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Contributions of the Insulin/Insulin-Like Growth Factor-1 Axis to Diabetic Osteopathy

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Abstract

Recent studies in diabetic humans and rodent models of diabetes have identified osteopathy as a serious complication of type 1 (T1D) and type 2 (T2D) diabetes. Accumulating evidence suggests that disruption of insulin and insulin-like growth factor 1 (IGF-1) homeostasis in the diabetic condition may be responsible for the observed skeletal deficits. Indeed, replacement of insulin or IGF-1 in rodent models of T1D results in significant improvement in bone healing despite ongoing moderate to severe hyperglycemia. Insulin and IGF-1 act through distinct receptors. Mice in which the receptor for insulin or IGF-1 is selectively deleted from osteoblast lineages show skeletal deficits. Despite acting through distinct receptors, insulin and IGF-1 exert their cellular activities via conserved intracellular signaling proteins. Genetic manipulation of these signaling proteins, such as insulin receptor substrate (IRS)-1 and -2, Protein Kinase B (Akt), and MAPK/ERK kinase (MEK), has uncovered a significant role for these signal transduction pathways in skeletal homeostasis. In addition to effects on skeletal physiology via canonical signaling pathways, insulin and IGF-1 may crosstalk with wingless-int. (Wnt) and bone morphogenic protein 2 (BMP-2) signaling pathways in cells of the osteoblast lineage and thereby promote skeletal development. In this review, a discussion is presented regarding the role of insulin and IGF-1 in skeletal physiology and disruptions of this axis that occur in the diabetic condition which could underlie many of the skeletal pathologies observed.

Introduction

It is now well established that humans with type 1 (T1D) or type 2 (T2D) diabetes have an increased risk of fracture [1-6]. While decreased bone density and a state of low bone turnover have been described in those with T1D, T2D is not associated with osteopenia or osteoporosis. However, recent studies have reported that subperiosteal porosity is increased in T2D patients who fracture [7,8].

The underlying mechanisms involved in skeletal deficits observed in both T1D and T2D are poorly understood. While it is likely a multi-factorial process that contributes to skeletal compromise in diabetes, as is the case with most diabetes-related complications, insulin and its homolog, insulin-like growth factor 1 (IGF-1), have been implicated in the pathogenesis of skeletal deficits attributable to diabetes (i.e., diabetic osteopathy) [8]. For instance, in humans with diabetes, there is a positive correlation between bone mineral density (BMD) by dual-energy x-ray absorptiometry (DXA) and insulin dose [9,10] or urinary C-peptide excretion, a measure of endogenous insulin production [9], suggesting that endogenous and exogenous insulin may affect skeletal homeostasis in diabetes. IGF-1 concentrations are lower in diabetic patients with osteopenia, compared to those without osteopenia, and decreased serum markers of bone formation in diabetes are associated with lower IGF-1 concentrations [11-15]. Patients with T2D who have higher IGF-1 concentrations also have higher BMD and decreased vertebral fractures [12,16]. Recently, we have shown in individuals with T1D that measures of endogenous insulin, exogenous insulin dose, and serum IGF-1 concentrations all positively correlate with osteocalcin, a marker of bone formation [17].

In this review, we will explore the current knowledge, obtained in rodent models, of how insulin and IGF-1, through their cognate receptors, regulate normal skeletal physiology, and how the actions of these peptide hormones may be critical to understanding the pathogenesis and potential treatment of diabetic osteopathy.

