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Non-invasive assessment of human brown adipose tissue: development of robust imaging methods to facilitate clinical translation

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Chapter 1

General introduction

Historically, the primary function of brown adipose tissue (BAT) was thought to be to maintain core body temperature in infants and hibernating mammals. In 2009, it was discovered that BAT is still present, and is functionally active during adulthood[1–3]. Since then, the presence of metabolically active BAT in human adults has been irrefutably confirmed. Reports from preclinical- and clinical studies have shown that enhanced BAT activity, as assessed with [^{18}F]fluorodeoxyglucose PET-CT scans relates to insulin sensitivity and inversely relates to cardiovascular events[4–8], which hold promise for future treatment strategies against obesity and cardiometabolic diseases. Non-invasive methods to determine the activity of human BAT are under constant development. This thesis focusses on the improvement of non-invasive methods for BAT detection to facilitate research on treatment strategies (in)directly targeting BAT.

1.1 BROWN ADIPOSE TISSUE PHYSIOLOGY AND MORPHOLOGY

In mammals, there are two main types of adipose tissue: BAT and white adipose tissue (WAT), that each have their own physiology, morphology and function. The main function of WAT is energy storage, and as a result, white adipocytes contain a single large lipid droplet. In contrast, the main function of BAT is to regulate core body temperature by converting chemical energy stored in glucose and lipids into heat. To facilitate this, BAT contains numerous smaller lipid droplets. In addition, BAT has a high abundancy of mitochondria and uncoupling protein (UCP)-1 expression and is more vascularized and innervated compared to WAT9. Cold exposure stimulates cation channels in the skin, which stimulate afferent nerves that transfer the cold perception to the hypothalamic temperature regulation center10. Once the signal is received by the hypothalamus, via efferent sympathetic outflow, norepinephrine is released from nerve endings in BAT. Norepinephrine subsequently binds to β-adrenergic receptors that are located on the cell surface of brown adipocytes, which induces a signaling cascade via the messenger cyclic adenosine monophosphate (cAMP) that, via protein kinase A (PKA) activation, promotes the hydrolysis of triglycerides within the lipid droplets to liberate fatty acids11. These fatty acids then enter the mitochondria where they are broken down by β-oxidation into substrates to enter the citric acid cycle. This activates the electron transport chain to induce a proton gradient across the inner membrane of mitochondria, which in most tissues is used to drive the synthesis of adenosine triphosphate (ATP) via complex V. In BAT, the uncoupling protein (UCP-1) dissipates the proton gradient to generate heat instead of ATP by forming a pore in the inner mitochondrial membrane resulting in release of energy as heat. Subsequently, intracellular lipids are replenished by extracting fatty acids and glucose from the systemic circulation via cluster of differentiation protein 36 (CD36) and fatty acid transport protein (FATP), and glucose transporters (GLUT 1 and 4), respectively12,13.

The extracted glucose is amongst others used for the synthesis of fatty acids, which is referred to as de novo lipogenesis. A schematic representation of human BAT physiology is presented in Fig. 1. In infants, brown adipocytes are clustered and mostly separated from white adipocytes. In adults, brown adipocytes are dispersed in WAT, making the depot much more heterogeneous in composition. The average amount of BAT volume in human adults is 50-150 mL14. BAT depots are typically located in the deep cervical, supraclavicular, supra-aortic and perirenal regions, with the supraclavicular BAT depot (scBAT) being the largest BAT depot in human adults (**Fig. 1**).

