ABSTRACTS
Doxorubicin Provokes Autophagy and Necrotic Cell Death of Ventricular Myocytes.

Rimpy Dhingra, Victoria Margulets, Inna Rabinowitz-Nikitin and Lorrie A. Kirshenbaum

The Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Centre, Department of Physiology and Pathophysiology, Rady College of Medicine, Max Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada R2H 2H6.

Herein we provide novel evidence that chemotherapeutic agent doxorubicin (dox) triggers maladaptive autophagy and necrotic cell death of ventricular myocytes. While vehicle treated cells displayed normal cardiac morphology, cells treated with dox displayed severe mitochondrial abnormalities including, disrupted cristae, swelling and extensive vacuolization. Concordantly, the Bcl-2/adenovirus E1B 19kDa interacting protein 3 (Bnip3) was localized to mitochondria in cells treated with dox. This coincided with increased ROS, mPTP, and loss of mitochondrial ΔΨm. Further, phosphorylation of the mitochondrial fission protein DRP-1 pSer616 coincided with mitochondrial Parkin recruitment and loss of SQSTM1 p62 and autophagy in cells treated with dox. Notably, recruitment of Parkin to damaged mitochondria was dependent upon Bnip3. Importantly, autophagy induced by dox resulted in LDH release, loss of nuclear HMGB1 immunoreactivity indicative of necrotic cell death. Conversely, inhibition of autophagy with 3-MA or knock-down of Atg7 suppressed autophagy and necrotic cell death induced by doxorubicin. To our knowledge our data provide the first direct evidence that mitochondrial perturbations induced by doxorubicin are functionally coupled to autophagy and necrotic cell death of ventricular myocytes. Interventions that mitigate abnormal autophagy clearance may provide beneficial in suppressing necrotic cell death and cardiac dysfunction in cancer patients treated with dox.
CARDDIC SPHEROIDS AS NOVEL 3D IN VITRO MODELS OF CARDIOTOXICITY
Carmine Gentile, Liudmila Polonchuk, Mamta Chabria, Michael J. Davies, Gemma A. Figtree.

aSydney Medical School, University of Sydney, Sydney, Australia; bHeart Research Institute, Newtown, Australia; cBeth Israel Deaconess Medical Center - Harvard Medical School, United States; dRoche Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., Basel, Switzerland; eUniversity of Copenhagen, Denmark.

Anthracycline-induced toxicity can result in severe late-onset dilated cardiomyopathy. This has been suggested to occur via nitric oxide (NO) synthesis, but its mechanism is not fully understood yet. To better evaluate these toxic effects, we have treated our in vitro 3D model of the human heart, that we call “cardiac spheroids”, in presence or absence of doxorubicin (DOX), a widely used anthracycline. Cardiac spheroids have been generated by co-culturing human endothelial cells (ECs), cardiac myocytes (CMs) and fibroblasts (CFs) in hanging drop cultures and showed to better recapitulate the in vivo microenvironment of the human heart compared to existing in vitro models (Polonchuk et al., 2017). We have treated cardiac spheroids in either presence or absence of DOX and evaluated its toxic effect by measuring: i) cell viability/toxicity by calcein-AM and ethidium homodimer for live and dead cells, respectively; ii) NO synthesis with DAF-FM diacetate; iii) apoptosis by active caspase 3 expression. NO formation was inhibited by pharmacological (L-NIO) and genetic means (NOS3 shRNA). First, our analyses showed that DOX-induced toxic effects in cardiac spheroids were dependent on NO synthesis and both pharmacological and genetic NO inhibition reduced this toxicity. Then, the EC-forming microvascular network played a protective role against DOX-induced toxic effects. Finally, most of the DOX-induced toxic effects occurred via NO derived from CFs. In conclusion, cardiac spheroids present biochemical, morphological and pharmacological features typical of the human heart and have the potential to be used to unveil novel mechanisms regulating toxic effects on the human heart.
Fetal or neonatal heart arrhythmias in humans are a common disorder. While the type and severity of congenital arrhythmias vary, some are life-threatening. Only limited genetic mutations leading to fetal arrhythmias have been identified.

Here, we present an animal model with fetal arrhythmias and reveal that the activation of the β2-adrenergic receptor-protein kinase A (β2AR-PKA) signaling pathway contributes to the Rnd3 deficiency-mediated arrhythmic phenotype. Rnd3, a small Rho GTPase, is involved in the regulation of cell actin cytoskeleton dynamics, cell migration and proliferation.

This study uncovers a new biological function of Rnd3. We provide evidence that the downregulation of Rnd3 is sufficient to initiate the activation of PKA signaling in vivo in animal hearts and in vitro in both cardiac and non-cardiac cells, suggesting a general mechanism for Rnd3-mediated PKA regulation. We further determine that Rnd3 is a regulator in the β2AR ubiquitination regulatory complex. Rnd3 regulates β2AR ubiquitination, mediated by the physical interaction between both proteins. The lack of Rnd3 prevents the ubiquitination of β2AR, resulting in the accumulation of β2AR protein. Excess β2AR promotes the activation of PKA signaling, which then contributes to the dysfunction of RyR2 calcium release channels. The β2AR antagonist treatment significantly reduced arrhythmia and improved cardiac contractility.
The Dawn of Epitranscriptomic Medicine

Medicine is at the crossroads of expanding disciplines. Prompt adaptation of medicine to each rapidly advancing research field, bridging bench to bedside is a key step towards health improvement. Cardiovascular disease still ranks first among the mortality causes in the western world, indicating a poor adaptation rate of cardiovascular medicine, albeit the gigantic scientific breakthroughs of this century. This urges the cardiovascular research field to explore novel concepts with promising prognostic and therapeutic potential. My talk will introduce the newly emerging field of “epitranscriptomics” (or else known as “RNA epigenetics”) to cardiovascular researchers and clinicians summarizing its applications on health and disease. The intermediate carrier of genetic information, RNA, is dynamically subjected to more than 140 different kinds of chemical modifications determining its fate, which may profoundly impact the cellular responses and thus both health and disease course. Which are the most prevalent types of these RNA modifications, how are they catalyzed, how are they regulated, which role may they play in health and disease and which are the implications for the cardiovascular medicine are few important questions that will be discussed in my talk.
PPARβ/δ signaling in the stressed heart

Peroxisome proliferator-activated receptors, members of the nuclear receptor transcription factor superfamily, are critical regulators of cardiac metabolism and function in health and disease. Although PPARβ/δ is the most prevalent subtype in the myocardium, it is the least studied member of the PPAR subfamily. Accumulating evidence indicates the role of PPARβ/δ in many physiological functions, ranging from enhanced fatty acid catabolism and improved insulin sensitivity, to inflammation inhibition and mitochondrial biogenesis, and highlights its protective role in the improvement of cardiac function under diverse pathological settings. Selective agonism in rat hearts provided evidence of the protective potential of PPARβ/δ against post-ischemic cardiac dysfunction. PPARβ/δ is implicated in the regulation of cardiac redox balance through effects on transcriptional regulation of antioxidant enzymes or other effectors that could modulate oxidative stress. Furthermore, PPARβ/δ activation attenuates cardiac remodeling and improves cardiac dysfunction in the diabetic heart by normalizing glucose metabolism and modulating autophagy. Thus, PPARβ/δ might serve as a therapeutic target to improve cardiac function in several cardiac pathologies.
Ira J. Goldberg

Fat in the blood, fat in the artery, fat in the heart

Production of triglycerides is how we evolved to circulate calories from the gut, to the liver, to the tissues. Triglyceride supplies our heart and skeletal muscles with fuel, and allows our adipose to efficiently store fat. Each triglyceride molecule supplies more than ten times the energy of a molecular of glucose; failure to transport, acquire and use triglyceride leads to energy deficiency and often death. But as is often the case, we can have too much of a good thing. In the blood excess triglyceride leads to pancreatitis and in the heart excess triglyceride causes cardiomyopathy. My laboratory has focused on the transport, uptake, storage and pathological consequences of too much fat in the wrong places. 1) In the circulation, triglyceride is transported in lipoproteins and cleared via the actions of lipoprotein lipase (LpL) and hepatic triglyceride lipase. By inhibiting these enzymes we showed that triglyceride metabolism regulates LDL and HDL; hepatic lipase inhibition shifts LDL to larger, more buoyant particles and LpL inhibition reduces HDL cholesterol by >50%. 2) Genetic variations that regulate the activity of LpL correlate with cardiovascular risk, as do circulating triglyceride levels. LpL is expressed by macrophages within the arterial wall and, in contrast to its anti-atherosclerotic effects when expressed in muscle, macrophage LpL deficiency reduces macrophage function and atherosclerosis. 3) In the heart, oxidation of fatty acids from triglyceride produces the majority of ATP. Cardiomyocyte-specific LpL deletion leads heart failure with aging and with increased afterload. However, excess lipid accumulation can cause lipotoxic heart failure and ventricular fibrillation. By defining the pathways mediating heart lipid uptake, we will test whether these forms of heart failure can be prevented. Heart disease(s) are about more lipids than cholesterol.
Myocardial injury causes an irreversible loss of vital myocardium. Regenerative approaches might represent new therapeutic strategies for heart failure patients. This especially important as the prognosis for patients with terminal heart failure is still poor. Human engineered heart tissue (hEHTs) can be generated from induced pluripotent stem cell derived cardiomyocytes. Transplantation of hEHTs could partially remuscularize injured hearts in a cryo-injury model in guinea pigs. Histological analysis revealed large human grafts (indicated by a positive staining for human Ku80). A detailed analysis showed that the grafts consisted of densely packed, elongated human cardiomyocytes with regular sarcomere assembly. Human cardiomyocytes were smaller in size than the host cells. Left-ventricular function improved after transplantation of hEHTs, whereas transplantation of cell-free constructs had no effect. We are currently working on the optimization of hEHT-geometry, and the establishment of GMP-like culture conditions, to evaluate the transplantation of hEHTs in a large animal model and advance this concept from a "proof of principle"-study towards a clinical application.
Regenerative approaches to post-MI heart failure using engineered cardiac patch

Transplantation of engineered tissue patches with either progenitor cells or cardiomyocytes for cardiac repair is emerged as an exciting treatment option for post infarction LV remodeling. Beneficial effects may be due to direct remuscularization or paracrine mechanisms leading to mobilization and/or activation of endogenous progenitors with subsequent promotion of neovascularization, remuscularization and inhibition of apoptosis, and thus attenuation of disease progression. Participants will be able to discuss and explain the current understanding the major roadblocks in cardiac cell therapy, and the potential approaches to overcome these problems. One of the major objective is to make the cell products become the treatment options in the future. Participants will also share their knowledge and interests in pursuing the novel applications in this emerging field of tissue engineering and cell therapy.
Factors affecting metabolic flux remodeling in the diseased heart

The metabolic adaptations of the heart to pathological stress include maladaptive shifts toward reduced long chain fatty acid (LCFA) oxidation, impaired lipid storage dynamics, and inefficient glucose metabolism. We have mediated PPAR alpha activation via availability of LCFA to hypertrophied rat hearts, with oleate being more protective than palmitate for preserving PPAR alpha target gene expression, lipotoxic ceramide accumulation, and contractile function. Echocardiography reveals that rats fed an oleate-based diet versus a palmitate-based diet display an attenuated decline in cardiac function in response to transverse aortic constriction (TAC).