Insulin and IGF-1 physiology

Insulin and IGF-1 are small peptide hormones (~7.5 kD) which share a high degree of homology with proinsulin. Each possesses the ability to increase glucose disposal, insulin being significantly more potent than IGF-1 [18]. Unlike insulin, which is produced by pancreatic β-cells, IGF-1 is produced predominantly by the liver, with other tissues producing smaller amounts, and circulates at high concentrations in serum [18,19]. Insulin and IGF-1, beyond their metabolic effects, can be growth-promoting peptides which influence cellular proliferation and differentiation [18]. Unlike insulin, the interaction of IGF-1 with cell-surface receptors is tightly regulated by at least six distinct high affinity carrier proteins, the IGF-binding proteins (IGFBPs), and possibly by several low-affinity IGFBP-like molecules [18,20]. The interaction of IGF-1 with IGFBPs can prevent untoward IGF-1 effects, such as uncontrolled cellular proliferation or hypoglycemia. Conversely, disruption of the IGF:IGFBP complex is a prerequisite for IGFs to exert their mitogenic and metabolic effects through the IGF-1 receptor (IGF1R) [21].

Downstream mediators of both insulin and IGF-1 signaling pathways are important in promoting osteogenesis

Insulin and IGF-1 signaling pathways utilize many of the same cellular proteins to achieve various cellular outcomes (Figure 1). Each ligand, through its cognate receptor, can mediate events via insulin receptor substrate (IRS)-1 and IRS-2 phosphorylation and subsequent activation of phosphatidylinositol (PI) 3-kinase [22] and by activation

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of the mitogen-activated protein (MAP)/ERK kinases [23]. A direct link between insulin and/or IGF-1 signaling and bone formation *in vivo* is supported by transgenic models which manipulate various proteins in these downstream signaling pathways (Figure 1). The growth factor receptor-bound protein 2 (Grb-2)–associated binder 1 (Gab1) is a scaffolding protein that is involved in both ERK activation as well as in regulating the PI3K-Akt signaling pathway. Osteoblast-specific elimination of Gab1 results in decreased trabecular bone, diminished bone formation, reduced strength, and reduced MAP/ERK and Akt activation in response to insulin or IGF-1 [24]. Mice null for IRS-1 and IRS-2 develop unique bone phenotypes; *in vivo*, IRS-2 appears to maintain dominance of bone formation over bone resorption, while IRS-1 regulates bone turnover [25,26]. Bone healing is also impaired in IRS-1 deficient mice and can be corrected with re-expression of IRS-1 within the fracture site [27]. Because IRS molecules mediate insulin and IGF receptor signaling, cross-talk downstream of IRS molecules may take place via insulin and IGF signaling in osteoblasts. Akt and forkhead transcription factors (FoxO) proteins are downstream mediators of IRS signaling within the PI3-kinase pathway. Mice null for Akt1 or both Akt1 and Akt2 have significant skeletal deficits which include delayed ossification, and even dwarfing [28,29]. In contrast, elimination of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), an antagonist of PI3-kinase activity, is associated with increased bone mineral density [30]. Activated Akt can phosphorylate several forkhead transcription factors (FoxO1, 3 and 4) and inactivate their transcriptional activity by excluding them from the nucleus to the cytoplasm. This mechanism is a major pathway in many cell types and tissues through which insulin and IGF-1 regulate gene transcription of many genes involved in cell growth, differentiation and metabolism. FoxO1, 3, and 4 are expressed in osteoblasts [31]; however, their regulation of skeletal homeostasis remains unclear. Elimination of FoxO1 in osteoblasts has the affect of decreasing bone mass and multi-tissue knockdown of FoxO1, 3 and 4 proteins decreases bone formation [32,33]. In contrast, newer data shows that deletion of FoxO1, 3, and 4 specifically in osteoblast progenitors results in an increase in vertebral and femoral BMD, as well as a striking increase in femoral cortical thickness [34,35]. In keeping with these *in vivo* findings, *in vitro* FoxO1 has been shown to directly interfere with the activities of runt-related transcription factor 2 (RUNX2), which is considered a "master regulator" of osteoblast development and whose expression is essential for normal bone formation [36,37]. In addition to the PI3K/Akt/FoxO pathway, activation of the MAP/ERK kinase pathway may also mediate insulin and IGF-1 events in bone. For instance, osteoblast specific expression of a constitutively active form of the MAP kinase, MEK1, is associated with accelerated skeletal development, enhanced skeletal size, and mineralization; whereas mice expressing a dominant negative form of MEK1 display delayed skeletal development, and these outcomes are believed to be mediated by effects on RUNX2 phosphorylation and transcriptional activity [38]. Taken together, these studies demonstrate that disruption of signal transduction pathways shared by insulin and IGF-1 receptors result in abnormalities of normal skeletogenesis.

