-3-Rbx1-KCTD10 complex controls endothelial barrier function via K63 ubiquitination of RhoB.* J Cell Biol 217, 1015-1032.

69. Krejcik, J., Frerichs, K.A., Nijhof, I.S., van Kessel, B., van Velzen, J.F., Bloem, A.C., Broekmans, M.E.C., Zweegman, S., van Meerloo, J., Musters, R.J.P., et al. (2017). *Monocytes and Granulocytes Reduce CD38 Expression Levels on Myeloma Cells in Patients Treated with Daratumumab.* Clin Cancer Res 23, 7498-7511.

70. Kroon, J., Schaefer, A., van Rijssel, J., Hoogenboezem, M., van Alphen, F., Hordijk, P., Stroes, E.S.G., Stromblad, S., van Rheenen, J., and van Buul, J.D. (2018). *Inflammation-Sensitive Myosin-X Functionally Supports Leukocyte Extravasation by Cdc42-Mediated ICAM-1-Rich Endothelial Filopodia Formation.* J Immunol 200, 1790-1801.

71. Kupreishvili, K., Fuijkschot, W.W., Vonk, A.B., Smulders, Y.M., Stooker, W., Van Hinsbergh, V.W., Niessen, H.W., and Krijnen, P.A. (2017). *Mast cells are increased in the media of coronary lesions in patients with myocardial infarction and may favor atherosclerotic plaque instability.* J Cardiol 69, 548-554.

72. Kupreishvili, K., Meischl, C., Vonk, A.B.A., Stooker, W., Eijsman, L., Blom, A.M., Quax, P.H.A., van Hinsbergh, V.W.M., Niessen, H.W.M., and Krijnen, P.A.J. (2017). *Arterial Blood Pressure Induces Transient C4b-Binding Protein in Human Saphenous Vein Grafts.* Ann Vasc Surg 41, 259-264.

73. Kupreishvili, K., Stooker, W., Emmens, R.W., Vonk, A.B.A., Sipkens, J.A., van Dijk, A., Eijsman, L., Quax, P.H., van Hinsbergh, V.W.M., Krijnen, P.A.J., et al. (2017). *PX-18 Protects Human Saphenous Vein Endothelial Cells under Arterial Blood Pressure.* Ann Vasc Surg 42, 293-298.

74. Lagerweij, T., Dusoswa, S.A., Negrean, A., Hendrikx, E.M.L., de Vries, H.E., Kole, J., Garcia-Vallejo, J.J., Mansvelder, H.D., Vandertop, W.P., Noske, D.P., et al. (2017). *Optical clearing and fluorescence deep-tissue imaging for 3D quantitative analysis of the brain tumor microenvironment.* Angiogenesis 20, 533-546.

75. Lanters, E.A.H., Yaksh, A., Teuwen, C.P., van der Does, L., Kik, C., Knops, P., van Marion, D.M.S., Brundel, B., Bogers, A., Allessie, M.A., et al. (2017). *Spatial distribution of conduction disorders during sinus rhythm.* Int J Cardiol 249, 220-225.

76. Leisegang, M.S., Fork, C., Josipovic, I., Richter, F.M., Preussner, J., Hu, J., Miller, M.J., Epah, J., Hofmann, P., Gunther, S., et al. (2017). *Long Noncoding RNA MANTIS Facilitates Endothelial Angiogenic Function.* Circulation 136, 65-79.

77. Leite-Moreira, A.M., Almeida-Coelho, J., Neves, J.S., Pires, A.L., Ferreira-Martins, J., Castro-Ferreira, R., Ladeiras-Lopes, R., Conceicao, G., Miranda-Silva, D., Rodrigues, P., et al. (2018). *Stretch-induced compliance: a novel adaptive biological mechanism following acute cardiac load.* Cardiovasc Res 114, 656-667.

78. Li, D.Y., Busch, A., Jin, H., Chernogubova, E., Pelisek, J., Karlsson, J., Sennblad, B., Liu, S., Lao, S., Hofmann, P., et al. (2018). *H19 Induces Abdominal Aortic Aneurysm Development and Progression.* Circulation 138, 1551-1568.

