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Review

Bone and glucose metabolism: A two-way street

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ABSTRACT

Evidence from rodent models indicates that undercarboxylated osteocalcin (ucOC), a product of osteoblasts, is a hormone affecting insulin production by the pancreas and insulin sensitivity in peripheral tissues, at least in part through enhanced secretion of adiponectin from adipocytes. Clinical research to test whether this relationship is found in humans is just beginning to emerge. Cross-sectional studies confirm associations between total osteocalcin (OC), ucOC and glucose metabolism but cannot distinguish causality. To date, longitudinal studies have not provided a consistent picture of the effects of ucOC or OC on fasting glucose and insulin sensitivity. Further exploration into the physiological and mechanistic effects of ucOC and OC, in rodent models and clinical studies, is necessary to determine to what extent the skeleton regulates energy metabolism in humans.

Introduction

It is well known that altered metabolism, as seen in diabetes, is a risk factor for bone loss and fractures in humans [1–13] and rodent models [14–19]. However, recent results from rodent models suggest an entirely new role for bone, namely, that bone is the source of a hormone affecting energy metabolism, insulin resistance, obesity and development of diabetes. In these models, a bone product, osteocalcin, in particular the undercarboxylated form of osteocalcin (ucOC),[1] had a positive effect on both insulin production and insulin sensitivity [20]. These exciting findings suggest new possibilities for the prevention of obesity and diabetes although there is still much work to be done in mouse models to clarify the pathways connecting ucOC and energy metabolism. Translating these findings to clinical research is just beginning. It is not known if these mechanisms operate in humans, and, if they do, to what extent physiological levels of ucOC influence the pancreas or insulin sensitivity in other tissues. Initial cross-sectional studies have reported associations consistent with the rodent models but the limited longitudinal data are conflicting. In this review, we summarize the key findings to date from mouse models and clinical studies.

Synthesis of osteocalcin and undercarboxylated osteocalcin

Osteocalcin (also known as bone Gla protein, BGP) is a secreted 5 kDa protein that is the most prevalent noncollagenous protein in bone [21]. It is synthesized exclusively in certain cells of the osteoblast lineage: mature osteoblasts and osteocytes [22,23]. Most OC is found in bone, but small amounts circulate in the blood, and serum levels are considered a marker of bone formation [24]. However, the role of OC in bone is not entirely understood. OC is not required for bone formation, because $OC^{-/-}$ mice actually have increased bone density, failure load, and mid-diaphyseal hardness [23,25]. It has been postulated that OC normally functions in bone to inhibit mineralization [23], perhaps in order to prevent osteocytes from becoming completely embedded in mineral [22,26]. OC gene transcription is regulated in part by levels of 1,25-dihydroxyvitamin D [27].

Post-translational modification of osteocalcin (gamma-carboxylation at three Gla residues) allows it to tightly bind the calcium ions in hydroxyapatite (HA) [28–30]. Carboxylation occurs through vitamin-K dependent carboxylase activity, although the mechanism is not completely understood [31]. Undercarboxylated OC (ucOC) has fewer than three carboxylated residues, ranging from none to two, and therefore has a lower affinity for bone. The fully carboxylated and undercarboxylated forms of OC are both found in bone and in serum [32]. However, a higher proportion of ucOC is found in the circulation, while a higher proportion of fully carboxylated OC resides in the bone matrix.

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[1] *Abbreviations used:* ucOC, undercarboxylated osteocalcin; OC, osteocalcin; HA, hydroxyapatite; HFD, high fat diet; GTG, gold thioglucose; *Ins1*, *Insulin1*; *Ins2*, *Insulin2*; ATF4, activating transcription factor 4; FoxOs, Forkhead family transcription factors; HAP, hydroxyapatite; UTV, ultrasonic transmitted velocity; TOC, total OC; FG, fasting glucose; HT, hormone therapy; SERM, selective estrogen receptor modulator; PEPI, Postmenopausal Estrogen/Progestin Intervention Study; MORE, Multiple Outcomes of Raloxifene Evaluation; PTH, parathyroid hormone; BMI, body mass index; DXA, dual X-ray absorptiometry; PAI, plasminogen activator inhibitor.

