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Understanding the Pathology and Mechanisms of Type I Diabetic Bone Loss

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Abstract Type I (T1) diabetes, also called insulin dependent diabetes mellitus (IDDM), is characterized by little or no insulin production and hyperglycemia. One of the less well known complications of T1-diabetes is bone loss which occurs in humans and animal models. This complication is receiving increased attention because T1-diabetics are living longer due to better therapeutics, and are faced with their existing health concerns being compounded by complications associated with aging, such as osteoporosis. Both male and female, endochondrial and intra-membranous, and axial and appendicular bones are susceptible to T1-diabetic bone loss. Exact mechanisms accounting for T1-diabetic bone loss are not known. Existing data indicate that the bone defect in T1-diabetes is anabolic rather than catabolic, suggesting that anabolic therapeutics may be more effective in preventing bone loss. Potential contributors to T1-diabetic suppression of bone formation are discussed in this review and include: increased marrow adiposity, hyperlipidemia, reduced insulin signaling, hyperglycemia, inflammation, altered adipokine and endocrine factors, increased cell death, and altered metabolism. Differences between T1-diabetic- and age-associated bone loss underlie the importance of condition specific, individualized treatments for osteoporosis. Optimizing therapies that prevent bone loss or restore bone density will allow T1-diabetic patients to live longer with strong healthy bones. J. Cell. Biochem. 102: 1343–1357, 2007.

Key words: diabetes; bone; osteoblast; metabolism; insulin; marrow; fat; density

The skeleton is a dynamic system. Targeted bone remodeling through the activities of osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells) maintains blood calcium levels within a critical range while keeping bone strong at sites where support is needed. This dynamic process requires intricate regulatory pathways, which under disease conditions can be altered and lead to reduced bone density and increased fracture risk. This review will focus on the effect of type I (T1) diabetes, a metabolic disease, on the skeleton and potential mechanisms accounting for its associated osteoporosis. While the T1-diabetic bone phenotype has similarities with age associated osteoporosis, its cause stems from reduced bone formation rather than increased bone resorption. This difference marks the importance of future development and selection of osteoporosis treatments that are specific for different types of bone loss.

DIABETES

Diabetes affects over 20 million people in the United States, roughly 7 percent of the population, and is the result of decreased insulin signaling and the inability of insulin sensitive cells to take up glucose. Therefore, hyperglycemia (non-fasting plasma glucose levels greater or equal to 200mg/dl [Kuzuya et al., 2002]) is one criteria used in the diagnosis of diabetes. Two main forms of this metabolic disease exist and their prevalence is increasing world wide. T1 diabetes, also called insulin dependent diabetes mellitus

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(IDDM), is characterized by little or no insulin production and weight loss and affects more than 800,000 people in the United States. Autoimmune, genetic and environmental factors can contribute to the development of T1-diabetes. Type II (T2) diabetes, also called non-insulin dependent diabetes mellitus (NIDDM), is caused by cells becoming resistant to insulin signaling and accounts for more than 90% of diabetes in the United States. Diet, obesity, and reduced physical activity are a few of the factors that are thought to contribute to the development of T2-diabetes. Improved glucose monitoring, insulin delivery methods, and pharmacologic treatments are increasing patient lifespan, but increasing the risk of complications from extended exposure to diabetic conditions. Well known diabetic complications include retinopathy, neuropathy, and nephropathy; however, recently attention has focused on diabetic bone pathology [Levin et al., 1976; Auwerx et al., 1988; Buysschaert et al., 1992; Bouillon et al., 1995; Krakauer et al., 1995; Munoz-Torres et al., 1996; Tuominen et al., 1999; Kemink et al., 2000]. T2-diabetic bone pathology is marked by increased fracture risk but effects on bone mineral density (BMD) are controversial [Karsenty, 2006; Rosen and Bouxsein, 2006; Zhao et al., 2007]. In addition, bone itself may influence insulin sensitivity and T2-diabetes onset [Karsenty, 2007]. On the other hand, T1-diabetes is clearly associated with bone loss and suppressed bone formation. Thus, the T1-diabetic bone pathology serves as a more straight forward system to understand the effect of suppressed insulin signaling, hyperglycemia, and metabolic abnormalities on the regulation of bone formation.

