Phase 1 Study: Detection of Brown Adipose Tissue in Normal Volunteers Using a 3 Tesla (3T) Magnetic Resonance Imaging System and Hyperpolarized (HP) Xenon Gas

NCT number NCT02220426
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SPECIFIC AIMS: In spite of the many efforts to raise awareness on the health complications of obesity, our obesity epidemic keeps growing, adding an immense cost to our health care system. At a fundamental level obesity is the result of an imbalance between energy intake and energy expenditure[1]. The latter is very difficult to quantify and recent work suggests that in humans it is partially modulated by the activity of Brown Adipose Tissue (BAT)[2,3]. BAT is a tissue with the unique capacity to regulate energy expenditure by a process called thermogenesis during which this tissue burns fat to produce heat[4]. By preventing excessive accumulation of triglyceride in non-adipose tissues such as muscle and liver, this tissue also helps to protect against insulin resistance and type 2 diabetes[5,6].

As ways to harness this tissue to address obesity and diabetes are being investigated, its non invasive detection in humans remains an unmet need. The current gold standard for the detection of BAT activity is FDG-PET. Unfortunately this technique has obvious limitations: 1) It cannot be used for longitudinal studies in healthy subjects due to concerns over radiation exposure, 2) It is not reproducible, and 3) It fails in detecting BAT in the target population, i.e. in overweight and obese subjects[3]. Other imaging modalities like MRI and CT have been tried, but aside from lacking specificity and their inability to detect BAT activity, they all share the same insensitivity to the lipid rich BAT present in overweight and obese subjects.

The goal of the proposed application is to provide a non-invasive imaging tool that is suitable to detect mass and metabolic activity of BAT in both lean and obese human subjects. Our group was the first to demonstrate the use of hyperpolarized xenon gas MRI to specifically and directly detect BAT mass and thermogenic activity in lean and obese mouse phenotypes, with unprecedented sensitivity in the latter. The objective of the current proposal is to validate this technique in humans. To this end we propose the following aims:

Specific Aim 1: Demonstrate BAT mass detection in human subjects. For this aim we will implement hyperpolarized xenon imaging on our clinical scanner and we will compare the images obtained before stimulation of BAT thermogenesis with those obtained during stimulation of BAT thermogenesis. Our working hypothesis is that, during stimulation of BAT thermogenesis the lipophilic gas will accumulate in the highly perfused BAT tissue enabling us to clearly delineate it.

Specific Aim 2: Demonstrate detection of BAT thermogenesis in humans. For this aim we will measure BAT temperature by measuring the chemical shift of lipid-dissolved xenon. Our working hypothesis is that, during stimulation of BAT thermogenic activity, changes in BAT temperature would be detected as a shift in the resonance frequency of lipid-dissolved xenon.

By the end of the proposed project we expect to have demonstrated the feasibility of HP xenon

MRI detection of human BAT mass and thermogenic activity. These studies will also provide preliminary data for powering a future clinical trial with which we aim to investigate the incidence and activity of this tissue in the human population.

SIGNIFICANCE: Most of our knowledge on BAT comes from animal studies[8]. In small animals we know that BAT prevents obesity and improves and reverses insulin insensitivity[6]. In humans the role of this tissue is less clear[9,10]. This is because human BAT studies have been limited mainly to retrospective studies on cancer patients due to lack of safe and reliable detection techniques to study the small quantities of diffusely distributed BAT depots present in humans *in vivo*[11]. Fusion FDG-PET/CT remains the gold-standard imaging modality for non-invasive studies of metabolically active BAT. However, this technique is intrinsically insensitive to BAT metabolism as only small amounts (less than 10%) of glucose are taken up by activated BAT[11]. The rest of the substrate (more than 90%) is made by fatty acids released from intracellular lipid storages[10]. As such, the activity of this tissue is clearly underestimated by FDG-PET scans[7,12]. This is especially true for overweight and obese subjects, the target population for BAT[3,10], in whom the intracellular lipid storage is more abundant (brown fat cells are swollen with fat) than in lean subjects. Therefore, this tissue relies even less on circulating glucose for thermogenesis, further limiting its detectability by FDG-PET scans[7,12,13]. More importantly, the elevated risk of ionizing radiation and radionuclide tracer radiation exposure from these scans precludes repetitive BAT screening in healthy and young subjects, scans which are needed to correctly estimate the true prevalence of BAT, to determine the physiological relevance of BAT in humans[14,15], and to test the efficacy of new anti-obesity treatments. With this proposal we aim to fulfill this need. HP xenon MRI is extremely sensitive to the more lipid rich and less thermogenically active BAT of obese phenotypes (see comparison with FDG-PET in Fig 1) and, by being non invasive, can be repeatedly used on healthy subjects to track BAT function over time. In addition it allows direct measurement of BAT cell temperature (Fig. 2) and therefore represents the only direct way we have to monitor BAT thermogenic activity.

