A34 The Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model: Part I—Intraskeletal Variability and Regional Differences Detected Via Micro-Computed Tomography (Micro-CT)

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Learning Overview: The goal of this presentation is to introduce a novel longitudinal model for studying the effects of prolonged opioid exposure on cortical bone remodeling in an animal—the rabbit—that remodels its cortical bone in a manner comparable to humans. The ultimate goal is to describe how analgesic drugs, particularly morphine and fentanyl, affect microscopic structures of cortical bone used in histological age-estimation methods in forensic anthropology. Related objectives include: (1) characterizing, for the first time, 3D cortical bone microstructural changes in a rabbit opioid model; and (2) demonstrating intraskeletal and regional effects in the rabbit femur and tibia due to opioid exposure.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by describing how histological age-at-death estimation methods derive their accuracy from predictable cortical bone remodeling. While opioid abuse is thought to dysregulate bone remodeling, its longitudinal impact on bone microstructure has not been well defined. We must further understand the underlying biological processes to adapt histological age-estimation methods and scientific standards within the field of forensic anthropology for application to chronic opioid users.

Current evidence suggests that opioid use upsets the balance of bone remodeling toward more destruction and less formation of bone. Experimental studies have been limited by the fact that small laboratory animals traditionally used in bone research (mice and rats) do not exhibit spontaneous cortical bone remodeling, making them a poor animal model for this subject. Thus, rabbits were selected as an animal model system since they have a shortened remodeling period and relatively quick skeletal maturation. Male New Zealand White rabbits were divided into three groups of seven animals each: morphine, fentanyl, and control. Following the acclimation period, the experimental treatments for the opioid groups (morphine and fentanyl) were initiated and continued for eight weeks. All animals underwent bi-weekly subcutaneous injection with a bone-labeling fluorochrome, calcein, to facilitate *ex vivo* dynamic histomorphometry following euthanasia. It was hypothesized that opioids would significantly alter bone porosity and pore morphometry, producing substantial alterations to bone microarchitecture.

Cortical porosity was visualized in mid-shaft femora and tibiae using a SkyScan 1172 laboratory micro-Computed Tomography (μCT) system at The University of Akron's National Polymer Innovation Center. An imaging workflow was developed that included a 5.49μm pixel size, 100uA, medium camera selection, and aluminum filter. A proprietary image processing suite automatically processed all μCT data through macros written for ImageJ (National Institutes of Health [NIH]) and CTAnalyzer (Bruker). Workflow involved tomographic reconstruction, anatomical orientation, longitudinal alignment, extraction of total area and cortical area masks, and low-contrast filter extraction of the pore network. Pore morphometric variables, broadly associated with pore density, volume, connectivity, orientation, and cross-sectional geometry of the cortical area were assessed using CTAnalyzer.

Opioid exposure desensitizes skeletal elements to localized mechanical demands, which control intraskeletal variation in pore morphometry in healthy animals. While femoral porosity significantly exceeded tibial porosity in controls, this intraskeletal variation was silenced in morphine and fentanyl groups. This was due both to increased tibial porosity and decreased femoral porosity under drug treatment. Within the tibia, both fentanyl and morphine rabbits possessed increased total percent porosity and pore volume compared to controls. Morphine rabbit tibiae further developed individual pore systems that were significantly larger in diameter, longer, and more highly branched. The femora of morphine rabbits concurrently displayed a significant decrease in these same metrics, compared to controls.

The effects of opioids on cortical porosity were regionally concentrated in both rabbit femora and tibiae. Elevated porosity in the tibiae of opioid rabbits was localized in the anterior (fentanyl) and lateral regions (morphine and fentanyl). In the femur, decreased porosity in both morphine and fentanyl groups was significantly concentrated in the anterior and medial regions. In the tibia, drug treatment additionally silenced regional variation in metrics of pore size, pore density, and pore branching complexity. As in the whole bone analysis, drug treatment appeared to physiologically desensitize aspects of pore morphometry that were normally differentiated by regional mechanical control.

Following eight weeks of opioid administration, these results confirmed the hypothesis that opioid exposure significantly alters bone porosity and pore morphometry, producing substantial alterations to bone microarchitecture. Conventional histomorphometry is underway that will further characterize cortical bone differences among groups. Knowledge gained from these histology experiments will provide the basis for developing powerful new histological aging techniques in forensic anthropology.

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