Author's response to reviews

Title: Semi-automated segmentation and quantification of adipose tissue in calf and thigh by MRI: a preliminary study in patients with monogenic metabolic syndrome

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Version: 2 Date: 26 July 2006

Author's response to reviews: see over
July 26th, 2006

Dear Iratxe Puebla,

Please find attached our responses to the reviewer comments for the research article we submitted to BMC Medical Imaging: “Semi-automated segmentation and quantification of adipose tissue in calf and thigh by MRI: a preliminary study in patients with monogenic metabolic syndrome” (revised title). We have responded to all reviewer comments in a detailed manner (see attached summary). The paper is greatly improved as a result and we trust that you will find everything in order.

Thank you for your consideration. Please let us know immediately if there is anything that we can assist you with.

Sincerely,

Salam A. Al-Attar,
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and

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Connectivity Analysis Tool Enables SEMI-Automated Segmentation and Quantification of Adipose Tissue Magnetic Resonance Images in Patients with Monogenic Metabolic Syndrome

Response to Reviewers’ Comments

REVIEWER 1

We thank the reviewer for the thoughtful comments.

Major Compulsory Comment 1: “Have phantom studies been employed to assess accuracy of method? This would add weight to the technique. Can the method be adapted to estimating preperitoneal and skeletal muscle fat.”

Response to comment 1: We agree with the reviewer that additional studies to assess the accuracy of the method would certainly be desirable. At present, phantom studies – including the manufacture of an appropriate tissue phantom - have not been undertaken, but we agree that this might be a good idea for the future. Instead, we have performed replicate analyses of the in vivo data in order to define intra- and inter-observer variability using Pearson correlation, which is the standard approach for characterizing performance of a diagnostic method and which has demonstrated a high degree of reproducibility and accuracy, as indicated by significant high correlation coefficient values. With respect to adapting this method to measure other fat stores, we agree that these are important applications that could be part of future research endeavours, but were not the focus of this study.

Minor Comment 1: “Discussion too long by 25%.”

Response to minor comment 1: We have reduced the Discussion from 950 to 780 words (~20%). We could not reduce further because we had numerous reviewer comments that had to be addressed.
“Connectivity Analysis Tool Enables SEI-M-Automated Segmentation and Quantification of Adipose Tissue Magnetic Resonance Images in Patients with Monogenic Metabolic Syndrome”

Response to Reviewers’ Comments

REVIEWER 2

We thank the reviewer for the thoughtful review, together with the helpful comments and suggestions.

Major Compulsory Revisions

Comment 1: “Page 6, Magnetic resonance imaging: the acquisition procedure should be better detailed. Authors should provide FOV, flip angle, NEX, and image size (rowsxcolumns). How the images in figure 1 were obtained? Are simple a reconstruction from transaxial images or was really acquired? Mode details should be provided because the authors refer to these images in the results section.”

Response to comment 1: We have reviewed our paper and have added the following details to the Methods subsection outlining the MRI acquisition procedure (Magnetic resonance imaging and image analysis; page 6, lines 11-15). Note the flip angle was not specified as Spin Echo pulse sequence generally implies a $90^\circ$ and $180^\circ$ flip angle.

The images in Figure 1 were obtained via coronal cross sections made of the different body segments. Mid-slices from the coronal stack images were selected and pasted together to provide the “whole body MRI” profile presented in the figure. We agree with the reviewer that more details should be provided for Figure 1. Thus we have added the following sentence to the end of the Method section ‘Magnetic resonance imaging and image analysis’: “The whole body scans (Figure 1) are a composite of four mid-slice coronal stack images acquired at four stations (head/neck, thoracic/abdominal, pelvic/thigh, lower leg).”

Comment 2: “Page 7, line 13: It is not clear what $\text{\oea}$ distance in pixel set to 1.00 mm$\text{\oei}$ means. If the image processing is performed in 3D, this may be the image stack was interpolated to obtain 1mm cubic voxels$\text{\oei}$.”

Response to comment 2: We agree with the reviewer the units chosen should be clarified. The change in scale to 1.00 represents the number of pixels per mm (i.e. 1.00 pixel/mm). Raw images from different scans come with different scale settings (e.g. 0.640, 1.113). Therefore the change to 1.00 standardizes all images analyzed to one setting. We have changed the reported units in our method section from mm to the more accurate units of pixel/mm.
“Connectivity Analysis Tool Enables SEMI-Automated Segmentation and Quantification of Adipose Tissue Magnetic Resonance Images in Patients with Monogenic Metabolic Syndrome”

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Comment 3: “Standardization to 8 bit reduce image dynamic and may reduce the accuracy in SAT detection. The used software seems to be able to work on the original DICOM 16-bit images (http://rsb.info.nih.gov/ij/features.html), so 8bit down-sampling should be avoided.

