Effects of Glucose on Bone Markers: Overview of Current Knowledge with Focus on Diabetes, Glucose, and Bone Markers

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Abstract

Diabetes mellitus is associated with an increased risk of fracture. However, in patients with diabetes the bone mineral density does not explain this. Bone turnover markers give information on bone formation and bone resorption and may explain the decreased bone material competence in patients with diabetes. Diabetes mellitus is characterized by the lack of a fasting condition, which also may affect the general bone turnover and be reflected in the bone turnover markers. This chapter focuses on the relation between bone turnover markers and plasma glucose, and bone turnover markers in diabetes subjects. In clinical trials, an oral glucose tolerance test (OGTT) decreased bone resorption markers in both patients with type 2 diabetes and healthy individuals. During an OGTT, bone formation markers were decreased in healthy individuals, but the markers were not investigated in patients with diabetes. An intravenous glucose tolerance test decreases the bone resorption marker C-terminal cross-linked telopeptide of type-I collagen (CTX) but not as much as the OGTT. Therefore a gastrointestinal interaction may affect the relation between glucose and bone turnover markers. In patients with diabetes, both CTX and the bone formation marker osteocalcin were decreased compared to controls. However, heterogeneity was present in the markers, which may be due to differences in glycemic status. In vitro studies show direct effects of glucose on the bone cells: osteoblasts, osteoclasts, and osteocytes. Hyperglycemia had detrimental effects on osteoblasts and osteoclasts and increased the sclerostin production in osteocytes; thus both bone resorption and formation seemed to decrease during hyperglycemia. However, in the mild hyperglycemia with a glucose level of 11–15 mmol/l, the osteoblasts increased the mineralization. Thus, hyperglycemia may hypermineralize the bone, so the bone mineral density is increased relatively to the bone material competence due to a relative decrease in non-mineralized matrix, e.g., collagen.

Further, investigations are needed to determine if the glucose bone turnover marker interaction may be a prognostic marker of fracture in patients with diabetes.

Keywords

Glucose • Hyperglycemia • Bone turnover markers • Bone turnover • Diabetes mellitus • Osteoblasts • Osteoclasts • Osteocytes • Hypermineralization

List of Abbreviations

BAP	Bone-specific alkaline phosphatase
BMD	Bone mineral density
BSP	Bone sialoprotein
CA/P	Calcium/phosphate
CTX	C-terminal cross-linked telopeptide of type-I collagen
FGF-23	Fibroblast growth factor-23
FRAX	The fracture risk assessment tool
GIP	Gastric inhibitory peptide

GLP-1	Glucagon-like peptide-1
GLP-2	Glucagon-like peptide-2
HbA1c	Glycated hemoglobin A1c
hMSC	Human mesenchymal stem cells
hMSC-TERT	Human mesenchymal stem cells telomerase-immortalized
IGF-1	Insulin-like growth factor-1
IVGTT	Intravenous glucose tolerance test
NTX	N-terminal cross-linked telopeptide of type-I collagen
OGTT	Oral glucose tolerance test
OPG	Osteoprotegerin
P1NP	Procollagen type 1 N-terminal propeptide
PTH	Parathyroid hormone
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor Activator of Nuclear factor Kappa beta Ligand
Runx2	Runt-related protein 2
TRAP	Tartrate resistant acid phosphatase

Key Facts

Key Facts of Diabetes Mellitus and Related Bone Disease

- Diabetes is an extremely common disease throughout the world, with an estimated 592 million cases in 2035.
- Type 1 diabetes is characterized by a near complete loss of insulin production.
- Type 2 diabetes is characterized by a decrease in insulin production relative to insulin sensitivity.
- Diagnosis of diabetes mellitus can be made by measuring fasting plasma glucose above 7 mmol/l, a 2 h oral glucose tolerance test plasma glucose value above 11 mmol/l or an HbA1c of $\geq$ 48 mmol/mol.
- Apart from the well-known microvascular and macrovascular complications, diabetes is also related to poor bone health.
- The risk of hip fracture is suggested to be sevenfold increased in patients with type 1 diabetes and twofold increased in patients with type 2 diabetes.
- Bone mineral density is increased in type 2 diabetes and slightly decreased in type 1 diabetes, but the lower bone mineral density in type 1 diabetes cannot explain the increased risk of fracture.
- Patients with diabetes display lower C-terminal cross-linked telopeptide of type-I collagen and osteocalcin levels representing lower bone resorption and bone formation.
- The mechanisms behind the increased risk of fractures in diabetes are still unclear but could be related to lack of insulin, disturbed glucose metabolism, medication use, renal impairment, falls or other factors.

Key Facts of Bone Remodeling

- Bone consists of a mineralized matrix, mainly hydroxyapatite, a non-mineralized matrix, mainly collagen, and a cellular compartment.
- The cellular component of bone consist of osteoclasts that resorb bone tissue, osteoblasts that form new bone tissue, and osteocytes that are thought to regulate the bone turnover process.
- Bone remodeling is a highly coordinated process of degradation of old bone and creation of new bone.
- Bone remodeling consists of three phases: A bone resorption phase maintained by the osteoclasts, a reversal phase where the bone is prepared for the osteoblasts, and a bone formation phase where matrix is produced by osteoblasts and subsequently matured and mineralized.
- When the bone remodeling is out of balance, typically with degradation exceeding creation of bone, osteoporosis can arise.
- Bone turnover markers are biomarkers that reflect bone remodeling.
- Bone turnover markers can easily be measured in blood and are a useful tool in assessing bone remodeling. See Table 1.

