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Metabolic interventions in heart failure

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Chapter 7
Discussion

Discussion

Survival for heart failure (HF) is still worse than most malignancies, despite the development of new therapeutic strategies for HF.[1] To improve the outcome of HF therapy, better insight in its pathophysiology is needed. In **part 1** of this thesis we investigated whether β-blocker therapy should be continued after successful revascularization in patients with CAD. Coronary artery disease (CAD) is still a major cause for HF, although treatment has improved with the introduction of revascularization strategies. The question remains whether traditional pharmacotherapy remains appropriate in this changing landscape. We also investigated whether revascularization for CAD reduces the propensity of diabetic patients to develop HF. **In part 2** of the thesis we studied the role of A kinase interacting protein 1 (AKIP1) in the cardiac response to stress by generating a mouse model with autonomous cardiomyocyte specific overexpression of AKIP1. First, we investigated whether AKIP1 could modulate the acute and chronic cardiac stress response, and then we also studied the role of AKIP1 in the heart in relation to physical exercise.

Metabolic interventions after revascularization

Revascularization strategies have altered the course of CAD in many patients dramatically. Previously, HF with reduced ejection fraction was common after myocardial infarction (MI). However, as treatment efficacy is becoming more and more efficient, the incidence of post-MI HF is declining. For instance, a recent trial in ST-elevation MI patients that had received a primary percutaneous coronary intervention reported that the average left ventricular ejection fraction (LVEF) after MI was close to normal (54%).[2] In this changing landscape, pharmacological therapy has been remarkably consistent. β-blocker therapy has been the cornerstone of pharmacotherapy of CAD for decades, but recent evidence suggests that this central role may not be justified in patients that are at relatively low risk, have good control of their cardiovascular risk factors and are receiving evidence-based therapy.[3-5] In patients receiving coronary artery bypass graft surgery (CABG), pre-operative β-blocker therapy has been reported to be as high as 80-93% over the last few years.[3] β-blockers are effective in reducing symptoms, but their effects on prognosis in patients with a preserved LVEF is unclear. **In chapter 2**, we performed an explorative analysis to determine if β-blocker therapy was associated with a reduction in the incidence of cardiovascular events after CABG. We showed that β-blocker therapy in fact was not related to a decreased risk of recurrent angina or cardiovascular events after CABG, in low risk patients with CAD and preserved cardiac function. Our results were consistent across different types of analyses and subgroups, including propensity matched and time-dependent analyses, suggesting that the lack of association between β-blocker therapy and clinical outcome is robust. We must stress that this study focused on low

risk patients with a low event-rate, thus the results may be different in the general CABG population which will include many patients with a previous MI and overt cardiac dysfunction. Nevertheless, our data fuel the hypothesis that these agents should not be continued indiscriminately in low risk patients. Our results are in line with the recently published Study assessInG the morbidity-mortality beNefits of the If inhibitor ivabradine in patients with coronarY artery disease (SIGNIFY) which randomized 19,102 patients with stable CAD and normal cardiac function to the selective sinus node inhibitor ivabradine. In SIGNIFY, Ivabradine also did not influence clinical outcome compared to placebo.[6] Of note, heart rate reduction is considered to be the most important mode of action of β-blockers in CAD. The neutral results of SIGNIFY therefore provide an additional line of evidence supporting that concept that modulation of the sympathetic tone is not generally effective after revascularization in low-risk patients. Although β-blockers are still important drugs for the treatment of symptomatic angina, recent MI and patients with left ventricular (LV) dysfunction,[7-9] their efficacy in other indications is under scrutiny.

HF and CAD are both common in patients with diabetes. Diabetes predisposes to severe CAD and an increased incidence of subsequent MIs, which may cause HF. Nevertheless, diabetes may also cause HF through direct toxic and / or metabolic effects on the heart that may cause a distinct diabetic cardiomyopathy (Figure 1). In **chapter 3**, we show that revascularization with CABG did not reduce the propensity of diabetic patients to develop HF. Interestingly, acute HF in these patients was not preceded by evidence of acute or clinically worsening chronic myocardial ischemia, suggesting that mechanisms beyond epicardial CAD were responsible. We should therefore be watchful of HF development in diabetic patients even if they are completely revascularized for CAD. Diabetes is believed to increase oxidative stress and activation of detrimental signal transduction pathways by glycosylation, AGEs and changes in mitochondrial metabolism.[10-13] These specific processes underlying diabetic cardiomyopathy are not influenced by revascularization and will therefore continue to exert their detrimental effects on the heart. Accordingly, these pathways could cause HF to develop after adequate revascularization (Figure 1).