T1-DIABETES—HUMAN BONE PATHOLOGY

T1-diabetic bone loss was identified at the beginning of the 1900's and now more than 50% of T1-diabetic patients are thought to have bone loss compared to healthy age matched subjects, and almost 20% of patients age 20–56 meet the criteria for being osteoporotic [Munoz-Torres et al., 1996; Kemink et al., 2000]. Correspondingly, T1-diabetes is a risk factor for fractures [Bouillon, 1991; Meyer et al., 1993; Forsen et al., 1999; Schwartz et al., 2001] and delayed fracture healing [Herskind et al., 1992; Folk et al., 1999; White et al., 2003]. Bone loss (at the radius and femur) and hip fracture risk are evident in both male and female T1-diabetics [Auwerx et al., 1988; Buysschaert et al., 1992; Miao et al., 2005; Hadjidakis et al., 2006; Strotmeyer et al., 2006]. However, the influence of gender on diabetic vertebral BMD is inconsistent, with reports indicating suppression only in males [Hadjidakis et al., 2006; Strotmeyer et al., 2006] or only in females [Auwerx et al., 1988]. Oral contraceptive use in women may offer some protection against T1-diabetic bone loss [Lunt et al., 1998; Hofbauer et al., 2007] and may contribute to study variability. Overall, combined study analyses calculate that T1-diabetes increases fracture risk by 1.3–2.3-fold at the lumbar spine, 1.4–2.6-fold at the femoral neck, and 1.8-fold for the distal radius [Hofbauer et al., 2007]. Bone loss can begin at the onset of diabetes in children [Gunczler et al., 1998; Lopez-Ibarra et al., 2001; Bechtold et al., 2006], but there are reports of children with T1-diabetes who do not exhibit bone loss [Pascual et al., 1998; Valerio et al., 2002]. A concern is that existing bone loss in T1-diabetic patients could compound the fracture risk associated with conditions such as menopause and aging.

Mechanisms contributing to T1-diabetic bone loss are unknown, but there are many theories (Fig. 1). Analysis of T1-diabetic bone remodeling serum markers suggests that resorption is unaltered [Bonfanti et al., 1997; Kemink et al., 2000] or decreased [Cloos et al., 1998; Gunczler et al., 1998]. Bone formation, on the other hand, is thought to be decreased as noted by reduced serum levels of osteocalcin [Bouillon et al., 1995; Kemink et al., 2000], a marker of osteoblast maturation and bone remodeling. This suggests that a decrease in osteoblast number and/or osteoblast differentiation contributes to the reduced bone formation.

T1-DIABETES—RODENT MODELS AND BONE DENSITY

Pharmacologic (i.e., alloxan or streptozotocin, STZ) and genetically predisposed (i.e., non-obese diabetic, NOD) rodent models of T1-diabetes allow further examination of the T1-diabetic bone phenotype and its mechanisms. Similar to T1-diabetic patients, T1-diabetic animal models exhibit significant and even more pronounced bone loss (Fig. 2) [Verhaeghe et al., 1990; Herrero et al., 1998; Verhaeghe et al., 2000; Shyng et al., 2001; Botolin et al., 2005; Thrailkill

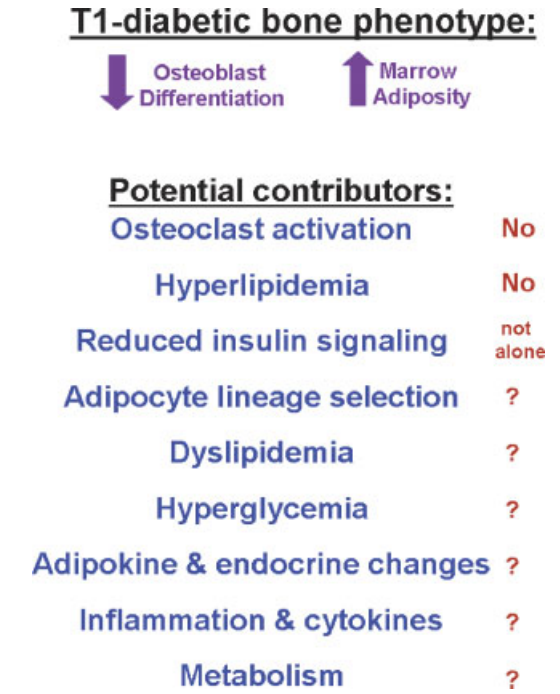

Fig. 1. Potential contributors to the T1-diabetic bone phenotype. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

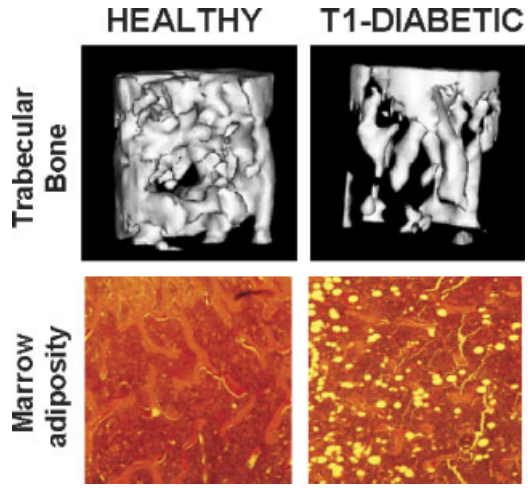

Fig. 2. T1-diabetes causes bone loss and increased marrow adiposity in mice. T1-diabetic mice exhibit significant bone loss 4 weeks after the confirmation of hyperglycemia (blood glucose levels greater than 300 mg/dl). Trabecular bone loss is evident in all bones examined regardless of location or derivation (endochondrial or intra-membranous). Shown is a three-dimensional computed tomography image of a region of tibial bone just beneath the growth plate in control and diabetic mice. Histology in this tibial region demonstrates an increase in marrow adiposity (white round cells) in diabetic compared to control mice. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

