In vivo Assessment of Cold Stimulation Effects on the Fat Fraction of Brown Adipose Tissue Using DIXON MRI

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Parts of this work were presented at the Annual Meeting of the ISMRM in 2015 and 2016.

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Grant Sponsor:
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.

Word Count:
4867

Running Title:
Fat Fraction of Brown Adipose Tissue

ABSTRACT

Purpose: To evaluate the volume and changes of human brown adipose tissue (BAT) *in vivo* following exposure to cold using MRI.

Materials and Methods: The clavicular region of 10 healthy volunteers was examined at a 3T MRI system. One volunteer participated twice. A cooling vest that was circulated with temperature-controlled water was used to expose each volunteer to a cold environment. Three different water temperature phases were employed: baseline (23°C, 20 min), cooling (12°C, 90 min) and a final warming phase (37°C, 30 min). Temperatures of the water in the circuit, of the body, and at the back skin of the volunteers were monitored with fiber optic temperature probes. Applying the 2-point DIXON pulse sequence every 5 min, fat fraction (FF) maps were determined and evaluated over time to distinguish between brown and white adipose tissue.

Results: Temperature measurements showed a decrease of (3.8 ± 1.0)°C of the back skin temperature, while the body temperature stayed constant at (37.2 ± 0.9)°C. Focusing on the two interscapular BAT depots, a mean FF decrease of (-2.9 ± 2.0)% / h ($p < 0.001$) was detected during cold stimulation in a mean absolute volume of (1.31 ± 1.43) ml. Also, a correlation of FF decrease to back skin temperature decrease was observed in all volunteers (correlation coefficients: $|r| = [0.51; 0.99]$).

Conclusion: We found that FF decreases in BAT begins immediately with mild cooling of the body and continues during long-time cooling.

Keywords:
MRI, Brown Adipose Tissue, Fat Fraction, DIXON, Fiber Optic Temperature Measurements

INTRODUCTION

Adipose tissues in the human body can be classified into brown adipose tissue (BAT) and white adipose tissue (WAT)(1). In newborns, BAT contributes approximately 5% of the body weight (2). In adults, WAT is the dominating type of adipose tissue and for several decades BAT was believed to be absent in the body of adult humans (3–5). In the early 1960s and 1970s, histomorphological studies found BAT in human infants that disappears with age within 10 - 60 years depending on its anatomical area (1, 6). After discovering active human BAT also in adults, its potential impact on the treatment of obesity and metabolic diseases has generated interest (4, 5, 7–10).

Operating as a heat generator, BAT induces fat burning, whereas white adipocytes serve as energy storage and thermal insulators (11). BAT has the ability to dissipate energy through non-shivering thermogenesis (NST), generating heat in response to cold exposure (7, 8, 11, 12). Heat is generated through the following biochemical mechanism: Fatty acid oxidation decouples from the adenosine triphosphate (ATP) synthesis, caused by the membrane protein thermogenin (UCP-1 uncoupling protein) (4, 13). BAT consists of much smaller lipid droplets and a higher amount of mitochondria than WAT. The iron-rich mitochondria give the brown adipocytes its color. In contrast to white adipose tissue, human BAT is typically a heterogeneous mixture of brown and white adipocytes and is therefore difficult to detect and to evaluate.

The first radiological imaging modality that was used to visualize BAT depots was ^{18}Fluorine-fluorodesoxyglucose (^{18}F-FDG) positron emission tomography (PET) (4). Since BAT existence in adults was detected in ^{18}F-FDG PET scans, it has been the most common method for BAT imaging. Activated BAT was identified at PET via a symmetrical high glucose uptake in the clavicular and supraclavicular region (3). BAT was found more frequently in women than in men (5) and more often during cold winters than in summers (14,

15). Imaging of human BAT by ^{18}F-FDG PET scans is limited to tissues with high glucose uptake. Therefore, ^{18}F-FDG PET scans show only activated BAT and are not sensitive for BAT in the non-activated thermoneutral state (14–16). Additionally, the use of ionizing radiation limits its applicability for volunteer studies. Since BAT imaging using ^{18}F-FDG PET/CT was only partially reproducible (15, 17), there is a great demand for an alternative method for BAT imaging, avoiding long-term side effects for the volunteers.

As a non-invasive examination method, MRI can be used for repetitive measurements of BAT in the thermoneutral state as well as under cold exposure, which improves reproducibility. Additionally, MRI enables higher spatial resolution than PET. Therefore, MR imaging is a potentially well-suited method for investigating localization and activation of BAT. Physiologically, BAT can be distinguished from WAT via its higher water content and the corresponding lower fat fraction (FF) (18, 19). In addition, BAT activity can be induced by cold stimulation. This has been confirmed by *in vitro* measurements of adipose tissue samples of mice (18, 19) and via blood-oxygen-level dependent functional MRI (BOLD fMRI) in humans (20–22).

