Oral Presentation: **Proteomic Analyses of Proteins Isolated From Mammalian Bone Using a Pressure Cycling Technology**

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Comprehensive proteomic analysis of tissues is only possible when the total protein constituency of cells and extracellular matrices is effectively isolated. The efficiency of sample preparation is therefore a critical component of the analytical process and is essential to reliable proteomic analyses. Cortical bone is an extensively calcified osseous tissue and represents a specific challenge in sample preparation. Historically, bone requires prolonged acid demineralization over several days to enable complete penetration of histochemical reagents to cellular components. Mammalian, avian, and reptilian samples were used to model protein and peptide release from compact bone. Formic, acetic, and hydrochloric acid were evaluated as demineralization reagents prior to the extraction of proteins from bone using a pressure cycling technology (PCT) in which the sample was rapidly cycled between 35,000 psi and atmospheric pressure. Differences in proteins and peptides released during demineralization with different acids were analyzed by protein assay and electrophoresis. Multiple exchanges of the demineralization reagent are required to effectively remove minerals from the samples, but if the demineralization solution is not reserved, there is significant and potentially selective loss of protein from the analysis . Without prior acid demineralization, PCT yielded more protein than demineralized samples. The ability to extract proteins from bone without prior demineralization offers an important advantage in time savings. Moreover, when working with fossilized bone, where it is assumed that protein remnants will be fragmentary or highly cross-linked, extensive demineralization may further contribute to molecular damage, loss, or modification. Finally, since the carryover of Ca and PO_4 from these tissues interferes with IEF, several strategies for the removal of these substances prior to two-dimensional gel electrophoresis were investigated.