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Regulation of Glucose Handling by the Skeleton: Insights From Mouse and Human Studies

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The skeleton is now recognized as an endocrine organ regulating a growing number of physiological processes. Its endocrine function came to light when it was realized that osteoblasts, the bone-forming cells, contribute to the regulation of energy homeostasis by favoring glucose metabolism, insulin sensitivity, and energy expenditure. Accumulating evidence from mouse studies demonstrated that the traditional bone formation marker osteocalcin secreted by osteoblasts, when undercarboxylated through a resorption- and insulin-dependent mechanism, is an active hormone that promotes insulin secretion and improves insulin sensitivity. This metabolic process is counterbalanced by sympathetic nervous system signaling in osteoblasts that under the control of leptin favors carboxylation and therefore inactivation of osteocalcin. These observations in mice triggered extensive studies exploring the cross talk between bone, pancreas, and fat and showed that, in turn, osteoblasts receive signals from endocrine organs during chronic hyperglycemia and insulin resistance that impact their fuel utilization and substrate availability. Clinical studies also have added support to the notion that bone regulates glucose metabolism in humans. This review highlights recent advances in our understanding of the endocrine functions of bone and explores their relationship to clinical observations.

DIABETES AND BONE

A link between the skeleton and glucose handling has long been suggested. Initially this relationship was mainly thought to be the consequence of metabolic dysregulation on bone health. Diabetes is a disease affecting glucose utilization principally, but not only, in muscle and adipose tissue. In reality the hyperglycemia impacts bone as it affects osteoblast differentiation, as well as the quality of the bone matrix. Impaired bone formation is often associated with increased marrow adiposity (1), which is a hallmark of patients both with type 1 and with type 2 diabetes (T1D and T2D). Importantly, in addition to compromised recruitment of osteogenic progenitors, osteoblast differentiation is impaired by chronic hyperglycemia likely contributing to an increased risk of vertebral, hip, and nonvertebral fractures in individuals with diabetes (2,3). Recently, the field has further expanded, and bone biology has been enriched by a number of studies providing evidence that bone is an endocrine organ regulating insulin secretion and glucose homeostasis.

THE SKELETON AS AN ENDOCRINE ORGAN

A conjunction of cell biological and clinical evidence led to the hypothesis that there may be a common regulation, endocrine in nature, of bone growth, glucose handling, and reproduction. This hypothesis implied that bone should receive signals from organs such as fat, pancreas, or even the brain but, perhaps more provocatively, it should also be an endocrine organ regulating glucose handling and reproduction. The first genetic evidence in mice and in humans that this tripartite regulation exists was the demonstration that in addition to its well-known action on suppressing appetite and favoring energy expenditure (4), the adipocyte-derived hormone leptin is a potent inhibitor of bone mineral accrual in rodents, sheep, and humans (5–8). The second aspect of the original hypothesis was verified when it was demonstrated that osteocalcin, a circulating peptide historically viewed as a bone formation biomarker, is also a hormone regulating glucose metabolism.

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Osteocalcin is a small protein synthesized by osteoblasts and osteocytes that is carboxylated by the vitamin K–dependent glutamate carboxylase at three glutamic acid residues. This postranslational modification confers to proteins high affinity for mineral ions and hydroxyapatite, which explains why osteocalcin accumulates in the bone matrix. In addition to the carboxylated form, a small but measurable amount of undercarboxylated osteocalcin (ucOC) exists. ucOC has low binding affinity to hydroxyapatite and thus is more readily released into the circulation, and it is the hormonally active form of the molecule. Osteocalcin decarboxylation and activity is regulated by insulin signaling in osteoblasts. Insulin binds to its receptor in osteoblasts and disengages it from its role as a substrate for a protein tyrosine phosphatase, an embryonic stem cell phosphatase (ESP). The activated insulin receptor subsequently activates a molecular pathway that induces bone resorption, acidification of the bone extracellular matrix, and thereby osteocalcin decarboxylation. The functional equivalent of ESP in human osteoblasts is protein tyrosine phosphatase 1B, a tyrosine phosphatase already known to inactivate the insulin receptor in other cell types (9).

This review intends to present in a synthetic manner the knowledge acquired in recent years through preclinical and clinical studies about the contribution of bone as an endocrine organ to glucose homeostasis.