INNOVATION: MRI with hyperpolarized xenon-129 is a technique that is been used extensively in humans to detect lung ventilation function[16]. Here we propose for the first time to use hyperpolarized xenon gas MRI to detect BAT. Xenon is an ideal BAT probe for several reasons: A) the solubility of xenon in fatty tissue is 10 times higher than in lean tissues or blood[17]. This means that circulating xenon naturally diffuses into BAT, enabling adequate SNR for imaging. B) Xenon is an ideal blood perfusion tracer, and therefore ideally suited to detect the increase in blood flow to BAT that occurs during stimulation of BAT thermogenesis. C)

Because xenon is not intrinsic to biological tissue, HP ^{129}Xe can produce background free maps of human BAT. D) The temperature sensitivity of lipid-dissolved xenon will enable us to directly measure BAT temperature and monitor thermogenic activity in real time.

This research is innovative as it uses an entirely different approach to investigate BAT. To our knowledge we are the very first group to use hyperpolarized xenon gas to detect BAT mass and to have demonstrated HP xenon MR thermometry of fatty tissues.

APPROACH: Aim1: Demonstrate BAT mass detection in human subjects.

Our extensive preliminary studies in rodents have shown that, upon stimulation of BAT thermogenesis, an enhancement of more than 10-fold of the MR signal from xenon dissolved in BAT is observed. This enhancement is such that a background free map of this tissue can be obtained in just few seconds (Fig. 1). In addition, when compared to FDG-PET images of BAT, hyperpolarized xenon gas images were able to clearly show BAT in both lean and obese mice and, in the latter, with superior sensitivity (Fig.1b-d).

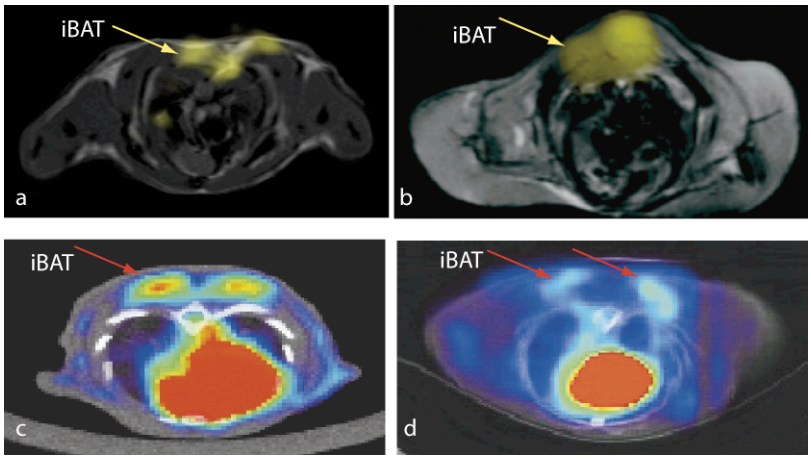

Fig. 1 Detection of interscapular BAT by hyperpolarized xenon gas MRI (a-b) and FDG-PET (c-d) in a lean (a-c) and obese (b-d) animal. Hyperpolarized xenon gas is clearly more sensitive to the lipid-rich BAT of obese animals (b) than FDG-PET. In d) glucose uptake by BAT is substantially reduced, making it difficult to differentiate BAT from the surrounding tissue.

The broad aim of this proposal is to validate this completely new approach to detect BAT in humans. To this end we will perform HP 129Xe-MRI of BAT on a 3T clinical magnet using a dual tuned xenon/1H surface coil recently purchased for human BAT studies. The coil will be accurately located above the supraclavicular area to increase sensitivity to the xenon signal dissolved in the tissue beneath. Conventional ^{1}H MRI, which will provide anatomical reference images, will be followed by HP 129Xe spectroscopy and imaging scans, similar to those used in our rodent studies. Before the acquisition of the xenon images, the subject will be asked to inhale a 1L bag of 100% hyperpolarized and enriched xenon from a Tedlar bag and to hold his breath for about 15-20s. During this time hyperpolarized xenon gas will diffuse from the lung airspaces to BAT in a perfusion-based manner. At the end of the 10 s from the beginning of gas inhalation we will trigger the acquisition of the MR image, which will last for about 10s. The

same protocol will be followed during stimulation of BAT thermogenesis, which will be achieved by an MR compatible water cooling-blanket (~15^{o}C circulating water temperature), which will be positioned on the lower abdomen/upper leg part of the subjects. The ability to detect BAT by hyperpolarized xenon gas relies on the enhancement in blood flow to this tissue that occurs during stimulation of thermogenic activity. The magnitude of this enhancement seems to be the same, irrespective of the species and of the modality of stimulation of BAT thermogenesis (NE injection of mild cold exposure). Therefore we expect to detect a similar enhancement in the MR signal from xenon dissolved in human BAT. In addition, the use of enriched xenon versus naturally abundant xenon will compensate for the possible loss in xenon polarization during blood transit time. Pre and post stimulation scans will be compared with Matlab to detect differences of more than 5% in the intensity of the dissolved phase xenon images. Detectable BAT volume will be calculated as the sum of pixels whose intensity rose by more than 5% during cold stimulation multiplied by the pixel volume (1 mm^3). Data will be analyzed using JMP Pro-9.0.0 software (SAS Institute). All P values presented will be two-tailed, and values less than 0.05 will be considered to indicate statistical significance. According to previous studies done in human brain[18,19] and considering the increase in blood perfusion that occurs in BAT during cold stimulation[20] as well as the higher solubility of xenon in fatty tissues, we expect to be able to obtain images with a resolution of 0.3x0.3cm, high enough to differentiate BAT from the surrounding tissues.

