

Functional Adaptation of Bone to Exercise and Injury

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Bone adapts to altered physical stimuli, dietary changes, or injury. Dietary calcium and vitamins play important roles in maintaining skeletal health, but high-fat diets are pervasive in western cultures and may contribute to the increasing prevalence of osteoporosis and incidence of related hip fractures. Exercise helps maintain bone mass and counter osteoporosis, but exercise can also have detrimental effects-particularly for immature bone. Some negative exercise effects may also be linked to diet. For example, insufficient dietary protein during exercise can impair bone development and remodeling. Bone remodeling is a potent example of tissue repair. Chronically altered loading after a joint injury, however, can result in remodeling processes that can be detrimental to the joint. Anterior cruciate ligament injury, for example, commonly leads to osteoarthritis. Early changes in the periarticular cancellous bone may play a role in the development of knee osteoarthritis. Although these factors influence skeletal health, the mechanisms remain unclear by which bone interprets its environment and responds to mechanical stimuli or injury. To understand why different levels of exercise are beneficial or detrimental or why altered joint loading leads to changes in periarticular bone structure, underlying mechanisms must be understood by which bone interprets its mechanical environment.

Introduction

Bone adapts to altered physical stimuli, dietary changes, or injury. Although these factors influence skeletal health, the mechanisms remain unclear by which bone interprets and responds to stimuli or injury. Here, we highlight our research that provides insights into bone adaptation processes.

Diet

Femoral neck and vertebral fractures in the elderly pose significant health risks and contribute to a considerable cost in health care. Many factors can increase fracture risk, but nutrition and metabolism are two of the predominant influences on bone morphology, mechanical properties (Hou et al., 1990b; Li et al., 1990; Salem et al., 1992; Zernicke et al., 1995a), and mineral content (Atteh and Leeson, 1984). Diets high in fat and sucrose are pervasive in western cultures, and high-fat, high-sucrose diets (HFS) can have deleterious effects on bone through both metabolic and nutritional mechanisms.

After only 10 wk, immature rats fed an HFS diet (Tables 1 and 2) generally had inferior bones compared with age-matched controls fed a low-fat complex carbohydrate diet (Hou et al., 1990b; Li et al., 1990; Salem et al., 1992; Zernicke et al., 1995a). There were, however, bone-to-bone differences in the changes to

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	Control	HFS
Casein	247.8	250.0
DL-Methionine	2.5	2.5
Sucrose	0.0	472.5
Corn Starch	649.0	0.0
Lard	17.7	190.0
Corn oil	18.0	20.0
Fibre (cellulose)	20.0	20.0
Vitamin/mineral mix	45.0	45.0

Table 1: Composition of experimental diets (g•kg⁻¹).

	Control	HFS
Fat	6.0	39.5
Sucrose	0.0	39.5
Complex Carbohydrate	68.0	0.0
Protein	26.0	21.0

Table 2: Energy composition of diets (%).

		Control		HFS	
L6 vertebra	Cross-sectional area [mm ²]	11.3	(1.7)	10.2	(1.2)
	Strain energy density [N•mm•mm ⁻²]	305	(134)	182	(53)*
	Energy at failure [mJ]	118	(65)	53	(25)*
Femoral neck	Cortical shell [%area]	79.8	(3.3)	74.2	(5.5)*
	Maximal stress [MPa]	36.8	(7.9)	26.4	(4.7)*
	Energy at failure [mJ]	35.5	(5.9)	26.8	(6.0)*
Tibia	Cross-sectional area [mm ²]	4.5	(0.5)	4.2	(0.4)
	Tensile yield stress [MPa]	146	(9.6)	135	(15.4)*
	Energy at failure [mJ]	89.3	(17)	58.7	(15)*
Metatarsus	Cross-sectional area [mm ²]	1.0	(0.1)	1.2	(0.1)#
	Tensile yield stress [MPa]	221	(27)	169	(17)*
	Energy at failure [mJ]	11.9	(3.4)	10.2	(2.6)

* HFS significantly reduced ($p < 0.05$) compared to control
HFS significantly greater ($p < 0.05$) than control

Table 3: High fat-sucrose (HFS) diet effects on immature rat bone morphology, material properties, and structural mechanical properties — values reported are mean (SD).

bone properties and morphology (Table 3). Deleterious HFS diet effects on the L6 vertebra and femoral neck were due to reductions in both bone material properties and structural morphology properties (Hou et al., 1990b; Salem et al., 1992). HFS L6 vertebrae were shorter than control vertebrae, and there was a reduction in strain energy density to failure in the HFS vertebrae. The morphological and material changes resulted in significant decrements in L6 structural properties (failure load and energy to failure). The HFS femoral neck

revealed no significant differences in total bone cross-sectional area, but the HFS femoral neck cross-section had a significantly larger trabecular core and a smaller cortical shell than the controls. That redistribution of bone, coupled with changes in material properties (yield and maximal stresses), led to significant reductions in structural properties.

