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

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Chapter 6

Summary, general discussion and future perspectives

6.1 SUMMARY

The primary aim of this thesis was to improve the overall methodology for assessing supraclavicular BAT (scBAT) activity in human adults to enable evaluation of BAT-targeted therapies against cardiovascular diseases. In **Chapter 1**, we introduced the physiology and morphology of BAT, as well as strategies for BAT activation and imaging techniques for BAT detection in human adults. In **chapter 2**, we assessed how body composition and scBAT activity influenced the human cold tolerance capacity such that cooling strategies may be further optimized to ensure maximal BAT stimulation. In **chapters 3**, **4** and **5**, we focused on the development of non-invasive methods to facilitate their translation to clinical research on BAT.

In **chapter 2**, we investigated the association of the shivering threshold time, as a proxy for the cold tolerance capacity, with body composition, scBAT activity and volume as measured with ^{18}F-FDG PET-CT, the perception of shivering and skin temperature in young adults. We showed that interindividual differences in body type attribute to the shivering response: the cold tolerance capacity was related to body composition in females, and to body size in males and females. However, we found that the amount of metabolically activated scBAT did not seem to associate with the cold tolerance capacity, indicating that an individual with a higher amount of metabolically active BAT, and thereby a potentially higher capacity of BAT thermogenesis, could elicit the same cold tolerance capacity as an individual with less metabolically active BAT. Our findings, therefore, suggest that body size and composition should be considered when applying cooling protocols in heterogenous study populations regardless of the amount of activated BAT.

In the next chapters, we shifted our focus towards non-invasive imaging modalities: IRT (**chapter 3**) and MRI (**chapter 4** and **chapter 5**). We developed an open-access semi-automated segmentation tool (the IRT-toolbox) in **chapter 3** for measuring skin temperatures in the thoracic area to estimate scBAT activity. The main features of the IRT-toolbox are image alignment and non-rigid image registration, which enable a full image overlap between all follow-up images and the baseline image within a participant's dataset. As a result, the drawing time the ROIs was substantially reduced to a single image per dataset, while maintaining the inter-user reliability as compared to manual segmentations.

Since IRT only provides two-dimensional surface temperatures, we focused on quantitative MRI fat fraction (FF) mapping for detecting scBAT FF *in vivo* in the next chapters. Previous MRI studies have consistently reported FF reductions after cold exposure, which is most likely attributed to the combustion of lipids with brown adipocytes. Yet, reports on the extent of FF changes vary in the literature. Differences in selected FF threshold ranges for scBAT segmentation could be an explanation for this. Indeed, in **chapter 4**,

we showed that after 2-h of personalized cold exposure, FF predominantly decreased in lipid-rich regions within the scBAT depot, whereas FF increased in lipid-poor regions. We demonstrated that by increasing the lower FF threshold level (e.g., from 30-100% to 50-100%), pre-and post-cooling FF differences became less pronounced, whereas estimated pre-and post-cooling scBAT volume differences became larger in magnitude. Due to the heterogenous nature of the scBAT depot in human adults, the use of FF thresholding influences the magnitude and direction of scBAT outcomes. Therefore, future studies should carefully consider the use of FF thresholds when analyzing scBAT in human adults.

In the study described in **chapter 4**, two MRI scans were acquired per individual: one timepoint before cooling and one timepoint after 2-hours of cooling. This does not provide insight into the time course of scBAT activity. In addition, since scBAT is located in the supraclavicular area, measurements can be prone to motion-induced variability. In **chapter 5**, we aimed to minimize this variability across dynamic FF measurements using a high-time resolution protocol for assessing FF dynamics in scBAT during cold exposure. We used breath-holds in our image acquisition protocol, and image registration and mutual FF thresholding in our analysis to minimize motion artefacts. We used mutual FF thresholding in our analysis to segment scBAT more accurately, and we applied a 30-100% FF range based on results from **chapter 4**. We demonstrated that the use of motion-correcting techniques, such as non-rigid image registration and mutual FF thresholding improve the stability of our data by 30%.

6.2 GENERAL DISCUSSION

To bring our results into perspective with the literature, we date back to 2009, where three different research groups identified metabolically active brown adipose tissue (BAT) in human adults using [^{18}F]FDG-PET-CT[1–3]. Since then, multiple studies have been performed that confirmed the presence of metabolically active BAT, reinvigorating interest of its implications in cardiovascular health[4–7]. While activation of BAT emerged as a potential strategy in cardiovascular health, research focused on the development of non-invasive and safer alternatives to [^{18}F]FDG-PET-CT to detect activated BAT, with the ultimate goal to facilitate clinical research on therapies (in)directly targeting BAT.