Increased activation of exogenous LCFA in hearts of mice with overexpression of coenzyme A via acyl-coA synthetase long chain family member 1 (ACSL1) maintains baseline contributions of exogenous LCFA to oxidative metabolism and bioenergetic potential (phosphocreatine:ATP) following TAC. ACSL1 overexpression also normalized the prevented increases in lipotoxic ceramide species C16, C24 and C24:1, while increasing content of potentially benign C20 and C22 ceramides. Importantly, the preservation of metabolic state during the early response to TAC due to ACSL1 overexpression is associated with reduced progression of cardiac hypertrophy and impaired function.
The degradation of damaged mitochondria by mitophagy, a mitochondria-specific form of autophagy, is essential for the maintenance of healthy mitochondrial function. Mitophagy is often downregulated during chronic cardiac stress, whereas rescue of mitophagy delays the development of cardiac dysfunction. However, the molecular mechanisms through which mitophagy is activated during stress remains poorly understood in the heart. Using electron microscopy and mitochondria-targeted-Keima, a fluorescent dye that indicates the pH environment in which it is located, it has been observed that glucose deprivation (GD) and hypoxia (HO), stresses known to impose energy stress, induce mitophagy in cardiomyocytes (CMs). Although downregulation of Atg7, an intervention that abrogates a non-selective form of autophagy (hereafter conventional autophagy), does not affect GD- or HO-induced mitophagy, downregulation of Ulk1, one of the two mammalian orthologs of Atg1, markedly attenuates mitophagy in CMs. Autophagosomes observed in this form of mitophagy are associated with Rab9 but not LC3. Thus, mitophagy in CMs during energy stress is likely to be mediated by a mechanism distinct from conventional autophagy. In response to energy stress, Ulk1 and Rab9 form a complex, recruit Rip1, a Drp1 kinase, and induce Ser616 phosphorylation of Drp1 and mitochondrial fission. Through this mechanism, damaged portions of mitochondria in CMs are segregated and sequestered in Rab9-positive autophagosomes. In summary, mitophagy during energy stress is mediated by a molecular mechanism distinct from conventional autophagy in CMs and Ulk1 mediates mitochondrial fission and mitophagy through the coordinated actions of Rab9, Rip1, and Drp1, thereby playing a central role in degrading damaged mitochondria during energy stress. In the lecture, I will also discuss the functional significance of mitophagy mediated through the Ulk1-Rab9-Rip1-Drp1-dependent mechanism during pressure overload and myocardial ischemia.
**Title:** Preconception Exposure of Particulate Matter Leads to Adult Cardiac Dysfunction through Altering Myocyte Function and Ca2⁺ Signaling Pathways

**Objective:** Previous studies have demonstrated that particulate matter, or ambient particles less than 2.5 μm (PM₂.₅) in diameter, exposure during both in utero and postnatal developmental periods triggers electrical remodeling and cardiac dysfunction during adulthood. This shows that PM₂.₅ can reprogram hearts during the gestational period. Despite this evidence, cardiac effects from pre-gestational particulate matter exposure remain inconclusive. This study was performed to further investigate the potential priming effects of preconception exposure of PM₂.₅ on global cardiac dysfunction at adulthood.

**METHODS:** Male and female FVB mice were exposed separately to either filtered air (FA) or PM₂.₅ at a concentration (within the annual average range of 15 μg/m³ according to the National Ambient Air Quality Standards (NAAQS)) for 3 months. Mice were then crossed into two groups: (1) FAm X FAf (both parents were FA exposed) and, (2) PMm X PMf (both parents were PM exposed). Offspring born to these crosses (PC FA and PC PM₂.₅) were analyzed at 3 months of age for in vivo cardiac function via echocardiography, followed by in vitro cardiomyocyte functional and molecular analyses.

**RESULTS:** Echocardiography identified increased LVESd (2.58 ± 0.13 PC FA, 2.93 ± 0.13 PC PM₂.₅, P=0.1) and reduced PWTs (1.70 ± 0.06 PC FA, 1.44 ± 0.08 PC PM₂.₅, P=0.05) dimensions in PC PM₂.₅-exposed mice. Morphological alterations were associated with lower systolic function indicated by reduced fractional shortening % (35.09% ± 1.34 PC FA, 29.05% ± 1.25 PC PM₂.₅, P=0.03) and ejection fraction % (64.86% ± 1.76 PC FA, 56.27% ± 1.95 PC PM₂.₅, P=0.03) in PM₂.₅-exposed mice. Cardiomyocytes isolated from PC PM₂.₅ mice showed reduced peak shortening %PS (12.93% ± 0.42 PC FA, 11.10% ± 0.41 PC PM₂.₅, P=0.002). -dL/dt (-10.60 ± 0.82 PC FA, -8.50 ± 0.60 PC PM₂.₅, P=0.05), TPS90 (0.08 ± 0.003 PC FA, 0.07 ± 0.002 PC PM₂.₅, P=0.05) and slower calcium reuptake (tau, 0.42±0.06 s FA, 0.67±0.09 s PC PM₂.₅, P=0.05). qPCR analyses revealed decreased MURC, MyBPC3 and increased Mypt1 expression and western blot analyses demonstrated modified NCX, SERCA and PLN expression in PC PM₂.₅-exposed mice compared to PC FA-exposed mice.

**CONCLUSION:** Similar to our previous study involving in utero exposure, preconception exposure to PC PM₂.₅ at real-world concentrations results in adult cardiac dysfunction. These results suggest that abnormalities in developmental potential are not limited to prenatal or postnatal period but can also be determined prior to conception.
Cardiovascular disease results from a complex interplay between heritable risk and environmental exposure. The integrator of these factors is the epigenome. Our laboratory studies basic principles of chromatin structure, investigating how genetic variation and pathological stimuli regulate the epigenome to control gene expression in the heart. Our work explores how chromatin structural proteins and histone modifying enzymes regulate transcription in the diseased heart to identify biomarkers and therapeutic targets. We are testing the hypothesis that chronic syndromes like heart failure involve global changes in genome accessibility to favor more plastic chromatin environments, thereby enabling pathologic gene expression. My lecture will present recent studies testing this hypothesis in the areas of endogenous chromatin structure and DNA methylation.
Fetal or neonatal heart arrhythmias in humans are a common disorder. While the type and severity of congenital arrhythmias vary, some are life-threatening. Only limited genetic mutations leading to fetal arrhythmias have been identified.

Here, we present an animal model with fetal arrhythmias and reveal that the activation of the β2-adrenergic receptor-protein kinase A (β2AR-PKA) signaling pathway contributes to the Rnd3 deficiency-mediated arrhythmic phenotype. Rnd3, a small Rho GTPase, is involved in the regulation of cell actin cytoskeleton dynamics, cell migration and proliferation.

This study uncovers a new biological function of Rnd3. We provide evidence that the downregulation of Rnd3 is sufficient to initiate the activation of PKA signaling in vivo in animal hearts and in vitro in both cardiac and non-cardiac cells, suggesting a general mechanism for Rnd3-mediated PKA regulation. We further determine that Rnd3 is a regulator in the β2AR ubiquitination regulatory complex. Rnd3 regulates β2AR ubiquitination, mediated by the physical interaction between both proteins. The lack of Rnd3 prevents the ubiquitination of β2AR, resulting in the accumulation of β2AR protein. Excess β2AR promotes the activation of PKA signaling, which then contributes to the dysfunction of RyR2 calcium release channels. The β2AR antagonist treatment significantly reduced arrhythmia and improved cardiac contractility.
Desmin Cytoskeleton in Proper SR-Mitochondrial Cross-Talk in Healthy and Failing Heart.

Desmin mutations in humans and Desmin deficiency in mice lead to mitochondrial defects, myocardial degeneration, with extensive inflammation and fibrosis, cardiomyopathy and heart failure. Our studies have linked desmin and some of its associated proteins to MAMs (the ER/SR-mitochondria-associated membranes contact sites) and components of the MICOS complex. MAMs are the sites of multiple vital cellular processes, including regulation of cellular metabolism, intracellular calcium homeostasis, mitochondrial membrane dynamics, cell death, autophagosome formation and inflammasome activation. Our current studies focus in the unraveling of the role of the desmin cytoskeletal network in the coordination and balance of these processes and how loss of this balance lead to heart failure. Our progress towards these goals will be presented. Supported by PENED 01ED371, EPAN YB-22 and PEP ATT-39 and ESPA 09SYN-21-965 and “Excellence II” ARISTEIA II 5342 grants from the Greek Secretariat for R&D to YC.

Emerging evidence suggests the importance of inter-organellar connections in cellular homeostasis. The aim of this proposal is to unravel the importance of the intermediate filament (IF) cytoskeleton in general, and the muscle specific desmin IF scaffold in particular, in the coordination of proper mitochondrial cross-talk with other membranous organelles, thus ensuring maintenance of cellular homeostasis and cytoprotection. Cardiac muscle will be the focus of this proposal, considering the plethora of mitochondria in this tissue and the importance of their proper biogenesis and homeostasis in the maintenance of healthy heart. Such studies have never been done before for any IF protein in any tissue except of our very recent studies which lead to the present hypotheses. The importance of the IF cytoskeleton is reflected by the explosion of the number of diseases (>50) that are caused or predisposed to by mutations in IF genes. IFs have been linking to different important functions; however, the exact mechanism leading to these diseases remains elusive. Desmin mutations in humans and Desmin deficiency in mice lead to mitochondrial defects, myocardial degeneration, with extensive inflammation and fibrosis, cardiomyopathy and heart failure. In addition to mitochondria, our studies have linked desmin to MAMs (the ER/SR-mitochondria-associated membranes contact sites), to lysosomes (and potentially autophagosomes through the desmin-associated TRIM-like protein myospryn) and to nuclei. The coordination of these connections seems to be very important and its disturbance leads to disease, but how it is achieved and if and how it is facilitated by the IF network is completely unknown. MAMs are the sites of multiple vital cellular processes, including regulation of cellular metabolism, intracellular calcium homeostasis, mitochondrial dynamics, cell death, autophagosome formation and inflammasome activation. Our hypothesis is that at least in muscle, the desmin IF network with some of its associated proteins, such as myospryn and αB-crystallin, might play an important role in the coordination and balance of these processes.

