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Bone and Glucose Metabolism: A Two-Way Street

Katherine J. Motyl^{a,b}, Laura R. McCabe^{a,b}, and Ann V. Schwartz^c

^a Department of Physiology, Biomedical Imaging Research Center, Michigan State University, 2201 Biomedical and Physical Sciences Building East Lansing, MI 48824, USA

^b Department of Radiology, Biomedical Imaging Research Center, Michigan State University, 2201 Biomedical and Physical Sciences Building East Lansing, MI 48824, USA

^c Department of Epidemiology and Biostatistics, University of California, San Francisco, 185 Berry St. Suite 5700, San Francisco, CA 94107, USA

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.

Keywords

undercarboxylated osteocalcin; insulin sensitivity; glucose metabolism

1. 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), 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

Corresponding Author: Ann V. Schwartz, PhD, Department of Epidemiology and Biostatistics, University of California San Francisco, 185 Berry Street, Suite 5700, San Francisco, CA 94107-1762, Tel: 415-514-8038, Fax: 415-514-8150, Aschwartz@psg.ucsf.edu.

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longitudinal data are conflicting. In this review, we summarize the key findings to date from mouse models and clinical studies.

2. 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.

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).

3. 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.

3.1. 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/hr) 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).

3.2. 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. 2007 and Ferron, et al. 2008 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 Figure 1.

3.3. 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. 2007 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 posttranslational (20). However, OC itself is not phosphorylated which implicates an unknown intermediary protein that links the phosphatase activity of OST-PTP to changes in the level of γ -carboxylation of OC. A recent report by Ferron, et al. 2010 suggests that OST-PTP does not directly affect OC processing, but rather is involved in osteoclast-dependent ucOC release from the bone mineral. Specifically, the authors demonstrated that OST-PTP promotes osteoprotegerin expression by suppressing osteoblast insulin signaling (43).

The closest homolog of the *Esp* gene in humans is a pseudogene that does not appear to produce a functional product (44). It is probable that one or more other PTP-ases provides the functions in human osteoblast differentiation that are fulfilled by OST-PTP in mice. Ferron, et al. 2010 demonstrated that dephosphorylation of the insulin receptor by OST-PTP in mice can be achieved by PTP1B in human cells (43). The exact mechanism of γ -carboxylation of OC will likely be an area of interest for bone researchers in the next few years (45).

3.4. Regulation of *Esp* by ATF4 and FoxO1

New insights in to the regulation of *Esp* expression have emerged over the last year. Activating transcription factor 4 (ATF4) is known to be involved in regulation of expression of multiple genes important for adult bone formation and development (46,47). Through osteoblast-specific knockout and overexpression studies, Yoshizawa, et al. 2009 demonstrated that ATF4 in osteoblasts regulates glucose metabolism similar to OST-PTP: ATF4 overexpression in bone reduced glucose tolerance and pancreatic insulin content (37). Alternately, osteoblast-specific *ATF4*^{-/-} mice had improved glucose tolerance and insulin sensitivity in target tissues (37). The authors also demonstrated direct binding of ATF4 to the *Esp* promoter and went further to demonstrate that an *Esp*^{-/-} metabolic phenotype could be elicited in mice heterozygous for both *Esp* and *Atf4*. Finally, the authors examined the γ carboxylation status of OC and found that mice deficient in ATF4 had higher ucOC compared to that of wild type mice, while mice overexpressing ATF4 had less ucOC, which when replaced by exogenous treatment returned the metabolic phenotype to normal (37).

Forkhead family transcription factors (FoxOs) have recently been shown to suppress oxidative stress and reduce osteoblast apoptosis (48,49). In addition to this activity, FoxO1 has been shown to regulate *Esp* expression in osteoblasts (45). Mice with an osteoblast-specific deletion of *FoxO1* (*FoxO1_{ob}*^{-/-}) have increased islet area, pancreatic β -cell mass, β -cell proliferation and improved glucose tolerance, reminiscent of *Esp*^{-/-} and *Atf4*^{-/-} mice. Interestingly, *FoxO1_{ob}*^{-/-} mice have two-fold more *OC* mRNA expression, in addition to approximately 30% more serum OC compared to wild type mice (45), which is contrary to the reduced bone formation observed in these mice (49). With the increased total OC, Rached, et al. 2010 also found increased uncarboxylated OC, which in addition to the metabolic phenotype led them to examine *Esp* expression (45). As expected, *Esp* was reduced in the *FoxO1_{ob}*^{-/-} mice and ChIP assay demonstrated that FoxO1 could bind to the *Esp* promoter (45).

