Author's response to reviews

Title: Effects of iodinated contrast agents on renal oxygenation level determined by blood oxygenation level dependent magnetic resonance imaging in rabbit models of type 1 and type 2 diabetic nephropathy

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Author's response to reviews: see over
Author's response to reviews

Title: Effects of iodinated contrast agents on renal oxygenation level by BOLD-MRI in a rabbit model of diabetic nephropathy

Authors:

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Version: 5 Date: 5 August 2014

Author's response to reviews: see over
Reviewer's report

Title: Effects of iodinated contrast agents on renal oxygenation level by BOLD-MRI in a rabbit model of diabetic nephropathy

Version:5 Date:24 July 2014

Reviewer: Robert Menzies

Reviewer's report:

This manuscript investigates the question of whether iodine based contrast agents can alter the BOLD signal. This is an important research question with potentially important clinical implications as has been previously explored by others, for example Heneder et al. (Invest Radiol. 2012 May;47(5):299-305). The authors present a large and commendable dataset with strong group numbers.

• Major Compulsory Revisions

The authors present a detailed description of type II and I diabetic models. However, blood and urine biochemical analysis cannot distinguish the two models. Can the authors provide a quantitative measure that clearly demonstrates insulin dependence/independence in the respective models?

Response: We wish to thank you for your valuable comment. We agree it is important to be able to differentiate between the two models. Therefore, we have added the results of laboratory tests for glutamic acid decarboxylase autoantibody (GAD-Ab) and insulin autoantibodies (IAA). As we would expect, the rabbits with experimentally induced type 1 diabetes were positive for both GAD-Ab and IAA, whereas the rabbits with type 2 diabetes were negative for both markers. These data confirm the suitability of the procedures and we have added these data to Table 2.

Page 5, Line 1: (see above comment on insulin resistance). If these indices rose to what level exactly? Allowing for error but above an acceptable normal rage? It is not clear that insulin sensitivity/insensitivity was significantly established between the two models. Rather these are two models of general ‘diabetes’. Do you have insulin measurements?

Response: In response to this comment, we have added the normal ranges for the biochemical
tests shown in Table 2, as follows:

“Normal ranges: blood glucose, 3.61–6.11 mmol/L; SCr, 44–133 µmol/L; BUN, 3.2–7.1 mmol/L; urinary microalbumin, <10 mg/L; fasting insulin, 5.8–18.6 µIU/mL.”

To provide further support for the differentiation of these models, we measured fasting insulin and added the results to Table 2. We hope that these data and the expression of autoantibodies provide reassurance that the animal models of type 1 and type 2 diabetes were successfully established.

Protocol 1 looks like a dose finding study but it is not a conclusive one. This is because no maximal R2* level is observed. What would happen at 400mgI/ml, or higher? I accept that BOLD MRI studies are expensive, and the dataset presented is already extensive. Therefore I propose that the following two limitations are fully addressed in the manuscript (ideally a more detailed limitations section). (1) The reason(s) why dose finding was performed on type II diabetic animals (and not controls or type I). (2) The reason(s) why a dose above 350mgI/ml was not used.

Response: In response to (1), there are several reasons why we did not examine the effects of different doses in control or type 1 diabetic rabbits. First, we have already reported the effects of iodinated contrast agents on non-diabetic rabbits (the manuscript had been under reviewed by other periodical office). Second, type 2 diabetes is much common than type 1 diabetes. Therefore, the results in type 2 diabetes will be of greater value in clinical settings. Accordingly, we believe it is more important to focus on type 2 diabetes. Nevertheless, we included Protocol 2 to compare the effects of a high dose of an iodinated contrast agent on both types of diabetes models, using the dose that had the greatest effect on renal oxygenation in Protocol 1.

In response to (2), we have now tested a higher dose (400 mg I/mL) in Protocol 1 and added the results to the manuscript. Because this dose had the greatest effects, we have repeated Protocol 2 using 400 mg I/mL.

There is some switching between active and passive voice, particularly in the discussion section. There are also many unclear sentence structures throughout the manuscript. I have tried to identify as many errors as possible as these issues do detract from the content and need to be addressed.

Response: We wish to thank you for carefully reading our manuscript. We have corrected these issues and the manuscript has been edited by a native-English-speaking scientific editor.
• Minor Essential Revisions

Page 2, line 2: for clarity change “…contrast agents with different concentrations…” to “…contrast agents containing increasing concentrations…”

Response: Change made as indicated by the reviewer.

P2, Line 5: for clarity change “performed on type II diabetic” to “performed on experimentally induced type II diabetic”

Response: Change made as indicated by the reviewer.

