Endocrine regulation of energy metabolism by bone

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Summary
The classical functions of bone are the maintenance of phosphorus-calcium homeostasis, damage repair, as well its structural function which allows locomotion and protects the vital organs. The recent discovery of new functions for bone in the regulation of energy metabolism suggest that bone may be an endocrine organ. In the last decade, different genetic and molecular studies carried out in mice have determined that osteocalcin increases the secretion of insulin, and sensitivity to it, by increasing the secretion of adiponectin, stimulates the proliferation and the better functioning of the beta cells, promotes the reduction of fatty mass and an increase in the consumption of energy. These findings demonstrate the existence of a reciprocal regulation between bone and energy metabolism, mediated by osteocalcin. The recognition of the metabolic role of osteocalcin is a significant discovery in the field of osteology and endocrinology, bringing the possibility of new therapies in the treatment and prevention of metabolic diseases such as diabetes mellitus, sarcopenia, obesity and osteoporosis.

Key words: osteocalcin, bone, endocrine organ, energy metabolism, insulin, diabetes mellitus, obesity.
Introduction
The classical functions of bone are the maintenance of phosphorus calcium homeostasis, the repair of damage to the bone, as well as a structural function which allows locomotion and protects vital organs. The bone is a dynamic tissue in constant change through bone remodelling, and which requires a great quantity of energy to perform this process.

Osteoporosis is the most frequent disease caused by changes in bone remodelling and is related to an increase in bone resorption or a decrease in its formation. The clinical observation that osteoporosis happens as a consequence of gonad failure and that being overweight protects against the development of osteoporosis suggests a hypothesis that appetite or body mass, reproduction and bone mass may have a hormonal regulation mechanism in common. This conjecture, and the recent discoveries of the new functions of bone in energy metabolism, suggest that bone may be an endocrine organ.

In the last few decades, numerous clinical trials have demonstrated that leptin, a hormone derived from adipocytes, regulates the appetite and exerts a bimodal antagonistic effect on bone remodelling. To achieve this, two different hypothalamic pathways are used, the sympathetic nervous system (SNS) and the cocaine and amphetamine regulated transcript system (CART), which act directly on the osteoblasts.

Lee et al., in different genetic and molecular studies carried out in mice, determined that osteocalcin increases the secretion of insulin and its sensitivity by raising the secretion of adiponectin; and that it also stimulates the proliferation and functioning of the beta cells, at the same time as promoting the reduction of fat mass and an increase in the consumption of energy. These findings demonstrate the existence of a regulatory relationship between bone and energy metabolism, mediated by osteocalcin (OC).

In some previous studies carried out in humans, different markers for low bone mass were detected in diabetic patients, among which was OC, but until very recently, research had not been carried out in these patients to determine its metabolic functions. Recently, an association has been found in adults these patients to determine its metabolic functions.

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Bone remodelling is a biphasic process which occurs sequentially and in a balanced way. It consists of destruction or resorption followed by formation of new bone matrix. This process allows the constant maintenance of bone mass throughout adulthood, and is essential for the maintenance of bone architecture to meet mechanical requirements, the repair of damage which occurs in daily exercise and the maintenance of the homeostasis of the phosphorous-calcium metabolism, such that remodelling constitutes a true homeostatic function. A great many homeostatic functions, such as appetite and reproduction, are controlled by the hypothalamus. It is not strange to imagine that remodelling could, at least in part, be controlled by a central mechanism.

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In the identification of hormones which may regulate the formation of bone, we start with two essential clinical facts. First, osteoporosis originates with gonad failure, and second, being overweight appears to protect against osteoporosis. These two observations suggest the existence of a common regulatory mechanism between appetite, reproduction and bone mass. In attempting to determine the regulatory hormone or hormones, it has been observed that leptin is the only one which significantly influences all three functions.

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It has been established that there are different phases in the central regulation of bone remodelling. The process starts with the emission of afferent signals from the adipocytes to the hypothalamus, in which leptin has an important role. It continues with a complex hypothalamic neural phase in which numerous neuropeptides participate, and from which originate efferent signals towards the adrenergic β2 receptors in the osteoblasts. As a consequence there are changes in transcription factors and clock genes which affect osteoblastogenesis. The last phase is the regulation by bone of the adipocytes, probably through the release of OC. The adipocytes may, in turn, regulate the proliferation and differentiation of the osteoblasts (Figure 1).
Osteocalcin (OC)

Bone and energy metabolism appear to have a regulation which is linked by a central component, which is fat. To validate this hypothesis, a mediator is necessary which, originating in bone, is able to regulate energy metabolism. This mediator is OC.

The strategy for demonstrating this theory requires the identification of genes specific to the osteoblasts, to produce mice with deletions and to study the metabolic phenotypes5. Lee et al., in different experiments in vitro, confirmed that osteoblasts secrete a substance which affects the pancreatic cells and the adipocytes, and which appears to regulate glucose metabolism1. The co-cultivation of osteoblasts of wild-type mice with pancreatic islets increased by up to 500% the expression of the gene for insulin and of the markers for cell cycle progression in the islets. Karsenty et al., were the first to propose, in 2006, that there was endocrine regulation of energy metabolism in the skeleton1.