3.5. Sympathetic nervous system regulation of OC carboxylation

What extracellular/intracellular signal regulates ATF4 and FoxO1 dependent transcription of *Esp* and apropos carboxylation of OC? Karsenty's group believes that leptin-dependent

sympathetic nervous system input in osteoblasts is responsible for *Esp* expression and ucOC levels (50). It has long been recognized that young leptin deficient mice (*ob/ob*^{-/-}) have increased insulin production and reduced blood glucose before insulin resistance and type 2 diabetes set in. These mice do not have different levels of total serum OC, but Hinoi, et al. found that they do have increased ucOC compared to wild type (50). In fact, osteoblast specific deletion of the β 2-adrenergic receptor increased the percent of ucOC without changing total levels as well. Targeted deletion of leptin receptor in the ventromedial hypothalamus, but not deletion in arcuate neurons, increased serum insulin levels (51), while deletion of leptin receptor in islet cells had no effect on insulin production. Alternately, recent findings from Karsenty's group indicate that leptin-dependent regulation of energy expenditure, appetite and bone mass is reliant on leptin's inhibition of brain stem derived serotonin (52)(53). Taken together, leptin seems to work at multiple levels to regulate metabolism. Leptin-dependent regulation of osteocalcin carboxylation, however, appears to be through the hypothalamus. The question remains as to how much hypothalamic SNS relay modulates *Esp* and OC carboxylation under physiologic conditions. One would expect that because leptin fluctuates drastically with food intake, that *Esp* expression and ucOC levels would fluctuate as well. Additionally, since *Esp* does not have a functional product in humans, there are likely multiple mechanisms of ucOC regulation that have not yet been uncovered.

3.6. Evidence for physiologic changes in OC/ucOC levels affecting metabolism in rodents

If osteocalcin is a major contributor to metabolic regulation, then it will be important to determine if changes are associated with altered metabolism. Most studies to date have only measured total osteocalcin levels, making it difficult to assess the contribution of ucOC to metabolic changes. However fluctuations in total OC levels, as seen with circadian rhythm, aging and diabetes, are to some extent associated with changes in metabolism. In mice (which are nocturnal), osteocalcin expression is highest during the day when they are sleeping (and insulin sensitivity is low) (54) and then decreases during the night (when glucose and insulin increase) (55). This is consistent with the human activity pattern of low levels of OC in the morning and peak levels at night (55–57). Rodent studies have not examined the form of circulating osteocalcin during this process.

Exactly how undercarboxylated osteocalcin is modulated with age and diabetes in mice remains unclear. Studies in mice suggest that total serum osteocalcin can go down with aging (58,59). One study in young mice found that total serum OC levels decrease as mice approach peak bone density (approximately 12 weeks of age), while bone OC levels increase (60), suggesting that more OC is carboxylated at this age, but little is known about how OC is modified with long term aging in mice. Similarly, diabetes, a disease of low insulin secretion and/or insulin resistance and hyperglycemia, is associated with reduced serum osteocalcin levels in rodent models (2,4,18,61), but levels of ucOC have not yet been reported. However, a recent study by Fulzele, et al. demonstrates that mice lacking insulin receptor expression in osteoblasts have lower circulating ucOC, decreased bone formation, and develop metabolic syndrome characterized by glucose intolerance and insulin resistance (62). While bone loss and insulin resistance were not demonstrated in a mouse model of broader insulin receptor depletion, the latter mouse model exhibited hyperinsulinemia which likely increased IGF-1 receptor signaling and may have compensated for the lack of insulin receptor signaling (63). Interestingly, treatment with ucOC improves the metabolic dysfunction of the osteoblast specific insulin receptor knockout mice (62). Thus, the study by Fulzele, et al supports a role for ucOC in metabolic regulation and further supports the existence of a bone-pancreas regulatory loop.