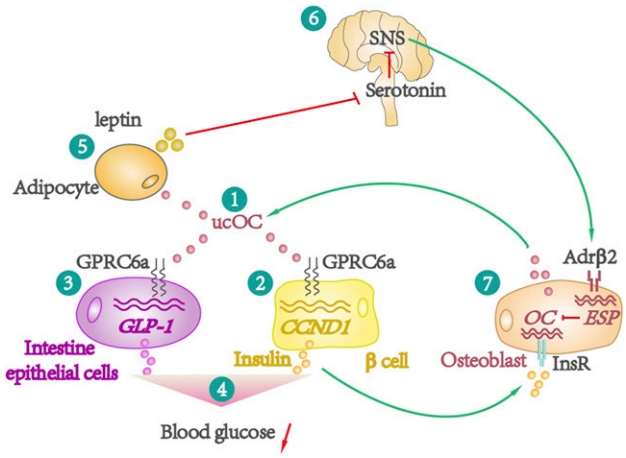

Figure 1—An integrative view on the role of bone in the regulation of energy homeostasis. The bioactive form of osteocalcin (ucOC) (1) binds to its Gprc6a receptor expressed on pancreatic β-cells (2) and intestinal epithelial cells (3), leading to the proliferation of β-cells and the release of GLP-1, thus stimulating insulin secretion (4). This action is counterbalanced by leptin signaling in the SNS: leptin crosses the blood-brain barrier (5) and binds to serotonergic neurons in the brain stem and inhibits serotonin synthesis (6), thereby diminishing the inhibitory effect of serotonin on sympathetic tone. With the increase of SNS activity, a larger amount of catecholamines can bind to Adrβ2 present on osteoblasts to increase Esp expression, thus decreasing insulin signaling on osteoblasts (7). Green lines indicate stimulation; red lines indicate inhibition. CCND1, cyclin D1.

THE ROLE OF BONE IN GLUCOSE METABOLISM: AN OVERVIEW OF LESSONS FROM MOUSE GENETICS

Evidence implicating osteocalcin as a regulator of glucose metabolism originated from observations that homozygous osteocalcin-deficient ($Ocn^{-/-}$) mice are hyperglycemic and hypoinsulinemic and have reduced pancreatic β-cell mass due to decreased β-cell proliferation (10). Conversely, delivery of ucOC to wild-type mice stimulates β-cell proliferation and insulin production, improves glucose homeostasis and increases energy expenditure, and protects against diet-induced obesity (11).

In direct contrast to mice lacking *Esp* in all cells ($Esp^{-/-}$) or only in osteoblasts ($Esp_{osb}^{-/-}$), $Ocn^{-/-}$ mice were glucose intolerant and insulin resistant (10). Remarkably, $Esp_{osb}^{-/-}$ mice display higher serum levels of ucOC. Normalization of their osteocalcin levels rescued the metabolic phenotype of $Esp^{-/-}$ mice, indicating that $Esp^{-/-}$ mice are a gain-of-function model of osteocalcin activity. Subsequent in vitro and in vivo studies demonstrated that *Esp* regulates glucose metabolism by indirectly suppressing the decarboxylation of osteocalcin. These results suggested that osteocalcin promotes insulin secretion and improves insulin sensitivity in mice and that osteocalcin decarboxylation, specifically in Glu13 (Glu17 in humans), is an important determinant of its metabolic activity (Fig. 1). Results of insulin tolerance tests performed in mice lacking osteocalcin or its receptor only in β-cells and results of euglycemic-hyperinsulinemic clamps performed in $osteocalcin^{-/-}$ mice are compatible with the notion that osteocalcin exerts a favorable influence on insulin sensitivity through mechanisms that are not elucidated.

Studies in mice have revealed that carboxylation of osteocalcin is modulated by two means: transcriptionally in osteoblasts and posttranslationally during bone resorption. In osteoblasts, two transcription factors, forkhead box O and activating transcription factor 4, suppress osteocalcin activity by favoring *Esp* expression (12) (Fig. 2). The biochemical mechanism whereby decarboxylation of osteocalcin occurs is intimately linked to insulin signaling in osteoblasts, which has a dual effect: it favors osteocalcin production by suppressing the expression of the Runx2 inhibitor, Twist2, but more notably it limits the production of the antiosteoclastogenic cytokine osteoprotegerin (OPG) (13), thus increasing osteoclast numbers and bone resorption. This proresorptive action of insulin signaling in osteoblasts facilitates the decarboxylation (activation) of osteocalcin stored in the bone matrix because only an acidic environment such as the one present in the resorption lacuna (pH 4.5) can decarboxylate proteins in the extracellular milieu. In agreement with these observations, $InsR_{osb}^{-/-}$ mice, which lack the insulin receptor in osteoblasts, have low circulating ucOC levels and are glucose intolerant and insulin resistant on a normal diet. Thus, in a feed-forward loop, insulin signaling in osteoblasts facilitates the production of ucOC, the active form