Aim 2: Demonstrate detection of BAT thermogenesis in humans.

Dynamic spectroscopy data have shown the possibility to track BAT temperature over time by measurement of the chemical shift of lipid-dissolved xenon. We demonstrated for the first time MR thermometry by hyperpolarized xenon gas. This method is based on the temperature dependence of the chemical shift of lipid dissolved xenon, which we measured for the first time *in vivo* to be -0.2ppm/^{o}C,(Fig 2.a), i.e. 20 times larger than that of water protons currently used for MR thermometry in lean tissues. This higher sensitivity of lipid-dissolved xenon to temperature, coupled with the lower gyromagnetic ration of xenon (which makes xenon less sensitive to possible magnetic field in homogeneities present in fatty tissues), allowed us to detect BAT thermogenic activity in mice (Fig.2.b). To this end we will acquire dynamic spectroscopy data from xenon dissolved in BAT during stimulation of BAT thermogenesis by mild cold exposure. During these scans we will track the resonance frequency of lipid-dissolved xenon to detect changes in BAT temperature during thermogenic activity. Although in humans we expect temperature changes less than 2 ^{o}C, the high sensitivity of xenon to temperature and the narrow line width can allow us to detect changes as small as 0.2^{o}C.

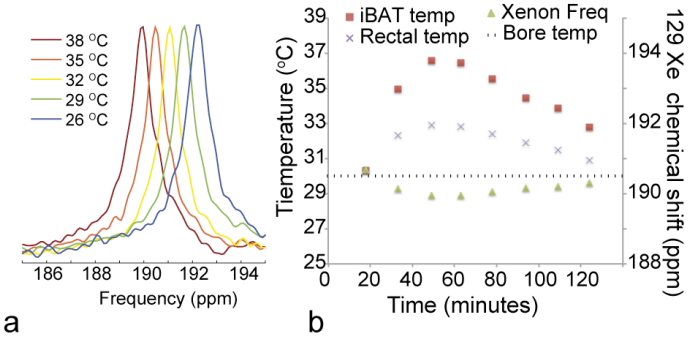

Fig.2 a) Linear temperature shift of the lipi-dissolved xenon frequency as acquired *in vivo* in an obese mouse. b. Thermogenic activity as detected by hyperpolarized xenon gas. The graph shows the resonance frequency shift of lipid-dissolved xenon and the corresponding shift in BAT temperature right after injection of NE.

For these studies the subject will inhale 4 doses of 1L each of xenon during mild cold exposure, achieved with a water cooling blanket. After inhalation, the subject will be asked to hold his breath for 15-20 seconds, during which we will trigger the acquisition of xenon spectra. The spectra will then be fitted with homemade Matlab routines to extract lipid-dissolved xenon resonance frequency that will be used to detect any change in BAT temperature.

The change in BAT temperature will then be analyzed as function of BMI to establish whether or not there is an association between BMI and BAT temperature increase (Pearson correlation and Spearman correlation coefficient calculation).

This feasibility study will allow us to estimate the standard deviation for the increase in signal from xenon dissolved in BAT and for the increase in BAT temperature that will then be used to power future trials to investigate the incident of this tissue in the healthy human population and the association between BMI and BAT thermogenic activity. For these aims we plan on enrolling a total of 24 healthy subjects (12 for aim 1 and 12 for aim 2) with a BMI ranging between 18 and 28. Twelve is the minimum sample size recommended in the absence of prior information on which to base our sample size calculation[21]. With 12 subjects per group we should be able to obtain positive results (with a 95% confidence interval) on at least 9 subjects if the true prevalence of BAT is 90%[22] (expected prevalence), and on at least 3 subjects if the true prevalence of BAT is much smaller and close to 50% (estimated by FDG-PET scans[7,23]).

By the end of the study we expect to have fully assessed the potential of HP xenon to detect BAT mass and activity and to have developed an imaging protocol that can be used in studies aimed to investigate the prevalence of this tissue in humans, its connection with other metabolic diseases such as diabetes, and to study the response to treatments aimed to counteract obesity by increasing BAT mass and or function.

References

1 Himms-Hagen, J. Brown adipose tissue thermogenesis, energy balance, and obesity. *Canadian Journal of Biochemistry and Cell Biology* **62**, 610-617, doi:10.1139/o84-081 (1984).

2 Wang, J. *et al.* Ablation of LGR4 promotes energy expenditure by driving white-to-brown fat switch. *Nat Cell Biol* **15**, 1455-1463, doi:10.1038/ncb2867 http://www.nature.com/ncb/journal/v15/n12/abs/ncb2867.html - supplementary-information (2013).

3 Vijgen, G. H. E. J. *et al.* Brown Adipose Tissue in Morbidly Obese Subjects. *PLoS ONE* **6**, e17247, doi:10.1371/journal.pone.0017247 (2011).

