Bone as a regulator of glucose metabolism

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ABSTRACT

For a long time the only functions attributed to the skeleton were locomotion and calcium storage. Over the last decade, this view has changed. Genetic studies in mice have shown that bone metabolism is regulated by the autonomic nervous system and interacts with energy metabolism and reproduction. Osteocalcin, one of the main organic ingredients of the bone matrix, was discovered to stimulate insulin production by the pancreas, as well as energy expenditure and insulin sensitivity. Administration of recombinant osteocalcin to mice on a high fat diet decreased weight gain and insulin resistance. These unanticipated results stimulated studies on osteocalcin and glucose metabolism in humans. This review will discuss these clinical studies and their perspective for the future.

KEYWORDS

Bone metabolism, glucose metabolism, insulin, osteocalcin, review

INTRODUCTION

For a long time the only functions attributed to the skeleton were locomotion and calcium storage. Over the last decade, this view has changed. Genetic studies in mice have shown that bone metabolism is regulated by the autonomic nervous system and interacts with energy metabolism and reproduction (reviewed in references 1-5). This review will focus on the interaction between bone metabolism and glucose metabolism and will highlight animal experimental research with potential towards clinical application.

INSULIN AND BONE

It is well recognised that diabetes patients have an increased fracture risk. However, bone mineral density is affected differently in diabetes type 1 and 2 patients.[6] Diabetes mellitus type 1 (DM1) patients have a lower bone mineral density[7] whereas diabetes mellitus type 2 (DM2) patients have a higher bone mass than healthy individuals.[8] The mechanisms for these differences are not completely understood, but one of the hypotheses is that insulin is an anabolic factor for bone. As a consequence, DM1 patients, with a lack of insulin, do not attain their peak bone mass which leads to lower bone mineral density and a higher risk of fracture while DM2 patients are hyperinsulinaemic which stimulates bone accrual. This hypothesis has been strengthened by in vitro experiments showing that osteoblasts express the insulin receptor and addition of insulin to osteoblast cultures promotes survival[9] and collagen synthesis.[10] Although bone mineral density is increased in DM2 patients, the quality of the bone is probably lower, possibly due to the hyperglycaemia, leading to an increase in fracture risk.[11] Furthermore, the risk of falls promoting fracture is increased especially in DM2 patients; the reasons for this include medication use, hypoglycaemic episodes and gait instability because of neuropathy and visual impairment.[12]

BONE AND INSULIN

Karsenty *et al.* were the first to hypothesise that bone exerts a reciprocal influence on insulin metabolism. They reasoned that the skeleton is a very large organ and its maintenance consumes vast amounts of energy, making a link between the skeleton and energy supply plausible. By screening for bone-specific genes and subsequently generating knockout mice of these genes to study the metabolic phenotypes, osteocalcin and embryonic stem

cell phosphatase (Esp) became likely candidate genes involved in energy metabolism.[13-15] Osteocalcin is one of the main organic ingredients of the bone matrix and exists in an undercarboxylated and carboxylated form. Carboxylation of its glutamic acid residues increases its affinity for hydroxyapatite, facilitating its engraftment in the bone matrix. Osteocalcin knockout mice, which have been studied before in the context of bone metabolism, turned out to be obese and poor breeders. Metabolically, these mice exhibited hyperglycaemia, low insulin levels, low beta cell mass, low insulin sensitivity and low energy expenditure. The phenotype of heterozygous Esp knockout mice posed a mirror image of the osteocalcin knockout mice. Esp encodes the enzyme osteotesticular protein tyrosine phosphatase (OST-PTP), Esp is expressed solely in osteoblasts and Sertoli cells and OST-PTP inactivates the insulin receptor in the osteoblast. Therefore in Esp knockout mice the insulin receptor in the osteoblast is constitutively active. Esp knockout mice had increased osteocalcin concentrations, were lean with high energy expenditure, and had increased glucose tolerance and insulin sensitivity. At the same time, the research group of Clemens reported the phenotype of the osteoblast-specific insulin receptor knockout mouse which turned out to be osteopenic with low osteocalcin serum concentrations, obese and insulin resistant.[16]