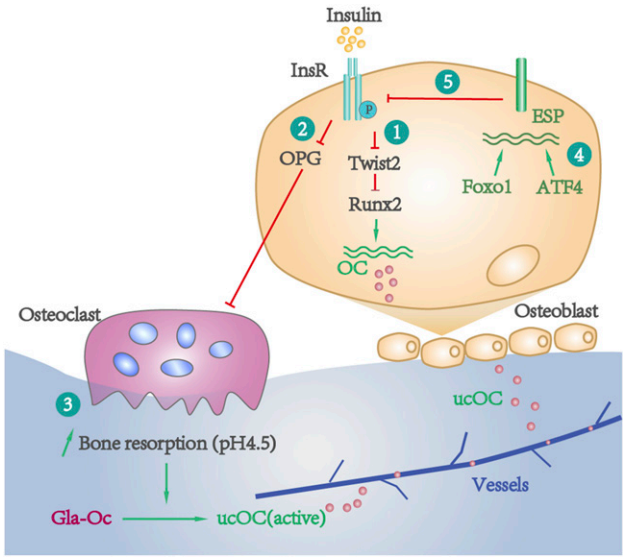

Figure 2—Insulin signaling in osteoblasts facilitates the formation of metabolic active form of osteocalcin (ucOC). Insulin signaling on osteoblasts suppresses the expression of Twist2, a Runx2 inhibitor, thus favoring the production of osteocalcin (1), and, at the same time, it limits the production of OPG and enhances osteoclast activity (2), thus providing an acidic environment in the resorption lacuna for the formation of ucOC (3). Transcription factors forkhead box O (FoxO1) and activating transcription factor 4 (ATF4) act synergistically to suppress the bioactivity of osteocalcin by upregulating the expression of Esp (4), which can dephosphorylate the insulin receptor and inactivate its signaling on osteoblasts (5). Green lines indicate stimulation; red lines indicate inhibition. Gla-Oc, carboxylated osteocalcin.

of osteocalcin that in turn acts on pancreatic β-cells to stimulate insulin synthesis and secretion (Fig. 2).

If the activity of this feed-forward loop were not limited, it would lead to hypoglycemia. The process of enhanced insulin production through the osteoblasts is counterbalanced at least in part by a leptin-dependent central control of osteocalcin carboxylation (Fig. 1). Leptin secreted by adipocytes crosses the blood-brain barrier and inhibits bone mineral accrual by suppressing brain serotonin synthesis and thereby increasing the activity of the sympathetic nervous system (SNS). In turn, the SNS acts through *Adrβ2* expressed in osteoblasts to increase *Esp* expression, thus decreasing insulin signaling in osteoblasts, osteocalcin bioactivity, and therefore insulin secretion (14).

How does osteocalcin signal into pancreatic β-cells? It had previously been demonstrated that the G-protein–coupled receptor Gprc6a could mediate osteocalcin signaling in Leydig cells of the testes. This receptor is also expressed in pancreatic islets, and its inactivation in the β-cell lineage ($Gprc6a_{Pdx}^{-/-}$ mice) leads to glucose intolerance, decreased insulin production, and diminished β-cell area and mass (15). $Gprc6a_{Pdx}^{-/-}$ mice showed reduced cyclin D1 expression in islet extracts, which is consistent with the decreased β-cell proliferation. The promoting effect of osteocalcin/Gprc6a signaling on β-cell mass accrual is active as early as during the perinatal peak of β-cell proliferation (15,16). This is however not the only mechanism whereby osteocalcin contributes to glucose homeostasis. It was recently shown that osteocalcin favors glucose uptake in myoblasts and that by this mechanism it contributes to glucose homeostasis since mice lacking Gprc6a in myofibers are hyperglycemic (17). Gprc6a is also expressed in epithelial cells of the small intestine (18), a site responsible for the secretion of glucagon-like peptide 1 (GLP-1). GLP-1 fulfills multiple physiological functions including stimulating insulin secretion in a glucose-dependent manner. Since ucOC administration to mice through intraperitoneal, oral, or intravenous routes can increase serum GLP-1 level, it is possible that part of the stimulatory effect of ucOC on insulin secretion is mediated in part by GLP-1 (18).

