Author's response to reviews

Title: Lipomatous Metaplasia Identified in Rabbits with Evolving Reperfused Myocardial Infarction: 3.0T Magnetic Resonance Imaging and Histopathology Findings

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Version: 2  Date: 18 February 2013

Author's response to reviews: see over
February 15, 2013

Dear Prof. Adrian Aldcroft, the Editor-in-Executive of BMC Medical Imaging,

Thank you for your encouraging letter about our submitted manuscript entitled “Lipomatous Metaplasia Incidentally Identified in Rabbits with Evolving Reperfused Myocardial Infarction: 3.0T MRI and Histopathology Findings”.

The comments from the three reviewers have been carefully addressed point-by-point both in the manuscript and in this revision letter (see the appended pages).

We hope that this revised version should meet the requirements for being published in BMC Medical Imaging and eventually contribute to the ever-enhancing IF of your journal.

Sincerely yours

With my best regards,

Yicheng Ni
Responses to the Reviewer Francisco Ridocci’s Comments (FR)

**Reviewer’s report:**

It is an interesting study that shows the utility of 3T MRI and histological visualization in vivo in an animal model of acute myocardial infarction and chronic.
As the authors point out the main limitation of the study is that only 3 rabbits were studied at 9 months and that detection of lipomatous metaplasia was not an objective of the study
Nevertheless findings deserve publication. The images and sequences are of high quality.

We are grateful to the reviewer for his appreciation of our work. Indeed, the lipomatous metaplasia here in rabbits represents an accidental finding, which is however of potentially high value for experimental cardiology or cardiac imaging research, we believed.

Minor Essential Revisions:

Q 1: Although lipomatous metaplasia was discovered incidentally, the findings are consistent and the term “incidentally” should be deleted in the title.

A 1: The term “incidentally” has been deleted in the title accordingly.

Q 2: Results Longitudinal cMRI findings during 9 months The first sentence should be modified, taking into account that data from only 3 animals only permit the characterization of the sample without assuming that “EF “increased apparently “. In addition, LVEDV enlarged

A 2: According to your advice, we have modified this sentence to specify the functional changes only on three cases.

Q 3: Discussion Second sentence: the term ictus should be replaced by “myocardial ischemia”

A 3: The term “ictus” has been replaced by “myocardial ischemia” accordingly.

Responses to the Reviewer Satoshi Okayama’s Comments (SO)

**General comments:**

This manuscript describes that 3.0 T cardiac MRI is useful for the evaluation of lipomatous metaplasia. I am very interested in the fact that the image characteristics of lipomatous metaplasia can change as time goes on. This study is considered to take a lot of time and effort. However the interest of this result is not sufficiently conveyed to readers unfortunately.

GA: We are grateful to the reviewer for his interest in our work and ready to follow his advice for making improvement.

**Major Compulsory Revisions**

Q 1: This manuscript contains many grammatical and other errors, and thus should be proofread. The description is redundant and lengthy. The useful messages to the readers of BMC imaging are not clearly described in discussion.
A1: According to your advice, we have made efforts to significantly shorten the text and to make it more focused. The manuscript has been proofread by two native English speaking researchers.

Minor Essential Revisions

Q1: Title is lengthy. I consider that “incidentally” is unnecessary, and prefer the following title. “Lipomatous Metaplasia Identified in Rabbits with Reperfused Myocardial Infarction by 3.0 T Magnetic Resonance Imaging and Histopathology”.

A1: We have adopted the title that the reviewer recommended.

Q2: Line 2-9, page 4, background. Please refer to the following sentences. “The moderate size rabbit model of occlusion/reperfusion-induced myocardial infarction (MI) has been frequently evaluated by 1.5 T magnetic resonance imaging (MRI) scanner. 3.0T MRI scanner with 8 channels or more cardiac array coils can obtain cardiac images with higher signal-to-noise-ratio (SNR), temporal and spatial resolutions, and shorter acquisition time compared to the conventional 1.5 MRI scanner, however there are few reports on the evaluation of rabbit model of occlusion/reperfusion-induced myocardial infarction by 3.0T scanner.”

