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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.

54 ***Declaration of interest***

55 None.

56 ***Keywords***

59

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).

89   Estimates of BAT prevalence in adult humans varies, but larger studies using

90   PET/CT report point prevalence of between 1.1% (14) and 8.5% (11), and 5.3% in

91   our own population (15) – all of which are certainly underestimates. Cumulative

92   prevalence (based on repeated PET/CT scanning of 145 patients) is estimated at

93   64% (11), while small dedicated prospective PET/CT studies report point prevalence

94   as high as 95.8% (16) and 100% (17). However, the high radiation burden, costly

95   radiotracer and relatively low spatial resolution of PET/CT limit its role as a research

96   tool in large populations (18).

97   MRI may offer a non-invasive and safe alternative for BAT quantification, as it can

98   differentiate between brown and white adipose tissue (WAT) based on differences in

99   fat (19) and mitochondrial content (20). Therefore MRI has the potential to identify

100 BAT regardless of its metabolic state (21),

101 Significantly lower fat fractions (FF) have been reported in BAT than WAT in rodents

102 (18,19,22-24) and human infants (20,25,26). Human studies have tended to focus on

103 infants in whom BAT is more prevalent and extensive. Lower BAT FF has been

104 reported in small numbers of human adults (20,27), although differences decrease

105 with age (20). We previously reported a case in whom we were able to identify BAT

106 using MRI, which was subsequently confirmed histologically (28). Gifford *et al* (29)

107 identified probable BAT based on FF in two adult humans. For the most part,

108 however, it has not been possible to extrapolate these findings to prospectively

109 identify BAT in adult humans solely on the basis of MRI imaging findings.

110 The aim of this study was to determine whether there was a significant difference in

111 FF between BAT and WAT in adult humans, and to identify an optimal threshold for

112 differentiating between these tissues on the basis of FF in vivo.

113 Our secondary aims were to determine whether FF within BAT and WAT fluctuates

114 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.

143   IDEAL fat- and water-only images were post-processed using ImageJ (30) to

144   generate FF maps according to the formula:

145
$$Fat\ fraction = \frac{Fat\ only}{Fat\ only + Water\ only}$$

146   **2.5 Image analysis**

147   Non-fatty tissues were excluded from analysis by thresholding FF maps with a lower

148   limit set at 50% FF. PET/CT scans were registered to the MRI scans using Mirada

149   XD 3.4 image fusion software (Mirada Medical Ltd, Oxford, UK) using both rigid and

150   non-rigid registration techniques, with manual placement of fiducial markers as

151   necessary.

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

153   1. Transposition from PET/CT: ROIs were drawn around ^{18}F-FDG BAT deposits

154   semi-automatically by defining iso-contours set at a standardized uptake

155   value (SUV) of 2.5 g/ml on the PET/CT scan, which we felt provided the best

156   compromise between capturing the extent of ^{18}F-FDG uptake within BAT ,

157   whilst minimizing artefactual bleeding into adjacent tissue. These ROIs were

158   then transposed onto the co-registered FF map.

159   2. Regional segmentation of supraclavicular fat: BAT, when active on PET/CT,

160   most likely occurs within the supraclavicular fossa (12,31). Therefore to

161   determine whether BAT could be identified without the benefit of *a priori*

162   knowledge from PET/CT scans, ROIs were drawn freehand around fat in the

163   supraclavicular fossa on contiguous slices. Morphological edge erosion was

164   then applied to minimize edge effects from adjacent tissue interfaces using

165   the 'eroded range limited' technique described by Lundström *et al* (32,33).

