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CLINICAL UPDATE

Bone and Fat

Old Questions, New Insights

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Until recently, adipose tissue was considered to serve only as a triglyceride reservoir and was relegated to a passive endocrine role. With the discovery of leptin and other adipokines, adipose tissue is now recognized as an active participant in systemic metabolism. This review focuses on the complex relationship existing between adipose tissue and bone metabolism and differentiation. It explores the paradigms that have shaped the past decade's research and what these findings forecast for the future. Particular attention is given to the multipotent adult stem cell populations that reside within bone and fat. These adult stem cells have critical importance to the emerging field of tissue engineering and regenerative medicine.

Key Words: Adipocyte; adult stem cell; bone; differentiation; osteoblast; stromal cell.

Introduction

Physicians do not routinely consider bone and fat as tissues with related physiologic functions. This review article will present the case that shared metabolic pathways regulate these tissues by controlling the differentiation of multipotent adult stem cells found in the bone marrow and in adipose tissue depots. The existence of adult stem cells and the mechanisms controlling their differentiation in health and disease have important implications for clinical practice and the differential diagnosis of endocrine disorders.

Structure and Function: Fat and Bone

The differences between adipose tissue and bone are striking (Table 1). They are the prototypical "soft" and "hard" tissues, respectively. While one serves as a mechanical cushion, the other provides structural support. Bone provides the location for hematopoietic activities, while adipose tissue serves as an energy reservoir. Yet there are similarities. Both are storage sites; adipose tissue stores energy as lipid and bone stores calcium as hydroxyapatite crystals. Peripheral adipose tissue is metabolically active, storing or releasing triglycerides and fatty acids in response to the body's energy demands. Bone metabolism is similar, undergoing continually balanced resorption and remodeling, resulting in the release of calcium, phosphate, magnesium, and other minerals into the circulation.

The cells within both adipose tissue and bone are endocrine targets (Table 2). Preadipocytes and adipocytes respond to growth hormone, insulin, and thyroid hormones. Osteoblasts and osteocytes respond to insulin-like growth factor, parathyroid hormone, and vitamin D_3. At the same time, both tissues release growth factors and cytokines with systemic, endocrine-like functions. For example, bone is a source of bone morphogenetic proteins and transforming growth factor β. Likewise, adipocytes are the major cell source of secreted leptin and adiponectin; the serum levels of each of these cytokines displays a circadian rhythm, similar to that seen for glucocorticoids *(1,2)*. These studies suggest that there is a linkage between adipocyte function and the hypothalamic–pituitary–adrenal axis, resembling that of classical endocrine organs.

Bone can be categorized based on its embryologic origin and its morphology. Embryologists recognize both intramembranous and endochondral bone. Intramembranous bone arises within the embryo directly from connective tissue; in contrast, endochondral bone develops from sites already occupied by developed cartilage. Anatomists classify bone as either cancellous (spongy, trabecular) or compact, based on the density of extracellular and mineral matrix *(3)*.

Adipose tissues can be classified in a similar manner based on function (Table 3) *(4)*. The most well-characterized form is "white adipose tissue" (WAT), which serves as an energy storage reservoir throughout the body. "Brown adipose tissue" (BAT) is found in the newborn human as a thermogenic organ. Located around the major organs and the interscapular region, BAT expresses a unique "uncoupling protein" that allows energy produced in the mitochondria to be converted directly into heat rather than ATP. This protects the individual during infancy; with advancing age,

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Table 1
Structure–Function

Adipose	Bone
Soft tissue	Hard tissue
Energy reserve	Skeletal support
Mechanical cushion	Calcium storage
Insulation	Hematopoiesis

Table 2
Endocrine Roles

	Adipose	Bone
Responds to	Insulin (Diabetes)	PTH
	Growth hormone	1,25 Vit D3
	Thyroid hormone	IGF
Secretes and synthesizes	Leptin	
	Adiponectin	BMP
	Angiotensinogen	TGF beta

Table 3
Types of Adipose Tissue

Tissue Type	Function
White	Energy storage
Brown	Nonshivering heat generation
Mammary	Lactation
Mechanical	Weight-bearing stress protection
Bone Marrow	Space occupying (passive), lipid metabolism, hematopoiesis, osteogenesis (active)

