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Measurement of Brown Adipose Tissue Activity Using Microwave Radiometry (MRAD) and FDG PET/CT

John P Crandall[1], Joo H O[2], Prateek Gajwani[3], Jeffrey P Leal[3], Daniel D Mawhinney[4], Fred Sterzer[4], Richard L Wahl[1, 3]

[1]Mallinckrodt Institute of Radiology, Washington University in St. Louis, St. Louis, Missouri;

[2]Department of Radiology, College of Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea;

[3]The Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins Medical Institutions, Baltimore, Maryland;

[4]MMTC, Inc., Princeton, New Jersey

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Corresponding author:

Richard L Wahl
Washington University School of Medicine
Mallinckrodt Institute of Radiology
510 S. Kingshighway Blvd, Campus Box 8131
St. Louis, MO 63110
Phone: (314) 362 – 7100
Fax: (314) 361 – 5428
Email: rwahl@wustl.edu

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Abstract

Objectives: The aim of this study was to evaluate the operating characteristics of a microwave radiometer system in the non-invasive assessment of activated and non-activated brown adipose tissue (BAT) and normal tissue temperatures, reflecting metabolic activity in normal human subjects. The radiometer data was compared with FDG PET/CT images in the same subjects.

Materials and Methods: Microwave radiometry (MRAD) and FDG PET/CT were sequentially performed on nineteen participants who underwent a cold intervention to activate BAT. The cold intervention involved the participant intermittently placing their feet on a cold ice block while sitting in a cool room. Participants exhibiting BAT activity qualitatively on PET/CT were scanned again with both modalities following a BAT minimization protocol (exposure to a warm room and a 20 mg dose of propranolol). Radiometry was performed every five minutes for two hours prior to PET/CT imaging during both warm and cold interventions. A grid of 15 - 20 points was drawn on the participant's upper body (data was collected at each point) and a photo was taken for comparison with PET/CT images.

Results: PET/CT identified increased signal consistent with BAT activity in 11 of 19 participants. In 10 of 11 participants with active BAT, radiometry measurements collected during the cold study were modestly, but significantly higher on points located over areas of active brown fat on PET/CT, than in points not exhibiting BAT activity ($P < 0.01$). This difference lessened during the warm studies: 7 of 11 participants showed radiometry measurements that did not differ significantly between the same set of points.

The mean radiometry result collected during BAT maximization was 33.2 ± 1.5 ˚C at points designated as active and 32.7 ± 1.3 ˚C measured at points designated as inactive ($P < 0.01$).

Conclusions: Passive microwave radiometry was shown to be feasible and, with substantial improvements, has the potential to non-invasively detect active brown adipose tissue without a radiotracer injection.

Keywords: 18F-FDG, brown adipose tissue, microwave radiometry, thermogenesis

Introduction

Until recently, the extent to which brown adipose tissue (BAT) is present and active in adult humans was unclear. The introduction of imaging modalities such as positron emission tomography combined with CT (PET/CT) and selective tissue sampling have confirmed that functional BAT exists into adulthood *(1-3)*. Additionally, the inverse correlation between active BAT and overall adiposity may imply that BAT plays a role in the regulation of energy expenditure *(4)*. Thus, BAT has been proposed as a potential target for modulating metabolic activity in humans. However, current approaches for evaluating BAT presence and activity do not allow for a true assessment of BAT's role in energy homeostasis because they require activation of BAT (generally using a cold intervention) and only provide information on BAT activity during a limited timeframe. Developing a method capable of monitoring BAT activity for extended periods of time and under normal daily conditions is critical for understanding the full extent to which brown adipose tissue impacts human metabolism.

The preferred method to investigate the presence and activity of BAT has been PET/CT using 2-deoxy-2-[18F] fluoro-D-glucose (FDG). Increased uptake of FDG in BAT indicates greater glucose utilization and has been shown, using correlative studies with 15O-oxygen and 11C-acetate, to be associated with increased oxidative metabolism and thermogenesis in BAT *(5-7)*. Although this method offers excellent sensitivity in detecting active BAT, the use of ionizing radiation limits its application on healthy human participants in research. In addition, because FDG uptake is a mainly irreversible process as FDG is phosphorylated, obtaining information about the dynamics of BAT

activation, such as on/off switching, is difficult. Furthermore, FDG PET/CT is limited to detecting active BAT during the imaging session while BAT activity levels may vary substantially throughout the day. To complement these shortcomings, additional non-invasive methods are needed.

Thermographic imaging techniques are of interest as BAT is a thermogenically active tissue and has been shown to be significantly warmer than surrounding tissue *(8).* Infrared thermography (IRT) is one such method, which is used to produce thermograms based on detection of radiation in the long-infrared range of the electromagnetic spectrum. Lee et al tested this method in 87 individuals and found skin temperature differences between the supraclavicular fossae (SCV) and mediastinal regions, which became more pronounced following a meal and cold exposure *(9).* Other studies have assessed the ability of IRT to detect activated BAT, with FDG PET/CT imaging used as a gold standard, and found higher supraclavicular temperatures in BAT-positive individuals *(10,11).* Though IRT avoids the cost and radiation limitations of FDG PET/CT, it is still limited to detecting BAT only during the imaging session. In addition, IRT can only detect skin temperatures at the surface while BAT is a subcutaneous tissue.