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Vitamin K is a co-factor for the enzyme glutamate carboxylase required for carboxylation of the Gla-containing proteins in the coagulation cascade and for carboxylation of osteocalcin [33]. Lower dietary levels of vitamin K are associated with increased levels of ucOC, and vitamin K supplementation reduces ucOC [34]. Warfarin's action as an anti-coagulant is based on its inhibition of the vitamin-K dependent carboxylase, preventing post-translational carboxylation of factors in the coagulation cascade and of OC. Thus, warfarin results in increased levels of ucOC. However, warfarin also regulates mRNA expression of osteocalcin, which makes interpretation of warfarin treatment studies more complex [35].

Bone regulation of metabolism: evidence from rodent models

The work of G. Karsenty's group has dominated this field since 2007 when the publication entitled "Endocrine regulation of energy metabolism by the skeleton" was the first to suggest that bone could influence glucose homeostasis by acting as an endocrine organ [20]. Karsenty's initial notion that bone could regulate glucose homeostasis came from an observation that mice deficient in an osteoblast-lineage specific protein osteocalcin ($OC^{-/-}$) were a little fat. Further examination revealed that these mice presented with some metabolic abnormalities: they had higher blood glucose, lower serum insulin, impaired glucose-stimulated insulin secretion (GSIS) and poor glucose tolerance when compared to wild type mice [20]. The low serum insulin resulted from a near 50% decrease in pancreatic β-cell mass and insulin content. Interestingly, osteocalcin-knockout mice had reduced serum adiponectin, suggesting a role for osteocalcin in insulin sensitivity as well as insulin secretion. The idea that deletion of this bone-specific protein could radically alter whole-body metabolism was unheard of, to put it lightly.

Evidence for systemic ucOC regulation of glucose homeostasis

Systemic ucOC (purified from bacteria) treatment has the opposite effect on metabolism as that in the $OC^{-/-}$ mice. Metabolic assays demonstrate that chronic infusion of ucOC (0.3–3.0 ng/h) reduces blood glucose, increases serum insulin, improves GSIS and glucose tolerance [36]. Interestingly, ucOC treatment had anti-diabetic properties: it attenuated high fat diet (HFD) and gold thioglucose (GTG)-induced obesity and diabetes [36]. In the pancreas, these mice had increased Ki67 positive islet cells [36], but it is important to point out that ucOC also has profound effects on insulin sensitivity of target tissues (muscle, adipose tissue, and liver) [20,36]. Serum adiponectin (an adipocytokine that influences insulin sensitivity) levels were increased in ucOC treated mice [20]. Warfarin treatment, which inhibits γ-carboxylation, increases the amount of ucOC and reduces blood glucose in wild type mice but has no effect on blood glucose of $OC^{-/-}$ mice, demonstrating the effect is osteocalcin-specific [20,37].

Evidence for ucOC directly regulating pancreatic β-cell function

OC is a small, secreted protein, and it is assumed that it binds an unknown receptor on pancreatic β-cells under normal circumstances, although this has never been demonstrated. Lee et al. and Ferron et al. demonstrated that ucOC has the ability to induce *Insulin1* (*Ins1*), *Insulin2* (*Ins2*), *CyclinD1*, *CyclinD2*, and *Cdk4* expression in islet cultures, although it is unclear whether this effect is specific to uncarboxylated OC, or if it could be elicited to some extent by undercarboxylated or carboxylated OC [20,36].

