



## The skeleton in diabetes – involvement and interaction

VESNA KUŠEC

**Correspondence:**

Prof.dr.sc. Vesna Kušec  
Department of Laboratory Diagnostics  
University Hospital Centre Zagreb  
Kišpatićeva 12, 10000 Zagreb, Croatia  
E-mail: vkusec@kbc-zagreb.hr

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### Abstract

*Diabetes mellitus (DM) is characterized by high blood glucose; impairment of the skeleton is among its other deleterious effects. Adverse effect upon the bone tissue is primarily altered bone quality and consequently increased bone fragility. In DM 1, bone mass may be low and in DM2 normal or even increased. Therapy for DM2 also affects bone metabolism as thiazolidine-diones cause differentiation shift of mesenchyme precursors from osteoblasts into adipocytes. Association of bone with glucose metabolism was discovered in the last decade indicating that insulin is necessary for synthesis of undercarboxylated osteocalcin which acts in a hormonal fashion to promote insulin secretion in the pancreas, adiponectin in adipocytes, and increases insulin sensitivity in target tissues. Undercarboxylated osteocalcin is also secreted from resorbed bone. Thus insulin is required for normal bone metabolism and bone acts as an endocrine organ. Additional role of osteocalcin is in promoting testosterone secretion in testes.*

### INTRODUCTION

Diabetes mellitus (DM, both type 1 and 2) has long been recognized as disease which affects skeletal integrity. Although different etiologies and disease mechanisms are involved in diabetes type 1 and 2, certain similarities regarding the outcome upon skeleton exist, i.e. greater bone fragility and greater risk of fractures, and this includes also hypovitaminosis D. The common mechanism of impaired mineral metabolism includes hyperglycemia which contributes to increased urine calcium excretion, poor bone quality due to formation of advanced glycosylation end products that permanently affect bone matrix, low bone turnover, increased bone fragility and increased fracture risk arising from altered bone quality. The differences in etiologies of DM1 and DM2 are also reflected on their effect upon bone metabolism. The onset of DM1 is usually at an earlier age and very likely to affect achievement of peak bone mass with low bone mass. In DM1, lack of insulin affects all aspects of development and growth. Body mass index may also be low in these patients, with low muscle mass and low fatty tissue which both have a negative impact upon peak bone mass and its maintenance. In patients with DM2 with predominantly late onset, bone mass may be normal or even increased (1, 2).

The underlying disorder of DM2 is insulin resistance and hyperinsulinemia. As insulin is an anabolic agent, together with increased IGF1, insulin contributes to bone mass accrual. Increased body mass, very frequent in DM2 patients, also favors bone mass preservation. Hypovitaminosis D which is common in both DM1 and DM2 may be due to general poor health and compromised quality of life in particular in DM1 patients whose glucose levels are not optimally controlled. Vitamin

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D sequestration in fatty tissue in both DM1 and DM2 patients is recognized as additional risk for skeletal impairment. Bone quality in DM2 deteriorates as indicated by increased fractures of the hip and spine despite preserved bone mass (3). Assessment of skeletal features, including bone quality and not only bone mass, will become a prerogative in monitoring and treating DM2 patients as DM2 incidence is increasing in aging societies and requiring also fracture risk assessment. Basic characteristics regarding skeletal involvement in DM1 and DM2 are summarized in Table 1.

### ASSOCIATION OF BONE AND ENERGY METABOLISM

It was the crucial discovery of Lee et al reported in 2007 (4) which brought a new insight of association of bone metabolism and energy homeostasis (Figure 1). Os-

teocalcin, i.e. undercarboxylated osteocalcin was recognized as a key factor in coupling of these otherwise diverse systems. Conclusions of Karsenty's group on new aspects of glucose metabolism which were consistent with reports of other investigators on glucose, osteocalcin, adiponectin, fat mass and other factors in humans, have so far enabled new concepts of the entire metabolism (4). In summary, insulin acting through its receptor on osteoblasts activates protein tyrosine phosphatase (in humans protein tyrosine phosphatase 1B) to produce undercarboxylated osteocalcin as opposed to osteocalcin with post-translational three glutamic acid residues. This modification of osteocalcin is important because of its calcium binding properties. Thus osteocalcin containing glutamic acid residues has high affinity for bone matrix. Decarboxylation reduces the affinity of osteocalcin (undercarboxylated osteocalcin) for hydroxyapatite in bone matrix. This process occurs during bone resorption. This form of