BAT MR imaging utilizes the fact that BAT FF is lower compared to WAT FF and decreases under cold exposure (18, 19). To characterize BAT more accurately, determination and quantification of BAT properties, e.g. BAT volume and activity, is needed.

Until now, MR studies examining cold-activated BAT performed measurements before and after cold exposure of healthy volunteers (7, 8, 20, 21).

The aim of this study was to evaluate volume and activity of human BAT *in vivo* under cold exposure. Using Dixon water-fat-separated MRI, changes in BAT FF were monitored and quantified over time in interscapular BAT depots.

MATERIALS AND METHODS

Examinations of volunteers in this study were approved by the ethical review board of the University of Heidelberg. Written informed consent was obtained from all volunteers.

Five healthy female and five healthy male volunteers were enrolled (mean age 27 ± 2 years; mean body mass index (BMI) 23 ± 2, Table 1). The ten subjects were annotated as S01 to S10. S10 participated twice within three weeks to examine reproducibility. The two measurements are labeled with S10A and S10B. Detailed results of the measurement of S10B were added to the Supplemental Measurement S10B.

All volunteers were examined on a 3T whole-body MR system (*Biograph mMR, Siemens Healthcare, Erlangen, Germany*) at an air-conditioning-controlled room temperature of (23.6 ± 1.0)°C. The temperature was measured with a digital thermometer (*Precision Pocket Thermometer GTH 175/PT, Greisinger electronic GmbH, Regenstauf, Germany*). To investigate the clavicular region, a spine-array coil with 8 elements, a flexible 3 × 3-body-matrix array coil, and a 16-channel head/neck coil (*Siemens Healthcare, Erlangen, Germany*) were used. To optimize SNR, only receive elements close to the examination area were used: the neck element with four channels of the head/neck coil, three elements of the spine array and three elements of the body array.

For activation of the examination area, all volunteers were dressed with a cooling vest (*Polar Products Inc., OH, USA*), supplied with temperature-controlled water by a water circulator (*Haake F6 Circulator, Artisan Scientific, IL, USA*). At the two lateral sides, the vest could be closed with a hook-and-loop fastener, yielding a tight contact of the body surface and the cooling tubes of the vest. A thin cotton shirt was worn under the vest for hygienic reasons. The experimental setup is illustrated in Figure 1. Firstly, the water temperature was maintained at 23°C (approximately room temperature) during an initial phase of 20 min baseline measurements, followed by the cooling phase with a water temperature of 12°C for a

period of 90 min to induce a mild cold stimulation of the clavicular area. Finally, a warming phase of 30 min followed with a water temperature of 37°C (approximately body temperature). Time intervals were chosen by making sure that the volunteers felt well and did not shiver especially during the cooling phase and that the whole measurement time was appropriate. Contact to all volunteers was constantly maintained during the MR measurements by means of the intercom system of the MRI system.

An in-house developed software tool for temperature monitoring and control implemented in Python (*Python Software Foundation, Delaware, USA*) was used to adjust the water temperature during the experiment automatically.

Temperature Measurements

Magnetic field independent data were obtained by a 4-channel fiber-optic thermometer (*LumaSense Technologies Inc., CA, USA*) (23). The fiber-optic probes were calibrated in a vessel filled with ice water before each experiment. According to the manufacturer, the device-specific error of the fiber-optic thermometer was ±0.5°C. The length of the tubes from the water circulator to the cooling vest as well as the length of the temperature probes was 10 m. To monitor the water temperature and the temperatures of the volunteers, the probes were positioned as described in Figure 1. Water temperature inside the tank of the water circulator was measured via an internal sensor of the water circulator. The device-specific error of the internal sensor given by the manufacturer was ±0.01°C. Temperature data were acquired with a rate of 1 Hz and were recorded using our own temperature monitoring and control software. Values of body temperature and back skin temperature were evaluated at the following time points: beginning of the measurement (T_{start}), transition of the initial phase to the cooling phase ($T_{in->cool}$), transition of the cooling phase to the warming phase ($T_{cool->warm}$), and end of the measurement (T_{end}).

MRI Sequence

The clavicular region was imaged at an isotropic spatial resolution of $(1.2\ mm)^3$. A 2-point DIXON (24, 25) pulse sequence (volumetric interpolated breath-hold examination [VIBE, (26)]; TR = 5.85 ms, TE_{in}/TE_{opp} = 2.46/3.69 ms, $\alpha = 10°$, slices = 64, slice thickness = 1.2 mm, FOV = 500 × 312 mm^2, matrix = 416 × 260, BW = 710 Hz / px, GRAPPA R = 2) was used to estimate the fat fraction. Each measurement was performed within 30 s, during a breath-hold in inhaled state. The sequence was applied every 5 min over a total measurement time of 140 min. Each measurement started and ended with automatic breathing commands given directly by the MR system. Before starting the true measurements a test run was performed to make the volunteers familiar with the breathing commands.