et al., 2005] marked by decreased bone volume and osseointegration in implants and distraction models [Verhaeghe et al., 1990; Sasaki et al., 1991; Takeshita et al., 1997; McCracken et al., 2000; Botolin et al., 2005; Thrailkill et al., 2005]. Decreased mineral apposition rate, serum osteocalcin levels, and expression of osteoblast markers (such as osteocalcin) in diabetic bones [Goodman and Hori, 1984; Krakauer et al., 1995; Botolin et al., 2005] support a mechanism of decreased osteoblast number and/or maturation in T1-diabetic bone loss, consistent with human studies. Histomorphometry and microcomputed tomography (μCT) analyses of tibias 4 weeks after the confirmation of STZ-induced diabetes demonstrate a significant decrease (>50%) in mouse tibia, femur, and vertebrae trabecular bone volume fraction (BVF) [Botolin et al., 2005; Martin and McCabe, 2007] similar to the bone pathology seen in diabetic rats [Locatto et al., 1993; Waud et al., 1994]. Bone loss is also seen in spontaneously diabetic NOD mice, confirming the STZ model as being useful for studying T1-diabetic bone Pathology [Botolin and McCabe, 2007b]. T1-diabetes also affects cortical bone density parameters and causes bone loss in the intra-membranous formed, unloaded skull bone [Martin and McCabe, 2007]. These results indicate that T1-diabetic bone loss affects all bones (not just endochondrial or loaded bone) and affects both trabecular and cortical bone.

T1-DIABETIC BONE LOSS—OSTEOCLAST ACTIVITY IS NOT INCREASED

Most studies examining osteoclast activity in T1-diabetic rodent models suggest no change or a decrease in activity based on histology (osteoclast number, erosion depth, erosion surface) and secretion in the urine of bone matrix breakdown products such as deoxypyridinoline [Locatto et al., 1993; Herrero et al., 1998; Verhaeghe et al., 2000] (see Fig. 3), consistent with human studies. Because urine measurements can be confounded by diabetic polyurea and nephropathy, additional serum measurements are important. In mice, 2 weeks of confirmed diabetes does not alter urine deoxypyridinoline levels [Botolin et al., 2005], osteoclast histological parameters, or TRAP5 and cathepsin mRNA levels in whole bone [Botolin et al., 2005; Botolin and McCabe, 2007a]. However, serum PYD and TRAP5b levels are

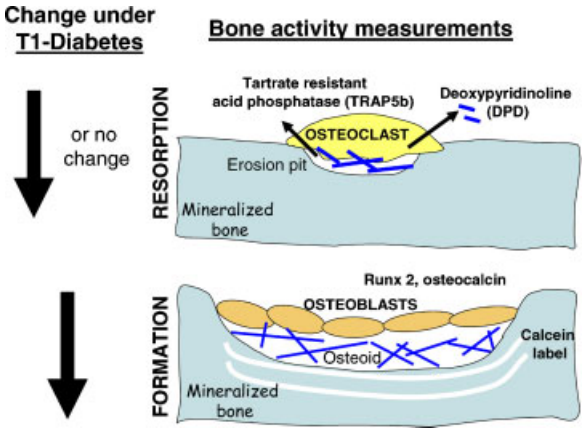

Fig. 3. Bone density is determined by the contributions of bone resorption by osteoclasts and bone formation by osteoblasts. Osteoclast activity can be assessed by a combination of approaches including histological measurement of the number of osteoclasts, the depth of visible resorption pits (erosion depth) the area of bone that is undergoing resorption (erosion surface); urine measurements of collagen fragments released from bone during resorption (i.e., deoxypyridinoline); serum markers specific for osteoclast activity (active TRAP5b); and mRNA or protein levels of markers of mature osteoclasts in bone homogenates. Osteoblast activity (bone formation) can be assessed through methods that include measuring bone mRNA levels of markers of osteoblast maturation (such as runx2 (early stage) and osteocalcin (late stage)); histomorphometric measurements such as osteoblast number and osteoid (unmineralized predominantly collagen I containing bone matrix) surface and area; and dynamic bone measurements utilizing two calcein injections to measure the rate of matrix mineralization (matrix apposition rate, MAR). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

suppressed at 2 and 3 weeks of diabetes [Halleen et al., 2000, 2002; Botolin et al., 2005; Botolin and McCabe, 2006b]. These findings indicate that increased osteoclast activity cannot account for T1-diabetic bone loss. The lack of a visible suppression of osteoclast activity in tibias (while systemic markers of resorption are decreased) suggests either that osteoclast activity is unaltered in tibia but is suppressed in other bones or that suppression of osteoclast activity is minimal in all bones and only becomes apparent in systemic measures that take into account the total skeletal resorptive activity. Decreases in osteoclast activity could contribute to decreased osteoblast maturation through reduced osteoclast production of osteoblast enhancing factors [Dai et al., 2004; Phan et al., 2004; Koh et al., 2005]. Alternatively, the reduction in osteoclast activity could be secondary to reduced osteoblast maturation and its associated decrease in osteoclast signaling [Phan et al., 2004]. Still, these findings indicate that increased osteoclast activity is not a mediator of bone loss in T1-diabetes and that suppression of osteoblast activity must account for the bone loss.