**TABLE 1**

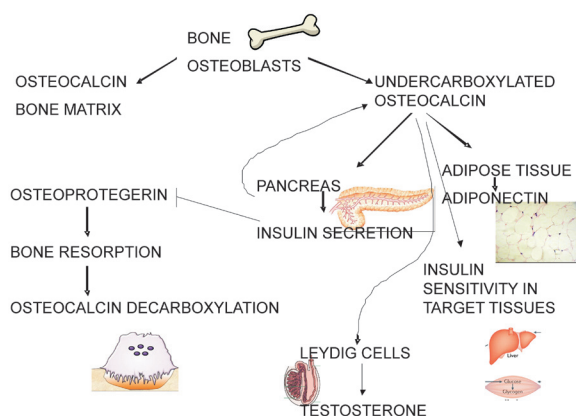
Summarized features of DM1 and DM2 regarding skeletal involvement.

	DM1	DM2
Endocrine mechanism	Insulin deficiency, hyperglycemia	Insulin resistance, increased IGF1, hyperglycemia
Age of onset	young	mature, old
Body mass	low	increased
Skeletal impairment	affect peak bone mass, bone mass low, bone turnover low, altered bone quality, increased risk of fractures, increased calcium excretion due to hyperglycemia, hypovitaminosis D	bone mass normal or increased, bone turnover low, altered bone quality, increased risk of fractures, increased calcium excretion due to hyperglycemia, hypovitaminosis D
Involvement of other systems	deleterious effect on blood vessels, neuropathy, visual impairment, kidney function deterioration	deleterious effect on blood vessels, neuropathy, visual impairment, kidney function deterioration

**TABLE 2**

Reports on association of skeletal metabolism with glucose metabolism.

Reference	Observation	Subjects/patients
36	Negative correlation of osteocalcin and blood glucose and parameters of atherosclerosis	DM2 adults
37	Negative correlation of undercarboxylated osteocalcin and blood glucose and fat mass, positive with adiponectin	DM2
38	Negative correlation of osteocalcin and blood glucose and fat mass	non-diabetic
39	Positive correlation of osteocalcin and insulin sensitivity, increase of osteocalcin after weight loss	obese adults
8	Negative correlation of osteocalcin with fasting glucose and insulin, insulin resistance, C-reactive protein, IL-6, BMI and fat mass; osteocalcin associated with change in blood glucose	DM2
40	Lower levels of osteocalcin in diabetics which increased with glycemic improvement	healthy and diabetics
41	Increase in undercarboxylated osteocalcin as a result of aerobic exercise	obese and diabetic men
42	Increase of osteocalcin, decrease of leptin and improvement of insulin sensitivity after weight loss	obese children
43	Positive correlation of osteocalcin and adiponectin	DM2
44	Positive correlation of undercarboxylated osteocalcin and adiponectin	postmenopausal women
45	No difference in undercarboxylated osteocalcin	DM1 and controls
46	Undercarboxylated osteocalcin positive correlated with insulin	healthy children



**Figure 1.** Presentation of interaction of bone metabolism and glucose regulation. Insulin promotes secretion of undercarboxylated osteocalcin in osteoblasts, which in turn stimulates insulin secretion in the pancreas, adiponectin in adipose tissue and energy expenditure, improves insulin sensitivity in target tissues and blocks osteoprotegerin synthesis with increased bone resorption and release of decarboxylated osteocalcin. Also, testosterone synthesis is stimulated in the Leydig cells of testes. Insulin deficiency has opposite effects, including poor osteocalcin synthesis and low bone mass.