In 2011, the potential of IRT as an indirect measure of supraclavicular BAT (scBAT) activity was first demonstrated in healthy adults[8]. That study reported a smaller reduction in supraclavicular skin temperature (-0.9°C) after cooling compared to a reference tissue (mediastinal region; -2.0°C) potentially reflecting the thermogenic capacity of BAT. Since then, IRT has been increasingly used for the assessment of BAT activity in response to cold, either in standalone settings[9–14] or in combination with other techniques, such as

[^{18}F]FDG-PET-CT[15–19]. In most studies, however, ROIs were manually drawn for analysis. This can be very time consuming in large datasets and prone to ROI drawing variability. We developed the semi-automated segmentation tool: *the IRT-toolbox* in **chapter 3** to address these limitations. The most important feature of our toolbox is non-rigid image registration: this post-processing technique enables a stepwise deformation of a target image to fully overlap with a given reference image. We applied this method to enable a full image overlap between all follow-up images and the baseline image within each participant's dataset. As a result, ROI drawing was solely required on the baseline image within each dataset. Our toolbox therefore substantially reduced the ROI drawing time to a single image per dataset, while maintaining the same user reliability compared to manual segmentations. The IRT-toolbox is freely available (https://github.com/AashleySD/IRT_toolbox) and ready for clinical applications.

While IRT has been more frequently applied over the years, MRI fat fraction (FF) imaging concurrently gained interest as an indirect readout for scBAT activity. In 2013, Hu et al[20] identified morphological differences between scBAT and WAT using both post-mortem and in vivo MRI in infants, adolescents and adults. Lower FFs were found in the scBAT depot with a more granular appearance compared to WAT. Similarly, van Rooijen et al[21] reported a FF of approximately 66% in supraclavicular and cervical depots in adults, which was 16.3% lower compared to the FF in white adipose tissue. That study additionally acquired [^{18}F]FDG-PET-CT images as a reference method, and showed that the FF of the supraclavicular/cervical regions did not correlate with the glucose uptake. Thereafter, several validation studies were performed that simultaneously used [^{18}F]FDG-PET-CT and MRI to assess the correlation between scBAT FF and [^{18}F]FDG uptake in scBAT. For instance, McCallister et al showed a good correlation between these parameters only when subjects showed a substantially high glucose uptake[22], while Fisher et al[23] and Sun et al[24] showed that only 40% of the variation in scBAT [^{18}F]FDG uptake was explained by MRI FF. These moderate correlations may reflect the underlying difference between the physiological responses of these readouts, as [^{18}F]FDG-PET-CT measures glucose uptake, and MRI-derived FF reflects lipid metabolism, and indirectly glucose metabolism due to de novo lipogenesis. Meanwhile, studies continued on exploring MRI as a standalone modality, quantifying the change after cold exposure with respect to warm conditions (scBAT ΔFF) as a measure for scBAT activity. These studies reported a relatively small reduction of scBAT FF after cold exposure and with varying magnitude (-0.4%, -4.7%)[25,26]. In line with the literature, we found a FF decrease of -3.5% after two hours of personalized cold-exposure in **chapter 4**. At the same time, we found that heterogenous changes occurred across the entire depot: lipid-rich regions in the scBAT depot decreased in fat fraction, whereas lipid-poor regions increased in fat fraction. Similar findings were reported by Coolbaugh et al[27] where regions in the scBAT depot that were grouped at lower baseline fat fractions (0-30%) exhibited a FF increase after cooling, whereas regions grouped at higher FFs (60-100%) showed a FF

decrease in response to cold. As such, the net balance in scBAT FF is most likely determined by the combustion- and uptake of lipids, which on average, causes a relatively small effect in cold-induced FF outcomes as reported in the literature. In addition, in **chapter 4**, we demonstrated that the selection of FF thresholds for scBAT segmentation influence the magnitude of scBAT MR outcomes. Here, we demonstrated that by increasing the lower FF threshold level (e.g., 30-100% → 50-100%), pre-and post-cooling FF differences became smaller, whereas estimated pre-and post-cooling scBAT volume differences became larger. As such, these results indicate that differences in FF segmentation thresholds could have contributed to the varying magnitude of scBAT FF changes in the literature, and thus care should be taken when selecting FF thresholds for analysis.

