Author’s response to reviews

Title: Left Ventricular Diastolic Dysfunction in Nonhuman Primate Model of Dysmetabolism and Diabetes

Authors:

Haihua Gu (guhaihua@crownbio.com)
Yongqiang Liu (liuyongqiang@crownbio.com)
Shuang Mei (shuang.mei@crownbio.com)
Bingdi Wang (Wangbingdi@crownbio.com)
Guofeng Sun (Sunguofeng@crownbio.com)
Xiaoli Wang (Wangxiaoli@crownbio.com)
Yong-Fu Xiao (xiaoyongfu@crownbio.com)
Michael Staup Staup (mstaup@crownbio.com)
Francine Gregoire (fmgregoire@crownbio.com)
Keefe Chng (keefechng@crownbio.com)
Yixin (Jim) Wang (yxwang@crownbio.com)

Version: 1 Date: 20 Aug 2015

Author’s response to reviews:

Dear Editor,

I am submitting the revision of the manuscript entitled ”Left Ventricular Diastolic Dysfunction in Nonhuman Primate Model of Dysmetabolism and Diabetes” for publication along with a point-by-point response to the comments by the reviewers. We thank the editors and reviewers for their constructive comments and suggestions. We have revised the manuscript accordingly.

Attached are the revised manuscript with and without tracing of the changes as well as the point to point rebuttal to the reviewers’ comments. We hope that we have addressed the reviewers’ concern properly and the revised manuscript is now acceptable for publication in Cardiovascular Disorders.
Response to the Reviewer #1

The authors present a novel and interesting study on cynomolgus monkeys. The idea, statistical analysis and results are logical and well-written. However, I suggest few improvements for its publication:

Thank the reviewer for the encouraging comments and constructive suggestions. We have addressed these comments point by point below and made corresponding modifications in the revised manuscript.

1. Animals considered in the diabetes group are worse on their lipid profile apart from their hyperglycemic state and definitions for diabetes. As they are older, dyslipidemic and more obese than the control group, perhaps it would be wiser to consider the group as animals with components of metabolic syndrome, as the authors correctly state "dysmetabolism" for the studied animals several times. However, we do not find much evidence and deduction for cardiac malfunction related to such lipid profile and the discussion is centered around effects of diabetes. The authors should either revise the discussion for dyslipidemia and cardiac effects or somehow perform additional analysis for their data which controls the confounding effects of lipid profile.

The reviewer raised a very important point that aging and diabetic patients often accompanied by dyslipidemia, although this study was focusing on diabetic, the contribution of dyslipidemia to cardiac dysfunction should not be undermined. We revised the discussion by adding the.

2. Definition of diabetes or suggested metabolic syndrome in cynomolgus monkeys should be more concise and have exact reference in the methods section.

There is currently no strict definition of diabetes in NHP model. In this paper, we have to reference human criteria to use FBG 125 mg/dL for diabetes. The definition for metabolic syndromes is even more complicated, including high blood pressure. Since we did not have full spectrum of all these measurements, it may be better not to use this nomenclature. That is why we named prediabetic/dysmetabolic for the animals between diabetic and control. Again, the criteria used in this study is still arbiter, since there is no consensus in the research community as
for exactly what levels should be used in the cynomolgus monkey model. We have stated it more clearly in the revised manuscript.

3. Few grammatical mistakes are found which need corrections.

Thank the reviewer pointed out this and the authors have carefully read through the revised manuscript and corrected some grammatical mistakes.

Response to the Reviewer #3

The manuscript "Left ventricular diastolic dysfunction in Nonhuman Primate model of dysmetabolism and diabetes" investigates the association between diabetes and LV diastolic dysfunction in 89 cynomolgus monkeys. The results of this study might be of interest in the research of the field however some major concerns should be taken in consideration by the authors.

Major concerns:

1. The selection: It must be stated how the monkeys were chosen to the study.

   This is an entire colony of the in-house existing monkeys at different dysmetabolic and diabetic stages. We scanned the entire colony without selection with the attempting to look at the relationship between the CV function and metabolic status. This background information has been added to the method section in the revised manuscript.

