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14. ABSTRACT Objectives: In this application, we propose to build upon our current work to determine the association between fatty acid synthase (FAS) overexpression and intraprostatic fat as measured by in-vivo imaging using proton magnetic resonance spectroscopy imaging in the prediction of prostate disease aggressiveness. Mechanisms linking fatty acid synthase overexpression, lipid accumulation, lipid oxidation, and tumor aggressiveness will be explored using metabolomics. Plan: Employing a cross-sectional design we will recruit 50 men with low-grade and 50 men with high grade prostate cancer post-diagnosis as determined prior to prostatectomy. Each patient will complete one proton magnetic resonance spectroscopy imaging session and provide access to his prostatectomy tissue. Study aims: Among men diagnosed with low grade (proposed as more indolent) and high grade (proposed as more aggressive) prostate cancer (as determined by Gleason scoring) we propose to: 1) Determine the correlation between FAS expression in prostatectomy samples and the amount of intraprostatic lipid using ¹ H magnetic resonance spectroscopic imaging (proton MRSI) with an endorectal coil. 2) Identify the association between FAS expression and FAS activity in prostatectomy samples, intraprostatic lipid as measured by MRSI and prostate tumor aggressiveness. 3) To quantify key metabolic intermediates involved in lipid metabolism, mitochondrial function, inflammation, and apoptosis in the prostatectomy samples.					
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INTRODUCTION: Mounting evidence suggests that dysregulation of fatty acid synthase (FAS), the rate limiting multienzyme in the de novo formation of free fatty acids, is an early and important step in carcinogenesis and transformation to aggressive prostate cancer. Excess production of free fatty acids by FAS occurs through enhanced synthesis of malonyl-coA from acetyl-CoA and leads to increased cellular triglyceride formation and deposition. Thus we **hypothesize** that increased intraprostatic lipid concentration as measured by ^1H Magnetic Resonance Spectroscopy (MRSI) will identify tissues with higher FAS activity, which in turn will be those that exhibit more aggressive disease. In more aggressive cancer tissues, we expect to find metabolic signatures of enhanced fatty acid oxidation. In showing an association between FAS protein overexpression by histology, in-vivo intraprostatic fat as measured by ^1H MRSI, metabolic signatures of lipid oxidation and metabolism, and prostate cancer aggressiveness, our **objective** is to provide support for the novel application of this imaging modality for use in the clinical setting to determine the proper management of newly diagnosed prostate cancer. Specifically, among men diagnosed with low grade (proposed as more indolent) and high grade (proposed as more aggressive) prostate cancer (as determined by the 2011 National Comprehensive Cancer Network (NCCN) guidelines [6]) we propose to 1) determine the correlation between the amount of intraprostatic lipid using ^1H magnetic resonance spectroscopic imaging (MRSI) with an endorectal coil obtained prior to prostatectomy with FAS protein expression measured in benign and cancer tissue from prostatectomy samples; 2) identify the association between FAS protein expression in prostatectomy samples, intraprostatic lipid as measured by ^1H MRSI, and prostate tumor aggressiveness; and 3) quantify the association between key metabolic intermediates involved in lipid metabolism, mitochondrial function, inflammation, and apoptosis in prostatectomy samples and FAS protein expression, intraprostatic lipid and tumor aggressiveness.

BODY: Department of Defense funding to allow initiation of this project was set up and received locally at the end of January/ beginning of February, 2013. From that point forward we have made progress in meeting the following items from our statement of work as described below (full SOW attached).

Task 1. Finalize clinical protocol and training (Shannon & Purnell) Months 1-6

1. Develop tracking system for recording patient recruitment, contact and consent information; laboratory and specimen receipt and analysis. **(Shannon)**

This task has been completed. All patient records are recorded using the Progeny system. Security is assured by maintaining all identifiers on the VA computer system with a crosswalk to a random unique ID maintained in the tracking program.