Although ucOC is clearly a novel and exciting regulator of glucose metabolism, it remains unclear to what extent it functions as a hormone in normal mice. Are there situations in which ucOC levels increase/decrease depending on energy demands of the skeleton? For instance, in a situation of high remodeling (and therefore high skeletal energy needs) such as growth in young mice or during fracture repair, do ucOC levels decrease so that more glucose can reach and be utilized in bone? Do all conditions that suppress osteocalcin expression in mice result in metabolic syndrome? This is probably not the case since chronic obstructive pulmonary disease, inflammatory bowel disease, and ethanol consumption are not typically thought to cause metabolic syndrome but are associated with decreased serum osteocalcin levels. It is possible that in these low osteocalcin secretion conditions, serum ucOC is maintained at normal levels despite a reduction in total OC levels. Interestingly, spaceflight, known to cause bone loss and suppress osteoblast activity and osteocalcin expression, has been suggested to decrease insulin secretion and increase blood glucose levels [38,39]. While the exact mechanism of γ-carboxylation (and biological inactivation) of OC remains unknown, in recent years Karsenty has identified several osteoblast-specific regulators of ucOC, and therefore energy balance, and these are summarized in Table 1 and Fig. 1.

γ-carboxylation of OC through an OST-PTP-dependent mechanism

The product of the *Esp* gene (osteotesticular protein tyrosine phosphatase, OST-PTP) is a receptor-like protein tyrosine phosphatase expressed only in osteoblasts and Sertoli cells [40]. *In vitro* and *in vivo* evidence suggests that OST-PTP is important for osteoblast maturation and skeletogenesis and responsive to parathyroid hormone [40–42], although the specific protein directly modified by OST-PTP is unknown.

Karsenty's group found that deletion of the *Esp* gene results in a phenotype opposite that of the $OC^{-/-}$ mice: lower blood glucose and higher insulin, glucose-stimulated insulin secretion, glucose tolerance, pancreatic insulin content and β-cell proliferation when compared to wild type mice [20]. $Esp^{-/-}$ mice are also protected from HFD and GTG-induced obesity and diabetes, similar to ucOC treated mice [20,36]. Alternately, bone-specific overexpression of OST-PTP resulted in a phenotype identical to $OC^{-/-}$ mice. Therefore, Lee et al. hypothesized that OST-PTP was responsible for inactivating OC through γ-carboxylation [20]. In support of this hypothesis, deleting one *OC* allele in $Esp^{-/-}$ mice ($Esp^{-/-}$,$OC^{+/-}$) returns the metabolic phenotype of the $Esp^{-/-}$ mice back to normal [20]. In other words, deletion of one *OC* allele reduces the amount of total OC, and presumably the amount of ucOC, thereby mimicking OST-PTP activity (which is absent in $Esp^{-/-}$,$OC^{+/-}$ mice).

Genetic deletion of *Esp* does not alter *OC* gene expression or total serum levels, but it does reduce the amount of HA-bound OC by approximately 15%, demonstrating that the effect of OST-PTP must be post-translational [20]. However, OC itself is not phosphory-

Table 1
Metabolic phenotypes of mouse models compared to wild type.

Increased insulin secretion and sensitivity	Similar insulin secretion and sensitivity[a]	Reduced insulin secretion and sensitivity
Warfarin	$OC^{-/-}$ + warfarin	$OC^{-/-}$
ucOC	Atf4-TG_{ob} + ucOC	Esp-TG_{ob}
$Esp^{-/-}$	Atf4-TG_{ob};$Atf4_{ob}{}^{-/-}$	Atf4-TG_{ob}
$Atf4_{ob}{}^{-/-}$	$Foxo1_{ob}{}^{-/-}$;$OC^{+/-}$	
$Foxo1_{ob}{}^{-/-}$		
$Esp^{+/-}Atf4_{ob}{}^{+/-}$		
$Esp^{+/-}Foxo1_{ob}{}^{+/-}$		

TG_{ob} – osteoblast specific overexpression from the type I collagen promoter.
$ob^{x/x}$ – osteoblast-specific genetic deletion through *Cre* expression from the type I collagen promoter.
[a] Rescue of metabolic phenotype.