4 Foster, D. O. Quantitative contribution of brown adipose tissue thermogenesis to overall metabolism. *Can J Biochem Cell Biol.* **62**, 618-622 (1984).

5 Silva, F. J. *et al.* Metabolically Active Human Brown Adipose Tissue Derived Stem Cells. *STEM CELLS* **32**, 572-581, doi:10.1002/stem.1595 (2014).

6 Gunawardana, S. C. & Piston, D. W. Reversal of Type 1 Diabetes in Mice by Brown Adipose Tissue Transplant. *Diabetes* **61**, 674-682, doi:10.2337/db11-0510 (2012).

7 Lee, P., Greenfield, J. R., Ho, K. K. Y. & Fulham, M. J. A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. *American Journal of Physiology - Endocrinology And Metabolism* **299**, E601-E606, doi:10.1152/ajpendo.00298.2010 (2010).

8 CANNON, B. & NEDERGAARD, J. Brown Adipose Tissue: Function and Physiological Significance. *Physiological Reviews* **84**, 277-359, doi:10.1152/physrev.00015.2003 (2004).

9 Nedergaard, J., Bengtsson, T. & Cannon, B. Unexpected evidence for active brown adipose tissue in adult humans. *American Journal of Physiology - Endocrinology And Metabolism* **293**, E444-E452, doi:10.1152/ajpendo.00691.2006 (2007).

10 Ouellet, V. r. *et al.* Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *The Journal of Clinical Investigation* **122**, 545-552 (2012).

11 Nedergaard, J. & Cannon, B. The Changed Metabolic World with Human Brown Adipose Tissue: Therapeutic Visions. *Cell Metabolism* **11**, 268-272.

12 Lee, P. *et al.* High Prevalence of Brown Adipose Tissue in Adult Humans. *Journal of Clinical Endocrinology & Metabolism* **96**, 2450-2455, doi:10.1210/jc.2011-0487 (2011).

13 Cannon, B. & Nedergaard, J. Yes, even human brown fat is on fire! *The Journal of Clinical Investigation* **122**, 486-489, doi:10.1172/jci60941 (2012).

14 Brix, G. *et al.* Radiation Exposure of Patients Undergoing Whole-Body Dual-Modality 18F-FDG PET/CT Examinations. *J Nucl Med* **46**, 608-613 (2005).

15 Huang, B., Law, M. W.-M. & Khong, P.-L. Whole-Body PET/CT Scanning: Estimation of Radiation Dose and Cancer Risk1. *Radiology* **251**, 166-174 (2009).

16 Fain, S. B. *et al.* Functional lung imaging using hyperpolarized gas MRI. *Journal of Magnetic Resonance Imaging* **25**, 910-923, doi:10.1002/jmri.20876 (2007).

17 Cherubini, A. & Bifone, A. Hyperpolarised xenon in biology. *Progress in Nuclear Magnetic Resonance Spectroscopy* **42**, 1-30, doi:citeulike-article-id:4275368 doi: 10.1016/S0079-6565(02)00052-3 (2003).

18 Kilian, W., Seifert, F. & Rinneberg, H. Dynamic NMR spectroscopy of hyperpolarized 129Xe in human brain analyzed by an uptake model. *Magnetic Resonance in Medicine* **51**, 843-847, doi:10.1002/mrm.10726 (2004).

19 Kilian, W., Seifert, F. & Rinneberg, H. in *International Society of Magnetic Resonance in Medicine* (Honolulu, Hawaii, 2002).

20 Orava, J. *et al.* Different Metabolic Responses of Human Brown Adipose Tissue to Activation by Cold and Insulin. *Cell Metabolism* **14**, 272-279 (2011).

21 Julious, S. A. Sample size of 12 per group rule of thumb for a pilot study. *Pharmaceutical Statistics* **4**, 287-291, doi:10.1002/pst.185 (2005).

22 van Marken Lichtenbelt, W. D. *et al.* Cold-Activated Brown Adipose Tissue in Healthy Men. *New England Journal of Medicine* **360**, 1500-1508, doi:doi:10.1056/NEJMoa0808718 (2009).

23 Saito, M. *et al.* High Incidence of Metabolically Active Brown Adipose Tissue in Healthy Adult Humans: Effects of Cold Exposure and Adiposity. *Diabetes* **58**, 1526-1531, doi:10.2337/db09-0530 (2009).

The present project will be HIPAA compliant and will be performed under a protocol approved by the UNC Chapel Hill Institutional Review Board (IRB) and under an FDA IND approval.

A. Proposed involvement of human subjects

All research involving human subjects will be carried out at the University of North Carolina at Chapel Hill. All MRI experiments will take place in the Biomedical Research Imaging Center, where the PI's lab for hyperpolarized 129Xe gas MRI and the 3T Siemens MRI scanner is located. A medical technician, with knowledge and skills consistent with those provided in the American Heart Association's Advanced Cardiac Life Support training will observe the subjects whenever they are breathing xenon gas. Supplemental oxygen (adjusted to flow at 4 liters/minute), suction source, bag valve mask, ventilation device and an airway support device will be available in the MR scanner room. During scanning, a MRI compatible pulse oximeter will be used to monitor the subject's heart rate and blood oxygenation.