To address this hypothesis we will elucidate how desmin and its partners facilitate the formation of proper contact sites and cross-talk between organelles, focusing on ER/SR-mitochondria-lysosomes, and whether they serve as platforms that could facilitate the autophagy initiation (phagophore, omegasome formation), bridge the damaged mitochondria to it, help in maturation and final docking and fusion with lysosomes, and efficiently regulate the antagonizing role of autophagy on inflammasome activation, by regulating lipid and protein complex assembly at the MAM sites. Successful studies in disease models under investigation will have a great impact on our understanding and fighting of diseases caused by damaged mitochondria.

Desmin mutations in humans and Desmin deficiency in mice lead to myocardial degeneration, cardiomyopathy and heart failure. We have shown the importance of desmin in mitochondrial homeostasis, lysosome behavior and correct nuclear shape and positioning. Recently we demonstrated an interplay between desmin and its associated chaperon protein αB-crystallin in mitoprotection and cardioprotection through the association of both of them with the ER/SR-mitochondria contact sites and components of the MICOS complex. Emerging evidence suggests the importance of interorganellar connections in cellular homeostasis and our hypothesis is that the IF network in general and muscle specific Desmin IF in particular, together with αB-crystallin, might play an important role in their formation and function. To address this hypothesis we will elucidate how desmin, together with αB-crystallin, facilitates the formation of proper contact sites and cross-talk between organelles, focusing on the mechanisms by which they maintain proper: a) protein and lipid targeting; b) formation and stabilization of mitochondrial complexes and their supercomplexes with ER/SR and association with lysosomes; d) mitochondrial fission/fusion balance; e) communication and molecular shuttling between mitochondria and nuclei. To achieve that, we will: a) determine which aspects of mitochondrial and other organelles defects seen in Desmin null mice are first rescued by overexpressing αB-crystallin and compare them with the hearts of overexpressing mitochondrial targeted αB-crystallin; b) compare the proteome of the affected organelles in Desmin-null to that of wt or “rescued” animals at early and later stages; c) assess whether, and how, the expression, distribution or function of specific proteins crucial for mitochondrial function and for communication with the nucleus and the ER/SR are affected in Demin null cardiomyocytes, d) test the dependence of Desmin’s effects on the recently identified

Desmin-associated proteins, such as VDAC, mitofilin and other MICOS’ components and elucidate the mechanism of these associations; e) apply the above strategy to TNFα heart failure model.
Renal Sympathetic Denervation Protects the Failing Heart

**Background:**
Overactivity of the sympathetic nervous system plays a critical role in the pathogenesis of heart failure. We investigated the effects of renal denervation (RDN) on left ventricular (LV) function and natriuretic peptide levels in both rodent and porcine models of ischemic heart failure.

**Methods:**
Spontaneously hypertensive rats (SHR) were subjected to 45 min. of coronary artery ligation and reperfusion (Rep) for 12 weeks. At 4 weeks post-Rep, SHR underwent either bilateral radiofrequency RDN (n=9) or sham RDN (n=8) procedure. Transthoracic 2D echocardiogram was performed (Visualsonics Vevo 2100) biweekly. Plasma peptides including were quantified at 12 weeks post-Rep. In additional studies we evaluated the effects of RDN on cardiac remodeling and left ventricular function in Yucatan mini-swine subjected to coronary balloon occlusion and ischemic heart failure.

**Results:**
RDN significantly preserved LV ejection fraction (*Figure A*) and LV stroke volume (318 ± 24.5 vs. 215 ± 17.8 μl, p < 0.05) at 12 weeks vs. sham. Heart weight/tibia length was also reduced in the RDN group (41 ± 2.4 vs. 49 ± 1.2 mg/mm, p < 0.05). Interestingly, Plasma BNP, ANP, and BK levels were significantly elevated in the RDN treated group compared to sham (*Figure B*). LV pressures were also recorded and demonstrated significant improvements in positive and negative dP/dT.

**Conclusion:**
Radiofrequency RDN improved LV function and significantly increased circulating cardioprotective natriuretic peptides during heart failure. We conclude that RDN therapy exerts beneficial effects in heart failure in part via increasing circulating cardioprotective natriuretic peptides. Current studies are aimed at the elucidation of the mechanisms responsible for increases BNP, ANP, and BK following RDN.
β1-adrenergic receptors (β1ARs) mediate catecholamine actions in cardiomyocytes by coupling to both Gs/cAMP-dependent and Gs-independent/growth-regulatory pathways. Structural studies of the β1AR define ligand-binding sites in the transmembrane helices and effector docking sites at the intracellular surface of the β1AR, but the extracellular N-terminus, which is a target for post-translational modifications, typically is ignored. We have identified β1AR N-terminal O-glycosylation at Ser37/Ser41 as a mechanism that prevents β1AR N-terminal cleavage. We used an adenoviral overexpression strategy to show that both full-length/glycosylated β1ARs and N-terminally truncated glycosylation-defective β1ARs couple to cAMP and ERK-MAPK signaling pathways in cardiomyocytes. However, a glycosylation defect that results in N-terminal truncation stabilizes β1ARs in a conformation that is biased toward the cAMP pathway. Our studies identify O-glycosylation and N-terminal cleavage as novel structural determinants that influence β1AR responsiveness in cardiomyocytes and can be exploited for therapeutic advantage.
How we will practice Medicine in the future: Predicting and preventing coronary disease

Cardiovascular disease remains the single largest cause of morbidity and mortality in the developed and developing world. Identifying populations and individuals at risk is necessary to appropriately target therapies with defined costs, risks of side effects and benefits. However translating population risk to the individual patient is challenging. Primary (preventing the first event) and secondary prevention (preventing recurrent events) pose distinct problems - the latter introduces the concept of “residual risk” in those on optimal therapy. This residual risk potentially relates to unknown factors, as well as residual hyperlipidaemia, inflammatory processes and thrombotic risk. It is unlikely that we will be able to progressively add therapeutic classes of drugs to routine treatment given the cost implications and the risks of polypharmacy, especially in the elderly. Quantifying the most important therapeutic targets for individual patients will most likely require integration of established clinical phenotypes with sophisticated metabolomic, proteomic and genomic evaluations.
Despite current therapies, heart failure morbidity and mortality remain significant, mandating the continued development of new therapeutics. The scaffold protein muscle A-kinase anchoring protein β (mAKAPβ/AKAP6) is a critical regulator of pathological cardiac remodeling that organizes multimolecular signaling complexes at the nuclear envelope of the cardiac myocyte. We have published that p90 ribosomal S6 kinase 3 (RSK3) is associated with mAKAPβ signalosomes in cardiac myocytes, and that inhibition of RSK3-mAKAPβ anchoring using a competing peptide inhibits the hypertrophy of cultured neonatal rat ventricular myocytes. In addition, we have shown that genetic targeting of either the mAKAPβ or RSK3 genes will prevent the pathological remodeling associated with pressure overload in mice due to transverse aortic constriction. More recently, we have found that conditional mAKAPβ knock-out also decreases the remodeling due to myocardial infarction following permanent ligation of the left coronary artery. In order to develop new therapeutics based upon mAKAPβ signalosomes targeting, we have designed two new gene therapy vectors, self-complementary, serotype 9 adeno-associated viruses (scAAV) that express under the control of the cardiac troponin T promoter either a mAKAP-specific small hairpin RNA (mAKAP-shRNA) or a RSK3 anchoring disruptor peptide based upon mAKAPβ protein sequence (RBD). We now present results demonstrating the efficacy of these biologics in both pressure overload disease and myocardial infarction in mice.
Mitochondrial calcium signaling in cellular differentiation

When the heart is injured quiescent fibroblasts differentiate into contractile, synthetic myofibroblasts. Initially myofibroblast formation and fibrosis is reparative, but when chronic it becomes maladaptive and contributes to heart failure progression. Cytosolic Ca\(^{2+}\) (\(\text{cCa}^{2+}\)) signaling is reported to be necessary for myofibroblast differentiation, yet the role of mitochondrial Ca\(^{2+}\) (\(\text{mCa}^{2+}\)) exchange has not been explored. \(\text{Ca}^{2+}\) signaling is rapidly integrated into the mitochondrial matrix via the \(\text{mCa}^{2+}\) uniporter channel (MCUc). To examine the contribution of \(\text{mCa}^{2+}\) in cardiac fibrosis, we generated conditional, fibroblast-specific 
Mcu knockout mice (KO) to ablate \(\text{mCa}^{2+}\) uptake. In this lecture I will discuss new molecular mechanisms regulating mitochondrial calcium uptake and it’s impact on cellular metabolism, epigenetics and differentiation. Our results suggest that alterations in \(\text{mCa}^{2+}\) uptake and bioenergetic pathways are necessary for the induction of the myofibroblast gene program and cellular differentiation.
Autophagy represents the most important renovation system in cells, responsible for degradation and turnover of dysfunctional or misfolded proteins as well as aged and/or damaged organelles. In this study, we assessed autophagic activity in pig myocardium subjected to ischemia-reperfusion (IR). Occlusion of the left anterior descending (LAD) artery was performed in anesthetized pigs to induce ischemia for 60 minutes followed by reperfusion for 120 minutes. In order to assess autphagic flux, chloroquine was administrated intravenously at the start of reperfusion in one group of animals. Biopsies from the ischemic and remote myocardial tissue were collected in vivo from each animal before LAD occlusion (baseline), after 60 minutes of ischemia, and subsequently at 20-, 60- and 120 minutes of reperfusion. Time-matched biopsies were collected from control pigs subjected to a sham procedure. Immunoblotting of tissue lysates and immunofluorescent staining of tissue sections with the autophagy markers LC3 and p62/SQSTM1, showed an elevated level of LC3 II and p62 and an increased number of co-localized LC3 and p62 puncta, respectively in ischemic myocardium after 120 minutes of reperfusion. Super-resolution imaging of the stained tissue sections displayed membrane staining in close association with co-localized LC3 and p62 puncta. Furthermore, correlative light-electron microscopy (CLEM) of the stained tissue sections revealed the ultrastructure of co-localized LC3 and p62 puncta as autophagosomes. Taken together, our results show an increase of autophagosomes in ischemic pig myocardium after 120 minutes of reperfusion, possibly due to inhibition of the autophagic flux.
Left and right heart failure is a complex clinical syndrome resulting in a reduced quality of life as well as patients’ prognosis. The most frequent disorders leading to left heart failure are hypertensive heart disease (HHD), ischemic (ICM), valvular (VCM) and dilated (DCM) cardiomyopathy. Right heart failure frequently occurs as a consequence of pulmonary hypertension (PH) due to left heart disease and a variety of additional underlying pathologies (different PH groups). All these cardiovascular disorders are characterized by both, vascular and myocardial remodelling including a fetal reorganisation of the extracellular matrix. This process entails the re-expression of fetal variants of the cell adhesion modulating proteins fibronectin (Fn) and Tenascin-C (Tn-C), which are virtually absent in healthy adult hearts. Thus, these molecules qualify as excellent novel biomarkers and also therapeutic targets, e.g., by using human recombinant antibodies fused with cytokines (immunocytokines). We descriptively analysed the fetal ECM reorganisation in patients suffering from different cardiovascular disorders both in tissue and in serum and tested their value as biomarkers. Moreover, we established two representative animal models, a heart transplantation model that mimics myocardial and vascular remodelling as well as a model of pulmonary hypertension (PH) specifically focussing on vascular remodelling in the lung and consecutive right heart failure.

