

# Analyzing body fat in mice using $\mu$ CT

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## Aims

The aim of this study was to analyze changes in the amount of body fat in transgenic mice using the Skyscan 1076 in vivo  $\mu$ CT system. As a test system, we used conditional knockout mice for the WT1 gene (wER mice).

## Method

Ten-week old mice were anaesthetized using gas anesthesia and scanned using the Skyscan 1076 in vivo scanner. We performed two scans on each mouse; the first scan before the tamoxifen treatment, and the second 9 days later. Injection of tamoxifen in these mice results in the rapid induction of the Cre enzyme which then excises the floxed WT1 gene. Cre negative mice were used as controls. Scans were performed at a resolution of 35  $\mu$ m with the X-ray source set to 40kV, 250  $\mu$ A and using a 0.5mm Al filter. We tested a range of settings for the rotation step and frame average settings. Although a rotation step size around 0.8° combined with no frame averaging lead to very fast scan times, the resulting images were relatively noisy and this lead to problems with the thresholding during the analysis. We decided on a step size of 0.4° combined with frame averaging set to 3. This resulted in images that were relatively straightforward to threshold (see fig. 1), and an acceptable scan time of 9 min per scan.

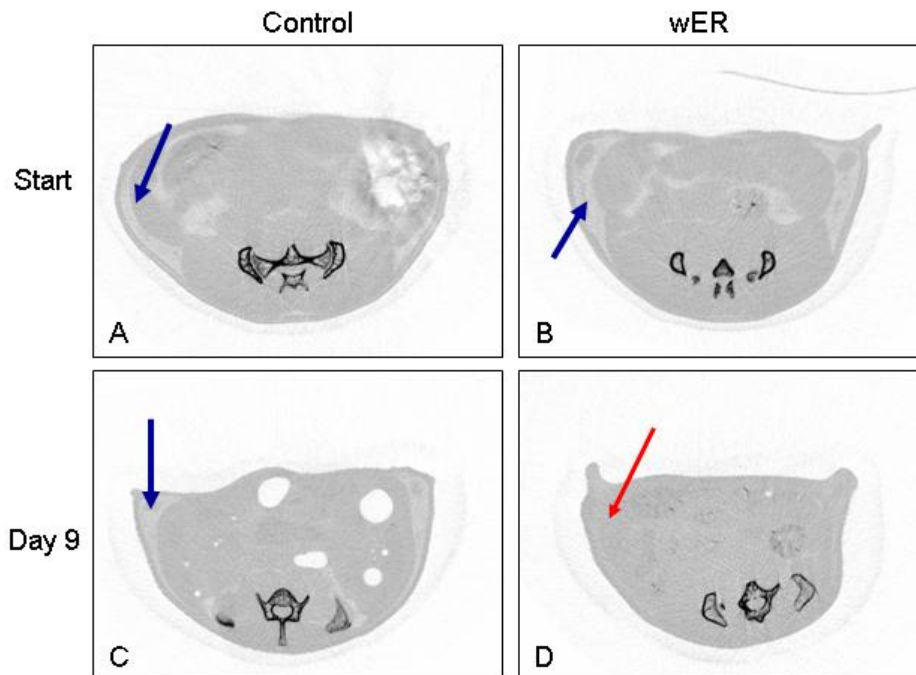


Figure1: Imaging of fat by  $\mu$ CT shows rapid loss of fat mass in wER mice. Mice were scanned just before the tamoxifen treatment, and 9 days later. Blue arrow indicates fat pad, red arrow indicates lack of fat pad.

## Results

The  $\mu$ CT imaging clearly showed very rapid fat loss in the wER mice after the inactivation of the WT1 gene (figure 1). Quantitative analysis though, suffered from artifacts in the image caused by gas and X-ray dense particles in the gut of the mice, as can be seen in fig 2. A scan of the food pellets revealed relatively large grains of chalk, which were added as a source of calcium. A change to a different make of food containing finer chalk particles largely solved the problems caused by these particles. However, the problems caused by moving gas in the gut have as yet not been solved.