Conversely, tibial morphology was not affected by the diets (Li et al., 1990). The significant decreases in HFS tibial structural properties (maximal load and energy) were related to the reduction in bone material properties (tensile yield stress). The HFS metatarsus, in contrast to the femoral neck and L6 vertebra, had a cross-sectional area that was greater than controls (Li et al., 1990). Thus, though there were significant reductions in HFS metatarsal bone material properties (tensile stress, and elastic modulus), there were no differences in metatarsal structural properties (maximal load and energy).

Thus, diet differentially affected the immature rat skeleton. In high-metabolism regions containing trabecular bone (L6 vertebra and femoral neck) decrements in bone structural properties were due to a combination of reduced bone material properties and changes in bone morphology. In cortical bones of the appendicular skeleton, decrements in structural properties were due to reductions in bone material properties.

Several mechanisms may have contributed to these diet-related changes in bone. High-sucrose diets in the rat can elevate blood insulin levels and induce hyperinsulinemia (Holl and Allen, 1987), which in turn produces hypercalciuria (Holl and Allen, 1987; DeFronzo et al., 1975) by inhibiting renal tubular reabsorption of calcium (DeFronzo et al., 1975; Lemann et al., 1970). Calcium deficiencies and high insulin levels produce poor bone mineralization and adversely influence bone mechanical properties. Furthermore, high glucose levels can diminish renal reabsorption of magnesium (Lemann et al., 1970), which results in poor bone crystallization (Cohen and Kitzes, 1981) and reduced bone turnover (Jones et al., 1980). If the skeleton does not effectively adapt, low bone turnover for an extended time can result in osteopenia (Zernicke et al., 1995a).

In a long-term study, the HFS diet did not stimulate adaptation to arrest progressive osteopenia (Zernicke et al., 1995a). After two years, HFS rats had significantly greater body mass than the control rats (low-fat, complex carbohydrate diet), but HFS femoral necks and L6 vertebra were mechanically inferior. Thus, the bone did not appear to compensate for either short-term or long-term ingestion of the HFS diet.

Exercise

Regular, prolonged exercise helps maintain bone mass and counter osteoporosis (Dalen and Olsson, 1974; Pirnay et al., 1987). Also, active children have a higher peak bone mass in their early adulthood than sedentary children (Grimston et al., 1993). Exercise, however, can have either beneficial or detrimental effects on bone. Strenuous exercise, particularly in the immature skeleton, can delay collagen cross-link maturation in joint connective tissues (Pedrini-Mille et al., 1988) and retard long-bone growth (Matsuda et al., 1986).

To investigate the effects of strenuous exercise on immature bone, we studied immature female rats exercised 5 d/wk for 10 wk on a treadmill (75-80% $\text{VO}_{2\text{max}}$). Generally, the strenuous exercise had a negative effect on rat limb-bone mechanical properties (Table 4), but, as with dietary effects, there were differential

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		Control		Exercise	
RAT					
L6 vertebra	Cross-sectional area [mm ²]	11.3	(1.7)	11.0	(1.3)
	Maximal stress [MPa]	35.3	(7.5)	33.2	(6.1)
	Energy at failure [mJ]	118	(65)	97	(62)
Femoral neck	Cortical shell [%area]	79.8	(3.3)	74.0	(5.5)*
	Maximal stress [MPa]	36.8	(7.9)	27.9	(5.8)*
	Energy at failure [mJ]	36	(6)	36	(12)
Tibia	Area moment of inertia [mm ⁴]	2.1	(0.54)	1.8	(0.2)*
	Tensile yield stress [MPa]	133	(24)	134	(19)
	Energy at failure [mJ]	90	(18)	71	(23)*
Metatarsus	Dorsal cortical thickness [mm]	0.47	(0.05)	0.52	(0.04)#
	Tensile yield stress [MPa]	221	(27)	188	(29)*
	Energy at failure [mJ]	12	(3)	12	(4)
ROOSTER					
Tarsometatarsus	Anterior cortical thickness [mm]	0.78	(0.15)	1.22	(0.32)#
	Tensile yield stress [MPa]	112	(21)	89	(15)*
	Energy at failure [mJ]	1322	(254)	1058	(205)

* HFS significantly reduced ($p < 0.05$) compared to control
HFS significantly greater ($p < 0.05$) than control

Table 4: Strenuous exercise effects on immature rat bone morphology, material properties, and structural mechanical properties — values reported are mean (SD).

effects on the skeleton (Hou et al., 1990a; Li et al., 1991). For example, tibiae from the exercised rats were shorter and had smaller middiaphyseal cross-sectional areas, but there were no significant changes in tibial cortical bone material properties (Li et al., 1991). The reduction in tibial cross-sectional measures produced significant decreases in tibial structural properties. Conversely, the metatarsus experienced a significant increase in cortical thickening, but there was a significant decrease in bone material properties (yield tensile stress, and elastic modulus) (Li et al., 1991). The combination of increased morphological parameters and decreased bone material properties resulted in no net changes in metatarsal structural properties. Similar effects were found in the immature rooster tarsometatarsus. After similar strenuous exercise protocols, the tarsometatarsus increased middiaphyseal thickness, but there was a significant reduction in tarsometatarsus material properties (e.g., tensile yield stress) (Table 4) (Matsuda et al., 1986).