Using these models and in vitro experiments, we evaluated the newly identified fetal cECM components as therapeutic target molecules for both, functional inhibition and antibody based delivery of diagnostic (e.g., radionuclides) or therapeutic (cytokines) agents directly to the site of disease.
The metabolic syndrome (MetS) is a cluster of clinical disorders such as dyslipidemia, diabetes and obesity which are associated with increased risk for cardiovascular disease. The etiology of MetS is poorly understood and effective therapies are urgently needed. Our main goal was to monitor global changes in the expression of hepatic genes and circulating miRNAs in ApoE3L.CETP mice that were used as a model of MetS. Male mice were fed either a High (HFD) or a Low (LFD) Fat Diet for different time periods. Liver RNA was analyzed on Affymetrix Mouse Gene 2.0 ST arrays followed by bioinformatical analysis. Total miRNAs were isolated from serum and quantitated by RT-qPCR. The microarray analysis identified an increasing number of differentially expressed transcripts during MetS development in the liver. A comparison of the HFD vs the LFD fed mice revealed 114, 200 and 358 differentially expressed transcripts upon 4, 8 and 12 weeks feeding by applying conservative thresholds (absolute fold change 1.5 and Two-Way Anova p-value < 0.05). Functional analysis of the differentially expressed genes revealed a number of biological processes, and networks related mostly to lipid metabolism, steatosis and inflammation. The top list of affected pathways includes Ppar signaling, AMPK signaling and cholesterol biosynthesis that probably mediate the observed metabolic dysregulation of the MetS. Finally, miRNAs previously correlated to metabolic diseases were detected in the serum of ApoE3L.CETP mice and their levels were increased in response to a 12 week HFD administration. Our findings are indicative of characteristic hepatic gene and plasma miRNA signatures during the MetS development in ApoE3L.CETP mice which could be exploited further for diagnostic or therapeutic purposes.
Mitochondrial hyperacetylation in failing heart from patients with obesity is partly mediated by a reduction in SIRT-3: involvement of the mitochondrial permeability transition pore.

Elena C. Castillo, PhD\textsuperscript{a}, José A. Morales, MSc\textsuperscript{a}, Héctor Chapoy-Villanueva, PhD\textsuperscript{a},\textsuperscript{b}, Christian Silva-Platas, MSc\textsuperscript{a}; Keith Youker, PhD, Noemi Garcia\textsuperscript{a,b,\textsuperscript{c}}; Guillermo Torre-Amione, MD, PhD\textsuperscript{a,b,\textsuperscript{c}} and Gerardo Garcia-Rivas, PhD\textsuperscript{a,b,*}.

\textsuperscript{a} Cátedra de Cardiología y Medicina Vascular, Escuela de Medicina, Tecnológico de Monterrey. Monterrey, México.


\textsuperscript{c} Methodist DeBakey Heart & Vascular Center, The Methodist Hospital, Houston, Texas. USA.

**Background:** Cyclophilin D (CypD) mediates mitochondrial permeability transition pore (mPTP) opening that contributes to mitochondrial dysfunction. CypD is regulated by their acetylation/deacetylation state that depends on SIRT3 a mitochondrial deacetylase. Since obesity and metabolic syndrome (MS) decrease SIRT3 activity and expression, we tested the hypothesis that CypD hyperacetylation promotes mitochondrial dysfunction under this pathophysiological state, which is associated with diastolic dysfunction and heart failure (HF).

**Methods and Results:** Accordingly, mitochondria from animals with obesity and MS were 2.5-fold prone to mPTP opening compared with controls. SIRT3 mitochondrial expression decreased 22%, concomitantly with hyperacetylated mitochondrial profile including CypD. Additionally, SIRT3 expression in human biopsies from failing hearts showed 35% decrease expression level in patients with obesity in comparison to non-obese patients. Remarkably, Body mass index (BMI) was associated with protein acetylation (0.627, p=0.035) suggesting that acetylation profile in failing heart from patients with obesity is partly mediated by a reduction in SIRT3, which also was associated with higher BNP levels (-0.636, p=0.042).

**Conclusions:** Our results indicate that obesity and MS reduces SIRT3 expression and that CypD hyperacetylation increase the mPTP opening, suggesting that activation of SIRT3 might be a potential target for ventricular dysfunction and HF progression.
Inflammatory activation and metabolic impairment, are important mediators of ARVC pathophysiology

I. Sfyroera¹, A. Varela¹, I. Kostavasilis², I. Karaktitsios², C. Davos², Y. Capetanaki¹, C.D. Anagnostopoulos² M. Mavroidis¹*

*Correspondence: Tel: +30-210-6597057, emavroeid@bioacademy.gr

Introduction: Inflammatory activation and metabolic impairment are becoming the focus of research as novel therapeutic targets in heart failure. We recently demonstrated in a genetic model of arrhythmogenic cardiomyopathy (desmin-deficient mice, Des-/-) that modulation of innate immunity through elimination of complement C5a receptor (C5aR) resulted in impressive improvement of cardiac function.

Aim: To analyze the role of the second C5a receptor (C5L2) that has been linked to energy metabolism and inflammation as a novel therapeutic target in desmin deficient cardiomyopathy.

Materials/Methods: We generated C5L2-/-Des+/- mice by crossing C5L2-/- with Des-/- mice. Histology, electron microscopy, echocardiography, RNAseq and ¹⁸F-FDG microPET/CT were performed in 12 months old animals (n=10) and parameters related to cardiac structure, function and myocardial glucose consumption were compared with those of wild type (WT) controls of similar age.

Results: C5L2-/-Des+/- mice progressively developed severe cardiac dysfunction compared to WT controls (Fractional shortening 22.89±2.52 vs. 46.94±0.67, p<0.0001). Histology revealed increased fibrosis in C5L2-/-Des+/- compared to WT (Fibrosis index, 1.5±0.21, vs. 0.4±0.34, p<0.01). Electron microscopy showed severe mitochondrial and T-tubules abnormalities in C5L2-/-Des+/- compared to WT. Additionally, cardiac tissue RNAseq analysis demonstrated altered expression of several genes involved in metabolic pathways, indicating a “metabolic switch” in C5L2-/-Des+/- from fatty acid to glucose oxidation compared to WT. This was also confirmed by the higher myocardial metabolic rate of glucose values in C5L2-/-Des+/- compared to WT animals (168.6 ±55.2 vs. 39.8±3.3μmol/min/100g, p<0.05).

Conclusions: Our results highlight the detrimental consequences on cardiac structure and function of C5L2 receptor elimination in arrhythmogenic cardiomyopathy and support the hypothesis of its implication in the metabolic impairment, which occurs in this pathological entity.
Magnesium Supplementation Improves Cardiac Mitochondrial and Diastolic Function

Rationale: In congestive heart failure and type 2 diabetes, hypomagnesemia has been found in the majority of patients, and supplementation of Mg\textsuperscript{2+} has improved heart function and insulin resistance, respectively. Recently, we have shown that diabetes can cause cardiac diastolic dysfunction. Therefore, we hypothesized that Mg\textsuperscript{2+} supplementation would benefit diastolic function.

Methods and Results: High fat diet (HFD)-induced diabetic mouse hearts showed increased mitochondrial reactive oxygen species (ROS), myofilament cardiac myosin binding protein C (cMyBP-C) oxidation, and diastolic dysfunction. Dietary Mg\textsuperscript{2+} administration (450 mg/day/kg) for 6 weeks increased plasma Mg\textsuperscript{2+} concentration (1.49 ± 0.04 mg/dL in HFD+Mg vs. 0.80 ± 0.04 mg/dL in non-treated HFD, P<0.001), and the incidence of diastolic dysfunction was significantly reduced (incidence of DD: 90% in HFD vs 20% in HFD+Mg, P<0.05). Mitochondrial ROS was decreased significantly, mitochondrial membrane potential was repolarized, and mitochondrial ATP production was improved by Mg\textsuperscript{2+} supplementation in diabetic mice.

Conclusion: These results indicate that Mg\textsuperscript{2+} supplementation improved mitochondrial function, reduced oxidative stress, and prevented diastolic dysfunction in diabetes.
KLF5 Regulates the miR-30 Family in Ischemic Cardiomyopathy

Hoffman, M.1,2, Walker, L.1,2, Pol, C.1,2, Kyriazis, I.1,2, Brown, B.1,2, Kurian, J.2,3, Khan, M.2,3, Drosatos, K.1,2

Author Information:
1: Temple University, Center for Translational Medicine
2: Temple University, Center for Metabolic Disease Research
3: Temple University, Department of Physiology

Ischemic cardiomyopathy is a leading cause of heart failure associated morbidity, mortality, and healthcare expenditure. Impaired cardiac energetics resulting from mitochondrial dysfunction and switch in substrate preference is characteristic of and contributes to disease progression and worsening of cardiac function. We previously showed that mice with cardiomyocyte specific-ablation of Krüppel-like factor (KLF)5 (αMHC-Klf5−/−) had lower cardiac fatty acid oxidation rates and progression to dilated cardiomyopathy at six months of age. We therefore sought to identify novel regulatory targets for cardiac KLF5 that contribute to myocardial disease. We performed miR array analysis in αMHC-Klf5−/− heart tissue and identified differentially expressed miRs predicted to target genes involved in cardiac metabolism. Through this analysis, we identified the miR-30-5p family, which was elevated in αMHC-Klf5−/− heart tissue and is predicted to regulate PGC1α. Gene expression analysis of isolated cardiomyocytes from αMHC-Klf5−/− mice confirmed that miR-30a-5p, miR-30b-5p, miR-30c-5p, miR-30d-5p, and miR-30e-5p are increased three-fold, whereas analysis of cardiomyocyte-restricted doxycycline-inducible KLF5 transgenic mice (αMHC-rtTA-TRE-KLF5), which we generated, showed decreased levels of these miRs. We further observed that KLF5 transgenic mice exhibit cardiac dysfunction beginning 2-weeks post dox resulting in increased mortality. To elucidate the regulatory changes in KLF5 and miR30 family members which occur in ischemic cardiomyopathy, we investigated the LAD ligation induced rodent model of MI 1-day and 2 weeks post-surgery. One day post-MI, we found a reduction in KLF5 protein levels in isolated cardiomyocytes which may contribute to the reported induction in miR30 family members during ischemia. Beginning 2 weeks post-MI, we found induction of cardiomyocyte KLF5 mRNA and protein, and a reduction in all miR30 family members. Because miR30 inhibition has been shown to be pro-hypertrophic, we propose KLF5 as a novel regulator of ischemic cardiac dysfunction via a mechanism that involves altered expression of miR-30 family members.
The MEF2 transcriptional target DMPK induces loss of sarcomere structure and cardiomyopathy

Amin Damanafshan, Ies Elzenaar, Benoit Samson-Couterie, Ingeborg van der Made, Meriem Bourajjaj, Maarten M. van den Hoogenhof, Henk A. van Veen, Daisy I. Picavet, Abdelaziz Beqqali, Elisabeth Ehler, Leon J. De Windt, Yigal M. Pinto, Ralph J. van Oort

Department of Experimental Cardiology, Academic Medical Center, Amsterdam, The Netherlands.