IGF-1 affects on the skeleton

Research over several decades has supported a primary role for IGF-1 in anabolic bone formation [39]. However, it has only been in recent years that an essential role of IGFs in normal bone development has been confirmed through the elimination of IGF-1 and the IGF1 receptor in mice via homologous recombination [40,41]. In these animals, profound growth retardation as well as growth plate abnormalities and decreased bone calcification were observed. Later studies have further refined how elimination of IGF-1 affects bone physiology not only through the dwarfing of bones, but also by significantly decreasing bone formation rate and cortical thickness, resulting in more compact bone [42]. Studies specifically designed to examine how relative degrees of peripheral (i.e, hepatic production) IGF-1 deficiency affect bone formation have revealed only small decreases in cortical periosteal bone growth [43]. Further reductions in circulating IGF-1 concentrations (to 10-15% of controls) achieved by crossing the previously described animals with animals made null for the acid-labile subunit (ALS) of the IGFBP-3/IGF-1 complex (ALS, IGFBP-3 and IGF-1 form the major 150 kD complex responsible for carrying IGFs in the vascular compartment in mammals), results in a 10% decrease in bone mineral density and a 35% decrease in periosteal circumference and cortical thickness [43]. Thus, together these studies demonstrate that circulating (i.e., endocrine) IGF-1 can have a significant effect on several parameters of bone density and formation, as well as the overall size of the bone. Exactly how these IGF-1-mediated effects may involve anabolic effects on osteoblast activity has been recently elucidated by Zhang et al [44], who, through tissue-specific gene targeting, ablated the

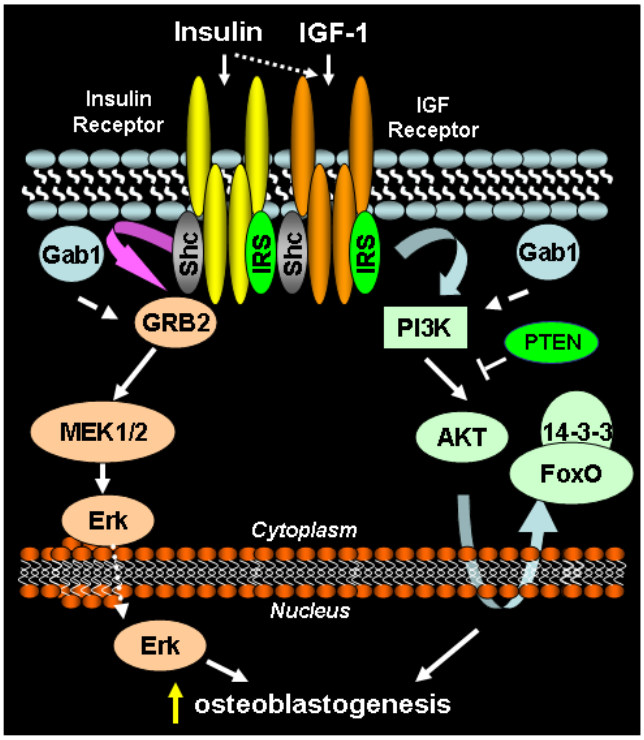

Figure 1: Pro-osteoblastogenic signal transduction by insulin and IGF1 receptors. Receptors at the plasma membrane bind ligands within extracellular domains, triggering receptor autophosphorylation on tyrosine residues within the cytoplasmic domain. Phosphorylated receptors recruit scaffolding proteins [src homology 2 domain-containing transforming protein C (Shc), IRS], which are subsequently phosphorylated by the receptor kinase domain. Recruitment of growth factor receptor bound protein 2 (GRB2) to the receptor-Shc complex initiates phosphorylation and activation of the MEK/ERK pathway. MEK/ERK signaling culminates with phosphorylation of osteoblastogenic transcription factors in the nucleus. IRS proteins recruit phosphatidylinositol 3-kinase to the receptor complex, resulting in kinase activation, which generates phosphatidylinositol (3,4,5) triphosphate (PIP3). PIP3 recruits AKT to the plasma membrane, resulting in AKT activation. Activated AKT phosphorylates numerous proteins, among them FoxO1, resulting in retention of FoxO1 in the cytosol, where it is unable to perform its function as a transcription factor.

