Identification of an optimal threshold for detecting human brown adipose tissue using receiver operating characteristic analysis of IDEAL MRI fat fraction maps

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Abstract

Purpose

Lower fat fraction (FF) in brown adipose tissue (BAT) than white adipose tissue (WAT) has been exploited using Dixon-based Magnetic Resonance Imaging (MRI) to differentiate these tissues in rodents, human infants and adults. We aimed to determine whether an optimal FF threshold could be determined to differentiate between BAT and WAT in adult humans in vivo.

Methods

Sixteen volunteers were recruited (9 females, 7 males; 44.2±19.2 years) based on BAT uptake on ^{18}F-FDG PET/CT. Axial 3-echo TSE IDEAL sequences were acquired (TR(ms)/TE(ms)/matrix/NEX/FoV(cm) = 440/10.7-11.1/512x512/3/30-40), of the neck/upper thorax on a 3T HDxt MRI scanner (GE Medical Systems, Milwaukee, USA), and FF maps generated from the resulting water- and fat-only images. BAT depots were delineated on PET/CT based on standardized uptake values (SUV) >2.5 g/ml, and transposed onto FF maps. WAT depots were defined manually within subcutaneous fat.

Receiver operating characteristic (ROC) analyses were performed, and optimal thresholds for differentiating BAT and WAT determined for each subject using Youden's J statistic.

Results

There was large variation in optimal FF thresholds to differentiate BAT and WAT between subjects (0.68–0.85), with great variation in sensitivity (0.26-0.84) and specificity (0.62-0.99). FF was excellent or good at separating BAT and WAT in four cases (area under the curve [AUC] 0.84-0.92), but poor in 10 (AUC 0.25-0.68).

Conclusion

Although this technique was effective at differentiating BAT and WAT in some cases, no universal cut-off could be identified to reliably differentiate BAT and WAT in vivo in adult humans on the basis of FF.

Declaration of interest

None.

Keywords

Brown adipose tissue; Human; Magnetic resonance imaging; Positron-emission tomography

1. Introduction

Obesity is a major global public health problem (1); the prevalence of obesity has doubled between 1980 and 2008, with approximately 1.4 billion adults being overweight (*i.e.* BMI > 25kg/m^2), of whom 500 million are obese (*i.e.* BMI > 30 kg/m^2) (2).

Brown adipose tissue (BAT) may have a role in the aetiology and management of obesity. BAT is a thermogenic organ occurring exclusively in mammals. It produces heat through non-shivering thermogenesis by dissipating chemical energy (in the form of fatty acids and glucose) in response to cold exposure without the need for shivering or locomotor activity (3).

There is compelling evidence of a link between defective BAT and obesity (4,5). In rodents, BAT dysfunction leads to impaired non-shivering thermogenesis and a propensity for obesity (6). Furthermore BAT ablation has been shown to induce obesity in mice (7). *Post mortem* studies in humans have shown a correlation between larger BAT accumulations and lower BMI (8).

Stimulation of non-shivering thermogenesis through BAT manipulation has been posited as a means of reducing elevated triglycerides and combating obesity (9,10). The evaluation of such interventions would require a reliable imaging biomarker to quantify BAT. As a result there has been resurgence in interest in BAT imaging.

Positron-emission tomography with computed tomography (PET/CT) has limitations as a means of quantifying BAT as it relies on the uptake of the radiolabelled tracer 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F-FDG) by metabolically active tissue. ^{18}F-FDG is taken up to a much lesser degree in metabolically inactive BAT. Consequently BAT detection on PET/CT is opportunistic, limiting its reproducibility (11), although we previously reported that the pattern of BAT distribution (when active) remains fairly consistent across serial PET/CT scans within individuals (12). BAT-specific PET probes have been developed, including ^{18}F-BODIPY (boron-dipyrromethane), which show significant accumulation in metabolically active BAT, although uptake in unstimulated BAT is relatively low (13).

Estimates of BAT prevalence in adult humans varies, but larger studies using PET/CT report point prevalence of between 1.1% (14) and 8.5% (11), and 5.3% in our own population (15) – all of which are certainly underestimates. Cumulative prevalence (based on repeated PET/CT scanning of 145 patients) is estimated at 64% (11), while small dedicated prospective PET/CT studies report point prevalence as high as 95.8% (16) and 100% (17). However, the high radiation burden, costly radiotracer and relatively low spatial resolution of PET/CT limit its role as a research tool in large populations (18).