Fat Fraction Data Analysis

This work was focused on quantifying the fat fraction at the mediastinal area, a known BAT site in humans (4, 11, 27). The evaluation of the acquired data was performed with MATLAB (*The MathWorks, Natick, AM, USA*) and MITK (*Medical Imaging Interaction Toolkit, German Cancer Research Center, Heidelberg, Germany*) (28, 29).

FF data were quantified for each time point and evaluated over time**.** For comparison, FF in subcutaneous adipose tissue (SAT) and muscle tissue was also evaluated. The regions of interest (ROI) for subcutaneous adipose and muscle tissue were manually segmented under assistance of a board-certified radiologist (M.T.F.) with 6 years of experience in MRI, focusing at the upper thoracic aperture where less movement of the volunteers during the total measurement time was expected.

To compensate for changes in breath-hold positions over time, all DIXON water images (W) were registered to a water target data set. To choose a reliable target data set, a set of slices in the lung region was taken and merged in time to a video to check for a data set with a medium breath-hold position. Data sets with extreme breath-hold positions such as a

very large or very small inhale volume were discarded as potential target data sets.

For registration, the *Insight Tookit* (itk)-based (30) and MITK integrated Implementation of the deformable Fast Symmetric Forces Demons algorithm (FSFDemons) was employed (31, 32). Finally, the resulting transformations for every time point were applied to the corresponding fat images (F). Afterwards, FF data for each time point were determined voxel by voxel (equation 1)

$$FF = F / (F + W). \quad (1)$$

To detect potential BAT depots, the following criteria were applied on the 4D data. Only voxels fulfilling each criterion were evaluated as supposed BAT**:** a) threshold for the baseline FF at the end of the initial phase: $0.6 \leq FF_{base} \leq 0.8$, b) lower threshold for the FF at the end of the cooling phase: $FF_{cool} \geq 0.3$, c) a negative gradient of the FF during the cooling phase (19), d) a minimum bilateral region of interest (ROI) size of $N \geq 15$ px (0.03 ml).

To filter voxels with a negative FF gradient out of the data during the cooling phase, a linear fit (optimizer: Levenberg-Marquardt algorithm, cost function: difference squares, implementation based on MITK) was applied pixel by pixel over time. The first data point of the cooling phase was excluded to ensure that the water temperature had reached 12°C. Thus, the linear fit was applied to 18 data points from time point 25 min to 110 min. The resulting FF maps with the assumed BAT depots were median filtered in a 3-by-3-by-1 neighborhood. Median filtering eliminates isolated noise, does not influence linear greyscale trends and does not have any antialiasing effects.

The FF Map with the assumed BAT depots was overlaid on the fat image of the target data. Under assistance of a board-certified radiologist (M.T.F., 6 years of experience in MRI), it was evaluated if the FF Map showed feasible results at anatomical sites were BAT depots are expected. Focusing on the interscapular depots (27), where less sensitivity to registration errors was expected at the back, frontal parts of the chest area were excluded to prevent

evaluation of regions prone to registration errors.

FF changes in BAT during the initial and warming phase were also investigated by applying a linear fit and results were compared for each temperature phase. BAT results were subsequently compared to ROIs of SAT and muscle tissue. SAT ROIs were manually segmented by V.S. under the assistance of M.T.F. on the fat image of the target data set and muscle ROIs on the corresponding water target image. Both ROIs were selected in the dorsal area of the thorax taking into account that there was less motion of the volunteers during the measurement time. ROIs were evaluated with regard to $FF_{start,}$ FF_{base}, FF_{cool}, FF_{end} and FF change rates resulting from the linear fits and the volume of the evaluated ROIs. For data analysis, the object was segmented excluding lungs using MITK.

To estimate an error value of the data registration, performance of the FSFDemons algorithm was evaluated with the help of the board-certified radiologist (M.T.F.). Detailed results are shown in the Supplemental Registration Evaluation.

Statistical Analysis

To determine significance of the FF change rates in BAT during the initial phase (from FF_{start} at time point t = 0 min to FF_{base}), cooling phase (FF_{base} to FF_{cool}) and warming phase (FF_{cool} to FF_{end} at time point t = 140 min), a paired t-test with a significance level of 5% was applied and p-values $p_{initial}$, p_{cool} and p_{warm} were determined. Therefore, for each temperature phase mean differences $\Delta d_{initial/cool/warm}$ of FF_{base} to FF_{start}, FF_{cool} to FF_{base} and FF_{end} to FF_{cool}, and the corresponding standard deviations ($SD_{\Delta d,initial,.cool,warm}$) were computed. The 95% confidence intervals (CI) for each phase were estimated using the equation $[\Delta d_{initial/cool/warm}\ d \pm t^* \times SD_{\Delta d,initial,.cool,warm} / \sqrt{N}]$. The scalar for a normal distribution is denoted by t* and N is the number of voxels in the evaluated BAT ROIs. For SAT and muscle tissue, p-values p_{SAT} and p_{muscle} were also determined by applying a paired-test to determine significance of FF values.

