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### 32. Ontogenetic Changes in Regional Collagen Fiber Orientation Suggest a Role for Variant Strain Stimuli in Cortical Bone Construction. John G. Skedros, Tony Y. Kuo,\* Bone and Joint Research Lab, Orthop. Surgery, Univ. of Utah, Salt Lake City, UT.

There is evidence that bone tissue can differentiate between specific mechanical strain features during developmental and maintenance phases of skeletal ontogeny. This study examines the idea that variant mechanical factors (i.e., those whose magnitudes are dependent upon a coordinate system), if recognized as being important in cortical bone construction, would be seen during development as progressive differences in preferred collagen fiber orientation (CFO) between regions that are habitually loaded in compression, tension, and shear. Transverse sections were obtained from the midshaft sheep radii from 4 age groups [10-20 day (n=10), 4-5 month (n=7), 8-10 month (n=7), adult (2-3 yr., n=9)]. A horse radius mid-diaphysis was used as a 'control' since *in vivo* strain data show that it has a customary tension, compression, and shear (neutral axis, NA) distribution. Undecalcified sections were embedded in methacrylate, micromilled (100-150 microns), and viewed in circularly polarized light. Preferred CFO is expressed as the mean graylevel in two microscopic fields (each 2.3mm<sup>2</sup>) in the mid-cortex of each location [cranial ('tension'), caudal ('compression'), medial (NA), and lateral (NA)]. Newborns were the only group that did not demonstrate significant differences between 'compression'/'tension' cortices (all other groups were significant:  $p < 0.05$ ). The control bone showed significant CFO compression/tension differences (similar to 8-10 m. sheep). Differences along the NA were minor in all groups ( $p > 0.1$ ).

TABLE: Graylevel (Collagen Fiber Orientation) Data, Means and (S.D.)

Larger numbers = more oblique-to-transverse ('compression') CFO

	cranial	caudal	% diff.	medial	lateral	% diff.
Newborn	114.5(16.2)	124.1(14.7)	8%	109.4(19.9)	110.6(19.5)	1%
4-5 month	119.0(17.4)	143.0(26.7)	16%	119.0(36.7)	116.0(39.5)	3%
8-10 month	99.0(13.6)	155.1(28.3)	36%	103.3(30.6)	97.9(32.1)	5%
Adult	131.7(37.4)	160.2(30.8)	18%	122.6(35.9)	137.5(40.8)	11%

Preferred CFO corresponding to a customary tension/compression strain distribution, and absence of important differences along each NA (shear), supports the hypothesis that specific variant strain stimuli may be an important influence in these growth-related modifications of tissue construction. These material variations may serve to enhance mechanical properties (e.g., toughness) for fatigue-related disparities between tension, compression, and shear loading.

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### Collagen Fiber Orientation in the Proximal Femur: Challenging Wolff's Tension/Compression Interpretation. John G. Skedros, P. E. Hughes,\* K. Nelson,\* H. Winet, Orthopaedic Hospital and U. Southern Cal., L.A., CA.

Conventional wisdom teaches that the proximal femur customarily receives a tension/compression strain distribution. This results from bending applied across the femoral neck and proximal diaphysis. This hypothesis remains untested since *in vivo* strains have not been adequately measured in these regions. This study used methods shown to be sensitive in identifying strain-mode-specific (e.g., tension vs. compression) adaptations. Undecalcified sections from proximal femora were embedded in methacrylate, micromilled to 100-150 microns, and viewed under circularly polarized light to determine if regional variations in preferred collagen fiber orientation (CFO) correspond to the assumed strain distribution. Sections included: femoral neck (n=29); inferior base lesser trochanter (n=12); proximal diaphysis (n=12) (age range: 18-95 yrs.). Using published methods, preferred CFO was quantified as the mean graylevel in microscopic images taken in cortical octants. The lesser trochanter and proximal diaphysis sections exhibited expected variations: relatively more longitudinal CFO in the antero-lateral ('tension') cortex, and more oblique-to-transverse CFO in the postero-medial ('compression') cortex. However, the so-called 'tension' (superior) cortex of the femoral neck exhibited relatively more oblique-to-transverse CFO than the 'compression' (inferior) cortex, and this variation was consistent over the age range. Cortical thickness variations were not correlated with preferred CFO.

TABLE: Graylevel (Collagen Fiber Orientation) Data, Means and (S.D.)