In conclusion, we observed no hints that continuation of a β-blocker therapy in a-symptomatic CAD patients with preserved cardiac function is of benefit. Diabetic patients with CAD should be monitored (more) closely because they appear to develop HF, also in the absence of CAD recurrence.
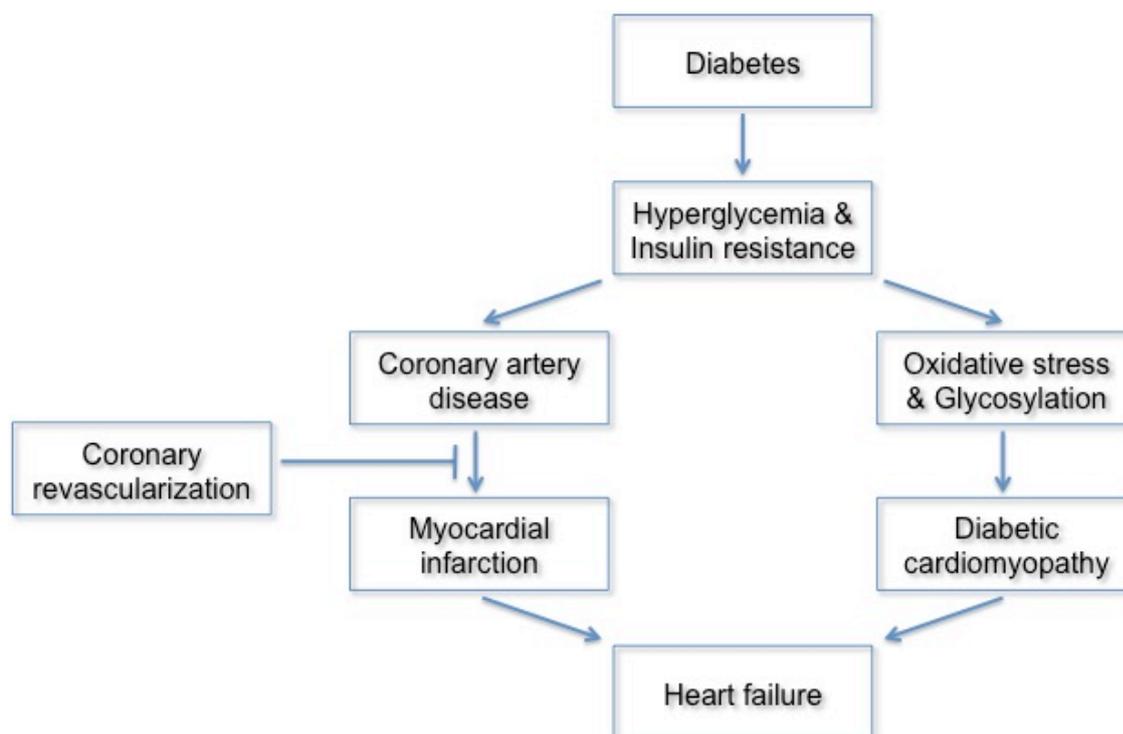
AKIP1 in cardiac stress

Myocardial hypertrophy decompensation is a main characteristic of HF development.(chapter1) Hypertrophy is a compensatory response to increased wall stress, but ultimately fails. It has long been a goal to dissect the adaptive features of hypertrophy from it's deleterious effects. We previously performed a genome wide transcription study and showed that AKIP1 was consistently upregulated in several *in vivo* and *in vitro* models of HF and pathological hypertrophy.[15] AKIP1 has previously been described in several malignant cell lines and the role of AKIP1 may vary in different cell types.[16-21] Previous findings in cultured cardiomyocytes and other cell types suggested that AKIP1 could be an important protein with protective properties under various forms of cardiac stress.[22-24] It might induce cardiomyocyte hypertrophy *in vitro* by activating AKT and protect from ischemic injury in several *in vitro* and *ex vivo* models. Furthermore, we showed that AKIP1 improves mitochondrial efficiency, by increasing mitochondrial energy production while decreasing mitochondrial reactive oxygen species (ROS) production.[22]