4. CLINICAL STUDIES

The intriguing findings from rodent models have naturally led to an interest in assessing whether OC and/or ucOC levels affect glucose or lipid metabolism in humans. Historically, clinical data on ucOC and OC have focused on skeletal outcomes, reviewed briefly below. A more limited body of research has assessed the effects of diabetes and hyperglycemia on bone turnover markers, including OC. More recent studies, also discussed below, have examined the effects of ucOC and OC on energy metabolism.

4.1. Determination of undercarboxylated osteocalcin and osteocalcin

Interpretation of results from clinical studies is complicated by the difficulties in accurately measuring circulating ucOC levels and by controversy regarding the optimal assay for ucOC. Two methods have been mostly used to measure the degree of circulating undercarboxylated osteocalcin (ucOC), the hydroxyapatite (HAP) binding assay (64,65) and direct determination by an ELISA specific for uncarboxylated osteocalcin (Takara, Shuzo Co, Ltd, Japan). The HAP binding assay is based on the lower affinity of ucOC for HAP compared to fully carboxylated OC. Before and after incubation with HAP, osteocalcin is measured with an immunoassay that detects with a similar affinity carboxylated and undercarboxylated osteocalcin. The ucOC levels are expressed as a percentage of the total osteocalcin levels measured before incubation with HAP.

Although the HAP method is not completely specific for undercarboxylated osteocalcin, this assay has been shown to provide discrimination between patients with and without treatment with the vitamin K antagonist, warfarin, and is sensitive to changes in carboxylation status with vitamin K supplementation (66). This HAP method was also used by Lee, et al. (20) and others (67–69) to assess the percentage of undercarboxylated osteocalcin in rodent models. As discussed by Gundberg, et al. (66), the ability of the assay to identify ucOC depends on the concentration of total OC. To adjust for this dependence, it is recommended that the ucOC levels be expressed as a percentage of the total osteocalcin levels (%ucOC) measured before incubation with HAP and should be compared to a control group matched for the same total OC. Assays of total OC recognizing only the intact and the N-terminal 1–43 fragment are preferable.

The direct ELISA for ucOC is highly specific for fully ucOC (66). However, it cannot be used in conjunction with total OC assays to determine %ucOC because of differences in standardization and sensitivity to circulating fragments (3). Takara also offers a combination kit that will measure fully carboxylated osteocalcin (gla-type) in parallel with uncarboxylated osteocalcin (glu-type). These assays can provide some estimation of the %ucOC. However, interpretation of results is complicated by the fact that read-out antibodies have differing epitopes, potentially leading to differential measurement of known fragments (70). Furthermore, compared to direct measurement, a hydroxyapatite binding step results in up to a tripling of the measured ucOC concentration in the sample, suggesting serum interference with the ELISA assay (71).

4.2. Correlates of ucOC

OC has a circadian rhythm with peak levels around 4AM and lowest concentrations in the afternoon (72). Sokoll et al. examined ucOC levels and reported a similar circadian rhythm for absolute ucOC values, but no change in %ucOC over 24 hours (73). Average differences from the afternoon low to the peak at about 4AM were 28% for total OC and 49% for ucOC.

Children and adolescents have higher levels of total OC, reflecting increased bone formation, and also have higher levels of ucOC (66). During the increased bone formation required for

fracture healing, total OC is dramatically increased although data are not available on ucOC levels (72). Osteocalcin is modestly increased in postmenopausal women but this increase is associated with net bone loss as bone formation is coupled to increased bone resorption (74, 75). For ucOC, most (76–78), but not all (79), studies have reported increased levels with older age. UcOC is negatively correlated with endogenous estradiol levels and is increased in postmenopausal women (79,80). A study of bilateral oophorectomy in premenopausal women reported increased levels of ucOC after surgery (81). Use of hormone replacement therapy is associated with decreased levels of ucOC (79,82). Taken together, the above studies, on their own, suggest an association between increased ucOC and conditions of bone loss.

