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Year in School: Junior
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Funding Source: Life Sciences Undergraduate Research Opportunity Program

Quantitative real-time RT-PCR determining extracellular matrix protein expression in osteogenesis imperfecta murine (oim) thoracic aortas

Primary components of the thoracic aorta critical for tissue integrity are collagen and elastin. Collagen, a rod-like protein contributes to aortic strength and stiffness, while elastin, a highly extensible protein contributes to aortic compliance. Type I collagen, the predominate collagen in aortic tissue, is normally a heterotrimeric molecule composed of two proalpha 1(I) chains and one proalpha 2(I) chain. The osteogenesis imperfecta murine (oim) model is an exceptional system to study type I collagen's affect on aortic integrity because it is a functional null for the proalpha 2(I) collagen gene, synthesizing only homotrimeric type I collagen molecules composed of three proalpha 1(I) chains. Our biomechanical studies of oim mice demonstrate that the absence of proalpha 2(I) collagen chains significantly reduces thoracic aortic breaking strength and stiffness. Histological analysis suggested reduced collagen staining in oim/oim and heterozygote aortas. To further investigate the mechanism of reduced collagen staining, HPLC analysis was done to determine total collagen and crosslinking content. Results demonstrated a significant reduction of collagen content per tissue content and an increase of collagen crosslinks in oim/oim and heterozygote aortas compared to wildtype. The reduced collagen content and increased collagen crosslinks of oim/oim and heterozygote aortas prompted us to examine the pre-translational amounts of aortic extracellular matrix protein mRNAs. We determined the COL1A1, COL1A2, COL3A1, ELASTIN, LYSYL OXIDASE, and TUBULIN mRNA levels in thoracic aortas of oim/oim, heterozygote, and wildtype mice at 3, 8, and 18 months of age using quantitative real-time RT-PCR.