In **chapter 4** we describe how mitochondrial efficiency becomes compromised during advanced HF, leading to an energy deficit of the heart. Also, increased mitochondrial ROS production during HF damages structures in the mitochondrial membrane, leading to further loss of mitochondrial energy production and even more oxidative stress. To investigate whether the effects of AKIP1 on hypertrophy and efficient energy metabolism translate to

108

beneficial effects of AKIP1 *in vivo*, we generated a transgenic mouse line with cardiomyocyte-autonomous overexpression of AKIP1 (AKIP1-TG).

In **chapter 5** we showed that, in contrast to our *in vitro* studies, a stable 40-fold increase in cardiac AKIP1-protein expression did not cause a spontaneous cardiac phenotype *in vivo*. The only detectable difference between AKIP1-TG mice and their wild type (WT) littermates was a minor reduction in cardiomyocyte diameter after 4 months. To investigate whether overexpression of AKIP1 could be beneficial in HF, we subjected AKIP1-TG mice to two well-established HF models; transverse aortic constriction (TAC) through banding of the aorta between the carotid arteries and permanent myocardial infarction through ligation of the left coronary artery. In contrast to our expectations, AKIP1 overexpression did not affect left ventricular hypertrophy, nor did it influence left ventricular dysfunction after TAC. After permanent coronary artery ligation, AKIP1-TG mice displayed a 1/3rd reduction in myocardial infarct size and modest reduction in cardiomyocyte size. However, this did not translate into improvements in cardiac function. To investigate whether AKIP1 could reduce infarct size after ischemia/reperfusion (I/R), we subjected our mice to temporary coronary artery ligation. We found that infarct size after 45 minutes of ischemia followed by 24 h of reperfusion was significantly reduced in AKIP1-TG mice and resulted in marked reduction in myocardial infarct formation. Most studies have shown that the primary role for AKIP1 in cells is the modulation of PKA and NF-κB activity. As these proteins are key mediators of myocardial reperfusion injury, we first hypothesized that the protective effect of AKIP1 could be explained by modulation of these proteins. In contrast to studies in other cell types, AKIP1 did not bind to NF-κB or PKA and also did not influence the activity of these signaling molecules. Furthermore, in contrast to our findings in cultured cardiomyocytes,[23] AKT phosphorylation was not influenced by AKIP1 overexpression after I/R injury. AKIP1, however, did localize to mitochondria, where it was found to associate with ATP-synthase. ATP-synthase has been identified as a key component of the mitochondrial permeability transition (MPT) pore, which is a key effector of necroptosis.[25] To test whether the salutary effects of AKIP1 could be explained by reductions in MPT pore formation, we performed calcium induced mitochondrial swelling analysis. Interestingly, mitochondrial isolated from AKIP1-TG mice displayed markedly reduced calcium-induced swelling, indicative of reduced MPT pore formation. These last findings indicate that AKIP1 attenuates myocardial I/R injury by reducing MPT pore formation, which could be explained by its interaction with ATP-synthase.

While previous *in vitro* evidence suggested that AKIP1 could induce a physiological type of cardiac hypertrophy,[23] we investigated this hypothesis in more detail in **chapter 6,** where we describe the results of a voluntary wheel running exercise. AKIP1-TG and WT mice were subjected to voluntary wheel running or regular housing without running wheel. AKIP1-TG and WT mice ran