Page 3 Line 10-12: This sentence is misleading. Renal insufficiency/AKI/CKD are not necessarily the ‘most important’ risk factors for CIN (e.g. hypercholesterolemia). They are of course important, as is pointed out in the manuscripts you reference. Please revise to be explicit about this.

Response: In accordance with this comment, we have revised this sentence as follows:

“According to the studies by Pakfetrat et al. [6] and Toprak and Cirit [7], renal insufficiency and chronic kidney disease are major risk factors for contrast-induced nephrotoxicity (CIN), especially in patients with diabetes.”

P3, Line 15/16: This is colloquialism. Consider changing the last sentence of the paragraph to “Early clinical detection is therefore challenging”

Response: Change made as indicated by the reviewer.

P3, Line 17: Sentence unclear, allows more than what? Biopsy? Consider changing “MR imaging allows more” to “MR imaging can facilitate”

Response: Change made as indicated by the reviewer.

P3, Line 17: MR defined on line 18 but used previously.

Response: Change made as indicated by the reviewer.
P3, Line 22: space missing between Wistar Kyoto  
**Response:** Change made as indicated by the reviewer.

P3, Line 22: studied THE effects  
**Response:** Change made as indicated by the reviewer.

Page 4, line2: procedures WERE permitted  
**Response:** Change made as indicated by the reviewer.

P4, Line 2: University AND complied  
**Response:** Change made as indicated by the reviewer.

P4, Line 15: sentence structure is confusing. “After 2-3 weeks, the type I diabetes...” change to  “The development of type I diabetes mellitus (approximately 2-3 weeks) was considered…”  
**Response:** Change made as indicated by the reviewer.

P4, Line 22: delete “for a concentration of” and place dilution in brackets  
**Response:** Change made as indicated by the reviewer.

P4, Line 2: Sentence structure. “..., blood WAS COLLECTED FROM RABBITS VIA THE AURICULAR VEIN for biochemical analysis…”  
**Response:** Change made as indicated by the reviewer.

Page 5, Line 4: delete ‘Overall’, delete ‘kinds of’  
**Response:** Change made as indicated by the reviewer.

Page 5 Line 10: Language: ‘suffering’. Consider changing to induced with type I/II diabetes?  
**Response:** Change made as indicated by the reviewer.

Page 5 Line 17: change “most influence” to “largest effect”
Response: Change made as indicated by the reviewer.

Page 6, line 3: through THE rabbit’s auricular
Response: Change made as indicated by the reviewer.

Page 6 Line 3: In rabbits is a 2ml IV injection likely to induce volume expansion? Which can change R2* value in rats (J Magn Reson Imaging. Apr 2011; 33(4):898–901.)
Response: The reviewer is correct and we have deleted the section.

Page 6 Line 11: coronary? Do you mean coronal sections?
Response: In order to prevent the misunderstanding, we had deleted “coronary”.

Page 6 Line 22: change “following criteria according to the injury scoring” to “following injury scoring criteria”. Please include a sentence explaining the injury scoring criteria.
Response: Change made as indicated by the reviewer.

Page 7, line 4: ‘random pictures’ to ‘randomly selected images’
Response: Change made as indicated by the reviewer.

Response: Change made as indicated by the reviewer.

Page 8: It would be valuable to explicitly point out that your induction protocol successfully induced some form of diabetes (typII/I v.s. control). Then go into the description that (1) there was no difference in blood/urine results between type I/II and (2) bodyweight was constant in all groups.
Response: In accordance with this comment, we have revised this paragraph as follows:
“The bodyweights of rabbits in all of the study groups were similar during the study (Table 1). Types 1 and 2 diabetes were successfully induced and rabbits could be differentiated based on their fasting insulin, glutamic acid decarboxylase autoantibody, and insulin autoantibody levels
(Table 2). There were no differences in blood glucose, serum creatinine, blood urea nitrogen, and urinary microalbumin levels between rabbits with type 1 and type 2 diabetic nephropathy (Table 2).”

Page 9, line 5: change ‘reflected’ to ‘determined’
Response: Change made as indicated by the reviewer.

Page 9 line 11: “tubules cells” should read “renal tubule cells” or something similar
Response: Change made as indicated by the reviewer.

P9 line 16: use of plural in ‘injuries’
Response: Change made as indicated by the reviewer.

Page 10, line 1: study OF rabbit models of
Response: Change made as indicated by the reviewer.

Page 10Line 6: remove first comma
Response: Change made as indicated by the reviewer.

Page 10Line 6: full stop should be after the reference.
Response: Change made as indicated by the reviewer.

Page 11, Line 5: change ‘were’ to ‘was’
Response: Change made as indicated by the reviewer.