Table 1 Overview of commonly used bone turnover markers

Bone turnover marker	Secreted from	Marker of
CTX	Product of collagen degradation	Bone resorption. Is a marker of collagen degradation
NTX	Product of collagen degradation	Bone resorption. Is a marker of collagen degradation
P1NP	Product of collagen formation	Bone formation. Is cleaved from collagen and a marker of collagen production
Osteocalcin	Osteoblasts	Formation of the bone matrix
Bone-specific alkaline phosphatase	Osteoblasts	Calcification of bone mineral matrix
Tartrate resistant acid phosphatase	Osteoclasts	Reflects osteoclast number and activity
RANKL	Osteoblasts and osteocytes	Stimulates osteoclasts and bone resorption through the RANK pathway
Osteoprotegerin	Osteoblasts and osteocytes	Is the antagonist of RANKL and is thus a marker of decreased bone resorption
Sclerostin	Osteocytes	Antagonist of the Wnt pathway. Inhibits bone formation and osteblastogenesis

C-terminal cross-linked telopeptide of type-I collagen (*CTX*), N-terminal cross-linked telopeptide of type-I collagen (*NTX*), Procollagen type 1 N-terminal propeptide (*P1NP*), Receptor activator of nuclear factor kappa-B (RANK), Receptor Activator of Nuclear factor Kappa beta Ligand (*RANKL*)

Key Facts of Glucose and Bone Turnover

- C-terminal cross-linked telopeptide of type-I collagen decreases within 20 min of an oral glucose tolerance test.
- When glucose is given intravenously, a decrease in C-terminal cross-linked telopeptide of type-I collagen is seen; only it is delayed by 1 h compared to the oral glucose tolerance test.
- The decrease in C-terminal cross-linked telopeptide of type-I collagen seen in an intravenous glucose tolerance test is significantly lower than that of the oral glucose tolerance test.
- In healthy males, a hyperglycemic clamp has been shown to induce a decrease in osteoprotegerin, whereas no change was seen during euglycemia.
- Procollagen type 1 N-terminal propeptide has both been reported to be stable and to decrease during an oral glucose tolerance test.
- Hyperglycemia may decrease osteoblast differentiation and bone formation and impair bone resorption by increasing osteoprotegerin.
- Hyperglycemia decreases the number of osteoclasts, inhibits osteoclastogenesis and osteoclast differentiation, and impairs the ability of osteoclasts to resorb mineralized matrix.
- Osteocytes react to hyperglycemia by increasing sclerostin production, which in turn inhibits the Wnt pathway and thereby bone formation.

Definitions of Words and Terms

Bone formation	The process of producing new bone tissue.
Bone resorption	The process of degrading old bone tissue.
Bone turnover	The process of old and damaged bone tissue being degraded and replaced by new bone tissue.
Euglycemia	The state with a normal concentration of glucose in the blood.
Hyperglycemia	The condition of having a higher than normal concentration of glucose in the blood.
Hyperglycemic clamp	A technique used in experiments where a constant but varying amount of glucose is infused intravenously in accordance to insulin secretion and glucose metabolism to keep blood glucose at a constant high level. Same technique can be used for achieving euglycemia (euglycemic clamp).
Hypermineralization	A state where bone is over-mineralized relative to its collagenous matrix.
Mineralization	The process of impregnate mineral in the matrix of the bone. The mineral content of bone is primarily hydroxyapatite, which primarily consists of calcium and phosphate.
Osteoblasts	The cell type responsible for bone formation.

Osteoclastogenesis	The development of osteoclasts.
Osteoclasts	The cell type responsible for bone resorption.
Osteocytes	The most common cell type in bone tissue, thought to be encased osteoblasts that control the activity of osteoblasts and osteoclasts through mechanosensory mechanisms.

Introduction

Diabetes Mellitus

Diabetes Mellitus is a highly prevalent condition throughout the world with an estimate of 592 million suffering from it in 2035 (International Diabetes Federation). Diabetes is characterized by a relatively decreased insulin production, with a complete lack of insulin production in type 1 diabetes and a decreased insulin production relatively to the insulin resistance in patients with type 2 diabetes. The decreased insulin production causes unstable fasting conditions and patients with diabetes may be diagnosed by increased fasting plasma (p-) glucose of >7 mmol/l, an 2 h value of >11 mmol/l at an oral glucose tolerance test (OGTT) (American Diabetes Association 2012) or an elevated glycated hemoglobin A1c (HbA1c) level of $\geq$ 48 mmol/mol. The glycemic regulation in patients with diabetes is disturbed and fasting p-glucose levels do not follow the same pattern as in non-diabetes subjects.

Bone Remodeling

Bone remodeling is the process of degradation of old bone and creation of new bone (Hadjidakis and Androulakis 2006; Khosla and Riggs 2005). Under optimal circumstances the degradation (bone resorption) and creation (bone formation) of bone are balanced. In osteoporotic individuals, the rate of resorption is higher than the rate of formation (Khosla and Riggs 2005) which diminish bone mass and bone mineral density (BMD). Bone is constructed by a mineralized matrix consisting of mainly hydroxyapatite, a non-mineralized matrix consisting of primarily collagen, and a cellular compartment consisting of the bone cells osteoclasts, osteoblasts, and osteocytes. Mechanical resistance is provided by the hydroxyapatite crystals whereas stability and elasticity are provided by the network of type I collagen (Boskey 2013).

Osteoclasts are the bone resorping cells and osteoblasts are the bone forming cells. The osteocytes may serve as main regulators of the bone remodeling as they react to mechanical stress. The osteocytes produce and secrete sclerostin, a Wnt pathway inhibitor that decreases bone formation, and fibroblast growth factor-23 (FGF-23) that stimulate phosphate excretion in the kidneys. Furthermore, Receptor Activator of Nuclear factor Kappa beta Ligand (RANKL), a promoter of bone resorption, and its antagonist osteoprotegerin (OPG) are secreted by osteocytes.

However, the main production of OPG and RANKL are from the osteoblasts (Hadjidakis and Androulakis 2006).

The bone remodeling is divided into three phases; a bone resorption phase performed by the osteoclasts, a reversal phase where the bone is prepared for the osteoblasts, and finally the bone formation phase that consists of the production, maturation, and mineralization of the matrix. The resorption phase may take 2 weeks, whereas bone formation may continue for as long as 4 months. In healthy subjects, the production of matrix and mineralization of matrix are at the same rate (Hadjidakis and Androulakis 2006). Figure 1 depicts the systems involved in bone remodeling.