Further investigations[17-21] into the relation between osteocalcin, OST-PTP and glucose metabolism showed that insulin, upon binding to the insulin receptor on the osteoblast, promotes osteocalcin gene expression and decreases the expression of the gene osteoprotegerin (OPG). OPG normally impedes osteoclast differentiation; therefore, insulin signalling on the osteoblast stimulates bone resorption by the osteoclast. During bone resorption, osteoclasts create an acidic environment to dissolve bone matrix. Osteocalcin is released from the bone matrix and because of the low pH, the glutamic acid residues on osteocalcin become decarboxylated and the concentration of undercarboxylated osteocalcin in the circulation rises. Finally, binding of undercarboxylated osteocalcin to the receptor GPCR6a on the pancreatic beta cell stimulates insulin secretion (*figure 1*).

Infusion of recombinant osteocalcin into wild-type mice indeed improved glucose tolerance and increased insulin secretion. Furthermore, when infused in mice on a high fat diet, osteocalcin reduced weight gain and insulin resistance.[22,23]

CLINICAL STUDIES

Glucose metabolism

Following the discovery of osteocalcin as a regulator of glucose metabolism in mice, many researchers started reporting on the association between osteocalcin levels and measures of glucose metabolism in humans. Since osteocalcin deficient mice are hyperglycaemic, it was expected that humans with lower osteocalcin levels would have higher indices of glucose metabolism, such as fasting plasma glucose, insulin and HOMA index. Several studies indeed confirmed this inverse relation in postmenopausal women,[24] obese patients,[25] men[26-28] and older patients.[29] In addition, a compensatory increase in osteocalcin was shown in prediabetes[30] and lower osteocalcin predicted the development of diabetes over ten years of follow-up in men with an increased risk of diabetes.[31] Additional studies showed the same inverse relation between osteocalcin and the metabolic syndrome,[32-34] coronary atherosclerosis,[35] fat mass and intima-media thickness[36] and non-alcoholic fatty liver disease.[37]

From these studies, there seems to be an association between osteocalcin and glucose or insulin metabolism, which is compatible with the mouse models. However, in all of the reported studies, total osteocalcin was measured and in the studies that measured both total and undercarboxylated osteocalcin the relation was observed for total osteocalcin only, whereas the studies in mice centred on undercarboxylated osteocalcin. To solve this inconsistency, it would be necessary to prospectively evaluate the effect of osteocalcin on glucose metabolism in an intervention study. However administration of recombinant osteocalcin to humans has not been reported yet.
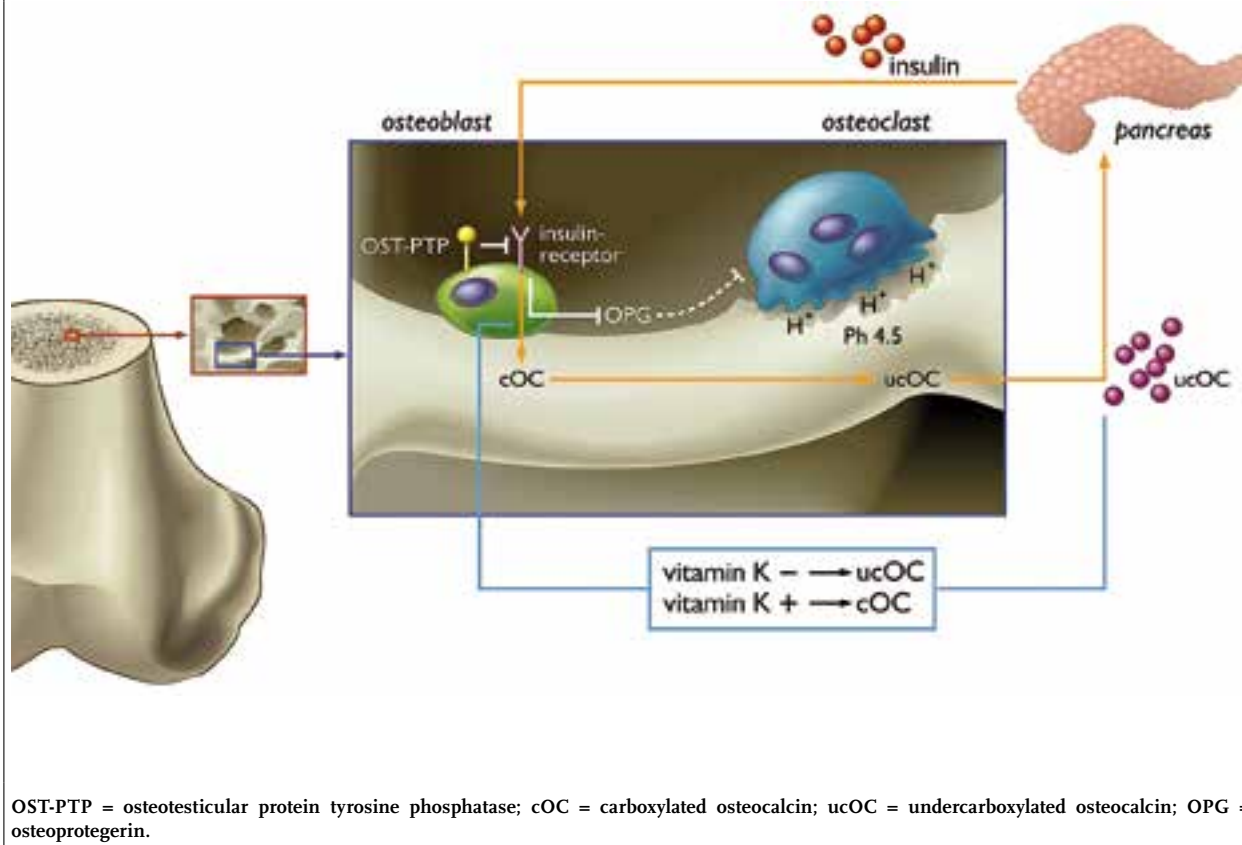
Bone metabolism