A2: According to your suggestion, we have rephrased this part to make the sentences more precise.

Q3: Line 23-27, page 4, background. The purpose of this study is unclear, and I recommend that third paragraph starts with the sentence of “The purposes of this study were as follows: ” Line 23-27, page 4, background. I understand that the purposes are as follows.
(a) Longitudinal evaluation of myocardial infarction by 3.0 T MRI scanner
(b) Comparison of myocardial infarction on acute phase between MRI findings and histopathology
(c) Comparison of lipomatous metaplasia on chronic phase between MRI findings and histopathology

A3: According to your advice, we rephrased the last paragraph in the background in consideration of your suggested purposes.

Q4: Methods.
Time schedule of the experiments should be added, which will make it easy to understand the methods.

A4: Accordingly a graph of time schedule for the experiments has been added.

Q5: Line 28, page 4, Methods. There are too many abbreviations in this manuscript. We consider that “TA” is less commonly used as an abbreviation for “acquisition time”.

A5: According to your advice, we choose not to use any abbreviation for “acquisition time”.

Q6: Line 28, page 5, Methods. In many human studies, both late enhancement and cine
imaging use no fat suppression. Lipomatous metaplasia can be thus detected as hyperintensity area on late enhancement images, although cannot be differentiated from myocardial fibrosis. For avoiding misunderstanding, “3D delayed-enhancement imaging with fat suppression” should be used.

A6: We agree with your opinion that normally fat suppression is not used in cMRI, and thus the LM should have been detected as hyperintense area even without delayed enhancement. However, with late enhancement cMRI, the signal intensity of the lesion dramatically evolved during the entire pathological process. Retrospectively we noticed that the MI from acute phase till 2 months when myocardial fibrosis formed was hyperenhanced on DE-cMRI, whereas the healed MI, especially when old scar became the LM 9 months later, could not be hyperenhanced on DE-cMRI as shown in our study. Since we did not anticipate the presence of the LM, we failed to apply all cMRI sequences with and without fat suppression, although they should have been used. This drawback has been addressed as a study limitation.

Q7: Line 1, page 7, Methods. There are too many abbreviations in this manuscript. We consider that “MIS” is less commonly used as an abbreviation for “myocardial infarction size”.

A7: According to your advice, we used “MI size” instead of “MIS” in the text.

Q8: Line 1, page 7, Methods. Correct the sentence, as follows. “The global MI size were represented as the percentage of left ventricular mass volume (%LV).”

A8: It has been corrected.

Q9: Line 12, page 8, Results. Add the value of r2.

A9: It has been added.

Q10: Line 15-16, page 8, Results. Correct the sentence, as follows. “A hyperenhanced transmural zone with sporadic hypointense spots was observed at the anterior and lateral wall on DE-MRI (Fig 2A).”

A10: It has been corrected.

Q11: Line 4, page 11, Discussion. Add edema to the components of myocardial infarction.

A11: It has been added.

Q12: Line 4, page 20-27, Discussion. The main findings of the study should be described in the first paragraph in discussion, which will make it easy to understand the intension.

A12: We appreciate your advice. In order to maintain the integrity of the context, actually the main findings of the study are described in the second paragraph instead.

Q13: Legend of figure 4 and 5. Acute phase, early chronic phase, and chronic phase were not defined. For avoiding misunderstanding, these should be replaced with 48 hours, 2 weeks, and 2 months after the onset of myocardial infarction, respectively.

A13: The time points of 48 hours, 2 weeks and 2 months of MI correspond to the acute phase,
early chronic phase, and chronic phase, which indeed have now been defined within the text.

Q 14: Table 1. “True-FISP” is an abbreviation for “true fast imaging with steady-state precession”.

A 14: It has been corrected.

Q 15: Table 2. Please show the data of the remaining rabbits (No. 4-10) 48 hours after myocardial infarction.

A 15: According to your advice, we provided the left ventricle functional data of all the animals on 48 hours and 9 months on table 2.