2. Obtain IRB approval from Portland Veterans Affairs Medical Center (PVAMC) **(Shannon)** and Oregon Health & Science University (OHSU) **(Purnell)**

*This task has been completed. Portland VA Medical Center (PVAMC) IRB approval was received 9/9/2012. Oregon Health & Science University (OHSU) IRB approval was received on 12/28/2012. **2014 Update:** We initiated a move to the joint IRB (PVAMC+OHSU) in order to streamline all project modifications, assure we operated under the same protocol at all times as well as to minimize paperwork submission on 9/3/2013; our move was approved on 10/31/2013. **2015 Update:** JOINT IRB approval remains intact.*

3. Finalize and review services with Clinical and Translational Research Center (CTRC) bionutrition staff. **(Shannon & Purnell)**

We are not utilizing the CTRC bionutrition staff at this time. We are, however, utilizing the CTRC core laboratory to process our urine and blood specimens since the first subject's enrollment onto this study on March 8th, 2013. This SOW point has been modified to reflect this change in our study plan.

4. Arrange meetings between research coordinator and Advanced Imaging Research Center staff in order to: **(Purnell)**
 - a. Identify point of contact cascade
 - b. Collaboratively develop study-specific Standard Operating Procedures
 - c. Train all staff on following research protocol exclusively
 - d. Gather regulatory documents

*These tasks have been completed. Monthly research meetings are held with all study staff and the interdisciplinary investigational team. Point of contact has been identified for each step in the research process and an SOP has been developed for consistent recruitment of subjects and exchange of data from the urologist to the research coordinator to the MRSI technician and investigator to the pathologist (see Appendix 2; biopsy and MRSI measurement report form). Research protocol training has been completed with Ms. Farris and Mr. Stoller as well as all participating investigators. All regulatory documents are stored per VA protocol. **2014 Update:** As our clinical radiologist, Dr. Fergus Coakley, has emphasized, the accomplishment of assembling a multi-disciplinary team to meet on a monthly basis is of great import. We discuss the research process, its progress, provide quality improvement and care as well as maintain all aspects of the study as a cohesive group. Communication is clear, consistent and concise.*

We have new, supporting coordination staff; one recruiting participants from OHSU (Ms. Martinez), another from PVAMC (Ms. Palma). The addition of staff has increased our capacity for assuring subjects have project staff with them/ in the vicinity for the research visit at all times. Our coordinating staff assures the post-procedure handout for complications is provided, and is a reliable escort through campus and the imaging facility back to familiar ground. Additional procedures have been identified, streamlined and instituted over the course of the past year both at our monthly meetings as well as with subgroups intimately involved with subjects. This includes such procedures as 1) enemas must be completed a full hour prior to research MRSI with endorectal probe, 2) scheduling each subjects' imaging research visit for a full 2 hours in order to accommodate multiple steps in support of image capture process, 3) taking pictures of the prostate at the time of pathology processing and 4) assuring clinical radiology interpretation not only goes back to the imaging investigators but to the respective urologic surgeon as well. All changes to procedure were reviewed and approved by the IRB prior to implementation.

***2015 Update:** During more than one of our monthly, multi-disciplinary team meetings, we decided that it would be important to request a No-Cost Extension for this project.*

5. Review protocol and procedures with clinical staff; establish pathology residents' formal independent contracts **(Shannon)**

*Review of protocol and procedures has been an ongoing monthly task. Optimization of procedures has been ongoing and we have recruited 10 men whose data will only be utilized during this optimization time period. Independent contracts with the pathology residents occurred during the months of March and April 2013. **2014 Update:** Since our project's initial review and first*

DOD annual report, we have submitted 5 modifications and 1 continuing review. Please see 'Key Research Accomplishments' section for a full list and related descriptions. **2015 Update:** In the previous year, we submitted 3 modifications and 1 continuing review. Please see 'Key Research Accomplishments' section for a full list and related descriptions.

Task 2. Initiate subject recruitment and testing Months 6-30

1. Identify potentially eligible patients, contact men and initiate recruitment (**Shannon**)
2. Complete consenting process and confirm eligibility for interested men (**Shannon**)
3. Conduct fasting blood collection, magnetic resonance spectroscopy imaging (MRSI) visits and prostatectomy tissue processing (**Purnell**)

Progress towards completion of these tasks is ongoing; as of September 01, 2015, we have enrolled and consented a total of 51 men to the study. Since our last year's annual progress report, 20 men successfully completed an MRSI with no complications. We have three pending MRSIs, one withdrawal and six screen fails (that is, no successful MRSI). We have collected specimens on 42 men. At the time of our 2014 annual report, we estimated an average recruitment rate from OHSU of 2 men per month; in the past year, all of our 20 men were recruited from OHSU. Despite this fact, project recruitment has not reached the total level we hoped and planned for. However, it is notable that we were not able to begin our project until we received funding from the DOD, which did not occur until 6 months post-award notification. Therefore, we have submitted a request for a 1-year No-Cost Extension in order to recruit an additional 10 men to the project.