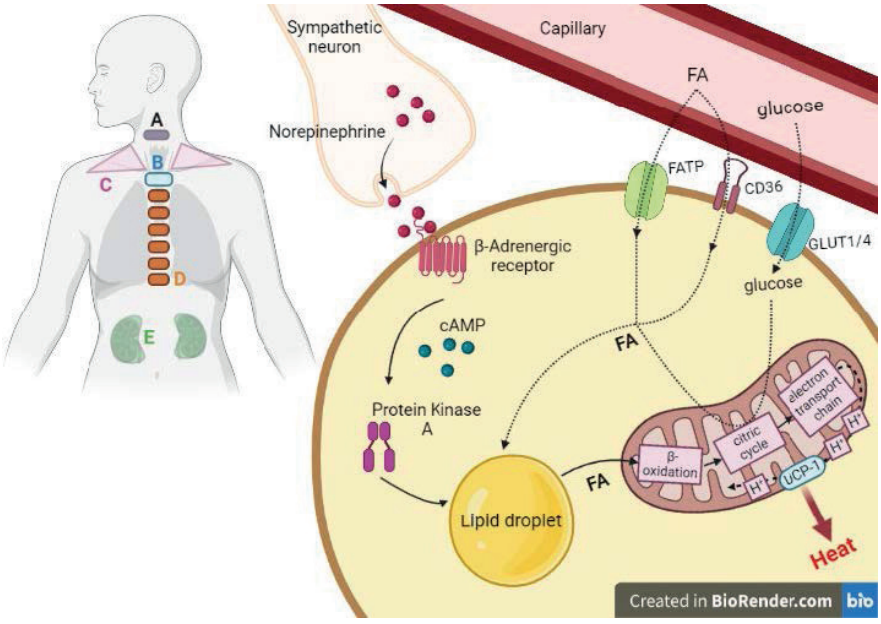

Figure 1. A schematic representation of brown adipose tissue (BAT) morphology and physiology.
Human BAT is located in (A) cervical, (B) supra-aortic, (C) supraclavicular, (D) paravertebral and (E) perirenal areas. Adrenergic stimulation of brown adipocytes by norepinephrine leads to intracellular lipolysis via the messenger cyclic adenosine monophosphate (cAMP) that activates protein kinase A (PKA). Liberated fatty acids subsequently enter the mitochondria where they are broken down by β-oxidation into substrates to enter the citric acid cycle and activate the electron transport chain to generate a proton gradient over the inner mitochondrial membrane. The uncoupling protein (UCP-1), uniquely present in BAT, increases the conductance of the inner mitochondrial membrane, which diverts the proton gradient from adenosine triphosphate (ATP) synthesis to generation of heat. Subsequently, intracellular lipids are replenished by extracting fatty acids and glucose from the bloodstream via cluster of differentiation protein 36 (CD36) and fatty acid transport protein (FATP), and glucose transporters (GLUT 1 and 4), respectively.

1.2 IMAGING MODALITIES FOR THE ASSESSMENT OF BROWN ADIPOSE TISSUE ACTIVITY

Since BAT is activated by the sympathetic nervous system, plasma norepinephrine levels in blood have been used as a proxy of BAT activation[15]. The cold-induced change in resting energy expenditure (ΔREE), up to the shivering temperature, has been used as another measure of the thermogenic BAT response[16–18], where REE is defined as the amount of energy an individual uses for maintaining essential body functions at rest. These methods, however, estimate BAT on whole-body level, and are less sensitive to assess changes in the BAT depot. Imaging methods that assess properties of BAT directly could solve this issue.

The most commonly used imaging technique to assess BAT activity is by quantifying the glucose uptake of a radioactively-labelled glucose analog, [^{18}F]fluorodeoxyglucose (FDG), using positron emission tomography/computed tomography (PET-CT)[19] in the supraclavicular area (scBAT). However, this imaging modality is invasive and depends on harmful radiation, which increases the participation burden and hampers longitudinal studies. In addition, BAT is an insulin sensitive organ, and since [^{18}F]FDG-PET-CT relies on quantifying the glucose uptake, this technique could produce unreliable measurements of scBAT activity in individuals who are insulin resistant. Moreover, it has been shown that BAT predominantly combusts fatty acids rather than glucose during cold exposure[20].

Safer and non-invasive alternatives have been explored for the assessment of scBAT activity. For instance, infrared thermography (IRT) is an imaging technique that converts emitted infrared energy from an object into a two-dimensional surface temperature map[21]. Since heat is generated by BAT, IRT has been used for assessing skin temperature changes near the scBAT depot[22–25]. Although IRT is readily available and cheaper compared to PET-CT, it only quantifies surface temperatures, such that changes inside the scBAT depot cannot be assessed. In addition, IRT could produce misleading results in obese subjects as the amount of subcutaneous fat in the area of interest influences this read-out[26].