By applying a two sample t-test for independent samples, mean values for female and

male volunteers were compared and significant differences were determined. The 95% CI were estimated using the equation $[(m_{female} - m_{male}) \pm t^{*}\times \sqrt{\frac{\hat{s}^2_{female}}{n_{female}} + \frac{\hat{s}^2_{male}}{n_{male}}}]$ with the mean values $m_{female,male}$, variances $\hat{s}^2_{female,male}$ and the number $n_{female,male}$ of both gender populations.

Investigation of Pearson's correlation coefficient (38) was performed to examine correlations of FF values in BAT and back skin temperature (r_{FFbs}) as well as between r_{FFbs} and BAT volume ($r_{FFbs<->vol}$), subject age ($r_{FFbs<->age}$) and BMI ($r_{FFbs<->BMI}$). For each investigated correlation the two parameters were plotted in one diagram. For the investigation of r_{FFbs}, back skin temperatures were averaged each 5 min in a span of 60 data points. Correlations between subject BMI and BAT volume ($r_{BMI<->vol}$), and between subject age and BAT volume ($r_{age<->vol}$) were also examined. Data were classified as correlated if $|r| > 0.5$.

RESULTS

Temperature Measurements

Figure 2 (a) shows exemplarily the temperature evolution of a healthy volunteer (subject S01). The black dashed line in Figure 2 (a) shows the set water temperatures, defining the three temperature phases: initial phase, cooling phase, and final warming phase.

The measured water temperatures followed the set water temperatures at the water circulator with mean delays of $\Delta t_{in->cool}$ = (1.6 ± 0.4) min when passing the initial phase to the cooling phase and $\Delta t_{cool->warm}$ = (11.9 ± 0.5) min when passing the cooling phase to the warming phase.

During the initial and the cooling phase, the outlet water temperature was higher than the inlet temperature with mean temperature differences of ΔT_{inital} = (0.7 ± 0.1)°C and ΔT_{cool} = (1.3 ± 0.1)°C, respectively. Detailed calculations on this resulting heat transfer from the volunteers into the water circuit are delineated in the Supplemental Heat Transfer Determination.

The estimated body temperature based on a measurement under the armpit (blue line) stayed constant during the full measurement time with a mean value of T_{Body} = (37.2 ± 0.9)°C. During the first measurement of S03, the probe under the armpit detached and moved. S02 reported that the probe felt loose when moving in-between measurements during the experiment. Therefore, the data of S02 and S03 were considered as invalid and excluded. Additional medical tape was used for the following volunteers to address this issue.

Figure 2 (b) illustrates the individual back skin temperature decreases of all volunteers. During the initial phase, the temperatures at the back skin of the volunteers, showed a mean slight decrease from T_{start} to $T_{in->cool}$ of (0.8 ± 0.4)°C and of (3.8 ± 1.0)°C from $T_{in->cool}$ to $T_{cool->warm}$ during the cooling phase. During the warming phase, the back skin

temperatures increased by (3.7 ± 1.3)°C from the end of the cooling phase at $T_{cool->warm}$ to the end at T_{end} (excluding S05). No recovering to the initial values T_{start} was observed.

After the time point $T_{cool->warm}$, a continuous slight decrease of the back skin temperatures was observed before another increase during the warming phase. No subject reported shivering during the measurement. Mean room temperature during all measurements in the scanner room was (23.6 ± 0.1)°C.

Fat Fraction Data Analysis

Figure 3 shows a FF map of volunteer S01. Positions of the evaluated ROIs for BAT, SAT and muscle tissue are marked. Figure 4 illustrates the FF values of the evaluated BAT, SAT and muscle tissue ROIs for each subject.

Individual slopes $slope_{initial/cool/warm}$ in BAT ROIs, mean FF values and evaluated BAT volume as well as mean FF values and resulting slopes $slope_{SAT/muscle}$ for SAT and muscle ROIs are illustrated in Figure 5.

According to the paired t-test, three of ten volunteers (S07, S09, S10A) showed a slight mean FF decrease during the initial phase of (-1.9 ± 0.5) %/h ($p < 0.001$) in the evaluated BAT depots. During the cooling phase, a mean FF decrease in the evaluated BAT depots of (-2.9 ± 2.0) %/h ($p < 0.001$) was detected in all volunteers. In S01, the largest change from FF_{base} to FF_{cool} was observed. During the warming phase, significant changes ($p < 0.001$) between FF_{cool} and FF_{end} were observed in 7 subjects. Two subjects (S07 and S09) showed a FF increase, whereas a continuing FF decrease in five subjects (S01, S03, S06, S08 and S10A) was observed.

No significant FF change during the whole measurement time was observed in SAT (mean FF_{SAT} = (76 ± 8) %, mean $slope_{SAT}$ = (0.1 ± 0.4) %/h, $p_{SAT} < 0.01$) and muscle tissue (mean FF_{muscle} = (7 ± 2) %, mean $slope_{muscle}$ = (0.1 ± 0.1) %/h, $p_{muscle} < 0.03$) except for S09 ($p_{SAT,S09} > 0.05$, $p_{muscle,S09} > 0.05$). Corresponding individual quantifications on BAT, SAT and

muscle tissue are given in the supplementary Table. Individual p-values for BAT, SAT and muscle tissue are delineated in Table 2.