We presented one technical abstract at this year's International Society for Magnetic Resonance in Medicine annual meeting (1). During which, we reported quantitative correction for intraprostatic MRSI lipid spectra. It is well known that periprostatic lipid content is significantly higher than that from inside the prostate gland. Even though different, advanced techniques have been proposed to minimize the signal contamination from periprostatic lipid to intraprostatic lipid spectra, a contamination free approach is yet to be identified. In fact, our results indicate that the contamination from clinically-available sequences can be so great that it could render proton MR spectroscopy near the vicinity of lipid peak, which includes that for lactate, uninformative. This is because the function peaks, e.g., lactate, often have more than three magnitude weaker MR spectroscopy signals. Thus, a very small fraction of residual lipid peak can still be significantly larger than that of lactate. Based on this finding, we added up to six single voxel spectroscopy (SVS) data acquisitions into our MRSI data acquisition protocol in 2015. Most SVS voxel locations are within the respective MRSI slices, so a direct comparison of MRSI signal to the SVS signal is potentially feasible. In addition and in order to facilitate co-registration between MRSI slices and histological tissue slice, the IRB approved in vivo inking of the prostate just prior to prostatectomy. As stated in the 2015 IRB and Research Updates, below. The full research team is in the midst of compiling all variables in support of interim – and eventually, final – data analyses.

4. Compensate men for their participation in study (**Shannon & Purnell**)

Within a month of a man participating on our trial, they have either been compensated for a successful MRI and/or travel reimbursement. **2104 Update;** this task is ongoing and will continue until recruitment is complete. **2015 Update;** compensating men for their participation is an ongoing,

important task that will continue until the end of the project (into the requested No-Cost Extension year).

Task 4. Conduct immunohistochemistry analyses (Shannon) Months 12, 24, 32 (3 batches)

Dr. George Thomas requested that we submit prostatectomy specimens for immunohistochemistry analyses (*fatty acid synthase staining*) throughout the life of the project v. in large batches. Therefore, Dr. Thomas and Mr. Stoller have been meeting regularly to read research slides, submit specimens for cutting and staining and track Dr. Thomas' scoring, all in support of eventual alignment with all other variables and, ultimately, data analyses.

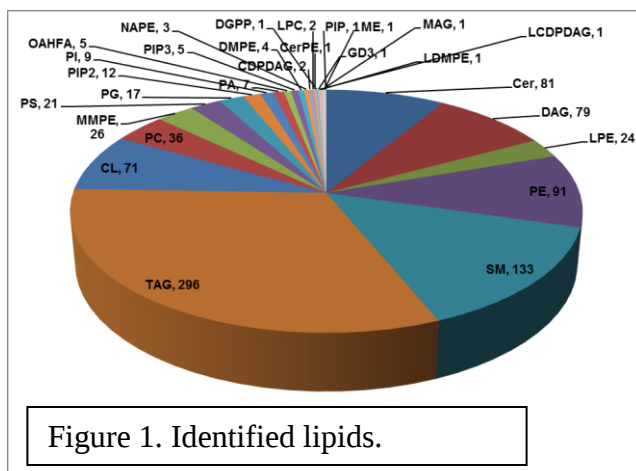
Task 5. Conduct metabolomics analyses (Purnell) Month 30-34

A subcontract for metabolomics analyses is in place with Drs. Arun Sreekumar and Nagireddy Putluri at Baylor Medical School. All tissue collected to date has been shipped to Dr. Sreekumar's metabolomics team. Baylor investigators will utilize our first 10 [test] subjects' specimens in order to optimize the quantification of fatty acid synthase expression and intraprostatic lipid accumulation in prostate cancer process but this data will not be used in final analyses. Additional specimens will be sent at the time of final subject recruitment.

