

# Long term quantitative evaluation of muscle and bone wasting induced by botulinum toxin in mice using microCT

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## Aims

Muscle and bone masses are highly correlated and muscles impose large loads on bone. Long term immobilization or prolonged exposure to microgravity induce a severe muscle wasting and bone loss. Muscle wasting that accompanied bone loss has been poorly investigated mainly due to the lack of simple analytical methods to quantify muscle loss. We have previously shown that a single injection of botulinum toxin (BTX) in the right quadriceps muscle induces a transient paralysis and a rapid bone loss due to disuse<sup>1-3</sup>. However, muscle changes have not been considered because of their radiolucency<sup>4</sup>.

## Method

21 female mice were spread into 7 groups. At day 0, 18 mice received a BTX injection in the quadriceps muscle to induce paralysis of the right hind limb; the left contralateral side was used as control. Mice were sacrificed at 7, 14, 21, 28, 56 and 90 days post-BTX (3 mice per group). The remaining group was not injected and sacrificed at day 0. Trabecular bone volume was determined by microCT at the proximal tibial metaphysis on both sides. Hind limbs were immersed in a contrasting solution containing HgCl<sub>2</sub> for muscle visualization by microCT<sup>5</sup>. On the 2D sections, the surface area, circularity and aspect ratio were measured for M. quadriceps at mid-tigh and M. gastrocnemius at mid-leg. These muscles were then carefully dissected by trained anatomists and weighed (anatomical method).

## Results

### *MicroCT and anatomical analysis of muscle evolution*

In order to obtain a scan of the whole hindlimb, the multi-scan mode of the Skyscan 1172 microCT was used. The whole musculature in a complete mouse leg was easily "stained" by the HgCl<sub>2</sub> solution (Fig. 1). 2D sections provided a sharp contrast between the muscles and tendons and images were similar to MRI in humans. The surface area of M. gastrocnemius and M. quadriceps were significantly lower in the paralyzed limb from 7d; the decrease was maximum at resp. 21 day (-46.9%) and 28 day (-47.9%). No difference in geometric form-factor parameters were found between the paralyzed and non-paralyzed limb indicating that the global shape of the muscles was respected. Significant correlations were obtained between the surface area obtained by microCT and weight data obtained by the anatomical method.

### *MicroCT analysis of bone loss*

Bone volume decreased in the paralyzed side at the femur and tibia. Bone loss was maximal at 56 day followed by recuperation at 90 day. Bone loss is observed in the metaphysis and the epiphysis<sup>6</sup>.

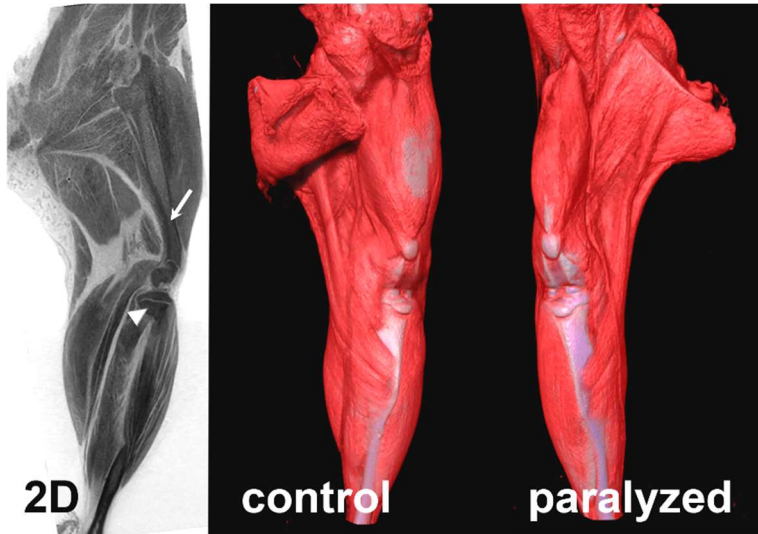


Figure 1: MicroCT analysis of the muscle anatomy of a full leg after contrast enhancement with  $HgCl_2$ . A) 2D section of a leg showing the different muscle bodies impregnated with Hg (arrow indicates the femur, arrowhead indicates the proximal tibia extremities). To improve visualization and contrast, the image is inverted. B) 3D reconstruction image of the left (control) side of a mouse at 21 days; C) 3D reconstruction image of the right (paralyzed) side of the same animal which received a BTX injection in the right M. quadriceps. 3D images produced with CTVox with a look-up table emphasizing muscle in red and bone in white.

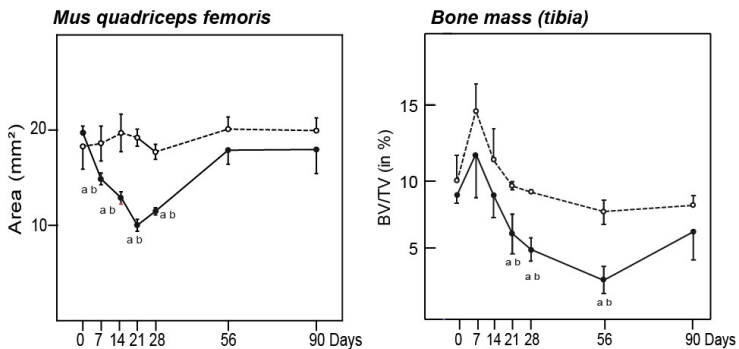


Figure 2: Left: MicroCT analysis of M. quadriceps evolution during the time course of the study in cross sections images of the tibia. Right: evolution of trabecular bone volume at the proximal tibial extremity. ○ identifies the left side and ● the paralyzed right side with BTX. Significance: <sup>a</sup> Same limb, different from t0 ; <sup>b</sup> Same time, different from opposite limb.

## Conclusion

The use of a metallic contrast agent opens new perspectives to better understand the changes occurring within soft tissues<sup>7</sup>. A quantitative analysis of muscle loss and muscle recovery was possible by a microCT technique in the BTX animal model. MicroCT and fine anatomical dissection were correlated. Loss of bone secondary to muscle wastage induced by BTX and recovery showed a parallel evolution for bone and muscles. This study reports for the first time a quantitative analysis of muscle loss and muscle recovery by a microCT technique. Although the number of animals is limited in each group, the sensitivity of the methods used here (microCT after Hg “staining” and fine anatomical dissection) provided similar results which are consistent with previous studies. The study also confirmed the associated loss of bone secondary to muscle wastage induced by BTX and their parallel evolution. Contrast enhancement of muscular structures can be useful to provide a quantitative approach of sarcopenia in other animal models.

## References

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