14 T NMR and 7 T MRI *in vitro* investigation of cold stimulation of abdominal WAT, inguinal WAT and BAT

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PURPOSE: Based on its unique thermogenic capacity, brown adipose tissue (BAT) shows very high potential to serve as a therapeutic node in the treatment of metabolic disorders, e.g., obesity. Recently, the BOLD effect T2*-weighted MRI during cold stimulation was used to detect cold-activated BAT in human individuals [1]. In this work, we compared 14 T NMR spectra and, for the first time, water fat fraction (WFF) [2], T1, T2 values measured *in vitro* by 7 T MRI between BAT, inguinal white adipose tissue (iWAT) and abdominal white adipose tissue (aWAT) in cold-stimulated mice and normal mice.

MATERIALS AND METHODS:

Tissue Preparation: Wild-type C57Bl6 mice (Charles River Laboratories, Koeln, Germany) were housed at room temperature (22°-24°C). Half of the animals (n = 8-10) were kept at 4°C for two weeks prior to pooling to stimulate thermogenesis. BAT, iWAT and aWAT were surgically removed from mice, pooled and washed with D_2O (MSD Isotopes, Montreal, Canada). Tissue probes were measured immediately after preparation (for sample denotation see Fig. 1).

NMR Spectroscopy: ^{1}H NMR spectra were acquired using a Bruker Avance II NMR spectrometer (Bruker, Karlsruhe, Germany) equipped with a 5-mm inverse-configuration probe at a field strength of 14.1 T. Mouse fat samples were partitioned into small pieces to be easily introduced into the 5-mm NMR tubes with some D_2O filling the void spaces for lock and shim purposes. Spectra were referenced externally to trimethylsilyl propanoic acid (δ = 0 ppm). WFF_{14T} was calculated from the magnitude ratio of the water peak to the fat peak.

Relaxometry and Water Fat Fraction Images: Datasets for T1, T2 and WFF determination were acquired on a 7 T whole body system (Magnetom 7 T, Siemens Healthcare, Erlangen, Germany) using a 24-channel head coil (Nova Medical, Wilmington, MA, USA). Data evaluation was done with MATLAB (The MathWorks, Natick, AM, USA). For T1, T2 values a non-linear least-squares curve fitting of the signal functions (see below, M_0 = magnetization, N = noise) to every image pixel was employed. Statistical analysis was done using R (R Foundation for Statistical Computing, Vienna, Austria).

- T1: standard inversion-recovery spin-echo (IRSE) sequence; imaging parameters: TR = TI_n + 3000 ms, TE = 13 ms, TI_n = 40/60/80/100/130/160/190/230/20/320/370/440/530/660/900 ms, α = 90°, slice thickness = 10 mm, FOV = 96 × 128 mm^2, matrix = 96 × 128, BW = 797 Hz/px; signal function: $S_{IRSE} = M_0(1 - (1-\cos\alpha)e^{-TI/T1} + e^{-TR/T1}) + N$
- T2: standard CPMG sequence; imaging parameters: TR = 3000 ms, TE_n = n × 14.8 ms, $\{\boldsymbol{n} \in \mathbb{N} | \mathbf{1} \leq \boldsymbol{n} \leq \mathbf{32}\}$, α = 180°, slice thickness = 10 mm, FOV = 96 × 128 mm^2, matrix = 96 × 128, BW = 797 Hz/px; signal function: $S_{CPMG} = M_0 \cdot e^{-TE/T2} + N$
- Fat and water images were measured by a Dixon-VIBE pulse sequence. Imaging parameters: TR = 13.9 ms, TE_{in}/TE_{opp} = 3.06/5.61 ms, α = 15°, slices = 35, slice thickness = 0.3 mm, FOV = 185 × 185 mm^2, matrix = 512 ×512, BW = 425 Hz/px. Fat pixels were selected from the image slices by histogram-based thresholding and subsequently, WFF_{7T} was calculated.