79. Li, J., Zhang, D., Wiersma, M., and Brundel, B. (2018). *Role of Autophagy in Proteostasis: Friend and Foe in Cardiac Diseases.* Cells 7.

80. Lindqvist, J., van den Berg, M., van der Pijl, R., Hooijman, P.E., Beishuizen, A., Elshof, J., de Waard, M., Girbes, A., Spoelstra-de Man, A., Shi, Z.H., et al. (2018). *Positive End-Expiratory Pressure Ventilation Induces Longitudinal Atrophy in Diaphragm Fibers.* Am J Respir Crit Care Med 198, 472-485.

81. Lynch, T.L.t., Kuster, D.W.D., Gonzalez, B., Balasubramanian, N., Nair, N., Day, S., Calvino, J.E., Tan, Y., Liebetrau, C., Troidl, C., et al. (2017). *Cardiac Myosin Binding Protein-C Autoantibodies are Potential Early Indicators of Cardiac Dysfunction and Patient Outcome in Acute Coronary Syndrome.* JACC Basic Transl Sci 2, 122-131.

82. Maack, C., Lehrke, M., Backs, J., Heinzel, F.R., Hulot, J.S., Marx, N., Paulus, W.J., Rossignol, P., Taegtmeyer, H., Bauersachs, J., et al. (2018). *Heart failure and diabetes: metabolic alterations and therapeutic interventions: a state-of-the-art review from the Translational Research Committee of the Heart Failure Association-European Society of Cardiology.* Eur Heart J 39, 4243-4254.

83. Manavski, Y., Abel, T., Hu, J., Kleinlutzum, D., Buchholz, C.J., Belz, C., Augustin, H.G., Boon, R.A., and Dimmeler, S. (2017). *Endothelial transcription factor KLF2 negatively regulates liver regeneration via induction of activin A.* Proc Natl Acad Sci U S A 114, 3993-3998.

84. Manavski, Y., Lucas, T., Glaser, S.F., Dorsheimer, L., Gunther, S., Braun, T., Rieger, M.A., Zeiher, A.M., Boon, R.A., and Dimmeler, S. (2018). *Clonal Expansion of Endothelial Cells Contributes to Ischemia-Induced Neovascularization.* Circ Res 122, 670-677.

85. Medina, R.J., Barber, C.L., Sabatier, F., Dignat-George, F., Melero-Martin, J.M., Khosrotehrani, K., Ohneda, O., Randi, A.M., Chan, J.K.Y., Yamaguchi, T., et al. (2017). *Endothelial Progenitors: A Consensus Statement on Nomenclature.* Stem Cells Transl Med 6, 1316-1320.

86. Meekel, J.P., Groeneveld, M.E., Bogunovic, N., Keekstra, N., Musters, R.J.P., Zandieh-Doulabi, B., Pals, G., Micha, D., Niessen, H.W.M., Wiersema, A.M., et al. (2018). *An in vitro method to keep human aortic tissue sections functionally and structurally intact.* Sci Rep 8, 8094.

87. Meijering, R.A.M., Wiersma, M., Zhang, D., Lanters, E.A.H., Hoogstra-Berends, F., Scholma, J., Diks, S., Qi, X., de Groot, N.M.S., Nattel, S., et al. (2018). *Application of kinomic array analysis to screen for altered kinases in atrial fibrillation remodeling.* Heart Rhythm 15, 1708-1716.

88. Monsuur, H.N., van den Broek, L.J., Koolwijk, P., Niessen, F.B., and Gibbs, S. (2018). *Endothelial cells enhance adipose mesenchymal stromal cell-mediated matrix contraction via ALK receptors and reduced follistatin: Potential role of endothelial cells in skin fibrosis.* J Cell Physiol 233, 6714-6722.

89. Montag, J., Kowalski, K., Makul, M., Ernstberger, P., Radocaj, A., Beck, J., Becker, E., Tripathi, S., Keyser, B., Muhlfeld, C., et al. (2018). *Burst-Like Transcription of Mutant and Wildtype MYH7-Alleles as Possible Origin of Cell-to-Cell Contractile Imbalance in Hypertrophic Cardiomyopathy.* Front Physiol 9, 359.

