**Supplementary Materials**

**Methods and Materials**

***Imaging sequence and data analysis***

A three-dimensional multi-echo gradient-echo sequence was performed on a 7T MR system (Bruker) using a four-channel head coil. The animals were placed in the coil headfirst in the supine position. The anesthesia was maintained with isoflurane (1–2% isoflurane, oxygen flow rate: 1 L/min). The MRI scan was performed with following parameters: TR = 50 ms; TE1/∆TE/#TE = 1.7 ms/1.3 ms/1 ms; matrix size = 90 × 90 × 60; voxel size = 0.4 mm × 0.4 mm × 0.4 mm and flip angle = 20°. The coronal plane of interscapular region was acquired using a respiratory trigger.

The in-phase echoes (second, fifth, and eighth echoes) were used to calculate initial  and field maps. Then, the  -IDEAL with the obtained initial  and field maps as initialization was conducted using all echoes to obtain water, fat, , and field maps. QSM was calculated from the final field map using Laplacian boundary value (LBV) for background removal and morphology enabled dipole inversion (MEDI) for dipole inversion. Water and fat maps were used for FF calculation. The maximum three regions of interest (ROIs) of BAT and WAT were manually drawn on FF images within the interscapular region, avoiding the surrounding vessel. ROIs were then manually transferred onto QSM and  maps at the same anatomical location, and the mean QSM, FF, and  were recorded.

***Statistical analysis***

All data were presented as mean ± SD if not otherwise stated. Differences between control and cold stimulated groups in BAT or WAT regarding QSM, FF, , and UCP1 expression were done on normally distributed data using one-way ANOVA followed by Bonferroni *post hoc* tests with SPSS 21.0 (IBM Corp, Armonk, NY, USA), and independent-samples *t*-test was performed for three-group comparison of BAT and WAT. If the normal distribution was not satisfied, Kruskal–Wallis and Mann–Whitney *U* tests were used for comparison among groups. Pearson correlation coefficients were calculated to assess the linear relationship between QSM, FF, , and UCP1 expression. Significance was accepted at \**P* < 0.05, †*P* < 0.01, and ‡*P*<0.001.

**Results**

**Supplementary Table 1: Comparison of measurement results of parameters among different groups of BAT and WAT.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Items** | **Parameters** | **Control (*n* = 5)** | **Cold (12 h) (*n* = 5)** | **Cold (24 h) (*n* = 5)** | ***P*-value** |
| BAT |  |  |  |  |  |
|  | QSM (ppm) | 0.32 ± 0.06 | −0.04 ± 0.05 | 0.06 ± 0.05 | <0.001‡ |
|  | FF (%) | 46.40 ± 5.28 | 12.27 ± 4.06 | 13.98 ± 2.29 | <0.001‡ |
|  |  (S−1) | 175.27 ± 14.82 | 100.83 ± 13.73 | 120.83 ± 23.94 | <0.001‡ |
|  | UCP1/GAPDH | 0.35 ± 0.08 | 0.56 ± 0.06 | 0.57 ± 0.11 | 0.002† |
| WAT |  |  |  |  |  |
|  | QSM (ppm) | 0.55 ± 0.09 | 0.19 ± 0.12 | 0.21 ± 0.13 | 0.001† |
|  | FF (%) | 85.98 ± 5.63 | 84.97 ± 2.02 | 81.25 ± 3.83 | 0.102 |
|  |  (S−1) | 48.93 ± 17.88 | 65.13 ± 6.45 | 76.60 ± 26.39 | 0.105 |

Values are expressed as mean ± standard deviation. Significance was accepted at \**P*<0.05, †*P*<0.01, ‡*P*<0.001.

BAT: Brown adipose tissue; FF: Fat fraction; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; QSM: Quantitative susceptibility mapping; UCP1: Unique uncoupling protein 1; WAT: White adipose tissue.

**Supplementary Table 2: Comparison of measurement results of parameters among different groups of BAT and WAT.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Items** | **Groups** | **BAT** | **WAT** | ***P***-**value** |
| QSM (ppm) |  |  |  |  |
|  | Control | 0.32 ± 0.06 | 0.55 ± 0.09 | 0.002† |
|  | Cold (12 h) | −0.04 ± 0.05 | 0.19 ± 0.12 | 0.005† |
|  | Cold (24 h) | 0.06 ± 0.05 | 0.21 ± 0.13 | 0.041\* |
| FF (%) |  |  |  |  |
|  | Control | 46.40 ± 5.28 | 85.98 ± 5.63 | 0.008† |
|  | Cold (12 h) | 12.27 ± 4.06 | 84.97 ± 2.02 | <0.001‡ |
|  | Cold (24 h) | 13.98 ± 2.29 | 81.25 ± 3.83 | <0.001‡ |
| R2\* (S−1) |  |  |  |  |
|  | Control | 175.27 ± 14.82 | 48.93 ± 17.88 | <0.001‡ |
|  | Cold (12 h) | 100.83 ± 13.73 | 65.13 ± 6.45 | 0.001† |
|  | Cold (24 h) | 120.83 ± 23.94 | 76.60 ± 26.39 | 0.024\* |

Values are expressed as mean ± standard deviation. Significance was accepted at \**P*<0.05, †*P*<0.01, ‡*P*<0.001. BAT: Brown adipose tissue; FF: Fat fraction; QSM: Quantitative susceptibility mapping; WAT: White adipose tissue.

**Limitations**

Our study of quantifying adipose tissue has several limitations. First, relatively limited samples for data have been used. However, the analysis identified significant differences between activated or inactive BAT and WAT. Second, the content of iron and fat in the tissue are not quantitatively measured on histology, and a further quantitative comparative analysis of QSM and histology in BAT is needed. Additionally, intermittent cold exposure or other treatment to activate the metabolism of BAT may be more stable for further exploration for perfection.



**Supplementary Figure 1:** Experiment design. Rats were divided into control group and cold stimulation groups (12 h or 24 h). MRI scan was performed immediately after cold stimulation. Rats were finally sacrificed for histological analysis and special protein expression quantification.



**Supplementary Figure 2:** Regression analysis. Linear correlation coefficient in BAT between QSM and UCP1 expression (A) measured by western blotting, FF and UCP1 expression (B), R2\* and UCP1 expression (C). All of QSM, FF and R2\* showed good negative correlations with UCP1 expression. BAT: Brown adipose tissue; FF: Fat fraction; QSM: Quantitative susceptibility mapping; UCP1: Unique uncoupling protein 1.