T1-DIABETIC BONE LOSS—OSTEOBLAST ACTIVITY IS DECREASED

Examination of osteoblast and bone formation markers (see Fig. 2) in T1-diabetic tibias indicates a decrease in markers of mature osteoblasts: osteocalcin mRNA, serum osteocalcin levels, and mineral apposition rate [Verhaeghe et al., 1990; Epstein et al., 1994; Herrero et al., 1998; Botolin et al., 2005; Botolin and McCabe, 2007a]. While short and long term diabetes consistently result in suppression of osteocalcin mRNA levels [Botolin et al., 2005; Botolin and McCabe, 2006b, 2007a], suppression of runx2 mRNA levels is evident at early time points but is not always seen in long term studies [Botolin et al., 2005; Botolin and McCabe, 2006b, 2007a]. This could indicate osteoblast dedifferentiation, transdifferentiation, or death at the onset of diabetes followed by continued suppression of osteoblast maturation under chronic conditions. Thus, T1-diabetes likely influences both osteoblast number and maturation. Diabetes-associated suppression of osteoblast maturation is also suggested by: (1) reduced bone implant/healing of diabetic rat calvarial defects [Shyng et al., 2001], (2) decreased bone formation around titanium or hydroxyapatite bone implants [Takeshita et al., 1997; Giglio et al., 2000; Siqueira et al., 2003], and (3) decreased alveolar (jaw) bone formation [Mishima et al., 2002]. In bone marrow ablated mice, the ability of T1-diabetic mice to make bone was decreased (marked by decreased runx2 and osteocalcin mRNA levels) compared to controls at 4 and 6 days after ablation [Lu et al., 2003]. This early suppression of runx2 and osteocalcin expression is similar to what is seen in early stages of STZ-induced diabetic mice.

T1-DIABETIC BONE LOSS—THE ROLE OF MARROW ADIPOSITY

Bone marrow stromal cells (BMSC) not only give rise to osteoblasts, but also to adipocytes. In vitro studies demonstrate that promoting adipocyte maturation of mesenchymal cells reduces the number of mature osteoblasts in

culture [Diascro et al., 1998; Lecka-Czernik et al., 2002; Ahdjoudj et al., 2004; McBeath et al., 2004]. While the regulation of lineage selection is complex, key aspects of lineage selection and differentiation are well known (Fig. 3). For example, factors such as TGFβ/BMPs, TAZ, cytokines/adipokines (such as TNFα and leptin), Msx2, thyroid hormone, metabolic stress, and wnt signaling are thought to contribute to the regulation of osteoblast versus adipocyte lineage selection [Thomas et al., 1999; Kato et al., 2002; Little et al., 2002; Cheng et al., 2003; Bennett et al., 2005; Hong et al., 2005; Kindblom et al., 2005; Irwin et al., 2007]. A reciprocal relationship between bone adiposity and BMD has been recognized with conditions of osteoporosis including: age related and disuse-associated osteoporosis [Jilka et al., 1996; Kajkenova et al., 1997; Verma et al., 2002; Moerman et al., 2004; Nuttall and Gimble, 2004], suggesting that lineage selection could regulate bone density. When cells commit to the adipocyte lineage, there is an early elevation in a transcription factors called CCAAT/enhancer binding protein (C/EBP)β followed by an increase in peroxisomal proliferator-activated receptor (PPAR)γ2 and C/EBPα [Rosen and Spiegelman, 2000]. Activation of PPARγ by its binding to fatty acid ligands and exogenous ligands such as thiazolidinediones (including troglitazone and rosiglitazone; antidiabetic compounds that lower hyperglycemia, hyperinsulinemia, and hypertriglyceridemia in T2-diabetics) [Ferre, 2004; Knouff and Auwerx, 2004] induces adipocyte differentiation and transcription of genes such as adipose-specific fatty acid binding protein (aP2; intracellular fatty acid transport protein). PPARγ2 over expression or activation can stimulate marrow adiposity, suppress osteoblast maturation and in some cases cause bone loss [Lecka-Czernik et al., 1999; Picard and Auwerx, 2002; Rzonca et al., 2004; Ali et al., 2005], whereas mice deficient in PPARγ exhibit an increase in BMD [Akune et al., 2004; Cock et al., 2004].

Similar to age related and disuse bone pathology, male and female T1-diabetic mouse tibias, femurs, and even calvaria exhibit increased bone marrow adiposity, increased adipocyte markers (such as PPARγ2 and aP2 mRNA) and increased numbers of lipid-dense adipocytes in the bone marrow (Fig. 2) [Botolin et al., 2005; Martin and McCabe, 2007]. In contrast, peripheral subcutaneous fat stores are depleted in T1-diabetic mice. A similar switch in adiposity from periphery to bone marrow occurs in spontaneously diabetic mice [Botolin and McCabe, 2007b] indicating that this phenomenon is specific to the T1-diabetes condition. This indicates that marrow lipogenesis/adiposity is regulated differently from subcutaneous adipose stores, perhaps as a result of local differences in the levels of factors such as cytokines and growth factors.

