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Insulin, fat, and bone: multiple interactions lead to complex biology

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Bone marrow stromal cells (MSCs) include a population of multipotent cells that, under appropriate conditions, can differentiate along osteogenic, chondrogenic, adipogenic, or fibroblastic paths.¹ This population can be readily enriched by flow cytometry, using expression of the STRO-1 antigen to sort marrow cells.² Expression of the transcription factor RUNX2 initiates development along the osteoblastic lineage,³ while transcriptional changes induced by PPAR γ drives differentiation toward adipogenesis.⁴ The conditions favoring each of these paths reciprocally inhibits the other *in vitro*,⁵ leading to the hypothesis that skewing differentiation toward adipogenesis is one of the mechanisms underlying the observation of increased fracture risk among thiazolidinedione users.

In this issue of *Translational Research*, Beck and colleagues address this hypothesis. They report a pair of experiments examining the differentiation potential of human MSCs. In the first experiment, MSCs obtained from bone marrow aspirates of normal subjects were cultured, either in the absence or presence of rosiglitazone at several concentrations. Rosiglitazone is a PPAR γ agonist, and the *in vitro* data showed that treatment enhanced adipogenic differentiation. However, the expected reciprocal inhibition of osteogenesis was not observed, with rosiglitazone having no observable impact on the ability of the MSCs to form mineralized nodules or express markers of the osteogenic lineage. In the second experiment, the *in vitro* differentiation of bone marrow aspirates from subjects with type 2 diabetes treated with pioglitazone or placebo for 26 weeks was studied. The MSCs from the pioglitazone treated subjects displayed decreased osteogenesis as well as increased adipogenesis, in contrast to the results of the first experiment.

Multiple alternative mechanisms might explain the apparent discrepancy between these experiments. These provide an opportunity to briefly review the extensive and growing literature on cross-talk between fat and bone.

Leptin produced by adipocytes inhibits trabecular bone osteogenesis in mice. This is mediated by leptin receptors in the hypothalamus, which then signals the bone via the β 2 adrenergic system.^{6,7} *Lep^{ob/ob}* mice, which harbor a null mutation of leptin, demonstrate increased vertebral trabecular bone mass relative to wild type mice, and this is reversed by hypothalamic leptin infusion. In contrast, *Lepr^{db/db}* mice, which lack functional leptin

receptors demonstrate a similar phenotype to *Lep^{ob/ob}* mice, but cannot be rescued by leptin infusion. Moreover, the hypothalamic populations affecting feeding behavior and sympathetic inhibition of trabecular bone formation are distinct.

Because endocrine signaling pathways feature feedback control, bone-derived signaling to the adipose tissue has been sought. Undercarboxylated osteocalcin has been proposed to serve this role, acting to increase insulin secretion by pancreatic β cells and adiponectin secretion by adipocytes.⁸ Insulin would then act on adipocytes, favoring their ability to take up glucose and store energy. Evidence supporting this indirect effect of osteocalcin production was obtained by the finding that targeted ablation of osteoblasts reduced the mass of the gonadal fat depots in mice.⁹ Osteoblasts express insulin receptors, and insulin signaling in these cells promotes osteogenesis and limits accumulation of fat mass.¹⁰ The generalizability of these mouse findings to human biology is supported by the finding that circulating undercarboxylated osteocalcin is inversely related to fat mass and serum glucose in diabetics.¹¹

Taken together, the findings summarized above suggest the existence of a classical hormonal feedback pathway, with greater osteogenic activity favoring fat accumulation via endocrine osteocalcin signaling, with the resulting increased fat mass feeding back to suppress further osteogenesis via the leptin-hypothalamic-sympathetic pathway. Additional pathways complicate this model, however. Fat also provides mixed osteoblastic and osteoclastic stimulation via adiponectin¹² and insulin.¹³ Yet, adiponectin has also been reported to inhibit osteoclastogenesis.¹⁴ Furthermore, a leptin-induced hypothalamic neuropeptide, cocaine amphetamine related transcript (Cart),¹⁵ inhibits osteoclastogenesis.¹⁶ These additional findings show that the known signaling pathways operate at multiple levels, working simultaneously to exert opposite effects on bone and fat mass. Given the complexity of the biology, it is unsurprising that Beck and colleagues' data do not conform to a simple model of reciprocal control of bone and fat mass.

Growing interest in the application of body composition analysis to obesity has provided new clinical data that bear on the issue. Although it has long been recognized that high body weight is generally protective against fracture, lean mass appears to be a better predictor of bone strength than total body mass.¹⁷ There is growing appreciation that bone mass and muscle mass are highly correlated (see reference 18 for review¹⁸) and appear to share common genetic determinants.¹⁹

The observed correlation between muscle and bone mass and function fits nicely with present understanding of the mechanisms by which bone adapts to its habitual level of mechanical loading. It has long been known that elite racket sport athletes have markedly increased bone and muscle mass in their dominant arms.²⁰ Conversely, decreased loads mechanical loading, as occurs with spaceflight,²¹ prolonged bed rest,²² or spinal cord injury,²³ leads to loss of skeletal and bone mass.

The concept that bone modeling mirrors skeletal loading has been formalized as the mechanostat hypothesis.^{24,25} According to this model, bone modeling is a physiological response to the strain experienced by bone during the course of activity. Key predictions of

the hypothesis have been confirmed and extended over the past generation. *In vivo* mechanical loading and unloading experiments have demonstrated that modeling occurs in response to loading and that the response is greatest at the bone surfaces subjected to the greatest strains.²⁶ Inbred mouse strains are known to differ in their responsiveness to experimentally imposed loading,^{27,28} bone mineral density,²⁹ and long bone diaphyseal geometry.^{29,30} Because locomotion and muscle contraction produce forces that are multiples of body mass, skeletal adaptation is more closely related to muscle mass and strength than to overall body mass.

Some of the molecular and cellular details of the response to mechanical loading in bone are known, although understanding is still incomplete. There is ample evidence that osteocytes are the primary mechanically responsive cells and that fluid flow and shear stress are each sufficient to elicit the downstream response.^{31,32} However, osteoblasts and osteoblast-like tissue culture cells also display robust mechanical responsiveness to shear stress.^{33–35} The mechanical signal is communicated among osteoclasts and to osteoblasts via gap junctions.^{36,37} Disruption of the ciliary protein polycystin-1, a mechanosensing protein in the kidney impairs the bone response to mechanical loading,^{38,39} which is mediated via the primary cilium.^{38,39}

The interplay of bone, muscle, and fat leads to complex biology, illustrated schematically in figure 1, whose understanding will require that each be appreciated as a dynamic tissue, exerting an impact on whole body metabolism. Diseases such as type 2 diabetes, and drugs used to treat them, such as thiazolidinediones add further layers of complexity. Beck et al's work in this issue represents a credible step toward understanding off-target drug effects.

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Fig 1.
Signaling among (clockwise from top left) beta cells, fat, brain, muscle, and bone.