Microwave radiometers are instruments for measuring thermally generated microwave noise emissions, the intensity of such emissions being proportional to the absolute temperature of the emitting body *(12).* Microwaves, unlike visible or infrared radiation, can penetrate through clouds and microwave radiometers are therefore widely used for the remote sensing of earth from satellites *(13).* Also, unlike infrared and visible radiation, microwaves can penetrate deeply into tissues and microwave radiometers can

therefore be used to non-invasively detect increased temperatures of deep-seated tissues. This feature of microwave radiometers has found, in the past, several medical applications including the detection of breast cancers and carotid inflammations, as well as hyperthermia treatments of cancer *(14-16)*.

In medical applications of microwave radiometry (MRAD), a microwave antenna is placed on the skin above the volume of subsurface tissue whose temperature one wants to estimate and the microwave noise over a chosen bandwidth reaching the antenna is amplified and rectified. A major difficulty in relating the amount of microwave noise reaching the antenna to the temperatures of the subsurface target tissues is that the thermally generated microwave noise emitted by the target subsurface tissues must pass through overlying tissues before reaching the antenna. These overlying tissues not only attenuate the thermal microwave noise emissions from the target tissues, but also contribute thermal microwave noise emissions of their own. Choosing the bandwidth of the radiometer to be centered at low microwave frequencies can minimize the losses in the overlying tissues since loss of microwaves traversing tissues decreases with frequency. However, using a lower the operating frequency band reduces spatial resolution.

The aim of this study was to evaluate the operating characteristics of a non-invasive microwave radiometer system in the assessment of BAT and normal tissue temperatures, reflecting metabolic activity in normal human subjects. The radiometer data was compared with FDG PET/CT images in the same subjects.

Materials and Methods

This prospective study was approved by the Johns Hopkins Medicine Institutional Review Board (IRB approval NA_00050285) and conducted according to the principles expressed in the Declaration of Helsinki. Written, informed consent was obtained from all participants. Healthy participants between the ages of 18 to 35 with a body mass index less than 25 kg/m^2 were eligible for the study. Pertinent exclusion criteria included known diabetes mellitus, the use of beta–blockers, a history of cold-related injury, and the use of tobacco.

Microwave radiometry data was collected before and during both protocols (outlined below) using a dual band radiometer system (Figure 1, MMTC, Inc., Princeton, NJ). The basic principles of MRAD have been described previously (*12, 17, 18*). Briefly, MRAD measures electromagnetic radiation produced by deep tissue at microwave frequencies. The intensity of the radiation is proportional to the absolute temperature of the tissue. The electromagnetic radiation is detected non-invasively using a small microwave antenna (approximately 2 cm in diameter) placed at the surface of the skin. The depth of measurement is mostly determined by the measurement frequency band of the radiometer used. In this study, radiometric measurements were made using the 3.7 to 4.2 GHz frequency band, a range that minimizes potential interference from terrestrial communication. The radiometer was calibrated for antenna mismatches and temperature variations at the beginning of every study visit that included radiometry data collection.

All PET/CT images were acquired using the Discovery ST PET/CT system (GE Healthcare). Participants were instructed to fast for no less than 6 hours prior to administration of FDG (PETNET Solutions INC., Knoxville, TN). Intravenous injection

of FDG (mean injected dose of 258.4 ± 36.4 MBq) was followed by an uptake period of approximately 60 minutes.

All participants first underwent a "BAT maximization" cooling protocol followed immediately by whole-body FDG PET/CT imaging (Figure 2). Radiometry data was collected from about 5 minutes prior to the start of the BAT minimization protocol until the start of PET imaging. Those participants showing active BAT on PET/CT following the BAT maximization protocol were invited back for an additional session during which a "BAT minimization" protocol was conducted followed immediately by whole-body FDG PET/CT imaging. Again, radiometry data was collected from about 5 minutes prior to the start of the BAT maximization protocol until the start of PET imaging. BAT maximization and minimization sessions were conducted at least 7 days apart.