90. Mundi, S., Massaro, M., Scoditti, E., Carluccio, M.A., van Hinsbergh, V.W.M., Iruela-Arispe, M.L., and De Caterina, R. (2018). *Endothelial permeability, LDL deposition, and cardiovascular risk factors-a review.* Cardiovasc Res 114, 35-52.

91. Nauta, T.D., van den Broek, M., Gibbs, S., van der Pouw-Kraan, T.C., Oudejans, C.B., van Hinsbergh, V.W., and Koolwijk, P. (2017). Identification of HIF-2alpha-regulated genes that play a role in human microvascular endothelial sprouting during prolonged hypoxia in vitro. Angiogenesis 20, 39-54.

92. Nederhoed, J.H., Ebben, H.P., Slikkerveer, J., Hoksbergen, A.W.J., Kamp, O., Tangelder, G.J., Wisselink, W., Musters, R.J.P., and Yeung, K.K. (2017). Intravenous Targeted Microbubbles Carrying Urokinase versus Urokinase Alone in Acute Peripheral Arterial Thrombosis in a Porcine Model. Ann Vasc Surg 44, 400-407.

93. Neumann, P., Jae, N., Knau, A., Glaser, S.F., Fouani, Y., Rossbach, O., Kruger, M., John, D., Bindereif, A., Grote, P., et al. (2018). The lncRNA GATA6-AS epigenetically regulates endothelial gene expression via interaction with LOXL2. Nat Commun 9, 237.

94. Nijenkamp, L., Bollen, I.A.E., van Velzen, H.G., Regan, J.A., van Slegtenhorst, M., Niessen, H.W.M., Schinkel, A.F.L., Kruger, M., Poggesi, C., Ho, C.Y., et al. (2018). Sex Differences at the Time of Myectomy in Hypertrophic Cardiomyopathy. Circ Heart Fail 11, e004133.

95. Nolte, I.M., Munoz, M.L., Tragante, V., Amare, A.T., Jansen, R., Vaez, A., von der Heyde, B., Avery, C.L., Bis, J.C., Dierckx, B., et al. (2017). *Genetic loci associated with heart rate variability and their effects on cardiac disease risk.* Nat Commun 8, 15805.

96. Nowak-Sliwinska, P., Alitalo, K., Allen, E., Anisimov, A., Aplin, A.C., Auerbach, R., Augustin, H.G., Bates, D.O., van Beijnum, J.R., Bender, R.H.F., et al. (2018). Consensus guidelines for the use and interpretation of angiogenesis assays. Angiogenesis 21, 425-532.

97. Papp, Z., Radovits, T., Paulus, W.J., Hamdani, N., and Seferovic, P.M. (2018). *Molecular and pathophysiological links between heart failure with preserved ejection fraction and type 2 diabetes mellitus*. Eur J Heart Fail 20, 1649-1652.

98. Parbhudayal, R.Y., Garra, A.R., Gotte, M.J.W., Michels, M., Pei, J., Harakalova, M., Asselbergs, F.W., van Rossum, A.C., van der Velden, J., and Kuster, D.W.D. (2018). *Variable cardiac myosin binding protein-C expression in the myofilaments due to MYBPC3 mutations in hypertrophic cardiomyopathy.* J Mol Cell Cardiol 123, 59-63.

99. Paulus, W.J. (2018). H2FPEF Score. Circulation 138, 871-873.

100. Paulus, W.J., and Dal Canto, E. (2018). *Diastole Tracks Cardiometabolic Risk*. JACC Heart Fail 6, 326-328.

101. Paulus, W.J., and Dal Canto, E. (2018). Distinct Myocardial Targets for Diabetes Therapy in Heart Failure With Preserved or Reduced Ejection Fraction. JACC Heart Fail 6, 1-7.

102. Paulus, W.J., and Dal Canto, E. (2018). *Reply: Diabetes and Heart Failure: Are We in the Right Direction to Find the Right Morsel for Success?* JACC Heart Fail 6, 270-271.

103. Paulus, W.J., and Waddingham, M.T. (2017). *Racial Tension in Hypertension: Divergent Effects of African and European Ancestry on Cardiac Remodeling.* JACC Heart Fail 5, 166-168.