IGF1 receptor in mature osteoblasts using the osteocalcin promoter-*Cre* construct. These studies showed that mice deficient in IGF1R in mature osteoblasts were of normal size, yet demonstrated a marked decrease in cancellous bone volume, connectivity, and trabecular number, as well as a striking decrease in the rate of mineralization of osteoid matrix. Recently, the IGF1R has been eliminated in osteoblast progenitors using the osterix promoter-*Cre* construct. In contrast to mice lacking the IGF1R only in mature osteoblasts, those lacking the IGF1R throughout osteoblast development are growth retarded [45]. Furthermore, these mice display decreased BMD and metaphyseal deficits [45]. Thus, a significant amount of the bone-forming and mineralization actions of IGF-1 appear to be mediated via the osteoblast. Indeed, transgenic over-expression of IGF-1 *in vivo* under the control of the osteocalcin promoter, results in a phenotype in which bone mineral density, as measured by DXA and quantitative computed tomography, is significantly increased in transgenic mice compared with controls. Furthermore, histomorphometric measurements reveal a marked increase in femoral cancellous bone volume in mice overexpressing IGF-1 compared with controls [46]. Therefore, any alteration in the IGF system may have profound effects on anabolic bone formation.

Insulin affects on the skeleton

While many studies have clearly demonstrated that IGF-1 functions as an anabolic agent in bone, only a few studies have examined the specific role that insulin and its cognate receptor (IR) may play in regulating osteoblast physiology. Studies *in vitro* have shown that physiological doses of insulin promote osteoblast proliferation [47,48], collagen synthesis [49-51], alkaline phosphatase production [52,53], and glucose uptake [54,55]; nevertheless, these studies do not clarify what receptors or pathways insulin may use to promote osteogenesis. The insulin receptor is expressed in normal bone and in regenerating bone *in vivo* [56,57]. IR expression is detected throughout differentiation, from pre-osteoblast to mature osteoblast, in MC3T3-E1 cells (Figure 2A). IR expression is detected in early bone progenitor cells (bone marrow stromal cells and C3H10T1/2 cells) as well as in *ex vivo* bone cell cultures (mouse calvarial cells) (Figure 2B). Furthermore, insulin and IGF-1 can activate the PI3K and MEK/ERK pathways in osteoprogenitor cells and in osteoblastic cells (Figure 2C and 2D, respectively), suggesting that insulin signaling is operative in osteoblastic cells at various stages of differentiation. While elimination of the IR in all skeletal elements has been reported to result in no skeletal abnormalities or in diminished trabecular architecture [57,58], two recent reports queried specifically the importance of insulin meditated events in osteogenesis. Both studies knocked down IR expression specifically in osteoblasts using *Cre*-mediated recombination [57,59]. It was observed that knock-down of IR in osteoblasts resulted in altered bone formation [59] and in abnormal trabecular architecture [57]. While both models support a role for insulin in osteoblast development, Ferron *et al* reported only a partial (60%) knock-down of the IR using the Col1a1-*Cre* mouse, while Fuzele *et al* eliminated the IR only in mature osteoblasts using the osteocalcin-*Cre* construct [57,59]. Thus, additional informative models are needed to fully appreciate the role that insulin signaling via the IR may play throughout osteoblastogenesis and skeletal development.