The BAT maximization protocol utilized in this study was based on the method described previously by Saito et al and consisted of cold stimulation in the form of a cooled room (18.1ºC - 20.0ºC) as well as having the participants intermittently place their feet on a block of ice (*4*). Once cold stimulation began, participants were asked to place their feet on the block of ice for 4 minutes, followed by 1 minute of rest. The ice block was covered with a disposable blue paper pad ("chuck") so the skin did not make direct contact with the ice. Participants wore a hospital gown and thin cotton hospital pants. The cold stimulation went on for a target of 1 hour until the administration of FDG and continued during the FDG uptake period of about 1 hour (i.e. approximately 2 hours total of cold stimulation). A primary goal of this protocol was to minimize shivering, which

could potentially confound the results of the radiometer and the PET/CT. To that end, volunteers were clinically monitored throughout the session for signs of shivering. If shivering was detected by the researcher or reported by the volunteer, the cooling was modified by reducing the amount of time the participant's feet were on the ice until shivering ceased. Cold stimulation did not continue during PET/CT imaging. During the BAT minimization condition, fasted participants were seated in a warm room (24ºC – 28ºC) and given a 20 mg dose of a β–blocker (propranolol). They were in this room for the same time period as during the BAT maximization procedure, wore similar clothing but were given a warm blanket. Participants were injected with FDG after 60 minutes of warming.

Systematic collection of radiometry data was performed beginning 5 minutes prior to the start of BAT maximization or minimization and continued until the start of PET/CT imaging. A grid of 15 – 20 points was drawn on the participant's upper body (see figure 3 for an example of the pattern used) and a photo was taken for comparison with PET/CT images and so the grid could be replicated during subsequent sessions. Beginning every 5 minutes, radiometry data was collected by placing the device probe on each grid point until the signal stabilized (approximately 3 seconds). The result was then recorded and the probe was moved to the next grid point. The probe was an approximately 0.5 meter long by 0.05 meter wide hand-held cylinder with the detector attached to one end. Two researchers were always present for this procedure with one researcher systematically holding the radiometry probe over the pre-specified grid points in a consistent order and the other recording the results. The second researcher recorded

the results and the research assigned to maneuver the probe was always blinded to the radiometer result.

Radiometry and PET/CT Image Analysis

A single board-certified nuclear medicine physician used a clinical imaging workstation (Mirada XD3, Mirada Medical, Denver, CO) for determination of SUV values and to qualitatively analyze the PET/CT images. Areas expected to contain activated BAT were identified and SUV (adjusted for lean body mass) determined. A value of 1.2 or greater was used as the cutoff to identify active BAT. The qualitative analysis consisted of comparing the PET/CT image to the photograph of the grid drawn on each participant. A determination was then made as to whether each grid point on the surface of the skin was positioned over an area of active brown fat seen on the PET/CT image. Each grid point was given a binary result of either positive or negative for position over areas of active BAT. Positive grid points were then compared with negative grid points within each subject. The grid points were also compared between the BAT maximization and minimization sessions.

Statistical Considerations

Paired and unpaired t-tests were applied as well as Fisher's exact test. Receiver operating characteristic (ROC) analysis was applied to absolute MRAD values, using FDG PET/CT as gold standard, and a sensitivity/specificity report was generated. In all analyses, a *P* value of less than 0.05 was considered statistically significant. Descriptive statistics were

calculated using Microsoft Excel and further analyses were performed with Prism4.0 (Graphpad Software).

Results

Nineteen participants were prospectively enrolled between March, 2012 and March, 2013 at the Johns Hopkins medical campus in Baltimore, MD (39.296˚ N, 76.592˚ W). Participant characteristics are described in Table 1. All 19 participants underwent the BAT maximization procedure followed by PET/CT imaging. Eight participants did not exhibit BAT activity qualitatively on PET/CT and subsequently did not undergo the follow-up BAT minimization procedure. For the 11 remaining participants, the median and range between BAT maximization and minimization sessions was 20 days and 7 – 42 days, respectively. Shivering was observed or reported in 4/11 BAT positive participants and was then minimized or eliminated in all 4 participants using the modified cooling protocol. All sessions were performed during months with cooler temperatures (i.e. November – May), with the exception of one performed in August, which did not result in activated brown fat during the BAT maximization session. Mean outdoor temperature during months in which BAT was successfully activated was 7.7 ˚C and 9.9 ˚C during months in which BAT was not successfully activated ($P = 0.587$).

Brown fat activity was not visualized on FDG PET/CT in participants undergoing the BAT minimization procedure of exposure to warmth and a low dose of the β-blocker propranolol. Figure 4 shows a representative pair of images displaying extensive BAT activation following maximization procedures and absent BAT activity following the minimization procedures.

Mean radiometry temperature measurements at all pre-specified grid locations were consistently lower during BAT maximization sessions than during minimization sessions in the 11 BAT-positive participants. Mean radiometry value during maximization and minimization sessions was 32.8 ˚C and 35.6 ˚C, respectively (P = 0.005) in these 11 participants. Oral body temperature was significantly different on paired t-test between the two sessions with a mean of 97.7 during BAT maximization sessions and 98.0 during minimization (P = 0.047).