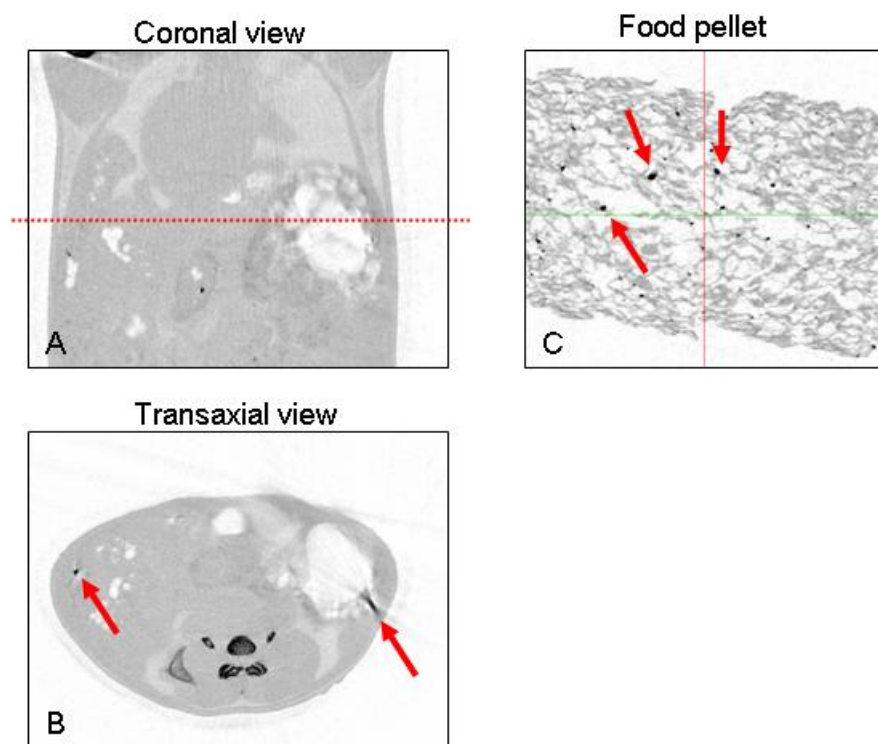


Figure 2: Image artifacts caused by chalk particles and gas in the gut.

A shows a coronal view of the abdomen of a mouse. The fat pads are clearly visible as light grey areas. On the transaxial view of the level indicated by the dashed red line in A, panel B clearly shows the artifacts caused by X-ray dense particles in the gut, and a smudging of the image caused by moving gas bubbles. The scan of a food pellet in panel C clearly shows the presence of relatively large chalk grains.

The areas showing problems caused by the gas bubbles were manually removed using a ROI in CTAn (Fig 3A). Thresholding unfiltered datasets resulted in a very noisy binary image (3B). We therefore employed a median filter with a relatively large radius of 4 on the input images. This removed most of the noise while preserving the shape of the fat pads (3C). The subsequent threshold generated a much cleaner binary image (3D), which was then further cleaned up by a morphological open (size 2) followed by a despeckle operation (Fig 3E). This removed the outline of the body and any remaining small artifacts. The final binary image stack was used to calculate fat volume and to create a 3D model of the fat tissue. Skeletal elements and total body volume 3D models were obtained by using appropriate threshold settings on the median filtered image stack. Figures 3F and 3G show the combined models for the abdomen of a mouse at the start of the experiment (F) and 9 days after the tamoxifen treatment (G).