The mean absolute volume of the both evaluated BAT ROIs in all subjects was (1.31 ± 1.43) ml with a fixed number of voxels N in the range of 38 to 2736, depending on the individual ROI sizes of the volunteers. The mean volume of the SAT and muscle tissue ROIs was (9.45 ± 8.66) ml. For S05, the two last fitting data points are missing since the measurement was stopped after 105 min.

Correlations between FF decrease and back skin temperature decrease for the initial (green), cooling (blue) and warming (red) phase are shown in Figure 6 (a). For each subject, a negative correlated slope (Figure 6 (b), Table 2) during the cooling phase was observed with a mean value of $slope_{FFbs,mean}$ = (-1.42 ± 1.24) %/°C. Since only during the cooling phase, each volunteer showed a significant FF decrease ($p < 0.001$), no correlated slope values were determined for the initial and the warming phase. Correlation coefficients were in the range of $|r_{FFbs}| = [0.51; 0.99]$ (Table 2).

No further correlations were observed neither between $slope_{FFbs}$ and BAT volume ($|r_{FFbs<->vol}| = 0.15$), subject age ($|r_{FFbs<->age}| = 0.45$) and subject BMI ($|r_{FFbs<->BMI}| = 0.06$) nor between subject BMI and BAT volume ($|r_{BMI<->vol}| = 0.40$) and subject age and BAT volume ($|r_{age<->vol}| = 0.36$). Supplementary Figure 7 illustrates the corresponding plots. Since the temperature got out of place during the measurement of S02 and S09, and S05 stopped early, those subjects were excluded for further correlations of $r_{FFbs<->vol/age/BMI}$.

For female volunteers, mean values $FF_{SAT,female}$ and $FF_{muscle,female}$ were significantly higher compared to $FF_{SAT,male}$ and $FF_{muscle,male}$ ($p < 0.001$). For mean values in BAT ($slope_{initial/cool/warm}$ and evaluated volume) and for correlated slopes $slope_{FFbs}$ no significant differences were observed ($p > 0.5$, Table 3).

DISCUSSION

In this study, acute activation of human BAT was induced by cold-stimulation in 10 healthy adult volunteers including one reproducibility measurement, and FF values were evaluated with a temporal resolution of 5 min. Temperatures of the cooling system and the volunteer's body and skin were monitored continuously with 4 temperature probes. FF decreased in regions where human interscapular BAT was expected and was observed to correlate with subject's back skin temperature decrease.

Localization of BAT in adults based on the ratio of water and fat using MRI has been reported by several groups (20, 21), who studied mostly supraclavicular depots. The two interscapular BAT depots, that are mainly known from mammals (11, 18), were also studied in humans (27). Cold activation of supraclavicular BAT in humans was induced with a similar cooling vest within 60 min using water with a temperature between 13°C and 16°C (20). In our study the water temperature was decreased to 12°C and cold activation was extended to 90 min. Another approach (21) used a water-perfused suit and a subject-specific cooling protocol for NST. Subjects were exposed to thermoneutral conditions for 30 min, cooling until shivering, warming for 5 min and mild cold conditions (20.4°C) for 60 min. In all studies, MRI was performed once before and once after cold exposure. In our work, images were acquired continuously every 5 min, allowing for time-resolved evaluation of FF changes during the cooling phase as well as during the initial and warming phase. This yields more information on the characteristics of the FF change during each temperature phase and enables a more reliable fitting of the slope of the FF changes. Our findings of a decreased FF due to an increased water signal at detected potential BAT sites correspond well to other findings (19) where the water content in BAT increased after cold stimulation. This increase in water content indicates that the detected regions contain activated BAT.

In these BAT depots, the increase in water content was presumably caused by increased perfusion of BAT, given that blood flow in BAT has been shown to increase with cold exposure (39). The relatively short time-frame excludes tissue re-modelling as explanation for the change in water content, which are more long-term cold adaptations including the reduction of fat content and changes in cellular morphology (11, 40, 41). As expected, no FF change in non-activated tissue such as subcutaneous adipose (19, 42) and muscle tissue was found.

For the BAT depots evaluated in this study, no ^{18}F-FDG-PET/CT data existed for comparison. PET/CT data would have been helpful for evaluation of the FF data during the cooling phase. However, PET/CT data show only activated regions and cannot identify BAT depots in the thermoneutral state. In contrast, as a non-invasive and ionizing-radiation-free modality, MRI is a promising method for examining human BAT. The applied criteria for BAT identification in this work are well studied (19, 33–35) and allow to assess BAT depots in the human body.

