ExiTron™ BAT, a novel contrast agent visualising activated brown adipose tissue (BAT) by CT imaging

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Introduction

In 2016 more than 1.9 billion adults, classified as 18 years of age or above, were considered overweight, with 650 million of them falling into the category ‘obese’ (World Health Organisation, 2017). Obesity develops when calorie uptake exceeds energy expenditure, with the excess energy being stored in fat. Researchers have identified adipose tissue (i.e., fat) not simply as an energy storing depot, but as an essential player in weight regulation with at least two distinguishable forms: white adipose tissue (WAT), and brown adipose tissue (BAT). In mammals, BAT has been identified as the primary tissue involved in energy expenditure and non-shivering thermogenesis, and its amount and level of activation allow for regulation of the utilisation of excessive calories for thermogenesis rather than storage in WAT, which results in weight gain [1].

Various biomedical imaging techniques have been utilised in the study of the morphology and function of BAT *in vivo* such as positron emission tomography (PET), computed tomography (CT), magnetic resonance imaging (MRI), contrast-enhanced ultrasound (CEUS), or various optical techniques [1-3].

BAT activity *in vivo* is commonly measured by PET-CT scans using 2-[18F]-fluoro-2-deoxy-D-glucose (18F-FDG) to assess glucose utilisation linked to BAT mitochondrial respiration [2,3]. The use of 18F-FDG however presents exposure to radioactivity. Additionally, different degrees of BAT activation in combination with the poor reproducibility of FDG PET-CT in measuring BAT glucose metabolism leads to a high variability of results [4].

In our study we develop a CT method that involves the use of the novel CT contrast agent, ExiTron™ BAT (Viscover™, nanoPET Pharma GmbH, Berlin, Germany) for measuring BAT activity in vivo. This novel contrast agent specifically accumulates in BAT, allowing for easy visualisation and quantification of this tissue without radioactive tracers.

Methods

In a pilot study, two groups of mice were compared, with each group containing both young (8 weeks) and old (1+ year) mice. One group underwent short-term cold exposure (4°C for 12h) with the aim of activating the BAT, while the other group did not.

CT imaging was performed using the nanoScan® PET/CT scanner (Mediso) with fixed parameters for all groups (helical scan, 360 projections, 900 ms exposure time, 1:4 binning, Butterworth filter). Mice were anaesthetised using isoflurane and kept at 37°C using a heated bed.

CT scans of the upper mouse body (head and thorax) were performed prior and at specific time points after intravenous injection of the mice with ExiTron BAT (dose 1050 mg iodine / kg body weight, i.e., 125 µL per 25 g mouse).

Post analysis was performed by placing a region of interest (ROI) in the lower neck region where BAT is located and comparing the signal intensity to that found in ROIs located in lung tissue as control.