the same distance over the course of 4 weeks. We showed that AKIP1 overexpression caused an increase in heart weight during wheel running, while cardiac function was preserved. This was associated with increased AKT-activity in AKIP1-TG mice after running (Figure 2). Together, this shows that AKIP1 is not essential for development of pathological hypertrophy, but we observed it may promote physiological hypertrophy and reduce infarct size formation after an acute ischemic injury.
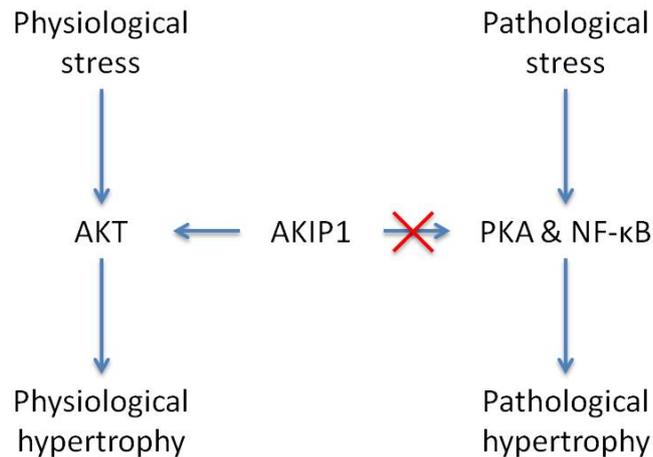
Figure 2 AKIP1 does promote physiological hypertrophy, but does not alter development of pathological hypertrophy.

In summary, we conclude that AKIP1 does not change myocardial hypertrophy decompensation or HF development, but elevated AKIP1 levels do enhance myocardial hypertrophy after exercise. Furthermore, cardiac-specific AKIP1 overexpression reduced infarct size, presumably via its interaction with ATP-synthase and inhibition of MPT pore formation.

Future perspectives.
As treatment for CAD continues to improve, the classical type of ischemic HF with reduced ejection fraction will become less prevalent. In patients with acute coronary syndromes, rapid revascularization therapy is extremely effective as it minimizes injury, reduces scar formation and preserves cardiac function. The suitability of the temporary pharmacological regimen after MI should therefore be reconsidered in patients with small infarcts. More importantly, data from studies such as **chapter 2** will hopefully change our approach to the treatment of stable CAD. Currently β-blockers are continued for decades in patients with stable CAD, without any evidence for their efficacy in the absence of angina. Furthermore, common comorbidities in patients with CAD such as hypertension and diabetes also predispose to HF through mechanisms beyond CAD and can therefore cause non-ischemic HF in patients with CAD. **Chapter 3** underscores this concept because new HF in diabetic patients was increased in the absence of CAD recurrence. Therefore we should

be mindful that patients with diabetes are at increased risk of developing non-ischemic HF. We should focus future research towards the development of therapeutic targets in non-ischemic HF.

Overexpression of AKIP1 did protect from reperfusion injury. Simulating or stimulating the effect of AKIP1 might therefore be a putative target for pharmacotherapeutics during acute MI. Our research suggests that AKIP1 reduces infarct size through inhibition of MPT pore opening. The MPT pore forms during acute stress and although its structure is not clear, it is composed of at least ATP-synthase, mitochondrial translocator TSPO and cyclophilin-D.[26] Of note, the TSPO inhibitor TRO40303 did not cause reductions in infarct size in the clinical setting.[27] We might need to target another part of the MPT pore. Therefore, we need to look further into the interaction of AKIP1 with the MPT pore to elucidate what part of AKIP1 and the MPT pore do interact. Subsequently, we could potentially design a pharmacotherapeutic or small peptide that resembles the functional domain of AKIP1 and test this in experimental setup. An important question is whether a pharmacotherapeutic would only be beneficial when given before ischemia, or that it would also be beneficial when given at reperfusion. The latter would be more promising as a future therapy. AKIP1 appears to have a specific role in mitochondria. In addition, many signaling molecules have different functions in different cellular compartments, necessitating specific mitochondrial targeting. There are several ways to improve the delivery of a pharmacotherapeutic to the right cellular compartment. For instance, we could add a molecular group including a cation to the pharmacon that targets directly to mitochondria.[28]

