INTRODUCTION AND GOALS

Bone remodelling is the conjoining activity of bone-resorbing cells (osteoclasts) and the bone-forming cells (osteoblasts). Distinct factors can impair the balance of bone remodelling, causing cellular dysfunction and altered bone deposition, ultimately leading to bone fragility. Hyperlipidaemia – the disequilibrium between the low-density lipoprotein cholesterol (LDL-C) and the high-density lipoprotein cholesterol (HDL-C) in the blood stream – was recently linked to altered bone metabolism, which could lead to osteopenia/osteoporosis.

The aim of this study is determine whether hyperlipidaemia influences the bone development process using an embryonic chick femur ex vivo model.

MATERIALS AND METHODS

- **Organotypic culture**: Chick fertilized eggs were sacrificed at day 11. The femora were isolated and cultured in an air/liquid interface with α-Minimal Essential Medium and ascorbic acid. After 6 days, the regular medium was replaced by medium containing bovine serum albumin and palmitic acid at 0, 50, 200 and 500 μM concentrations. Femora were fixed after 5 days.

- **Microtomography evaluation (μCT)**: Samples were scanned (Skyscan 1276) and reconstructed using NRecon software. The photos were taken using CTVox software (all from Brucker).
- **Histological staining**: Fixed femora were embedded in paraffin blocks and cut in 5 µm-thick sections. Samples were stained using Alcian blue and Sirius red. Photos were taken using Zeiss AxioLab 5 microscope and an Axiocam 5 Colour Camera.

RESULTS

μCT images: Control and 500 μM samples exhibited a smaller calcified sagittal section, as well as a thinner and less trabeculated transversal section compared to the 50 μM and the 200 μM. In addition, 500 μM samples presented the dimmest brightness among samples, suggesting a bone mineral density depletion. No differences between 50 μM and 200 μM samples were observed (n=4, scale 500 μm).

Histological staining: It is possible to observe the matrix' organization - the inner layer of the bone is made of cartilage, stained by Alcian blue, while the surrounding layer was stained by Sirius red, showing the presence of collagen, one of osteoid's main elements. No differences regarding the organization can be observed, however, 200 μM samples were shown to be the ones with the thickest osteoid portion. The arrows point out possible palmitic acid incorporation zones.

CONCLUSION

The gathered evidence suggests that palmitic acid may be deposited in the embryonic femur organotypic model, and that mild concentrations (50–200 μM) may stimulate the bone remodelling cycle. However, higher concentrations (500 μM) seem to induce deleterious effects, which indicates that the exposure to high fatty acid levels may induce morphological alterations and compromise bone deposition and calcification. Therefore, the present work suggests that hyperlipidemic conditions may lead to bone fragility and have a clinically relevant impact on bone functionality.

REFERENCES