Interpolation to 1mm voxels increment the partial volume effect, especially in z direction. This may reduces the accuracy in the SAT detection. I don’t see any reason to perform this operations instead to use original data. I suspect that the software used may be not able to work on non-cubic voxels. I appreciate the use of a free software and, because the software is free, some limitation may be accepted. However, the authors should elaborate this point.”

Response to comment 3: We agree with the reviewer that 16-bit images would be more advantageous to work with. Unfortunately, the change to 8-bit was necessary for the creation of the connectedness map. As such, the change to 8-bit is a limitation of the software. The interpolation to 1mm voxels was a method of standardization as discussed in our response to comment 2.

Comment 4: “Page 10. The Pearson correlation coefficient was used to assess the inter- and intra-observer variability. Clearly, a dependence may exist across data acquired from the same subject. If a simple linear regression is used, results are not valid since the data contain 17 samples from 4 subjects with no correction for multiple dependent/correlated samples. More advanced statistics (two-way anova) should be used instead.”

Response to comment 4: The standard method for evaluating assay performance attributes such as intra- and inter-observer variability is through correlation analysis. Thus, we believe that this was the appropriate statistical test to assess these attributes of our method. In addition to assessing assay performance, the Pearson correlation coefficient was used to compare the relationship between mean subcutaneous fat areas and overall fat volume (page 11, lines 12-13). In this instance we would agree that these two variables are interdependent, and that more advanced statistics might perhaps be used. Our analysis, however, was simply illustrative, to emphasize the fact that the two measures are essentially the same and interchangeable, implying that future studies might only require a single measurement.
Connectivity Analysis Tool Enables SEMI-Automated Segmentation and Quantification of Adipose Tissue Magnetic Resonance Images in Patients with Monogenic Metabolic Syndrome

Response to Reviewers' Comments

Minor Comment 1: “Page 2, Abstract, Results: The fact that percentages of SAT are calculated with respect to the whole extremities size should be clarified in order to make the abstract self-consistent.”

Response to minor comment 1: In order to clarify that the percentage SAT was calculated from a defined region of the leg we have added the word “segments” to the abstract results section as follows: “MR images revealed significant differences in the amounts of subcutaneous adipose tissue in lower limb segments of FPLD3 and FPLD2 subjects:…”


Response to discretionary comment 1: The references have been added to the Background section as suggested.

Discretionary Comment 2: “Page 8, line 3: In my opinion, the average percent adipose tissue/slice index doesn’t make so many sense. It is just a (wrong) approximation of the percent volume defined below that should be used instead.

Response to discretionary comment 2: The average percent adipose tissue/slice was used as an approximation of a mid-slice value. We noted that the mid-slice of each section had a very similar value to that of the mean adipose tissue/slice (see table below). This shows us that we can use mid-slice values in the future to minimize analysis time, MRI acquisition time, and efficiency of experimentation. However, we felt that including this additional information would unnecessarily burden the reader, without adding much value to the report, and so we elected not to include it in the results section. However, for the reviewer’s information, we present the tabulated results of the direct comparison of the two measurements in the Table below.
“Connectivity Analysis Tool Enables SEMI-Automated Segmentation and Quantification of Adipose Tissue Magnetic Resonance Images in Patients with Monogenic Metabolic Syndrome”

Response to Reviewers’ Comments

Table (exhibit for review only): Comparison of mean sc+inf volume/slice vs. mid-slice sc+inf adipose tissue (note: bolded values were not included in the manuscript and represent single slice (slice 9) sc+inf volumes)

<table>
<thead>
<tr>
<th></th>
<th>GL2784</th>
<th>GL2990</th>
<th>GL0658</th>
<th>GL0096</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-calf Mean sc+inf volume / slice (%)</td>
<td>26.3±1.1</td>
<td>34.8±1.5</td>
<td>19.2±1.7</td>
<td>N/A</td>
</tr>
<tr>
<td>Slice 9 (mid-slice) (%)</td>
<td>25.3</td>
<td>34.0</td>
<td>19.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Mid-thigh Mean sc+inf volume / slice (%)</td>
<td>44.3±2.1</td>
<td>56.1±1.5</td>
<td>34.4±2.5</td>
<td>24.3±3.7</td>
</tr>
<tr>
<td>Slice 9 (mid-slice) (%)</td>
<td>43.4</td>
<td>55.6</td>
<td>33.4</td>
<td>23.6</td>
</tr>
</tbody>
</table>

Discretionary Comment 3: Discussion: The error committed due to the inclusion of some VAT in the measurement may be quantified by the used software by manual definition of SAT region.