Results

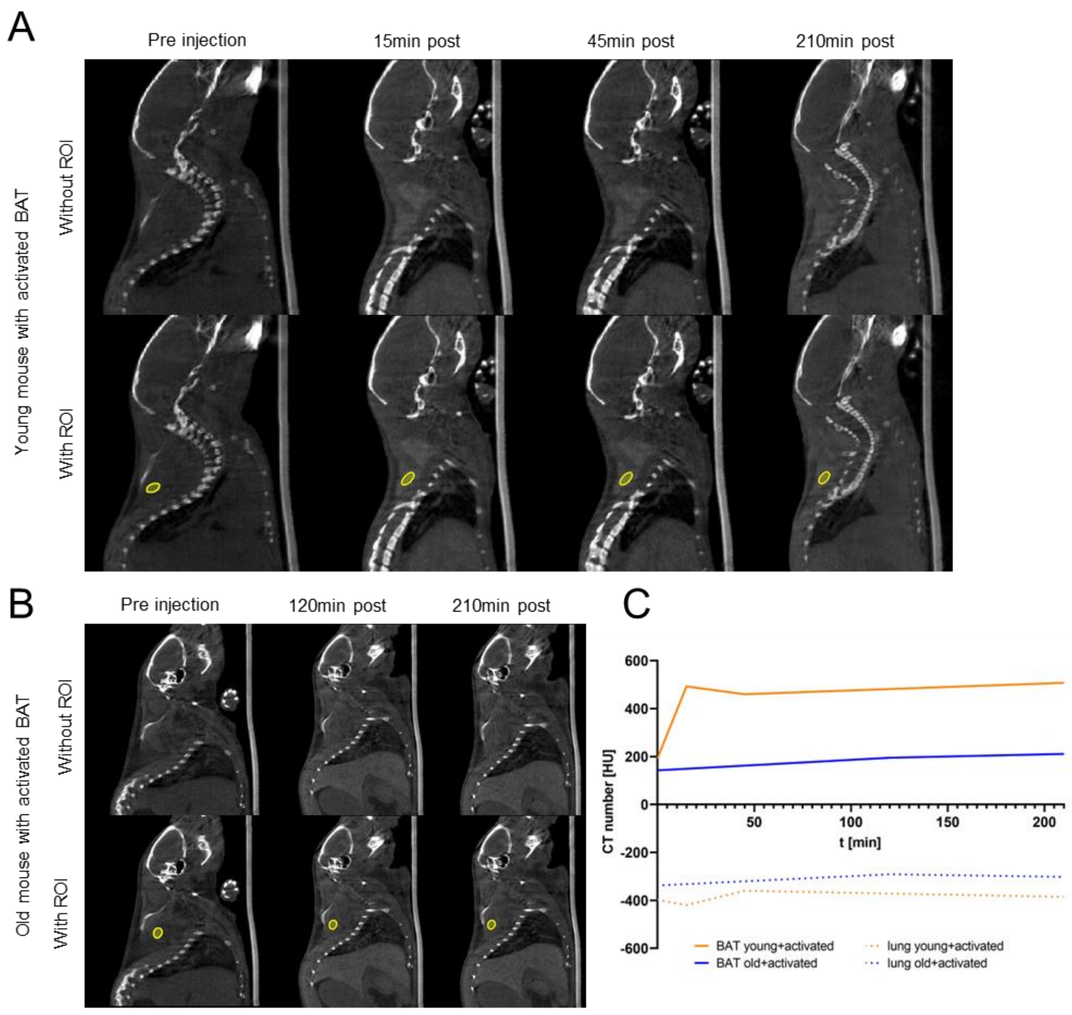

Figure 1: Visualisation of BAT after cold exposure using ExiTron™ BAT and CT imaging
(A) Sagittal CT images of the upper body of a young mouse (8 weeks) after BAT activation through short-term cold exposure (12h) pre and post intravenous injection of ExiTron™ BAT (125 µl / 25 g), compared to corresponding CT images of an old mouse (1+ year) (B). (C) Representative time course of the contrast agent uptake in the BAT region (marked in yellow in A and B) and lung tissue (control) of a young mouse compared to an old mouse after BAT activation over 210min

After injection of ExiTron BAT, BAT in young mice activated via short-term cold exposure could be clearly visualised (Figure 1A). The results showed a rapid increase in signal intensity within the BAT region during the first 15 minutes post injection. This signal then increased slowly but progressively over the time course of the imaging study, with the maximum enhancement being reached at 210 min, the last measured timepoint (Figure 1C, orange line). As expected, the lung tissue acting as control did not show uptake of the contrast agent (Figure 1C, orange dotted line).

In old mice, however, the uptake of ExiTron BAT after activation via short-term cold exposure was only slightly increased compared to the younger counterpart (Figure 1B and 1C blue line). This finding was expected, as BAT has been found to be more prevalent in young individuals [5]. Again, the lung tissue acting as control did not show uptake of the contrast agent (Figure 1C, blue dotted line).