Q 16: References. Refer to the following articles.

A 16: They have been cited as ref. 14 and 15.

Discretionary Revisions

Q 1: Please compare the lipomatous metaplasia volume (%LV) between T1-weighted imaging and histopathology, if possible.

A 1: The size of LM on T1WI and TTC has been added to the results of the manuscript. Regarding the limited number LM cases, there was no statistic comparison given, though.

Q 2: Please longitudinally measure the signal intensity ratio of, if possible. I consider that quantitative evaluation is better than visual evaluation.

A 2: According to your advice, we have added another figure (Fig. 5 now) about signal intensity ratio of lipomatous metaplasia / non-infarct area in the results.

Responses to the Reviewer Giovanni Donato D Aquaro’s Comments (GDDA)

Reviewer’s report:

Feng and colleagues evaluated the evolution of myocardial infarction using a rabbit model of occlusion/reperfusion-induced myocardial infarction. Authors repeated several MRI examination on 3T magnetic resonance scanner in 3 rabbits after myocardial infarction describing the evolution from acute infarction, scar and finally fat metaplasia. Other 7 rabbits were sacrificed immediately after first MRI scan. On the results, authors found a good
correlation between DE and histology. Then in the 3 rabbits sacrificed at 9 months, one rabbit had large sign of fat metaplasia. The remaining 2 rabbits had no clear signs of fat at MRI but histological analysis demonstrated small spot of fat metaplasia.

Major Compulsory Revisions

Q1: Actually, I did not understand the rationale of this study. The accuracy of late enhancement in animal models of myocardial infarction, compared with histology, was demonstrated by several study in the last years. Then, this point lacks of novelty. Moreover, the manuscript was entitled "Lipomatous Metaplasia Incidentally Identified in Rabbits with Evolving Reperfused Myocardial Infarction", then why to perform histopathological evaluation after the first MRI?

A1: A model of occlusion/reperfusion acute myocardial infarction (MI) in rabbits has been successfully established in our laboratory. Our purpose of this study was further validated the usefulness of this experimental setting as a platform for pre- and post-mortem imaging co-localization on the acute and the chronic phase of MI. Cardiac lipomatous metaplasia (LM) was incidentally encountered in rabbits with healed MI, which we did not design at the beginning.

Although animal models of MI has been comprehensively studied on pathology, imaging the beating heart of small animal on clinic MR scanners is challenging and seldom been reported. Rabbit heart has a perfect size that an entire transverse section of the heart can be mounted on an ordinary slide for microscopy, which makes planimetric histomorphology study correlated well with in vivo MR imaging. We believe the images in this article are of high quality, which has never been reported in rabbits. In addition, cardiac LM commonly occurs in patients with chronic ischemic heart disease and heart failure, however there is a lack of animal models and research platform for further study on the mechanisms and possible therapeutic interventions of LM after MI. The novelty of this study is based on the fact it is the first time that the LM was found in rabbits and described in evolving details using in vivo and ex vivo imaging techniques. Moreover, the present experimental setup has enabled cardiac imaging research in rabbits with enhanced applicability, accuracy and cost effectiveness.

Q2: To describe only an "incidental finding of fat metaplasia" occurred in MRI performed for other purpose (what purpose?) is absolutely not interesting and definitely not novel.

A2: The LM is an important phenotype in human chronic heart disease, but its mechanisms are unclear up to now and it has never been reported in rabbits as a common experimental animal. By this article we offer a novel research platform for studying the LM.

Q3: Instead, the description of the pathological changes in myocardial infarction evaluated in vivo by repeated MRI examinations, from the acute phase to 9 months after AMI, may be interesting. However, this was performed in only 3 animals and MRI was performed only after 48h post MI, after 2 weeks, 2 months and 9 months. I believe the authors should add at least other 7 rabbits completing the full protocol. MRI examination should be repeated also after 4-6 months at least.

