

# Supplemental material

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## PET/MRI of glucose metabolic rate, lipid content and perfusion in human brown adipose tissue

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### Materials and methods

Mean fat fraction (FF) of suspected BAT (sBAT-FF) was estimated from segmentations obtained with a modified version of a previously presented method<sup>1</sup>, implemented in MATLAB. The method was atlas-based with the atlas sBAT depots defined according to anatomical criteria. The main adjustments to the published method are provided below in 1) *Generation of atlases* – 4) *Fine adjustment and refinement of segmentations*.

- 1) Generation of atlases – Initially, an atlas was created from the baseline scan of each subject (denoted *atlas 1*). The atlas consisted of a water signal image and a fat signal image (instead of a water fraction and an R<sub>2</sub>\* map as previously described<sup>1</sup>) with an associated crude sBAT volume of interest (VOI) segmentation. Prior to image registration, pre-processing of the water and fat signal images was carried out, which aimed for removal of voxels outside the body (background and water piping). Segmentation of the crude sBAT VOI was performed manually from anatomically defined criteria as previously described<sup>1</sup>. The two outmost slices, both in superior and inferior direction of the image data, were removed to reduce possible effects related to non-optimal slab profiles at the edges of the 3D image volume.
- 2) Image registration – A single-atlas approach, instead of the previously described multi-atlas approach<sup>1</sup>, was used. Prior to image registration, the water and fat signal images of the rest of the scans of each subject were pre-processed as described above for the baseline scan in *Generation*

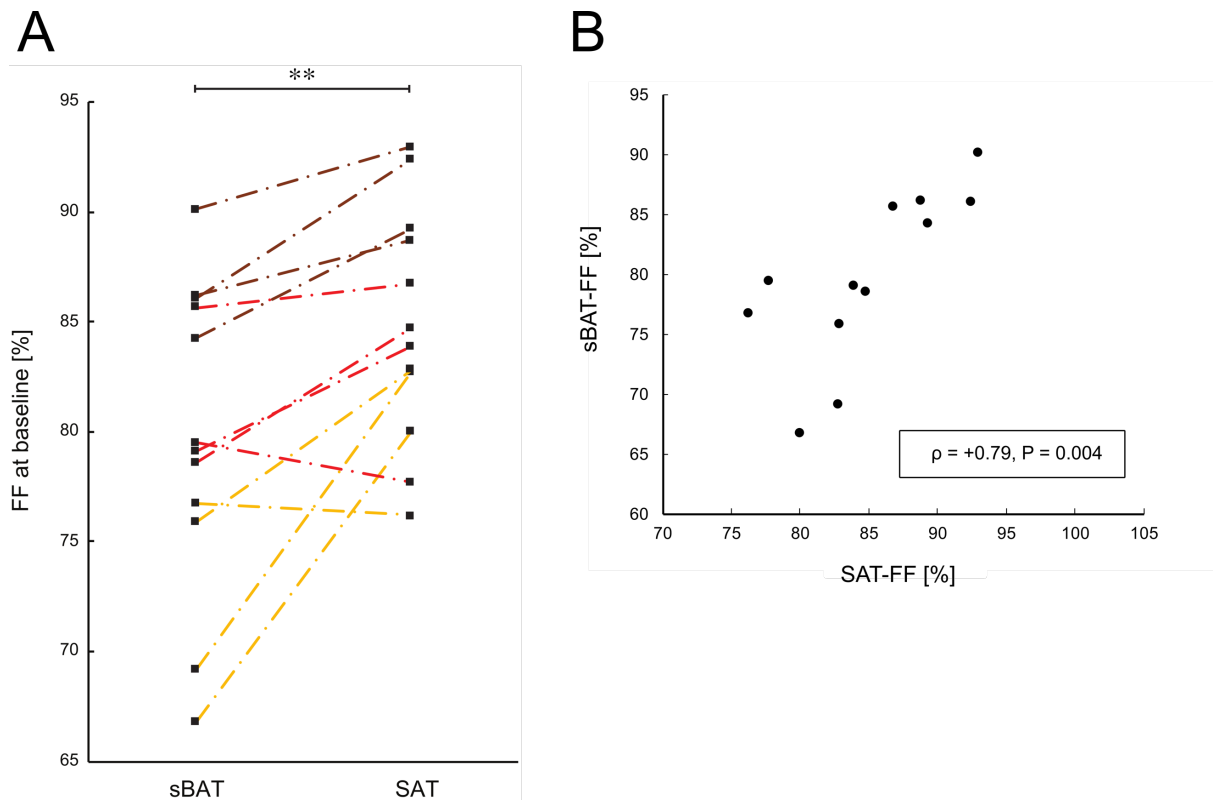
of *atlases*. For each subject, a pairwise intra-subject image registration between atlas 1 and cold scan 1 (target) was carried out in line with the previously described method<sup>1</sup>. An initial affine registration was performed between the water signal images and a subsequent elastic registration was performed between both the water and fat signal images.