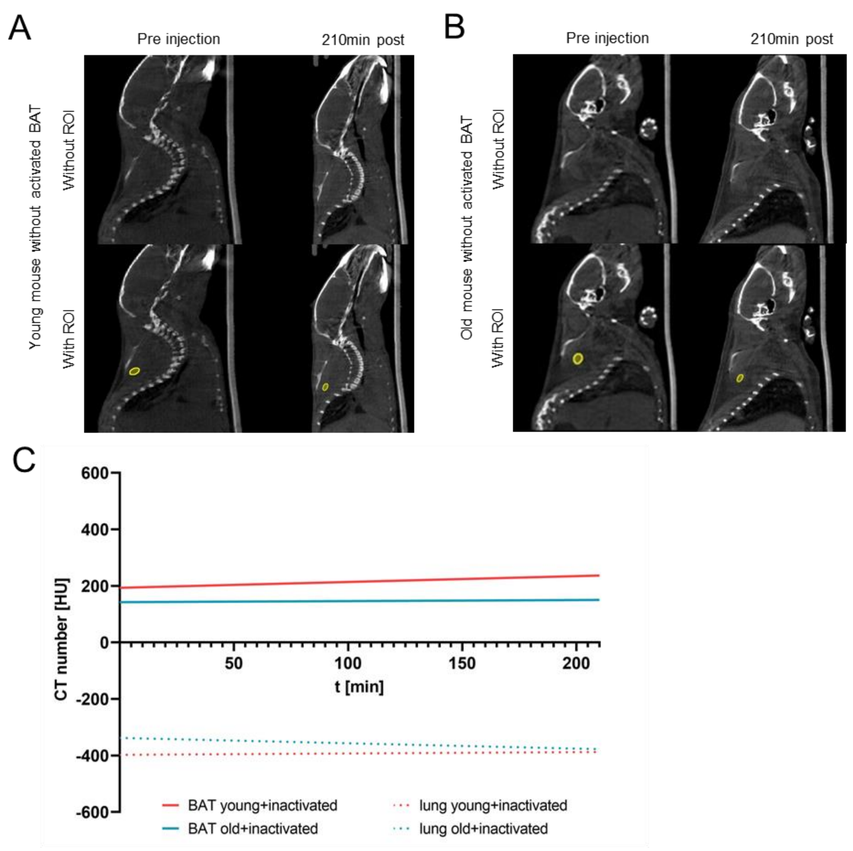

Figure 2: Visualisation of BAT after cold exposure using ExiTron™ BAT and CT imaging
(A) Sagittal CT images of the upper body of a young mouse (8 weeks) without BAT activation pre and post intravenous injection of ExiTron™ BAT (125 µl / 25 g), compared to corresponding CT images of an old mouse (1+ year) (B). (C) Representative time course of the contrast agent uptake in the BAT region (marked in yellow in A and B) and lung tissue (control) of a young mouse compared to an old mouse without BAT activation

In mice that did not undergo BAT activation via short-term cold exposure, BAT was unable to be clearly visualised, irrespective of age (Figures 2A & 2B). Even though the signal intensity in BAT was low in all animals that did not undergo cold exposure, it was found to be higher than in the control tissue and was found to progressively increase over time for the duration of the imaging experiment (Figure 2C). Furthermore, BAT signal enhancement was slightly higher in young mice than in old mice again confirming that BAT seems to be more prevalent in younger individuals.

Besides providing signal enhancement in BAT, ExiTron BAT also resulted in enhancement of other tissues including the liver. Liver contrast was increased equally in all mice, irrespective of age or whether steps were taken to activate BAT and indicated that the contrast agent is cleared predominantly via the hepatic clearance route.

Conclusion

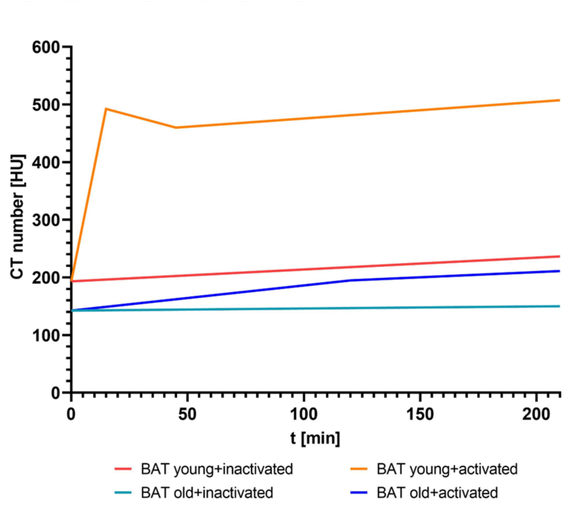

Figure 3: Representative time course of ExiTron™ BAT uptake in the BAT region of all treatment groups

Our study explores a CT method involving a novel contrast agent to visualise BAT *in vivo* in mice. The agent shows specific accumulation in BAT, a high contrast efficiency, biocompatibility, and clearance properties. Using the CT method combined with ExiTron BAT allows for longitudinal studies of BAT morphology and offers an innovative tool for monitoring BAT in small animals to evaluate its role in the treatment of obesity.

Bibliography

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