Response to discretionary comment 3: We agree with the reviewer that it would be possible to determine the SAT with the manual removal of all potential VAT. However, since there are no absolute defining boundaries between subcutaneous and infiltrated fatty septa, subcutaneous tissue was considered to be that region which is adjacent to the skin/outer perimeter of the leg, as well as that connected to the seed point lying within. Also by utilizing the Connected Threshold Grower tool, we were able to minimize user manipulation and errors in the data analysis.
Response to Reviewers’ Comments

REVIEWER 3

We thank the reviewer for the thorough review and comments.

Major comments and responses
(note Responses to comments below are in regular font).

Methods:
“Page 5, line 15: 23.5 vs. 24.8 is comparable but not well matched!”

We agree that it would be ideal to have a perfect match, but we feel that the difference here is minimal.

“Page 5, line 16: same for the age, 50 and 63 is not matched!”

We agree with the reviewer that these subjects are not matched as well as they could have been for age. Given that this is a pilot study on FPLD patients, we had small numbers of study subjects to compare, and thus we were limited in our subject pairing. However, we feel that the data from these subjects provide some value and these preliminary results are of interest.

“Page 6, line 10ff: is this the description of whole body imaging? What’s the rationale for whole body imaging, when only lower extremities are relevant for this study?”

The information presented on page 6, line 10ff, is a description of the transaxial images that were used for SAT analysis. The coronal images obtained, as extra images, were simply compiled together as a “whole body” MRI composite to show the location of the transaxial segments used for analysis (figure 1, blue rectangles) as well as to provide an overall picture of fat depots in our subjects.

“Page 6, line 16f: are the measurement parameters for mid-calf and mid-thigh the same as for whole body imaging?”

Yes, the same MR image acquisition parameters were used for both the mid-calf/mid-thigh and the whole body image composite. No fat measurements were made on the whole body image.
Response to Reviewers’ Comments

“Page 7, line 2ff: how was the threshold between lean tissue and fat determined? By determination of a histogram? Automatically or manually by taking the signal intensity of the seed point? Please add this information!”

The threshold range chosen for the lean tissue and fat tissue was determined manually by surveying the respective tissue type of interest. This was described in the method section, page 7, lines 21-23: “Total tissue and adipose tissue threshold value ranges were obtained by manually sampling the image intensity in each image stack.”

“Page 7, line 7ff: Why are fatty septa added to subcutaneous adipose tissue? This is a different lipid compartment and should be separated from subcutaneous adipose tissue!”

Since there are no absolute accepted defining boundaries between subcutaneous and infiltrated fatty septa, subcutaneous tissue was considered that which is adjacent to the skin/outside perimeter of the leg, as well as all connected infiltrated fat. We feel that our strategy minimized the use of human judgment, which would represent a new source of analytical variation.

“Page 8, line 4: the average of 17 slices was reported as the average percent adipose tissue/slice. Seems to be the average percent adipose tissue/region!? It is not clear what is being asked here.”

The region is “mid-calf” or “mid-thigh” region. Hence, these measurements are essentially the average percent adipose tissue/slice for a specific region.

“Page 8, line 13: how many observers were involved in the evaluation? This information should also be given here!”

This information has been added to the methods section, page 7 line 6: “Analysis of the MRI stack images and measurements of subcutaneous adipose tissue was done by a single observer…”

RESULTS:

“Page 9, line 20f: increased visceral fat in the FPLD2 subject compared with the FPLD3 subject. As you did not quantify visceral adipose tissue and
Response to Reviewers’ Comments

**the images in Figure 1 stem from different coronal slices (spine is visible in FPLD3 but not in FPLD2 patient) this statement should be omitted!”**

Done.

**“Page 10: determination of intra- and inter-observer correlation imply manual determination of threshold, otherwise the correlation should be 1! What about inhomogeneities of the signal distribution? Are they corrected?”**

Yes, manual determinations of threshold were made. As for the inhomogeneities in signal distribution, all images were adjusted using the Auto-brightness tool prior to analysis.