A3: We appreciate your suggestion. Actually, we did not intend to find LM in rabbits at the beginning of this experiment. To follow up cMRI scanning on animal MI during 9 months has never been reported. In this study, the first time to our knowledge, the LM was detected in rabbits, which will inspire further experimental research with a full protocol in the future.
Q4: In the discussion, author stated that "this study was not by intention to address the LM, and therefore only some cMRI sequences were applied for detection of fat. The LM was only recognized incidentally at 9 months after MI induction". Actually, what's the aim of this study? Is this an "incidental finding" report? it is known that 33% of old myocardial infarction had fat metaplasia, then what is the novelty?

A4: As we mentioned above, the LM was an important phenomenon in human chronic heart disease, but its mechanisms are not clear up to now and it has never been detected on rabbit heart, which can serve as an experimental platform to conduct further research on the LM.

Q5: in the conclusion, authors stated that "The in vivo cMRI corresponded well to ex vivo MRI and histomorphology, suggesting a promising animal model and research platform for further study on the mechanisms and possible therapeutic interventions of LM." I've some concerns about this sentence: 1) "corresponded well", is this a qualitative statement, I've found no comparison between in-vivo and ex-vivo and histological measurement of extent of fat metaplasia. Then we cannot say whether correspond well or not.2) "promising animal model and research platform for further study...metaplasia", actually we cannot say this, because only three animals completed the protocol and only in one the fat was found at in-vivo MRI but all the three of them had fat (small island in two) at histology, then, the model should be evaluated by further studies. 3)"possible therapeutic interventions of LM", why? LM is the normal evolution of fibrosis in 33% of old myocardial infarction. In humans it is described in >60% of myocardial infarction older than 5 years, then it is intrinsically associated to a good prognosis. Therapy is not necessary.

A5: 1) We added one case of the largest LM size on T1WI and TTC after 9 months of MI. Although the animal numbers were insufficient for comprehensive statistic analyses, our data showed that a close match between cMRI and TTC. Since the small size and moving artifacts on the heart, sporadic fat was difficult detected by cMRI. We agree that the other cases with small fat infiltration on cMRI was not matched clearly with histology, however, cMRI provided the possibility to monitor in vivo the evolution of the entire lesion.

2) Animal models play important roles in the continuum of experimental activities that make up translational medical research. Translation of human disease to experimental animal models is deemed very important and useful for further improvement of diagnostic and therapeutic methods.

3) Further research on the etiology and pathophysiology of the LM may expose more clues for gene or stem cell based regenerative therapies after ischemic heart disease. Thus, therapy is not necessary today but possible and necessary in the future.

Minor essential revisions

Q1: Authors stated that "the 3.0-T superconducting system with a higher magnetic field, cardiac array coils with 8-channels or more and upgraded hardware enables obtaining images with higher signal-to-noise ratio (SNR), temporal and spatial resolutions, and speed of imaging acquisition as compared to the". This is correct. However, at 3 Tesla cardiac imaging is characterized by more susceptibility artifact, higher chemical shift artifact (then more indian ink in cine imaging), more turbulence artifact, making cardiac imaging more difficult in pediatric patients as well as small animals as rat and rabbits. Please discuss this.
A1: The purpose of this experiment was not focus on the physical studies of MRI sequences. The detailed parameters were provided on Table 1 and the nice MR imaging was shown on Figures, which confirmed that a 3.0T clinic MR scanner can be successfully used for cMRI in rabbits.

Q2: Image: in the figure 5, authors showed in-vivo and ex-vivo MRI images. From these images it is very hard to confirm the presence of sub-epicardial fat metaplasia in in-vivo images. Instead, in the ex-vivo FSE images it is easy. How was performed the image analysis? were Investigators blinded of the results of histopathology and ex-vivo MRI? Please discuss this.

A2: In the figure 5 (now Fig 7), the LM was hardly detectable by cMRI, but confirmed by photomicrographs on sporadic adipose deposition. We did not perform any image analysis on this case. We performed statistic analysis on acute MI size between MRI and pathology, while on chronic phase, we just described the phenomenon of LM in 3 rabbits, as an incidental finding.