Department of Cardiology, Maastricht University, Maastricht, The Netherlands.

Electron Microscopy Centre Amsterdam, Department of Medical Biology, Academic Medical Center, Amsterdam, The Netherlands.

Cardiovascular Division, King’s College, London, United Kingdom.

Rationale: Heart failure is characterized by poorly contracting and dilated ventricles. This is associated with lengthening of cardiomyocytes and loss of sarcomeres. While it is known that the transcription factor myocyte enhancer factor 2 (MEF2) is involved in this cardiomyocyte remodeling, the underlying mechanism remains to be elucidated.

Objective: We aim to mechanistically link MEF2 target genes with loss of sarcomeres during cardiomyocyte remodeling.

Methods and Results: Neonatal rat cardiomyocytes overexpressing MEF2 lost their sarcomeric structure. We identified myotonic dystrophy protein kinase (DMPK) as direct MEF2 target gene involved in this process. Overexpression of DMPK E, the isoform upregulated in heart failure, resulted in severe loss of sarcomeres in vitro and disruption of sarcomere structure and cardiomyopathy in vivo. Moreover, we found a decreased expression of several sarcomeric genes following DMPK E gain-of-function. These sarcomeric genes are targets of the transcription factor serum response factor (SRF) and we found that DMPK acts as inhibitor of SRF transcriptional activity.

Conclusions: Our data indicate that MEF2-induced loss of sarcomeres is mediated by DMPK via a decrease in sarcomeric gene expression by interfering with SRF. These results demonstrate an unexpected role for DMPK as a direct mediator of adverse cardiomyocyte remodeling and heart failure.

This project is supported by grant 2012T094 from the Dutch Heart Foundation (NHS).
Validation and downregulation of circRNAs in iPSC-derived cardiomyocytes
Lucia Cocera Ortega, Guillermo R. Griffith, Yolan J. Reckman, Anouk van den Bout, Mischa Klerk, Ingeborg van der Made, Yigal M. Pinto, Anke J. Tijsen

1 Experimental Cardiology, Academic Medical Centre, Amsterdam, The Netherlands

Circular RNAs are a type of RNAs formed by backsplicing of an upstream donor sequence to a downstream acceptor sequence. Recent developments in deep sequencing techniques combined with novel bioinformatics led to the discovery of a surprisingly large amount of circRNAs in many different tissues. Subsequently, circRNAs have been demonstrated to act as miRNA sponges, regulate transcription, interfere with pre-mRNA splicing or even serve as a template for protein synthesis. However, a general role of circRNAs has not been elucidated.

In the present study we selected 28 circRNAs previously detected in human heart by circRNA profiling on ribosomal depleted RNA. Selection was based on the assumption that circRNAs derived from cardiac genes might have an important function in the heart, which resulted in 25 selected circRNAs arising from excitation-contraction coupling related genes and 3 circRNAs from TTN. We determined the expression of the 28 circRNAs in induced pluripotent stem cell derived cardiomyocytes (iPSC-CM) by PCR with divergent primers, which detected 24 of the circRNAs. We validated their circular characteristic by showing their resistance to RNAse R treatment.

To elucidate the role of the circRNAs in iPSC-CM we will downregulate them by shRNAs. Therefore, we designed shRNAs that specifically target the backsplice junction and expressed them lentivirally in iPSC-CM. Currently, we confirmed successful downregulation of 17 circRNAs by PCR with divergent primers. For 8 of these circRNAs, we confirmed that they do not induce toxicity by sequence-specific activation of the interferon response. Furthermore, we show that these 8 circRNAs are specific for the backsplice junction and do not downregulate the linear counterpart.

In conclusion, we developed shRNAs to specifically downregulate circRNAs expressed in iPSC-CMs and we will determine the effect of the loss of these circRNAs on the phenotype of the iPSC-CMs by immunostainings, Ca-handling and electrophysiological experiments.
Histone acetyltransferase-dependent signaling pathways mediate the up-regulation of endothelin-1 and markers of vascular dysfunction in experimental diabetes

Alexandra-Gela Lazar, Mihaela-Loredana Antonescu, Ioana Madalina Fenyo, Adrian Manea, Simona-Adriana Manea

Institute of Cellular Biology and Pathology “Nicolae Simionescu”, Bucharest, Romania

Background. Excess production of endothelin -1 (ET-1) induces vascular deleterious effects in diabetes. The mechanisms of ET-1 up-regulation are not entirely elucidated. Alterations of epigenetic mechanisms involving histone acetyltransferase (HAT) and histone deacetylase enzymes are associated with cardiovascular disorders. The precise implication of histone acetylation system in mediating diabetic vasculopathies and, in particular in the regulation of ET-1 remains elusive.

Purpose. The aim of this study was to investigate the existence of HAT-dependent regulatory mechanisms of ET-1 expression in experimental diabetes.

Methods. Streptozotocin-induced diabetic C57BL/6J mice were randomized to receive vehicle or CPTH2 (HAT inhibitor). Human umbilical vein endothelial cells EAhy926 (ECs) were exposed to normal or high levels of glucose in the absence/presence of HAT inhibitors (CPTH2, C646). Real-time PCR, Western blot, ELISA, luciferase reporter, and chromatin immunoprecipitation assays were employed.

Results. We found that p300 protein along with ET-1 expression levels were significantly elevated in the aortas of diabetic mice compared to non-diabetic animals. Treatment of diabetic mice with CPTH2 mitigated the aortic expression of ET-1, NADPH oxidase, intercellular adhesion molecule-1, and monocyte chemotactic protein-1. High glucose concentrations up-regulated the levels of H3K27 acetylation and HAT1 in cultured ECs. CPTH2/C646 dose-dependently reduced the high glucose-augmented expression of ET-1 in ECs. Transient overexpression of p300 augmented the ET-1 gene promoter activity and the activities of NF-kB, STAT, and C/EBP transcription factors in ECs. Physical interaction of p300/HAT1 with ET-1 gene promoter was detected at the sites of active transcription in ECs. High glucose induced histone H3K27 acetylation enrichment and the recruitment of p300/HAT1 at the promoter of ET-1 gene in ECs.

Conclusion. HAT subtypes mediate ET-1 up-regulation in experimental diabetes. Pharmacological inhibition of HAT may reduce vascular dysfunction in diabetes, possibly by negative regulation of ET-1, oxidative stress, and pro-inflammatory molecules expression.

Acknowledgements. Work supported by UEFISCDI (PN-III-P4-ID-PCE-2016-0665, PN-III-P2-2.1-PED-2016-1308).
Pharmacological inhibition of NADPH oxidase down-regulates the expression of pro-inflammatory markers in classically-activated macrophages in vitro: potential implication in human atherosclerosis

Mihaela Loredana Antonescu¹, Alexandra Gela Lazar¹, Simona-Adriana Manea¹, Monica Raicu¹, Horia Muresian², Maya Simionescu¹, Adrian Manea²

¹Institute of Cellular Biology and Pathology “Nicolae Simionescu”, Bucharest, Romania
²Cardiovascular Surgery Department, University Hospital of Bucharest, Bucharest, Romania

Background. Oxidative stress microenvironment shapes the phenotype of monocyte (Mon)-derived macrophage (Mac) in atherosclerosis. Two major Mac populations with different major phenotypes have been described: the pro-inflammatory (M1) and anti-inflammatory (M2). Down-regulation of proinflammatory molecules expression in M1-Mac and/or active induction of a specific M2-Mac subset may be therapeutically relevant for the outcome of atherosclerosis. NADPH oxidases (Nox) are major sources of reactive oxygen species (ROS) in Mac contributing to atheroma formation. The role of Nox-derived ROS in Mac polarization via redox-sensitive mechanisms is not known.

Purpose. This study aimed at investigating the implication of Nox enzymes in mediating Mac polarization in atherosclerosis.

Methods. Non-atherosclerotic (superior thyroid artery) and atherosclerotic (carotid artery) samples obtained as discarded tissues from patients undergoing carotid endarterectomy were used. Human THP-1 Mac were polarized into M1 and M2-Mac. The cells were further exposed (24 h) to M1/M2 polarization factors in absence/presence of vehicle or GKT137831, a clinically approved Nox1/4 pharmacological inhibitor.

Results. We found that Nox1, Nox2, Nox4, and Nox5 were significantly up-regulated in human atherosclerotic plaques compared to non-atherosclerotic samples. Immunohistochemical staining of human atherosclerotic lesions revealed that Nox proteins are up-regulated within CD68⁺/CD45⁺ Mac-rich areas. Significant increases in Nox1, Nox2, Nox4, and Nox5 expression levels were detected in M1-Mac as compared to resting Mac (M0) and M2-Mac in vitro. Pharmacological inhibition of Nox greatly reduced the expression markers defining the M1-Mac phenotype (TNFα, MCP-1, TLR2, TLR4, NOS2) and produced a significant up-regulation of CD163, a M2-Mac marker, in both M1-Mac and M2-Mac.

Conclusion. Our data indicate the existence of a new redox-sensitive mechanism whereby ROS generated by activated Nox contribute to Mac polarization. Pharmacological inhibition of Nox may be an attractive therapeutic strategy to reduce the overproduction ROS and inflammatory mediators derived from M1-activated Mac in atherosclerosis.