MRI may offer a non-invasive and safe alternative for BAT quantification, as it can differentiate between brown and white adipose tissue (WAT) based on differences in fat (19) and mitochondrial content (20). Therefore MRI has the potential to identify BAT regardless of its metabolic state (21),

Significantly lower fat fractions (FF) have been reported in BAT than WAT in rodents (18,19,22-24) and human infants (20,25,26). Human studies have tended to focus on infants in whom BAT is more prevalent and extensive. Lower BAT FF has been reported in small numbers of human adults (20,27), although differences decrease with age (20). We previously reported a case in whom we were able to identify BAT using MRI, which was subsequently confirmed histologically (28). Gifford *et al* (29) identified probable BAT based on FF in two adult humans. For the most part, however, it has not been possible to extrapolate these findings to prospectively identify BAT in adult humans solely on the basis of MRI imaging findings.

The aim of this study was to determine whether there was a significant difference in FF between BAT and WAT in adult humans, and to identify an optimal threshold for differentiating between these tissues on the basis of FF in vivo.

Our secondary aims were to determine whether FF within BAT and WAT fluctuates over time.

2. Materials and methods

2.1 Sample

Sixteen volunteers were recruited (mean age 44.2 ± 19.2 years; 9 females, 7 males) on the basis of showing ^{18}F-FDG standardized uptake values (SUV) >2.5 g/ml within adipose tissue (*i.e.* CT attenuation below -100 Hounsfield units) on PET/CT scans consistent with BAT ('^{18}F-FDG BAT'). In addition six age (± 5 years) and sex-matched controls were recruited on the basis of showing consistently absent ^{18}F-FDG BAT uptake across serial PET/CT scans (Figure 1).

The PET/CT scans were performed as part of patients' routine clinical care.

2.2 Ethical approval

Ethical approval was obtained from the Birmingham East, North and Solihull Research Ethics Committee (NHS REC reference 11/H1206/3).

2.3 PET/CT scanning technique

Scanning was performed on a combined GE Discovery STE PET/CT scanner (General Electric Medical Systems, Milwaukee, USA). Patients were routinely fasted for 6 hours prior to scanning. Following administration of ^{18}F-FDG (mean injected dose 362 MBq, range 103 - 505 MBq), patients rested for 60 minutes within the PET/CT suite, where ambient temperature was maintained at 24°C.

Emission data were obtained for 3 minutes in each bed position from skull base to mid thighs, and reconstructed using CT data for attenuation correction.

2.4 MRI scanning technique

Axial 3-point 2D TSE IDEAL (iterative decomposition of water and fat with echo asymmetry and least-squares estimation) images were acquired on a GE 3T HDxt MRI scanner (General Electric Medical Systems, Milwaukee, USA) using a quad-channel cardiac receiver coil placed anteriorly across the upper thorax and neck (slice thickness = 2.5 – 5 mm, repetition time = 440 ms, echo time = 10.7 – 11.1 ms, acquisition matrix = 512 x 512, number of excitations = 3, and field of view = 300 - 400 mm.

IDEAL fat- and water-only images were post-processed using ImageJ (30) to generate FF maps according to the formula:

$$Fat\ fraction = \frac{Fat\ only}{Fat\ only + Water\ only}$$

2.5 Image analysis

Non-fatty tissues were excluded from analysis by thresholding FF maps with a lower limit set at 50% FF. PET/CT scans were registered to the MRI scans using Mirada XD 3.4 image fusion software (Mirada Medical Ltd, Oxford, UK) using both rigid and non-rigid registration techniques, with manual placement of fiducial markers as necessary.

Two methods of BAT segmentation were then performed, illustrated in Figure 2:

1. Transposition from PET/CT: ROIs were drawn around ^{18}F-FDG BAT deposits semi-automatically by defining iso-contours set at a standardized uptake value (SUV) of 2.5 g/ml on the PET/CT scan, which we felt provided the best compromise between capturing the extent of ^{18}F-FDG uptake within BAT , whilst minimizing artefactual bleeding into adjacent tissue. These ROIs were then transposed onto the co-registered FF map.
2. Regional segmentation of supraclavicular fat: BAT, when active on PET/CT, most likely occurs within the supraclavicular fossa (12,31). Therefore to determine whether BAT could be identified without the benefit of *a priori* knowledge from PET/CT scans, ROIs were drawn freehand around fat in the supraclavicular fossa on contiguous slices. Morphological edge erosion was then applied to minimize edge effects from adjacent tissue interfaces using the 'eroded range limited' technique described by Lundström *et al* (32,33).