BAT stores decrease. Adipose tissue in mammary tissue develops later in life, expanding during puberty. This energy reservoir is closely associated with pregnancy, parturition, and lactation. During pregnancy and lactation, mammary adipose tissue stores decrease as the ductal epithelium expands. When the mother weans her child, the ductal epithelium undergoes apoptosis (programmed cell death) and the adipocyte numbers and volume increase dramatically. Adipose tissue can also serve a biomechanical function in sites such as the palms of the hands, the soles of the feet, and the infrapatellar and retro-orbital fat pads. At each of these locations, adipose tissue cushions the underlying skeletal or orbital tissues against traumatic injury.

There is an additional type of adipose tissue that is usually overlooked—bone marrow fat. The function of the marrow adipocyte remains controversial. Some have postulated that bone marrow adipocytes serve a passive role, simply occupying space that is no longer required for hematopoiesis. Consistent with this are the observations that the degree of marrow fat can be modulated in response to erythropoietic demands—hypertransfusion increased and phlebotomy/anemia decreased the marrow fat stores in murine models *(5–8)*. Other data support the hypothesis that marrow fat plays a more active role in energy metabolism. In non-human species, marrow adipose cells avidly take up chylomicrons, clearing circulating triglycerides *(9,10)*. With starvation or anorexia nervosa, the composition of the marrow changes significantly and fat stores are replaced by a gelatinous material *(5a,11,12)*. Further roles include the possibilities that marrow adipocytes are a source of cytokines regulating hematopoietic and osteogenic events and/or provide an energy reservoir available to respond to emergencies such as bone regeneration following a traumatic fracture. Indeed, marrow adipocytes may serve all of these needs, both active and passive, during the lifetime of the individual; each of these nonexclusive functions needs to be considered.

Bone Marrow Derived Stromal Cells or Mesenchymal Stem Cells

Nearly a century of research on bone marrow adipocytes has led to a number of landmark discoveries. Detailed autopsy studies performed in the 1930s on bone marrow from patients of all ages first documented the relationship between aging and marrow adipose stores *(13,14)*. At birth, little if any marrow fat exists; as individuals age, adipogenesis or fat differentiation occurs gradually until, by the third decade, the majority of the femoral marrow cavity is occupied by fat. Recent, noninvasive MRI studies have confirmed these initial observations *(15)*. The Nobel Laureate Charles Huggins and his colleagues first demonstrated that adipose tissue increased in the marrow of the appendages (femur, radius), rather than the core (vertebra, ribs), and this was associated with a temperature gradient *(16–18)*. This suggested that lower temperatures promoted adipocyte differentiation within the marrow *(19)*. Consistent with this were novel observations made in the banded armadillo, an animal with a bony exoskeleton *(20)*. In the summer, the exoskeleton displayed a "red" or erythropoietic marrow; during the winter months, when the ambient temperature was low, the marrow cavity was "yellow" or fatty *(20)*. Epidemiological observations in the 1970s suggested that a relationship existed between marrow adipocytes and bone formation *(21)*. Meunier and colleagues made the classic correlation between the number and size of adipocytes and the degree of bone loss in the marrow biopsies from patients with osteoporosis *(21,22)*. They found that the number of adipocytes and their individual volume was increased in the marrow of osteoporotic patients *(21,22)*. One explanation for this observation was that the pathophysiology of osteoporosis promoted osteoblasts (or their progenitors) to differentiate into the adipocyte lineage. Alexander Friedenstein laid the foundation for the cellular basis of this phenomenon in work dating back to the 1960s *(23)*. He discovered the presence of bone marrow fibroblasts capable of differentiating along multiple pathways, including adipocytes and