A total of 24 healthy subjects, without any metabolic disease, of ages 18 to 40 will be recruited to participate. All subjects will undergo hyperpolarized xenon-129 MR. Healthy subjects are considered those who have never being diagnosed with metabolic diseases, and have no history of any chronic lung disease such as asthma, COPD, or CF.

Since our aim is devoted to test the efficacy of the new methodology for the detection of BAT, there is no justification to scan children under 18 years old. Pregnant or lactating women will be excluded due to potential health risks. All subjects must be able to undergo an MRI examination (e.g., no MRI incompatible implanted devices or other MR contra-indications).

Dr. Branca will be present during all hyperpolarized xenon-129 MR scans.

<u>Practice xenon inhalation for subjects before placement into the MR scanner:</u>

Individual subject sensitivity to xenon inhalation will be practiced before MR imaging. The subject will be directed to breathe 1-L of undiluted xenon (natural isotopic abundance) and to hold his breath for 15-20s. Oxygen saturation, heart rate, and blood pressure will be continuously recorded. If the effects of xenon inhalation are deemed intolerable by the subject the amount of xenon will be lowered. If the blood oxygen saturation drops significantly (5%) below the subject's baseline and/or the pulse rate rises by 40 bpm, the practice session will be interrupted to explore the cause. If the cause cannot be identified and rectified, the subject will be excluded from the study.

<u>Hyperpolarized xenon-129 MRI:</u>

Subjects will be positioned supine within the MR scanner. The dual tuned xenon/proton surface coil will be positioned above the supraclavicular area. After breathing normally for a few minutes, the subject will exhale completely to residual volume, and then inhale a 1-L volume of undiluted hyperpolarized xenon-129, followed by a breath-hold lasting from 10-15 s during and after which imaging will be performed. The subject will then exhale fully and breathe normal air. Xenon doses will be administered no less than 15 minutes apart. During the MRI exam, oxygen saturation, heart rate, and blood pressure will be continuously recorded. These parameters, along with respiratory rate and subjective symptoms, will also be assessed after each dose. Immediately after inhalation, the subject will also be asked to report any symptoms or effects from xenon.

Source of material

The research material obtained from the subjects will be the results of MR studies. This information will be used solely for research.

Subject recruitment

Subjects will be recruited through advertisements, posters, and/or flyers in clinics and/or newspapers. The research procedure will be fully described and potential volunteers will be offered the opportunity to ask questions and read the protocol and consent form. No individual in a supervisory relationship with an investigator will be a participant (employees of an investigator, students in classes with the investigator, or those pursuing a degree under the investigator's direction). Candidates for participation will be provided with our informed consent form and given sufficient time to read it. A physician or a nurse coordinator will verbally review the major points and ask for questions. Those who wish to participate will give their consent by signing the form.

B. Potential risks

B1. Risks of the MR scan:

For subjects that are properly screened for the specific contraindications to MR imaging, it is generally believed at this time that the health risks associated with the short-term exposure to radiowaves and high magnetic fields as required for an MR imaging examination are insignificant.

B2. Risks of xenon inhalation:

Xenon is a general anesthetic when breathed continuously for long periods of time (minutes) at concentrations greater than 50% (anesthetic dose ~70%). There is also the theoretical risk that HP 129Xe, if given in sufficiently large volumes in forms that do not include oxygen, could cause or exacerbate hypoxia in a subject. However, a recent clinical trial performed at Duke University, were the same protocol was used to image lung ventilation function, has not caused hypoxia but only mild, transient, xenon-related symptoms, consistent with its known anesthetic properties, such as dizziness, paresthesia, euphoria, and hypoesthesia that have resolved soon after discontinuing gas administration(*1*).

B3. Purity of laser polarized xenon:

The purity of the laser polarized gases is very high because:

1. The initial source of xenon has a purity of at least 99.99%;
2. Only nitrogen is added to the gas before laser polarization;
3. Only room air and oxygen are added to the gas after laser polarization;
4. All chambers into which the gases are transferred are always pumped to a vacuum before gas is transferred into them; and
5. The rubidium vapor used during the polarization process is removed by allowing the polarized gas to pass through either a Teflon filter or a cold trap.

Before xenon is laser polarized, the polarization chamber and connecting tubes are pumped to a vacuum to ensure there are no contaminants that would interfere with the polarization process. For noble-gas laser polarization, xenon, together with nitrogen, which acts as a buffer gas, are then added to the polarization cell. The xenon gas is supplied from specially obtained gas cylinders with a minimum purity of 99.99%. Already present in the polarization chamber is a thin layer of solid rubidium. During the laser polarization, the polarization chamber is heated in order to create an appropriate vapor pressure of rubidium. The laser then excites the rubidium which then exchanges its energy with the noble gas (xenon) to create the laser polarized noble gas. Once polarized, the noble gas is allowed to expand out of the polarization chamber into a glass storage cell, through a Teflon filter or cold trap that removes any remnant rubidium. Before the storage cell is used, however, it is pumped out to a vacuum to remove any gaseous contaminants that might reduce the lifetime of the polarization. Polarized xenon-129 gas is then transferred into a previously evacuated plastic bag. The subject then breathes the undiluted xenon gas through a disposable one-way valve and a connecting tube to the plastic bag.