osteocalcin, i.e. the undercarboxylated osteocalcin, is the active or hormonal form as it promotes insulin synthesis and secretion in pancreatic beta cells and adiponectin secretion from adipocytes. Additionally, insulin sensitivity is improved, energy expenditure increased and fat mass decreased. The regulation goes through ESP gene which encodes for the protein tyrosine phosphatase, and leptin also acts to increase its action with resulting secretion of osteocalcin with three glutamic residues, i.e. the non-active osteocalcin (or the non-hormone form). Leptin also decreases insulin secretion in the pancreas. Undercarboxylated osteocalcin is also released from bone matrix during bone resorption, as insulin inhibits osteoprotegerin synthesis and thus promotes osteoclast bone degradation. The work of Lee et al. (4) will probably require a change in the traditional concept of endocrinology as the skeleton or bone tissue becomes an endocrine organ involved in regulation of energy metabolism.

This brings new light to understanding skeletal involvement in diabetes. In DM1 with impaired insulin secretion, opposite metabolic effects are observed compared to those described previously for the normal insulin secretion. Also, insulin deficiency is the cause of low osteocalcin synthesis and poor bone formation, with the result of low bone mass and impaired bone quality. In rodents, low osteocalcin is also associated with low testosterone synthesis in testicular Leydig cells, but it apparently lacks a similar effect on ovaries.

Endocrine interaction of bone (i.e. osteoblasts) and energy metabolism (i.e. pancreas) includes two counter-

acting key factors: leptin (the bad guy) and insulin (the good guy). Insulin signalling in osteoblasts is regulated through its receptor tyrosin kinase, a substrate of tyrosine phosphatase (encoded by ESP gene) (2). Binding of insulin to its osteoblast receptor results in decrease of osteoprotegerin secretion. This alters the balance of osteoblast-osteoclast cross-talk and enables osteoclast activity. Bone resorption and acidification of bone microenvironment releases decarboxylated osteocalcin from bone matrix into extracellular fluid. Body fluids and plasma contain both carboxylated and undercarboxylated osteocalcin. Bone resorption favors decarboxylation of osteocalcin by creating acidic environment. Release of osteocalcin and its decarboxylation yield undercarboxylated osteocalcin which acts as a hormone on the pancreas, adipose tissue, liver and the testes (4). In adolescent boys, a positive association between osteocalcin and testosterone would traditionally be attributed to pubertal growth spurt. Puberty is characterized by several-fold higher levels of bone markers, both formation and resorption markers, as compared to adults. Bone markers reflect intensive and accelerated bone growth during puberty which will level-off toward adulthood, i.e. approximately toward the twenties. With regard to new data on bone and gonads, it can be speculated that this association of osteocalcin and testosterone also reflects a direct interaction of the endocrine bone and gonad function (5).

Data on clinical studies supporting causative relationship between different aspects of bone metabolism and glucose homeostasis are summarized in Table 2. These results also indicate the key role of osteocalcin (or rather undercarboxylated osteocalcin) in coupling bone and energy metabolism (6).

Systemic administration of undercarboxylated osteocalcin in experimental models has anti-diabetic properties by lowering blood glucose, increasing insulin secretion and insulin sensitivity of target tissues (muscle, adipose tissue, and liver) (7). In experimental animal models (primarily rodents), gross alterations of osteocalcin and undercarboxylated osteocalcin were reported. In studies on humans, the differences are mostly within a normal physiological range and correlations with fasting glucose and insulin sensitivity were reported. Not surprisingly, diabetes in humans is associated with lower total osteocalcin. In clinical studies, improved glycemic control have resulted in higher osteocalcin levels. More human studies are necessary to confirm the results obtained in animal studies. For example, studies in patients where effects of undercarboxylated osteocalcin or osteocalcin on glucose metabolism were investigated have provided conflicting results. In some studies (8), improved fasting glucose could be predicted, but in others (9) (with vitamin K supplementation for decrease of undercarboxylated osteocalcin) opposite results were found on insulin sensitivity compared to those predicted from rodent models.