- 3) **Propagation of segmentations** – For each pairwise image registration, the resulting deformation field was applied to transfer the crude sBAT VOI from atlas 1 to the target. Two additional crude segmentations were created from the deformed crude sBAT VOI and the metabolic rate of glucose ( $MR_{glu}$ ) map. A crude high  $MR_{glu}$  sBAT VOI (crude sBAT<sub>HI</sub>) was obtained by including only sBAT VOI voxels with  $MR_{glu} > 11 \mu\text{mol}/100 \text{ cm}^3/\text{min}$ . The crude low  $MR_{glu}$  sBAT VOI (crude sBAT<sub>LO</sub>) contained sBAT VOI voxels with  $MR_{glu} \leq 11 \mu\text{mol}/100 \text{ cm}^3/\text{min}$ . The crude sBAT, sBAT<sub>HI</sub> and sBAT<sub>LO</sub> segmentations together with the water and fat signal images of cold scan 1 formed a new single atlas (denoted *atlas 2*) that was used to automatically segment the rest of the scans from both visits, using the same registration method as described above in *Image registration*. The  $MR_{glu}$  threshold limit of  $11 \mu\text{mol}/100 \text{ cm}^3/\text{min}$  aimed to separate active sBAT and inactive subcutaneous adipose tissue (SAT), and was determined empirically.
- 4) **Fine adjustment and refinement of segmentations** – After the crude segmentations had been transferred to all targets, an automated fine adjustment was applied to the crude sBAT VOI, for isolating the adipose tissue and reducing the partial volume effects from adjacent non-adipose tissues. This fine adjustment comprised a voxel threshold on FF ( $\geq 40\%$ ), a six-neighbourhood erosion, a voxel threshold on  $R_2^*$  ( $\leq 120 \text{ s}^{-1}$ ) and attempts to remove residual voxels in air and bone, based on thresholding on the sum of the water and fat signal images with subsequent steps of dilation, filling of holes and erosion. The threshold limits were determined empirically. In addition, the outmost slice, both in superior and inferior direction, was excluded from the segmentation. This fine adjustment was also applied to the crude sBAT<sub>HI</sub> and sBAT<sub>LO</sub> by merging the final sBAT VOI with the crude sBAT<sub>HI</sub> and sBAT<sub>LO</sub> VOIs. The final segmentations are referred to as sBAT, sBAT<sub>HI</sub> and sBAT<sub>LO</sub>.

Posterior SAT was segmented from the same water-fat image slices used for segmenting sBAT, according to the following. A body mask was segmented from the background using thresholding on the sum of the water and fat signal images. The threshold level was manually adjusted if deemed necessary. Holes in the mask were filled. A muscle mask was created by removing voxels with  $R_2^* > 100 \text{ s}^{-1}$  (skin and bone) and  $FF \geq 40\%$  (adipose tissue and bone). A SAT mask was created by excluding voxels with  $FF < 40\%$  from the body mask, and also by excluding voxels enclosed within the muscle mask to remove voxels in bones and non-SAT. The area superior of the centre of gravity of the body mask (in anterior direction), determined slice-wise, was removed from the SAT mask. A six-neighbourhood erosion was performed to decrease the partial volume effects. Voxels with  $R_2^* > 120 \text{ s}^{-1}$  (residual skin and bones) were removed from the SAT mask. Finally, some minor manual corrections were performed. Regions of high and low  $MR_{glu}$  within posterior SAT were obtained using the same threshold level ( $MR_{glu} = 11 \mu\text{mol}/100 \text{ cm}^3/\text{min}$ ) as for sBAT. The final segmentations are referred to as SAT, SAT<sub>HI</sub> and SAT<sub>LO</sub>.

## Results

Comparison and correlation between mean sBAT-FF and SAT-FF, estimated at baseline for each subject, are shown in Fig. S1. Results from correlation of sBAT-FF and SAT-FF with  $MR_{glu}$  and  $MR_{glu2}$ , estimated within the same segmentation, are presented in Table S1. The  $MR_{glu}$  and  $MR_{glu2}$  were derived from different plasma glucose assessments,  $C_{glu}^P$  and  $C_{glu}^{P2}$ , respectively (see details in manuscript). The FF, perfusion,  $V_A$ ,  $MR_{glu}$  and segmented volume of different subregions of sBAT and SAT, obtained from the Cooling-reheating and Control protocols, are summarized in Table S2.