4.3. UcOC associated with lower bone density and increased fracture risk

In older adults, total OC levels predicted increased fracture risk in a few but not most cohort studies, in contrast to the more robust predictive value of resorption markers (83). Higher ucOC levels also appear to be associated with lower bone density and increased fracture risk. Vergnaud et al. reported a negative correlation between ucOC levels and femoral neck BMD (84). Liu et al. did not find a correlation with femoral neck BMD ($r = -0.09$; $p\text{-value} = 0.176$) but did report a modest negative correlation between ucOC and ultrasonic transmitted velocity (UTV) at the os calcis ($r = -0.16$; $p\text{-value} 0.017$) in age-adjusted models (76). In a study of older women with type 2 diabetes, osteoporosis was associated with higher levels of ucOC, and %ucOC was negatively correlated with spine BMD Z score (85).

Studies have demonstrated that higher levels of ucOC are associated with hip fracture risk in older institutionalized (86) and community-dwelling (84) women. A smaller retrospective case-control study of hip fracture in older women also reported higher levels of ucOC in fracture cases but the differences were not statistically significant (76). An increased risk of all clinical fractures in older men and women with lower levels of fully carboxylated OC has also been reported (87). However, trials of vitamin K supplementation have generally failed to demonstrate an effect on fracture risk (88). Similarly, treatment with warfarin, a potent vitamin K antagonist that increases ucOC levels, has generally not been found to decrease BMD or bone strength in animal models (69,89,90). Clinical studies investigating warfarin use have had mixed results with some reporting increased risk of fracture (91,92) or lower bone density (93,94) but others reporting no discernable effect (95,96). These results suggest that higher ucOC levels are not directly linked to fracture risk, but may be associated with fracture as a marker of increased bone turnover or poor nutritional status.

In spite of this body of research, the exact functions of OC and ucOC in bone metabolism remain unclear. The conflicting clinical results for ucOC and fracture suggest that there is either additional complexity or no relationship at all. As noted above (Section 2), the skeletal phenotype of osteocalcin-knockout mice is surprisingly normal. The minor differences from wild type mice suggest that OC plays a role in bone remodeling by limiting bone formation and by regulating mineralization (23,97). A distinct role for ucOC in skeletal health has not been clearly identified.

4.4. OC, ucOC, glucose metabolism and diabetes in humans

While most clinical research has focused on osteocalcin's role in bone metabolism, there have also been efforts to identify the effects of diabetes, hyperglycemia and insulin on bone metabolism, including osteocalcin. Most recently, the effects of OC and ucOC on energy metabolism in clinical studies have become a topic of interest with the findings from the Karsenty group. In the sections below we review our current knowledge of the relationships between ucOC, OC and energy metabolism.

4.4.1. Cross-sectional—Type 1 and type 2 diabetes are associated with lower levels of total OC (98–102). Among those with diabetes, total OC is associated with lower fasting glucose and A1C (103,104). Interestingly, total OC also appears to be associated with glucose metabolism and insulin resistance in those without diabetes. A study in 149 non-diabetic men reported a positive correlation between total OC and insulin sensitivity, assessed with the frequently sampled intravenous glucose tolerance test (105). In a study of older Swedish men, OC was negatively correlated with fasting glucose, fasting insulin and HOMA-IR in those without diabetes (101). In a study of postmenopausal Korean women, including 9% with diabetes, OC was associated with fasting glucose and A1C, but not with fasting insulin or HOMA-IR (102). In older Australian men, OC was negatively correlated with fasting glucose, fasting insulin and HOMA-IR (106). In a study of Chinese patients with newly diagnosed diabetes (N=253) and with normal glucose tolerance (N=247), OC was negatively associated with fasting glucose and A1C, positively associated with fasting insulin and HOMA-%B, but not associated with HOMA-IR (107). In sum, these studies suggest higher total OC levels are associated with improved metabolic parameters, but whether OC affects metabolism, or vice versa, is unclear.