For comparison ROIs were manually defined in dorsal subcutaneous WAT on MRI FF maps.

2.6 Statistical analysis

Differences in mean FF for transposed BAT and WAT were compared on a slice-by-slice basis using analysis of variance (ANOVA) with Bonferroni's *post hoc* test for

pairwise comparisons using GraphPad Prism 7.00 for Mac OS (GraphPad Software, La Jolla, California, USA). Significance was defined as $p < 0.05$.

Receiver operating characteristic (ROC) curves were generated to plot true positivity rate (sensitivity) against false positivity rate (1-specificity) for various FF cut-off points. Area under the curve (AUC), a measure of the accuracy of the test, was derived, and optimal thresholds for differentiating BAT and WAT calculated using Youden's J statistic (34).

Temporal changes in mean FF for BAT and WAT across serial scans (12 scans in 6 subjects; A, D, E, F, I and K) were compared using one-way ANOVA, with Bonferroni's post test.

Differences in mean FF between subjects with and without evidence of BAT on PET/CT (subjects K-V) were compared using two-way ANOVA with Bonferroni's *post hoc* analysis, and ANCOVA to control for differences in baseline WAT FF and mean daily temperature. ANCOVA analysis was performed on IBM SPSS Statistics for Macintosh, version 24.0 (IBM Corp., Armonk, N.Y., USA).

3. Results

3.1 Demographics

Twenty-two participants were recruited; 16 with evidence of BAT on PET/CT, and 6 age-and sex matched controls without evidence of BAT activity on multiple PET/CT scans.

The mean age of participants with BAT activity on PET/CT was 44.3 ± 19.3 years at initial scanning (Table 1). Nine were female (56.3%) and seven male.

For subjects with BAT activity on PET/CT (subjects A-P) there was a statistically significant difference in FF between males and females for both subcutaneous WAT (0.767 ± 0.058 and 0.817 ± 0.037 respectively; $p < 0.001$) and transposed BAT (0.669 ± 0.065 and 0.778 ± 0.061; $p < 0.001$).

FF within subcutaneous WAT showed no correlation with body mass index (r = 0.10).

3.2 Difference in FF between subcutaneous WAT and transposed BAT

One-way analysis of variance showed a statistically significant difference in FF between transposed BAT and subcutaneous WAT, $F(31, 560) = 60.03$, $p < 0.001$. *Post hoc* Bonferroni's test for multiple comparisons (Table 2) showed FF to be statistically significantly lower in transposed BAT than subcutaneous WAT in 10/16 subjects (62.5%), and higher in a single subject (E, Figure 3).

3.3 Receiver operating analysis to determine an optimal threshold to differentiate transposed BAT and subcutaneous WAT

The optimal cut-off to separate transposed BAT and subcutaneous WAT varied considerably between subjects (Table 3), ranging from 0.681 to 0.853.

There was also considerable variation between subjects in the accuracy of these optimal FF cut-offs (area under the curve [AUC] 0.248 – 0.924), resulting in wide variation in sensitivity (0.264 – 0.844) and specificity (0.616 – 0.986). Tissue separation was excellent or good in 4 subjects (B, C, D and F, *i.e.* AUC > 0.8), fair in 2 (M and N, *i.e.* AUC 0.7 - 0.8), and poor in 10 subjects (*i.e.* AUC < 0.7, Figure 3).

3.4 Temporal FF changes in subcutaneous WAT and transposed BAT

One-way ANOVA for the six subjects who had repeat MRI scans showed a statistically significant difference in FF between initial and subsequent MRI scan for both subcutaneous WAT and transposed BAT, $F(23, 250) = 39.44$, $p < 0.0001$. *Post-hoc* analysis with Bonferroni's test for multiple comparisons (Table 4) showed a statistically significant temporal change in FF for transposed BAT in subject I ($p = 0.0003$), and for subcutaneous WAT in subject D ($p < 0.0001$). The remainder showed no significant difference in FF for either transposed BAT or subcutaneous WAT between serial MRI scans (Table 4 and Figure 5).