**Figure S1.** Baseline FF measurements from the Cooling-reheating protocol. **(A)** Mean sBAT-FF and SAT-FF for each individual. Yellow, red and brown indicate the four subjects with the highest, intermediate and lowest sBAT-MR<sub>glu</sub>, respectively. Difference between sBAT and SAT at \*\*P < 0.01. **(B)** Correlation between sBAT-FF and SAT-FF.  $\rho$ , Spearman rank-order correlation coefficient.

|             | Correlation with MR <sub>glu</sub> |           |       |           | Correlation with MR <sub>glu2</sub> |       |           |       |           |    |
|-------------|------------------------------------|-----------|-------|-----------|-------------------------------------|-------|-----------|-------|-----------|----|
| Measurement | ρ                                  | (P)       | r     | (P)       | n                                   | ρ     | (P)       | r     | (P)       | n  |
| Baseline FF |                                    |           |       |           |                                     |       |           |       |           |    |
| sBAT        | -0.88                              | (< 0.001) | -0.81 | (0.001)   | 12                                  | -0.87 | (< 0.001) | -0.85 | (< 0.001) | 11 |
| SAT         | -0.83                              | (0.002)   | -0.71 | (0.009)   | 12                                  | -0.90 | (< 0.001) | -0.71 | (0.014)   | 11 |
| Cold 1 FF   |                                    |           |       |           |                                     |       |           |       |           |    |
| sBAT        | -0.89                              | (< 0.001) | -0.90 | (< 0.001) | 11                                  | -0.91 | (< 0.001) | -0.92 | (< 0.001) | 11 |
| SAT         | -0.80                              | (0.005)   | -0.69 | (0.020)   | 11                                  | -0.87 | (< 0.001) | -0.76 | (0.006)   | 11 |
| Cold 2 FF   |                                    |           |       |           |                                     |       |           |       |           |    |
| sBAT        | -0.90                              | (< 0.001) | -0.91 | (< 0.001) | 10                                  | -0.90 | 0.002     | -0.93 | (< 0.001) | 9  |
| SAT         | -0.83                              | (0.006)   | -0.69 | (0.029)   | 10                                  | -0.80 | 0.014     | -0.70 | (0.035)   | 9  |
| Reheated FF |                                    |           |       |           |                                     |       |           |       |           |    |
| sBAT        | -0.87                              | (< 0.001) | -0.87 | (< 0.001) | 12                                  | -0.85 | 0.002     | -0.89 | (< 0.001) | 11 |
| SAT         | -0.83                              | (0.001)   | -0.74 | (0.006)   | 12                                  | -0.88 | (< 0.001) | -0.74 | (0.009)   | 11 |

**Table S1.** Correlation between FF and MR<sub>glu</sub> and between FF and MR<sub>glu2</sub> in sBAT and SAT during the Cooling-reheating protocol.  $\rho$ , Spearman rank-order correlation coefficient. r, Pearson correlation coefficient. n, number of observations. Statistical significance (P < 0.05) in bold font.