Alternatively, magnetic resonance imaging (MRI) is increasingly being used for estimating scBAT activity *in vivo* using sequences such as Dixon's method for quantitative fat fraction (FF) mapping[27,28]. MRI produces images, which map the distribution of hydrogen-containing molecules, predominantly water and lipid. The hydrogen nuclei in lipid and water precess at slightly different frequencies (Df= 440 Hz on a 3 Tesla scanner). This can be detected as a difference in the phase (Dϕ) of the detected MRI signals from water and fat,

$$Df = 2\pi\Delta f\tau \qquad 1$$

where τ is the time at which the signal is measured. For example, in a 2-point Dixon sequence, two MR images are acquired, one in which water and fat signals are in-phase at $\theta = k\pi$, where k is an even integer, and the other out-of-phase where k is an odd integer. Water and fat signals can then theoretically be produced by addition and subtraction from these in-and out-of-phase images[29]. However, in practice there are several other factors which need to be considered, including non-uniformities in the main magnetic field (which by themselves cause spatially-dependent phase differences) and noise. In order to account for these factors, multi-point Dixon sequences are run, in which more than two images are acquired at different τ values. Since the magnitude of each signal is proportional to exp(-t/T2*), where T2* is a characteristic relaxation time related to tissue structure and magnetic field homogeneity, there is a limit to how many time points are acquired in practice for the best fitting to be performed. Iterative methods are used to estimate each of the parameters, water fraction, fat fraction, static field homogeneity and T2*. Finally, quantitative fat fraction (FF) maps are produced on a pixel-by-pixel basis. An example of images that can be obtained with the three imaging modalities: [^{18}F]FDG-PET-CT, IRT and MRI are visualized in **Fig. 2A-C** in three different participants.

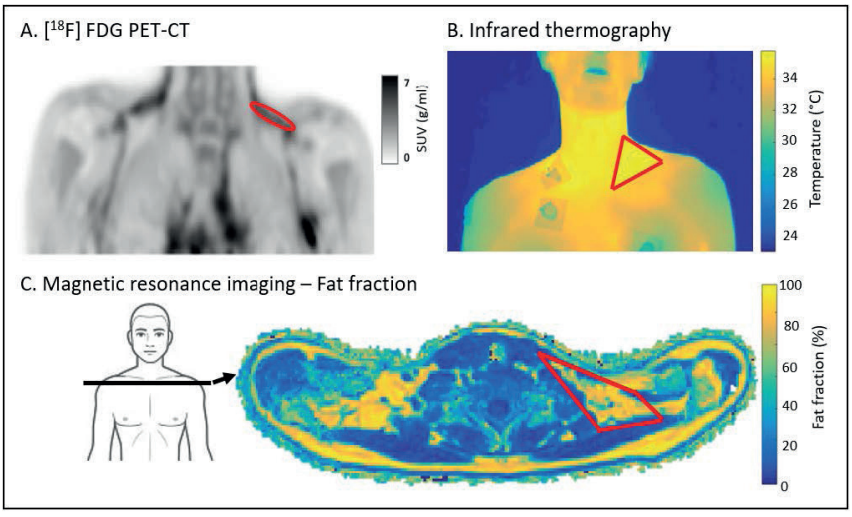

Figure 2. Examples of imaging modalities for assessing supraclavicular BAT activity.
A) [^{18}F]FDG-PET-CT indirectly quantifies metabolic scBAT activity based on the glucose uptake. B) Infrared thermography is used to assess changes in supraclavicular skin temperature. C) Magnetic resonance imaging using fat fraction mapping estimates scBAT activity based on changes in the relative fat content. Regions of interest (ROIs) are drawn in the scBAT area and shown in red. A different participant is shown for each technique. SUV, standardized uptake value.