3.5 Characterisation of subcutaneous WAT and supraclavicular fat

For the subgroup of age-and sex-matched subjects (K-V), FF within supraclavicular fat was compared against subcutaneous WAT using two-way ANOVA to determine whether BAT status (*i.e.* presence or absence of BAT activity on PET/CT) affected FF.

There was a statistically significant interaction between the effects of tissue type (i.e. supraclavicular fat and subcutaneous WAT) and BAT status on PET/CT on FF, $F(11, 616) = 13.93$, $p < 0.0001$, $\eta_p^2 = 0.024$. Simple main effects analysis showed a statistically significant difference in FF between supraclavicular fat and WAT, $F(11, 616) = 467.9$, $p < 0.0001$, $\eta_p^2 = 0.792$. There was also a significant (albeit small) difference in FF between subjects with and without BAT activity on PET/CT $F(1, 616) = 579.5$, $p < 0.0001$, $\eta_p^2 = 0.089$. *Post hoc* Bonferroni's test for multiple comparisons showed FF within supraclavicular fat was significantly lower than WAT in all but one case (Figure 6).

To evaluate differences in supraclavicular fat FF between subjects with (K-P) and without BAT activity (Q-V) on PET/CT, whilst controlling for variation in both baseline subcutaneous WAT FF and environmental temperature, a one-way ANCOVA was performed with baseline subcutaneous WAT FF and mean daily temperature as covariates.

There was a significant effect of PET/CT BAT status on FF after controlling for WAT FF and temperature, $F(1,316) = 12.537$, $p < 0.001$, although the effect size was small ($\eta_p^2 = 0.038$). Mean FF within supraclavicular fat was significantly lower in

subjects with BAT activity on PET/CT (corrected mean FF 0.739, 95% CI 0.734 – 0.743) than those without BAT activity on PET/CT (0.750, 95% CI 0.746 – 0.755), although the differences were small (corrected mean difference 0.011, 95% CI 0.005 – 0.018).

The predicted main effect of subcutaneous WAT FF upon supraclavicular fat FF was statistically significant [$F(1,316) = 948.455$, $p < 0.005$, $\eta_p^2 = 0.750$], as was mean daily temperature [$F(1,316) = 26.723$, $p < 0.005$, $\eta_p^2 = 0.078$].

4. Discussion

In this study, a retrospective analysis of MRI scans, we sought to differentiate between BAT and WAT in adult humans on the basis of differences in FF, and whether this fluctuated within individuals over time. We also sought to determine whether there was a difference in FF within the supraclavicular fossae (the area most likely to contain BAT) between individuals with and without evidence of BAT on PET/CT.

Mean FF was statistically significantly lower within BAT than subcutaneous WAT (as determined by one-way ANOVA) in 10 out of 16 subjects. A single subject showed a significantly higher FF within BAT (subject E), although this may be a spurious as the WAT FF (0.596) is unusually low. Although subject E's BMI was within the normal range (25.2 kg/m^2), they had a muscular body habitus and consequently the volume of subcutaneous fat was low which may make it susceptible to measurement error or volume averaging artefact. We observed no significant correlation between BMI and subcutaneous WAT FF.

This accords with findings from other studies which have shown lower FF within BAT using Dixon chemical-shift techniques (35) and spectroscopy (36). The lower fat content within BAT may be attributed to morphological differences between BAT and WAT adipocytes (37,38).

Studies evaluating BAT on MRI in humans have tended to focus on infants in whom BAT is more extensive, with far fewer studies involving adults. Although these show FF to be significantly lower in BAT than WAT the difference diminishes with age; FF within BAT tends to be lower in infants (24,26) than adults (20,33,39).

There was considerable variation in both BAT and WAT FF between individuals, and as a result the optimal FF to differentiate BAT and WAT varied between 0.681 and 0.853. Using these heuristic thresholds to identify BAT produced variable results between subjects, with high accuracy in 4 subjects, moderate in 2, and poor accuracy in 10. Therefore, a single FF to differentiate BAT from WAT could not be identified.

For the second element of the study, a subset of six subjects underwent a second MRI scan to determine whether FF changed over time. Comparison of the two MRI scans using ANOVA revealed a significant difference in mean BAT FF between scans, although post hoc tests showed that the change in mean BAT FF was only statistically significant in a single subject. Similarly the change in mean WAT FF between scans was only statistically significant in a single subject.