| Variable          | Baseline                                |                                                      |                                                       | Cold 1                                  |                                                       |                                                       | Cold 2                                  |                                                      |                                                       | Reheated                                |                                                       |                                                       |
|-------------------|-----------------------------------------|------------------------------------------------------|-------------------------------------------------------|-----------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-----------------------------------------|------------------------------------------------------|-------------------------------------------------------|-----------------------------------------|-------------------------------------------------------|-------------------------------------------------------|
|                   | sBAT<br><i>n</i> =12<br>( <i>n</i> =10) | sBAT <sub>HI</sub><br><i>n</i> =11<br>( <i>n</i> =9) | sBAT <sub>LO</sub><br><i>n</i> =12<br>( <i>n</i> =10) | sBAT<br><i>n</i> =11<br>( <i>n</i> =11) | sBAT <sub>HI</sub><br><i>n</i> =10<br>( <i>n</i> =10) | sBAT <sub>LO</sub><br><i>n</i> =11<br>( <i>n</i> =11) | sBAT<br><i>n</i> =10<br>( <i>n</i> =11) | sBAT <sub>HI</sub><br><i>n</i> =9<br>( <i>n</i> =10) | sBAT <sub>LO</sub><br><i>n</i> =10<br>( <i>n</i> =11) | sBAT<br><i>n</i> =12<br>( <i>n</i> =11) | sBAT <sub>HI</sub><br><i>n</i> =11<br>( <i>n</i> =10) | sBAT <sub>LO</sub><br><i>n</i> =12<br>( <i>n</i> =11) |
| FF                | 79.8±7.1<br>(79.6±8.1)                  | 79.5±7.5<br>(79.0±8.7)                               | 80.8±5.9<br>(80.9±6.5)                                | 77.7±8.7<br>(80.3±7.6)                  | 76.9±9.2<br>(79.5±7.7)                                | 79.6±7.2<br>(81.7±6.1)                                | 76.8±8.5<br>(80.3±7.9)                  | 75.4±8.5<br>(80.3±9.0)                               | 78.6±6.8<br>(81.4±6.5)                                | 78.6±7.7<br>(81.0±8.1)                  | 77.8±7.9<br>(81.2±9.6)                                | 79.9±6.3<br>(82.2±7.0)                                |
| Perfusion         | 13.2±9.3<br><i>n</i> =12                | <i>na</i>                                            | <i>na</i>                                             | 18.3±5.9<br><i>n</i> =12                | <i>na</i>                                             | <i>na</i>                                             | <i>na</i>                               | <i>na</i>                                            | <i>na</i>                                             | 21.6±12.4<br><i>n</i> =12               | <i>na</i>                                             | <i>na</i>                                             |
| V <sub>A</sub>    | 2.9±2.0<br><i>n</i> =11                 | <i>na</i>                                            | <i>na</i>                                             | 7.3±3.8<br><i>n</i> =12                 | <i>na</i>                                             | <i>na</i>                                             | <i>na</i>                               | <i>na</i>                                            | <i>na</i>                                             | 2.1±1.5<br><i>n</i> =11                 | <i>na</i>                                             | <i>na</i>                                             |
| MR <sub>glu</sub> | <i>na</i>                               | <i>na</i>                                            | <i>na</i>                                             | 21.5±17.1<br><i>n</i> =12               | 28.7±14.4<br><i>n</i> =11                             | 5.9±2.2<br><i>n</i> =12                               | <i>na</i>                               | <i>na</i>                                            | <i>na</i>                                             | <i>na</i>                               | <i>na</i>                                             | <i>na</i>                                             |
| Volume            | 42.5±31.4<br>(40.4±28.0)                | 17.0±12.0<br>(20.2±13.8)                             | 26.9±37.2<br>(22.2±33.2)                              | 45.5±36.7<br>(45.8± 32.3)               | 16.0±12.1<br>(18.5±13.7)                              | 30.9±42.0<br>(29.0±38.7)                              | 37.2±31.0<br>(45.5±33.2)                | 16.0±11.5<br>(17.9±12.3)                             | 22.8±33.6<br>(29.2±39.7)                              | 40.3±33.2<br>(45.9±33.7)                | 14.8±11.4<br>(18.4±13.2)                              | 26.7±37.7<br>(29.2±40.1)                              |