Effects of insulin and IGF-1 on diabetic osteopathy

In rodent models of T1D, skeletal architecture, bone quality and bone integrity are compromised [see reference [60] for review of studies]. These alterations are associated with poor bone formation and regeneration [56]. Furthermore, at a molecular level, T1D impacts bone formation by down-regulating RUNX2 [61,62], and genes known to be targets of RUNX2 activity (e.g., osteocalcin, matrix metalloproteinases 9 and 13, integrin-binding sialoprotein, collagen, phosphate regulating endopeptidase homolog, X-linked (PHEX), dentin matrix acidic phosphoprotein 1 (DMP-1), alkaline phosphatase, osteopontin, vitamin D receptor, and ameloblastin) [62]. These

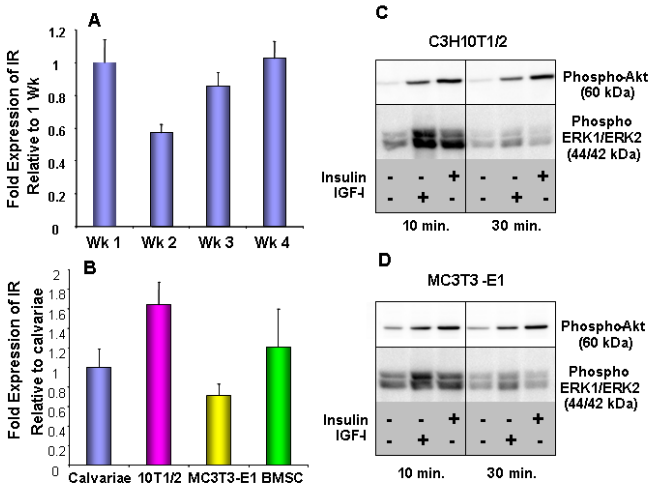

Figure 2: Insulin and IGF1 signal transduction is functional in osteoblast precursors *in vitro*. IR mRNA levels measured by quantitative RT-PCR in MC3T3-E1 cells after 1, 2, 3, or 4 weeks of differentiation with ascorbate and β-glycerol phosphate (**A**) and in primary calvariae, C3H10T1/2, MC3T3-E1, and bone marrow stromal cell cultures **(B).** Western blot detection of Akt and ERK1/2 phosphorylation in C3H10T1/2 (**C**) or MC3T3-E1 **(D)** cell lysates prepared after treating cells with 10 ng/ml of insulin or IGF-1 for the indicated times.

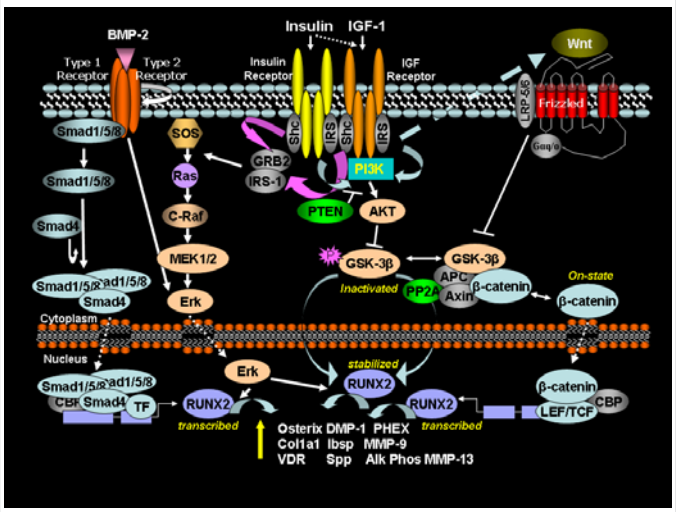

Figure 3: Potential cross-talk between insulin/IGF-1, BMP2, and Wnt signaling pathways. BMP2 receptor signaling and the MEK/ERK branch of the insulin/IGF-1 signaling pathway converge on ERK1/2 kinase, which can modulate the activity of the master osteoblastogenic transcription factor, Runx2. Wnt and the PI3-K/Akt branch of the insulin/IGF-1 signaling pathway converge on GSK-3b, inhibiting GSK-3b kinase activity and reducing its constitutive phosphorylation of β-catenin. Unphosphorylated β-catenin is not readily degraded and can accumulate in the nucleus, where it acts as a pro-osteoblastogenic transcription factor by regulating transcription of Runx2 and other genes.