2. It is stated in the manuscript that the typical value of FBG is about 20mg/dl however in table 1 even in the group without diabetes the levels are about 66mg/dl. Are these monkey somehow special? This needs to be stated and described in the methods.

   The age of these monkeys are more than 10 years old, above the normal, lean and healthy monkeys commonly used for PK or toxicity studies, thus, the average FBG in control group may be a little bit higher. Currently, there is no consensus in the scientific community of the exact criteria to define diabetes and “normal FBG”. Therefore, in this study, we have to use the human criteria as a reference and set the arbiter FBG <85 for control, 85-125 for pre-diabetic/dysmetabolic and >125 mg/dL for diabetic. There is no reference that can be found in setting such criteria in NHP model. We have added the above information in the revision.

3. The authors decide to give cut-offs for the diabetes in the monkey. These cut-offs are at 4 to 5 folds higher than the normal levels of FBG in a normal monkey-population. If the population you choose has levels of FBG that are very high why don’t compare the tertiles or quartiles? Is there any reference for the chosen cut-offs? Was the choice of cut-offs made
before or after the echocardiography-measurements? There must be a clear statements in this points in order to better understand the analyses.

The cut-offs for these analysis are arbiter numbers without any references since there is no consensus in the research community with a standard criteria or definition in NHP model. To simplify the analysis, we just made 1 cut-off line for all the parameters. No like clinical studies commonly including hundreds or thousands patients, this preclinical study included only 89 monkeys. Thus, to further subgroup them to tertiles or quartiles would not only make the analysis overly complicated, but also makes the number of animals in each subgroup too little for statistical power. According to reviewer’s suggestion, we have modified the statement to make it more clearly in the revision.

4. P. 10 In the first paragraph the association between low EF and age, and HbA1c is done using cut-offs not described previously in the methods. It should be done before presenting these results.

According to the suggestion, we have made more detailed description of the cut-offs and moved it from the results to the method section.

Minor concerns:

1. The use of the abbreviations in the abstract make it difficult thread and to follow the redline.

Due to the space limitation, we have to use the abbreviations to describe the data. To help the readers follow the abbreviation, there is not only a list of the abbreviations attached but also fully explained when first appears in the abstract.

2. A multivariable logistic regression analysis is mentioned in the abstract but it is not stated whether the association between DM and LV diastolic dysfunction was significant after adjustments. This is not mentioned in the results

3. The sentence of the aim at p 5 second paragraph must be more clear and probably shorter.

The abstract stated the correlation of LV function with aging and hyperglycemia, which are showed in the left (age) and middle (hyperglycemia) panels of the figure/result 3, so as the HbA1c. In order to be more specific, we changed “highly” to “significantly” correlate in the abstract in revision. Furthermore, the aims of the study have been rewritten for more clear with shorter sentences.

4. Was the diagnosis DM based in one measurement?

The diagnosis of diabetes and dysmetabolism is not just based on 1 measurement of FBG, it is based on multiple measurements over the progress of history for at least 3 months, along with other metabolic parameters such as intravenous glucose tolerance test (ivGTT), body fat
composition measured by dual-energy X-ray absorptiometry (DEX); HbA1c, insulin and lipids levels, etc. These details have now added in the revision.

5. P 10 It is stated that gender seemed not an independent predictor of LV dysfunction: While the result are not shown still a gender stratified analyses is necessary in this context as the impact of diabetes in humans is very different with regard to the sex. Since the experiment included very small female population, the power of the analysis is weak. That is why we just made a soft statement of the insignificant correlation without showing the data.

6. p10 last paragraph: it is stated that 2 representative monkeys with DM ...How were they chosen? That needs a clearer statement on why you chose them. These 2 animals were not selected, who died naturally with unknown reasons, such as aging, diabetes and its complications, etc. A statement has been added in the revision.