In the exercised rat femoral neck, there was no significant change in total bone cross-sectional area, but the cortical-to-trabecular bone ratio was significantly reduced—the trabecular bone core was significantly increased at the expense of the cortical shell (Hou et al., 1990a). Strenuous exercise also resulted in a significant decrease in femoral neck material properties. The altered bone structure and reduced material properties resulted in a significant reduction of energy at the proportional limit in the exercised rat femoral neck. The differences in bone sensitivity to mechanical stimuli may have been related to different strain environments in the bones or differential sensitivity of specific bone cells to systemic factors.

We found that exercise has differential effects on different bones—even in the same limb—and especially in different regions of the body (appendicular skeleton versus axial skeleton). While moderate, prolonged exercise can be beneficial to bone health, very strenuous exercise (particularly in immature bone) may have detrimental consequences for the skeleton.

Exercise and Diet

Some negative exercise effects on bone may be compounded by diet. Dietary protein plays a significant role in maintaining bone health, particularly in conjunction with exercise (Zernicke et al., 1995b). Chronic protein deficiency in normal, growing, sedentary rats induces hypercalciuria, negatively affects skeletal morphology (Orwoll et al., 1992), decreases bone mineral content (Likimani et al., 1992), and significantly reduces cortical-bone mechanical properties (Ferretti et al., 1991). To assess the interactive role of diet and exercise, immature male rats were strenuously exercised and fed either a high-protein diet or recommended protein diet. The high-protein diet coupled with exercise produced significant increases in femoral neck material and structural properties (Zernicke et al., 1995b).

Post-traumatic Osteoarthritis

Bone remodeling is a potent example of tissue repair. Chronically altered loading after a joint injury, however, can result in a maladaptation of bone remodeling. Anterior cruciate ligament (ACL) injury of the knee commonly leads to osteoarthritis (OA). Past research has focussed on changes to the soft tissues in the knee (cartilage and ligaments), but post-injury changes in periarticular bone may also play a contributory role in OA.

While periarticular and subchondral bone changes have been reported for patients with late-stage development of OA (Grynpas et al., 1991; Kamibayashi et al., 1995; Odgaard et al., 1989), little is known about the changes that occur shortly after joint injury. Understanding the early bone changes may provide insight into the mechanisms responsible for the development of OA and lead to new strategies for prevention of the disease. Thus, we studied the canine model of experimental knee OA (Adams and Pelletier, 1988) with a unilateral ACL transection (ACLX). Long-term studies have confirmed that the canine model develops—over a period of four years—symptoms similar to the human disease (Brandt et al., 1991). These changes are associated with altered kinematics and loading characteristics (O'Connor et al., 1989; Korvick et al., 1994), as well as physiological stimuli such as altered blood flow (Judex et al., 1997a) in the knee following the joint injury.

We analyzed skeletally mature, mixed-breed dogs assigned to one of two experimental groups—3 or 12 wk post-ACLX. We assessed changes in the distal femur and proximal tibia using quantitative computed tomography (qCT) to measure bone mineral density (BMD - bone mineral content per unit volume of bone) (Carter and Hayes, 1977). BMD decreased rapidly after ACLX, with the distal femur more profoundly affected (Boyd et al., 2000a). An exemplar qCT scan reveals density variations between ACLX and contralateral control (Figure 1). After 3 wk, BMD decreased in both proximal tibia and distal femur, but only the posterior medial femoral condyle showed a significant decrease in BMD (-14.9%). After 12 wk, however, all regions in the tibia and femur exhibited significant decreases in BMD, and the average decrease was greatest in the femur where the