C. Adequacy of protection against risks

C1. MR Safety Considerations:

All potential subjects will be familiarized with the MR scanning procedure and will be excluded if they are bothered by the associated noise and confined spaces, or based on the standard exclusion criteria on our MR screening forms. All MR pulse sequences will conform to standard safety guidelines in terms of radio-frequency (RF) power deposition and rate of change of magnetic field (dB/dt).

C2. Xenon Inhalation:

The xenon dosing strategy is designed to minimize the previously described xenon physiological effects from the inhalation. In addition, a licensed medical professional will observe the subject whenever they are breathing xenon, and MR compatible monitoring equipment and supplemental oxygen and other equipment for artificial ventilation will be available. In addition, there will be practice sessions to assess the subject's response to the gas mixture. If there is any significant discomfort by a subject, he/she will be excluded from the project.

C3. Volunteer Confidentiality:

All MRI images, as well as all records of research, will contain only a study code number and no subject identifying information. Research records will be kept in password protected computer files and will only be available to investigators. The key between subject name and study number will be kept on a flash drive that is stored in a locked file cabinet.

D. Potential benefits of the proposed research to subjects and others and importance of the knowledge to be gained

Subjects will receive no direct benefit from participating in this study. Nonetheless, the risks to the subjects from participating are very low as discussed above. This very low, but non-zero, risk to the study subjects is reasonable considering that success of proposed research will yield high societal benefit. Hyperpolarized-gas MRI has already shown substantial potential for providing medically-relevant information, unmatched by any other modality, about important lung diseases such as COPD and asthma. If successful, the proposed research will open a completely new venue for hyperpolarized xenon-129 MRI which, by virtue of producing well defined maps of human BAT and by measuring its temperature non invasively, can provide insights on the mass and function of this tissue in human population. This study will lay the foundation for future application of this technique for purposes such as (i) improving our

understanding of the physiological relevance of this tissue in human populations and its connection with other metabolic diseases. (ii) monitoring BAT function in response to obesity interventions.

E. Inclusion of Women and Minorities

In keeping with the NIH policy to provide equal participation opportunities for women and for members of minority and ethnic groups, we will ensure that recruitment announcements are accessible to a diverse range of potential subjects, for instance by posting announcements at women's health sites and in Spanish.

No specific gender or racial or ethnic group will be excluded or favored. Based on the racial and ethnic composition estimates for the University of North Carolina at Chapel Hill area it is anticipated that roughly 10% of the study subjects will be from racial minorities and roughly 6% will be of Hispanic or Latino origin. We are aware of no evidence to suggest that the outcome of our evaluations of these new imaging methods would be affected by the racial or ethnic composition of the study population and thus, while we will take steps to ensure equal participation opportunities as discussed above, there is no scientific basis for targeting the enrollment of a specific group in numbers higher than those represented in our underlying population.

F. Number Justification

A total of 24 subjects will be enrolled in this study. 12 to complete specific aim 1 and 12 to complete specific aim 2. Twelve is the minimum sample size recommended in absence of prior information on which to base our sample size calculation(*2*). With 12 subjects per group we should be able to obtain positive results (with a 95% confidence interval) on at least 9 subjects if the true prevalence of BAT is 90%(*3*) (expected prevalence), and on at least 3 subjects if the true prevalence of BAT is much smaller and close to 50% (estimated by FDG-PET scans(*4*).

1. B. Driehuys *et al.*, Chronic Obstructive Pulmonary Disease: Safety and Tolerability of Hyperpolarized 129Xe MR Imaging in Healthy Volunteers and Patients. *Radiology* **262**, 279 (January 1, 2012, 2012).
2. S. A. Julious, Sample size of 12 per group rule of thumb for a pilot study. *Pharmaceutical Statistics* **4**, 287 (2005).
3. W. D. van Marken Lichtenbelt *et al.*, Cold-Activated Brown Adipose Tissue in Healthy Men. *New England Journal of Medicine* **360**, 1500 (2009).

4. M. Saito *et al.*, High Incidence of Metabolically Active Brown Adipose Tissue in Healthy Adult Humans: Effects of Cold Exposure and Adiposity. *Diabetes* **58**, 1526 (July 1, 2009, 2009).

Program Director/Principal Investigator (Last, First, Middle): Branca, Rosa, Tamara

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 06/01/2014	THROUGH 05/31/2015

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	PD/PI							
Research Assistant		4	4		24,025	7,600	1,040	8,640
SUBTOTALS →						7,600	1,040	8,640

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* 3T scan time Enriched Xenon gas mixture Volunteer time	19,968 12,240 1,440
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		**$** 42,288
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 42,288

Budget Justification

Personnel Justification:

Zhang Le,Graduate Research Assistant (4 calendar months). The graduate Assistant will be involved in data collection and data analysis.