In conclusion, studies in mice have uncovered an intricate regulation of glucose homeostasis by the skeleton that comprises two interacting and counterbalancing loops. In a positive feed-forward process, osteocalcin produced by osteoblasts and activated via bone resorption to become ucOC acts on pancreatic β-cells to increase insulin production. In turn, insulin signaling feeds back to bone, favoring the formation of ucOC. On the other hand, a fat–brain–SNS negative loop under the control of leptin suppresses osteocalcin decarboxylation at the level of the osteoblasts. Going forward, a fundamental question that needs to be addressed is whether ucOC is an insulin-sensitizing molecule or whether it favors glucose uptake on its own in vivo.

HYPERGLYCEMIA AND OBESITY SIGNALING IN OSTEOBLASTS

The relationship between bone and glucose metabolism is more complex and involves also, in pathological circumstances, the deleterious influence of hyperglycemia on osteoblast biology. Dynamic histomorphometric analyses have demonstrated that diabetic bone disease is associated with a profound reduction in bone formation often accompanied by increased marrow adiposity (19). This association has led to the hypothesis that in diabetes mesenchymal progenitor allocation favors differentiation of mesenchymal cells into the adipogenic rather than the osteoblastic lineage. Importantly, in addition to compromised recruitment of osteogenic progenitors, the differentiating function of the osteoblast is also impaired by chronic hyperglycemia (3).

During bone modeling and remodeling, ATP demand drives the type of substrate utilization that is operative, thus requiring the upregulation of context-specific transcription factors. Early osteogenic progenitors and pluripotent stem cells utilize glucose as their primary fuel, even in aerobic states (i.e., the Warburg effect). For instance, during LRP5-induced osteoblastogenesis, glucose is diverted from the tricarboxylic acid cycle to glycolysis (20). In contrast to that in myoblasts, glucose uptake in osteoblasts is not insulin dependent. Glut1 expression predominates in osteoblasts at all stages of differentiation,

and glucose uptake through Glut1 increases accumulation of Runx2, a key transcriptional regulator of osteoblast differentiation, and of type I collagen (21). Both pathways downstream of glucose uptake recruit AMPK (22). Evidence suggests that as osteoblasts differentiate, they may rely less on oxidative phosphorylation of fatty acids and may require distinct ATP needs (e.g., collagen synthesis vs. matrix mineralization). Similarly, autophagy may also be enhanced (23). Importantly, metformin has been shown to stimulate osteoblast differentiation through upregulation of AMPK in a time-dependent manner. Hence, it is possible that mechanical loading, which also activates AMPK, may favor osteobastogenesis and/or collagen synthesis and bone formation during exercise.

In high glucose states and/or obesity with insulin resistance, several events could impair bone formation and lead to diabetic bone disease, a syndrome associated with frequent fractures, particularly of the distal extremities, in both T1D and T2D patients (1,2,24). First, a high-fat diet enhances free intracellular saturated fatty acids, leading to ubiquitination and subsequent degradation of the insulin receptor in mice (25). This in turn reduces bone resorption and therefore the production of ucOC, which impacts insulin secretion and glucose homeostasis. Second, at least in vitro, high ambient glucose concentrations can lead to enhanced glycolysis and suppression of oxidative phosphorylation. If such a fine balance between these two energy-driven processes exists in vivo, then osteoblast function could be impaired by suppression of fatty acid oxidation. Third, excess bone marrow adipogenesis may contribute to osteoblast dysfunction. Preadipocyte progenitors and newly differentiated adipocytes primarily use mitochondrial respiration to supply energy for their metabolic needs. The process of glucose entry and fatty acid oxidation through the Krebs cycle generates more molecules of ATP per mole of glucose (36:1) than glycolysis (2:1), but it comes at a cost as mitochondrial respiration leads to the generation of reactive oxygen species (ROS) from the electron transport chain. ROS compounds can further suppress mitochondrial respiration and promote an adipogenic program. Excess ROS in adipocytes may also cause mitochondrial DNA damage or further changes to complex I in the electron transport chain leading to metabolic dysfunction. Further support for that premise comes from studies using positron emission tomography/computed tomography scanning in T2D showing that bone marrow adipocytes exhibit insulin resistance and may have impaired free fatty acid uptake. Insulin-resistant adipocytes in the marrow may influence nearby osteoblasts by secreting inflammatory cytokines thereby further impairing osteogenesis. Unlike what is the case for other depots, marrow adipose tissue increases during high-fat diet and caloric restriction (26). *ob/ob* mice that lack leptin exhibit high marrow adipose tissue and excessive peripheral fat. Conversely, central or peripheral administration of leptin reduces bone marrow adiposity in a manner analogous to lipolysis in peripheral adipose depots (27). Sympathetic activation by β-adrenergic agents or cold induction reduces marrow adiposity but without any browning of marrow adipocytes (28).