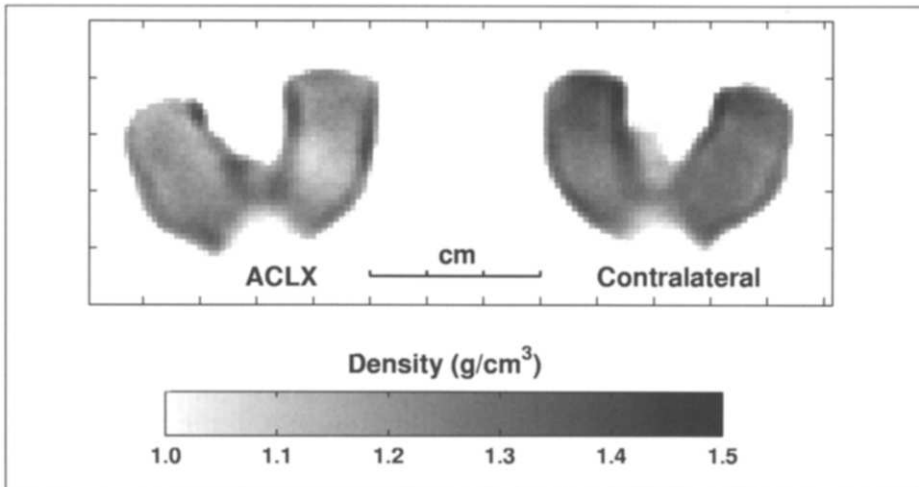


Figure 1: Exemplar quantitative computed tomography image of canine femoral condyles at 12 wk after ACL transection (ACLX). All regions in the ACLX tibial and femoral condyles had significantly reduced bone mineral density compared to the contralateral tibial and femoral condyles ($p < 0.05$).

postero-medial region decrease was greatest (-42.4%). The regions of high periarticular cancellous BMD adaptation corresponded to regions of observed focal cartilage defects (Matyas et al., 1995).

A limitation of qCT was that the BMD measure was a scalar quantity averaged over the voxel examination volume ($0.75 \times 0.75 \times 3.00 \text{ mm}^3$). A more detailed analysis of the bone structure was required to measure changes to the cancellous bone architecture. Architectural changes in diseased bone can provide detailed information about trabecular adaptations that lead to altered bone material properties at the structural level. The periarticular cancellous regions of greatest change as determined from the qCT study were the medial tibial and femoral condyles, thus we examined the micro architecture of these regions with high-resolution computed tomography (μ CT) (Rüegsegger et al., 1996).

With μ CT, we assessed excised cylindrical bone cores (6 mm diameter, 12 mm length) from the medial aspect of the canine knee joint (Boyd et al., 2000b). From the high-resolution scans (34 μm), morphological data sets were determined: bone volume ratio (BV/TV), bone surface ratio (BS/BV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and trabecular number (Tb.N) using model-independent measurement techniques (Hildebrand and Rüegsegger, 1997). The anisotropy (indicating bone directionality) was calculated using mean intercept length (MIL) (Harrigan and Mann, 1984). An unbiased measure of the trabecular connectivity was calculated using the Euler characteristic (Odgaard et al., 1993).

The tibia and femur exhibited microarchitectural changes in the periarticular bone at 3 and 12 wk post-ACLX (Figure 2). BV/TV and Tb.Th decreased (maximal changes -42% and -33%, respectively), BS/BV and Tb.Sp increased (maximal changes 74% and 19%, respectively), but Tb.N did not change. A trend toward decreased anisotropy was observed in both the tibia and femur after ACLX and became significant in the tibia 12 wk post-ACLX. Changes in anisotropy indicated that while the primary mechanism of bone loss following a joint injury was

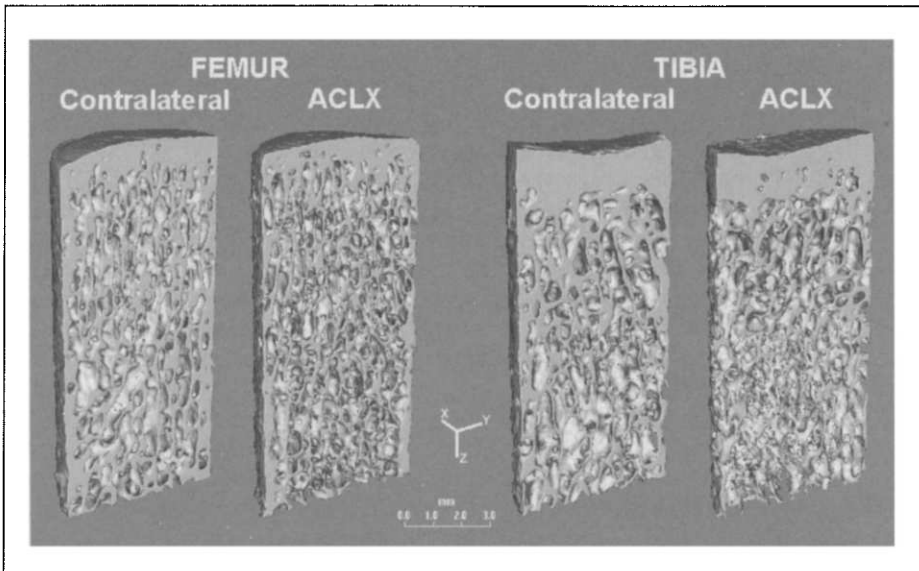


Figure 2: Exemplar high-resolution computed tomographic images of 6mm diameter bone cores, including the subchondral plate and underlying cancellous bone, from the medial femoral and tibial condyles of ACL transected and contralateral limbs at 12 wk after ACL transection. There were significant decreases in morphometric measures BV/TV and Tb.Th, increases in BS/BV and Tb.Sp, and no change in Tb.N. There was also a trend toward decreased anisotropy and an increase in connectivity (likely due to an increase in fenestrations in trabecular plates).

decreasing Tb.Th, trabecular tissue loss did not happen uniformly in all directions (Boyd et al., 2000b). One reason for this non-uniform loss could be the altered loading distributions across the knee joint, leading to altered contact patterns and stress distributions.