AKIP1 increased cardiac hypertrophy after exercise i.e. physiological hypertrophy, but pathological hypertrophy was not affected. Since both types of hypertrophy are a response to increased cardiac workload, it is an interesting question whether we could superimpose physiological hypertrophy to pathologically remodelled hearts. Physiological hypertrophy is the result of an intermittent pattern of increased cardiac workload and cardiac structure and organization remains normal.[29] As physiological hypertrophy is accompanied with improvements in cardiac performance in healthy individuals, this may suggest that physiological hypertrophy is also helpful in the failing heart. While studies show that exercise training in HF patients can improve exercise capacity,[30,31] the benefits on survival rate are limited. The HF-ACTION trial, which randomized 2331 HF patients to exercise or control, only showed a trend towards decreased mortality or hospitalizations after a thorough exercise training program during a median follow up of 2.5 years.[30] Although cardiac hypertrophy, either physiological or pathological, was not assessed in this trial, the results are supportive for the suggestion that stimulation of physiological hypertrophy of the heart has a slight benefit in patients with HF. Nevertheless, it is unlikely that AKIP1 would offer us possibilities to physiologically remodel

pathological hypertrophy because AKIP1 overexpression did not affect pathological remodeling in our model.

Several screens have been reported which compare the molecular characteristics of physiological and pathophysiological hypertrophy. It would be useful if we could identify and modulate a key factor that promotes physiological hypertrophy. The AKT-signalling pathway is implicated as such a key factor.[32] The challenge in translating the concept of physiological hypertrophy to the clinical setting might be that the line between physiological and pathological hypertrophy is blurry. Whereas physiological hypertrophy is regarded to regress after deconditioning from training, some athletes retain characteristics like enlarged left ventricular cavity dimensions.[33,34] It might therefore be difficult to hit the sweet spot for pharmacological stimulation of physiological hypertrophy in HF.

Several screens were performed that improved our understanding of physiological and pathological gene expression.[35-37] Some of these screens used up to 5 models of hypertrophy. While some used only genetic models, others used also more clinical models of HF. To improve the previously mentioned screen performed by our laboratory,[15] it would be useful to shift focus from the currently combined *in vitro/in* vivo models to *in vivo* models, include more in vivo models, add a comparison from pathologic and physiologic models and include a comparison of human samples.

References

1. Stewart S, MacIntyre K, Hole DJ, Capewell S, McMurray JJ. More 'malignant' than cancer? Five-year survival following a first admission for HF. Eur J Heart Fail 2001;3:315-322.

2. Lexis CP, van der Horst IC, Lipsic E, et al. Effect of metformin on left ventricular function after acute myocardial infarction in patients without diabetes: the GIPS-III randomized clinical trial. JAMA 2014;311:1526-1535.

3. Brinkman W, Herbert MA, O'Brien S, et al. Preoperative beta-Blocker Use in Coronary Artery Bypass Grafting Surgery: National Database Analysis. JAMA Intern Med 2014;174:1320-1327.

4. Bangalore S, Steg G, Deedwania P, et al. beta-Blocker use and clinical outcomes in stable outpatients with and without coronary artery disease. JAMA 2012;308:1340-1349.

5. Bouri S, Shun-Shin MJ, Cole GD, et al. Meta-analysis of secure randomised controlled trials of beta-blockade to prevent perioperative death in non-cardiac surgery. Heart 2014;100:456-464.

6. Fox K, Ford I, Steg PG, Tardif JC, Tendera M, Ferrari R. Ivabradine in Stable Coronary Artery Disease without Clinical HF. N Engl J Med 2014;371:1091-1099.

7. Kawanishi DT, Reid CL, Morrison EC, et al. Response of angina and ischemia to long-term treatment in patients with chronic stable angina: a double-blind randomized individualized dosing trial of nifedipine, propranolol and their combination. J Am Coll Cardiol 1992;19:409-417.

8. Andersson C, Merie C, Jorgensen M, et al. Association of beta-Blocker Therapy With Risks of Adverse Cardiovascular Events and Deaths in Patients With Ischemic Heart Disease Undergoing Noncardiac Surgery: A Danish Nationwide Cohort Study. JAMA Intern Med 2014;174:336-344.