The first MRI studies that were performed to assess scBAT usually acquired MRI scans one timepoint before- and one timepoint after cold exposure. Several studies switched to dynamic scanning where MRI scans were repeatedly performed to obtain insight into the time evolution of scBAT activity during cold exposure[27–31]. Most of these studies performed dynamic MRI measurements without the use of motion-correcting methods such as breath-holds or image registration. Since scBAT is located in the supraclavicular area, and thus prone to motion, scBAT FF results may become more variable when no motion correction is applied. Given the small decrease in scBAT MRI FF upon cold exposure, any variability due to motion should be minimized to ensure a reliable detection of scBAT. We addressed this in **chapter 5**, where we showed a low inter-image variability of less than 0.1% across 1-minute time resolution MRI measurements of scBAT FF changes during cold exposure. To obtain this low variability, we used breath-holds in our acquisition, and non-rigid image registration and an optimized scBAT segmentation procedure in our analysis pipeline. At the same time, we demonstrated that scBAT FF changes can be overestimated by two-fold when motion-induced variability is not taken into account. A lack of these motion-correcting techniques could therefore have produced larger scBAT FF reductions in studies that did not use breath-holds (-4.7%, -1.94)[27,29] or non-rigid image registration (-3.0%)[30,31]. Our results therefore highlight the importance of using breath-holds, non-rigid image registration and mutual thresholding in tissues such as scBAT that are prone to movement artefacts.

The small reduction in scBAT FF upon cold exposure additionally requires that the applied stimuli sufficiently activate BAT to measure a detectable FF change. In **chapter 2**, we showed that differences in body size and composition may invoke different cold perceptions between individuals. As a consequence, the magnitude of scBAT FF changes upon cold exposure could be smaller in individuals that experience less cold compared to others due to e.g., better insulation traits. Moreover, results from **chapters 4** and **5** suggest that the amount of activated BAT may depend on the applied cooling procedure. For instance, we used a personalized cooling protocol for BAT activation in **chapter 4**,

resulting in a cold-induced scBAT FF difference of -3.5%, whereas this change was almost 6 times lower (-0.61%) when we applied standardized mild-cooling (18 °C) in **chapter 5**. Interestingly, Oreskovich et al[31] also applied a mild-cooling procedure at 18°C, but used a liquid-conditioned suit instead of a water circulating blanket. They reported a scBAT FF reduction of -3.0%, which is substantially higher than the results from **chapter 5** (-0.61%). Besides differences in cooling garments, lower cooling intensities most likely cause a larger reduction in scBAT FF. As such, a previous study by Stahl et al[28] reported a scBAT FF reduction of -2.9% after 1.5 hours of standardized cooling at 12°C. Coolbaugh[27] and Deng[30] also reported a FF reduction of -4.7% and -3.0% after applying one hour of personalized cold exposure (down to a minimum temperature of 10°C and 13°C, respectively). Altogether, these results suggest that cooling protocols should be carefully selected to ensure maximal scBAT activation, thereby enhancing the chance for scBAT detection, and especially among individuals with varying body types.

While promising efforts have been made towards the use of IRT and MRI as alternatives for [^{18}F]FDG PET-CT, tracers other than the glucose analogue [^{18}F]FDG have been evaluated as well. For instance, U din et al[32] used the radiotracers [^{15}O]O_2, [^{15}O]H_2O, and [^{18}F]FTHA to measure oxygen consumption, blood flow, and non-esterified fatty acids (NEFA) uptake, respectively. That study reported an almost two-fold increase in oxygen consumption and blood flow as well as an increase in NEFA uptake in scBAT after cold exposure. The advantages of the [^{15}O]O_2 tracer are its independency on substrate availability, as well as its non-invasiveness as the tracer is administered via a mask. However, the [^{15}O]O_2 tracer has a very short half-life of approximately two minutes, which could hamper measurements due to logistical reasons. Moreover, the [^{18}F]FTHA tracer reflects the uptake of free fatty acids, whereas BAT mainly extracts fatty acids from triglyceride-rich lipoproteins. As such, the development of a lipoprotein-derived FA tracer would be very valuable in this line of research as it could provide a better representation of the lipid uptake by scBAT. Combining this tracer with MRI FF mapping may allow a higher accuracy in detecting the uptake and combustion of lipids considering the high resolution of MRI and its ability to detect lipid changes across the entire scBAT volume (**chapters 4 and 5**).