RESULTS AND DISCUSSION: Based on the 14 T NMR spectra (cf. Fig 2), there is a difference for WFF_{14T}(BRT) = 47% to WFF_{14T}(B4C) = 83% and for WFF_{14T}(IRT) = 36% to WFF_{14T}(I4C) = 50% indicating cold-activation. In contrast, WFF of aWAT remains unchanged (WFF_{14T}(ART) = WFF_{14T}(A4C) = 30%), i.e., there is no cold-activation. A significant change in T1-values by cold-stimulation was only observed for BAT (T1(BRT) = 61 ms vs. T1(B4C) = 76 ms, p-value < 0.05, cf. Tab. 1). Hence, cold-stimulation in iWAT could not be confirmed by T1 relaxometry. Differences in T2 values of all mouse fat samples were found to be very small (≤ 15 ms) and therefore do not allow to detect cold-stimulation. For 7 T MRI, a trend to higher mean value differences between WFF_{7T}(BRT) and WFF_{7T}(B4C) was observed similarly to 14 T NMR (cf. Fig. 2 and 3). For iWAT and aWAT, no difference in WFF_{7T} was measured. However, the noise of the data prevents an unambiguous statement. This measurement needs to be repeated, using larger mouse fat samples and/or human individuals.

CONCLUSION: We successfully demonstrated the possibility to detect cold stimulation of BAT in mice with standard 14 T NMR and 7 T MRI *in vitro* measurements. This information can be used for further experiments in mice and/or human individuals similar to previous studies [1].

REFERENCES: [1] Y-C I Chen et al., *J. Nucl. Med.,* 54(9):1584-1587 (2013) [2] H H Hu et al., *Magn. Reson. Imaging*, 30(3):323-329 (2012)

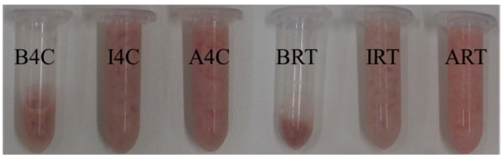

Fig. 1: Photo of the tissue samples. B4C/I4C/A4C denote BAT/iWAT/aWAT pooled from cold-stimulated mice kept at 4°C, BRT/IRT/ART from normal mice kept at room temperature.

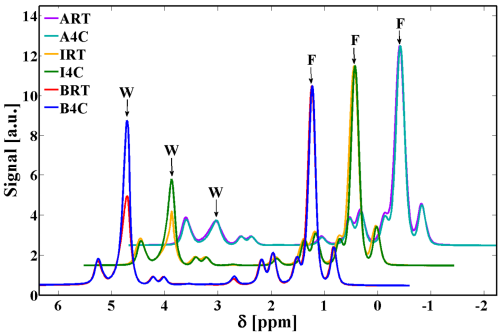

Fig. 2: 14 T NMR spectra of all mouse fat tissue samples. W and F indicate the peaks used for WFF determination which was done by division of the peak maximum values: $WFF_{14T} = 100 \cdot W/F$

T1[ms]	4C	RT	p-value
B	411±69	373±45	0.008
I	433±20	434±23	0.665
A	431±31	426±21	0.114

Tab. 1: Mean T1 values of all mouse fat tissue samples with standard deviation. The last column indicates p-values from a two sample Student's t-test between fat tissue of cold-stimulated and normal mice.

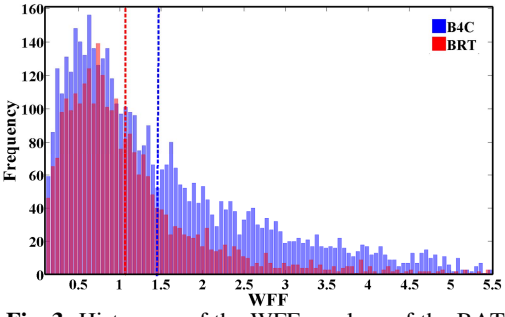

Fig. 3: Histogram of the WFF_{7T} values of the BAT samples from cold-stimulated and normal mice. The dotted lines mark the mean values of the distributions.

This work was funded by the Helmholtz Alliance ICEMED - Imaging and Curing Environmental Metabolic Diseases, through the Initiative and Networking Fund of the Helmholtz Association.