**“Page 10, line 11: is this the result for inter-observer correlation(?), as intra-observer correlation is given above.”**

This is the result for inter-observer correlation. We have made the correction in the text. Thank you for pointing this out to us.

**“Page 10, line 21ff: no subcutaneous connectedness map of fat. There is no subcutaneous fat â€“ but in the Methods you told about also including the fatty septa in the musculature. Was this not included in this patient?”**

The seed point selection is made from regions of fat depots that run along the perimeter of leg region, adjacent to the skin, which we have defined as the subcutaneous compartment. Since no continuous band of adipose tissue was present along the circumference of the legs it was considered to be zero by this study’s internal definition of subcutaneous fat.

**DISCUSSION:**

**“Page 12, line 1f: clinical assessment of adipose tissue distribution â€“ is crude. There is an increasing number of studies dealing with quantification of total body adipose tissue and its distribution. So soften this statement!”**

Done.

**“Page 13, line 9f: controls did not provide the ideal BMI and age-matching criteriaâ€“ Here you confirm that the data are not matching very well. It should be possible to select subjects with really matching anthropometric data (however, it will not change the results dramatically).”**
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Response to Reviewers’ Comments

We agree with this statement. Please refer to our responses above to the Methods, major comments 1 and 2.

“Page 13, line 20: intra- or intermyocellular depots: these can be determined by proton MRS (add Schick et al, MRM 1993, Boesch et al., MRM 1997 and e.g. Brechtel et al., JMRI 2000, where a patient with Acquired generalized lipoatrophy was assessed by fat selective MRI and MRS)”

These references have been included in the discussion section as follows (page 13 lines 20-22): “…could be more specifically evaluated using proton magnetic resonance spectroscopy (MRS) and/or fat selective MRI [11, 12, 13].”

Table 1:
“What’s the rationale for differentiation between mean sc+inf volume / slice and Overall sc+inf volume (%)? What’s the difference between these values?”

The rationale for listing these two numbers is to illustrate the similarity in percentage between volumetric and slice measurements. Due to the high degree of similarity, we infer that it is only necessary to measure one slice (i.e. we anticipate mainly focusing attention on the mid-slice of selected region for future studies, as opposed to acquiring 17 highly correlated image slices.) Thus, this will reduce both time in MRI and efficiency in analysis of a larger number of subjects.

Figure 1
“Survey images of lower extremities are sufficient as the other body parts are not subject to the quantitative evaluation. Statement about visceral adipose tissue content can not be made from these images!”

From the rationale that these coronal segments are not identical in location, we agree. Please refer to our response to Results major comment 1.

Figure 2
“Thigh image of patient GL0096 is cutted! Please show the complete image!”

This was an acquisition problem which led to a small segment in the thigh stack of images to be missing. Due to the clinical condition of the patient
“Connectivity Analysis Tool Enables SEMI-Automated Segmentation and Quantification of Adipose Tissue Magnetic Resonance Images in Patients with Monogenic Metabolic Syndrome”

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and discomfort, our image in this case had to be compromised, and we have not had an opportunity to obtain a repeat image.

Figure 3

“sc + inf image seems to be different from the original image (compare infiltrated fat). Please show corresponding images from the same slice! Furthermore it seems that fatty bone marrow of the tibia was removed, but not bone marrow of fibula! Please describe in the text whether bone marrow was included in the quantification or not!”

The image referred to represents a mid-thigh slice. As such, neither bone is present. In rare cases, if bone marrow was included, it could be manually deleted, with reference to the original MRI image.

Minor comments and responses

Abstract:

“Page 2, line 22, 23: FPLD2/3 patient instead of patients!”

Done

“Page 3, line 1: semi-automated quantification of adipose tissue instead of semi-automated adipose tissue imaging”

Done

Background:

“Page 4, line 5ff: sentence is not complete! ‘The salient clinical and biochemical’”

Thank you for noting this point we have corrected this mistake: “…salient clinical and biochemical manifestations.” (page 4 lines 7,8)

“Page 4, line 11: LMNA: please write it out”

This is defined in the sentence: LMNA = lamin A/C.
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Response to Reviewers’ Comments

Discussion: “Page 11, line 14: ‘and mid-thigh sections of FPLD patients compared to’ Remove ‘in’ after patients.”

Done

“Page 12, line 14: ‘The method has instead of does have’”

Done

“Page 12, lines 23: ‘this patient instead of these patients!’”

Done

“Page 13, line 11: ‘factors such as difference in age, add ‘does’ remove ‘does’!”

The sentence has been removed.