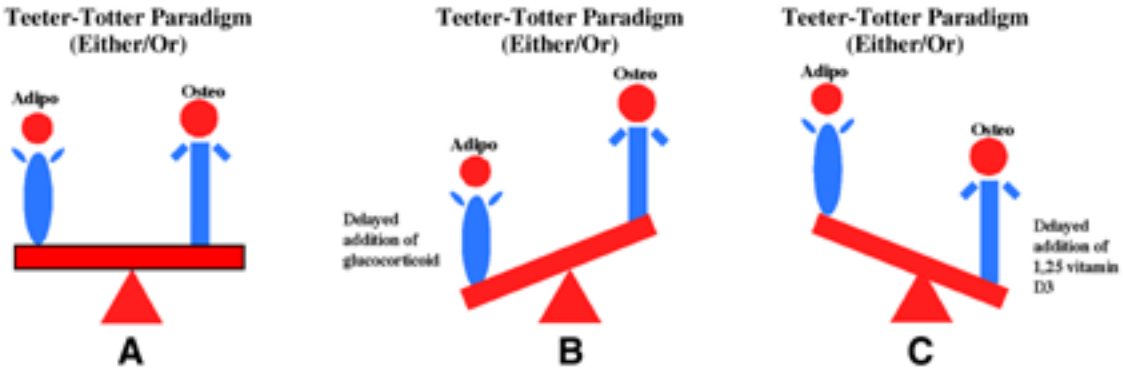

Fig. 1. The "teeter–totter" ("see–saw") model describing the relationship of bone marrow stromal cell adipogenesis and osteogenesis based on the observations of Beresford et al. *(30)*.

osteoblasts. Later studies in the late 1980s and 1990s extended this by demonstrating that cells with these multipotent properties could be cloned *(24–26)*. The number of these "adult stem cells" decreases in frequency during the first years of life, suggesting a relationship between age and bone forming capacity *(27)*, although the frequency remains relatively constant in patients 20–70 yr of age *(28,29)*.

The See–Saw Paradigm—Applications

A pivotal study by Beresford and colleagues *(30)* synthesized a concept that has become the paradigm driving much of the research during the past decade. In a seminal observation, these investigators showed that stromal cells derived from bone marrow displayed an equal propensity for both adipocyte and osteoblast differentiation; however, if culture conditions were modified, by either the delayed addition of a glucocorticoid or the continuous presence of vitamin D_3, the stromal cells preferentially differentiated along the adipocyte or osteoblast pathway, respectively. These authors concluded that an inverse relationship existed between the adipocytes and osteoblasts of the bone marrow. Their findings have been confirmed by our group and others *(31)*. The inverse relationship model, depicted in Fig. 1 as a balanced "see–saw," has become the basis for many investigations exploring the mechanisms regulating bone marrow stromal cell differentiation. Several examples outlined below reflect how this paradigm has directed drug discovery efforts for osteoporosis.

The thiazolidinedione compounds, used to treat diabetes, bind to the transcription factor peroxisome proliferator activated receptor γ (PPARγ) *(32)*. Initial in vitro studies demonstrated that these compounds induced bone marrow stromal cell adipogenesis *(33)*. At the same time, these agents inhibited osteoblast differentiation *(34)*. Separate signal transduction pathways or intracellular mechanisms mediated the distinct actions on adipogenesis and osteogenesis *(35)*. In vivo, mice treated chronically with thiazolidinediones increased their bone marrow fat and, with the compound rosiglitazone (Avandia®), this was accompanied by a decrease in bone mass *(36,37)*. To date, these studies support the "see–saw" paradigm.

A similar pattern may be emerging for the Wnt signaling pathway. This transmembrane-receptor-mediated system was first described in the *Drosophila* (fruit fly) model and is proving to be an important regulator in human cells. In vitro studies first demonstrated that Wnt ligands inhibited adipocyte differentiation *(38,38a)*. The receptor for the Wnt ligand involves a protein closely related to the LDL receptor, known as LRP5. Clinical genetic studies uncovered two very different mutations in the *LRP5* gene with profoundly different effects on bone formation. In families with the dominant negative form of *LRP5*, probands suffer from osteoporosis-pseudoglioma and have defective bone accrual *(39)*. In contrast, families with a constitutively active mutation of the *LRP5* gene display osteosclerotic bone and rarely fracture bones, even with severe trauma *(40,41)*.