104. Peters, E.L., van der Linde, S.M., Vogel, I.S.P., Haroon, M., Offringa, C., de Wit, G.M.J., Koolwijk, P., van der Laarse, W.J., and Jaspers, R.T. (2017). *IGF-1 Attenuates Hypoxia-Induced Atrophy but Inhibits Myoglobin Expression in C2C12 Skeletal Muscle Myotubes.* Int J Mol Sci 18.

105. Pronk, M.C.A., van Bezu, J.S.M., van Nieuw Amerongen, G.P., van Hinsbergh, V.W.M., and Hordijk, P.L. (2017). *RhoA, RhoB and RhoC differentially regulate endothelial barrier function.* Small GTPases, 1-19.

106. Ramsey, J., Butnor, K., Peng, Z., Leclair, T., van der Velden, J., Stein, G., Lian, J., and Kinsey, C.M. (2018). *Loss of RUNX1 is associated with aggressive lung adenocarcinomas.* J Cell Physiol 233, 3487-3497.

107. Reinhard, N.R., Mastop, M., Yin, T., Wu, Y., Bosma, E.K., Gadella, T.W.J., Jr., Goedhart, J., and Hordijk, P.L. (2017). *The balance between Galphai-Cdc42/Rac and Galpha12/13-RhoA pathways determines endothelial barrier regulation by sphingosine-1-phosphate.* Mol Biol Cell 28, 3371-3382.

108. Rol, N., de Raaf, M.A., Sun, X.Q., Kuiper, V.P., da Silva Goncalves Bos, D., Happe, C., Kurakula, K., Dickhoff, C., Thuillet, R., Tu, L., et al. (2019). *Nintedanib improves cardiac fibrosis but leaves pulmonary vascular remodelling unaltered in experimental pulmonary hypertension.* Cardiovasc Res 115, 432-439.

109. Schaefer, A., van Duijn, T.J., Majolee, J., Burridge, K., and Hordijk, P.L. (2017). *Endothelial CD2AP Binds the Receptor ICAM-1 To Control Mechanosignaling, Leukocyte Adhesion, and the Route of Leukocyte Diapedesis In Vitro.* J Immunol 198, 4823-4836.

110. Seferovic, P.M., Petrie, M.C., Filippatos, G.S., Anker, S.D., Rosano, G., Bauersachs, J., Paulus, W.J., Komajda, M., Cosentino, F., de Boer, R.A., et al. (2018). *Type 2 diabetes mellitus and heart failure: a position statement from the*

Heart Failure Association of the European Society of Cardiology. Eur J Heart Fail 20, 853-872.

111. Smeets, M.W., Bierings, R., Meems, H., Mul, F.P., Geerts, D., Vlaar, A.P., Voorberg, J., and Hordijk, P.L. (2017). *Platelet-independent adhesion of calcium-loaded erythrocytes to von Willebrand factor.* PLoS One 12, e0173077.

112. Smeets, M.W.J., Mourik, M.J., Niessen, H.W.M., and Hordijk, P.L. (2017). *Stasis Promotes Erythrocyte Adhesion to von Willebrand Factor.* Arterioscler Thromb Vasc Biol 37, 1618-1627.

113. Smit, B., Smulders, Y.M., de Waard, M.C., Oudemans-van Straaten, H.M., Girbes, A.R.J., Eringa, E.C., and Spoelstra-de Man, A.M.E. (2017). *Hyperoxia does not directly affect vascular tone in isolated arteries from mice.* PLoS One 12, e0182637.

114. Smit, B., Smulders, Y.M., Eringa, E.C., Gelissen, H., Girbes, A.R.J., de Grooth, H.S., Schotman, H.H.M., Scheffer, P.G., Oudemans-van Straaten, H.M., and Spoelstra-de Man, A.M.E. (2018). Hyperoxia does not affect oxygen delivery in healthy volunteers while causing a decrease in sublingual perfusion. Microcirculation 25.

115. Smit, B., Smulders, Y.M., Eringa, E.C., Oudemans-van Straaten, H.M., Girbes, A.R.J., Wever, K.E., Hooijmans, C.R., and Spoelstra-de Man, A.M.E. (2018). *Effects of hyperoxia on vascular tone in animal models: systematic review and meta-analysis.* Crit Care 22, 189.