The observation that a mouse deficient in protein specific to the osteoblast cell line, OC, (OC-/-), showed an abnormal quantity of visceral fat, led to the hypothesis that this was the hormone secreted by the osteoblasts which affected glucose metabolism1. The expression of adiponectin and its molecular modifications include the excision of a pre-propeptide or insulin-sensitising enzymes, as well as for adiponectin and insulin, were reduced in those mice without OC4. In mice without Esp an up to three-fold increase was observed in the mitochondrial area and in proteins associated with biogenesis. In spite of insulin being a lipogenic hormone these mice were thinner due to an increase in energy use, evidenced by an increase in the expression of PGC1-alpha and of the uncoupling protein 1 (UCP 1), without the appetite being affected. The reduction in fat mass was restricted to visceral fat, and triglyceride levels were lower.

In order to confirm that the deletion of Esp would protect against the development of diabetic symptoms studies were carried out in mice in which hyperphagia was induced through hypothalamic structural lesions, destruction of beta pancreatic cells or feeding with a fatty diet. In the mice without Esp, it was confirmed that there was a lower weight gain and that no glucose intolerance or insulin resistance were developed9.

The fact that the mice without OC have a phenotype contrary to those without Esp suggests that the two genes are in the same pathway, although they do not physically interact (Table 1). Lee reported that 90% of the OC was bonded with hydroxyapatite, while in the mice without Esp this was only 74%. This fact raises the possibility that OST-PTP, regulated by the Esp gene, would be in some way involved in the gamma-carboxylation of OC2.

OC is secreted by the osteoblasts following many posttranslational modifications. These modifications include the excision of a pre-propeptide and gamma-carboxylation, dependent on vitamin K, of the three glutamic residues in the Gla residues2. This gamma-carboxylation offers an opportunity for regulation41. Surprisingly, although it is the most abundant non-collagen protein (15% in bone), it is not involved in bone formation17.

Mice without OC have high levels of glucose, low levels of insulin and a reduction in insulin secretion stimulated by glucose, in comparison with those with the wild genotype20,21 (Figure 2). In the pancreas, the size of the islets, the mass of the beta cells and the amount of insulin were reduced. In addition, fat mass, the number of adipocytes and levels of triglyceride were increased. The expression of adiponectin and its molecular targets were reduced, and a subcutaneous infusion of recombinant OC in wild mice would produce an increase in insulin and in its sensitivity, and improve the expression of the insulin genes1.

Subsequently, mice were obtained which had an absence of genes which are expressed preferentially in the osteoblasts. The first gene was Esp, which codes for the receptor of the tyrosine phosphatase protein (OST-PTP) present in mother cells, Sertoli cells and in osteoblast, and which are not expressed in the beta cells of the pancreas or in adipose tissue10. Those mice without Esp (Esp-/-) had larger and more numerous pancreatic cells than wild mice, proliferating by 60% to 300% in mice from the age of 5 days to one month. The increase in levels of insulin and insulin sensitivity was due to the capture of glucose in the muscle, brown fat, white fat and liver, and to the increase in adiponectin (the only adipokine affected, which was doubled in these mice), as well as an increase in the expression of other target genes of adiponectin, which increased threefold, such as the acyl-CoA oxidase gene, the gene for the receptor activator of the proliferation of alpha peroxisome (PPAR alpha), and the gene for the uncoupling protein 2 (UCP 2).

On the other hand, those genes which coded for insulin-sensitising enzymes, as well as for adiponectin and insulin, were reduced in those mice without OC4. OC is one of the few proteins specific to the osteoblasts which has numerous characteristics of a hormone. It is a molecule of low molecular weight (5,700 Da) produced by the osteoblasts1. It is present in all vertebrates, and is considered to be a marker for differentiation to mature osteoblasts. It is secreted into the circulation and, since its identification 30 years ago, has been considered to be the main constituent of the extracellular matrix, where it bonds with hydroxyapatite by means of three gamma-carboxylate residues, called Gla residues. This carboxylation offers an opportunity for regulation41. Surprisingly, although it is the most abundant non-collagen protein (15% in bone), it is not involved in bone formation17.

There is a high proportion of non-carboxylated OC in circulation, while a greater proportion of carboxylated OC is found in the bone matrix. However, not all the residues of the three glutamic acids are completely carboxylated and incorporated into the crystals, and the degree of carboxylation varies2. Vitamin K is a cofactor for the enzyme glutamate carboxylase which is required for the carboxylation of the proteins which contain Gla in the coagulation cascade and for the carboxylation of OC. Carboxylation or its absence...
makes OC susceptible to release by the osteoblasts into the circulation. Experiments in vitro carried out in isolated islets and in primary adipocytes, have revealed that the carboxylated form is inactive while the non-carboxylated form is the active form.