Juanita Ramirez,Study Coordinator/Research (2 calendar months). She will be responsible for subject recruitment and screening, and will contribute to preparation and updating of human subject IRB protocols for this project. She will also attend the entire study session. She will be paid by the PI discretionary funds.

Other Direct Costs

-Scan time ($19,968)

Imaging and spectroscopy experiments in humans will be performed in the Biomedical Research Imaging Center, in which the cost for 1h of scan time is set at $416. For each subject, the entire imaging session will last for about 2 hours. Therefore funding for a total of 48 hours of MRI scan time are requested. (($416/hour scan time x 3 hours MRI session x 10subjects)

-Subject study compensation ($1,920)

Each subject will receive a compensation of $60 for the entire 2 hours imaging session.

-Enriched Xenon gas mixture ($12,240)

During the imaging study session the subject will receive two doses of 1L of HP Xenon, spaced 40 minutes apart (40 minutes is the minimum time required to produce a 1L of hyperpolarized xenon gas). For the spectroscopy study the subject will receive a total of 4 0.5L of hyperpolarized xenon every 20 minutes. Funds are therefore requested for 4800L of 1% enriched 129Xe (quoted at $5,100 per 2000L cylinder by Linde specialty gas) that will lead to a total of 48L (2L per subject x 12 subjects) of enriched Xenon.

The cost of the non-enriched xenon gas as well as the cost of the other gases needed for the xenon polarizer will be covered in its entirety by the PI funds.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Branca, Rosa Tamara	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) **RTBRANCA**	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
La Sapienza University of Rome, Italy	BS	05/2002	Physics
La Sapienza University of Rome, Italy	PhD	06/2006	BioPhysics
Duke University, US	PostDoc	2006-2009	Chemistry-Biochemistry

A. Personal Statement

The goal of our proposed research is to validate the use of hyperpolarized xenon gas MRI for the detection of human BAT mass and function. My graduate research training was largely on Magnetic Resonance Spin Dynamics, with an emphasis on nonlinear effects, under the guidance of Dr. Warren S. Warren, first at Princeton University, and then at Duke University. I have been trained in hyperpolarized gas imaging during my postdoctoral years at Duke University, when I developed a unique imaging method based on the use of hyperpolarized gas and super paramagnetic iron oxide contrast agents to detect lung nodules with high specificity and sensitivity, which I have carried through to a funded R01 grant (R01 CA142842). The success of my work and my ability to conduct research independently was a major factor in my promotion to the rank of Assistant Research Professor in Chemistry at Duke. Two years ago, while still at Duke, I received an R21 grant to develop an MR-based method to detect BAT mass and function. During this time I discovered the possibility to detect this tissue using hyperpolarized gas. This methodology, for which an invention disclosure has been filed, can overcome major limitation of 1H MR and PET based methods such as partial volume effects. More interestingly, this methodology seems to be particularly suited to detect BAT in obese individuals, where brown adipocytes lose their characteristic morphology and become similar to white adipocytes. After moving to UNC at the beginning of the year, I continued my work on BAT imaging using hyperpolarized xenon gas MRI. To this end I used most of my startup funds to purchase a gas polarizer and xenon coils, with which we have obtained our preliminary results in mice and rats. A surface xenon coil for the 3T, that will be used for both rodents and humans, has also been purchased under the R21 grant and an IND submission has been filed to FDA to perform these studies in humans.

We are the first group to propose and use hyperpolarized xenon gas to image brown adipose tissue. My research experience in BAT and nuclear magnetic resonance make me a unique leader of the proposed project. I also believe that my research will seed a much broader interest in the application of hyperpolarized gas imaging at UNC.

Positions and Honors

Positions:

2002-2006 Graduate Research Assistant Dept. of BioPhysics, “La Sapienza”, Rome, Italy
2003-2006 Visiting Graduate Student, Dept. of Chemistry, Princeton University, Princeton, NJ
2006-2009 Post-doctoral Associate, Center for Molecular and Biomolecular Imaging, Duke University, Durham,NC
2009-2011 Assistant Research Professor, Dept. of Chemistry, Duke University, Durham, NC

2012- Assistant Professor, Dept. of Physics and Astronomy & Biomedical Research Imaging Center University of North Carolina at Chapel Hill, Chapel Hill, NC

Honors:
1996-2000 Undergraduate student grant, “La Sapienza” University, Rome, Italy
2000 Teaching Assistant, Physic Department, “La Sapienza” University, Rome, Italy
2002 “Summa cum laude”, BA Degree in Physics, “La Sapienza” University, Rome, Italy
2002 Research grant, “La Sapienza” University, Rome, Italy
2002 Doctoral research grant in Biophysics, “La Sapienza” University, Rome, Italy
2004 Student Travel Stipend Award, Experimental Nuclear Magnetic Resonance Conference
2004 Student Travel Stipend Award, International Society of Magnetic Resonance
2004 Best poster presentation award for imaging in-vivo NMR, International Society of Magnetic Resonance
2005 Student Travel Stipend Award, Experimental Nuclear Magnetic Resonance Conference
2005 Student Travel Stipend Award, Magnetic Resonance in Medicine
2005 Marie Curie grant, Experimental European Nuclear Magnetic Resonance Conference
2007 Student Travel Stipend Award, Experimental Nuclear Magnetic Resonance Conference
2007 Student Travel Stipend Award, Magnetic Resonance in Medicine
2008 Student Travel Stipend Award, World Molecular Imaging Conference