Connectivity of the trabecular structure increased more than 100% in the tibia and femur. Connectivity is often analyzed in studies for osteoporosis (Dempster et al., 1995; Lane et al., 1998) but is rarely investigated in OA. Both increased and decreased connectivity have been associated with loss of bone strength, and hence relations between connectivity and bone volume ratio are weak (Kabel et al., 1999). In the ACLX knee, as bone is lost, connectivity is altered either by the development of fenestrations (holes) in the trabecular plates or by breakage of trabecular rods. Because canine trabecular bone is plate-like, fenestration is the dominant process and net "connectivity" increases. Fenestrations may be repairable (via estradiol or parathyroid hormone therapy), but no solution exists for the repair of broken trabecular rods. This suggests that despite therapy, rods will remain unrepaired and a net loss of connectivity will persist leading to inferior mechanical properties.

Based on the changes observed with qCT and μ CT, bone appears to adapt quickly after ACL injury as changes have been measured as early as 3 wk and are significantly advanced by 12 wk post-ACLX. Therefore, the maintenance of mechanically normal cancellous bone following a joint injury may require an early interventional strategy.

Mechanical Environment

Bone is sensitive to its mechanical environment, but it is not clear which mechanisms control (re)modeling. To understand why different levels of exercise are beneficial or detrimental or why altered joint loading leads to changes in periarticular bone structure, underlying mechanisms must be understood by which bone interprets its mechanical environment.

It is generally accepted that bone cells respond to strain (relative deformation of the bone tissue) or strain-related phenomena (fluid flow or streaming potentials within bone tissue) (Judex et al., 1997a). At the tissue level, a number of mechanical parameters have been proposed to initiate bone formation including strain magnitude, strain rate, and strain gradients (Judex et al., 1997b). Specific parameters may have varying osteogenic effects on the immature versus mature skeleton.

In mature roosters, we measured the strain in the tarsometatarsus during strenuous running (Judex et al., 1997b). After 3 wk of running, fluorochrome-tagged sites of surface mineralization in the tarsometatarsus cross section correlated significantly with induced peak circumferential strain gradients (Figure 3). Peak strain magnitudes and rates were only weakly associated with the sites of periosteal activation. Structurally, these results seemed counterintuitive—it would be expected that the bone adaptation would reduce peak strain magnitudes. Physiologically, however, strain gradients may generate pressure differentials within bone and, thereby, contribute to fluid flow in bone (Qin et al., 1997).

In another study, immature roosters were exercised in a strenuous running protocol for three short sessions per day, rather than one long session, to determine if short bouts of the exercise-imposed mechanical milieu induced positive changes in cortical bone morphology, mechanics, and mineral ash content (Judex and Zernicke, 2000a). Strain gauge measurements of the roosters' tarsometatarsi indicated that, compared to walking, running elevated peak strain

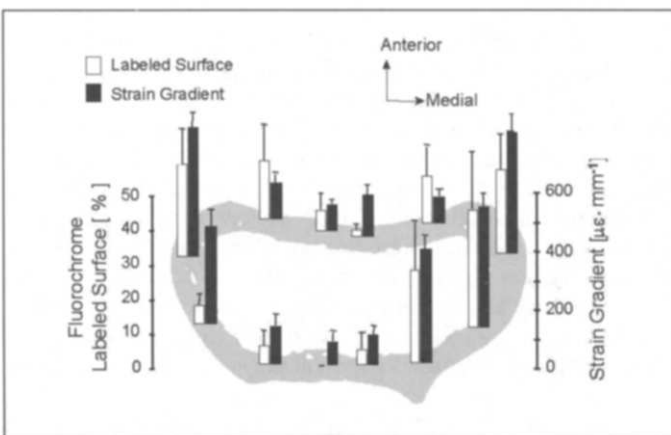


Figure 3: Distribution of peak circumferential strain gradients and percentage of labeled surface per sector superimposed on a middiaphyseal tarsometatarsus cross-section (mean+SE). Sectors with the largest gradients correlated highly ($r^2=0.63$) with the sectors that exhibited the largest regions of bone forming surfaces (adapted from Judex et al., 1997b).