Bone Turnover Markers

Bone turnover markers are biomarkers of the bone remodeling and a specific marker represents a specific phase of the bone remodeling. Bone turnover markers are released to the blood during the bone remodeling and are thus easily measured providing information on the bone turnover. Table 1 describes the most commonly used bone turnover markers. C-terminal cross-linked telopeptide of type-I collagen (CTX) and N-terminal cross-linked telopeptide of type-I collagen (NTX) are commonly used resorption markers that reflect collagen degradation. Tartrate resistant acid phosphatase (TRAP) reflects the activity of osteoclasts and is also a resorptive

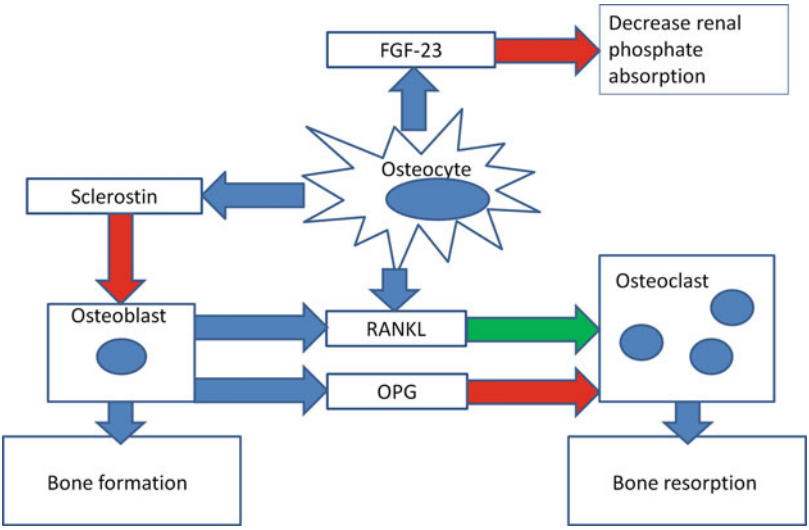

Fig. 1 ***Systems regulating bone remodeling.*** The osteocyte secretes sclerostin, which decrease bone formation and FGF-23 which increase phosphate excretion in the kidneys. The osteoblasts maintain bone formation and regulate the osteoclast through both OPG and RANKL. *Red arrows* are inhibitory actions, *green arrows* stimulatory actions, and *blue arrows* secreted products (With permission from the author (Starup-Linde 2015))

marker. Procollagen type 1 N-terminal propeptide (P1NP) is a formative marker, which is released during the cleavage of immature collagen. Osteocalcin, another formative marker, is an unmineralized matrix component and reflects the bone formation. Bone-specific alkaline phosphatase (BAP) is an enzyme produced during the mineralization phase of the bone formation (Starup-Linde 2013; Starup-Linde and Vestergaard 2015).

Analytical Factors

Bone turnover marker assays are offered by a large number of immunodiagnostic kit companies. Most are research-grade assays that are not intended for diagnostics use. Assays intended for diagnostic use are regulated by national and international bodies in terms of a range of validation parameters, particularly assay standardization (White 2011). This is in contrast to research-grade assays, which are unregulated and frequently missing assay characterization and standardization (Bowsher and Sailstad 2008). The above leads to considerable measurement differences between commercial assays and may result in conflicting research findings and slowing the implementation of bone turnover markers into routine clinical practice (Seibel et al. 2001; Whitham and Milford-Ward 2000). In this regard a recent publication stands out. The joint International Osteoporosis Foundation has recommended the use of CTX and P1NP as reference bone turnover markers in clinical trials and proposed strategies for standardization aiming for future inclusion in routine clinical practice and comparable values across assays (Vasikaran et al. 2011). Until such standardization has been attained, results and reference intervals from different assays should not be used interchangeably for clinical use, and care must be taken to address this important issue in research studies (Meier et al. 2009).

In addition to analytical issues, preanalytical factors are considered problematic with significant influence on measurements (Hannon and Eastell 2000). Preanalytical factors are factors such as sample handling, circadian, age, gender, menopausal status, and fractures. Of these, sample handling and circadian changes can be controlled by standardized sampling, sample handling, and collecting samples at the same time of day. Most other preanalytical factors cannot be controlled and their recognition is important in the interpretation of bone turnover marker results. Therefore, clinicians and researchers should be familiar with conditions where bone turnover levels are expected to be altered, for example in children, menopause, and after recent fracture (Seibel 2005). Another preanalytical factor commonly viewed as an obscuring factor but also of scientific interest and the focus of this review is the observed postprandial suppression of bone turnover levels (Clowes et al. 2003). This subject is further described in section "The Effect of Glucose Intake on Bone Turnover Markers in Humans."

Diabetes and Bone

Diabetes is related to microvascular and macrovascular complications (American Diabetes Association 2012). Until recently the increased risk of fracture was an

overseen complication. Thus, diabetes and bone may be related. The risk of hip fracture has been suggested to be sevenfold increased in patients with type 1 diabetes and twofold increased in patients with type 2 diabetes compared to non-diabetes individuals (Vestergaard 2007; Janghorbani et al. 2007). One would expect a similar lowering of BMD as it is the primary fracture predictive tool. However, BMD is increased in patients with type 2 diabetes and only slightly decreased in patients with type 1 diabetes and does not explain the increased fracture risk (Vestergaard 2007). Patients with diabetes have hypermineralized bone relative to their decreased bone material competence. When adding further fracture predictors to the model in The Fracture Risk Assessment Tool (FRAX), which also includes BMD, it underestimates both hip fracture risk and major osteoporotic fracture risk in patients with type 2 diabetes (Giangregorio et al. 2012). Furthermore, the increased fracture risk is not explained by either hypoglycemic events or the number of falls in patients with diabetes (Bonds et al. 2006; Vestergaard et al. 2005), or why the increased risk of fracture in diabetes patients seems to be bone related. The mechanism of the decreased bone quality and lack of fracture predictors is not well understood in diabetes; however, it may relate to the relative lack of insulin and disturbed glucose metabolism, but also factors as obesity, medication use, and renal impairment may affect the bone metabolism in patients with diabetes.

Glucose and Bone Turnover Markers

Clinical Studies

The Effect of Glucose Intake on Bone Turnover Markers in Humans

Patients with diabetes are at increased risk of fracture, thus their bone turnover may be altered. Clinical studies have investigated the effect of glucose on bone turnover markers. Table 2 presents the studies that have examined the effect of glucose ingestion on bone turnover markers. In men and women subjected to an OGTT, the bone resorption marker s-CTX and u-CTX decreased (Clowes et al. 2003; Henriksen et al. 2003; Bjarnason et al. 2002; Nissen et al. 2014; Chailurkit et al. 2008; Viljakainen et al. 2014; Paldanius et al. 2012; Schwetz et al. 2014; Karatzoglou et al. 2014). The decrease in s-CTX has been reported as early as 20 min after glucose ingestion (Clowes et al. 2003), whereas the decrease was delayed by one hour during the intravenous glucose tolerance test (IVGTT) (Bjarnason et al. 2002). The decrease in s-CTX was apparent in patients with type 2 diabetes but lower than in healthy controls (Chailurkit et al. 2008). Furthermore, the osteoclast specific marker TRAP decreased in both healthy obese and healthy non-obese individuals during OGTT (Viljakainen et al. 2014). A convincing effect of glucose intake on bone resorption markers was observed in these OGTT and IVGTT studies. The effect on bone formation markers was more unsettled, although both P1NP and osteocalcin have been reported to decrease. A decrease in s-osteocalcin has been shown two hours after an OGTT (Clowes et al. 2003; Viljakainen et al. 2014; Paldanius et al. 2012; Schwetz et al. 2014); however, other studies showed stable