IS MARROW ADIPOSITY LINKED TO T1-DIABETIC BONE LOSS?

Selection of adipogenesis over osteoblastogenesis is a common theme in bone [Meunier et al., 1971; Jilka et al., 1996; Kajkenova et al., 1997; Verma et al., 2002; Gimble et al., 2006] but there is an increasing number of studies in which this relationship does not hold [Tornvig et al., 2001; Lecka-Czernik et al., 2002; Lazarenko et al., 2006]. Exactly why marrow adiposity appears in T1-diabetes is not known, but if it is linked to bone loss then the regulation of adiposity is a key therapeutic target [Duque, 2003; Nuttall and Gimble, 2004]. Existing data cannot distinguish whether lipid sparse adipocytes that were always present in the marrow are accumulating lipid and becoming visible or whether mesenchymal pluripotent cells are becoming adipocytes. The latter could occur at the expense of osteoblast lineage selection or occur separate from osteoblast pathway selection (and not affect bone formation). It is unknown whether these adipocytes have a positive, negative or no effect on the bone [Gimble et al., 2006].

At least two possibilities can explain the function of PPARγ2 elevation in T1-diabetes: it could function as an inducer of adipogenesis or it simply represents a marker of maturing adipocytes. To test the role of PPARγ and marrow adiposity in T1-diabetic bone loss, PPARγ activity can be inhibited by treating mice with a PPARγ anagonist, bisphenol-A-diglycidyl ether (BADGE), which is demonstrated to prevent adipogenic cells from undergoing hormone-mediated differentiation [Wright et al., 2000] and can prevent rosiglitazone signaling in lung [Cuzzocrea et al., 2004]. While BADGE treatment did not prevent T1-diabetic hyperglycemia it did prevent marrow adiposity [Botolin and McCabe, 2006b], consistent with a role for active PPARγ in stimulating marrow adiposity [Picard and Auwerx, 2002; Rzonca et al., 2004;

Ali et al., 2005]. Despite this effect, BADGE treatment did not prevent T1-diabetic bone loss [Botolin and McCabe, 2006b], suggesting that the appearance of mature marrow adipocytes is not the cause of suppressed osteoblast maturation and bone formation. This is somewhat unexpected since congenital PPARγ deficiency results in enhanced BMD [Akune et al., 2004; Cock et al., 2004]. This difference may stem from the length of time that PPARγ is suppressed; in the BADGE treatment studies suppression is only for a 40-day period; extended treatment may be necessary to observe increases in BMD. Alternatively, the increased number of lipid dense adipocytes in T1-diabetes is not the result of changes in lineage selection, but changes in adipocytes maturation. Alternatively, BASGE treatment may leave marrow cells stuck at an early stage of adipocyte commitment prior to PPARγ activation in which case they cannot switch to the osteoblast lineage. Similar to the T1-diabetic mouse findings, PPARγ haploinsufficiency did not prevent bone loss in ovariectomized mice [Akune et al., 2004]. This suggests the possibility that PPARγ and adiposity may not be linked to all forms of bone loss. It may be that factors affected by diabetes are primary regulators of osteoblast differentiation and apoptosis, but also contribute to the regulation of lineage selection (see Fig. 4). For example, TGFβ treatment is effective at preventing bone loss and marrow adiposity in hind limb suspended mice [Ahdjoudj et al., 2002] and can decrease osteoblast and osteocyte cell death by more than 50% [Bodine et al., 2005]. Clearly more studies are needed to sort out the relationship between adiposity, regulators of adiposity and bone density.

LOSS OF INSULIN RECEPTOR (IR) SIGNALING DOES NOT CAUSE BONE LOSS

Two obvious potential contributors to T1-diabetic bone loss are low insulin levels/signaling and hyperglycemia. It is known that insulin treatment can prevent the negative effects of diabetes [Hough et al., 1981; Verhaeghe et al., 1992] and even enhance bone formation [Haffner and Bauer, 1993; Krakauer et al., 1995]. The requirement for insulin receptor (IR) in the metabolic actions of insulin is demonstrated by null mutation of the IR in mice or humans causing early postnatal death [Accili et al., 1996;