166   For comparison ROIs were manually defined in dorsal subcutaneous WAT on MRI

167   FF maps.

168   **2.6 Statistical analysis**

169   Differences in mean FF for transposed BAT and WAT were compared on a slice-by-

170   slice basis using analysis of variance (ANOVA) with Bonferroni's *post hoc* test for

171   pairwise comparisons using GraphPad Prism 7.00 for Mac OS (GraphPad Software,

172   La Jolla, California, USA). Significance was defined as $p < 0.05$.

173   Receiver operating characteristic (ROC) curves were generated to plot true positivity

174   rate (sensitivity) against false positivity rate (1-specificity) for various FF cut-off

175   points. Area under the curve (AUC), a measure of the accuracy of the test, was

176   derived, and optimal thresholds for differentiating BAT and WAT calculated using

177   Youden's J statistic (34).

178   Temporal changes in mean FF for BAT and WAT across serial scans (12 scans in 6

179   subjects; A, D, E, F, I and K) were compared using one-way ANOVA, with

180   Bonferroni's post test.

181   Differences in mean FF between subjects with and without evidence of BAT on

182   PET/CT (subjects K-V) were compared using two-way ANOVA with Bonferroni's *post*

183   *hoc* analysis, and ANCOVA to control for differences in baseline WAT FF and mean

184   daily temperature. ANCOVA analysis was performed on IBM SPSS Statistics for

185   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).

214 **_3.4 Temporal FF changes in subcutaneous WAT and transposed BAT_**

215 One-way ANOVA for the six subjects who had repeat MRI scans showed a

216 statistically significant difference in FF between initial and subsequent MRI scan for

217 both subcutaneous WAT and transposed BAT, $F(23, 250) = 39.44$, $p < 0.0001$. *Post-*

218 *hoc* analysis with Bonferroni's test for multiple comparisons (Table 4) showed a

219 statistically significant temporal change in FF for transposed BAT in subject I ($p =$

220 0.0003), and for subcutaneous WAT in subject D ($p < 0.0001$). The remainder

221 showed no significant difference in FF for either transposed BAT or subcutaneous

222 WAT between serial MRI scans (Table 4 and Figure 5).

223 **_3.5 Characterisation of subcutaneous WAT and supraclavicular fat_**

224 For the subgroup of age-and sex-matched subjects (K-V), FF within supraclavicular

225 fat was compared against subcutaneous WAT using two-way ANOVA to determine

226 whether BAT status (*i.e.* presence or absence of BAT activity on PET/CT) affected

227 FF.

228 There was a statistically significant interaction between the effects of tissue type (i.e.

229 supraclavicular fat and subcutaneous WAT) and BAT status on PET/CT on FF, $F(11,$

230 $616) = 13.93$, $p < 0.0001$, $\eta_p^2 = 0.024$. Simple main effects analysis showed a

231 statistically significant difference in FF between supraclavicular fat and WAT, $F(11,$

232 $616) = 467.9$, $p < 0.0001$, $\eta_p^2 = 0.792$. There was also a significant (albeit small)

233 difference in FF between subjects with and without BAT activity on PET/CT $F(1, 616)$

234 $= 579.5$, $p < 0.0001$, $\eta_p^2 = 0.089$. *Post hoc* Bonferroni's test for multiple comparisons

235 showed FF within supraclavicular fat was significantly lower than WAT in all but one

236 case (Figure 6).

237 To evaluate differences in supraclavicular fat FF between subjects with (K-P) and

238 without BAT activity (Q-V) on PET/CT, whilst controlling for variation in both baseline

239 subcutaneous WAT FF and environmental temperature, a one-way ANCOVA was

240 performed with baseline subcutaneous WAT FF and mean daily temperature as

241 covariates.

242 There was a significant effect of PET/CT BAT status on FF after controlling for WAT

243 FF and temperature, $F(1,316) = 12.537$, $p < 0.001$, although the effect size was

244 small ($\eta_p^2 = 0.038$). Mean FF within supraclavicular fat was significantly lower in

245   subjects with BAT activity on PET/CT (corrected mean FF 0.739, 95% CI 0.734 –

246   0.743) than those without BAT activity on PET/CT (0.750, 95% CI 0.746 – 0.755),

247   although the differences were small (corrected mean difference 0.011, 95% CI 0.005

248   – 0.018).

249   The predicted main effect of subcutaneous WAT FF upon supraclavicular fat FF was

250   statistically significant [$F_{(1,316)} = 948.455$, $p < 0.005$, $\eta_p^2 = 0.750$], as was mean

251   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.