Bone mass accrual is highly dependent on nutrients and in particular glucose intake. Indeed, glucose is a main regulator of both osteoblast differentiation and function (21), and any diet that affects glucose uptake will affect bone mass. This is best illustrated in children fed a ketogenic diet for intractable epilepsy, as a side effect of this treatment is that longitudinal growth is hampered (29). The paramount importance of glucose for osteoblast differentiation and bone formation explains why bone mass can decrease even in situations such as hypoleptinemia when blood glucose levels are low (21).

BONE AS A REGULATOR OF GLUCOSE HANDLING: LESSONS FROM CLINICAL STUDIES

As in rodents, both osteocalcin and ucOC are present in the general circulation in humans, and recent studies showed that decarboxylated osteocalcin stimulates insulin processing and secretion and β-cell proliferation in cultured human islets as well (30). Moreover, decarboxylated osteocalcin induced β-cell proliferation of human islets transplanted into immunocompromised mice. Notwithstanding limitations that have to do with the fact that some of these experiments were performed in an in vitro human islet culture model, these studies contribute to our understanding of these pathways in a human model by showing direct enhancement of β-cell function in human islets by osteocalcin and suggesting that the enhanced insulin response in osteocalcin-treated human islets was in part due to β-cell proliferation. Not surprisingly, the antidiabetes effect of ucOC observed in rodents has prompted numerous clinical studies that have overall suggested that serum osteocalcin level is a marker of glucose tolerance in humans. The evidence of a role for ucOC in promoting human insulin production and β-cell proliferation may stimulate interest in its use in clinical trials to treat diabetes, particularly in early T2D where ucOC could increase both insulin secretion and glucose homeostasis.

Cross-sectional Studies

While reviewing the clinical data, it is important to note that, unlike in mice, there is no available assay in humans that measures serum levels of bioactive osteocalcin, the form decarboxylated on the single Glu13 (corresponding to Glu17 in humans) residue (31). The most commonly used assays either measure total osteocalcin levels or, when a hydroxyapatite affinity assay is performed, quantify the levels of fully decarboxylated osteocalcin. We are not aware of any assays in humans that measure, in a manner that has been experimentally proven to be reliable, osteocalcin undercarboxylated at Glu13. These methodological limitations and the limited number of cases in some of these studies are a note of caution while interpreting current human cross-sectional studies. This is why we will next focus on studies describing associations between total osteocalcin and glucose, fat, or insulin resistance.

Higher serum levels of total osteocalcin (or ucOC) in adults, young adults, and children of both sexes are associated with lower plasma glucose levels, improved glucose tolerance, β-cell function, and insulin sensitivity in normal subjects and in patients with T2D, prediabetes, gestational diabetes, or metabolic syndrome (32). Higher circulating osteocalcin levels are also correlated with less total and visceral fat. Serum osteocalcin levels are positively associated with fat-free mass in premenopausal women after controlling for confounders that might influence osteocalcin levels such as age, circulating OPG, RANKL, estradiol, and glucose concentrations, as well as bone mineral density (33). Serum osteocalcin levels also increase significantly after 3 months of weight loss and are associated with lower BMI, less visceral fat mass, decreased leptin serum concentration, and lower HOMA score independently of serum adiponectin or ghrelin concentrations in healthy obese men. In addition, single nucleotide polymorphisms of osteocalcin, especially the G-C-G haplotype composed of three single nucleotide polymorphisms (rs2758605-rs1543294-rs2241106), are significantly associated with age- and sex-adjusted BMI and principal component scores of skinfold and circumference measurements, suggesting a genetic link between osteocalcin and obesity-related phenotypes in humans (34). Collectively, these clinical data supported the beneficial effects of osteocalcin in glucose and lipid metabolism.