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C. Selected Peer-reviewed Publications

Most relevant to the current application

1. **RT Branca**, T He, L Zhang, C Floyd, M Freeman, C White, “Detection of Brown Adipose Tissue Mass and Thermogenic Activity by Hyperpolarized Xenon MRI”, PNAS (under review).
2. **RT Branca**, WS Warren, A Khanna, S Degan, K Ugurbil, E Auerbach, R Maronpot, “In Vivo Noninvasive Detection of Human Brown Adipose Tissue through Non-Linear MRI”, PlosOne 8(9) (2013).
3. A. Khanna, **R.T. Branca**, “Detecting brown adipose tissue activity with BOLD MRI in mice”, Magn. Reson. in Medicine, Journal of Magnetic Resonance in Medicine, 68(4) 2012.PMID: 22231619
4. **R.T. Branca,** W.S. Warren, "In vivo brown adipose tissue detection and characterization using water-lipid intermolecular zero-quantum coherences", Magn Reson Med, 65 (2), 313-9 (2011). PMID: 20939093
5. **R.T. Branca,** W.S. Warren, "In vivo NMR detection of diet induced changes in adipose tissue composition", J Lipid Res, 52 (4), 833-9 (2011). PMID: 21270099
6. **R.T. Branca**, Z.I. Cleveland, B. Fubara, C. Kumar, C. Leuschner, R.R. Maronpot, W.S. Warren, B. Driehuys, “Molecular MRI for sensitive and specific detection of lung metastases”, PNAS 107(8):3693-7. PMID: 20142483

Additional recent publications of importance to the field (in chronological order)

7. **R.T. Branca**, E.R. Jenista, W.S. Warren, "Inhomogeneity-free heteronuclear iMQC", J Magn Reson, 209 (2), 347-51 (2011). PMID: 21316278
8. **R.T. Branca,** “MRI using intermolecular multiple-quantum coherences”, Methods Mol Biol. 771:241-52 (2011). PMID: 21874482
9. E.R. Jenista, **R.T. Branca**, W.S. Warren, "Absolute temperature imaging using intermolecular multiple quantum MRI", Int J Hyperthermia, 26 (7), 725-34 (2010) PMID: 20849265
10. E.R. Jenista, G. Galiana, **R.T. Branca**, P.S. Yarmolenko, A.M. Stokes, M. W. Dewhirst, W.S. Warren, “Application of mixed spin iMQCs for temperature and chemical-selective imaging”, J. Magn. Reson. 204 (2), 208-18 (2010). PMID: 20303808
11. W.S. Warren, E.R. Jenista, **R.T. Branca**, “Increasing hyperpolarized spin lifetimes through true singlet eigenstates”, Science 323(5922), 1711-1714 (2009). PMID: 19325112

12. **R.T. Branca,** W. S. Warren, "Solvent suppression without crosspeak attenuation in iZQC experiments", Chem. Phys. Lett., 470(4-6), 325-331 (2009).
13. **R.T. Branca**, Y. M. Chen, V. Mouraviev, G. Galiana, E. Jenista, C. Kumar, C. Leuschner, W. S. Warren, "iDQC anisotropy map imaging for tumor tissue characterization in vivo", Magn. Reson. in Med. 61(4), 937 - 943 (2009). PMID: 19215050
14. G. Galiana, **R.T. Branca**, E.R. Jenista, and W.S. Warren, "Accurate Temperature Imaging Based on Intermolecular Coherences in Magnetic Resonance," Science 322(5900), 421-424 (2008). PMID: 18927389
15. **R.T. Branca**, G. Galiana, W. S. Warren, "Enhanced Nonlinear Magnetic Resonance signals via Square Wave Dipolar Fields", J. Chem. Phys. 129, 054502 (2008). PMID: 18698909

D. Research Support

Ongoing Research Support

R21 DK090758 (role: PI) 09/20/2010–08/31/2013

Novel magnetic resonance approach to detect BAT distribution and temperature.

The goal of this project is to develop an MR-based method to detect both BAT mass and activity in rodents that can be easily translated to humans.

R01 CA142842-01A1 (role: PI) 04/01/2010–01/31/2015

Sensitive and specific molecular imaging of pulmonary nodules.
The goal of this project is to fully develop and characterize a method that combines hyperpolarized gas MRI with superparamagnetic contrast agent for the detection of lung metastases

Completed Research Support

(Pilot ½ Funding) Duke Cancer Center Branca (Investigator) 06/01/2008–12/31/2008
(Pilot ½ Funding) (5U24 CA092656-08) *Small Animal Imaging Resource Program* Johnson (PI)

Scanned breast tumor-bearing mice after they received an injection of our contrast agent LHRH-SPION. We utilized 10 breast tumor mice that had already been imaged with other modalities (CT and PET) and showed signs of metastatic cancer cells in lungs.
Role: Investigator