In situ BAT has been identified in rodents in both active (40) and inactive states (18,23,41). FF within BAT has been shown to fluctuate with functional status however, with significantly lower BAT FF reported in rats exposed to cold (50.9% at 16°C) than those kept warm (79.4% at 30°C) (42). A similar trend towards lower BAT FF at low temperatures has also been reported in adult humans (27,43). Lower lipid content has been described within activated BAT (44) which has been attributed to depletion of lipid stores (45).

In this study the mean interval between MRI scans was fairly short (1.7 ± 1.1 months), which may be insufficient to capture seasonal differences in BAT activation. Therefore it may be preferable to perform follow-up scans after 6 or 9 months. Franz et al (46) demonstrated that even in subjects with BAT activity on PET, the FF within supraclavicular fat remained stable across multiple MRI scans, albeit in a paediatric population.

In the third element of the study we compared FF within supraclavicular fat in 6 subjects with BAT uptake on PET/CT, and 6 age- and sex matched controls. We found that FF within supraclavicular fat was statistically significantly lower than subcutaneous WAT, which accords with the findings of Franz *et al* (46) albeit in a paediatric population. This difference was evident on both subjects with and without BAT activity on PET/CT, although there was a small statistically significant difference in supraclavicular FF between the two groups. WAT FF was the greatest factor influencing FF within supraclavicular fat, accounting for 75% of the variance.

Controlling for baseline differences in WAT FF and mean daily temperature, a statistically significant difference in mean supraclavicular FF persisted between the groups, although the effect size was small (mean difference in FF 0.011, 95% CI 0.005 – 0.018) accounting for only 3.8% of variance. This small effect size may be

due to the fact that even in subjects with BAT, supraclavicular fat is largely composed of WAT, which will increase the mean FF.

It is worthy of note that the mean FF between subjects K to P differed between the 'transpositional' and 'regional' techniques of the study. This is due to analyses being performed on different slices on the MRI scan, as BAT or supraclavicular fat depots were compared with WAT on contiguous slices a slice-by-slice basis. Nonetheless there was no significant difference in subcutaneous WAT FF between the techniques.

We are aware of several limitations to this study. Fat quantification based on a TSE acquisition may be biased due to T2 lengthening due to the removal of J-coupling-induced de-phasing by rapid application of the refocusing pulse (47). Therefore gradient-echo acquisitions may be more sensitive in detecting small differences between brown and white adipose tissue.

It is noteworthy that although slice thickness was not uniform (subjects A-J were scanned with a slice thickness of 5 mm, whilst subjects K-P and their corresponding age- and sex-matched controls Q-V were scanned at 2.5 mm), slice thickness did not have a significant effect upon FF for either transposed BAT ($P = 0.31$) or subcutaneous WAT ($P = 0.96$).

To segment out non-fat on MRI FF maps, we chose a lower threshold of 50% FF instead of the 40% threshold typically used in other studies. We felt that a threshold of 40% in adult humans was unnecessarily low, as FF within BAT (and in particular BAT in adult humans) is typically considerably higher than this. We found that adopting a higher threshold of 50% fat fraction segmented out more non-fat, without impacting on FF measurements within BAT.

The first element of the study used ROIs transposed from regions of ^{18}F-FDG uptake consistent with BAT (^{18}F-FDG BAT) on PET/CT onto MRI scans. BAT is highly dynamic, therefore uptake will fluctuate between PET/CT scans depending on its activation state. ^{18}F-FDG uptake within BAT may be activated or inhibited within as little as an hour before administration of ^{18}F-FDG (48). We identified subjects as lacking BAT based on sustained absence of BAT activity across serial PET/CT scans. Although the likelihood of detecting BAT increases the more PET/CT scans

an individual undergoes (15) this does not absolutely exclude the presence of inactive BAT, and there is still the potential to misclassify subjects as lacking BAT when it is merely quiescent. Currently, PET/CT represents the best and most widely accepted technique for imaging BAT, and although there was no cooling procedure to stimulate BAT, the standardised imaging protocol affords some uniformity across subjects. Thermography has been used as a means of non-invasively imaging BAT activity in humans (49) but is confined to imaging BAT in its activated state.