Longitudinal Studies

A few longitudinal studies have assessed the predictive value of osteocalcin with respect to glucose levels and insulin resistance or the occurrence of diabetes. In a 3-year longitudinal study involving 162 older men and women without diabetes, a higher baseline carboxylated osteocalcin was predictive of a smaller increase in HOMA of insulin resistance and fasting insulin and glucose levels after adjustment for multiple confounders. One post hoc analysis revealed that higher osteocalcin exposure level was a predictor of a lower rise in fasting plasma glucose at year 3 (35). In another cross-sectional and longitudinal study in older men with and without diabetes having more than three cardiovascular disease risk factors (hypertension, dyslipidemia, overweight, family history of premature cardiovascular disease), an increase in serum osteocalcin was associated with enhanced HOMA β-cell function, even after adjusting confounding factors like BMI, physical activity, presence of T2D, etc. The associations became stronger when subjects taking antidiabetes drugs were excluded possibly because antidiabetes drugs alter insulin secretion and insulin sensitivity.

In a retrospective follow-up study, 1,229 middle-aged men without diabetes were monitored regularly for a mean of 8.4 years to observe the development of T2D. This study showed that baseline osteocalcin levels were not associated with the development of T2D (36). In another nested case-control study, 63 men without diabetes at baseline who developed T2D during a 10-year follow-up period were compared with 63 age- and BMI-matched men without diabetes who did not develop diabetes. A reduced baseline serum osteocalcin level adjusted for fasting plasma glucose, age, BMI, and exercise was identified in this study as one of the independent risk factors for the development of diabetes. More recent clinical findings showed that higher bone turnover rate, as reflected by elevated bone formation and resorption markers, was associated with lower risk of developing T2D (37). Hence, the results of a majority of longitudinal investigations suggest that serum osteocalcin levels were positively associated with β-cell function but negatively correlated with insulin resistance and the subsequent development of T2D. Because of their nature, these studies could not demonstrate whether it is the decrease in osteocalcin levels that contributes to the compromised glucose metabolism or vice versa. Additional prospective studies are needed to better examine the association and contribution of either baseline or the changes of osteocalcin to the incidence of T2D.

The Role of Insulin and Glucose in Osteocalcin Function in Humans

Studies in mice have demonstrated that insulin signaling in osteoblasts leads to an increase in osteoclast activity, favoring the generation of ucOC (13). To determine the effect of insulin on bone in humans, a post hoc analysis in a previous glucose clamp study failed to show significant changes in bone turnover markers from baseline (38). These results need to be interpreted cautiously. It is not known for instance whether such an acute variation in insulin levels is long or sufficient enough to induce any alterations in bone remodeling. However, the same glucose clamp study (38) reported that insulin sensitivity parameters, e.g., steady-state glucose infusion rate and glucose disappearance rate, were positively associated with bone resorption and tended to be positively associated with ucOC levels.

A different study evaluated the effect of glucose on bone remodeling. An oral glucose tolerance test was performed in a small group of young normal subjects. After oral glucose intake, serum osteocalcin and bone formation and resorption biomarkers all decreased (39). Because this was not an insulin clamp study, it is unknown whether the suppressive effect of oral glucose load on bone remodeling markers is derived from the changes in glucose levels, insulin levels, or both. However, the improvement of glycemic control in T2D patients was associated with increased serum osteocalcin levels (40). Although more studies are needed, these reports indicate that acute variations of circulating insulin and glucose levels have little influence on bone turnover.

Impact of Antiosteoporosis Medications on Measures of Glycemia

If, as inferred by animal studies, bone resorption favors the release of activated osteocalcin stored in bone matrix, one could be concerned that antiresorptive drugs for treating

osteoporosis might have detrimental effects on the ability of bone cells to utilize nutrients.