9. Ozasa N, Kimura T, Morimoto T, et al. Lack of effect of oral beta-blocker therapy at discharge on long-term clinical outcomes of ST-segment elevation acute myocardial infarction after primary percutaneous coronary intervention. Am J Cardiol 2010;106:1225-1233.

10. Authors/Task Force Members, Ryden L, Grant PJ, Anker SD, Berne C, Cosentino F, et al. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). Eur Heart J 2013;34:3035-3087.

11. Clark RJ, McDonough PM, Swanson E, Trost SU, Suzuki M, Fukuda M, Dillmann WH. Diabetes and the accompanying hyperglycemia impairs cardiomyocyte calcium cycling through increased nuclear O-GlcNAcylation. J Biol Chem 2003;278:44230-44237.

12. Hartog JW, Voors AA, Bakker SJ, Smit AJ, van Veldhuisen DJ. Advanced glycation end-products (AGEs) and HF: pathophysiology and clinical implications. Eur J Heart Fail 2007;9:1146-1155.

13. Bugger H, Abel ED. Mitochondria in the diabetic heart. Cardiovasc Res 2010;88:229-240.

14. Dei Cas A, Fonarow GC, Gheorghiade M, Butler J. Concomitant diabetes mellitus and HF. Curr Probl Cardiol 2015;40:7-43.

15. Lu B, Yu H, Zwartbol M, Ruifrok WP, van Gilst WH, de Boer RA, Sillje HH. Identification of hypertrophy- and HF-associated genes by combining in vitro and in vivo models. Physiol Genomics 2012;44:443-454.

16. Gao F, Cheng J, Shi T, Yeh ET. Neddylation of a breast cancer-associated protein recruits a class III histone deacetylase that represses NFkappaB-dependent transcription. Nat Cell Biol 2006;8:1171-1177.

17. Kitching R, Li H, Wong MJ, Kanaganayakam S, Kahn H, Seth A. Characterization of a novel human breast cancer associated gene (BCA3) encoding an alternatively spliced proline-rich protein. Biochim Biophys Acta 2003;1625:116-121.

18. Leung TH, Ngan HY. Interaction of TAp73 and breast cancer-associated gene 3 enhances the sensitivity of cervical cancer cells in response to irradiation-induced apoptosis. Cancer Res 2010;70:6486-6496.

19. Zimmerman R, Peng DJ, Lanz H, Zhang YH, Danen-Van Oorschot A, Qu S, Backendorf C, Noteborn M. PP2A inactivation is a crucial step in triggering apoptin-induced tumor-selective cell killing. Cell Death Dis 2012;3:e291.

20. Lin C, Song L, Gong H, Liu A, Lin X, Wu J, Li M, Li J. Nkx2-8 downregulation promotes angiogenesis and activates NF-kappaB in esophageal cancer. Cancer Res 2013;73:3638-3648.

21. Lin C, Song L, Liu A, Gong H, Lin X, Wu J, Li M, Li J. Overexpression of AKIP1 promotes angiogenesis and lymphangiogenesis in human esophageal squamous cell carcinoma. Oncogene 2015;34:384-393.

22. Yu H, Tigchelaar W, Koonen DP, Patel HH, de Boer RA, van Gilst WH, Westenbrink BD, Sillje HH. AKIP1 expression modulates mitochondrial function in rat neonatal cardiomyocytes. PLoS One 2013;8:e80815.

23. Yu H, Tigchelaar W, Lu B, van Gilst WH, de Boer RA, Westenbrink BD, Sillje HH. AKIP1, a cardiac hypertrophy induced protein that stimulates cardiomyocyte growth via the Akt pathway. Int J Mol Sci 2013;14:21378-21393.

24. Sastri M, Haushalter KJ, Panneerselvam M, Chang P, Fridolfsson H, Finley JC, Ng D, Schilling JM, Miyanohara A, Day ME, Hakozaki H Petrosyan S, Koller A, King CC, Darshi M, Blumenthal DK, Ali SS, Roth DM, Patel HH, Taylor SS. A kinase interacting protein (AKIP1) is a key regulator of cardiac stress. Proc Natl Acad Sci USA 2013;110:E387-396.