Perturbations in Insulin/IGF-1 signaling resulting in diminished skeletogenesis							
Gene(s) modifed[1]	Genetic approach[2]	Phenotype					Ref.
		Dwarf	Trabecular Bone	Cortical Bone	BMD	Bone Strength	
Gab1	C (OC-*Cre*)	NR[3]	↓	↓	NR	↓	24
IRS1	G	Yes	↓	↓	↓	NR	26
IRS2	G	No	↓	↓	↓	NR	25
Akt1	G	Yes	↓	↓	↓	NR	29
Akt1 & Akt2	G	Yes	NR	NR	NR	NR	28
FoxO1	C (Col1a1-*Cre*)	No	↓	↓	↓	NR	32
FoxO1, 3 & 4	MT (Mx-*Cre*)	No	↓	↓	↓	NR	33
IGF1R	C (OC-*Cre*)	No	↓	↔	↓	NR	44
IGF1R	C (Osx-*Cre*)	Yes	↓	NR	NR	NR	45
IR	C (Col1a1-*Cre*)	NR	NR	NR	↓	NR	59
IR	C (OC-*Cre*)	NR	↓	NR	NR	NR	57
MEK1	TG (dominant/negative)	Yes	NR	NR	↓	NR	38
Perturbations in Insulin/IGF-1 signaling resulting in enhanced skeletogenesis							
MEK1	TG (active)	No (large)	NR	NR	↑	NR	38
PTEN	C (OC-*Cre*)	NR	↑	↑	↑	NR	30
FoxO1, 3 & 4	C (Osx-*Cre*)	NR	NR	↑	↑	NR	34

[1]Pathways presented in Figure 1
[2]Genetic Approach: C= conditional knockout; G=global knockout; MT = multiple tissue knockout; TG = transgenic over-expression
[3]NR = Not reported

Table 1: Various mouse models in which components of insulin and/or IGF-I signaling have been independently assessed in skeletogenesis.

data suggest that osteoblastogenesis is impaired from early stages of osteoblast commitment and may therefore explain the profound lack of new bone formation, compromised skeletal microarchitecture, and diminished bone strength observed in diabetic models.

In rodent models of diabetes, insulin therapy is consistently capable of improving many of the histomorphometric and biomechanical properties of bone, as well as the biochemical abnormalities observed in diabetic rodents [56,63-65]. Moreover, RUNX2 and RUNX2-regulated osteogenic genes are in large part normalized in insulin-treated diabetic animals, suggesting that insulin may directly or indirectly regulate bone formation through a pro-osteogenic pathway involving RUNX2 expression and RUNX2 downstream targets [62]. Critical to the argument that insulin may have direct effects on osteoblastogenesis during diabetes, local insulin delivery can normalize mineralization, callus bone content, and biomechanical properties in the healing fracture callus of the diabetic rat, despite persistent systemic hyperglycemia and systemic hypoinsulinemia [66].

While much research supports a primary role for IGF-1 in anabolic bone formation in rodents as just described [39,67,68], its role in the pathogenesis or reversal of diabetic osteopathy has been examined in only a limited way. In the Biobreeding (BB) rat model of diabetes, infusion with IGF-I dramatically increased tibial epiphyseal width and overall bone growth despite persistent hyperglycemia, suggesting that stimulatory effects on bone may be independent of metabolic affects of IGF-I [69]. Recently, we have explored the impact of IGF-I treatment in a mouse model of T1D on bone regeneration and bone strength. Regenerate bone was assessed by distraction osteogenesis [56]. IGF-I treatment significantly improved regenerate bone formation in this model. Furthermore, significant reductions in trabecular thickness, yield strength and peak force, were also improved with IGF-I treatment in diabetes [70]. These findings demonstrate that despite persistent hyperglycemia and insulinopenia, IGF-I therapy can promote new bone formation and improve biomechanical properties of bone in T1D.