Table 2 Studies that examine the effects of glucose ingestion and bone turnover marker response

Study and design	Population	Type of ingested glucose	Duration of the experiment	Result
Bjarnason et al. (2002) Randomized controlled cross-over	15 postmenopausal women, 12 premenopausal women, and 11 men	OGTT (75 g of glucose) and IVGTT (0.3 g glucose pr. kg)	24 h	Decrease in s-CTX after 1 h and 2 h for OGTT and IVGTT, respectively. Same pattern for u-CTX. S-osteocalcin decreased by the OGTT but not food intake
Chailurkit et al. (2008) Cross-sectional study	163 postmenopausal women (among these 54 with type 2 diabetes)	OGTT (75 g of glucose)	2 h	Decrease in s-CTX after 2 h. A significant decrease in OPG in non-diabetes women, but not in women with type 2 diabetes
Clowes et al. (2003) Randomized single blind cross-over	15 healthy subjects	OGTT (75 g of glucose) or placebo and in combinations with octreotide or saline infusion	4 h	OGTT decreased s-CTX, s-osteocalcin, and s-P1NP after 20 min and also decreased u-NTX. The effect of glucose on bone turnover markers was abolished by octreotide infusion, except for s-osteocalcin but with a delay
Clowes et al. (2002) Randomized double blind cross-over	16 healthy men	Euglycemic clamp (plasma glucose = 5 mmol/l) Hypoglycemic clamp (plasma glucose = 2.5 mmol/l). Similar hyperinsulinemia in both conditions	2 h	Euglycemic clamp did not change osteocalcin, s-CTX, or P1NP, whereas osteocalcin, s-CTX, and P1NP all decreased during the hypoglycemic clamp. PTH decreased in both groups but with partial recovery in the euglycemic group
Henriksen et al. (2003) Randomized controlled cross-over	10 healthy subjects	OGTT (75 g of glucose)	9 h	OGTT decreased s-CTX but not s-osteocalcin compared to fasting conditions

Holst et al. (2007) Cross-sectional	8 gastrectomized patients	OGTT (75 g of glucose)	3 h	OGTT decreased s-CTX and increased GLP-2. Osteocalcin remained unchanged by the OGTT
Karatzoglou et al. (2014) Cross-sectional	59 patients with Crohns disease and 45 healthy subjects	OGTT (75 g of glucose)	2 h	OGTT decreased s-CTX in both patients and healthy individuals, whereas P1NP remained unchanged
Knudsen et al. (2007) Randomized blinded cross-over study	9 healthy males	Hyperglycemic clamp (plasma glucose = 15 mmol/l) and euglycemic clamp (plasma glucose = 5 mmol/l)	4 h	OPG decreased during hyperglycemic conditions but not during euglycemia
Nissen et al. (2014) Cross-over	10 healthy males	Euglycemic (5 mmol/l) and hyperglycemic (12 mmol/l) clamps with co infusion of GIP or saline	90 min	Hyperglycemic clamp decreased CTX; however, a greater decrease was observed for the combination of GIP and hyperglycemia than for either GIP or hyperglycemia alone
Paldánius et al. (2012) Cross-sectional	23 healthy subjects	OGTT (75 g of glucose)	2 h	The OGTT decreased s-osteocalcin, s-CTX, s-TRAP, and s-P1NP
Schwetz et al. (2014) Cross-sectional	105 premenopausal women (of these 18 insulin resistant)	OGTT (75 g of glucose)	2 h	OGTT decrease CTX, P1NP, s-osteocalcin, and s-undercarboxylated osteocalcin in non-insulin resistant women, but only CTX in insulin resistant women
Viljakainen et al. (2014) Cross-sectional	34 obese individuals and 34 non-obese individuals- all non-diabetic	OGTT (75 g of glucose)	2 h	s-CTX, TRAP, BAP, osteocalcin, and P1NP all decreased during the OGTT in both groups. The osteocalcin decrease was significantly larger in the non-obese subjects compared to the obese subjects

Bone-specific alkaline phosphatase (*BAP*), C-terminal cross-linked telopeptide of type-I collagen (*CTX*), Gastric inhibitory peptide (*GIP*), Glucagon-like peptide 2 (*GLP-2*), Intravenous glucose tolerance test (*IVGTT*), N-terminal cross-linked telopeptide of type-I collagen (*NTX*), Oral glucose tolerance test (*OGTT*), Osteoprotegerin (*OPG*), Parathyroid hormone (*PTH*), Procollagen type 1 N-terminal propeptide (*P1NP*), Tartrate resistant acid phosphatase (*TRAP*)

osteocalcin levels when comparing to fasting conditions (Henriksen et al. 2003; Bjarnason et al. 2002; Holst et al. 2007). P1NP has both been reported to be stable (Karatzoglou et al. 2014) and to decrease (Clowes et al. 2003; Viljakainen et al. 2014; Paldanius et al. 2012; Schwetz et al. 2014) during an OGTT. The mineralization marker BAP decreased during OGTT in both obese and non-obese subjects (Viljakainen et al. 2014).

As the effects of OGTT and IVGTT are related to time, it was important to show whether time itself affected the bone turnover markers. Maintenance of a euglycemic p-glucose level of 5 mmol/l did not change the levels of s-CTX, osteocalcin, or P1NP (Clowes et al. 2002) when followed for two hours. Furthermore, when comparing fasting condition with OGTT, CTX decreased significantly more during the OGTT than during the fasting state (Henriksen et al. 2003). Thus, glucose intake has a time independent effect on bone turnover markers.