6.3 FUTURE PERSPECTIVES

Our results demonstrate the potential of using IRT and MR imaging as safer alternatives compared to [^{18}F]FDG-PET-CT, but future work is needed to establish their use in clinical research. More specifically, the target population for BAT-related research mainly consist of overweight individuals and individuals living with obesity. It remains questionable whether IRT can be used in such subject populations since it has been shown that the thickness of the subcutaneous fat layer inversely relates to skin temperature ouctomes[33].

Studies should therefore focus on evaluating the feasibility of IRT for the assessment of supraclavicular skin temperature in individuals with varying body types.

The advantage of MRI is that FF outcomes are not limited to the surface area since this technique enables a three-dimensional assessment of the entire depot. While research on MRI measurements in overweight and obese individuals is limited, Deng et al[30] reported a scBAT FF reduction of ~1.0% in four overweight and two obese individuals after personalized cold exposure. Albeit preliminary, this report demonstrates the potential of MRI in detecting small FF changes in overweight and/or obese individuals that have less active BAT compared to lean controls. Prior to extending these measurements towards larger (patient) populations, we would first recommended to further establish the validity of MRI for active BAT detection. Previous studies have compared scBAT FF outcomes to other methods like [^{18}F]FDG-PET-CT and whole body measures. Gashi et al investigated the correlation between scBAT FF and cold-induced changes in resting energy expenditure (REE)[34], where scBAT did not contribute to the change in REE. While BAT comprises of approximately 0.1% of body weight (~70 g), earlier work has estimated that 50 g of activated BAT can increase energy expenditure by 20%[1]. However, the contribution of BAT on whole body energy expenditure is under debate. For instance, Din et al showed that BAT contributes only ~1% of whole body energy expenditure[32], and that the remaining increase (range 2-47%) in whole-body energy expenditure in response to cold is predominantly mediated by shivering, which is the well-known form of thermogenesis produced by skeletal muscle.

In order to further elucidate the physiological mechanisms that may be associated to the supraclavicular MRI-derived FF changes upon cold exposure, MR imaging could be combined with repeated blood sampling. Since active BAT rapidly replenishes intracellular lipid stores by extracting lipids from the circulation, future work should compare scBAT FF changes to plasma lipid measurements.

In the literature, a wide variety of cooling protocols, imaging protocols and analysis pipelines have been described for both IRT and MRI, which hampers the comparison of BAT outcomes between research centers. In 2018, the BARCIST criteria were established to recommend on the use of protocols and analyses for assessing BAT activity using [^{18}F]FDG-PET-CT[35]. As a future prospective, it would be very useful to establish such criteria for IRT and MRI . Focusing on the target population for BAT-related research, this requires that cooling- and imaging protocols are optimized and tailored to individuals with different phenotypical features. For instance, in **chapter 2** of this thesis, 25 out of 110 participants did not report shivering during the personalized cooling procedure, and showed a higher whole-body adiposity compared to the participants that reported shivering. Personalized cooling protocols usually start at an initial temperature followed by a stepwise decrease in

temperature (e.g., 5°C every 10 minutes) until shivering occurs or a minimal temperature is reached. These temperature settings could be further optimized in different subgroups characterized by BMI and/or sex using e.g., larger step sizes when lowering the temperature or a lower minimum temperature for individuals that can preserve heat better compared to others. Another important aspect is to recommend on analysis methods that may affect BAT-related outcomes such as including co-registration in the analysis, or the selection of scBAT FFF segmentation thresholds. Altogether, standardizing protocols may contribute in facilitating the interpretation of BAT-related outcomes between research centers.

6.4 CONCLUSION

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. The studies in this thesis have improved the feasibility of using non-invasive methods such as IRT and MRI for the assessment of scBAT activity in human adults and have led to a better understanding of the physiological mechanisms that influence the cold tolerance capacity in human adults. We showed the amount of activated BAT could vary among individuals with different body types and that cooling protocols should be carefully selected, especially in heterogenous study populations. With regards to imaging, we strongly recommend to use motion-correcting methods such as non-rigid image registration to correct for motion-induced variability, and to reduce the analysis time. Finally, due to the heterogenous nature of the scBAT depot in human adults, the use of FF thresholds for analysis should be carefully considered.

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