Page 11, Line 7: it is not clear what you are trying to say in this sentence.
Response: In order to prevent the misunderstanding, we had deleted the sentence.

Page 11, Line 9: what do you mean by “explore the change regulation”? 
Response: We have replaced “explore the change regulation” with “observe the changes in R2*
values over time”.

Page 11, Line 11: Consider using the word ‘increase’ or ‘decrease’ instead of ‘progress’ as it is unclear what you mean by this.

**Response:** We have extensively rewritten this paragraph to improve its meaning.

Page 11, Line 12: “renal oxygenation level reached the lowest level...” –unnecessary repetition of ‘level’

**Response:** Change made as indicated by the reviewer.

**TABLE 2:** abbreviations need to be described

**Response:** Change made as indicated by the reviewer.

**TABLE 3:** A description of the measured P-value should be given in the legend

**Response:** Change made as indicated by the reviewer.

• Discretionary Revisions

Page 8, Line 7 (and onward): for clarity consider changing “R2* bar” to mean medulla/cortex R2* value of something similar.

**Response:** Change made as indicated by the reviewer.

P9 line 9-24: To facilitate readership, when presenting non-significant P-values consider writing N.S. (or similar) instead of P>0.05.

**Response:** Change made as indicated by the reviewer.

Page 11, Line 9-13: consider splitting this sentence, it is very long.

**Response:** We have rewritten this paragraph to improve readability, as follows:

“We found that the R2* values were higher after 1 h and reached peaks at 24 h after injection. The R2* values declined thereafter, reaching the lowest values at 72 h. These changes in R2* values suggest that the renal oxygenation was lowest at 24 h. Furthermore, it seems that the renal effects
of the iodinated contrast agents were greatest at 24 h and gradually declined by 72 h after injection. Nevertheless, the effects of the contrast agent persisted for at least 72 h because the R2* values remained higher than that in the control group.”

Figures 1-4: Presumably the final manuscript would be submitted in color. They are difficult to read in black and white print out (for offline readership), the authors may wish to address this.

**Response:** We agree that some of the images may be difficult to interpret after printing in black and white. Unfortunately, even when we convert the images to greyscale, it is difficult to visualize the changes. This is especially true for the BOLD-MRI figure (as indicated below). Therefore, we are unable to prepare figures in black and white at this time. However, if the reviewer has a suggestion on how we can address this, we would be willing to provide black and white figures as appropriate.

Original

![Image 1](image1.png)  ![Image 2](image2.png)  ![Image 3](image3.png)  ![Image 4](image4.png)

Greyscale

![Image 1](image1_greyscale.png)  ![Image 2](image2_greyscale.png)  ![Image 3](image3_greyscale.png)  ![Image 4](image4_greyscale.png)

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: I declare that I have no competing interests
Reviewer's report

Title: Effects of iodinated contrast agents on renal oxygenation level by BOLD-MRI in a rabbit model of diabetic nephropathy

Version: 5 Date: 3 August 2014

Reviewer: Matthew Bailey

Reviewer's report:

The authors have addressed all of my original comments in this new version of their manuscript. The study is much more focused and the control groups allow better discussion of the data.

Major compulsory Revision

The statistical approach is not clear. I have a comment on ROI (below). In addition, I think the statistical tests used should be included in the table heading and Figure legends. It is not clear to me why a combination of one-way anova (which post test?) and unpaired t-tests were used.

Response: We wish to thank you for your valuable comments. In response to these comments, we have consulted several statisticians and improved our understanding of multi-level ANOVA. We believe that multi-level ANOVA is more suitable than one-way ANOVA and t tests for the data reported in our manuscript because it essentially combines the functions of both statistical tests. Accordingly, we have reanalyzed the results using multi-level ANOVA all data.

Minor Essential Revisions

1. Can the authors detail how the 6 ROI for each region in each rat are processed to give the data shown in Table 3 and Figure 1? Are these values averaged to give a mean value per rat? If so, how is the variability between ROI taken into account?

Response: Using ADVANCE 4.4 Workstation software (General Electric Medical Systems, Milwaukee, WI, USA), when you use a fixed ROI, the software automatically calculates the R2* values. The values reported in Table 3 and Figure 1 are the mean values for all rabbits in each group. The ROI was fixed at 5 mm³ to avoid bias through different sizes of the ROI.

2. The authors include a quantitative scoring of renal injury and also of pimonidazole staining but
these are difficult to follow in the text- I think putting these data in a table would improve the manuscript.

Response: We wish to thank the reviewer for this suggestion. We have therefore added Tables 5 and 6 showing the pathological injury and pimonidazole staining scores, respectively.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: I declare that I have no competing interests