As we have already commented, the (Esp-/-) mice and mice treated with non-carboxylated OC resulted in a protector phenotype, as opposed to the (OC-/-) model. Alternatively, the overexpression of OST-PTP resulted in a phenotype identical to the (OC-/-) mice.

The metabolic changes in mice with a deletion of the insulin receptor in the osteoblasts provides evidence of a link between the action of the insulin in bone remodelling and glucose homeostasis. The signalling of the insulin in the osteoblasts inhibits carboxylation, which means that it is a positive regulator of metabolic activity. OST-PTP inhibits insulin signalling, such that the metabolic phenotype of mice without Esp is secondary to an increase in insulin signalling in the osteoblasts and to the secondary increase in non-carboxylated OC.

Insulin promotes the ability of osteoblasts to improve bone resorption. The expression of OPG is reduced, through Fox01, and this promotes bone resorption and, in particular, the expression of Tcigr 1 which codes for a proton pump subunit which contributes to the acidification of the extracellular bone matrix. The acidic pH of around 4.5 generated during bone resorption is sufficient to de-carboxylate and activate the molecules of OC (Gla-OCN) stored in the extracellular bone matrix. Sufficient quantities of non-carboxylated OC are produced to induce the expression of the beta cells and to stimulate in them the expression of insulin, just as with treatment with recombinant OC. This shows a pH-dependent activation mechanism for this hormone, and identifies insulin signalling as a critical link between bone remodelling and energy metabolism.

The data obtained in numerous studies in humans regarding the relationship of OC with glucose homeostasis and, on the one hand, energy metabolism and, on the other, bone metabolism, are highly significant. In the first area, the findings are very interesting. The mice models show OC to be a hormone which positively regulates the production of insulin and its sensitivity. Low levels of OC are associated with diabetes mellitus, glucose intolerance and insulin resistance. Additional studies have established that low levels of OC reduce glycosylated haemoglobin (HbA1c) and insulin resistance by means of the homeostasis model assessment (HOMA), independently of the presence of diabetes mellitus. These data suggest that an increase in the levels of OC could be a therapy for the treatment of diabetes in the future.

It has also been found that levels of OC in circulation are inversely correlated with parameters of adiposity, such as body mass index (BMI) or fat mass, and with levels of blood glucose in men and women of different ethnicities. Levels of circulating OC have also been associated with a number of lipid abnormalities, and are positively correlated with levels of adiponectin in postmenopausal women. More generally, obese people have less OC than those who are not obese, and those with diabetes mellitus type 2 have less OC than non-diabetics.

The dynamic production of OC as a marker for energy and glucose metabolism has begun to be tested. For example, it has been seen that a significant loss of weight causes a decrease in the
homeostasis model assessment for insulin resistance (HOMA-IR), as well as an increase in levels of OC in obese children. In non-diabetics, loss of weight through diet and exercise increases levels of OC in parallel with the decrease in visceral fat, and increases sensitivity to insulin. Lower levels of OC have also been correlated with acute myocardial infarction.

In terms of the second area, it has been widely reported that the alterations in glucose metabolism may be a risk factor in loss of bone mass and fractures in humans. In a transverse study it was found that osteoporotic women, after 6 months or more of treatment with alendronate had lower levels of non-carboxylated OC than those who had not been treated. There are scarcely any studies which establish the effects of bisphosphonates on the homeostasis of glucose or insulin sensitivity. Treatment with alendronate or raloxifene (selective estrogen-receptor modulator) for 12 weeks reduced the biochemical markers for bone remodelling, including OC.

It would be an important step to confirm whether the antiresorptive drugs, which are the main treatment for osteoporosis, may in some cases have a negative effect on glucose tolerance, as well as the effect of the anticoagulants which affect vitamin K.

Lastly, it is also worth highlighting the possible relationship which appears to exist between OC and the endocrine regulation of male reproduction through the regulation of androgen production.

Conclusions
Recent models in mice suggest a new role for bone in which it is the source of a hormone, non-carboxylated OC, which affects energy metabolism, insulin resistance, obesity and the development of diabetes. So, an organ with an endocrine function, such as fat, is mediated by the endocrine function of another organ, the skeleton. Studies which should be carried out, based on this physiological integration, should review other new areas which have not yet been studied.

There are increasing numbers of studies which are establishing that many aspects of the biology of OC are similar in mice and humans, and which are attempting to determine whether or not the endocrine system of mice is different to that in humans.

The possible role of adiponectin, leptin, OC and insulin, which appear to integrate the functions of the adipose, bone and pancreatic tissues to regulate the metabolism of glucose, bone remodelling, energy and lipid metabolism, remains to be clarified.

We may consider that the skeleton, as an endocrine organ, is involved in the overall co-ordination of energy use, through interaction with other tissues. The identification of OC, and its regulation and different effects, has provided the basis for the development of potential therapeutic applications for metabolic diseases such as diabetes, sarcopenia or obesity.

Both authors declare that there are no conflicts of interest.

Bibliography