The paradigm applies less well in other cases. One example is the bone morphogenetic proteins (BMPs). In vitro analyses of BMP actions first indicated that these cytokines inhibit bone marrow stromal cell adipogenesis *(42)*. Indeed, in the pre-adipocyte cell line, 3T3-L1, activation of the BMP receptors not only inhibited adipogenesis but enhanced osteogenesis *(43)*. However, other studies using bone marrow stromal cells in vitro found that activated BMP receptors promoted adipogenesis *(44,45)*. Although the basis of this discrepancy may relate to the use of different cell lines, further work is needed to fully understand the relationship of BMPs to adipogenesis. In the case of leptin, in vitro studies supported the "see–saw" paradigm; leptin inhibited bone marrow stromal cell adipogenesis and accelerated osteoblastogenesis in a paracrine manner *(46)*. Indeed, a correlation exists between serum leptin levels and bone density in children, although the significance of this remains uncertain

(47). But the actions of leptin may depend on its site and mode of action. When leptin was administered by direct injection into the brains of mice, it promoted bone loss through the activation of the sympathetic nervous system *(48,49)*. This suggests that the endocrine actions of leptin may have profoundly different consequences than its paracrine actions.

Using the "see–saw" paradigm, investigators have uncovered a number of important caveats that should be applied to future research:

1. In vitro studies do not always translate into in vivo findings.
2. Paracrine actions need to be distinguished from endocrine actions.
3. There may be species to species variations in the observed response to the same factors.

Despite its failure to be 100% accurate, the "see–saw" paradigm has been valuable. The model provides a simple "either/or" approach to experimental design and it will continue to be an important foundation for future analysis of osteogenic signal transduction pathways.

New Understanding of Adult Stem Cells

A variety of evidence indicates that the differentiation potential of bone marrow stromal cells is not limited solely to the adipocyte and osteoblast lineages. Friedenstein, Owen, Caplan, Bianco, Robey, and others have proposed that bone marrow stromal cells are multipotent adult stem cells, also known as a "mesenchymal stem cell or MSC"; these are capable of differentiating into adipocytes, chondrocytes, hematopoietic-supporting cells, myocytes, neuronal cells, osteoblasts, and possibly other lineages as well *(23,50–55)*. Until recently, differentiation was perceived as a linear process, where cells committed to a limited lineage phenotype early in development and underwent an irreversible "terminal differentiation" event at the organ level. Dogma stated that adult tissues did not contain stem cells; the only well-recognized exception was the hematopoietic stem cell.

Adipose Derived Adult Stem Cells

Adipose tissue is proving to be an abundant and accessible source of adult stem cells *(4)*. Pathologic conditions provide precedence for this finding. Patients with the rare inherited disorder, paroxysmal osseous heteroplasia, form ectopic bone complete with a hematopoietic marrow within the subcutaneous adipose layer of the skin *(56)*. Studies by Shore and colleagues *(57)* have identified mutations of *GNAS1*, a component of the G-coupled signaling pathway, as responsible for this condition. Our group and others have successfully isolated adipose-derived adult stem (ADAS) cells from liposuction aspirates, with yields of over 400,000 cells/mL of tissue *(58–66)*. The cells are isolated by a simple collagenase digestion, followed by centrifugation and expansion as an adherent culture population. The isolated ADAS cells display a remarkably homogeneous surface immunophenotype similar to, but not identical with, that of bone-marrow-derived MSCs *(65–68)*. When combined with hydroxyapatite and implanted subcutaneously in immunodeficient mice, human ADAS cells form osteoid, based on histologic analyses *(69,70)*. These cells are capable of differentiating along the adipocyte, cardiac myocyte, chondrocyte, hematopoietic supportive, myocyte, neuronal, and osteoblast lineages in vitro *(58–74)*. The human ADAS cells retain their multilineage differentiation potential (adipocytes, chondrocytes, neuronal cells, osteoblasts) at the clonal level, supporting their identification as "stem"-like cells (*65*; Lott, Awad, Gimble, Guilak, unpublished observations). Because adipose tissue is not in short supply and liposuction has gained acceptance by the general public, ADAS cells offer a reliable source of adult stem cells for future tissue engineering applications. In contrast, bone-marrow-derived MSCs may prove to be less readily available for general use owing to the reluctance of patients to subject themselves to bone marrow aspiration procedures. Indeed, enterprising surgeons in Spain have already begun to transplant autologous ADAS cells, expanded ex vivo, to repair a rectovaginal fistula in a patient suffering from Crohn's disease *(75)*. Future clinical applications in the United States will require the approval of the Food and Drug Administration.