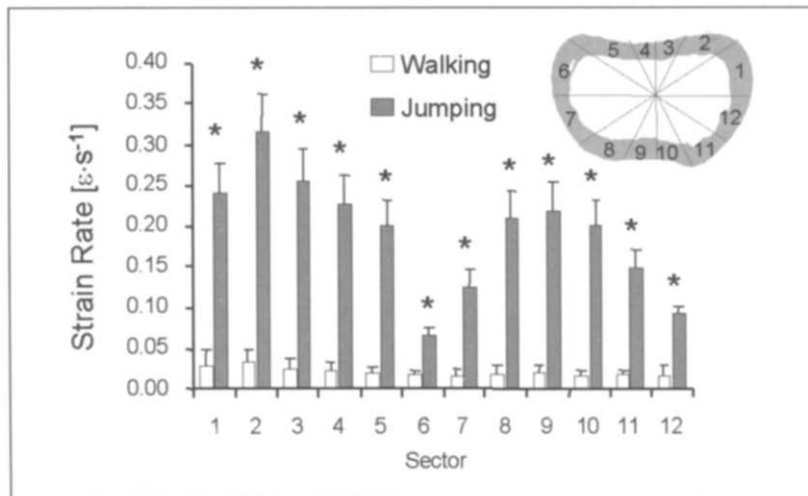


Figure 4: Distribution of peak strain (mean+SE) generated at the periosteal surface during walking and drop-jumping. Asterisks denote significant differences between drop-jumping and walking ($p < 0.05$) (adapted from Judex & Zernicke, 2000b).

magnitudes by 19%, peak strain rates by 136%, and peak strain gradients by approximately 18%. The exercise protocol successfully mitigated the negative effects seen in the long-session running regimens with immature roosters (Matsuda et al., 1986), but there was no significant increase in bone properties compared to walking roosters. We concluded that the moderate increase in strain magnitude in the presence of unchanged strain distributions and moderately elevated strain rates and strain gradients did not represent a stimulus capable of substantially enhancing bone formation/resorption and mechanical properties in the growing rooster tarsometatarsus. This was consistent with the tenet that mechanical milieus have to be substantially different from the habitual milieu to be osteogenic (Lanyon, 1996).

Thus, to alter significantly the mechanical milieu, immature roosters performed repeated high-impact drop jumps (Judex and Zernicke, 2000b). Compared to walking, the strain profile of the tarsometatarsus during jumping showed a significant increase in peak strain rate (+740% at impact) in the presence of moderately increased peak strain magnitudes (+30%) (Figure 4). In contrast to the running protocol with mature roosters (increased periosteal bone formation), the drop protocol produced significant bone formation rates at the endocortical surface of the immature tarsometatarsus that correlated with circumferential distribution of peak strain rates. Thus, brief exercise protocols that maximized strain rates while keeping load magnitudes at physiological levels might be osteogenically effective in immature bone.

In the aggregate, these studies suggest that searching for a single mechanical parameter to predict consistently the specific sites of bone formation under a variety of physiological conditions may be unrealistic. Rather, bone response to altered mechanical stimuli may be related to specific cell populations. One major difference among these models lies in the primary bone-forming surfaces in

growing animals and the primarily quiescent surfaces in adult animals. Osteocytes, as well as osteoblasts, bone lining cells, and osteoclasts can be responsive to mechanical loading, and we suggest that the sensitivity of a bone surface to a specific mechanical parameter can vary with the presence or absence of specific bone cell populations and their level of activity (Klein-Nulend et al., 1995; Owan et al., 1997; Xia and Ferrier, 1995).

Conclusions

Diet and exercise have potent effects on the skeleton, and the process of bone adaptation can be severely altered due to joint injury. Mechanisms that influence bone remodeling are beginning to be revealed, but a complete explanation remains elusive.