The air temperature in the scanner room and the initial water temperature were both lower than the back skin temperatures. This resulted in a mild cooling of the volunteers during the initial phase. An early activation of BAT could be observed in a slight significant FF decrease in three volunteers. During the warming phase, a significant FF increase was only observed in two volunteers. In all other volunteer´s the FF remained further decreased during the warming phase, suggesting the necessity of a longer warming phase for full recovery of initial BAT FF, i.e. reduction of local blood flow. This explanation is strengthened by the observation that 30 min of warming were just sufficient for the nearly complete recovery of the back skin temperature, indicating the eventual absence of the BAT activating stimulus

A lower bound of 0.6 and an upper bound of 0.8 for FF_{base} were chosen according to Rasmussen et al. (33), Hu et al. (34), and Lundström et al. (35). SAT FF values of 86% were

observed and determined to be higher as BAT FF by Hu et al. and Lundström et al., thus, FF_{base} was limited to 0.8 under thermoneutral conditions. Based on an analysis of *ex vivo* samples of rodents, Rasmussen et al. demonstrated correct BAT classification at an upper threshold of 60% FF, what led to our lower threshold of 0.6 before BAT activation. Furthermore, Graham et al. (36) and Bergström et al. (37) measured the water content in muscle tissue to be about 80%. Neither Rasmussen et al. (33) nor Hu et al. (34) observed BAT FF values far below 30%. To exclude non-fatty tissue for BAT detection the lower threshold of 0.3 for FF_{cool} was selected.

BAT detection correlates with outdoor temperatures and occurred more often in the winter time compared to other seasons (13–15, 17). Additionally, BAT mass was found to correlate with age and gender (15). In our study, a correlation between FF decrease in BAT depots and back skin temperature decrease of the volunteers under cold exposure could be observed. With the number of subjects included for this correlation determination, no further correlations to evaluated BAT volume, subject age and BMI were detected.

The observation of a significant higher mean value $FF_{SAT,female}$ compared to $FF_{SAT,male}$ corresponds well to the observations of (43), showing a higher body fat fraction for female volunteers than for male volunteers. Figure 4 also reflects this observation showing that for male subjects, mean values $FF_{SAT,male}$ are lower. Three male subjects (S05, S06, and S08) and female S09 showed very low values for FF_{SAT}. While these male subjects were very muscular and the BMI of S09 was very low, the observed thin SAT layer complicated the segmentation of SAT ROIs. Partial volume effects might have influenced the quantification of these SAT ROIs. Furthermore, S09 is the only subject showing non-significant p-values for FF changes in SAT and muscle tissue what also might be related to the mentioned partial volume effects.

Non constant standard deviations of BAT FF were observed. Especially S01, S04 and S09 showed an increase of standard deviations. Presumably, this observation might be due to FF changes, like increased perfusion, in the BAT ROI that do not occur evenly in each voxel.

The temperature measurements showed similar time courses in all volunteers. Body temperatures measured under the armpits stayed constant within their standard deviations for all volunteers (except the excluded ones S02 and S03). As expected, under the applied temperature challenge conditions all volunteers were able to defend their core body temperature by means of thermoregulatory responses, one of which is presumably BAT activation.

Several limitations have to be acknowledged. First, a 2-point DIXON-VIBE sequence under breath-hold was used. On the one hand, a multi-point DIXON sequence would be less susceptible to magnetic field inhomogeneities. On the other hand, due to varying breath-hold positions over the long total measurement time, this study was confined to the interscapular depots and the chest region including potential BAT depots in the supraclavicular region was excluded from evaluation to prevent registration errors. Furthermore, the short TR of the applied DIXON-VIBE sequence leads to T_1 weighting. Using the FLASH equation (44) and assuming a T_1 relaxation time of 900 ms and 400 ms for water and fat protons (45), respectively, the fat fraction was overestimated by less than 12% (Supplement Figure 8). However, long repetition times result in long acquisition times which were inappropriate for imaging during breath-holds. Secondly, since the resonance frequency of the water protons shows a slight temperature-dependent shift (-0.01 ppm/°C, (46)), major temperature changes can lead to erroneous FF estimates (47). The chemical-shift difference of water and fat methylene protons is much larger (3.3 ppm) than the temperature-dependent shift in the case of a temperature decrease of about 10°C (0.1 ppm). This results in a small error of approximately 3%. Thirdly, determination of FF values in BAT was not carried out during the

warming phase until each subject showed a significant FF increase in BAT. A longer warming phase would extend the measurement time for the subjects even though correlations that influence a FF recovering are not known at the moment. Fourthly, the study cohort needs to be increased. Although cold-activated supposed BAT was observed in each volunteer, large inter-subject variabilities make detailed investigation of BAT amount and activity difficult, especially regarding correlations between subject age and BMI and evaluated BAT volume plus correlated slope resulting from FF decrease and back skin temperature decrease. Statistical validations of BAT results concerning gender differences could also be improved. Furthermore, several measurements of more than one subject need to be performed to examine reproducibility and to investigate systematic and statistical aspects of repeated BAT activations as well as of the study design. However, cold-activated regions were observed in each volunteer, showing a significant FF decrease under cold exposure.