Glucose intake decreased the bone turnover markers, but a decrease was also observed during hypoglycemia where parathyroid hormone (PTH), P1NP, s-CTX, and osteocalcin decreased (Clowes et al. 2002). Bone turnover markers may decrease with p-glucose values both lower and higher than 5 mmol/l. Therefore the effect of glucose on bone turnover markers may be u-shaped with an optimal state in the normal healthy fasting condition. Although both IVGTT and OGTT decreased s-CTX, the decrease was significantly smaller during IVGTT (Bjarnason et al. 2002), which suggests that an additional component from the gastrointestinal tract affects bone turnover. Glucagon-like peptide-2 (GLP-2) increased while CTX decreased in gastrectomized patients (Holst et al. 2007), and this supports that the gastrointestinal absorption may affect bone turnover. Furthermore, the decrease in CTX during hyperglycemia was enhanced in combination with infusion of gastric inhibitory peptide (GIP) (Nissen et al. 2014), which suggests that the gastrointestinal hormones potentiate the effect of glucose on bone turnover. An intravenous injection of GIP and a subcutaneous injection of glucagon-like peptide-1 (GLP-1) did not affect s-CTX, whereas subcutaneous injection of GLP-2 decreased s-CTX (Henriksen et al. 2003).

The clinical studies show a strong relation between glucose intake and bone turnover, which may be either mediated or enhanced by gastrointestinal hormones. However, no direct pathway was established. The effect may be from an alteration of the OPG/RANKL pathway. During a hyperglycemic clamp OPG decreased in healthy males, while no change was observed during euglycemia (Knudsen et al. 2007). In type 2 diabetes women, OPG remained stable during an OGTT whereas it decreased in healthy women (Chailurkit et al. 2008). Thus, the OPG system may be altered in patients with diabetes compared to healthy subjects.

Bone Turnover Markers in Diabetes

Bone turnover markers in patients with diabetes have been examined in a meta-analysis (Starup-Linde et al. 2014). Both osteocalcin and CTX were decreased in patients with diabetes. compared to non-diabetes controls, whereas NTX was borderline significantly increased in diabetes patients. 25 hydroxy vitamin D levels were lower in diabetes patients, and phosphate levels were increased in patients with diabetes. PTH, calcium, and BAP were not different from controls in patients with

diabetes. When stratifying by diabetes type, patients with type 1 diabetes had lower 25 hydroxy vitamin D and osteocalcin, whereas patients with type 2 diabetes had lower phosphate levels and borderline decreased osteocalcin compared to non-diabetes subjects (Starup-Linde et al. 2014). Further studies add to a decreased bone turnover in both patients with type 1 and type 2 diabetes and report that BAP is not decreased, when other bone markers were decreased (Starup-Linde and Vestergaard 2015). Thus, bone turnover in diabetes is altered in comparison to non-diabetes individuals with lower CTX and osteocalcin levels representing lower bone resorption and bone formation. BAP, which represents mineralization, was not different, thus the bone matrix mineralization seems not to be impaired.

All bone turnover markers displayed heterogeneity between studies (Starup-Linde et al. 2014). The heterogeneity may be due to differences in patient characteristics, due to analytical and preanalytical factors, due to using different assays (no marker was evaluated with same method through all studies), or due to differences in p-glucose levels in the patients with diabetes. An in vitro study revealed that the decrease in bone turnover markers is not due to an immunochemical masking effect by bone marker glycation, as addition of glucose to serum samples with increasing dose and incubation time did not change P1NP, osteocalcin, and CTX (Starup-Linde et al. 2014). The heterogeneity among bone turnover markers also makes them unreliable fracture predictors as they may change depending on p-glucose. However, decreased osteocalcin levels and increased P1NP/CTX ratio have been associated with fractures in patients with type 2 diabetes (Starup-Linde and Vestergaard 2015).

In Vitro Studies

The Effect of Glucose on Osteoblasts

Osteoblast-like cells have been exposed to different hyperglycemic conditions, and indices of bone turnover have been assessed. Table 3 presents the studies that have evaluated the addition of glucose to osteoblast-like cells. In human osteoblast-like cells, hyperglycemia of both 12 mmol/l and 24 mmol/l for 7 and 14 days, respectively, increased the matrix calcification. The quality of the mineral was reduced with low calcium phosphate ratios (Garcia-Hernandez et al. 2012). Alkaline phosphatase activity increased at a glucose level of 12 mmol/l but decreased at a glucose level of 24 mmol/l (Garcia-Hernandez et al. 2012). Both bone formation markers osteocalcin and runt-related protein 2 (Runx2), and the bone resorptive marker RANKL increased while OPG decreased; this suggests an overall increased bone turnover (Garcia-Hernandez et al. 2012). Two other studies using a different human cell line showed decreased proliferation, alkaline phosphatase activity, and expression of OPG but with glucose concentrations from 16.7 mmol/l to 49.5 mmol/l (Terada et al. 1998; Shao et al. 2014). Continuous glucose levels of 49 mmol/l are life threatening in vivo and even sustained levels above 20 mmol/l are unphysiological and lead to ketoacidosis or hyperglycemic hyperosmolar nonketotic coma. Studies investigating murine osteoblast-like cells exposed to glucose have reported varying results. Increased proliferation and increased matrix mineralization have been

Table 3 Studies that examined the effects of in vitro added glucose on osteoblasts

Study	Cell line	Glucose dose	Duration	Results
Human cells				
Garcia-Hernandez et al. (2012)	Human alveolar bone-derived cells with osteoblastic phenotype	5.5 mmol/l, 8 mmol/l, 12 (hyperglycemia) mmol/l or 24 mmol/l (hyperglycemia)	24 h, 7 days, and 14 days	Hyperglycemia increased calcium deposits and biomineralization after 7 and 14 days; however, the quality of the mineral was decreased based on a lower Ca/P ratio. Alkaline phosphatase was increased at conditions with 12 mmol/l glucose but decreased at 24 mmol/l glucose. Hyperglycemia increased the expression of Osteocalcin, BSP, Runx2, and RANKL and decreased OPG after 7 and 14 days
Shao et al. (2014)	Human osteoblast-like cells MG63	5.5 mmol/or 16.7 mmol/l (hyperglycemia)	1, 3, 6, 7, 12, and 18 days	Hyperglycemia decreased cell proliferation, alkaline phosphatase activity, and expression of osteocalcin and OPG
Terada et al. (1998)	Human osteoblast-like cells MG-63 cells	5.5 mmol/l, 33.0 mmol/l (hyperglycemia) or 49.5 mmol/l (hyperglycemia)	7 days	Hyperglycemia decreased cell proliferation and decreased responsiveness to IGF-1
Murine cells				
Balint et al. (2001)	MC3T3-E1 mice like osteoblastic cells	5.5 mmol/l or 15 mmol/l (hyperglycemic).	30 days	More bone forming nodules were present in hyperglycemic conditions. The nodules were larger, irregular, and had a larger total calcified area in the hyperglycemic condition. However, calcium uptake was decreased, alkaline phosphatase activity increased, and osteocalcin unchanged in the hyperglycemic group
Batolomé et al. (2013)	MC3T3-E1 mice like osteoblastic cells	5.6 mmol/l or 25 mmol/l (hyperglycemic)	48 h	Hyperglycemic decreased the expression of Runx2, osterix, and osteocalcin and decreased the matrix mineralization
Botolin and McCabe (2006)	MC3T3-E1 mice like	5.5 mmol/l or 35.5 mmol/l (hyperglycemic)	29 days	Hyperglycemia increased alkaline phosphatase activity but did not change

