Author's response to reviews

Title: ACE-Inhibition, but not weight reduction restores cardiomyocyte response to beta-adrenergic stimulation in the Metabolic Syndrome

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Version: 2 Date: 27 May 2013

Author's response to reviews: see over
We would like to thank the Editor and Reviewers for the appreciation of the relevance of our work, as well as the constructive comments on our manuscript. We have modified our manuscript as recommended. We have added a section concerning Competing interests, Authors’ contribution and a list of abbreviations. The answers to the specific questions of the two reviewers are listed below.

Response to reviewer I (C.Pantos)

The aim of this study was to investigate cardiomyocyte contractility and Ca2+ handling in a mouse model of metabolic syndrome and to study the effect of in vivo treatment with diet or ACE-I. The authors used a double knock out (DKO) mouse model using LDLR-/- and leptin-deficient ob/ob mice. This model resulted in diabetes type 2, obesity, dyslipidemia and hypertension. Cardiomyocytes from DKO showed impaired relaxation at baseline and reduced responsiveness to isoproterenol or increased Ca++2. Diet and ACE-I improved relaxation in DKO but only ACE-I improved responsiveness to isoproterenol. Calcium transient amplitude in DKO was significantly reduced and associated with slower kinetics, while diet reversed this response. This is a well-written manuscript. The authors have considerable experience in the methodology used. The data provided give new insights about cardiomyopathy in metabolic syndrome.

We thank the reviewer for these comments.

Discretionary Revisions:

It is not clear why the authors did not study Ca2+ handling in DKO mice with ACE-I treatment

Both treatments (hypocaloric diet and ACE-inhibition) are widely used in patients with the metabolic syndrome. In the present manuscript, we have already presented a large set of experimental data that we think are of interest for investigators studying cardiomyopathy in the metabolic syndrome. Because of the time-consuming protocols for breeding and in vivo treatment, and the very demanding experimental protocol for cell isolation especially in DKO mice, with much lower yield of cells per tested hearts, we did not have the possibility to test Ca2+ handling in DKO mice with ACE-I treatment. This might be an interesting study for the future if breeding can be expanded sufficiently.

In the present manuscript, the authors did not investigate molecular changes (changes in calcium proteins or contractile proteins) in cardiac tissue in order to give more mechanistic insight. This issue should be added in limitations of the study

Regarding molecular changes in calcium handling proteins, we have shown previously in ventricular homogenates of DKO-mice that phosphorylation of phospholamban is decreased and associated with an increased SERCA2a-expression [Van den Bergh A, Vanderper A, Vangheluwe P, et al. Dyslipidaemia in type II diabetic mice does not aggravate contractile impairment but increases ventricular stiffness. Cardiovasc Res 2008;77:371-9]. Although these data provide some insights into contributing mechanisms to contractile myocardial dysfunction, we agree that further investigations on molecular
changes in calcium handling or contractile proteins are needed, especially in the treated groups. We have added this limitation to our manuscript.

Response to reviewer II (N. Manginas)

In this interesting paper the authors used a clear model of mouse metabolic syndrome, and examined in vitro the effects of diet and ACEI on Ca handling and β-adrenergic stimulation. They concluded that weight loss, although extreme, did not affect the attenuated response to extracellular Ca nor restored the response to β-adrenergic stimulation. In the contrary the latter was improved by ACEI.

We thank the reviewer for these comments.

Minor comments:

The authors rightly acknowledged the significant weight loss as a confounding factor. Would these results change with a moderate weight change?

Obesity is associated with an increased risk of developing heart failure [1]. Poirier et al showed that extreme weight loss e.g. starvation, has detrimental effects on cardiac performance [2]. In contrast, moderate weight reduction (10-15% loss of initial weight) in obese men is associated with reduction of cardiac hypertrophy, resulting in an improvement of cardiovascular performance [3,4]. Our DKO-mice experienced 41% loss of initial body weight after hypocaloric diet in our study, a severe weight loss that might have contributed to the results. Therefore, moderate weight loss (10-15% loss of initial body weight versus 41% in the present study) in our DKO-mice might have been preferable. Further experimental studies are needed to confirm this hypothesis. This severe weight loss was mentioned as a limitation of our study in the limitations section in the discussion (second paragraph).

References:

Please give your comments on the reason the effect of ACE-I on Ca handling was not studied.

Both treatments (hypocaloric diet and ACE-inhibition) are widely used in patients with the metabolic syndrome. In the present manuscript, we have already presented a large set of experimental data that we think are of interest for investigators studying cardiomyopathy in the metabolic syndrome. Because of the time-consuming protocols for breeding and in vivo treatment, and the very demanding experimental protocol for cell isolation especially in DKO mice, with much lower yield of cells per tested hearts, we did
not have the possibility to test Ca\(^{2+}\) handling in DKO mice with ACE-I treatment. This might be an interesting study for the future if breeding can be expanded sufficiently.

List of changes

- All e-mail addresses were included on the title page
- The limitations include now the fact that we did not investigate molecular changes to give more mechanistic insight
- List of abbreviations has been added
- Section on competing interest is added
- Author’s contribution section is added