INTRODUCTION  Osteocalcin (OC), also known as γ-carboxyglutamic acid-containing protein or BGP, is one of the most abundant noncollagenous proteins found in bone matrix. It has a strong affinity for hydroxyapatite (HA), but not for amorphous calcium phosphate, and is a potent inhibitor of HA formation (Price et al., 1976). OC is a biochemical marker of bone metabolism or turnover (Price et al., 1980), but its exact role is unclear. The hypotheses of the current study were that the concentration of osteocalcin along the length of a bone is reflected in both its Ca/P ratio, an indicator of the degree of mineralization present, and in its microstructural properties.

METHODS  The first, third, and fifth pairs of ribs were harvested from a fresh frozen Cyprinus carpio specimen. C. carpio rib bone was chosen for study primarily because of its unusually high content (~60%) of OC relative to other extractable proteins, like bluegill rib bone (Nishimoto et al., 1992). Each of the three pairs of ribs were cut into 14 pieces of approximately 5 mm each, producing segments of bone each representing about 6% of the overall length. Specimens were divided up such that alternating pieces were used for different tests for seven levels of discretization along the length of the rib. Specimens used for assays were prepared by extracting their mineral content with 10% formic acid over a period of 24 hours. Extracts of the specimen supernatants were used in all subsequent assays. The osteocalcin content was determined in triplicate by a radioimmunoassay specific for carp osteocalcin. Phosphate assays were performed in duplicate spectrophotometrically (Sigma Diagnostics, procedure 360-UV). Calcium assays were performed via atomic absorption spectrophotometry. Raw assay data were normalized to the initial sample weight. Specimens for nanoindentation were prepared by mounting in epoxy resin and polishing, and were tested using the Nano Indenter® II at Oak Ridge National Laboratory. The parametric, linear dependence between pairs of variables was determined by computing their correlation coefficient, with a significance level of 95%.

RESULTS  The parametric correlation tests revealed a positive linear relationship between OC concentration and molar Ca/P ratio ($p < 0.05$; $n = 19$). No significant relationship was found between OC and the concentrations of calcium or phosphate. A significant negative correlation ($p < 0.05$; $n = 21$) was found between OC concentration and the microstructural elastic modulus in the longitudinal direction ($E_L$) (Figure 1). Finally, significant correlations were found between $E_L$ and both the phosphate concentration ($p < 0.05$; $n = 20$) and the molar Ca/P ratio ($p < 0.05$; $n = 19$).
**DISCUSSION**  In this research a significant positive correlation was found between the concentration of OC and the degree of mineralization, as measured by the molar Ca/P ratio. Ducy et al. (1996) have shown OC-deficient transgenic mice to have increased bone formation without impairing bone resorption or affecting mineral content, suggesting osteocalcin prevents excessive bone formation. A significant positive correlation between the Ca/P ratio of OC-deficient and wild-type mice has also been reported by Boskey et al. (1998). In addition, a significant negative correlation between OC and the microstructural mechanical properties of bone was found in the current study. Ducy et al. (1996) found an increase in bending failure load in OC-deficient mice, which was attributed to the increase in bone mass, but found no differences in yield energy and stiffness. A negative correlation between OC concentration and the elastic modulus of bone has also recently been reported in core samples from the inferior region of the anterior/posterior axis of the human femoral head (Brown et al., 1999).


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![Figure 1. Indentation elastic modulus in the longitudinal direction as a function of osteocalcin concentration.](image-url)