Lipidomics Profiling of Case control samples

As described above, we have complemented lipidomics data for first test subjects using high resolution triple tof mass spectrometry. The samples were extracted using newly established protocol. We have spiked the equimolar 10uL (from 100pmol/uL stock) internal standard containing all lipids (17:0LPC, 17:0PC, 17:0PE, 17:0PG, 17:0 ceramide, 17:0SM, 17:0PS, 17:0PA, 17:0TG 17:0MG17:0 ChoE, D31 TAG and D5- DAG) Mix and 20uL 17:0PI (LM-1802), mix well. After extracted the samples were reconstitute with 100ul buffer B (85%IPA+5%H₂O+10%ACN) and inject 5uL (both positive and negative mode).

In order to quantify the lipids in frozen tissue specimens in an unbiased manner, we have undertaken extensive mass spectrometry-based method development employing independent data aquasions (IDA). Using this approach we have quantified 500 lipids across our first 10 [test] subjects' specimens (See Table 1 for class of lipids). The pie chart showing the % of each class of lipids, those were identified in the test subjects. These CVs are calculated as SD/Mean with the natural log transformed data. The values are much lower than the ones calculated with the original dataset because of the transformation. Total 222 lipids having less than 20% of CV across all the test samples.



Class of lipid	No of Lipids	Method
Cer	81	Positive mode
DAG	79	Positive mode
LPE	20	Positive mode
PE	48	Positive mode
SM	123	Positive mode
TAG	296	Positive mode
CL	71	Negative Mode
PE	43	Negative Mode
PC	36	Negative Mode
MMPE	26	Negative Mode
PS	21	Negative Mode
PG	17	Negative Mode
PIP2	12	Negative Mode
SM	10	Negative Mode
PI	9	Negative Mode
PA	7	Negative Mode
OAHA	5	Negative Mode
PIP3	5	Negative Mode
DMPE	4	Negative Mode
LPE	4	Negative Mode
NAPE	3	Negative Mode
CDPDAG	2	Negative Mode
LPC	2	Negative Mode
CerPE	1	Negative Mode
DGPP	1	Negative Mode
GD3	1	Negative Mode
LCPDAG	1	Negative Mode
LDMPE	1	Negative Mode
MAG	1	Negative Mode
ME	1	Negative Mode
PIP	1	Negative Mode

Table 1. Showing the identified lipids in test controls.

Key Metabolomics Research Accomplishments

- 1) Developed unbiased mass spectrometry methods to profile 500 lipids.
- 2) Completed lipidomics analysis of 9 test subjects.
- 3) Defined distinct class of lipids in tissue samples.

Metabolomics Research Conclusions; we have defined tissue-associated lipidomics profiles.

Task 6. Final Analyses and Report Writing (Shannon & Purnell) Months 30-36

Team members Dr. Xin Li (imaging), Dr. George Thomas (pathology) and Dr. Motomi Mori (biostatistician) are currently compiling all current variables in preparation of an interim statistical analysis of the immunohistochemistry and imaging measurements. This will be ongoing as we wrap up the final year of this project, particularly adding the metabolomics variables once completed.

Data Sources

An analytical data set was created by merging atty acid synthase (FAS) data and Gleason data by Subject ID. Because multiple single voxel spectroscopy (SVS) measures were made for each subject, the average SVS for tumor lesions and for normal lesions were calculated, then merged with FAS-Gleason data by Subject ID. The resulting analytical data set contained 41 subjects.

Data Summary

The target sample size is 100 subjects, 70 subjects with less aggressive (Gleason 3+4 or lower) prostate cancer and 30 subjects with aggressive prostate cancer (Gleason 4+3 or higher). By December 11, 2015, we have partial data from 41 subjects enrolled in the study (Table 2).

Table 2. Data availability as of December 10, 2015		
Variable	Data Available, N	% of Total (41)
Gleason Score	35	85%
FAS	23	56%
MRSI (SVS)	13	32%
Gleason, FAS and MRSI Available	3	7%
Metabolomics Data	0	0%

Among 35 patients with Gleason score, 19 patients (54%) had less aggressive cancer (Gleason 3+4), 16 patients (46%) had aggressive cancer (9 Gleason 4+3; 7 total Gleason 8 or higher). Because the study requires patients undergoing prostatectomy, none of the study subjects had a total Gleason score of 6 or less.

Metabolomics experiment will be performed for all samples at the same time to avoid a potential batch effect.