In this study we used subcutaneous WAT as a reference. Whilst it may be more appropriate to use non-avid fat in the supraclavicular fossae and mediastinum as a reference, these are areas in which BAT is most likely to occur. As MRI and PET/CT scans were not performed concurrently, absence of ^{18}F-FDG uptake within specific sites does not exclude inactive BAT. This limitation would be addressed by performing PET and MRI scans concurrently, ideally following a period of cold exposure to stimulate BAT activity.

There was considerable variation in WAT FF between subjects, which accounted for the majority of variation in BAT FF. FF within subcutaneous fat is not uniform, showing higher fat saturation in deep subcutaneous tissues than superficially (50). Furthermore WAT is not metabolically inert, and its composition and morphology varies with season and diet (51). This inter- and intra-subject variation in WAT FF may be problematic when using WAT as a comparator.

A further limitation is that the PET/CT and MRI scans were performed on different dates, often in different seasons. Therefore, it is possible that BAT activation status differed between the PET and MRI scans. This issue has been addressed in a small study involving hybrid PET/MRI scanning, which showed correlation between FF on MRI and areas of BAT on PET (52), albeit only in subjects with intense FDG uptake within BAT deposits. Furthermore no significant difference in FF was found in subjects with and without evidence of supraclavicular BAT on PET.

In addition to BAT having a lower FF than WAT, BAT is more heterogeneous due to the high mitochondrial content of BAT, which in turn shortens T2* time (18). The higher vascularity within BAT results in enhanced perfusion and oxygen consumption within BAT; basal oxygen consumption is approximately 300% higher than within

WAT (53) although this does fluctuate with the degree of BAT activity (54). This may be exploited using arterial spine labelling or blood-oxygen-level dependent (BOLD) imaging (55).

Accuracy may be improved by adopting a multi-parametric approach in which multiple MRI sequences are analysed. Identification of BAT based on neural network based automatic segmentation of multi-parametric MRI has shown high accuracy in identifying BAT in rats when compared with manual segmentation (56). Although there was histological and immunohistochemical confirmation, this has yet to be successfully employed in adult humans.

The development of a reliable and accurate radiological biomarker for identifying and quantifying BAT is an important prerequisite to developing novel therapies for BAT modulation. Most studies evaluating FF as a means of identifying BAT in humans have focussed on children and infants. This study provides valuable insights into the utility of FF in identifying BAT in adult humans.

5. Conclusions

We found a statistically significant difference in FF between BAT and WAT, although the optimal threshold to separate BAT and WAT varied between individuals.

An effective cut-off point to separate BAT and subcutaneous WAT was only possible in a quarter of cases, and consequently a universal cut-off to differentiate these two tissues on MRI could not be identified on these data.

Similarly, there was also a small but statistically significant difference in supraclavicular FF between those with and without BAT activity on PET/CT. Further studies would be required to achieve greater precision, in order that BAT may be identified on MRI without *a priori* knowledge of BAT uptake on PET/CT.

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Tables

Subject	Sex	Age at first scan (years)	BMI at first scan (kg/m^2)	Diagnosis
BAT uptake on PET/CT				
A	Male	65	22.8	Lymphoma
B	Male	67	20.9	Lung adenocarcinoma
C	Female	65	26.2	Colorectal carcinoma
D	Female	53	25.6	Melanoma
E	Male	26	25.2	Testicular seminoma
F	Male	21	23.1	Hodgkin's lymphoma
G	Male	61	21.4	Gastro-intestinal stromal tumour
H	Female	30	22.0	Hodgkin's lymphoma
I	Female	73	31.6	Gastro-intestinal stromal tumour
J	Female	27	19.4	Hodgkin's lymphoma
K	Female	24	23.4	Hyperparathyroidism-jaw tumour syndrome
L	Female	56	21.9	Mucinous rectal cancer
M	Male	27	19.0	Hodgkin's lymphoma
N	Male	18	20.9	Metastatic teratoma
O	Female	54	26.3	Colorectal cancer
P	Female	41	23.7	Solitary pulmonary nodule
No BAT uptake on PET/CT				
Q	Female	24	20.2	Lymphoma
R	Female	54	21.6	Submandibular gland carcinoma
S	Male	29	23.6	Seminoma
T	Male	23	25.9	Hodgkin’s lymphoma
U	Female	59	35.3	Non-Hodgkin’s lymphoma
V	Female	41	24.8	Vaginal carcinoma

Table 1: Demographics of subjects with BAT activity on PET/CT (n=16)