Future Directions

Primary human adult stem cells, from both bone marrow and adipose tissue, will grow in importance as a drug discovery tool for osteoporosis, diabetes, obesity, and other endocrine disorders. By using relatively un-manipulated human cells, rather than murine cell lines, investigators are less likely to be led astray due to subtle, but critical, interspecies variation in protein target molecular structures. A drug that works wonders in a mouse may fail in an expensive clinical trial. Adult stem cells can be used in high-throughput-screening approaches to identify small molecules capable of modulating signal transduction and intracellular biochemical pathways regulating cell differentiation and function. Future studies will need to explore the temporal and kinetic relationship involved in the small molecule action; when a drug is given during the course of a stem cell's lifespan or differentiation could be of critical importance to its clinical utility.

New therapies are likely to evolve from the emerging discipline of tissue engineering, and these efforts will be guided by principles defining practical, objective criteria for functional products *(76)*. Human adult stem cells provide one of the three fundamental building blocks for regenerative medicine, along with biomaterials and environmental modifiers (cytokines, biomechanical stimuli). The availability of large numbers of uncontaminated, well-characterized,

and safe adult stem cells remains a challenge to the stem cell biotechnology industry. The companies and their academic partners will need to develop new methods for large-scale cell expansion and purification. It is likely that this will involve the use of bioreactors similar to those now used to manufacture recombinant protein drugs. These cells can then be combined with appropriate biomaterials to repair acute or chronic defects in musculoskeletal and other tissues. Ultimately, the goal will be the safe, effective, and improved treatment of human conditions.

Paradigm Shifts

Intensive research over the past decade has established adipose tissue's role in disorders such as the "metabolic syndrome X," which links obesity and adipose tissue directly to cardiovascular disease, insulin resistance, and hypertriglyceridemia *(77)*. We now know that adipose tissue secretes a number of proteins with systemic metabolic activity. The classic example of these adipose-specific "adipokines" is leptin, originally described as an appetite suppressant and now recognized to have multiple activities consistent with the properties of a classical hormones *(78)*. Another is adiponectin, which has antiatherogenic, antidiabetic, and anti-inflammatory activities *(79)*. In addition, adipose tissue is a source of tumor necrosis factor-alpha (TNF-α), which has been suggested to play a role in the pathogenesis of insulin resistance *(80)*. The identification of adipose tissue as the major source of secreted adipokines with systemic actions is a paradigm shift. Fat tissue can no longer be considered simply as a passive, storage depot but now must be given greater prominence as a dynamic endocrine organ. Further work is needed to understand the interplay between the newly described adipokines and the classic endocrine hormones as they relate to bone metabolism.

Over this same decade of research, the "see–saw" paradigm proposed by Beresford and his colleagues has played a critical intellectual role in bone biology. This model must continue to evolve in the coming years. Based on the new discoveries relating to the multipotent phenotype of adult stem cells, we propose an alternative to the "either/or" "see–saw" model—the "both/and" "merry-go-round" approach (Fig. 2). Rather than think of the adult stem cell as defined by two differentiation options (adipocyte vs osteoblast), its choices may be far less limited. Depending on their environment and growth conditions, adult stem cells can also be manipulated to differentiate into chondrocytes, hematopoietic-supporting cells, myocytes, and neurons, among other lineages. Indeed, local concentrations of adipokines may play a role in regulating this process *(81)*. The challenge for basic scientists will be to think outside the box by exploring these new avenues and integrating our new understanding of adipose tissue's endocrine role. Physicians will also need to make a paradigm shift in order to translate these findings into clinical progress. As the field of regenerative medicine advances, we must begin to think of adult stem cells in the same therapeutic terms that we now ascribe to small molecules and protein hormones in the treatment of human disease.

Fig. 2. The "merry-go-round" model describing the differentiation potential of adult stem cells. Abbreviations: Adipo, adipocyte; Chondro, chondrocyte; Hemato, hematopoietic; Myo, myocyte; Neuro, neuronal cell; Osteo, osteoblast.

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