(*continued*)

Table 3 (continued)

Study	Cell line	Glucose dose	Duration	Results
	osteoblastic cells			mineralization. Osteocalcin and Collagenase3 decreased during hyperglycemia
Cunha et al. (2014)	MC3T3-E1 mice like osteoblastic cells	5 mmol/l, 30 mmol/l (hyperglycemic), 50 nmol/of insulin or the combination of high glucose and high insulin dose	24 h	Increased organic matrix production (10x) and cell differentiation in hyperglycemic conditions. Hyperglycemia increased OPG (30x) and RANKL (2–3x) production which was attenuated by insulin, whereas alkaline phosphatase activity and mineralization decreased
López-Herradón et al. (2013)	MC3T3-E1 mice like osteoblastic cells	5.5 mmol/l or 25 mmol/l (hyperglycemia)	5 days	Hyperglycemia decreased β-catenin levels and accumulation and downregulates the Wnt pathway
Liu et al. (2015)	MC3T3-E1 mice like osteoblastic cells	5.5 mmol/l, 15.5 mmol/l, 25.5 mmol/l or 35.5 mmol/l	24 and 72 h and 7 and 14 days	Cell proliferation and Runx2 were decreased in the 25.5 and 35.5 mmol/l and increased in the 15.5 mmol/l glucose group after 72 h. Alkaline phosphatase activity was increased in the 15.5 mmol/l and decreased in the 25.5 and 35.5 mmol/l glucose groups after 7 days. Mineralization was increased at 15.5, 25.5, and 35.5 glucose levels compared to the 5.5 mmol/l group. In the group with a glucose of 15.5 mmol/l osteocalcin, OPG, osterix, Runx2, and P-AKT were increased compared to the 5.5 mmol/l
Ma et al. (2014)	Primary rat osteoblasts	5.5 mmol/l or 16.5 mmol/l (hyperglycemia)	7 days and 14 days	Hyperglycemia decreased proliferation and decrease calcium accumulation
Wu et al. (2012)	Rat osteoblasts	5.5 mmol/or 22 mmol/l (hyperglycemia)	3 days	Hyperglycemia induced the highest proliferation rate and a decrease in alkaline phosphatase. Insulin receptor and vitamin D receptor mRNA decreased in hyperglycemic

(*continued*)

Table 3 (continued)

Study	Cell line	Glucose dose	Duration	Results
				conditions. Furthermore, hyperglycemia decreased osteocalcin and undercarboxylated osteocalcin
Zayzafoon et al. (2000)	MC3T3-E1 mice like osteoblastic cells	5.5 mmol/or 16.5 mmol/l (hyperglycemia)	24 h	Hyperglycemia decreased osteocalcin mRNA expression and increased collagen I mRNA expression and c-Jun expression
Zhen et al. (2010)	Rat primary osteoblasts	5.5 mmol/, 11 mmol/l, 22 mmol/or 44 mmol/l	48 h	High glucose levels inhibited cell proliferation. Alkaline phosphatase activity was increased at glucose levels of 11 mmol/l but decreased at higher glucose levels in comparison with the level of 5.5 mmol/l. Both the number of bone forming nodules, amount of calcium deposited, and matrix mineralized were increased at the 11 mmol/l but decreased at higher glucose levels in comparison with the level of 5.5 mmol/l. IGF-1 and Runx2 expression were increased in the 11 mmol/l glucose group

Tartrate resistant acid phosphatase (*TRAP*), Calcium/phosphate (*CA/P*), Bone sialoprotein (*BSP*), Osteoprotegerin (*OPG*), Receptor activator of nuclear factor kappa-B ligand (*RANKL*), Insulin-like growth factor-1 (*IGF-1*), Runt-related protein 2 (*Runx2*)

reported (Balint et al. 2001; Liu et al. 2015; Wu et al. 2012; Zhen et al. 2010); however, both decreased matrix mineralization (Zhen et al. 2010, Bartolome et al. 2013; Cunha et al. 2014; Ma et al. 2014) and unchanged matrix mineralization (Botolin and McCabe 2006) have also been reported. Alkaline phosphatase activity reflects the mineralization process and has been reported to be both decreased (Balint et al. 2001; Liu et al. 2015; Wu et al. 2012; Zhen et al. 2010; Cunha et al. 2014) and increased (Liu et al. 2015; Zhen et al. 2010; Botolin and McCabe 2006) during hyperglycemia. Runx2 is an important transcription factor in osteoblast differentiation and osteocalcin is a marker of osteoblasts activity. RANKL and osteocalcin have been reported decreased (Wu et al. 2012; Bartolome et al. 2013; Botolin and McCabe 2006; Zayzafoon et al. 2000) and increased (Liu et al. 2015; Zhen et al. 2010) in hyperglycemic circumstances. Furthermore, the OPG/RANKL pathway may be disturbed as OPG increased 30-fold during hyperglycemia and RANKL only

increased two- to threefold, suggesting an inhibitory effect on bone resorption (Cunha et al. 2014). The Wnt pathway was also downregulated during hyperglycemia by decreasing β-catenin accumulation (Lopez-Herradon et al. 2013). Thus, hyperglycemia may decrease osteoblast differentiation and bone formation and also impair the bone resorption by increasing OPG.

Very different doses of glucose have been used in the studies to induce hyperglycemia ranging from 11 mmol/l to 49.5 mmol/l. The studies by Liu et al. and Zhen et al. (Liu et al. 2015; Zhen et al. 2010; Li et al. 2007) use different levels of hyperglycemia and present the importance of the glucose levels as both studies reported increased alkaline phosphatase activity and mineralization at the lowest level of hyperglycemia (15.5 mmol/l and 11 mmol/l) whereas higher glucose levels (22 mmol/l, 25.5 mmol/l, 35.5 mmol/l, and 44 mmol/l) decreased alkaline phosphatase activity and did not increase mineralization at the lowest levels of hyperglycemia. The glucose levels of 22 mmol/l or more are very high and is life threatening if sustained for longer periods, whereas levels of 11 mmol/l or 15 mmol/l may be tolerated for a longer period. The studies reporting decreased mineralization have used glucose levels higher than 16 mmol/l. The effect of glucose on bone markers may thus depend on the glucose levels; small increases of glucose may increase alkaline phosphatase activity and increase mineralization, whereas supraphysiological levels of glucose may decrease mineralization and alkaline phosphatase activity.