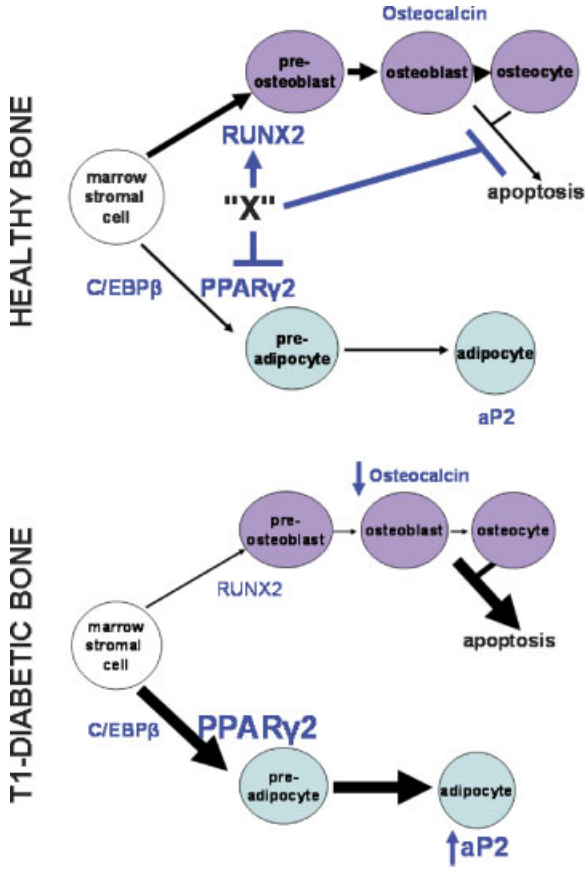

Fig. 4. Mechanisms regulating osteoblast number and maturation. Osteoblasts, the cells involved in bone formation, are derived from mesenchymal stem cells which can also give rise to adipocytes and a variety of other cell types. As cells commit to the osteoblast lineage, Runx2 is expressed. Runx2 is a key transcription factor required for osteoblast lineage selection and expression of osteoblast specific genes and osteoblast maturation. Subsequent to Runx2 induction, there is a selective expression of maturation, stage specific genes. Osteocalcin is expressed in mature osteoblasts and is used as a late stage marker of maturation [Lian et al., 1998; Winchester et al., 2000; Sasano et al., 2002]. During mineralization osteoblasts can either apoptose or become embedded in bone in which case they are called osteocytes. Apoptosis is another potential mechanism regulating the number of bone cells and perhaps bone formation. As mesenchymal cells commit to the adipocyte lineage PPARγ2 is expressed followed by other genes including aP2 (a fatty acid binding protein). The regulation of lineage selection is affected by a variety of factors (denoted ''X'' in the figure), including TGF-β, Wnts, TAZ, and BMPs. Many of these factors also contribute to the regulation of osteoblast maturation and osteoblast and osteocyte viability. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Nakae et al., 2001]. It is known that osteoblasts express IR as they differentiate and osteoblasts respond to insulin treatment [Kream et al., 1985; Levy et al., 1986], however osteoblasts are capable of differentiating in standard in vitro conditions that contain very low insulin levels

(~1 pM). Distinguishing the roles of insulin, hyperglycemia, and other factors in T1-diabetic bone loss is complex because modulation of one parameter will necessarily affect the other. Recently a mouse model was developed (the genetically reconstituted IR knockout mice (IRKO-L1) that are euglycemic as a result of human IR transgene expression in the pancreas, liver, and brain, but not in bone) which allows examination of the role of reduced insulin signaling in bone. Surprisingly, IRKO-L1 bones develop normally and exhibit similar (and trend toward greater) bone density compared to wild-type littermates [Irwin et al., 2006]. While osteoblast and osteoclast markers are similar in wild-type and IRKO-L1 bones, adipocyte markers and marrow adiposity are greatly decreased in IRKO-L1 bones consistent with IR inactivation impairing adipocyte differentiation [Accili and Taylor, 1991; Cinti et al., 1998; Entingh-Pearsall and Kahn, 2004] (although peripheral body fat content in IRKO-L1 mice was not reduced). Isolated BMSC from IRKO-L1 mice do not exhibit reduced adipogenic potentials in vitro suggesting that local or systemic factors secreted by other cell types likely play a role in the bone adiposity differences. One interesting finding was that IRKO-L1 bones exhibit upregulation of IGF-1 receptor expression; this may be an adaptive response since both IGF-1 and IRs signal through similar pathways involving Akt [Irwin et al., 2006] and IRKO-L1 mice have elevated insulin levels to increase IGF-1 signaling. A similar upregulation in IGF-1 receptor expression is seen in IRKO-L1 muscle [Shefi-Friedman et al., 2001]. The compensatory signaling of insulin and IGF-1 pathways also occurs in reverse as evidenced by the rescue of differentiation and mineralization of IGF-1 receptor deficient osteoblasts by insulin treatment [Fulzele et al., 2007]. Thus, sufficient signaling through either IR or IGF-1 is required for optimal bone mineralization.

IS HYPERLIPIDEMIA LINKED TO T1-DIABETIC BONE LOSS?