By comparing PET/CT images obtained following BAT maximization with the subject-specific diagram of points where radiometry data were collected (Figure 3), each radiometry collection point was designated as either “active” or “inactive” by an experienced nuclear medicine physician. The number of active and inactive points varied between subjects. The median number of active points was 8 and ranged from 6 – 13, while the median number of inactive points was 11 and ranged from 6 – 15. The mean radiometry result collected during BAT maximization was 33.2 ± 1.5 ˚C at points designated as active and 32.7 ± 1.3 ˚C measured at points designated as inactive (P < 0.01). During BAT minimization, the mean radiometry result was 35.7 ± 4.5 ˚C measured at active points, essentially identical to the 35.6 ± 4.8 ˚C measured at inactive points (P = NS; designation of “active” or “inactive” determined during BAT maximization session).

When individual subjects were considered separately, radiometry measurements taken during BAT maximization were significantly greater over areas of active BAT (as determined by PET/CT) than over areas not exhibiting BAT activity in 10 of 11 subjects (Figure 5). This difference lessened during the warm studies: 7 of 11 participants showed

an insignificant difference between the same set of points. These frequencies were compared using Fisher's exact test, which indicated a significant difference between BAT maximization and minimization results ($P = 0.012$). The area under the ROC curve for difference between maximization and minimization sessions at each data collection site was 0.88 ($P = 0.003$). At an MRAD-difference cut-off value of 0.48, sensitivity and specificity were 72.7% and 90.9%, respectively (Figure 6).

Discussion

The most commonly used technique for imaging brown adipose tissue is currently FDG PET/CT. Though this modality has been shown effective at imaging BAT, its use is limited by the associated cost and radiation exposure (*19*). Microwave radiometry is a non-invasive, low-cost method that may be useful for the detection of activated BAT. The current study is, to our knowledge, the first to test the feasibility of detecting activated BAT in human subjects using a microwave radiometry system.

Utilizing a cold-exposure technique previously described by Saito et al, BAT was activated in 11 of 19 healthy normal subjects. These 11 subjects subsequently underwent a BAT minimization session during which BAT activity was reduced completely in all subjects using a warm room and a low dose of propranolol, which has been shown to greatly reduce BAT activity (*20*). Microwave radiometry and FDG PET/CT were both performed during each session.

Based on the FDG PET/CT images acquired during the BAT maximization session, each point where radiometry data was collected was classified as "active" or

"inactive" (active points being those above areas of active BAT). The same points were compared during the maximization and minimization sessions. During maximization, the mean radiometry temperature of active points was modestly, but significantly, higher than the mean radiometry temperature of inactive points. The mean difference in temperature between these points was insignificant during BAT minimization. This is consistent with the microwave radiometer detecting a temperature increase over sites of activated brown fat.

The temperature of BAT has been studied indirectly in humans using temperature probes attached to the surface of the skin (*21, 22*). Van der Lans et al used a cooled room to activate brown fat and then compared supraclavicular surface temperature to the surface temperature of surrounding areas (i.e. the head and chest). Activation of BAT was then verified using PET/CT. During cold exposure, the mean supraclavicular temperature was 1.3 ˚C warmer than the surrounding areas. Similarly, in our study areas of activated BAT were compared with surrounding tissue and, during BAT activation, the mean difference in temperature between activated sites and surrounding sites was 0.58 ˚C. During BAT minimization, the same sites differed by only 0.15 ˚C. This difference is comparable to differences found using IRT. After a 2 hour cooling procedure, Jang et al found mean supraclavicular temperatures that were higher in BAT-positive volunteers than in those who were BAT-negative (1.0 ˚C over the left SCV and 0.6 ˚C over the right SCV) , though these differences were not statistically significant(8). Subjects with activated BAT were found by Gatidis et al to have significantly higher mean SCV skin temperatures than those without activated BAT (35.0±0.5 vs. 34.6±0.5 ˚C,

respectively)(*10*). Note the MRAD system is designed to detect the temperature of deeper tissues than the surface temperature assessment methods. The location of BAT in relation to major blood vessels is a possible confounding factor related to the temperature comparison. While the radiologist analyzing the PET/CT images and MRAD data was certainly aware of the location of BAT relative to large blood vessels, there is no way to be sure this early generation radiometer did not detect a fraction of its detected temperature from blood vessels.

Due to the design of the radiometer, human factors could contribute to measurement variability. In order to obtain consistent results, the probe needed to be held at the same angle at each data collection point, which is technically demanding. A change in the angle of the probe could result in collection of microwave radiation from different underlying tissues. In addition, the device is susceptible to interference from outside sources emitting microwave radiation in the same range in which data was collected. This potential confounding issue was addressed by choosing a range in which little else should be emitting and by calibrating the device prior to every use. Despite these precautions, interference may have occurred. Methods to minimize environmental interference may warrant further study.

Another issue was the limited number of data points able to be collected. Since the device required a researcher to hold the probe at each site and wait for the signal to settle, only about 20 data points could be collected once every 5 minutes. A significant increase in the number of data points could help verify the brown fat temperature signal. A significant design improvement would be a wearable system that uses multiple probes

(i.e. an array of several radiometer detectors) and collects data continuously and passively. This type of wearable system would need to overcome specific obstacles such as movement of the subject and potential interference from a constantly-changing background. Movement issues could be partially overcome by using an adhesive and placing the probes (which can be fabricated in relatively small dimensions) away from joints. Various fabrics are available that offer electromagnetic shielding and could be used to cover the probes and minimize background interference.