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References

- Adams, M. E., & Pelletier, J-P. (1988). The canine anterior cruciate ligament transection model of osteoarthritis. Greenwald, R. A. & Diamond, H. S. **Handbook of Models for Arthritis Research**. CRC Press, Boca Raton. pp.265-297.
- Atteh, J. O., & Leeson, S. (1984). Effects of dietary saturated or unsaturated fatty acids and calcium levels on performance and mineral metabolism of broiler chicks. **Poult Sci** **63** (11): 2252-2260.
- Boyd, S. K., Matyas, J. R., Wohl, G. R., Kantzas, A., & Zernicke, R.F. (2000a). Early regional adaptation of periarticular bone mineral density after anterior cruciate ligament injury. **J Appl Physiol** (in press).
- Boyd, S. K., Müller, R., Matyas, J. R., Wohl, G. R., & Zernicke, R.F. (2000b). Early morphometric and anisotropic change in periarticular cancellous bone in a model of experimental knee osteoarthritis quantified using micro-computed tomography. **Clin Biomech** (in press).
- Brandt, K. D., Myers, S. L., Burr, D., & Albrecht, M. (1991). Osteoarthritic changes in canine articular cartilage, subchondral bone, and synovium fifty-four months after transection of the anterior cruciate ligament. **Arthritis Rheum** **34** (12): 1560-1570.
- Carter, D. R., & Hayes, W. C. (1977). The compressive behavior of bone as a two-phase porous structure. **J Bone Joint Surg [Am]** **59** (7): 954-962.
- Cohen, L., & Kitges, R. (1981). Infrared spectroscopy and magnesium content of bone mineral in osteoporotic women. **Isr J Med Sci** **17** (12): 1123-1125.
- Dalen, N., & Olsson, K. E. (1974). Bone mineral content and physical activity. **Acta Orthop Scand** **45** (2): 170-174.
- DeFronzo, R. A., Cooke, C. R., Andres, R., Faloona G. R., & Davis, P. J. (1975). The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. **J Clin Invest** **55** (4): 845-855.
- Dempster, D. W., Birchman, R., Xu, R., Lindsay, R., & Shen, V. (1995). Temporal changes in cancellous bone structure of rats immediately after ovariectomy. **Bone** **16** (1): 157-161.
- Ferretti, J.L., Capozza, R., Cointy, G., Bozzini, C., Alippi, R. M., & Bozzini, C. E. (1991). Additive effects of dietary protein and energy deficiencies on diaphysis and bone tissue of rat femurs as determined by bending tests. **Acta Physiol Pharmacol Ther Latinoam** **41** (2): 253-262.
- Grimston, S. K., Willows, N. D., & Hanley, D. A. (1993). Mechanical loading regime and its relationship to bone mineral density in children. **Med Sci Sports Exerc** **25** (11): 1203-1210.
- Grynpas, M. D., Alpert, B., Katz, I., Lieberman, I., Pritzker, K. P. (1991). Subchondral bone in osteoarthritis. **Calcif Tissue Int** **49** (1): 20-26.
- Harrigan, T. P., & Mann, R. W. (1984). Characterization of microstructural anisotropy in orthotropic materials using a second rank tensor. **J Materials Science** **19**: 761-767.
- Hildebrand, T., & Rüeggsegger, P. (1997). A new method for the model-independent assessment of thickness in three-dimensional images. **J Microsc** **185** (1): 67-75.