In conclusion, potential BAT depots were evaluated in 10 healthy volunteers. A cooling vest was used for human BAT activation. Using DIXON water-fat MRI for FF mapping with a temporal resolution of 5 min over a total measurement time of 140 min allowed for detailed observation of FF values in potential BAT. Simultaneously, temperatures of the setup, of the back skin, and under the armpits as reference for body temperature of the volunteers were monitored. During cold activation, a FF decrease, correlated to back skin temperature decrease, in potential BAT depots was observed in each volunteer.

ACKNOWLEDGEMENTS

The authors thank Barbara Dillenberger and Christian Kindtner for technical support with the cooling vest and the water circuit. Valuable discussions on data evaluation with Dipl.-Phys. Jan Peter Karch (Institute of Physics, Johannes Gutenberg-University, Mainz, Germany) are also acknowledged.

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TABLES

Table 1 Gender, height, weight and body mass index (BMI) and age of the study participants.

Table 1

Subject	Gender	Height [m]	Weight [kg]	BMI	Age [yrs]
S01	f	1.73	70	23.4	29
S02	f	1.72	58	19.6	27
S03	f	1.70	68	23.5	28
S04	m	1.78	85	26.8	26
S05	m	1.75	67	21.9	23
S06	m	1.78	68	21.5	28
S07	m	1.98	93	23.7	30
S08	m	1.70	69	23.9	26
S09	f	1.60	51	19.9	27
S10	f	1.80	75	23.2	27

Table 2 Statistical results for each volunteer. For each temperature phase, the p-values for the determination of fat fraction (FF) changes in the evaluated brown adipose tissue (BAT) regions of interest (ROIs) as well as in the ROIs of subcutaneous (SAT) and muscle tissue were determined with a paired t-test (95 % confidence interval). Significant p-values and non-significant (n.s., determined as $p > 0.05$) are delineated. During the initial phase, three of ten volunteers (S07, S09, and S10A) showed a significant FF change in the BAT ROIs whereas during the cooling phase, a significant FF change was observed in each volunteer. FF changes during the warming phase were significant except for S02 and S04. No data was available for S05. In SAT and muscle tissue, no significant FF change was observed expect for S09. Investigation of correlations between FF decrease in BAT during the cooling phase and back skin temperature decrease of the volunteers showed a negative correlated slope ($slope_{FFbs}$) as well as a correlation $\left| r_{FFbs} \right| > 0.5$ for each volunteer.

Table 2

	p-values					Correlations	
	$p_{initial,\ BAT}$	$p_{cool,BAT}$	$p_{warm,BAT}$	p_{SAT}	p_{muscle}	$slope_{FFbs}$ [% / °C]	$\lvert r_{FFbs} \rvert$
S01	n.s.	<0.001	<0.001	<0.001	<0.001	-1.99±0.52	0.69
S02	n.s.	<0.001	n.s.	<0.01	<0.001	-1.73±0.35	0.77
S03	n.s.	<0.001	<0.001	<0.001	<0.001	-1.03±0.22	0.76
S04	n.s.	<0.001	n.s.	<0.001	<0.001	-0.69±0.29	0.51
S05	n.s.	<0.001	n.a.	<0.001	<0.001	-2.55±0.56	0.99
S06	n.s.	<0.001	<0.001	<0.001	<0.001	-0.34±0.12	0.57
S07	<0.001	<0.001	<0.001	<0.001	<0.001	-0.83±0.19	0.73
S08	n.s.	<0.001	<0.001	<0.001	<0.03	-0.35±0.06	0.80
S09	<0.001	<0.001	<0.001	n.s.	n.s.	-4.25±0.66	0.85
S10A	<0.001	<0.001	<0.001	<0.001	<0.001	-0.47±0.10	0.77

Table 3 Comparison of mean values (mean ± standard deviation) for female and male volunteers. Statistical significances were investigated with a two-sample t-test (95% confidence interval). In subcutaneous adipose tissue (SAT) and muscle tissue, female volunteers showed significant higher mean fat fractions (FF_{SAT} and FF_{muscle}) compared to male volunteers. No further significant differences were observed.

Table 3

	Interscapular BAT				**SAT**	**Muscle**	**Correlation**
	$Slope_{initial}$ [% / h]	$Slope_{cool}$ [% / h]	$Slope_{warm}$ [% / h]	Volume [ml]	FF_{SAT} [%]	FF_{muscle} [%]	$slope_{FFbs}$ [% / °C]
female	-1.9±2.6	-3.2±2.2	-1.9±11.8	1.53±1.85	78±9	8±2	-1.9±1.5
male	-1.3±0.9	-2.4±2.1	-0.9±4.4	1.10±1.03	73±5	7±1	-1.0±0.9
p-value	p = 0.62	p = 0.53	p = 0.89	p = 0.66	p < 0.001	p < 0.001	p = 0.23

FIGURE LEGENDS

Figure 1

Experimental setup. The cooling vest was worn by the volunteers and connected to a water circulator standing in the control room. Anatomical positions of human brown adipose tissue (BAT) depots in the activated clavicular region are shown in the magnified window that is adapted from (11). The 4 probes of the fiber-optic thermometer measured the water temperature directly in the water at the inlet (green) and at the outlet (yellow) of the cooling vest, the temperature at the back skin of the volunteer (red) and under the armpit of the volunteer (blue). Temperature data were read out via our own software.