Potential signaling pathways critical to insulin and IGF-1 induced bone formation in diabetic osteopathy

In the diabetic bone, other pro-osteogenic pathways may be disrupted, such as the Wnt-signaling pathway [71]. The current understanding of these anabolic pathways in skeletal development and homeostasis suggests that insulin and IGF-1 signaling could crosstalk with two major pro-osteogenic pathways that ultimately regulate RUNX2 activity: the canonical Wnt signaling pathway and the BMP-2 signaling pathway. The importance of the canonical Wnt signaling pathway in determination of bone mass has been extensively documented and confirmed in both animal models and in human genetic conditions in which loss-of-function or gain-of-function mutations in specific components of this pathway either disrupt or accentuate bone formation and/or osteoblastogenesis [72]. Key elements of this pathway are diagrammed in (Figure 3). Briefly, Wnts (secreted lipid modified proteins) are known to bind to a receptor complex consisting of lipoprotein receptor-related proteins 5 or 6 (LRP5/LRP6) with frizzled [72]. In the absence of Wnt ligand, a "destruction complex" consisting of glycogen synthase kinase 3 (GSK-3), Axin, and tumor suppressor adenomatous polyposis coli protein (APC) mediates the phosphorylation of β-catenin, resulting in its proteolysis. In the presence of Wnt ligand, phosphorylation and inactivation of GSK-3 occurs, inhibiting the constitutive phosphorylation and subsequent enzymatic degradation of β-catenin. β-catenin then accumulates in the cytoplasm, translocates into the nucleus, where it associates with the human T-cell factor 1 (TCF-1), mouse lymphoid enhancer factor (LEF-1) family of transcription factors and initiates the expression of Wnt target genes, including RUNX2. GSK-3 is a multifunctional kinase involved in numerous cellular functions, including regulation of insulin-dependent glycogen synthesis. Specifically, insulin, via the PI3K/AKT pathway, inhibits GSK-3 activity in skeletal muscle [73], promoting glycogen synthesis. While there remains debate over cross-signaling via the insulin/IGF-1 pathway and the Wnt signaling pathway through GSK-3 [74], studies have now shown that IGF-1 signaling can enhance Wnt protein production and activate

β-catenin [75]. Furthermore, inhibitors of Wnts can partially inhibit IGF-1 actions [75]. Bone morphogenetic protein (BMP)-2 induced osteogenesis is another major pathway contributing to bone formation (Figure 3). The interaction of BMPs with BMP receptors leads to the phosphorylation of Smads and ultimately to distal-less homeobox 5 (Dlx5) transcription. Dlx5 then independently regulates the osteogenic transcription factors, RUNX2. In mesenchymal stem cells, MAPK serves as a point of convergence for mediating up-regulation of osterix, a RUNX2 target gene and major promoter of osteogenesis, via BMP-2 and IGF-1 signaling [76]. Thus, these examples serve to demonstrate the great potential for insulin and IGF-1 signaling to synergize with other signaling pathways involved in promoting osteogenesis.

Summary and Significance

Insulin and IGF-1 may exert independent effects on skeletal homeostasis, yet they are highly homologous peptides and can cross-signal through insulin receptors and IGF-1 receptors, and they share downstream mediators. (Table 1) summarizes various mouse models in which components of insulin and/or IGF-I signaling have been independently assessed in skeletogenesis. Furthermore, both insulin and IGF-I may crosstalk with other pro-osteogenic pathways (e.g., Wnt and BMP-2). The relative contributions of insulin dysregulation (i.e., hypoinsulinemia in T1D vs. hyperinsulinemia in T2D) and/or IGF-1 deficiency (T1D) to skeletal integrity in diabetes remains largely unexplored. Thus, investigations into the specific effects of insulin and IGF-1 on osteoblastogenesis and bone formation as well as the relative contribution of insulin and IGF-1 to diabetic osteopathy are critical to understanding how manipulation of these hormones may improve skeletal health in persons with diabetes.

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