25. Zhang T, Zhang Y, Cui M, Jin L, Wang Y, Lv F, Liu Y, Zheng W, Shang H, Zhang J, Zhang M, Wu H, Guo J, Zhang X, Hu X, Cao CM, Xiao RP. CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis. Nat Med 2016;22:175-182.

26. Giorgio V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M, Glick GD, Petronilli V, Zoratti M, Szabó I, Lippe G, Bernardi P. Dimers of mitochondrial ATP synthase form the permeability transition pore. Proc Natl Acad Sci USA 2013;110:5887-5892.

27. Atar D, Arheden H, Berdeaux A, Bonnet JL, Carlsson M, Clemmensen P, Cuvier V, Danchin N, Dubois-Randé JL, Engblom H, Erlinge D, Firat H, Halvorsen S, Hansen HS, Hauke W, Heiberg E, Koul S, Larsen AI, Le Corvoisier P, Nordrehaug JE, Paganelli F, Pruss RM, Rousseau H, Schaller S, Sonou G, Tuseth V, Veys J, Vicaut E, Jensen SE. Effect of intravenous TRO40303 as an adjunct to primary percutaneous coronary intervention for acute ST-elevation myocardial infarction: MITOCARE study results. Eur Heart J 2015;36:112-119.

28. Porteous CM, Logan A, Evans C, Ledgerwood EC, Menon DK, Aigbirhio F, Smith RA, Murphy MP. Rapid uptake of lipophilic triphenylphosphonium cations by mitochondria in vivo following intravenous injection: implications for mitochondria-specific therapies and probes. Biochim Biophys Acta 2010;1800:1009-1017.

29. Bernardo BC, Weeks KL, Pretorius L, McMullen JR. Molecular distinction between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies. Pharmacol Ther 2010;128:191-227.

30. O'Connor CM, Whellan DJ, Lee KL, Keteyian SJ, Cooper LS, Ellis SJ, Leifer ES, Kraus WE, Kitzman DW, Blumenthal JA, Rendall DS, Miller NH, Fleg JL, Schulman KA, McKelvie RS, Zannad F, Piña IL; HF-ACTION Investigators. Efficacy and safety of exercise training in patients with chronic HF: HF-ACTION randomized controlled trial. JAMA 2009;301:1439-1450.

31. Piepoli MF, Conraads V, Corrà U, Dickstein K, Francis DP, Jaarsma T, McMurray J, Pieske B, Piotrowicz E, Schmid JP, Anker SD, Solal AC, Filippatos GS, Hoes AW, Gielen S, Giannuzzi P, Ponikowski PP. Exercise training in HF: from theory to practice. A consensus document of the HF Association and the European Association for Cardiovascular Prevention and Rehabilitation. Eur J Heart Fail 2011;13:347-357.

32. Dorn GW 2nd. The fuzzy logic of physiological cardiac hypertrophy. Hypertension 2007;49:962-970.

33. Pelliccia A, Maron BJ, De Luca R, Di Paolo FM, Spataro A, Culasso F. Remodeling of left ventricular hypertrophy in elite athletes after long-term deconditioning. Circulation 2002;105:944-949.

34. Maron BJ, Pelliccia A. The heart of trained athletes: cardiac remodeling and the risks of sports, including sudden death. Circulation 2006;114:1633-1644.

35. Aronow BJ, Toyokawa T, Canning A, Haghighi K, Delling U, Kranias E, Molkentin JD, Dorn GW. Divergent transcriptional responses to independent genetic causes of cardiac hypertrophy. Physiol Genomics 2001;6:19 −28.

36. Mirotsou M, Dzau VJ, Pratt RE, Weinberg EO. Physiological genomics of cardiac disease: quantitative relationships between gene expression and left ventricular hypertrophy. Physiol Genomics 2006;27:86 −94.

37. Kong SW, Bodyak N, Yue P, Liu Z, Brown J, Izumo S, Kang PM. Genetic expression profiles during physiological and pathological cardiac hypertrophy and HF in rats. Physiol Genomics 2005;21:34−42.