Larger numbers = more oblique-to-transverse ('compression') collagen

	'Tension'	'Compression'	% diff.	p value
Femoral Neck	114.0(22.3)	103.3(19.4)	9.9%	$p < 0.01$
Lesser Troch.	116.3(19.6)	127.6(16.8)	9.3%	$p < 0.01$
Proximal Diaphysis	124.6(16.5)	133.8(21.4)	7.1%	$p = 0.03$

These results support consideration of alternative hypotheses. One explanation is that the intertrochanteric region is a transition zone of two loading regimes: 1) the femoral neck receives relatively prevalent, complex, compression/torsional stresses caused by the gluteus medius, other muscles, and inertial loading, and 2) the proximal diaphysis is customarily loaded in torsion with superimposed bending which is limited to some extent (//sup)but not eliminated by the iliotibial band.

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### Membrane-Bound Transferrin-Like Protein (MTf): Analyses of the Structure, Evolution and Expression during Chondrogenic Differentiation in Mouse Embryonic Cell Line ATDC5. K. Nakamasu,\* T. Kawamoto,\* M. Noshiro,\* Y. Kato,\* Department of Biochemistry, School of Dentistry, Hiroshima Univ., Japan.

Recently we showed that the level of rabbit membrane-bound transferrin-like protein (MTf) was much higher in cartilage than that in other tissues (Kawamoto, T. et al., Eur. J. Biochem. 256: 503-509, 1998). In this study, we cloned a cDNA for mouse MTf to examine the expression during chondrogenic differentiation of a mouse embryonic cell line ATDC5, and to examine a phylogenetic relationship between MTfs and other transferrins. The deduced amino acid sequence of mouse MTf was 82% identical to that of rabbit MTf. Mouse MTf, as well as other transferrins including the other MTfs, consists of two repeated domains. The similarity between the N-terminal half and the C-terminal half in MTfs is much higher than that in the other transferrin family members, even though amino acids required for iron-binding were conserved in both domains of transferrin family members except for the C-terminal half of MTfs. These structural differences suggest that a biological function of MTf differ from that of the other transferrins.

Among various adult tissues in mice, MTf was expressed only in the cartilage and testis by Northern blot analysis. The level of MTf mRNA in ATDC5 cells was markedly increased after the cells initiated chondrogenic differentiation. The increase of MTf mRNA level was in parallel with increases in the levels of type II collagen and aggrecan, whereas the level of MTf mRNA was very low and not changed in undifferentiated ATDC5 cells. These findings suggest that MTf is developmentally expressed in chondrogenic cells in the pattern commensurate with the onset of chondrogenesis, and that MTf plays a role in chondrocyte differentiation.

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### $\beta$ ig-h3: A Matrix Protein Important in Bone Formation? A. R. Derubeis,<sup>1</sup> S. C. Dieudonne,<sup>1</sup> I. S. Kim,<sup>2</sup> S. A. Kuznetsov,<sup>1</sup> P. Gehron Robey,<sup>1</sup> M. F. Young,<sup>1</sup> <sup>1</sup>CSDB, NIH, Bethesda, MD, <sup>2</sup>Biochemistry, Kyungpook National University, Taegu, Republic of Korea.

Human bone marrow stromal cells (hBMSCs) are pluripotent cells that can differentiate into bone, cartilage, and adipocytes. Treatment of hBMSCs with dexamethasone (Dex) triggers morphological changes, and increases ALP activity and matrix mineralization *in vitro*. In an attempt to investigate Dex-induced changes in gene expression, hBMSCs were cultured with and without Dex (10<sup>-8</sup>M). Total RNA was extracted and subjected to differential display analysis. Based on the resulting pattern, eight sequences were isolated and subcloned. Of the eight, a clone found to code for  $\beta$ ig-h3 (a gene associated with melorheostosis, a disease characterized by hyperostotic bone, sclerotic skin and joint deformities) was dramatically down regulated by Dex.

Northern blot analysis confirmed a striking decrease in  $\beta$ ig-h3 mRNA expression level after treatment of hBMSCs with Dex whereas there was relatively little change in stromal cell populations from other tissues (skin, thymus and spleen). In addition, analysis of tissue distribution of  $\beta$ ig-h3 mRNA in developing human bone showed high levels of  $\beta$ ig-h3 expression in the primary spongiosa and in subperiosteal preosteoblasts suggesting an involvement in the early stages of bone formation.

These results suggest that downregulation of this gene is required for bone formation to occur. Further analysis into the function of  $\beta$ ig-h3 may provide insight into the complex pathway that leads from a multipotential cell population (hBMSCs) to bone formation.