T1-diabetes is a metabolic disease where the lack of insulin results in decreased glucose utilization by insulin sensitive cells. As a consequence, cells require other energy sources. Mobilization of free fatty acids and triglycerides are able to provide needed cellular energy. In well controlled T1-diabetic patients dyslipidemia is found in about 20 and 50% of adolescent and adults, respectively, and is consistent with the general population [Cullen et al., 1999; Ford et al., 2003; Imperatore et al., 2004; Wadwa et al., 2005; Faulkner et al., 2006; Maahs et al., 2007]. With reduced metabolic control, T1-diabetic patients have an increased risk and severity of hyperlipidemia [Weidman et al., 1982; Winocour et al., 1986; Chaturvedi et al., 2001] which is linked to diabetic complications such as nephropathy and retinopathy [Cullen et al., 1999; Hadjadj et al., 2004]. Hyperlipidemia has also been reported in T1-diabetic BALB/c mice [Botolin and McCabe, 2006b], C57BL/6J mice [Pighin et al., 2005], and rats [Zhang et al., 2002b]. Marrow cells isolated from mice fed high fat diets fail to undergo osteoblast differentiation in vitro [Parhami et al., 1999], suggesting a key role for serum lipids in the activation of PPARγ2 and bone marrow cell lineage selection. However, in past studies where PPARγ activity was inhibited by BADGE treatment, hyperlipidemia was prevented in STZ-diabetic mice, but the mice still lost bone [Botolin and McCabe, 2006b]. This indicates that fat metabolism and adiposity are not actively linked to the bone loss seen in T1-diabetes. In addition to altering total lipid levels, T1-diabetes can causes changes in lipid profiles and increase the proportion of serum 18:1n-9 (oleic) and 18:2n-6 (linoleic acid) lipids relative to other lipids [Christeff et al., 1994]. These changes can increase the concentration of ligands for PPARγ [Christeff et al., 1994; Forman et al., 1995; Lecka-Czernik et al., 2002; Ferre, 2004; Knouff and Auwerx, 2004] and result in PPARγ2 activation and increase marrow adiposity. Certainly, treatment of osteoblast-like cells with serum that contains high levels of palmitic, oleic, and linoleic fatty acids, can activate PPARs and induce adipocyte-like differentiation [Diascro et al., 1998; Lecka-Czernik et al., 2002]. Thus, one could hypothesize that dyslipidemia associated with diabetes can compound and further bone loss by PPARγ activation and/or influencing osteoblasts directly. Further studies are needed to fully understand the role of lipids in diabetic bone loss.

ROLE OF HYPERGLYCEMIA

As noted previously, insulin treatment can prevent the negative effects of diabetes, but is

associated with restoration of euglycemia which in itself could be important for avoiding complications [Brownlee et al., 1984]. Reports in bone and other tissues indicate that hyperglycemia contributes to diabetic complications through a variety of mechanisms including increasing reactive oxygen species (ROS) [Wolff and Dean, 1987; Hunt et al., 1990], polyol-pathway activity [Gabbay, 1973; Inaba et al., 1997], protein kinase C activity [Wolf et al., 1991; Ceolotto et al., 1999], and non-enzymatic glycosylation of key proteins such as collagen I or IGF-1 [Brownlee et al., 1984; Bucala et al., 1984; Locatto et al., 1993; Katayama et al., 1996; McCarthy et al., 1997; McCarthy et al., 2001]. In addition, hyperglycemia can induce an osmotic response in cells such as osteoblasts that express low K_m glucose transporters, GLUT1 and GLUT 3 [Thomas et al., 1996] since glucose transport is maximal at a euglycemic state (glucose concentration of 3–5.5 mM). During osmoadaptation to extracellular hyperosmotic conditions, short-term signaling pathways (ion transporters) are activated to allow cells to undergo a volume change and shrink while long-term metabolic pathways work to draw water back into the cell to restore cell volume and intra-cellular solute concentration. Acute (24 h) hyperglycemia and its associated hyperosmolality, at levels seen in diabetic mice [Botolin et al., 2005], can modulate osteoblast signaling pathways and suppress expression of genes associated with osteoblast maturation including osteocalcin [Zayzafoon et al., 2000, 2002], similar to what is seen in T1-diabetic mice [Botolin et al., 2005; Botolin and McCabe, 2006b, 2007b]. Chronic hyperglycemic conditions (days to weeks) can also suppress osteocalcin expression [Botolin and McCabe, 2006a] and calcium uptake in osteoblast cultures [Balint et al., 2001], but this effect is independent of hyperosmolarity. This indicates different, time dependent osteoblast responses to hyperglycemia. Chronic hyperglycemia in vitro also raises PPARγ2 expression while suppressing MMP-13 expression, the latter appears to be in response to hyperosmotic conditions [Botolin and McCabe, 2006a]. These changes further suggest that hyperglycemia suppresses osteoblast differentiation and may promote an adipocyte-like phenotype, which could contribute to chronic T1-diabetic bone loss. Potential mechanisms include chronic dysregulation of redox state, activation of the polyol pathway, or utilization of glucose metabolism perhaps at the cost of utilizing other fuels. Increased glucose metabolism could lead to altered energy status and increased lactic acid synthesis. Osteoblasts express acid-sensitive channels [Jahr et al., 2005] and can respond to low pH conditions by decreasing mineralization and gene expression [Brandao-Burch et al., 2005]. While the profiles of acid-induced gene expression changes do not completely parallel those seen under hyperglycemic conditions, lower extracellular pH may contribute to some of the changes.