Acknowledgements. Work supported by UEFISCDI, PN-III-P4-ID-PCE-2016-0665.
Abnormally persisting KCNQ1 imprinting interferes with disease modeling of hiPSC-derived cardiomyocytes

Anke Tijsen1, Rami Shinnawi1, Gil Arbel1, Amira Gepstein1, Sara Selig1, Irit Huber1, Lior Gepstein2.

1 Rappaport faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel
2 Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Long QT syndrome type 1 (LQT1) is an inherited cardiac arrhythmia, caused by heterozygous KCNQ1 mutations, in which the balance between mutated and healthy alleles is a key determinant for disease severity. Imprinting is an epigenetic phenomenon leading to a parent-of-origin dependent gene expression. Due to imprinting KCNQ1 is only maternally expressed in all cells except cardiomyocytes. We hypothesized that epigenetic memory could lead to residual imprinting in iPSC-derived cardiomyocytes (iPS-CM).

We generated 10 iPSC-clones from a LQT1 patient with a maternally inherited KCNQ1 mutation. Cardiomyocytes derived from nine out of the ten clones showed residual imprinting characterized by mono-allelic KCNQ1 expression and methylation of IC2 (imprinting control region). Similarly, we detected persistent KCNQ1 imprinting in iPSC-CM of two independent non-LQT1 iPSC-lines. Electrophysiological analysis showed that imprinted LQT1 iPSC-CM had longer action potentials and a higher incidence of arrhythmias compared to the iPSC-CM of the one non-imprinted clone.

Our findings indicate that residual imprinting in iPSC-CM could lead to over/under-estimation of mutational effects in LQT1 and potentially influence phenotypes of other cardiac disease iPSC-CM. Furthermore, this phenomenon calls for awareness for similar caveats in non-cardiac iPSC models involving imprinted genes.
The ability of empagliflozin to prevent worsening cardiac dysfunction in nondiabetic mice with heart failure is associated with osmotic diuresis and increased circulating ketones

NIKOLE J. BYRNE, BSC [NJBYRNE@UALBERTA.CA]; DYONNE Y. VOS, BSC; JODY LEVASSEUR, BSC; NIRMAL PARAJULI, PHD; JAMIE BOISVENUE, BSC; GRANT MASSON, BSC; DONNA BEKER JASON RB DYCK, PHD [JASON.DYCK@UALBERTA.CA]

BACKGROUND: Recent work has shown that the anti-diabetic drug empagliflozin, an inhibitor of the renal-SGLT2 transporter, reduced hospitalization for heart failure (HF) and reduced cardiovascular-related deaths in diabetic patients. Although we have shown that empagliflozin prevents worsening cardiac dysfunction in nondiabetic mice with HF, the mechanisms responsible for its cardioprotective action are currently unknown. In the present study, we therefore used a mouse model of pressure-overload induced HF to investigate several proposed mechanisms.

METHODS/RESULTS: 8-week old, male C57Bl/6 mice were subjected to sham or transverse aortic constriction surgery to induce HF. Three weeks following surgery, mice with established HF (ejection fraction < 45%) were administered either vehicle or empagliflozin (10 mg/kg/day) by oral gavage daily for 2 weeks. Consistent with our previous results, the progressive worsening of cardiac function evident in vehicle-treated mice (p<0.01) was blunted in the empagliflozin-treated group (p=0.41). Despite causing a calorie deficit by glycosuria, there was no significant difference in body weight or composition of empagliflozin and vehicle-treated HF mice. Although, empagliflozin caused a trend toward reduced respiratory exchange ratio (p=0.07) and whole-body glucose oxidation (p=0.09) and increased whole-body fat oxidation (p=0.07) for several hours following treatment. Interestingly, there was a profound increase in urine volume of HF mice treated with empagliflozin compared to vehicle treated controls after the first treatment (p<0.01), an observation that was less apparent after 2 weeks of treatment. Furthermore, empagliflozin increased fasting levels of blood ketones 1.5-fold (p=0.02), despite no apparent effect on blood glucose levels.

CONCLUSION: These data indicate that the blunted decline in cardiac function in nondiabetic mice with HF is associated with increased urine excretion and increased circulating ketones. Altogether, these data suggest that osmotic diuresis and altered substrate availability may be two mechanisms involved in the effect observed in nondiabetic mice with HF treated with empagliflozin.
Re-activation of Notch signaling is required for cardiac valve regeneration

P. Kefalos1,2, A. Brito1, A. Agalou1, K. Kawakami4, D. Stainier3, D. Beis1.

1Zebrafish Disease Model lab, Center for Experimental Surgery, Clinical and Translational Research, Biomedical Research Foundation, Academy of Athens, GR11527, Greece.
2Department of Biology, University of Patras, GR26504, Greece.
3Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, 61231 Bad Nauheim, Germany
4Division of Molecular and Developmental Biology, National Institute of Genetics, Mishima 411-8540, Japan.

Email: dbeis (at) bioacademy.gr

Zebrafish is an ideal model to investigate cardiac valve development as it allows these studies to be carried out in vivo through non-invasive imaging. Valve development depends on intracardiac flow dynamics via the shear stress response of the endocardial cells. Cardiovascular Disease is a leading cause of morbidity and mortality, with valvular heart disease being the most common subtype. In addition, there is a strong age-related component that leads to valvular degeneration and calcification. The majority of diseased valves are not repairable, and the only possible therapy relies on surgical replacement. Recently, the clinical application of decellularized valves has shown promising results. However, complete recellularization of the implanted valves remains a challenge. Therefore, identification of cellular and molecular factors promoting valve regeneration is crucial to ensure growth potential, repair and effective response to cardiac function demands.

Mammals have limited cardiac regeneration capacity, after myocardial infarction and to date an unexplored potential of cardiac valve regeneration. In contrast, zebrafish retain their ability to regenerate their hearts throughout their lifetime. We used an inducible cardiac valve injury model, to show that cardiac valves regenerate. We analyzed the transcriptome of adult hearts following valvular ablation and identified Notch as one of the activated pathways. Valvular ablation at larval stages results in an increased reverse flow fraction of intracardiac hemodynamics, which ectopically induces the flow responsive transcription factor klf2a and notch1b. Re-activation of Notch signaling is required for cardiac valve regeneration since pharmacological inhibition of Notch, hinders the regenerating potential of cardiac valves.
Eleni Geladari

Arterial Hypertension and the Akt-mTOR Signaling Pathway in SHRs; Target Organ Damage and Antihypertensive Treatment
Emmanuel A. Andreadis, MD, PhD,1,2 Eleni V. Geladari, MD,1,2 Charalampia V. Geladari, MD1,2

1 Hypertension and Cardiovascular Disease Prevention Outpatient Center, Evangelismos General Hospital, Athens, Greece
2 Research Fellow, Hypertension and Vascular Medicine Clinic, Veterans Affairs Medical Center, Washington, DC, USA

Background: Hypertension (HTN) is the leading risk factor for cardiovascular (CV) morbidity and mortality. Cardiac hypertrophic signaling transduction pathways are dysregulated in HTN. The protein kinase C (PKC) and the mammalian target of Rapamycin (mTOR) pathway are important regulators of cardiac hypertrophy. mTOR is required for the initiation and full development of cardiac hypertrophy evoked by rising blood pressure in spontaneously hypertensive rats (SHR). Cardiac specific overexpression of Akt is linked to both physiological and pathological cardiac hypertrophy. Akt is also involved in endothelium dysfunction in HTN. Antihypertensives, such as angiotensin-receptor blockers (ARBs) and resveratrol (an mTOR inhibitor), targeting upstream or downstream the Akt, respectively, could highlight major treatment effects on reversing hypertrophy, fibrosis, and inhibiting autophagy in defective hearts.

Aim of the study: To examine combination treatment of ARBs and mTOR inhibitors on blood pressure (BP) levels and left ventricular remodeling in SHR.

Methods: Thirty male and female rats, aged ~15 weeks, body weight 200±50gr, will be randomly divided into 3 groups: (i) SHR-IR (treated with irbesartan, 10mg/kgBW) (ii) SHR-Res (treated with trans-resveratrol, 146mg/kgBW) (iii) SHR-IR-Res (treated with both drugs). Ten male Wistar Kyoto rats (WKY) will act as the control and they will receive no treatment. All treatments will be administered once daily from 16 to 32 weeks of age. Drug effects on BP and left ventricular mass will be studied. Also, molecular studies will help us elucidate the role of key signaling proteins in mediating cardiac hypertrophy and fibrosis among SHR.
Arterial Hypertension and the Akt-mTOR Signaling Pathway in SHRs; Target Organ Damage and Antihypertensive Treatment

Emmanuel A. Andreadis, MD, PhD, 1,2 Eleni V. Geladari, MD, 1,2 Charalampia V. Geladari, MD 1,2

1 Hypertension and Cardiovascular Disease Prevention Outpatient Center, Evangelismos General Hospital, Athens, Greece
2 Research Fellow, Hypertension and Vascular Medicine Clinic, Veterans Affairs Medical Center, Washington, DC, USA

Background: Hypertension (HTN) is the leading risk factor for cardiovascular (CV) morbidity and mortality. Cardiac hypertrophic signaling transduction pathways are dysregulated in HTN. The protein kinase C (PKC) and the mammalian target of Rapamycin (mTOR) pathway are important regulators of cardiac hypertrophy. mTOR is required for the initiation and full development of cardiac hypertrophy evoked by rising blood pressure in spontaneously hypertensive rats (SHR). Cardiac specific overexpression of Akt is linked to both physiological and pathological cardiac hypertrophy. Akt is also involved in endothelium dysfunction in HTN. Antihypertensives, such as angiotensin-receptor blockers (ARBs) and resveratrol (an mTOR inhibitor), targeting upstream or downstream the Akt, respectively, could highlight major treatment effects on reversing hypertrophy, fibrosis, and inhibiting autophagy in defective hearts.

Aim of the study: To examine combination treatment of ARBs and mTOR inhibitors on blood pressure (BP) levels and left ventricular remodeling in SHR.