## BONE IN DIABETES – FRAGILITY AND FRACTURE RISK

Bone fragility and risk of fractures with its consequences on life-style and independence is an important problem of aging population with increasing incidence of DM2. Bone loss occurs with aging in both men and women. This is more pronounced in postmenopausal women with accelerated bone loss after cessation of ovarian estrogen production. At an advanced age, the rate of bone loss is similar for both sexes. However, females are at a disadvantage compared to males because peak bone mass is less and postmenopausal bone loss occurs at an increased rate. Osteoporosis in older population is thus frequent, affecting approximately 15% of women and 5% of men older than 50 years. It is well established that bone mass, measured by the standard method of dual-photon densitometry, is highly related to fracture risk (10).

Skeletal integrity is impaired in diabetes; this applies to both types 1 and 2 and was established long ago. It is a serious health problem for diabetic patients because fracture risk increased and has consequences for individual patients and the entire community. In particular, studies have reported an increased risk of hip fractures which are particularly detrimental in older people, resulting in disability and even death from multi-organ failure.

Aging population is also affected by greater DM2 incidence, and both DM2 and also DM1 are characterized by increased fracture risk. Bone mass in DM2 is not necessarily decreased; on the contrary, it may be normal or increased but bone fractures occur despite evidence of non-compromised bone mass. The problem is in altered bone quality, a feature not assessed by densitometry which, in fact, measures bone mineral content. This was observed and reported for both the hip, vertebral and foot fractures in DM2 (11). In the case of DM2, the association of bone mineralization and fragility is not reliable for prediction of fracture risk. An apparent paradox is that greater risk of fracture in diabetics does not correspond to low BMD as it does in healthy population. Although BMD in DM1 patients is lower than in general population, the actual densitometry measurements do not account for this high fracture risk. In contrast, BMD is in DM2 patients better than in non-diabetic population i.e. densitometry results are higher, but fractures occur with increased frequency (12). This was not a universal observation and some investigators have found fracture risk to be similar to that (13, 14) in normal population.

In DM1, increased risk was found for hip fractures (11), but also for the spine and humerus (15). Epidemiological studies have indicated that the main cause of increased fracture risk in diabetes both type 1 and 2 is altered bone quality, and not decreased bone quantity. Poor bone quality also leads to fractures. Increased blood glucose in inefficient glycemic control generates advanced glycation end products which permanently alter bone matrix structure,

among other tissues. Thus, bone quality deteriorates by accumulation of advanced glycation end products, its integrity is not any more intact and fragility is increased. These compounds adversely affect collagen, osteocytes, and multipotent bone marrow stem cells (16, 17, 18).

Prolonged exposure to hyperglycemia and to the detrimental effects of advanced glycation end products on bone resulted in an increment in fracture risk that was reported approximately 12–14 years after the diagnosis of diabetes (11, 19).

Established therapy for blood glucose control in DM2 also contributes to secondary osteoporosis. The mechanism is based on altered bone remodeling balance with decreased bone formation and increased bone resorption. Advanced age and postmenopause are additional adverse risk factors contributing to osteoporosis and fracture risk.

Evidence from retrospective clinical studies have confirmed adverse effect of thiazolidine-diones in DM2, with emphasis on increased bone loss and risk of fractures in elderly women, although according to some studies with equal risk for both sexes, increased risk in patients with previous fracture history and positive correlation with duration of treatment.

Regarding bone safety in DM2 patients, combination therapy with other antidiabetic therapies or preventing further bone loss by administration of antiresorptive/ antiosteoporotic drugs might be an option to preserve skeletal integrity. An established and effective therapy for normalization of blood glucose in DM2 are thiazolidinediones. These compounds act on peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) protein, a key regulator of energy metabolism in fat tissue. However, the disadvantage is that PPAR $\gamma$  also interacts with bone cell proliferation and differentiation and the cytokine composition in the bone marrow extracellular fluid. Thiazolidine-dione in in-vitro studies and animal models probably acts on pluripotent mesenchymal stem cells in the bone marrow, shifting differentiation towards adipocyte precursors at the expense of osteoblast precursors (20).