- Holl, M. G., & Allen, L. H. (1987). Sucrose ingestion, insulin response and mineral metabolism in humans. **J Nutr** 117 (7): 1229-1233.
- Hou, J. C., Salem, G. J., Zernicke, R. F., & Barnard, R. J. (1990a). Structural and mechanical adaptations of immature trabecular bone to strenuous exercise. **J Appl Physiol** 69 (4): 1309-1314.
- Hou, J. C., Zernicke, R. F., & Barnard, R. J. (1990b). High fat-sucrose diet effects on femoral neck geometry and biomechanics. **Clin Biomech** 5: 162-168.
- Jones, J. E., Schwartz, R., & Krook, L. (1980). Calcium homeostasis and bone pathology in magnesium deficient rats. **Calcif Tissue Int** 31 (3): 231-238.
- Judex, S., Gross, T. S., Bray, R. C., & Zernicke, R. F. (1997a). Adaptation of bone to physiological stimuli. **J Biomech** 30 (5): 421-429.
- Judex, S., Gross, T. S., & Zernicke, R. F. (1997b). Strain gradients correlate with sites of exercise-induced bone-forming surfaces in the adult skeleton. **J Bone Miner Res** 12 (10): 1737-1745.
- Judex, S., & Zernicke, R. F. (2000a). Does the mechanical milieu associated with high-speed running lead to adaptive changes in diaphyseal growing bone? **Bone** 26 (2): 153-159.
- Judex, S., & Zernicke, R. F. (2000b). High-impact exercise and growing bone: relation between high strain rates and enhanced bone formation. **J Appl Physiol** 88 (6): 2183-2191.
- Kabel, J., Odgaard, A., Van Rietbergen, B., & Huiskes, R. (1999). Connectivity and the elastic properties of cancellous bone. **Bone** 24 (2): 115-120.
- Kamibayashi, L., Wyss, U. P., Cooke, T. D., & Zee, B. (1995). Trabecular microstructure in the medial condyle of the proximal tibia of patients with knee osteoarthritis. **Bone** 17 (1): 27-35.
- Klein-Nulend, J., van der Plas, A., Semeins, C. M., Ajubi, N. E., Frangos, J. A., Nijweide, P. J., & Burger, E. H. (1995). Sensitivity of osteocytes to biomechanical stress in vitro. **FASEB J** 9 (5): 441-445.
- Korvick, D. L., Pijanowski, G. J., & Schaeffer, D. J. (1994). Three-dimensional kinematics of the intact and cranial cruciate ligament-deficient stifle of dogs. **J Biomech** 27 (1): 77-87.
- Lane, N. E., Thompson, J. M., Haupt, D., Kimmel, D. B., Modin, G., & Kinney, J. H. (1998). Acute changes in trabecular bone connectivity and osteoclast activity in the ovariectomized rat in vivo. **J Bone Miner Res** 13 (2): 229-236.
- Lanyon, L. E. (1996). Using functional loading to influence bone mass and architecture: objectives, mechanisms, and relationship with estrogen of the mechanically adaptive process in bone. **Bone** 18 (1 Suppl): 37S-43S.
- Lemann, J. J., Lennon, E. J., Piering, W. R., Prien, E. L. J., & Ricanati, E. S. (1970). Evidence that glucose ingestion inhibits net renal tubular reabsorption of calcium and magnesium in man. **J Lab Clin Med** 75 (4): 578-585.
- Li, K. C., Zernicke, R. F., Barnard, R. J., & Li, A. F. (1990). Effects of a high fat-sucrose diet on cortical bone morphology and biomechanics. **Calcif Tissue Int** 47 (5): 308-313.
- Li, K. C., Zernicke, R. F., Barnard, R. J., & Li, A. F. (1991). Differential response of rat limb bones to strenuous exercise. **J Appl Physiol** 70 (2): 554-560.
- Likimani, S., Whitford, G. M., & Kunkel, M. E. (1992). The effects of protein deficiency and fluoride on bone mineral content of rat tibia. **Calcif Tissue Int** 50 (2): 157-164.
- Matsuda, J. J., Zernicke, R. F., Vailas, A. C., Pedrini, V. A., Pedrini-Mille, A., & Maynard, J. A. (1986). Structural and mechanical adaptation of immature bone to strenuous exercise. **J Appl Physiol** 60 (6): 2028-2034.
- Matyas, J. R., Adams, M. E., Huang, D., & Sandell, L. J. (1995). Discoordinate gene expression of aggrecan and type II collagen in experimental osteoarthritis. **Arthritis Rheum** 38 (3): 420-425.
- O'Connor, B. L., Visco, D. M., Heck, D. A., Myers, S. L., & Brandt, K. D. (1989). Gait alterations in dogs after transection of the anterior cruciate ligament. **Arthritis Rheum** 32 (9): 1142-1147.
- Odgaard, A., Pedersen, C. M., Bentzen, S. M., Jorgensen, J., & Hvid, I. (1989). Density changes at the proximal tibia after medial meniscectomy. **J Orthop Res** 7 (5): 744-753.
- Odgaard, A., & Gundersen, H. J. (1993). Quantification of connectivity in cancellous bone, with special emphasis on 3-D reconstructions. **Bone** 14 (2): 173-182.
- Orwoll, E. S. (1992). The effects of dietary protein insufficiency and excess on skeletal health. **Bone** 13 (4): 343-350.
- Owan, I., Burr, D. B., Turner, C. H., Qiu, J., Tu, Y., Onyia, J. E., & Duncan, R. L. (1997). Mechanotransduction in bone: osteoblasts are more responsive to fluid forces than mechanical strain. **Am J Physiol** 273 (3 Pt 1): C810-C815.
- Pedrini-Mille, A., Pedrini, V. A., Maynard, J. A., & Vailas, A. C. (1988). Response of immature

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- chicken meniscus to strenuous exercise: biochemical studies of proteoglycan and collagen. **J Orthop Res** **6** (2): 196-204.
- Pirnay, F., Bodeux, M., Crielaard, J. M., & Franchimont, P. (1987). Bone mineral content and physical activity. **Int J Sports Med** **8** (5): 331-335.
- Qin, Y. X., Otter, M. W., Rubin, C. T., & McLeod, K. J. (1997). Correlation of dilatational strain gradients to fluid flow and streaming potentials in an in vivo animal model. **Trans ORS** **43**: 112.
- Rüeggsegger, P., Koller, B., & Müller, R. (1996). A microtomographic system for the nondestructive evaluation of bone architecture. **Calcif Tissue Int** **58** (1): 24-29.
- Salem, G. J., Zernicke, R. F., & Barnard, R. J. (1992). Diet-related changes in mechanical properties of rat vertebrae. **Am J Physiol** **262** (2 Pt 2): R318-R321.
- Xia, S. L. & Ferrier, J. (1995). Calcium signal induced by mechanical perturbation of osteoclasts. **J Cell Physiol** **163** (3): 493-501.
- Zernicke, R. F., Salem, G. J., Barnard, R. J., & Schramm, E. (1995a). Long-term, high-fat-sucrose diet alters rat femoral neck and vertebral morphology, bone mineral content, and mechanical properties. **Bone** **16** (1): 25-31.
- Zernicke, R. F., Salem, G. J., Barnard, R. J., Woodward, J. S. J., Meduski, J. W., & Meduski, J. D. (1995b). Adaptations of immature trabecular bone to exercise and augmented dietary protein. **Med Sci Sports Exerc** **27** (11): 1486-1493.