Methods: Thirty male and female rats, aged ~15 weeks, body weight 200±50gr, will be randomly divided into 3 groups: (i) SHR-IR (treated with irbesartan, 10mg/kgBW) (ii) SHR-Res (treated with trans-resveratrol, 146mg/kgBW) (iii) SHR-IR-Res (treated with both drugs). Ten male Wistar Kyoto rats (WKY) will act as the control and they will receive no treatment. All treatments will be administered once daily from 16 to 32 weeks of age. Drug effects on BP and left ventricular mass will be studied. Also, molecular studies will help us elucidate the role of key signaling proteins in mediating cardiac hypertrophy and fibrosis among SHR.
Links of desmin cytoskeleton to mechanochemical signaling and cardiomyocyte differentiation, transdifferentiation and reprogramming.  
Tsikitis M, Srivastava D and Capetanaki Y

Given the limited regenerative capacity of the heart and the insufficient therapeutic approaches, much interest has been focused on cardiac regenerative medicine. Important steps towards possible therapeutic generation of cardiomyocytes were achieved with the direct reprogramming of fibroblasts into functional induced cardiomyocytes (iCM) through ectopic expression of three transcription factors GATA4, Mef2C and Tbx5 (GMT), however with rather low efficiency. Here, we show that desmin, the major muscle-specific intermediate filament protein, has the potential to increase reprogramming efficiency. This is of major interest since desmin is not the traditional transcriptional factor. Desmin forms a well organized cytoskeletal network that links the contractile apparatus to different membranous compartments and organelles, including the nucleus. We investigate the different levels where desmin may be important in cardiomyogenesis by further examine at the molecular and cellular level, the transdifferentiation potential of fibroblasts upon ectopic expression or absence of desmin. In addition to increasing the reprogramming efficiency, desmin appears to help iCMs proper maturation. We propose that desmin controls cardiac differentiation through its interacting proteins, thus we explore its interaction with Carp, a transcriptional regulator involved in cardiomyopathies. Carp is a target of the Yap pathway, a major signaling pathway that regulates among other, mechanotransduction and heart development and regeneration. This study raises the exciting possibility that desmin could be involved in cardiac differentiation and regeneration by regulating proper function of mechanosignaling pathways thus providing new avenues for the development of targeted therapeutic approaches for cardiac diseases.
Styliani Vakrou

Allele-specific differences in transcriptome, miRNome, and mitochondrial function in 2 Hypertrophic Cardiomyopathy mouse models at pre-disease stage suggest need for precision medicine approach to treatment

Vakrou S1, Fukunaga R1, Foster DB1, Sorensen L1, Liu Y1,2, Guan Y1, Woldemichael K1, Pineda-Reyes R1, Liu T1, Tardiff JC3, Leinwand LA4, Tocchetti CG1, Abraham TP1,2, O’Rourke B1, Aon MA1, Abraham MR1,2.

1Johns Hopkins University School of Medicine, USA 2Division of Cardiology University of California San Francisco, USA 3Department of Internal Medicine and Cellular and Molecular Medicine, University of Arizona, USA 4Department of Molecular, Cellular, and Developmental Biology and the BioFrontiers Institute, University of Colorado, USA

Background: Hypertrophic Cardiomyopathy (HCM) stems from mutations that elicit distinct biophysical sequelae, which may yield radically different intracellular signaling and molecular pathologic profiles. This factor remains unaddressed in clinical trials that have selected patients based on clinical diagnosis, irrespective of causal genotype. Determining the potential of precision medicine approaches requires a detailed mechanistic understanding of specific functional or signaling defects according to the HCM genotype.

Aim: To determine how two mouse models of HCM differ with respect to cellular/mitochondrial function and molecular biosignatures, at an early stage of disease. Methods: We examined the similarities/differences in the molecular cardiac phenotype, at the pre-disease stage, in 2 mouse models (R92W-TnT[troponin T] and R403Q-MHC[α-myosin heavy chain]) that span the spectrum of human HCM. We characterized 1) systolic/diastolic function, 2) the transcriptome and miRNome, 3) cellular redox, 4) mitochondrial (Mt) ROS (reactive O2 species), respiration, and number, in 5 week-old male mice. Studies were performed in parallel in mutant mice and littermate controls.

Results: TnT mutants exhibited higher ejection fraction, diastolic dysfunction, an oxidized redox environment, lower Mt matrix [Ca2+]free, lower [Ca2+] threshold for permeability transition pore (PTP) opening, lower Complex I Respiratory Control Ratio (RCR) and lower Mt-number compared to controls. In contrast, MHC mutants demonstrated similar systolic/diastolic function, a reduced redox environment, higher Mt matrix [Ca2+]free, similar [Ca2+] for PTP opening, higher Complex I RCR and similar Mt-number as controls. Pathway analysis of mRNA sequencing data and microRNA profiles indicate that TnT mutants exhibit a bio-signature consistent with activation of pro-fibrotic TGF-β signaling, and down-regulation of the anti-fibrotic miR-29.

Conclusion: Our results suggest that the oxidative environment and mitochondrial impairment in young TnT mice promote activation of TGF-β signaling that foreshadows a pernicious phenotype in young individuals. Of the two mutations, R92W-TnT is more likely to benefit from anti-TGF-β signaling effects conferred by angiotensin receptor blockers and may be responsive to mitochondrial antioxidant strategies in the early stage of disease. Molecular and functional profiling may therefore serve as aids to guide precision therapy for HCM.

Summary of multi-scale investigation in 2 mouse models at the pre-hypertrophic stage

- **Normal diastolic function**
  - No up-regulation of pro-fibrotic miRNAs or TGFβ signaling
  - Reduced Redox Environment
    - Up-regulation of TGFβ signaling
    - Dysregulation of TGFβ-miR29-collagen axis
  - Cardiac myocytes
  - Oxidized Redox Environment
    - Complex I RCR
    - Mitochondrial number
    - Complex I RCR Matrix [Ca2+]free
    - [Ca2+] threshold for PTP opening
  - Losartan
  - Therapeutic candidates predicted by IPA

- **Diastolic dysfunction**
  - Echocardiography
  - Cardiac mitochondria
  - Reduced GSH

- **R403Q-α-MHC**
  - R92W-TnT

**NADPH** Reduced GSH

**NADP**H Reduced GSH

**Reduced GSH**

**NADPH** Reduced GSH

**NADP**H Reduced GSH
**Effymia Maria Zacharia**

The combination of high levels of non-apoptotic and low levels of apoptotic circulating microparticles is correlated with an improved prognosis in patients with a recent ACS

Effimia Zacharia¹, Zoi Pallantza¹, Antigoni Miliou¹, Anastasios Kriebardis², Nikolaos Orologas³, Efthychie Valasiadi³, Dimitris Tousoulis⁴ - (1) Hippokration General Hospital, 1st Cardiology Department, Athens, Greece (2) Technological Educational Institution (T.E.I) of Athens, Athens, Greece (3) BD Biosciences BD Hellas, Athens, Greece

**Background:** Increased levels of apoptotic microparticles (MPs) have been correlated with increased incidence of adverse cardiovascular events in patients with acute coronary syndromes (ACS). The role of non-apoptotic MPs has not been determined yet.

**Purpose:** To determine the prognostic value of non-apoptotic MPs in patients with a recent ACS.

**Methods:** 40 patients with ST-elevation myocardial infarction/STEMI, 27 with non-STEMI/NSTEMI, 27 with unstable angina/UA, 15 with stable coronary artery disease/SCAD and 16 healthy subjects were enrolled. The levels of circulating endothelial CD144+/EMPs, red blood cell- CD253+/RMPs and platelet-derived CD41+/PMPs and their annexinV binding capacity (apoptotic/PS+MPs, non-apoptotic/PS-MPs) were determined by flow cytometry from peripheral blood samples. Patients with apoptotic MPs<median and non-apoptotic MPs>median of study group were categorized as having “excess non-apoptotic MPs”; the rest were categorized as having “excess apoptotic MPs”. The extent of myocardial damage was estimated from peak hs-troponin-I. Follow-up duration was 18 months.

**Results:** In STEMI and UA, peak hs-troponin-I levels were negatively correlated with non-apoptotic MPs (p<0.005 for all). PS+EMPs and PS+RMPs were positively correlated with the GENSINI scores across the study cohort (p=0.005). Patients with “excess apoptotic EMPs” had a higher incidence of re-hospitalization or death (42.9% vs 20%) and a shorter mean event-free survival (13.2 vs 16.2 months) compared to those with “excess non-apoptotic” EMPs (p=0.019).

**Conclusions:** Non-apoptotic MPs are negatively correlated with the extent of myocardial damage, while apoptotic MPs are positively correlated with GENSINI score. The combination of low apoptotic MPs and high non-apoptotic MPs was associated with an improved prognosis.
**Hsp20 phosphorylation modulates protein associations to regulate cytoskeletal dynamics and cardiac function**

Elizabeth Vafiadaki¹, Demetrios A. Arvanitis¹, Evangelia G. Kranias¹,², Despina Sanoudou¹,³
¹Molecular Biology Division, Biomedical Research Foundation of the Academy of Athens, Greece; ²Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, USA ³Clinical Genomics and Pharmacogenomics Unit, 4th Department of Internal Medicine, Medical School, National and Kapodistrian University of Athens, Greece.