Bone marrow space is filled with adipose tissue, bone turnover, i.e. regeneration, is impaired and bone loss results (21, 22). This also depletes the recruitment into osteoblasts, adversely affecting bone turnover. PPAR- $\gamma$  can regulate synthesis of many cytokines which support the hematopoietic-macrophage lineage, i.e. it supports osteoclastogenesis and bone resorption. Unfortunately, this needs to be confirmed in human studies, and other potential mechanisms, including an indirect negative effect on osteoblasts via enhanced secretion of adipocyte factors, remain to be elucidated. Also, an important aspect is the possible confounding effect of thiazolidine-diones on fracture healing (20).

There are more adverse effects of diabetes besides modifications of bone matrix due to advanced glycation end products. Mineralization of bone matrix may also be af-



ected. Additional risk factors for fractures in patients with diabetes include systemic consequences of uncontrolled glycemia which affect the nervous system, vision, kidney function and other systems, all contributing to risk of falls and poor bone healing. Diabetic neuropathy and neuromuscular impairment are major risk factors for falls (23). Severe hypoglycemia in patients on insulin therapy should also be considered a risk factor contributing to dizziness and falls.

## **OBESITY IN DIABETES – ENDOCRINE AND OTHER EFFECTS**

Obesity is not only a feature of DM2, but also a global epidemic of the Western world with affordable access to high calorie food and sedentary lifestyle. Body fat has an endocrine effect on the skeleton by secreting adipokines. For example, leptin – besides its effect on appetite and reproduction – acts centrally on the sympathetic nervous system and hypothalamic neurons, and has negative impact on bone mass by inhibiting bone accrual.

The pathway of central leptin effect is common to serotonin. Actually, leptin inhibits serotonin synthesis which in turn stimulates signalling of the sympathetic nervous system, and finally acting of beta adrenergic receptors on osteoblasts. Dual pathway acts by inhibition of osteoblast proliferation and also by promoting RANKL expression and osteoclast activation. Leptin also has peripheral anabolic action on bone mass, stimulating osteoblasts and inhibiting osteoclasts. The alternate pathway is through receptors of the arcuate nucleus neurons by increasing CART (cocaine and amphetamine regulated transcript) gene expression and further decreasing RANKL expression on osteoblasts (2,3).

Fatty tissue and its mass has long been established as a protective factor for bone mass, with main effect attributed to conversion of androgens into estrogens by an aromatase. The sheer weight of greater body mass is not a significant contributor to skeletal health. Adipose tissue has less specific weight in comparison to muscle tissue and thus proportionally has less impact. The effect of skeletal muscles, i.e. muscle contraction and strain during exercise, will exert considerable force on the bone. This change and increment of pressure-pull forces promote bone remodeling and bone formation, with the main goal of achieving a new biomechanical steady state with optimal force distribution (10,24). Modification of muscle work or exercise program can progressively stimulate further bone remodeling and bone growth to adapt to new and increasing load. Increased body weight in obese has a less pronounced beneficial effect upon the skeleton. Another recognized benefit, but of limited value, is the cushion effect of fatty tissue. These benefits are of moderate influence as mobility is with increasing body weight progressively hampered and muscle action on the skeleton reduced. Deleterious effects of obesity on other systems, e.g. cardiovascular, renal, locomotor and other, are well established.

Low body weight is a recognized risk factor for bone fracture, and obesity is characterized by high cortical bone mass. It is well known that adipose tissue insulates the skeleton, but also exerts increased load on the skeleton with enhanced mechanical signalling to osteocytes and the cortical bone. Also, excess body weight may be detrimental to the skeleton. Diseases associated with aging, e.g. metabolic syndrome and insulin resistance, are also linked to fragility fractures (10,24). Patients with DM1 and DM2 differ regarding body weight and accordingly in body mass index. These two disorders differ greatly in hormonal profiles. DM1 is characterized by insulin deficiency, low IGF1 and steroid hormones, while in DM2 there is increased insulin secretion, high estrogens and androgens, and normal IGF1 (3).