Preliminary Data Analysis

Because data are still actively collected and captured in the database, no statistical analyses are performed at this stage. Simple descriptive statistics are provided based on all available data (Table 3), and graphical comparison of tumor vs. normal lesions are shown for FAS and MRSI outcomes (Figures 2 and 3).

Table 3. Descriptive statistics of primary outcome variables				
Variable	N	Mean	ST. DEV.	Range
FAS_Tumor	20	218.5	61.7	110-300
FAS_Normal	23	118.3	43.2	50-200
MRSI_Tumor	10	0.208	0.005	0.015-0.034
MRSI_Normal	10	0.024	0.008	0.011-0.038

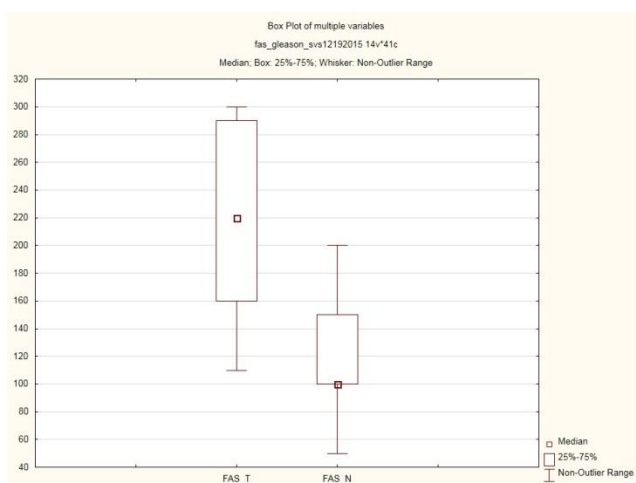


Figure 2. Box-plot of FAS protein expression in tumor (FAS_T) and normal (FAS_N) tissues

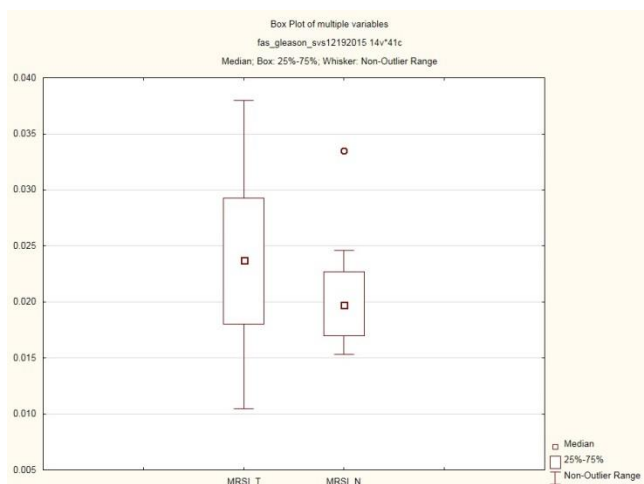


Figure 3. Box-plot of MRSI intraprostatic lipid in tumor (MRSI_T) and normal (MRSI_N) lesions

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

Year 01 – 2013

- Portland VA Medical Center (PVAMC) IRB approval as of 9/9/2012.
- Standing monthly investigational team meeting initiated 11/8/2012.
- Added Medical Monitor, Arthur Hung, MD to project 11/14/2012.
- Oregon Health & Science University (OHSU) IRB approval as of 12/28/2012.
- Initiation of enrollment; first participant consented to study 2/22/2013.
- Continuing review PVAMC IRB approval 3/12/2013.
- Modification to add safety ocular x-ray to study; PVAMC IRB approval 3/29/2013.
- Modification to add safety ocular x-ray to study; OHSU IRB approval 4/28/2013.
- Dr. Fergus Coakley, OHSU Diagnostic Radiology Chair agrees to collaborate, consult and share his MRI in prostate cancer expertise with the investigational team, 5/20/2013.
- Modification to exclude recently-prescribed statin users (i.e.: on statin drug for less than 6 months) from study, increase to number of men (to 140); PVAMC IRB approval 6/18/2013.
- As of August 29, 2013, 9 men consented to study; 1 pending MRSI, 6 successful MRSIs, 2 screen fails.