The Effect of Glucose on Osteoclasts

CTX is a marker of bone resorption and as CTX decreases during glucose intake, glucose may directly affect the osteoclasts. Table 4 presents the studies that have examined the effect of hyperglycemia on osteoclasts. Only three studies have examined this relationship and all on murine cells. Hyperglycemia was shown to have a detrimental effect when directly added to osteoclast-like cells. Hyperglycemia decreased the number of osteoclasts, TRAP expression, osteoclastogenesis, cell to cell fusion (which is an important step in creation of the multinucleated osteoclasts), and osteoclast differentiation (Wittrant et al. 2008; Xu et al. 2013, 2015). Furthermore, a decreased pit resorption area was observed during hyperglycemia. This reflects an impairment in the ability of the osteoclast to resorp mineralized matrix at elevated glucose concentrations (Xu et al. 2013). These in vitro studies clearly show a direct detrimental effect of hyperglycemia on osteoclasts. Thus, the hyperglycemia inhibits osteoclasts, this is in line with the clinical human studies where glucose ingestion decreased CTX (Bjarnason et al. 2002).

The Effect of Glucose on Osteocytes

Only a single study has assessed the effect of hyperglycemia on osteocytes. It is presented in Table 4. This study showed an increased expression of sclerostin protein, whereas RANKL was unchanged during hyperglycemia (Tanaka et al. 2015). The regulatory activity of osteocytes may thus also be affected by glucose, with an increased sclerostin production and thereby an inhibitory effect on the osteoblasts by blocking the Wnt pathway. Further research is needed to confirm the effect of glucose on osteocytes.

Table 4 Studies examining the effects of in vitro added glucose on osteoclasts and osteocytes

Study	Cell line	Glucose dose	Duration	Results
Osteoclasts				
Wittrant et al. (2008)	Murine RAW 264.7 monocytic cells	Differentiation medium using 15.5–30.5 mmol/l of D (+) glucose or L (−) glucose as control	6 days	High glucose levels inhibits RANKL induced TRAP expression, osteoclastogenesis, osteoclast differentiation, and cell migration in RAW 264.7 cells
Xu et al. (2013)	Mice osteoclasts derived from bone marrow cells	33.6 mmol/l (hyperglycemia)	4 days	Hyperglycemia decreased the number of mature osteoclasts and TRAP, RANK, and cathepsin k expression. Furthermore, hyperglycemia decreased TRAP activity, and the pit resorption area measured by absent mineral deposition
Xu et al. (2015)	Murine RAW 264.7 monocytic cells	5.6 mmol/l or 20.2 mmol/l	4–5 days	Hyperglycemia inhibited RANKL induced osteoclastogenesis, osteoclast differentiation, and decrease cell to cell fusion
Osteocytes				
Tanaka et al. (2015)	Mice osteocyte-like MLO-Y4-A2 cells	5.5 mmol/l or 22 mmol/l (hyperglycemia)	24, 48, and 72 h	Hyperglycemia increased sclerostin protein expression but did not affect RANKL

Tartrate resistant acid phosphatase (*TRAP*), Receptor activator of nuclear factor kappa-B (*RANK*), Receptor activator of nuclear factor kappa-B ligand (*RANKL*)

The Effect of Glucose on Mesenchymal Stem Cells

Both osteoblasts and osteoclasts are derived from the mesoderm, and mesenchymal stem cells may thus differentiate to both types. Only immortalized human mesenchymal stem cells proliferated during hyperglycemia. Primary human mesenchymal stem cells had lower proliferation rate but differentiated towards osteogenic cells during hyperglycemia over 4 weeks and had enhanced mineralization compared to cells exposed to lower glucose levels (Li et al. 2007). Murine mesenchymal stem cells decreased mineralization, TRAP, and alkaline phosphatase activity but increased collagen production when exposed to hyperglycemia (Dienelt and zur Nieden 2011). Glucose may thus affect the differentiation of mesenchymal stem cells to osteoblasts and osteoclasts (Table 5).

Table 5 Studies examining the effects of in vitro added glucose on mesenchymal stem cells

Study	Cell line	Glucose dose	Duration	Results
Dienelt and zur Nieden (2011)	Murine embryonic stem cells	1 g/l or 4.5 g/l (hyperglycemic)	30 days	Hyperglycemia decreased matrix calcification, TRAP, and alkaline phosphatase activity but increased collagen production
Li et al. (2007)	Human mesenchymal stem cells telomerase-immortalized (hMSC-TERT) and primary human mesenchymal stem cells (hMSC)	5.6 mmol/l, 11 mmol/l or 25 mmol/l (hyperglycemia)	4 days and 4 weeks	Hyperglycemia caused proliferation in the hMSC-TERT cell line. hMSC cells differentiated towards osteogenic cells in the hyperglycemic conditions with enhanced mineralization compared to lower glucose concentration.

Tartrate resistant acid phosphatase (*TRAP*), Human mesenchymal stem cells (*hMSC*), Human mesenchymal stem cells telomerase-immortalized (*hMSC-TERT*)

Glucose and Diabetic Bone Disease

Ingestion of glucose decreased both bone resorption markers and bone formation markers in healthy individuals and a link between glucose and bone turnover markers was established. In patients with diabetes the increased fracture risk was not explained by the apparent normal or increased BMD. This paradox suggests that BMD is not equal to bone quality in patients with diabetes. The mineralization marker BAP remained unchanged while CTX and osteocalcin were decreased in diabetes patients compared to non-diabetes subjects. Thus, a dissociative bone remodeling may be present in patients with diabetes with a decreased bone resorption and bone matrix formation whereas the mineralization is relatively increased. In this state, BMD is increased in comparison to the quality of the bone, which also is the case in some osteopetrotic patients (Starup-Linde and Vestergaard 2015). A study examining bone biopsies from patients with type 2 diabetes reports reduced bone formation rate and mineralizing surface but normal adjusted apposition rate in comparison to controls (Manavalan et al. 2012). Therefore the decreased mineralizing surface is due to a decreased bone formation. The in vitro studies on both human and murine cells report direct effects of glucose on bone cells. A physiological hyperglycemia (as in many patients with diabetes) with glucose levels of 11–15 mmol/l increased the mineralization and alkaline phosphatase activity by osteoblasts, whereas higher glucose levels decreased mineralization and alkaline phosphatase activity. Furthermore, hyperglycemia was mainly reported to decrease the Runx2 and osteocalcin expression and thus leading to decreased bone formation, although some in vitro studies also report

increased Runx2 and osteocalcin. Besides a direct effect on osteoblasts, hyperglycemia decreased osteoclastogenesis and osteoclast activity and thus impaired bone resorption and increased the osteocytes expression of sclerostin, which decrease bone formation by antagonizing the Wnt pathway.