The small heat shock protein 20 (Hsp20) has emerged as a key modulator of cardiac contraction and cardioprotection. These effects are directly linked to its phosphorylation on serine 16 (Ser16), which is regulated by α-adrenergic stimulation. In the present study, we determined the implications of Hsp20 phosphorylation on its interactions and downstream functions. Specifically, we demonstrate that Hsp20 binds to 14-3-3 in cardiac muscle, a cytoskeletal protein known to associate with coflin-2 (CFL2). The 14-3-3/Hsp20 association is tightly controlled by phosphorylation, since upon phosphorylation of Ser16, Hsp20 translocates from the cytosol to the cytoskeleton where it interacts with 14-3-3. This leads to dissociation of the 14-3-3/CFL2 complex, triggering CFL2 activity and enhanced F-actin depolymerization. Importantly, investigations of the first reported Hsp20 mutation in dilated cardiomyopathy patients, namely Hsp20-P20L, demonstrates the significance of Hsp20/14-3-3 association in regulation of F-actin dynamics. In particular, we show that Hsp20-P20L exhibits reduced binding to 14-3-3 due to diminished phosphorylation, with subsequent failure to translocate to the cytoskeleton and inability to dissociate the 14-3-3/CFL2 complex. This ultimately results in impaired regulation of F-actin dynamics, an effect implicated in loss of cytoskeletal stability and abrogation of the mutant’s anti-apoptotic function. Our findings reveal the contribution of Hsp20 in regulation of actin cytoskeleton dynamics, with significant implications in muscle physiology and pathophysiology.
PPAR agonists target hyperlipidemia (PPARα) and hyperglycemia (PPARγ). However, dual PPARα/γ agonists, such as Tesaglitazar (TESA), caused cardiac dysfunction in type 2 diabetes patients. Mice fed with diets containing TESA developed cardiac dysfunction associated with lower expression and increased acetylation (inhibition) of PGC-1α, along with lower mitochondria abundance and respiration in hearts and primary cardiomyocytes. The inhibitory effect on cardiac PGC1α expression was reproduced in vivo with combined administration of single PPARα and PPARγ agonists. PPARα and PPARγ competed for a PPAR element of the hPgc1α promoter. In vivo administration of dual PPARα/γ agonist decreased deacetylase SIRT1 levels and increased PGC1α acetylation in primary cardiomyocytes. Combined treatment with TESA and Resveratrol (SIRT1 activator) reduced PGC1α acetylation, corrected mitochondrial respiration in primary cardiomyocytes and prevented cardiac dysfunction in normal and diabetic mice. SIRT1 and PGC1α activation blunts cardiotoxicity of dual PPARα/γ agonists and improves their therapeutic potential. SIRT1-mediated activation of PGC1α blunts the cardiotoxic effect of dual PPARα/γ agonists and improves their therapeutic potential.
High-density lipoprotein (HDL) has anti-atherogenic functions, including antioxidant effects. Here, we evaluated the association between HDL antioxidant activity and acute ischemic stroke severity and outcome. We prospectively studied 199 consecutive patients admitted with acute ischemic stroke (42.7% males, age 78.6±6.5 years). The severity of stroke was assessed at admission with the National Institutes of Health Stroke Scale (NIHSS). The outcome was assessed with dependency rates at discharge (modified Rankin scale) and in-hospital mortality. The antioxidant capacity of HDL was evaluated with the dichlorofluorescein (DCF) assay, where an increase in fluorescence signal indicates reduced antioxidant potential. The DCF fluorescence signal correlated negatively with HDL-cholesterol levels ($r=-0.359$, $p<0.001$) and positively with NIHSS ($r=0.345$, $p<0.001$), while patients with severe stroke had higher DCF signal than patients with mild stroke ($p=0.004$). Independent risk factors for severe stroke were female gender (RR 2.74, 95% CI 1.38-5.43, $p=0.004$) and the DCF signal (RR 1.04, 95% CI 1.01-1.07, $p=0.013$). Patients who were dependent at discharge had higher DCF signal than patients who were independent ($p<0.001$). Independent predictors of dependency at discharge were age (RR 1.09, 95% CI 1.01-1.19, $p=0.031$) and NIHSS (RR 1.48, 95% CI 1.27-1.74, $p<0.001$). The DCF signal did not differ between patients who died during hospitalization and those who were discharged. The only independent predictor of in-hospital mortality was NIHSS (RR 1.15, 95% confidence interval 1.06-1.26, $p<0.001$). Overall, our analyses show that the impaired antioxidant activity of HDL is associated with more severe acute ischemic stroke and might predict a worse functional outcome in these patients.
Extraction of pacemaker leads with "Trouser-like" technique

C. Kontogiannis1, M. Kosmopoulos2, D. Tsilimigras2, E. Spartalis3, G. Georgiopoulos4, D. Vlastos2, M. Spartalis4, I. Paraskevaidis3, S. Chatzidou2 - (1) Alexandra University Hospital, Therapeutics, Athens, Greece (2) Athens Medical School, Athens, Greece (3) Athens Medical School, Laboratory of Experimental Surgery and Surgical Research, Athens, Greece (4) Onasis Cardiac Surgery Center, Division of Cardiology, Athens, Greece

Cardiac management devices consist an integral part of our armament for treatment of heart diseases. However, they are prone to a variety of morbidities which may mandate extraction of either the device or a lead. Especially when implantation timeframe exceeds 10 years, extraction can be particularly challenging due to the formation of adhesions between the lead and surrounding tissue. Laser-assisted lead extraction has become the preferred approach due to the low success rates of simple lead traction. However, its availability in Europe is limited and associated with high economic burden. With this in mind, we developed in our University Hospital a novel "trouser-like technique" for lead extraction without utilization of laser sheaths. Our technique is based on the lysis of adhesions through the transvenous application of two dilator sheaths over the leads. This technique carries the advantage of both isolating the lead from vascular lumen and concurrently maintaining lead visibility. It has been applied over the last 5 years in more than 40 cases where implantation occurred in a time-lapse exceeding 10 years. Success rates were non-inferior compared to reported outcomes of laser sheaths. Thus, "trouser-like technique" could pose a safe and feasible alternative to laser-assisted lead extraction.
Insights to the common molecular regulators of cardiovascular disease by plasma proteomics and bioinformatics analysis

Vasiliki Lygirou¹, Agnieszka Latosinska², Manousos Makridakis³, William Mullen³, Christina Vasilopoulou¹, Christian Delles³, Joost Schanstra⁴,⁵, Jerome Zoidakis², Burkert Pieske⁶,⁷, Harald Mischak²,³, Antonia Vlahou¹

1) Biotechnology Division, Biomedical Research Foundation, Academy of Athens, Athens, Greece
2) Mosaiques Diagnostics GmbH, Hannover, Germany
3) Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK
4) Institut National de la Santé et de la Recherche Médicale (INSERM), Institute of Cardiovascular and Metabolic Disease, Toulouse, France
5) Université Toulouse III Paul-Sabatier, Toulouse, France
6) Department of Internal Medicine / Cardiology, Deutsches Herzzentrum Berlin, Berlin, Germany
7) DZHK (German Centre for Cardiovascular Research) Partner Site Berlin, Berlin, Germany

Cardiovascular disease (CVD) is the leading cause of mortality worldwide. Despite the great number of studies on CVD and its etiology, its key modulators remain largely unknown. To this end, we performed a comprehensive proteomic analysis of blood plasma, to identify disease-associated changes, and generate a well characterized dataset for further use in CVD multi-omics integrative analysis. LC-MS/MS was employed to analyze plasma from 32 subjects (19 cases of various CVD phenotypes, 13 controls) in two steps: discovery (13 cases, 8 controls) and test (6 cases, 5 controls) set analysis. Pathway annotation confirmed the functional relevance of the findings (representation of complement cascade, platelet degranulation, etc.). Correlation of the relative abundance of the proteins identified in the discovery set with their reported concentrations in the Plasma Proteome Database was significant, confirming the validity of the quantification method. The discovery set analysis revealed 100 differentially expressed proteins between cases and controls, 39 of which were verified (≥ twofold change) in the test set. These included proteins already studied in the context of CVD (e.g. apolipoprotein B, alpha-2-macroglobulin), as well as novel findings (e.g. as low density lipoprotein receptor related protein 2 [LRP2], protein SZT2) for which a potential mechanism of action is suggested. This proteomic study provides a comprehensive dataset to be used for integrative and functional studies. The observed protein changes reflect known CVD-related processes (e.g. lipid uptake, inflammation) but also novel hypotheses for further investigation including a potential pleiotropic role of LPR2 but also links of SZT2 to CVD.
A systematic approach identifies lysine demethylase KDM5D as a potential therapeutic candidate for atherosclerosis

Marika Mokou, Julie Klein, Vasiliki Bitsika, Manousoos Makridakis, Jean-Sebastien Saulnier-Blache, Jerome Zoidakis, Burkert Pieske, Maria G. Roubelakis, Joost P. Schanstra, Antonia Vlahou

1. Biotechnology Laboratory, Centre of Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
2. Laboratory of Biology, University of Athens, School of Medicine, Athens, Greece
3. U1048, Institute of Cardiovascular and Metabolic Diseases, Institut National de la Santé et de la Recherche Médicale (INSERM), Toulouse, France
4. Université Toulouse III Paul-Sabatier Toulouse, Toulouse, France
5. Department of Internal Medicine and Cardiology, Charité University Medicine, Berlin, Germany
6. German Center for Cardiovascular Research (DZHK), Partner Site Berlin, Germany
7. Department of Internal Medicine and Cardiology, German Heart Center, Berlin, Germany

Atherosclerosis is a chronic, progressive disease with asymptomatic damage accumulating for many years before the clinical diagnosis. In this study, aiming to gain insight into the early disease mechanisms, a systematic approach based on mouse models, human tissues and in vitro studies, was used. In the first step, proteome analysis using thoracic aortas from animal models with diabetic (using streptozotocin) atherosclerosis and WT animals was performed. Two different diabetic atherosclerotic models were used to increase reliability of findings; Ldlr-/STZ (n=5) and ApoE-/STZ (n=3) mice with each group being compared to WT mice (n=5). This led to the identification of 262 differentially expressed proteins. Among the top 20 most significantly upregulated proteins in disease, were proteins associated with the respiratory chain (i.e. Ndufa10); cell migration (i.e. Stk25); adhesion (i.e. Kif26b); cytoskeleton dynamics (i.e. Cfl1) as well as proteins associated with histone modifications (i.e. Kdm5d). An upregulation of KDM5D (fold change 2.07, p<0.01) was also found in central and peripheral vessels from patients with atherosclerosis compared to controls. In agreement to this, a reduction of the trimethylated form of H3K4 which is the substrate of KDM5D was detected in the same samples using Western blot analyses. Inhibition of KDM5D in vitro led to a 38% reduction of the proliferation rate (p<0.05), a 52% decreased migratory capacity (p<0.05) and a 17% reduction of the angiogenic properties (p<0.05) of HUVEC cells compared to non-treated cells. Collectively, these data support that KDM5D may have some therapeutic impact on atherosclerosis meriting further investigation.
Dietary preferences and nutritional information needs of USA volunteer firefighters
Elena Deligianni², Maria Korre¹, Konstantina Sampani⁴, Stefanos N. Kales¹
¹Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, United States ²Department of Epidemiology and Medical Statistics, Medical School, University of Athens, Greece

Introduction
Evidence demonstrates a growing obesity problem in the US fire service which is associated with CVD risk, the leading cause of on-duty death. Poor diet contributes to this burden. Therefore, this study investigated US volunteer firefighters' dietary preferences and assessed their nutritional knowledge and needs.

Methodology
An online survey (SurveyMonkey) was conducted in collaboration with the National Volunteer Fire Council (NVFC). Invitations for the survey were emailed to NVFC members and data were collected in 2015. Analyses were restricted to participants reporting valid heights/weights.

Results
Among the 554 US volunteer firefighter respondents, the majority were obese with mean BMI 31 kg/m² (± 6.6). Most (73%) stated they do not follow a specific diet and 56% felt that they do not receive sufficient nutrition information from the fire service. Obese respondents reported more limited nutritional knowledge (p=.002) and greater dissatisfaction with their dietary habits (p<.001) compared to lean participants. Over 75% of the respondents showed willingness to learn more about healthy eating and 75% expressed interest for a free online nutrition learning platform. When presented with descriptive text regarding various popular diets, Mediterranean diet was rated most favourable as compared Paleo, Atkins, Therapeutic Lifestyle Changes, and the Esselsteyn Engine 2 diets.

Conclusion
Improved nutritional education could support positive lifestyle changes and weight control among volunteer firefighters. Among popular dietary choices Mediterranean diet was rated the most appealing to these US volunteer firefighters and could be used in platforms for nutritional education interventions.