1.3 CURRENT CHALLENGE FOR BROWN ADIPOSE TISSUE ACTIVATION

Cold exposure is the main physiological activator of BAT[30]. Different cooling protocols have been used in previous studies[31], which differ by duration, intensity, medium (i.e., water or air), garment and strategy (i.e., personalized or standardized). In standardized cooling protocols, the temperature is kept constant at a pre-determined value for a certain duration. In personalized cooling protocols, the temperature is gradually lowered and individualized to some participants' features, such as the onset of shivering or skin temperature. In addition to cold exposure, several pharmacological strategies have been evaluated for targeting BAT. For instance, Mirabegron, a drug used to treat overactive bladder, increases metabolic BAT activity, as assessed with [^{18}F]FDG PET-CT, while increasing resting energy expenditure[16,32]. Likewise, Exenatide and Spironolactone increase [^{18}F]FDG uptake by BAT after treatment[32,33]

It is important that the activation strategy sufficiently invokes the thermogenic BAT response since BAT can only be measured when it is metabolically activated. Despite the variety of cooling protocols used, there is no optimized procedure to activate BAT. Ideally, a cooling procedure should invoke a similar cold perception among individuals with different phenotypical features (e.g., sex, body size and composition), thereby ensuring maximal BAT stimulation. It has been shown that large individuals characterized by a small body surface area to volume ratio can preserve more heat compared to small individuals[34]. Moreover, individuals characterized by a higher fat mass and/or skeletal muscle mass could insulate heat better or produce more heat, respectively[35–37]. Currently, limited research focuses on the physiological mechanisms that drive the human cold tolerance capacity, which is defined as the ability to withstand cold temperatures. This hampers the optimization and standardization of cooling protocols among different research centers.

1.4 CURRENT CHALLENGES FOR QUANTIFYING BROWN ADIPOSE TISSUE ACTIVITY

While non-invasive and safe imaging modalities such as MRI and IRT are increasingly used to measure scBAT activity in humans, there are several technical challenges that need to be addressed before translating these techniques into clinical research. One of these challenges is the presence of motion. For instance, when moderate displacement is present within a repeated dataset consisting of e.g., baseline and follow-up images, regions of interest (ROIs) (**Fig. 1A**) need to be delineated on each image for analysis. This can be very time-consuming, and results can be variable due to differences in ROI sizes and manual ROI placement.

Moreover, recent MRI studies have used dynamic protocols where multiple MR scans were acquired during one session to detect scBAT FF changes during cooling[17,38,39]. Since scBAT is located in the supraclavicular area, and thus prone to motion-induced artefacts, results may be more variable. The influence of motion on the analysis time and data variability could be minimized by using non-rigid image registration. This technique enables a stepwise deformation of a target image towards a reference image to ensure a full image overlap[40]. Correct delineation of the active scBAT area is another challenge in the assessment of scBAT activity. Since "true" BAT (i.e., brown adipocytes) is mixed with other tissues, such as WAT, muscle and vessels, a micrometer resolution would be required to separate brown fat cells from white fat cells. The resolution of MRI is typically in the order of millimeters, and thus BAT cannot be separated from WAT in the supraclavicular area.