Year 02 - 2014 IRB and Research Updates

- As of last year's report, we have enrolled 30 additional men to this study. Our veteran participants number 12, OHSU's are 18; please note that the first 10 subjects will not be included in any data analyses (the first 10 subjects acted as our 'test' subjects while we optimized our research project's multi-level, multi-resource, multi-system processes). We have a total of 24 successful (and analyzable) MRSIs, 1 pending MRSI, 3 screen fails and 1 withdrawal.
- Based on the optimization work, a standardized protocol for imaging, obtaining and processing tissue and obtaining and storing biologic specimens was put into place and recruitment into the primary study began 11/22/2013.
- All scientific investigators, study staff and clinical investigators continued to meet monthly to discuss study progress, necessary changes and review the timeline for study analyses.
- PVAMC and OHSU IRBs joined forces to provide researchers with one system for human subjects review and monitoring for projects that operate at both institutions. We

submitted our modification to move the whole project to the 'joint' IRB as well as reconcile any remaining protocol differences; receiving JOINT IRB approval on 10/31/2013.

- Dr. Christopher Amling, OHSU Department of Urology Chair and surgical urologist agrees to collaborate and act as an addition recruitment site. Modification to add OHSU Department of Urology personnel (NP and Coord.) to the study; JOINT IRB approval on 11/7/2013.
- Due to confusion about the correct date to follow for continuing review, materials were submitted late for continuing review and the IRB approval lapsed for 3 weeks. All study activities were halted during this short time, regaining IRB approval on 1/21/2014.
- Dr. Mark Garzotto's conflict of interest form was incomplete and he was hence removed from the continuing review submission in order to rapidly re-gain IRB approval. Dr. Garzotto was re-added to the project on 3/24/2014.
- In order to communicate clearly and effectively with research participants, an enema instruction sheet was developed and approved by the JOINT IRB on 5/23/2014.
- At the time of the 2014 annual report update, a modification to add another OHSU Department of Urology clinician and update our post-MRI procedure handout is currently under JOINT IRB review.

Year 03 - 2015 IRB and Research Updates

- Mr. Wesley Stoller's name was added to our post-study visit handout in order to help triage any post-probe complications. We also added Dr. Theresa Koppie to the project, gaining JOINT IRB approval on 9/9/2014.
- Due to concerns raised by the OHSU pathology department over the availability of diagnostic tissue in men consented onto this study with small-focal tumor, we submitted a protocol modification at the time of our continuing review to narrow the eligibility criteria to men without singular small-focal tumor. In addition to this modification, we submitted paperwork to mark anatomical features of the prostate *in vivo* in order to better align the prostate with our MRI images during gross examination; one reviewing entity pushed back to remove this addition from our annual review paperwork. These modifications significantly delayed the continuing review submission. Therefore, our approval lapsed due to these issues; all study activities were halted during this short time, regaining JOINT IRB approval on 2/3/2015.
- We proposed inking the *in vivo* prostate in men (VA patients only) already sedated in preparation for prostatectomy. A second consent form for VA patients was approved. To date, we've had no VA patient consent to this research only process. We gained JOINT IRB approval for this option on 5/18/2015.
- Dr. Theresa Koppie left OHSU and was removed from the project; the JOINT IRB approved this personnel removal submission on 5/28/2015.

REPORTABLE OUTCOMES: None to date

CONCLUSION: *As of the time of this progress report, we are still awaiting word about our No-Cost Extension. Yet, at this stage, we believe that all the effort we've put into the development of processes and procedures to achieve our study aims is at its most efficient. We continue to meet regularly to discuss any subject difficulties and look forward to our interim analyses discussion. Completion of this portion of our project has laid the groundwork for successfully addressing our aims of correlating intraprostatic lipid as identified by MRSI with FAS protein expression in areas of high lipid content and with disease aggressiveness.*

REFERENCES:

1. X. Li, J. Shannon, M. G. Garzotto, C. Amling, W. J. Woodward, G. Thomas, E. Dacey, X. Wang, P. Farris, W. Stoller, A. M. Acevedo, A. Palma, M. Sammi, W. D. Rooney, F. V. Coakley, J. Purnell, "Intraprostatic Lipid Spectroscopic Imaging of the Prostate Cancer", Proc. Intl. Soc. Mag. Reson. Med. 23, 3838 (2015).

APPENDICES: None to date