## **THE ROLE OF VITAMIN D**

Vitamin D deficiency has been recognized almost as an epidemic ever since rapid and convenient measurement methods have become available. It is estimated that approximately 30-50% of children and adults are vitamin D-deficient with regard to concentration of 50 nmol/L for 25-hydroxyvitamin D in blood (25). This is the level of deficiency (or insufficiency) of 25-hydroxyvitamin D which causes a reactive increase of PTH secretion. In case of certain disorders, e.g., malignancies, the cut-off values higher than 50 nmol/L are required for optimal health benefits (e.g., 80 nmol/L). Epidemiological studies have indicated that hypovitaminosis D is associated with many disorders and malignancies (e.g., breast cancer, colon cancer, prostate cancer, leukemia), including both DM1 and DM2. In malignant disease, antiproliferative and pro-differentiating properties of calcitriol are probably involved. Circulating 25OH D levels have been found to be associated with mortality (all-cause) in patients with terminal kidney diseases and coronary artery diseases. Also, supplementation with vitamin D may reduce mortality. Normalization and increase of 25-hydroxyvitamin D was associated with reduced risk for DM, cardiovascular diseases and metabolic syndrome (26). Low vitamin D was linked to DM1 and also to insulin resistance in DM2 (27-29). This can be explained by the role of 1,25-dihydroxyvitamin D on target cells of insulin action, i.e. the liver, skeletal muscles and adipose tissue. Apparently it can improve pancreatic  $\beta$ -cell function. Immunomodulating function of 1,25-dihydroxyvitamin D might also be involved as it regulates immune cells and local immune response (30). The underlying mechanism in cardiovascular diseases is that hypovitaminosis activates renin-angiotensin system. In addition, vitamin D inhibits cardiac muscle hypertrophy (31).

Regarding DM1 and hypovitaminosis D, there are moderate data indicating associations of low vitamin D and poor glycemic control and insulin resistance. Apparently correction of hypovitaminosis D improves major

symptoms of DM1. Reasons for hypovitaminosis D in DM1 are multiple. Hypovitaminosis D observed in DM2 is a consequence of increased adipose tissue and storage of vitamin D in adipocytes. In DM2 low vitamin D is associated with decreased insulin secretion and insulin resistance. Correction of hypovitaminosis D is associated with improvement of pancreatic insulin secretion and insulin sensitivity. Receptors for 1,25-dihydroxyvitamin D have been found on pancreatic cells, which further supports these observations. Therapy with vitamin D restores insulin sensitivity in peripheral tissues through stimulation of its receptor expression. Several epidemiological studies have reported on decreased 25-OH D and incidence of DM2 (6).

Vitamin D receptors are also found on skeletal muscle cells. Their action is performed through alteration of gene expression by DNA synthesis but also through a rapid action on membrane receptors. This is important as skeletal mass (or lean mass) has a profound effect on bone maintenance and bone formation. Conclusions of these studies do not suggest that vitamin D deficiency is responsible for occurrence and development of DM2, but point toward further investigations of metabolic associations (6).

In addition to the effect of hyperglycemia on bone quality in diabetes, bone mineralization may be due to impaired vitamin D and calcium metabolism. Increased blood glucose interferes with calcium reabsorption in the kidney. Patients with diabetes also suffer from hypovitaminosis D (32, 33). DM2 patients are more likely to be vitamin D deficient, as their BMI is greater and this fat-soluble vitamin might be stored in adipose tissue. A recently published review by Madar et al. (34) demonstrated that currently there is insufficient evidence to recommend vitamin D supplementation in order to improve glycemia or insulin resistance in patients with diabetes, normal fasting glucose, or impaired glucose tolerance (34, 35).

Available data on clinical research of interaction of bone and glucose metabolism are limited and further studies are urgently required to investigate the exact role of osteocalcin in energy balance in humans. This would include research to assess how therapies that modulate undercarboxylated osteocalcin synthesis may alter and improve glucose and lipid metabolism.

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