An added benefit of the modified, wearable system would be the ability to collect radiometer data over an extended period of time. Brown fat activity is thought to vary considerably throughout the day depending on a variety of factors. Unfortunately, modalities such as FDG PET/CT are only capable of showing the amount of activity during the short span of time when the subject is undergoing the imaging study. A technique able to provide information on brown fat activity over a large span of time would significantly improve the understanding of BAT dynamics.

A challenge in measuring BAT activity in our study is that there are differences in the resolution of the microwave radiometer detector and of the PET scanner. The microwave radiometer used in our study was much larger than the resolution of a modern PET scanner. Thus areas without BAT signal were likely included in the signal obtained from the MRAD device, and in some instances, from the PET scanner. In addition, the PET and MRAD data acquisitions were sequential and not simultaneously performed, allowing for the potential for minor misalignments in the areas interrogated by the two methods. In future studies, to optimize mRAD, multiple smaller, high resolution

detectors as well as a precise alignment system to assure the same areas are assessed by PET and MRAD would be of relevance. We believe with such a system, more reliable collection and interpretation of the inherently continuous SUV and MRAD data may inform future, more refined studies linking these techniques.

Previous studies have either not included females or controlled for the thermoregulatory responses associated with the menstrual cycle (*23, 24*). A limitation of the current study is that most volunteers were female and the menstrual cycle was not monitored. However, the primary question considered here is whether the radiometry device can detect difference between areas of active and inactive or absent BAT. Since the effect of the menstrual cycle on thermoregulatory response should not vary substantially over the span of a single session (i.e. 3 hours), it should not have a significant impact on the primary aims of the study, though it may have affected baseline temperatures of our participants.

Utilizing a different cooling protocol may also help to improve the performance of the microwave radiometer. The cooling protocol utilized in the current study, based on a previously described method, has been suggested to be less effective than some other techniques (*19*). The individualized cooling method used by Vosselman et al may result in more consistently activated brown fat, which may have yielded larger signals than the current study (*25*). This individualized protocol involves precise cooling of a small room to a temperature usually close to 16.0 ˚C, which should result in a lower temperature at the surface of the skin and may improve the performance of the microwave radiometry system (*26*).

Conclusion

Brown adipose tissue has emerged as a potential target organ for the prevention and treatment of diabetes and obesity. In order to evaluate interventions aimed at modulating BAT activity or mass, it is important to have imaging methods capable of accurately assessing these parameters, ideally without the use of ionizing radiation, so that sequential and longitudinal studies can be performed. In the current study, we have demonstrated the feasibility of microwave radiometry in the assessment of brown adipose tissue activation in normal, lean, healthy participants. This modality is a noninvasive and potentially inexpensive technique that does not involve ionizing radiation and, with significant improvements, may potentially be a useful method for evaluating the presence, activity and response of BAT to various interventions.

Disclosures

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Tables

Table 1. Participant characteristics.

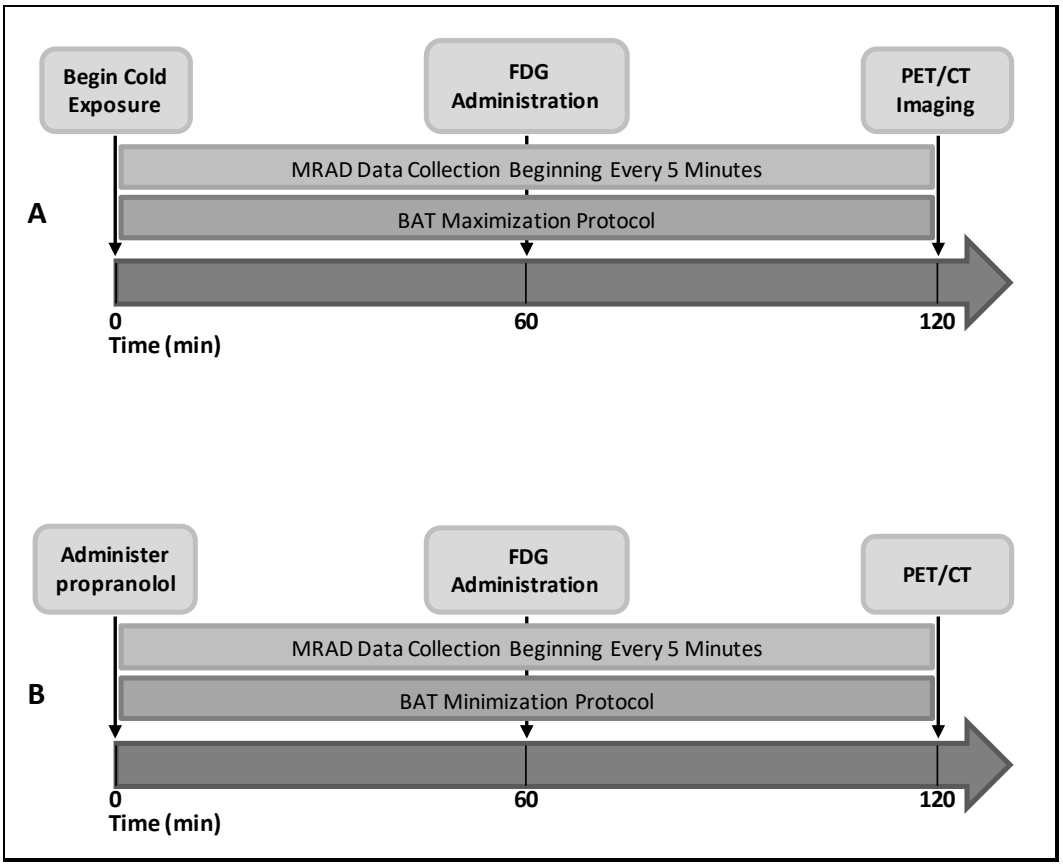
Characteristic	**Value**	**Range**
Gender (M/F)	2 / 17	
Age (y)	24.8 (2.9)	21-32
Body Mass, kg	56.5 (4.6)	48.0 - 63.0
Height, m	1.7 (0.1)	1.6 - 1.8
BMI, kg/m^2	19.7 (1.3)	17.0 - 23.1