Emerging evidence suggests that ucOC is also correlated with glucose metabolism, but the results are less consistent. A recent study in Japanese patients with type 2 diabetes reported that ucOC as well as total OC, was correlated with fasting glucose and A1C in men, and with A1C in postmenopausal women, but not with fasting C-peptide in men or women (104). In a study of Korean men with diabetes or pre-diabetes, higher ucOC was associated with lower fasting glucose and 2-hr glucose tolerance test and with higher HOMA- β , but not with HOMA-IR (108). In a study of children, %ucOC was positively associated with HOMA- β in leaner but not in heavier children (109). HOMA-IR was not associated with %ucOC. Higher ucOC was associated with higher levels of HMW adiponectin in the full cohort. In summary, cross-sectional studies generally report an association between lower total OC (TOC) and impaired glucose metabolism and insulin resistance. From studies to date, it seems that ucOC follows a similar pattern in relation to fasting glucose and insulin resistance. While these cross-sectional studies support an association between bone metabolism and energy metabolism, they cannot identify temporality or causality. Thus, these studies cannot clarify if higher ucOC or OC levels influence insulin secretion and insulin sensitivity, or vice versa.

4.4.2. Longitudinal: Effect of improved glycemic control on OC and ucOC—A number of previous longitudinal studies of glucose metabolism and OC have proceeded from the hypothesis that changes in glucose metabolism affect bone metabolism. Several published studies have reported the effects of improved glycemic control on bone markers. Most (110–113), but not all (114), of these studies reported an increase in OC in diabetic patients who entered each study with poorly controlled glycemia and achieved improved control. One of the studies reporting an increase in OC with improved control also had data on ucOC changes. Kanazawa et al. in a study of 50 poorly controlled diabetic patients reported a 15% increase in mean ucOC that was not statistically significant ($p=0.42$), and a decrease in the mean %ucOC ($p<0.01$) with the achievement of improved glycemic control (112). In sum, improved glycemic control appears to increase total OC but does not necessarily have the same effect on ucOC. Additional studies with ucOC as an outcome are needed to clarify the effects of improved glycemic control on this marker.

4.4.3. Longitudinal: Effect of total OC and ucOC on energy metabolism—There are few longitudinal reports on OC, ucOC and energy metabolism. A *post hoc* analysis by Pittas et al. using the placebo group for a trial of vitamin D supplementation, reported that lower mean TOC levels during follow-up were associated with larger increases in fasting glucose over 3 years, consistent with the rodent models (115). At baseline, the cohort of older adults (N=198) included about 66% with normal fasting glucose (FG), 29% with impaired fasting

glucose and 5% with diabetes. In unadjusted and adjusted regression analyses, mean OC concentration during the first 18 months of the trial was inversely associated with change in FG at 36 months. Similar results were obtained when the participants with diabetes were excluded. However, ucOC assays were not available in this study.

The only longitudinal study to date that has tested the effects of altered ucOC on glucose and lipid metabolism is a *post hoc* analysis of a trial designed to determine the effect of vitamin K supplementation on bone loss. Participants (N=355) randomized to receive 500 µg/day of phylloquinone had reduced levels of ucOC compared with the placebo group (116). The 36-month changes in mean %ucOC were about -19% in men and women. Measurements of fasting plasma glucose and insulin, and calculated HOMA-IR, were compared at the baseline, 6-month and 36 month visits. At 36 months, men in the vitamin K treated group had *improved* insulin sensitivity based on HOMA-IR, compared with placebo, in unadjusted ($p=0.05$) and adjusted ($p=0.01$) models. The adjusted models for this trial included postrandomization changes in weight and should therefore be considered speculative (117). There were no statistically significant differences across treatment groups in HOMA-IR for women, or in fasting glucose or fasting insulin for men or women. These results do not support the findings in rodent models but several limitations of the study should be noted. As noted above, clear statistical differences in HOMA-IR at 36-months in men were only achieved with adjustment for postrandomization changes. Regarding the lack of effect in women, the changes in ucOC with vitamin K supplementation in these healthy participants may have been too small to cause measurable differences in insulin sensitivity over 3 years. Finally, in addition to reducing ucOC, vitamin K also reduces inflammation, which may have a separate effect on insulin sensitivity.