281   For the second element of the study, a subset of six subjects underwent a second

282   MRI scan to determine whether FF changed over time. Comparison of the two MRI

283   scans using ANOVA revealed a significant difference in mean BAT FF between

284   scans, although post hoc tests showed that the change in mean BAT FF was only

285   statistically significant in a single subject. Similarly the change in mean WAT FF

286   between scans was only statistically significant in a single subject.

287   *In situ* BAT has been identified in rodents in both active (40) and inactive states

288   (18,23,41). FF within BAT has been shown to fluctuate with functional status

289   however, with significantly lower BAT FF reported in rats exposed to cold (50.9% at

290   16°C) than those kept warm (79.4% at 30°C) (42). A similar trend towards lower BAT

291   FF at low temperatures has also been reported in adult humans (27,43). Lower lipid

292   content has been described within activated BAT (44) which has been attributed to

293   depletion of lipid stores (45).

294   In this study the mean interval between MRI scans was fairly short (1.7 ± 1.1

295   months), which may be insufficient to capture seasonal differences in BAT activation.

296   Therefore it may be preferable to perform follow-up scans after 6 or 9 months. Franz

297   et al (46) demonstrated that even in subjects with BAT activity on PET, the FF within

298   supraclavicular fat remained stable across multiple MRI scans, albeit in a paediatric

299   population.

300   In the third element of the study we compared FF within supraclavicular fat in 6

301   subjects with BAT uptake on PET/CT, and 6 age- and sex matched controls. We

302   found that FF within supraclavicular fat was statistically significantly lower than

303   subcutaneous WAT, which accords with the findings of Franz *et al* (46) albeit in a

304   paediatric population. This difference was evident on both subjects with and without

305   BAT activity on PET/CT, although there was a small statistically significant difference

306   in supraclavicular FF between the two groups. WAT FF was the greatest factor

307   influencing FF within supraclavicular fat, accounting for 75% of the variance.

308   Controlling for baseline differences in WAT FF and mean daily temperature, a

309   statistically significant difference in mean supraclavicular FF persisted between the

310   groups, although the effect size was small (mean difference in FF 0.011, 95% CI

311   0.005 – 0.018) accounting for only 3.8% of variance. This small effect size may be

312 due to the fact that even in subjects with BAT, supraclavicular fat is largely

313 composed of WAT, which will increase the mean FF.

314 It is worthy of note that the mean FF between subjects K to P differed between the

315 'transpositional' and 'regional' techniques of the study. This is due to analyses being

316 performed on different slices on the MRI scan, as BAT or supraclavicular fat depots

317 were compared with WAT on contiguous slices a slice-by-slice basis. Nonetheless

318 there was no significant difference in subcutaneous WAT FF between the

319 techniques.

320 We are aware of several limitations to this study. Fat quantification based on a TSE

321 acquisition may be biased due to T2 lengthening due to the removal of J-coupling-

322 induced de-phasing by rapid application of the refocusing pulse (47). Therefore

323 gradient-echo acquisitions may be more sensitive in detecting small differences

324 between brown and white adipose tissue.

325 It is noteworthy that although slice thickness was not uniform (subjects A-J were

326 scanned with a slice thickness of 5 mm, whilst subjects K-P and their corresponding

327 age- and sex-matched controls Q-V were scanned at 2.5 mm), slice thickness did

328 not have a significant effect upon FF for either transposed BAT ($P = 0.31$) or

329 subcutaneous WAT ($P = 0.96$).

330 To segment out non-fat on MRI FF maps, we chose a lower threshold of 50% FF

331 instead of the 40% threshold typically used in other studies. We felt that a threshold

332 of 40% in adult humans was unnecessarily low, as FF within BAT (and in particular

333 BAT in adult humans) is typically considerably higher than this. We found that

334 adopting a higher threshold of 50% fat fraction segmented out more non-fat, without

335 impacting on FF measurements within BAT.