Data are reported as mean (SD)

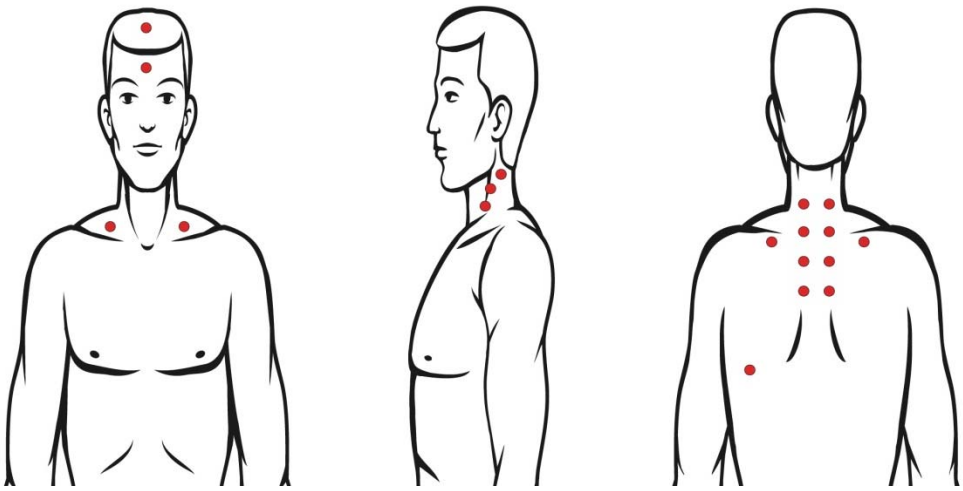
BMI indicates body mass index

Figures

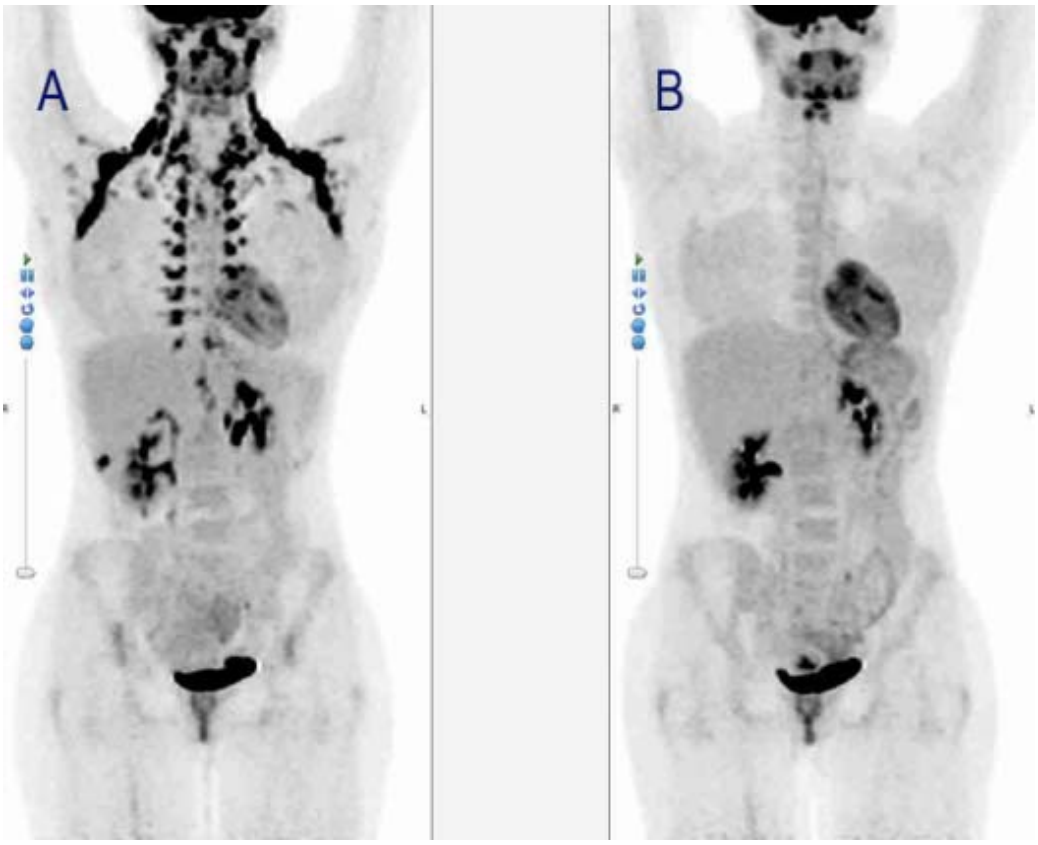
[**Figure 1. Microwave radiometer system.** This picture shows the microwave radiometry system used throughout the study. The probe used to collect radiometer data can be seen mounted on the right side of the picture.]