In an analysis of the placebo group (N = 162) from this same trial of vitamin K supplementation, Shea et al. considered whether baseline levels of OC, ucOC or carboxylated OC predicted changes over 3 years of follow-up in HOMA-IR, fasting insulin and fasting glucose (118). In contrast to the findings in rodent models and the clinical results reported by Pittas et al. (115), baseline levels of ucOC or total OC did not predict changes in these parameters. Instead, lower baseline levels of *carboxylated* OC and higher levels of %ucOC predicted greater increases in insulin resistance. These analyses were adjusted for baseline levels of the outcome variable as well as baseline and 3-year changes in percent fat mass and in serum 25-hydroxyvitamin D. During the study, the average changes in ucOC and %ucOC in this placebo group were not statistically significant. Total OC decreased by about 10% ($p<0.001$) and carboxylated OC by about 13% ($p=0.008$). These analyses support the hypothesis from rodent models that OC levels influence glucose metabolism, but suggest a role for carboxylated OC rather than ucOC.

Another longitudinal study considered the correlations between weight loss due to diet and/or exercise and changes in OC and glucose metabolism (105). Data on ucOC were not available in this trial. Obese women who followed a hypocaloric diet for 16 weeks (N=8) lost an average of 7.3% of body weight and had improved HOMA-IR ($p=0.066$), but did not have any change in OC. However, those who combined the diet with resistance training (N=11) lost a similar degree of body weight, an average of 8.7%, had an improvement in HOMA-IR ($p=0.029$), and had an increase in OC. When the two treatment groups and the control group were combined (N=26), the factors significantly correlated with change in OC in multivariable models were change in visceral fat ($r=-0.46$; $p=0.005$) and change in leg muscle strength ($r=0.51$; $p=0.002$). The correlation with change in HOMA-IR was modest and not statistically significant ($r=-0.12$; $p=0.94$).

Based on rodent models of ucOC, warfarin therapy, which inhibits carboxylation of OC and causes increased levels of ucOC, would be expected to result in increased insulin resistance and higher fasting glucose levels. To date, this hypothesis has not been investigated.

4.4.4. Osteoporosis treatments and development of insulin resistance or diabetes—If ucOC and/or OC affect glucose metabolism, then osteoporosis treatments that alter bone formation markers, including OC, would be expected to affect insulin sensitivity. Bisphosphonates are anti-resorptive treatments for osteoporosis that reduce bone formation markers, including osteocalcin (119). Total OC levels are reduced by 40–50%, and ucOC levels are likely reduced as well although only a few studies have investigated this directly. Reduced ucOC was found after one year of alendronate treatment (120). A cross-sectional study found that osteoporotic women with 6 months or more of alendronate use had lower ucOC than those who were not yet being treated (121). In contrast, a study of risedronate reported an increase in ucOC and a decrease in total OC with one year of treatment in 29 menopausal women. (122). If ucOC is indeed reduced by bisphosphonate treatment, then use of these agents might impact insulin sensitivity. However, studies assessing the effects of the bisphosphonates on glucose homeostasis or insulin sensitivity are not available.

Hormone therapy (HT) and raloxifene, a selective estrogen receptor modulator (SERM), are also anti-resorptive agents that result in lower OC (123). HT has been reported to lower ucOC (79,124); reports on raloxifene and ucOC are not available. In a randomized trial of HT, the Postmenopausal Estrogen/Progestin Intervention Study (PEPI), the women assigned to HT had reduced fasting glucose and fasting insulin but higher 2-hour glucose levels than women on placebo (125). In two other randomized trials of HT, HERS and WHI, women assigned to treatment had a lower rate of incident diabetes compared to placebo (126,127). Thus, although diabetes is associated cross-sectionally with lower OC in clinical studies, lowering OC and ucOC with HT appears to decrease rather than increase the incidence of diabetes. However, estrogen has a wide range of effects in addition to reduced OC and ucOC that may affect glucose homeostasis. In the Multiple Outcomes of Raloxifene Evaluation (MORE) trial, more women in the raloxifene (1.2%) than the placebo (0.5%) group reported new or worsening diabetes ($p=0.009$) (128). The raloxifene group also had an increased but not statistically significant proportion of women with at least 1 annual FPG > 126 mg/dL (6.4% raloxifene vs 5.7% placebo, $p=0.26$). Raloxifene assignment, however, was not associated with changes in FPG or A1C during the trial. There was an imbalance at baseline in the diabetic women, with higher levels of fasting glucose in the intervention group, that may account for the more frequent reports of worsening diabetes in the raloxifene group (129). However, given the rodent findings on ucOC and glucose metabolism, further research into the effects of SERMS on the incidence of diabetes and on insulin resistance is warranted.