In patients with diabetes the p-glucose level is cyclical depending on medication use and food intake. As the p-glucose level may rapidly increase, the bone formation and bone resorption may drop. However, the mineralization process seems to be spared, which may be caused by an increased mineralization in the p-glucose levels of 11–15 mmol/l. In patients with diabetes, the level of 11–15 mmol/l is a physiological level and may be sustained for longer periods. Furthermore, HbA1c was a positive effect modificator of BAP in a meta-regression analysis (Starup-Linde et al. 2014) and mineralization reflected by BAP may increase due to long-term hyperglycemia. The bone of patients with diabetes may be hypermineralized due to long-term hyperglycemia. Figure 2 presents the hypermineralization hypothesis. Hyperglycemia may decrease both bone formation and bone resorption by a direct inhibitory effect on osteoblasts and osteoclasts. During hyperglycemia, osteocytes produce more sclerostin, and osteoblasts may increase OPG production which will decrease bone formation and bone resorption, respectively. At p-glucose levels of 11–15 mmol/l, the hyperglycemia may increase the bone mineralization as reflected by both calcification and alkaline phosphatase activity but with low quality

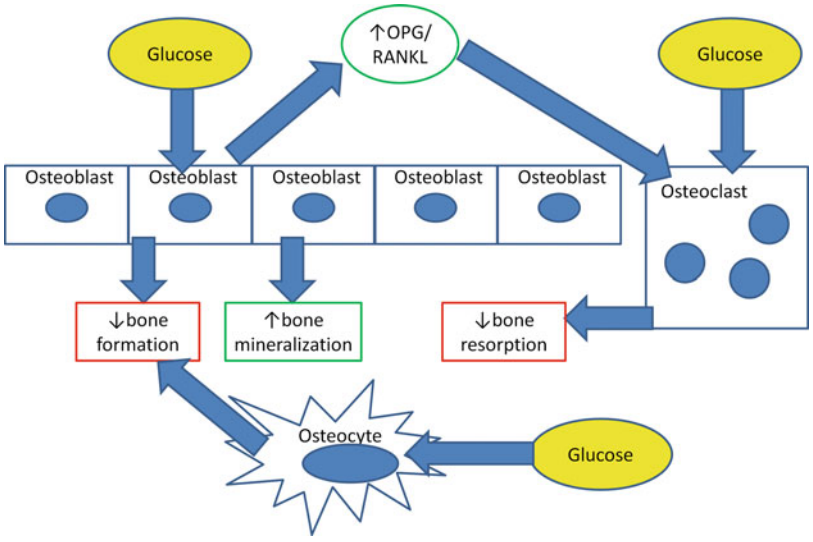

Fig. 2 ***The hypermineralization hypothesis.*** Hyperglycemia decreases the osteoclasts' ability to resorp bone directly and indirectly by increasing the OPG/RANKL ratio. Bone formation by the osteoblasts is directly inhibited by hyperglycemia reflected by decreased Runx2 and osteocalcin expression and indirectly by an increased production of sclerostin by the osteocytes. Both bone formation and resorption is decreased; however at hyperglycemic levels of 11–15 mmol/l the mineralization is increased by the osteoblast, whereas the quality of the mineralized matrix is decreased due to decreased calcium/phosphate ratio

mineralized material. Therefore, a state where the mineralization does not correspond to the bone turnover may exist in patients with diabetes, and the bone is hypermineralized relatively to the bone material strength.

Potential Applications to Prognosis, Other Diseases or Conditions

The interaction between glucose and bone turnover markers is a potential predictive and prognostic marker of fracture risk in patients with diabetes. The hyperglycemia, which characterizes patients with diabetes, may have detrimental effects on bone turnover, bone composition, and bone strength. The combination of bone turnover markers and continuous glucose monitoring may be of use to determine the bone turnover in patients with diabetes and evaluate whether this may be prognostic of fracture. No therapy of diabetic bone disease is available. Strict glucose control may be beneficial for bone health in patients with diabetes however, it is unknown whether antiresorptive therapies that decrease bone resorption are beneficial in a state already characterized by decreased bone resorption.

Future investigations may apply the advanced techniques of continuous glucose monitoring to determine the circadian rhythm of bone turnover in patients with diabetes and its relation to p-glucose levels, which may be very different from what is seen in non-diabetes subjects. Furthermore, additional studies investigating the effect of glucose on osteoblasts, osteoclasts, and osteocytes are needed. Translation of these results into animal models is also important. Randomized controlled trials are needed to determine whether a specific antidiabetic treatment is beneficial for bone health and if antiresorptive treatment may be of use.

Summary Points

- Diabetes mellitus patients have higher risk of bone fracture which cannot be explained by their bone mineral density.
- Bone turnover in patients with diabetes is altered in comparison to non-diabetes individuals as they display lower C-terminal cross-linked telopeptide of type-I collagen and osteocalcin levels representing lower bone resorption and bone formation.
- An oral glucose tolerance test induces a decrease in C-terminal cross-linked telopeptide of type-I collagen in healthy individuals, but the decrease is attenuated in patients with diabetes.
- An intravenous glucose tolerance test does not induce the same reduction in C-terminal cross-linked telopeptide of type-I collagen as an oral glucose tolerance test, indicating that gastrointestinal hormone release influence the glucose-bone interaction.
- Hyperglycemia may decrease osteoblast differentiation and bone formation and may also impair the bone resorption by increasing osteoprotegerin and inhibiting osteoclast activity directly.

- The effect of glucose on bone markers may be dependent on the glucose level, where small increases in blood glucose may increase alkaline phosphatase activity and mineralization, whereas supraphysiological levels decrease mineralization and alkaline phosphatase activity.
- The bone of patients with diabetes may be hypermineralized due to hyperglycemia that increase bone mineralization relatively to the bone turnover.

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