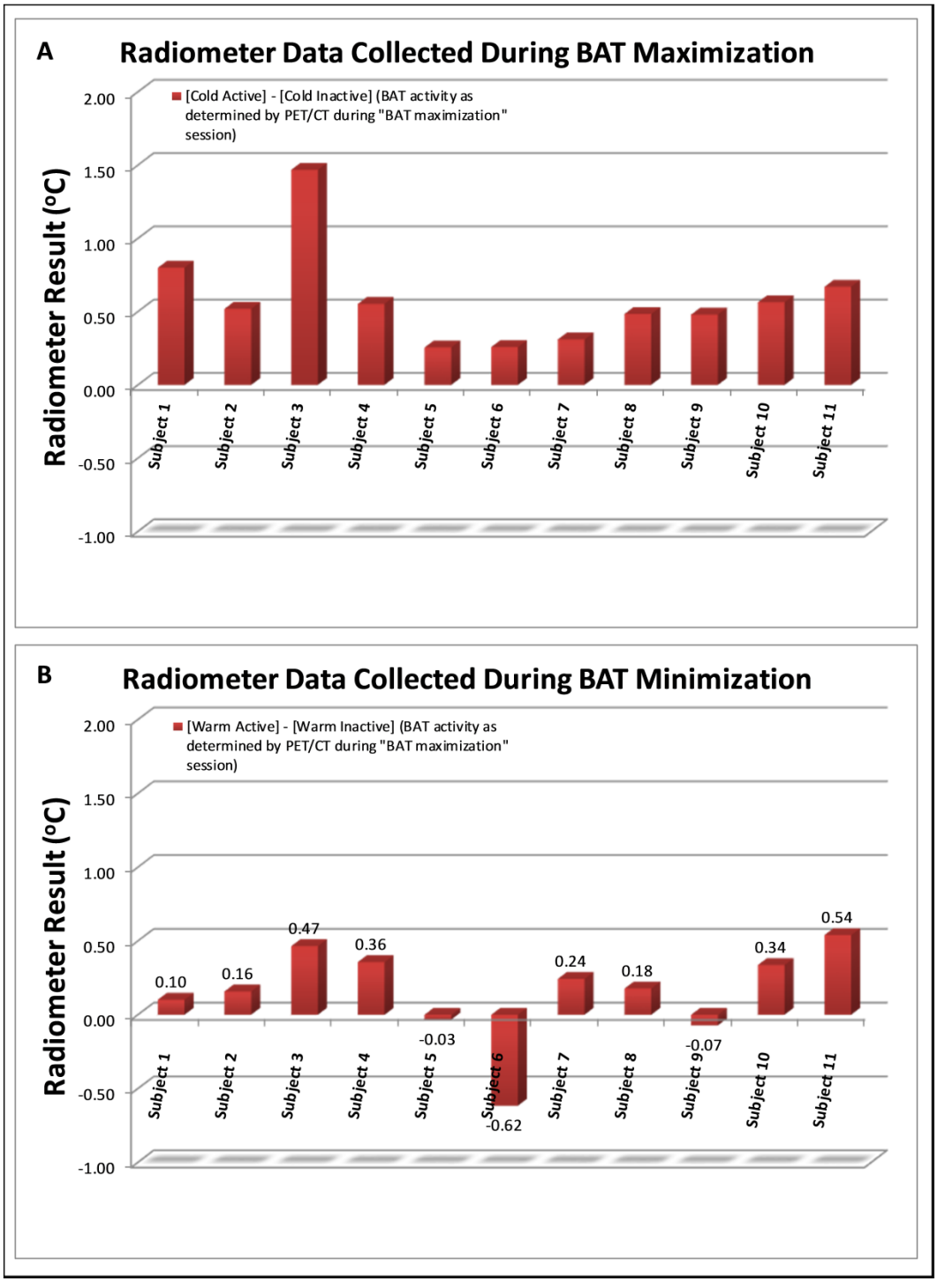
[Figure 2. BAT maximization (A) and minimization (B) schemas. During BAT maximization, each participant was exposed to cold for about two hours prior to PET/CT imaging. The BAT minimization protocol was organized in the same way with a 20 mg dose of propranolol replacing the start of cold exposure.]