| Variable          | Baseline                               |                                                    |                                                      | Cold 1                                 |                                                    |                                                      | Cold 2                                 |                                                    |                                                      | Reheated                               |                                                    |                                                      |
|-------------------|----------------------------------------|----------------------------------------------------|------------------------------------------------------|----------------------------------------|----------------------------------------------------|------------------------------------------------------|----------------------------------------|----------------------------------------------------|------------------------------------------------------|----------------------------------------|----------------------------------------------------|------------------------------------------------------|
|                   | SAT<br><i>n</i> =12<br>( <i>n</i> =10) | SAT <sub>HI</sub><br><i>n</i> =4<br>( <i>n</i> =3) | SAT <sub>LO</sub><br><i>n</i> =12<br>( <i>n</i> =10) | SAT<br><i>n</i> =11<br>( <i>n</i> =11) | SAT <sub>HI</sub><br><i>n</i> =3<br>( <i>n</i> =3) | SAT <sub>LO</sub><br><i>n</i> =11<br>( <i>n</i> =11) | SAT<br><i>n</i> =10<br>( <i>n</i> =11) | SAT <sub>HI</sub><br><i>n</i> =4<br>( <i>n</i> =3) | SAT <sub>LO</sub><br><i>n</i> =10<br>( <i>n</i> =11) | SAT<br><i>n</i> =12<br>( <i>n</i> =11) | SAT <sub>HI</sub><br><i>n</i> =3<br>( <i>n</i> =3) | SAT <sub>LO</sub><br><i>n</i> =12<br>( <i>n</i> =11) |
| FF                | 84.8±5.4<br>(85.2±5.1)                 | 79.5±3.7<br>(80.3±6.8)                             | 84.9±5.4<br>(85.2±5.0)                               | 86.0±5.0<br>(86.1±4.4)                 | 79.5±1.9<br>(82.0±5.6)                             | 86.0±4.9<br>(86.2±4.2)                               | 83.9±5.1<br>(85.5±4.9)                 | 80.4±3.4<br>(78.1±5.6)                             | 84.0±5.0<br>(85.6±4.8)                               | 84.4±5.8<br>(85.4±4.9)                 | 78.2±2.6<br>(79.4±6.7)                             | 84.5±5.7<br>(85.5±4.8)                               |
| MR <sub>glu</sub> | <i>na</i>                              | <i>na</i>                                          | <i>na</i>                                            | 3.6±1.6<br><i>n</i> =12                | 13.6±3.6<br><i>n</i> =4                            | 3.4±1.2<br><i>n</i> =12                              | <i>na</i>                              | <i>na</i>                                          | <i>na</i>                                            | <i>na</i>                              | <i>na</i>                                          | <i>na</i>                                            |
| Volume            | 130.1±114.7<br>(114.6±87.3)            | 2.8±5.6<br>(3.5±6.0)                               | 129.2±115.2<br>(113.6±87.9)                          | 140.4±119.8<br>(138.7±114.5)           | 4.0±6.8<br>(3.8±6.4)                               | 139.3±120.4<br>(137.6±115.3)                         | 116.7±100.8<br>(142.0±123.8)           | 2.9±5.7<br>(3.8±6.5)                               | 115.5±101.3<br>(140.9±124.6)                         | 123.7±110.2<br>(138.5±121.8)           | 3.9±6.7<br>(3.7±6.3)                               | 122.7±110.8<br>(137.5±122.4)                         |

**Table S2.** Summary of measurements in (A) sBAT and (B) SAT from the study population during the Cooling-reheating protocol, with corresponding measurements from the Control protocol in parenthesis when applicable. mean ± standard deviation. *n*, number of subjects or number of measurements in case measurements were lacking for some subjects. *na*, not applicable (measurements not conducted). Units: FF, %; perfusion, ml/100 cm<sup>3</sup>/min; V<sub>A</sub>, ml/100 cm<sup>3</sup>, MR<sub>glu</sub>, μmol/100 cm<sup>3</sup>/min; volume, ml. Measurements were based on all subjects for which data were available and the segmented volume non-zero.

Comparisons between the Cooling-reheating and Control protocols are provided in Table S3. sBAT-FF and SAT-FF estimated at baseline did not differ between the protocols.

| Variable                    | Cooling-reheating protocol | Control protocol | <i>P</i>     | <i>n</i>  |
|-----------------------------|----------------------------|------------------|--------------|-----------|
| sBAT FF ( <i>Baseline</i> ) | 79.25 ± 7.51               | 79.60 ± 8.09     | <i>0.625</i> | <i>10</i> |
| SAT FF ( <i>Baseline</i> )  | 84.79 ± 4.85               | 85.16 ± 5.07     | <i>0.625</i> | <i>10</i> |

**Table S3.** Differences in FF between the Cooling-reheating and Control protocols. mean ± standard deviation. *n*, number of observations used in statistical analyses. *P* values from Wilcoxon signed-rank test. Statistical significance (*P* < 0.05) in bold font.

## Discussion

According to Table S1, FF and glucose metabolic rate (estimated by  $MR_{glu}$  and  $MR_{glu2}$ ), within the whole sBAT and SAT depots, showed relatively strong negative correlations. The Spearman and Pearson correlation coefficients were similar for  $MR_{glu}$  and  $MR_{glu2}$ , although slightly stronger for  $MR_{glu2}$  overall. This could be due to the  $C_{glu}^{P2}$  measurement being more reliable and therefore more suitable than  $C_{glu}^P$  for estimating the metabolic rate of glucose. However, there was one missing data point for  $C_{glu}^{P2}$ , which further limits the statistical power in this cohort of 12 subjects. For this reason,  $C_{glu}^P$  and not  $C_{glu}^{P2}$ , was used in most analyses of the manuscript.

## References

- 1 Lundström, E. *et al.* Automated segmentation of human cervical-supraclavicular adipose tissue in magnetic resonance images. *Sci Rep* **7**, 3064, doi:10.1038/s41598-017-01586-7 (2017).