Subject	WAT FF (±SD)	Transposed BAT FF (±SD)	Adjusted *p* value
A	0.827 ± 0.006	0.750 ± 0.101	0.001
B	0.823 ± 0.010	0.725 ± 0.051	<0.0001
C	0.840 ± 0.009	0.703 ± 0.070	<0.0001
D	0.825 ± 0.014	0.708 ± 0.021	<0.0001
E	0.596 ± 0.040	0.672 ± 0.061	0.009
F	0.756 ± 0.012	0.599 ± 0.057	<0.0001
G	0.787 ± 0.011	0.738 ± 0.028	ns
H	0.812 ± 0.010	0.785 ± 0.063	ns
I	0.817 ± 0.007	0.768 ± 0.056	0.0001
J	0.778 ± 0.016	0.748 ± 0.052	0.026
K	0.815 ± 0.020	0.798 ± 0.031	ns
L	0.832 ± 0.006	0.810 ± 0.019	0.0595
M	0.760 ± 0.017	0.677 ± 0.025	<0.0001
N	0.739 ± 0.019	0.672 ± 0.047	<0.0001
O	0.815 ± 0.007	0.814 ± 0.022	ns
P	0.765 ± 0.018	0.738 ± 0.028	0.0054

Table 2: WAT and transposed BAT FF on initial MRI scan by subject, ns= not significant.

Subject	Optimal FF cut-off	AUC (95% CI)
A	0.810	0.678 (0.651 - 0.705)
B	0.801	0.842 (0.832 - 0.853)
C	0.830	0.899 (0.888 - 0.909)
D	0.800	0.924 (0.921 - 0.926)
E	0.718	0.248 (0.226 - 0.271)
F	0.681	0.838 (0.813 - 0.863)
G	0.748	0.599 (0.586 - 0.611)
H	0.783	0.560 (0.545 - 0.575)
I	0.779	0.662 (0.649 - 0.676)
J	0.732	0.578 (0.568 - 0.588)
K	0.783	0.500 (0.496 - 0.504)
L	0.792	0.533 (0.530 - 0.537)
M	0.749	0.762 (0.757 - 0.765)
N	0.688	0.710 (0.706 - 0.714)
O	0.853	0.557 (9.553 - 0.561)
P	0.712	0.577 (0.572 - 0.581)

Table 3: Receiver operating statistics (AUC = area under the curve) and optimal FF for WAT and transposed BAT discrimination for subjects with BAT activity on PET/CT (subjects A-P)

Subject	Scan 1	Scan 2	Adjusted *p* value
Transposed BAT FF			
A	0.750 ± 0.101	0.765 ± 0.051	ns
D	0.708 ± 0.021	0.730 ± 0.044	ns
E	0.672 ± 0.061	0.703 ± 0.017	ns
F	0.599 ± 0.057	0.609 ± 0.043	ns
I	0.768 ± 0.056	0.818 ± 0.055	0.0003
K	0.798 ± 0.031	0.819 ± 0.026	ns
WAT FF			
A	0.828 ± 0.006	0.829 ± 0.012	ns
D	0.825 ± 0.014	0.768 ± 0.016	< 0.0001
E	0.596 ± 0.040	0.581 ± 0.017	ns
F	0.756 ± 0.012	0.773 ± 0.004	ns
I	0.817 ± 0.007	0.818 ± 0.017	ns
K	0.815 ± 0.020	0.824 ± 0.017	ns

434 **Table 4: WAT and transposed BAT FF on serial scans (n=6 subjects)**

Figures

Figure 1: Flow chart of subjects

Figure 2: Regions of interest on axial FF map. Top: transposed BAT from PET/CT (red), with manually defined ROIs in subcutaneous WAT (blue), (b) ROIs within supraclavicular fossae (yellow)

Figure 3: Mean differences in FF between WAT and transposed BAT (with 95% confidence intervals) for subjects A-P. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.005$, *ns* = not significant

Figure 4: Summary receiver operating plot showing the sensitivity and specificity of the optimal FF cut-off for each subject (green = good/excellent tissue separation, yellow = fair, red = poor)

Figure 5: Temporal changes in WAT and transposed BAT FF (* = $p < 0.001$)**

Figure 6: Mean differences in FF between WAT and supraclavicular fat (with 95% CI) by subject (● subjects with BAT activity on PET/CT, ○ subjects without BAT activity on PET/CT, ns = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.005$)

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