336 The first element of the study used ROIs transposed from regions of ^{18}F-FDG uptake

337 consistent with BAT (^{18}F-FDG BAT) on PET/CT onto MRI scans. BAT is highly

338 dynamic, therefore uptake will fluctuate between PET/CT scans depending on its

339 activation state. ^{18}F-FDG uptake within BAT may be activated or inhibited within as

340 little as an hour before administration of ^{18}F-FDG (48). We identified subjects as

341 lacking BAT based on sustained absence of BAT activity across serial PET/CT

342 scans. Although the likelihood of detecting BAT increases the more PET/CT scans

343   an individual undergoes (15) this does not absolutely exclude the presence of

344   inactive BAT, and there is still the potential to misclassify subjects as lacking BAT

345   when it is merely quiescent. Currently, PET/CT represents the best and most widely

346   accepted technique for imaging BAT, and although there was no cooling procedure

347   to stimulate BAT, the standardised imaging protocol affords some uniformity across

348   subjects. Thermography has been used as a means of non-invasively imaging BAT

349   activity in humans (49) but is confined to imaging BAT in its activated state.

350   In this study we used subcutaneous WAT as a reference. Whilst it may be more

351   appropriate to use non-avid fat in the supraclavicular fossae and mediastinum as a

352   reference, these are areas in which BAT is most likely to occur. As MRI and PET/CT

353   scans were not performed concurrently, absence of ^{18}F-FDG uptake within specific

354   sites does not exclude inactive BAT. This limitation would be addressed by

355   performing PET and MRI scans concurrently, ideally following a period of cold

356   exposure to stimulate BAT activity.

357   There was considerable variation in WAT FF between subjects, which accounted for

358   the majority of variation in BAT FF. FF within subcutaneous fat is not uniform,

359   showing higher fat saturation in deep subcutaneous tissues than superficially (50).

360   Furthermore WAT is not metabolically inert, and its composition and morphology

361   varies with season and diet (51). This inter- and intra-subject variation in WAT FF

362   may be problematic when using WAT as a comparator.

363   A further limitation is that the PET/CT and MRI scans were performed on different

364   dates, often in different seasons. Therefore, it is possible that BAT activation status

365   differed between the PET and MRI scans. This issue has been addressed in a small

366   study involving hybrid PET/MRI scanning, which showed correlation between FF on

367   MRI and areas of BAT on PET (52), albeit only in subjects with intense FDG uptake

368   within BAT deposits. Furthermore no significant difference in FF was found in

369   subjects with and without evidence of supraclavicular BAT on PET.

370   In addition to BAT having a lower FF than WAT, BAT is more heterogeneous due to

371   the high mitochondrial content of BAT, which in turn shortens T2* time (18). The

372   higher vascularity within BAT results in enhanced perfusion and oxygen consumption

373   within BAT; basal oxygen consumption is approximately 300% higher than within

374   WAT (53) although this does fluctuate with the degree of BAT activity (54). This may

375   be exploited using arterial spine labelling or blood-oxygen-level dependent (BOLD)

376   imaging (55).

377   Accuracy may be improved by adopting a multi-parametric approach in which

378   multiple MRI sequences are analysed. Identification of BAT based on neural network

379   based automatic segmentation of multi-parametric MRI has shown high accuracy in

380   identifying BAT in rats when compared with manual segmentation (56). Although

381   there was histological and immunohistochemical confirmation, this has yet to be

382   successfully employed in adult humans.

383   The development of a reliable and accurate radiological biomarker for identifying and

384   quantifying BAT is an important prerequisite to developing novel therapies for BAT

385   modulation. Most studies evaluating FF as a means of identifying BAT in humans

386   have focussed on children and infants. This study provides valuable insights into the

387   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.

6. Acknowledgements

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)

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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

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

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418

419

420

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)

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

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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

435 **Figures**

436 **Figure 1: Flow chart of subjects**

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

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

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

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

447 **Figure 6: Mean differences in FF between WAT and supraclavicular fat (with**
448 **95% CI) by subject (● subjects with BAT activity on PET/CT, ○ subjects**
449 **without BAT activity on PET/CT, ns = not significant, * = *p* < 0.05, ** = *p* < 0.01,**
450 ***** = *p* < 0.005)**

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