Another approach is to investigate the effects of interventions in bone metabolism which affect osteocalcin concentrations on glucose metabolism. It was expected that a decrease in undercarboxylated osteocalcin as observed during bisphosphonate treatment would have a negative effect on glucose homeostasis. And vice versa, that treatment with parathyroid (PTH) hormone, increasing bone formation and osteocalcin concentrations, would protect against glucose metabolism derangements. But no difference in fasting glucose or the glucose/insulin ratio was observed comparing patients treated with alendronate or PTH, although the osteocalcin concentrations changed several-fold.[38] Contrary to the hypothesis, bisphosphonate users had a dose-dependent reduced risk of diabetes compared with matched controls with a dose-response effect.[39] In addition three large, randomised, placebo-controlled trials of alendronate (FIT trial), zoledronic acid (HORIZON-PFT) and denosumab (FREEDOM) showed no effect on fasting glucose, body weight or diabetes incidence.[40]

Vitamin K metabolism

Another possible interventional approach to modulate osteocalcin concentrations comes from vitamin K

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Figure 1. *Model of action of insulin and osteocalcin. Insulin activates the insulin receptor on the osteoblast and this stimulates production of osteocalcin. After vitamin K dependent carboxylation, carboxylated osteocalcin is incorporated into the bone matrix by the osteoblast. Furthermore, activation of the insulin receptor reduces the production of osteoprotegerin leading to an increase in osteoclastic bone resorption. The acidic pH in the resorption pit resolves the bone matrix and uncarboxylates osteocalcin. Undercarboxylated osteocalcin is released into the circulation and stimulates insulin production by the pancreas. Thus, the ratio of undercarboxylated and carboxylated osteocalcin is determined by osteoclastic bone resorption and vitamin K availability. Osteotesticular protein tyrosine phosphatase inactivates the insulin receptor and terminates the feedforward loop*

OST-PTP = osteotesticular protein tyrosine phosphatase; cOC = carboxylated osteocalcin; ucOC = undercarboxylated osteocalcin; OPG = osteoprotegerin.