Parathyroid hormone (PTH) and teriparatide treatments produce an anabolic response in bone, with large increases in total OC. The effect on ucOC has not been reported. A study of 25 postmenopausal women, designed to test the hypothesis that PTH might increase insulin resistance, found that six months of teriparatide treatment did not alter glucose metabolism or insulin sensitivity based on results of the 2-hour OGTT (130). However, this study may not have been large enough to identify an effect. As with antiresorptive therapies, studies of anabolic therapy, with OC and ucOC measurements, are needed to determine if these therapies cause changes in glucose metabolism and insulin sensitivity. In sum, *post hoc* analyses of trials of osteoporosis therapies are a potential, but so far underutilized, source of insights into the effects of altered ucOC and OC levels on glucose metabolism.

4.5. OC, ucOC, fat mass, and lipids in humans

In cross-sectional studies, total OC is negatively associated with body mass index (BMI) (101,104,131–133) and with fat mass measured by dual x-ray absorptiometry (DXA) (133) (131–133) (101) (104). In older men, OC was negatively associated with waist circumference and triglyceride levels, but was not associated with HDL (106). Total OC was also negatively associated with visceral fat in older adults with type 2 diabetes (104).

Cross-sectional investigations of ucOC have reported that waist circumference is inversely associated with ucOC in men, but not women (79). In older adults with type 2 diabetes, higher ucOC levels were associated with lower trunk fat and, in men, with lower visceral/subcutaneous fat ratio (104). Two studies have reported that there were no statistically significant associations between ucOC and serum triglycerides, total cholesterol or LDLc (79,134). Marginally significant associations were reported between HDLc and ucOC, a positive association in men ($p=0.09$) and a negative association in women ($p=0.08$) (79). Longitudinal data are not available on ucOC and changes in lipid levels or fat mass.

4.6. Adiponectin and undercarboxylated osteocalcin

As discussed above, Lee et al. demonstrated that adiponectin may be responsible for the effects of ucOC on insulin sensitivity (20). Leptin and resistin levels were not changed in either mouse model. Adiponectin is an adipocytokine that is higher in those with better insulin sensitivity and reduced with diabetes, in contrast to the inflammatory adipocytokines, such as TNF- α , IL-6 and plasminogen activator inhibitor (PAI)-1, that are elevated with insulin resistance (135). Adiponectin may also protect against development of diabetes (136,137). Positive correlations between serum adiponectin and osteocalcin levels have been reported for nondiabetic women (138) and men (105). However, a study in women with type 2 diabetes reported no association (139). Studies have reported no association between leptin and total OC (139,140). In patients with type 2 diabetes, adiponectin was correlated with ucOC/OC in men but not with OC or ucOC (104). In women, adiponectin was correlated with OC, but not ucOC or ucOC/OC. During improved glycemic control in diabetic patients, increases in OC ($r=-0.42$, $p<0.01$) but not in ucOC ($r=-0.21$, $p=0.16$) were associated with decreases in adiponectin (112).

5. CONCLUSION

In rodent models, the Karsenty group has determined that the uncarboxylated form of osteocalcin directly regulates insulin production in the pancreas and insulin sensitivity of target tissues (Figure 2). ucOC is capable of treating type 2 diabetes in rodents, which suggests it might be a novel therapy for diabetic patients. Future research tasks include confirmation of these exciting findings by other groups. If ucOC is working as a hormone affecting glucose metabolism under normal conditions, understanding how its levels are regulated is crucial. One factor that metabolically deactivates (indirectly) OC in mice is the *Esp* gene product OST-PTP. Transcriptional regulation of *Esp* may be dependent on leptin and sympathetic innervation to bone. However, there is no functional *Esp* gene in humans, suggesting that other mechanisms may regulate ucOC as well. The significance of energy regulation by osteocalcin will likely unfold within the next few years, as researchers compare ucOC levels to metabolic phenotypes and explore the metabolic effect of other osteoblast-specific genetic manipulations.