Figure 2

(a) Exemplary temperature profile (subject S01). Three different temperature phases are marked: initial (grey shaded background), cooling, warming (grey shaded background). The read out water temperature at the water circulator (purple), the water temperature at the inlet (green) and at the outlet (yellow) of the cooling vest followed the set water temperature course (black dashed-line) with a slight delay. The outlet temperature of the water was slightly higher than the inlet temperature. The temperature at the back skin of the volunteer (red) decreased especially during the cooling phase, whereas the body temperature measured under the armpit (blue) stayed constant. The time points of the two back skin temperatures at the phase transitions $T_{in->cool}$ and $T_{cool->warm}$ are marked with X. The time points T_{start} = 0 min and T_{end} = 137 min are not marked additionally. Data were acquired with a rate of 1Hz. (b) Illustration of the individual back skin temperature decreases of all volunteers. Start temperatures at T_{start} = 0 min were shifted to zero and averaged in a span of 111 data points to filter noise. Temperature decrease steps from -1°C to -7°C are marked with horizontal dashed lines. Back skin temperatures are deviating in absolute values but show a similar curve progression during each temperature phase. During the measurement of S02 and S09, the probe got out of place and S05 stopped the measurement after 105 min.

Figure 3

Axial fat fraction (FF) map of the first time point exemplarily shown for subject S01. FF = F / F + W were determined using water (W) and fat images (F) obtained by the 2-point DIXON-VIBE sequence. The anatomical positions of the evaluated regions of interest (ROIs) of brown adipose tissue (BAT, red colored), subcutaneous adipose tissue (SAT, blue circle) and muscle tissue (yellow circle) are illustrated.

Figure 4

Fat fraction (FF) values over time of the evaluated regions of interest (ROIs) in brown adipose tissue (BAT, red), subcutaneous adipose tissue (SAT, blue) and muscle tissue (yellow) for volunteer S01-S10A. A linear fit was applied to BAT results for each phase to estimate the FF change over time. It is exemplarily shown for the cooling phase during that each volunteer showed a significant FF decrease ($p_{cool} < 0.001$) over time. During the initial phase, three of ten volunteers showed a slight FF decrease ($p_{initial} < 0.001$) in BAT during the initial phase while during the warming phase, no immediate recovery of the FF decrease was observed except for two subjects (S07 and S09).

FF values in SAT and muscle tissue did not change during the experiment ($p_{SAT/muscle} < 0.001$).

Showing a very thin SAT layer, SAT depots in the male subjects S05, S06, S08 could hardly be evaluated. Additionally, male subjects showed less body fat in comparison to female subjects. Being the smallest and lightest volunteer, S09 also showed hardly a SAT layer for FF evaluation.

Individual data are delineated in the supplementary Table. Differentiation of the three temperature phases (initial, cooling, warming) is the same as in Figure 2 and colors are consistent with the marked ROIs in Figure 3.

Figure 5

Bar plot illustration of quantified fat fraction (FF) decreases over time estimated by a linear fit in brown adipose tissue (BAT) depots during the initial (a), cooling (b) and warming (c) phase shown for each volunteer. Corresponding mean FF values (mean ± standard deviation) in the evaluated BAT volume (d) at the beginning of the measurement (FF_{start}), at the phase transition to the cooling phase (FF_{base}) and to the warming phase (FF_{cool}) as well as at the end of the measurement (FF_{end}) are illustrated in (e). Mean FF values in subcutaneous adipose tissue (SAT) and muscle tissue (f) and the corresponding linear slopes during the whole measurement time (g) are also delineated. Quantified values for each volunteer in each subfigure are delineated in the supplementary Table.

Figure 6

(a) Plots of fat fraction (FF) values in evaluated brown adipose tissue (BAT) regions of interest (ROIs) and corresponding back skin temperatures (averaged in a span of 60 data points each 5 min) for the initial (green), cooling (blue) and warming (red) phase. Correlated slopes ($slope_{FFbs}$) were determined by a linear fit (black) on the significant FF changes ($p < 0.001$) during the cooling phase. While back skin temperatures of the volunteers decreased, a FF decrease in BAT could also be observed. FF changes during the initial and warming phase were not significant for each subject.

(b) Bar plot illustration of resulting negative values $slope_{FFbs}$. Correlations $|r_{FFbs}| > 0.5$ were observed for each volunteer. Individual data are delineated in Table 2.