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### Measuring *in vivo* Rate of Bone Collagen Synthesis in Growing Rats

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Measuring *in vivo* rate of bone collagen synthesis has so far been technically difficult and often subject to quite large errors. In the present study, bone collagen synthesis rate was measured using a precursor-product method, based on the exchange of  $^2\text{H}_2\text{O}$  into amino acids. Mass isotopomer abundances in amino acids from bone collagen were analyzed by gas chromatography/mass spectrometry. The  $^2\text{H}_2\text{O}$  labeling protocol consisted of an initial intraperitoneal injection of 99.9%  $^2\text{H}_2\text{O}$ , to achieve ca. 2.5% body water enrichment followed by administration of 4%  $^2\text{H}_2\text{O}$  in drinking water for 9 weeks. Body  $^2\text{H}_2\text{O}$  enrichments were stable at 2.7-3.0%. In growing rats, bone collagen synthesis rate ( $k_s$ ) was  $0.215 \text{ w}^{-1}$ .

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### Measurement of Age-related Changes in Bone Collagen Synthesis Rate in Mice Using Stable Isotope-mass Spectrometric Technique

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Age-related changes in bone metabolism is well established, as evidenced by biochemical markers of bone collagen in serum and urine, but analysis of the residual bone collagen, which is still turning over has not been investigated. In the present study, we measured *in vivo* rate of bone collagen synthesis using a precursor-product method based on the exchange of  $^2\text{H}_2\text{O}$  into non-essential amino acids. 4%  $^2\text{H}_2\text{O}$  was administered to mice in drinking water after intraperitoneal (i.p) bolus injection of 99.9%  $^2\text{H}_2\text{O}$ . Mice were assigned to the following two groups: group A (for growing young mice) were administered with 4%  $^2\text{H}_2\text{O}$  for 12 weeks after ip bolus injection at age of 5 week; group B (for adult mice) started 4%  $^2\text{H}_2\text{O}$  drinking 8 weeks later than group A and continued for 4 weeks. Mass isotopomer abundance in alanine from bone collagen was analyzed by gas chromatography/mass spectrometry. Body  $^2\text{H}_2\text{O}$  enrichments were stable at 2.5-3.0% over labeling period. Bone collagen fractional synthesis rates ( $k_s$ ) of growing young mice and adult mice were  $0.348 \pm 0.002 \text{ w}^{-1}$  and  $0.064 \pm 0.001 \text{ w}^{-1}$ , respectively. These results demonstrate bone collagen synthesis rate decreases with age presenting a direct evidence of age-related changes in bone collagen synthesis.