Bisphosphonates (e.g., alendronate), hormone replacement therapy, and selective estrogen receptor modulators (e.g., raloxifene) are effective antiresorptive therapies for osteoporosis. Some clinical studies have evaluated the relevance of such changes to metabolic phenotypes in humans. Those studies conducted in postmenopausal women with or without T2D reported either no effect on glucose metabolism (41) or, in contrast, a 30–50% or more risk reduction for the development of T2D in users of antiresorptive drugs compared with nonusers, especially in long-term users (42). Moreover, recent epidemiological data in the prospective population-based Bruneck Study showed that high serum concentration of soluble RANKL was a significant and independent risk predictor of T2D manifestation and that antiresorptive therapy with anti-RANKL blockade has beneficial effects on glucose metabolism, as it improved insulin sensitivity and ameliorated or even normalized plasma glucose concentrations and glucose tolerance in genetic and nutritional mouse models of T2D (43). Results from two large clinical trials, The Women's Health Initiative (WHI) and The Heart and Estrogen/progestin Replacement Study (HERS), indicated that hormone replacement therapy could improve insulin secretion and insulin sensitivity in postmenopausal women with hyperinsulinemia, independently of its antiresorptive action (44–46). This effect could be mediated through the hypocholesterolemic activity of the drugs or, as studies in rodent models of T2D have suggested, through protective effects of estrogen against β-cell lipogenesis and failure (47).

Human studies involving similar or different types of antiresorptives cannot be directly compared with studies in mouse models since their design is intrinsically different. Several issues need to be considered. First, with the exception of one study that ablated osteoclasts entirely, mouse studies have not tested the effect of decreasing bone resorption in glucose metabolism. Second, the effects of antiresorptives on glucose metabolism may point to the same direction as animal studies do, but if those effects are too small, they may not have a clinical outcome. Discrepancies between observations in humans and observations made in genetically modified mice may be the result of a much greater suppression of osteocalcin in a mouse model compared with a human receiving antiresorptive therapy.

For a bone anabolic agent, such as parathyroid hormone (PTH) (1-34), a positive correlation between osteocalcin and glucose metabolism was observed. Three months of PTH (1-34) treatment in 64 postmenopausal women with osteoporosis was associated with a median 1.7-fold elevation in total osteocalcin serum concentration and a decrease in body weight and fat mass (41). No changes in glucose and insulin levels were observed in the PTH treatment group. These observations in humans are in agreement with observations in an obese T2D rat model, in which an increase in serum osteocalcin concentration after 4 weeks of treatment with PTH (1-34) was accompanied by a reduction in serum glucose level without changes in serum insulin concentrations (48). These findings imply a positive influence of bone health on glucose handling in humans.

CONCLUSIONS AND PERSPECTIVE

In general, cross-sectional human studies indicate a positive association of osteocalcin with insulin secretion, insulin sensitivity, glucose tolerance, and glucose control. These studies are at this time largely descriptive and associational and lack the conclusive indications conferred by genetic observations or intervention studies. However, recent observations that osteocalcin stimulates β-cell mass and function of human islets are in support of the translational value of the studies in mice (30). In addition, concurrent work addressing the role of osteocalcin in male fertility has shown that a mutation in the osteocalcin receptor in men is associated with infertility (49). Therefore, additional genetic or epidemiological studies in humans are needed to identify the cause-and-effect relationship between osteocalcin levels and glucose homeostasis in humans.

In summary, the discovery of bone as a regulator of glucose homeostasis opens a new field to investigate the cross talk between bone and whole-body physiology. In the reverse feedback, fuel utilization and substrate availability in osteoblasts are impacted during chronic hyperglycemia and insulin resistance. Even though more studies of various kinds are needed, the notion that there is a cross talk between bone and glucose handling in mice and in humans is progressively gaining more attention. By understanding all the molecular aspects of this relationship, it is possible that novel treatments for diabetic bone disease may be formulated. In addition, with more evidence accumulated from experimental and clinical studies, a clearer picture of the complicated and delicate novel network comprising the skeleton, pancreas, adipose tissue, and perhaps other glucose-regulating organs will emerge.

In parallel with the regulatory role of osteocalcin in glucose metabolism to be studied in preclinical studies, and more precisely delineated in humans, more questions arise that are worth of future investigation: is there a "threshold" and/or "limit" for osteocalcin and/or ucOC to affect glucose metabolism? In support of this hypothesis, a recent clinical study suggested that the skeleton may normalize subtle increases of HbA_{1c} in glucose-tolerant individuals and subjects with prediabetes by enhancing osteoblast and osteoclast activities (50). Since osteocytes can sense the mechanical loading imposed on bone, is it possible that skeleton can "feel" the subtle variations of glucose level in the surrounding milieu? Since bone constantly renews itself, is there an optimal "priming window" or "timing period" for skeleton to preserve or exert its impact on glucose metabolism? It is likely that we do not have yet a full understanding of the extent of the functions of bone and that additional studies on osteocalcin and other hormones and cytokines made by bone cells

are needed to better understand the importance of skeletal hormones.

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