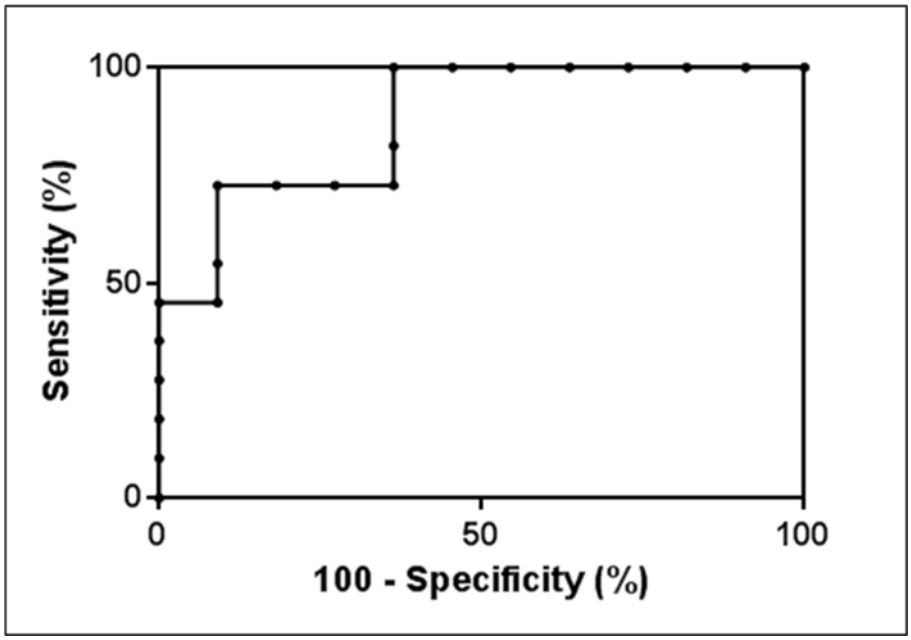
[Figure 3. Representative radiometry data collection diagram. A surgical marker was used to draw a set of points where radiometry data was collected. Radiometry data was collected from multiple points on both sides of the neck. The same grid was drawn during both BAT maximization and minimization sessions and a photo was taken for comparison with PET/CT images.]



[Figure 4. Representative BAT maximization and minimization images. Anterior FDG MIP views: (A) Extensive brown fat activity following BAT maximization procedures (i.e. cold stimulation). (B) In the same participant, 8 days later, BAT activity is not seen after exposure to warmth for 2 hours and a 20 mg propranolol dose at the outset of warming. Normal activity in the brain, salivary glands, heart, and bladder are consistent between the two images.]


A
Radiometer Data Collected During BAT Maximization
[Cold Active] - [Cold Inactive] (BAT activity as determined by PET/CT during "BAT maximization" session)
Radiometer Result (°C)
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
Subject 1
Subject 2
Subject 3
Subject 4
Subject 5
Subject 6
Subject 7
Subject 8
Subject 9
Subject 10
Subject 11
B
Radiometer Data Collected During BAT Minimization
[Warm Active] - [Warm Inactive] (BAT activity as determined by PET/CT during "BAT maximization" session)
Radiometer Result (°C)
0.10
0.16
0.47
0.36
-0.03
-0.62
0.24
0.18
-0.07
0.34
0.54

[**Figure 5. Differences in radiometry measurements between maximization and minimization procedures.** Radiometry measurements are shown to vary significantly between points over active and inactive BAT during BAT maximization sessions (A), while the same points tend to vary insignificantly during BAT minimization sessions (B). Each result shown is the difference between the mean of radiometry measurements taken over areas of active BAT and the mean of radiometry measurements collected over inactive BAT (designation of “active” and “inactive” was determined during BAT maximization session and the same points are compared in the two charts). The *P* values are noted above each bar with significant results in red.]



[**Figure 6. Reciver operating characteristic curve.** Using FDG PET/CT as the gold standard, the area under the ROC curve was 0.88 (*P* = 0.003). At an MRAD-difference cut-off value of 0.48 (i.e. the difference between the maximization and minimization sessions at each data collection site), the MRAD sensitivity and specificity for detecting activated BAT were 72.7% and 90.9%, respectively, and the likelihood ratio was 8.0.]