116. Smits, J., Tasev, D., Andersen, S., Szulcek, R., Botros, L., Ringgaard, S., Andersen, A., Vonk-Noordegraaf, A., Koolwijk, P., and Bogaard, H.J. (2018). *Blood Outgrowth and Proliferation of Endothelial Colony Forming Cells are Related to Markers of Disease Severity in Patients with Pulmonary Arterial Hypertension.* Int J Mol Sci 19.

117. Sorop, O., Heinonen, I., van Kranenburg, M., van de Wouw, J., de Beer, V.J., Nguyen, I.T.N., Octavia, Y., van Duin, R.W.B., Stam, K., van Geuns, R.J., et al. (2018). *Multiple common comorbidities produce left ventricular diastolic dysfunction associated with coronary microvascular dysfunction, oxidative stress, and myocardial stiffening.* Cardiovasc Res 114, 954-964.

118. Strutz, J., Cvitic, S., Hackl, H., Kashofer, K., Appel, H.M., Thuringer, A., Desoye, G., Koolwijk, P., and Hiden, U. (2018). *Gestational diabetes alters microRNA signatures in human feto-placental endothelial cells depending on fetal sex.* Clin Sci (Lond) 132, 2437-2449.

119. Szulcek, R., Bollensdorff, C., Hordijk, P., and Gabriel, M. (2018). *The covalently immobilized antimicrobial peptide LL37 acts as a VEGF mimic and stimulates endothelial cell proliferation.* Biochem Biophys Res Commun 496, 887-890.

120. Tasev, D., Dekker-Vroling, L., van Wijhe, M., Broxterman, H.J., Koolwijk, P., and van Hinsbergh, V.W.M. (2018). *Hypoxia Impairs Initial Outgrowth of Endothelial Colony Forming Cells and Reduces Their Proliferative and Sprouting Potential.* Front Med (Lausanne) 5, 356.

121. Ter Horst, E.N., Hahn, N.E., Geerts, D., Musters, R.J.P., Paulus, W.J., van Rossum, A.C., Meischl, C., Piek, J.J., Niessen, H.W.M., and Krijnen, P.A.J. (2018). *p47phox-Dependent Reactive Oxygen Species Stimulate Nuclear Translocation of the FoxO1 Transcription Factor During Metabolic Inhibition in Cardiomyoblasts.* Cell Biochem Biophys 76, 401-410.

122. Theodorou, K., and Boon, R.A. (2018). Endothelial Cell Metabolism in Atherosclerosis. Front Cell Dev Biol 6, 82.

123. Trippel, T.D., Van Linthout, S., Westermann, D., Lindhorst, R., Sandek, A., Ernst, S., Bobenko, A., Kasner, M., Spillmann, F., Gonzalez, A., et al. (2018). *Investigating a biomarker-driven approach to target collagen turnover in diabetic heart failure with preserved ejection fraction patients. Effect of torasemide versus furosemide on serum C-terminal propeptide of procollagen type I (DROP-PIP trial).* Eur J Heart Fail 20, 460-470.

124. Turaihi, A.H., Bakker, W., van Hinsbergh, V.W.M., Serne, E.H., Smulders, Y.M., Niessen, H.W.M., and Eringa, E.C. (2018). *Insulin Receptor Substrate 2 Controls Insulin-Mediated Vasoreactivity and Perivascular Adipose Tissue Function in Muscle.* Front Physiol 9, 245.

125. Turaihi, A.H., van Poelgeest, E.M., van Hinsbergh, V.W., Serne, E.H., Smulders, Y.M., and Eringa, E.C. (2017). *Combined Intravital Microscopy and Contrast-enhanced Ultrasonography of the Mouse Hindlimb to Study Insulin-induced Vasodilation and Muscle Perfusion.* J Vis Exp.

126. van den Berg, M., Hooijman, P.E., Beishuizen, A., de Waard, M.C., Paul, M.A., Hartemink, K.J., van Hees, H.W.H., Lawlor, M.W., Brocca, L., Bottinelli, R., et al. (2017). *Diaphragm Atrophy and Weakness in the Absence of Mitochondrial Dysfunction in the Critically Ill.* Am J Respir Crit Care Med 196, 1544-1558.