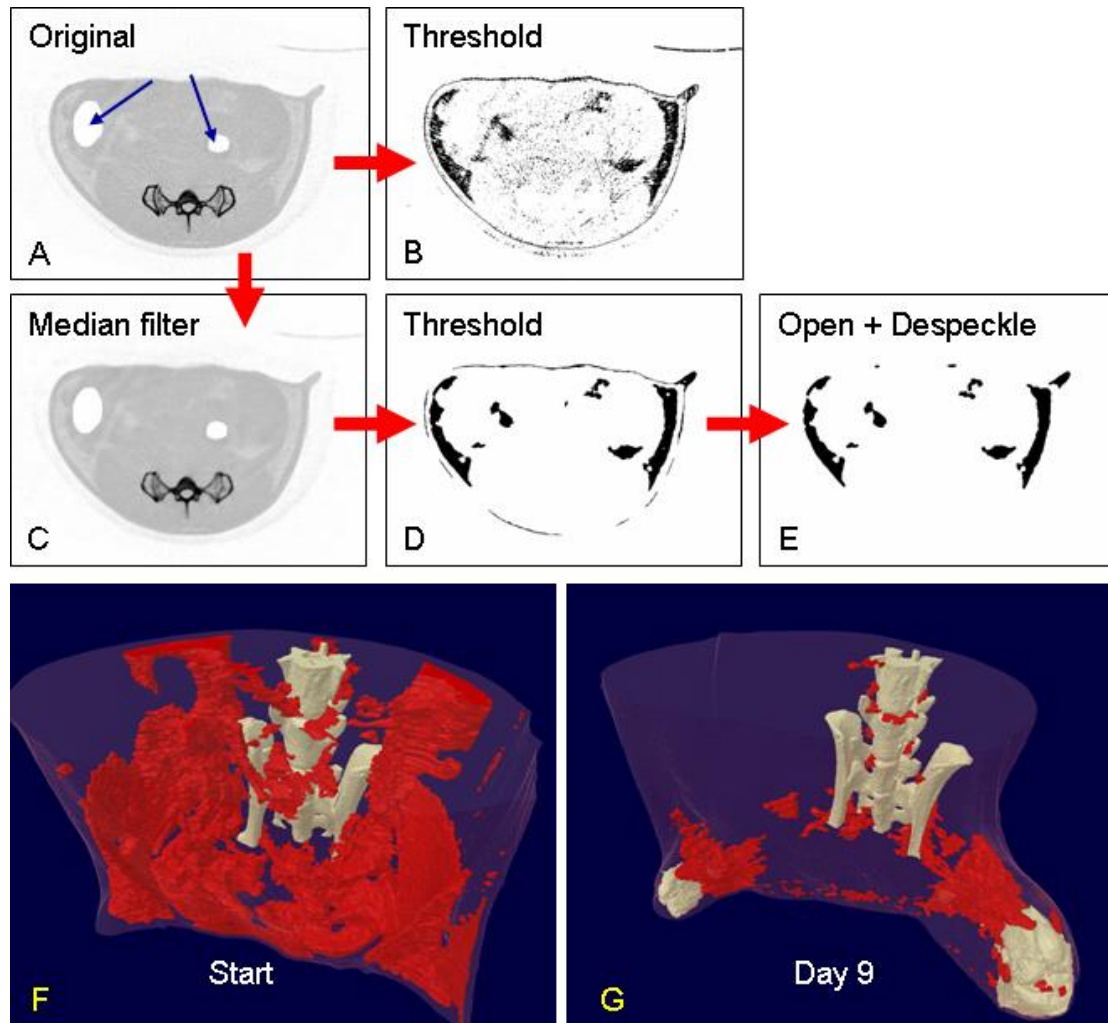


Figure 3: Analysis of Fat volume.

The blue arrows in A indicate areas where artifacts in the gut were removed. A and B show noisy result of threshold on a unfiltered image. Panels C to E show the result of processing a median filtered image. Panels F and G show 3D models of the abdomen with the skeleton in yellow, fat in red and body volume in transparent pink.

### Conclusion

Imaging of fat tissue using  $\mu$ CT is relatively straightforward. However, performing the analysis using live mice is slightly more complicated due to artifacts in the images caused by moving gas bubbles in the gut. By far the most time consuming part of the analysis is the removal of these artifacts by the drawing of ROIs in CTAn. We are currently investigating different diets to reduce intestinal gas.