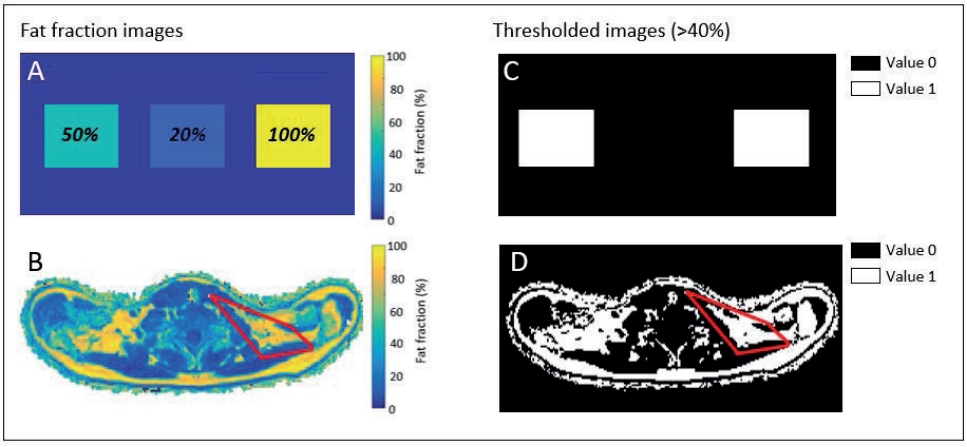

Figure 3. A simplified fat fraction (FF) image (A) and an MRI FF image (B) are shown in the left column. After applying a 40% FF threshold, values below this threshold are given a value of 0, whereas values above this threshold are given a value of 1 (C,D). FF values that are located within the ROI shown in red and that correspond to a value of 1 (white regions; D) are only included for analysis

This results in a segmentation challenge that hampers the extraction of "true" BAT within the supraclavicular depot. Post-processing techniques such as image thresholding could be used to exclude non-fatty tissue from the analysis. Image thresholding is a technique that is used to segment structures from an image, where image values above a certain threshold (e.g., 40%; see **Fig. 3A,C**) are set to 1 and values below this threshold are given a value of 0 or vice versa. Previous MRI studies[38,41,42] have used image thresholding on FF maps. This is illustrated in **Fig. 3B,D** where values below 40% FF are given a value of 0 and values above 40% are given a value of 1. Only the FF values that are located within the delineated scBAT ROI and correspond to a value of 1 (white regions) are included in the analysis. The remaining FF values are usually averaged across the thresholded ROI, and the difference between the average baseline and post-cooling FF is used to estimate the

cold-induced scBAT FF change. Reports on the extent of cold-induced scBAT FF changes, however, vary in the literature [-0.4%,-4.7%][27,28]. An explanation for this inconsistency may be the differences in applied FF thresholds (e.g., 0-100%, 40-100%, 50-100%)[38,41,42].

1.5 AIM OF THIS THESIS

The primary aim of this thesis was to improve the overall methodology for assessing scBAT activity in human adults to enable evaluation of therapies (in)directly targeting BAT. First, we aimed to gain insight into the role of body composition and BAT activity on the human cold tolerance capacity. This will facilitate the optimization of cooling protocols to ensure maximal BAT stimulation in heterogenous study populations. In **chapter 2**, we therefore investigated the association between the shivering threshold time, as a proxy for the human cold tolerance capacity, with body composition, cold perception and scBAT activity and volume, quantified using [^{18}F]FDG-PET-CT.

In the next chapters, we shifted our focus towards the non-invasive imaging modalities, IRT (**chapter 3**) and MRI (**chapter 4** and **chapter 5**), where we aimed to improve several technical challenges in order to provide reliable estimates of scBAT activity. IRT imaging has been previously used to study cold-induced changes in supraclavicular skin temperature. Most of these studies, however, still rely on manual segmentations, which can be very time consuming in large datasets and subjected to drawing variability. In **chapter 3**, we developed an open-access semiautomated segmentation tool (the IRT-toolbox) for measuring skin temperatures in the thoracic area to estimate scBAT activity, and compared it to manual segmentations.

In chapter 4 and 5, we focused on MR imaging for estimating scBAT FF in healthy adults. More specifically, since BAT cannot be easily distinguished from WAT, we aimed to explore the effect of different FF threshold ranges on different MRI-based outcomes in **chapter 4**, where we scanned once before and after cooling. Pre- and post-cooling assessments, however, do not provide sufficient insight into the time course of cold-induced scBAT FF changes. In addition, scBAT is located in the supraclavicular area, and thus prone to motion-induced variability. As such, in **chapter 5**, we aimed to minimize this variability by applying breath-holds and non-rigid image registration in a dynamic MRI protocol with a high temporal resolution for assessing FF changes in scBAT during cold exposure. In **chapter 6**, we summarized the main findings of this thesis, brought our results into perspective with the literature, and provided suggestions for future research.

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