127. van der Heijden, D.J., van Leeuwen, M.A.H., Janssens, G.N., Lenzen, M.J., van de Ven, P.M., Eringa, E.C., and van Royen, N. (2017). *Body Mass Index Is Associated With Microvascular Endothelial Dysfunction in Patients With Treated Metabolic Risk Factors and Suspected Coronary Artery Disease.* J Am Heart Assoc 6.

128. van der Laarse, W.J. (2017). *Heart heat separation.* J Physiol 595, 4579-4580.

129. van der Pijl, R., Strom, J., Conijn, S., Lindqvist, J., Labeit, S., Granzier, H., and Ottenheijm, C. (2018). *Titin-based mechanosensing modulates muscle hypertrophy.* J Cachexia Sarcopenia Muscle 9, 947-961.

130. van der Velden, J., Tocchetti, C.G., Varricchi, G., Bianco, A., Sequeira, V., Hilfiker-Kleiner, D., Hamdani, N., Leite-Moreira, A.F., Mayr, M., Falcao-Pires, I., et al. (2018). *Metabolic changes in hypertrophic cardiomyopathies: scientific update from the Working Group of Myocardial Function of the European Society of Cardiology.* Cardiovasc Res 114, 1273-1280.

131. van der Zwaard, S., Brocherie, F., Kom, B.L.G., Millet, G.P., Deldicque, L., van der Laarse, W.J., Girard, O., and Jaspers, R.T. (2018). *Adaptations in muscle oxidative capacity, fiber size, and oxygen supply capacity after repeated-sprint training in hypoxia combined with chronic hypoxic exposure.* J Appl Physiol (1985) 124, 1403-1412.

132. van der Zwaard, S., van der Laarse, W.J., Weide, G., Bloemers, F.W., Hofmijster, M.J., Levels, K., Noordhof, D.A., de Koning, J.J., de Ruiter, C.J., and Jaspers, R.T. (2018). *Critical determinants of combined sprint and endurance performance: an integrative analysis from muscle fiber to the human body.* FASEB J 32, 2110-2123.

133. van der Zwaard, S., Weide, G., Levels, K., Eikelboom, M.R.I., Noordhof, D.A., Hofmijster, M.J., van der Laarse, W.J., de Koning, J.J., de Ruiter, C.J., and Jaspers, R.T. (2018). *Muscle morphology of the vastus lateralis is strongly related to ergometer performance, sprint capacity and endurance capacity in Olympic rowers.* J Sports Sci 36, 2111-2120.

134. van Driel, B., Michels, M., and van der Velden, J. (2018). *Molecular autopsy.* Neth Heart J 26, 471-472.

135. van Essen, G.J., Te Lintel Hekkert, M., Sorop, O., Heinonen, I., van der Velden, J., Merkus, D., and Duncker, D.J. (2018). *Cardiovascular Function of Modern Pigs Does not Comply with Allometric Scaling Laws.* Sci Rep 8, 792.

136. van Hinsbergh, V.W. (2017). *Osteoglycin's embracement of VEGF receptor-2 limits angiogenesis and collateralization.* Cardiovasc Res 113, 10-12.

137. Verkaik, M., Juni, R.P., van Loon, E.P.M., van Poelgeest, E.M., Kwekkeboom, R.F.J., Gam, Z., Richards, W.G., Ter Wee, P.M., Hoenderop, J.G., Eringa, E.C., et al. (2018). *FGF23 impairs peripheral microvascular function in renal failure.* Am J Physiol Heart Circ Physiol 315, H1414-H1424.

138. Verkaik, M., Oranje, M., Abdurrachim, D., Goebel, M., Gam, Z., Prompers, J.J., Helmes, M., Ter Wee, P.M., van der Velden, J., Kuster, D.W., et al. (2018). *High Fibroblast Growth Factor 23 concentrations in experimental renal failure impair calcium handling in cardiomyocytes.* Physiol Rep 6, e13591.

139. Verkaik, M., van Poelgeest, E.M., Kwekkeboom, R.F.J., Ter Wee, P.M., van den Brom, C.E., Vervloet, M.G., and Eringa, E.C. (2018). *Myocardial contrast echocardiography in mice: technical and physiological aspects.* Am J Physiol Heart Circ Physiol 314, H381-H391.