While rodent models assess animals with dramatically altered levels of OC and ucOC, clinical research is generally limited to differences that are within a normal physiological range. However, there is a substantial body of cross-sectional evidence that OC, and possibly ucOC, are correlated with fasting glucose and insulin sensitivity. Diabetes is associated with lower total OC, and, in several populations, total OC is negatively correlated with A1C, fasting glucose and insulin resistance. Cross-sectional data on ucOC, the active form of OC in rodent models, and glucose or lipid metabolism in humans remain limited, and more studies are needed to determine whether ucOC, as well as OC, levels are correlated with indicators of energy metabolism. These cross-sectional studies are consistent with both the clinical findings that improved glycemic control results in higher OC and with the rodent findings that higher OC levels result in better insulin sensitivity and reduced fasting glucose. To disentangle whether

OC promotes improved insulin sensitivity in humans requires longitudinal data preferably under conditions with a wide range of ucOC and OC levels.

The few longitudinal studies to date that have considered the effects of ucOC or OC on glucose metabolism have provided conflicting results. One study reported that higher average OC levels predict improved fasting glucose over three years, consistent with the rodent models (115). However, in a trial of vitamin K supplementation that reduced ucOC levels, the effects on insulin sensitivity in men were the opposite of those predicted from rodent models while there were no effects in women (118). The few currently available studies do not provide a sufficient body of longitudinal data for us to determine whether OC or ucOC affects glucose metabolism in humans. At this juncture, additional observational studies are needed to assess correlations with ucOC and to determine if ucOC levels predict longitudinal changes in glucose metabolism. Research is needed to assess how therapies that alter ucOC affect glucose and lipid metabolism. Post hoc analyses of trials of osteoporosis therapies could provide useful insights. In addition, studies of warfarin therapy and of vitamin K supplementation could help to establish the effects of ucOC in humans. Improvements in the available assays for ucOC, as well as a better understanding of the strengths and limitations of the available assays, would be an important advance for this field.

The finding in rodent models that osteocalcin, produced by osteoblasts, influences insulin secretion and sensitivity has potentially opened up new avenues for understanding and preventing diabetes. However, the limited clinical research to date, designed to determine if osteocalcin plays a role in energy balance, has not been conclusive. Further clinical studies are sorely needed to determine whether, and how, osteoblasts affect glucose metabolism in humans.

RESEARCH HIGHLIGHTS

1. Bone effects on glucose metabolism in mice and humans
2. Undercarboxylated osteocalcin (ucOC) promotes insulin secretion and sensitivity in mice
3. Lower OC and insulin resistance associated in cross-sectional clinical studies
4. Longitudinal studies needed to determine if OC affects glucose metabolism in humans

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Figure 1. Proposed regulation of *Esp* expression and osteocalcin carboxylation in mice
 Abbreviations: HA, hydroxyapatite; K, vitamin K; SNS, sympathetic nervous system; OC, osteocalcin; ucOC, uncarboxylated osteocalcin.

Figure 2. Regulation of glucose metabolism by bone

In mouse models, undercarboxylated osteocalcin (ucOC) produced by osteoblasts increases insulin production and sensitivity and decreases fat mass, and diabetes risk. Increases in adiponectin accompany increased ucOC, suggesting that adiponectin is a pathway for the effect of ucOC on insulin sensitivity.

Table 1

Metabolic phenotypes of mouse models compared to wild type.

Increased insulin secretion and sensitivity	Similar insulin secretion and sensitivity *	Reduced insulin secretion and sensitivity
warfarin	<i>OC</i> ^{-/-} + warfarin	<i>OC</i> ^{-/-}
ucOC	Atf4-TG _{ob} + ucOC	Esp-TG _{ob}
<i>Esp</i> ^{-/-}	Atf4-TG _{ob} ; <i>Atf4</i> _{ob} ^{-/-}	Atf4-TG _{ob}
<i>Atf4</i> _{ob} ^{-/-}	<i>Foxo1</i> _{ob} ^{-/-} ; <i>OC</i> ^{+/-}	
<i>Foxo1</i> _{ob} ^{-/-}		
<i>Esp</i> ^{+/-} <i>Atf4</i> _{ob} ^{+/-}		
<i>Esp</i> ^{+/-} <i>Foxo1</i> _{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.

* rescue of metabolic phenotype.