metabolism. Vitamin K is essential for the carboxylation of glutamic acid residues in several proteins, including osteocalcin. Vitamin K deficiency increases undercarboxylated osteocalcin and supplementation of vitamin K reverses this effect.[41] Therefore, supplementation of vitamin K was expected to have a negative effect on glucose metabolism. Several randomised controlled trials demonstrated that vitamin K supplementation decreased undercarboxylated osteocalcin, but the effect on glucose metabolism varied from an increase in insulin sensitivity in younger men,[42] no effect on glucose metabolism in women,[43,44] to an increase in insulin concentrations in older men.[43]

Osteoid osteoma
Finally, a recent case report on osteoid osteoma patients was considered a proof of principle of the action of osteocalcin in humans. Osteoid osteoma is a benign osteoblastic tumour shown to secrete osteocalcin.[45] Two young male patients, who had this tumour removed, were compared with two matched patients undergoing knee surgery and with three healthy controls. Surgical resection of the tumour was followed by a decrease in serum total osteocalcin accompanied by an increase in serum glucose and insulin, representing some degree of insulin resistance in the osteoma patients but not in the two control groups. Undercarboxylated osteocalcin concentrations were not reported.

CONCLUSION AND DISCUSSION

The association of diabetes with impaired bone metabolism has been longstanding. Strong evidence from experimental studies in genetically modified mice indicates that the bone derived hormone osteocalcin interacts with glucose and insulin secretion and possibly insulin action. This led to the proposal of the

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bone-pancreas endocrine axis and spurred a wealth of studies investigating the mechanism in humans. So far, many post-hoc analyses of observational studies have confirmed the inverse relation between osteocalcin and parameters of glucose and insulin metabolism. However, only a few studies measured the undercarboxylated form of osteocalcin, which is known to be the hormonally active form in mice. Furthermore, the inverse relation between osteocalcin and glucose was not observed in several interventional studies. Since observational studies do not prove causality and the interventional studies do not support the hypothesis, the question remains whether osteocalcin has the same role in the regulation of energy metabolism in mice as in humans.

One of the possible explanations for the difference in mice and humans could be a genetic difference; humans have only one osteocalcin gene whereas mice have three. The protein sequence is conserved for 60% in mice compared with humans. In humans, the promoter of the osteocalcin gene is upregulated by vitamin D whereas the mouse gene is downregulated (reviewed in reference 46). Another explanation concerns the mouse model used in these experiments; knockout mice have a total lack of osteocalcin, whereas in human physiology osteocalcin levels may vary, but will never be completely absent. This will probably make it more difficult to pick up subtle effects. On top of this, serum osteocalcin exhibits diurnal variation and is increased during growth and skeletal maturation, ageing and menopause[47,48] which could influence the associations obtained in cross-sectional research designs.

Furthermore the role of vitamin K should be considered. Since the bone-pancreas axis is controlled by osteocalcin released from the bone and decarboxylated by the acidification of osteoclasts, the vitamin K dependent carboxylation in the circulation could influence the feedback loop. In humans the percentage of undercarboxylated circulating osteocalcin is supposed to be a marker of vitamin K intake and most studies did not take into account vitamin K concentrations or intake.[46,48] This is also an important limitation of the osteocalcin infusion studies in mice, since any clinically relevant change should be compared with the changes in concentrations with vitamin K intake. In addition, the measurement of osteocalcin and its carboxylated and undercarboxylated form is still a challenge and the interpretation could bias the results.[49,50]

Therefore, the question remains whether changes in osteocalcin mediate an effect on glucose metabolism or, the other way around, whether rising glucose concentrations and changing insulin concentrations in diabetes affect bone metabolism by influencing osteocalcin concentration. The evidence from the current studies is inconclusive to answer this question definitively. Finally,

an enticing question is whether it is 'just' osteocalcin and glucose metabolism or whether there are additional bone hormones which could influence not only glucose but also other processes of energy metabolism.

Notwithstanding these limitations, the unravelling of a new endocrine axis involving bone and glucose metabolism is very exciting and the prospect of novel therapeutic options for the treatment of obesity and diabetes is worth the effort.

ACKNOWLEDGEMENT AND DISCLOSURE

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