140. Vila Cuenca, M., Ferrantelli, E., Meinster, E., Pouw, S.M., Kovacevic, I., de Menezes, R.X., Niessen, H.W., Beelen, R.H.J., Hordijk, P.L., and Vervloet, M.G. (2018). Vitamin D Attenuates Endothelial Dysfunction in Uremic Rats and Maintains Human Endothelial Stability. J Am Heart Assoc 7, e008776.

141. Waddingham, M.T., and Paulus, W.J. (2017). *Microvascular Paradigm in Heart Failure With Preserved Ejection Fraction: A Quest for Proof of Concept.* Circ Heart Fail 10.

142. Wagner, J.U.G., Chavakis, E., Rogg, E.M., Muhly-Reinholz, M., Glaser, S.F., Gunther, S., John, D., Bonini, F., Zeiher, A.M., Schaefer, L., et al. (2018). *Switch in Laminin beta2 to Laminin beta1 Isoforms During Aging Controls Endothelial Cell Functions-Brief Report.* Arterioscler Thromb Vasc Biol 38, 1170-1177.

143. Westerhof, B.E., Saouti, N., van der Laarse, W.J., Westerhof, N., and Vonk Noordegraaf, A. (2017). *Treatment strategies for the right heart in pulmonary hypertension.* Cardiovasc Res 113, 1465-1473.

144. Wiersma, M., Beuren, T.M.A., de Vrij, E.L., Reitsema, V.A., Bruintjes, J.J., Bouma, H.R., Brundel, B., and Henning, R.H. (2018). *Torpor-arousal cycles in Syrian hamster heart are associated with transient activation of the protein quality control system.* Comp Biochem Physiol B Biochem Mol Biol 223, 23-28.

145. Wiersma, M., Meijering, R.A.M., Qi, X.Y., Zhang, D., Liu, T., Hoogstra-Berends, F., Sibon, O.C.M., Henning, R.H., Nattel, S., and Brundel, B. (2017). *Endoplasmic Reticulum Stress Is Associated With Autophagy and Cardiomyocyte Remodeling in Experimental and Human Atrial Fibrillation.* J Am Heart Assoc 6.

146. Woerdeman, J., Del Rio, D., Calani, L., Eringa, E.C., Smulders, Y.M., and Serne, E.H. (2018). *Red wine polyphenols do not improve obesity-associated insulin resistance: A randomized controlled trial.* Diabetes Obes Metab 20, 206-210.

147. Woerdeman, J., van Poelgeest, E., Ket, J.C.F., Eringa, E.C., Serne, E.H., and Smulders, Y.M. (2017). *Do grape polyphenols improve metabolic syndrome components? A systematic review.* Eur J Clin Nutr 71, 1381-1392.

148. Wust, R.C., Helmes, M., Martin, J.L., van der Wardt, T.J., Musters, R.J., van der Velden, J., and Stienen, G.J. (2017). *Rapid frequency-dependent changes in free mitochondrial calcium concentration in rat cardiac myocytes.* J Physiol 595, 2001-2019.

149. Yeung, K.K., Bogunovic, N., Keekstra, N., Beunders, A.A., Pals, J., van der Kuij, K., Overwater, E., Wisselink, W., Blankensteijn, J.D., van Hinsbergh, V.W., et al. (2017). *Transdifferentiation of Human Dermal Fibroblasts to Smooth Muscle-Like Cells to Study the Effect of MYH11 and ACTA2 Mutations in Aortic Aneurysms.* Hum Mutat 38, 439-450.

150. Zhang, D., Hu, X., Li, J., Hoogstra-Berends, F., Zhuang, Q., Esteban, M.A., de Groot, N., Henning, R.H., and Brundel, B. (2018). *Converse role of class I and class IIa HDACs in the progression of atrial fibrillation.* J Mol Cell Cardiol 125, 39-49.

151. Zuo, L., Zhou, T., Malatesta, D., Lanzi, S., Millet, G.P., Pogliaghi, S., Paterson, D.H., Murias, J.M., van der Zwaard, S., Jaspers, R.T., et al. (2018). *Commentaries on Viewpoint: Vo2peak is an acceptable estimate of cardiorespiratory fitness but not Vo2max.* J Appl Physiol (1985) 125, 966-967.

VUmc