Chronic elevation of extracellular glucose levels can also lead to glycosylation of proteins and other cell components (including DNA) by a non-enzymatic process, the products are called advanced glycation end products (AGE). The role of receptor for AGE products (RAGE) has been implicated in diabetic bone loss [Lalla et al., 2000] although its activation results in increased osteoclast formation [Ding et al., 2006] which does not occur early in development of T1-diabetes, but could be a factor at later stages. Addition of advanced glycosylation end-products to culture medium attenuates osteoblast differentiation [Kume et al., 2005] and osteoblasts cultured on AGE-collagen exhibit decreased maturation and nodule development [Katayama et al., 1996; McCarthy et al., 2001], suggesting a potential role for AGE in chronic hyperglycemia induced bone loss.

ROLE OF ADIPOKINES, CYTOKINES, AND OTHER ENDOCRINE FACTORS

This review would not be complete with out discussion of the contribution of other endocrine factors, adipokines, and cytokines to T1-diabetic bone loss. T1-diabetic patients and animal models can exhibit dysregulation of a variety of endocrine factors including reduced IGF-1 [Jehle et al., 1998; Clark, 2004] and amylin [Horcajada-Molteni et al., 2001]. It is well known that IGF-1 signaling is positively correlated with BMD and its role in T1-diabetes and bone loss has been reviewed [Rosen, 2004; Niu and Rosen, 2005]. Thus, low serum IGF-1 could contribute to reduced BMD, osteoblast differentiation, and increased marrow adiposity [Zhang et al., 2002a; Rosen et al., 2004]. In fact, restoration of IGF-1 serum levels in diabetic rats can, in part, correct decreases in bone formation [Verhaeghe et al., 1992]. Amylin is another hormone secreted from pancreatic beta

cells that can be decreased in T1-diabetes. Treatment with an amylin agonist improves bone indices in STZ-induced diabetic rats [Horcajada-Molteni et al., 2001]. The above treatments work by restoring critical endocrine effects and/or by enhancing anabolic activity in bone, and underlie how anabolic approaches can be of benefit to T1-diabetic patients. In addition, dysregulation (increase, decrease, or no change) of adipokines such as leptin, ghrelin, and adiponectin have been reported in T1-diabetic humans [Hanaki et al., 1999; Luna et al., 1999; Alexopoulou et al., 2006; Karaguzel et al., 2006; Martos-Moreno et al., 2006] and mouse models. For example, serum leptin levels are suppressed in both male and female diabetic mice compared to age matched controls, consistent with reduced peripheral fat depots [Martin and McCabe, 2007]. While not as effective in leptin-replete mice, leptin treatment can be an effective therapeutic to increase bone density in leptin-deficient mice [Hamrick et al., 2005] and in hind limb suspended mice [Martin et al., 2005]. Given the bone regulatory capabilities of adipokines, perhaps restoration of adipokines (like leptin) to normal levels could reduce T1-diabetic bone loss; future studies will determine this. Inflammation may be another contributor to bone loss, since T1-diabetes is the result of inflammatory autoimmune destruction of the pancreas and therefore cytokine profiles and cytokine signaling pathways are likely to be altered during acute and chronic stages of T1-diabetes. Many T1-diabetic complications have been shown to have an inflammatory component. For example, studies in T1-diabetic patients and rodent models with retinopathy indicate that levels of cytokines such as IL-1 are increased in the vitreous fluid [Carmo et al., 1999; Yuuki et al., 2001]. While elevation of cytokine levels can activate osteoclast bone resorption (not a big factor in diabetic bone loss), they can also suppress osteoblast differentiation and bone formation and could contribute to bone loss in T1-diabetics. Future understanding of osteo-immune signaling and regulatory pathways affected by T1-diabetes will also contribute to the development of optimal therapeutics for bone loss.

IN SUMMARY

T1-diabetic bone loss does not exhibit preference with regard to bone location or type, mechanical loading, or gender. Associated increases in marrow adiposity may be secondary to bone loss based on findings that suppression of adiposity does not prevent bone loss and that marrow adiposity is not present in vertebrae. Many of the factors involved in suppressing adipogenesis (such as TGFβ and Wnts) are also involved in promoting osteogenesis and protecting osteoblasts and osteocytes from apoptosis. Therefore, if these signaling pathways are modulated in T1-diabetes, prevention of adiposity alone would not be sufficient to overcome defects in the regulation of the other pathways (osteoblast maturation and viability). Certainly more studies are needed to examine these signaling defects. Decreased insulin signaling, increased PPARγ activity and hyperlipidemia do not alone account for T1-diabetic bone loss. Because the T1-diabetic bone defect is predominantly the result of a decrease in bone formation (rather than increased resorption) anabolic therapies will likely be the most effective treatment. Future studies directed at understanding the mechanism of T1-diabetic bone loss, possibly focusing on alterations in metabolism, cytokines, adipokines, hormones, and growth factors, will contribute to the development of novel therapeutics that allow T1-diabetic patients to live longer with healthier bones.

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