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Review Article

Osteocalcin: an emerging biomarker for bone turnover

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ABSTRACT

Osteocalcin (OC) is produced by osteoblasts during bone formation. OC is excreted into urine by glomerular filtration and can be found as fragments in urine. The presence of three vitamin K-dependent γ-carboxyglutamic acid residues is critical for osteocalcin's structure, which appears to regulate the maturation of bone mineral. Recent bone biology research have highlighted the importance of bone not only as a structural scaffold to support the human body, but also as a regulator of a metabolic processes that are independent of mineral metabolism. Circulating osteocalcin is present either as carboxylated or as undercarboxylated forms. Increased serum level of osteocalcin is linked with increased bone mineral density. The importance of the bone–kidney relation in physiologic regulation of mineral ion has also been extensively studied and documented. Several workers have uncovered the role of insulin as an additional factor involved in the skeletal remodelling process. Evidences are available which shows that osteoblastic insulin signalling is important for glucose metabolism. The measurement of OC in urine samples could be used as an index of bone turnover in monitoring bone metabolism. In this review, we have tried to explain different roles of OC, however further studies are required to elucidate the metabolic and hormonal role of OC in human body.

Keywords: Osteocalcin, Urinary Osteocalcin, Bone turn over, Bone resorption

INTRODUCTION

Osteocalcin (OC) is a major and most commonly characterized non-collagenous protein of mature human bone. Osteocalcin has a high affinity for calcium and exhibits a compact calcium dependent alpha helical conformation, in which the γ-carboxyglutamic acid (Gla) residues binds and promote absorption to hydroxyapatite in bone matrix, in this manner bone mineralization takes place.[1] OC, a bone-specific protein synthesized by the osteoblasts in bone. It has a molecular weight of 5,800 Da and contains 49 amino acids, including 3 gamma carboxyl glutamic acid residues that facilitate the binding of OC to hydroxyapatite in bone.[2]

The OC fraction, which undergoes imperfect gamma-carboxylation, is referred as undercarboxylated osteocalcin (ucOC). Serum ucOC concentrations are a marker of both; bone turnover and vitamin K status in bone.[3] Also, osteocalcin is also released from the bone matrix into blood during bone resorption, which suggests that osteocalcin is also a marker of bone turnover.[4]

In 1972, the discovery of the specialized calcium-binding amino acid, Gla6, as the mechanism for the activation of the vitamin K-dependent clotting factors, started the search for calcium-binding proteins that could regulate mineralized tissues.[5] In 1974, 2 groups of researchers independently isolated a small vitamin K-dependent protein from bone that contained 3 Gla residues and named it Bone Gla Protein and osteocalcin.[6,7] ^{1}H-NMR and X-ray crystallography studies demonstrated a 3-dimensional structure containing 3 helical regions, a C-terminal hydrophobic core, and N terminus. All 3 Gla residues are present in the first helical region and interact with the inter-calcium space in the HAP lattice. The C

terminus extends outward and is accessible to neighbouring cells as well as endogenous proteinases.[8,9]

Serum OC levels may be detected by various tests, such as assays using monoclonal antibodies against OC N-mid and N terminal OC fragments. The deficiencies of calcium and phosphorus in osteoporotic women lower the formation of the hydroxyapatite crystals, which make the free osteocalcin to circulate in the blood. This may explain the increased concentrations of serum osteocalcin levels in the osteoporotic post-menopausal women.[10]

The distribution of osteocalcin in human osteons (the basic unit of structure of compact bone) changes with sex and age, and local reductions of osteocalcin in bone are associated with reduced cortical remodeling.[11] Since, OC is useful in the diagnosis and follow-up of high turnover osteoporosis, it can be considered as an important and highly sensitive marker of bone turnover.

CIRCULATING OSTEOCALCIN

In humans, various forms of osteocalcin circulate. Osteoblastic synthesis contributes towards the circulating osteocalcin. Studies during 1990's confirmed that smaller fragments derived from the action of cathepsin K and matrix metalloproteinases during bone resorption have also been identified.[12,13] These fragments are rapidly cleared by the kidneys. ucOC has been considered as a measure of vitamin K nutrition. Vitamin K is a post-translational cofactor for the γ-carboxylation of vitamin K-dependent proteins, including osteocalcin.

Osteocalcin synthesis is regulated by several hormones and growth factors but not by vitamin K. Rather, vitamin K influences the degree of carboxylation of osteocalcin.[14] In most of the species, all Gla sites are fully carboxylated, but osteocalcin in human bone and serum is incompletely carboxylated, these variations in carboxylation status are presumed to be due to differences in vitamin K intake.[15]

According to a study, serum OC seems to be derived exclusively from newly synthesized protein rather than from bone resorption, because within 3 hrs of exposure to vitamin K-antagonism by warfarin, serum osteocalcin is devoid of Gla, while bone osteocalcin is fully gamma carboxylated.[16] Low dietary intake of vitamin K is related to elevated serum ucOC levels. Moreover, increased serum ucOC levels have been associated with an increased risk of hip fracture, and low bone mass density (BMD) of the hip and spine in pre and postmenopausal women.[17,18]

ROLE OF CARBOXYLATED OSTEOCALCIN IN BONE HEALTH

Several scientific groups tried to elucidate the role of vitamin K in the prevention of osteoporotic fractures based on its role in carboxylation of osteocalcin. A plethora of observational studies are available which examined the association of ucOC and outcomes for bone health, including BMD, qualitative ultrasound of the heel, and hip fracture risk.[19] Although, the preponderance of evidence suggested vitamin K-dependent association between osteocalcin and measures of bone, especially in the elderly, the high correlation between ucOC and total osteocalcin may have confounded the findings.

Vitamin K's role in the carboxylation of osteocalcin indirectly increased osteoblastic activity; changes in total osteocalcin indirectly suggest benefits to bone formation in response to vitamin K. These divergent findings reflect a changing equilibrium between bone-bound and circulating osteocalcin as it attains greater γ-carboxylation in response to vitamin K supplementation. Some studies examined the effects of manipulating dietary vitamin K on bone resorption markers, which in conjunction with markers of bone formation, such as total osteocalcin; provide a measure of the coupling of bone formation and resorption.

Several reports show that a high percent ucOC was associated with greater risk of low BMD.[20,21] Food sources of vitamin K are primarily limited to green leafy vegetables and plant oils, which are markers of healthy diets and predictive of healthy lifestyles.[22] Therefore, the epidemiologic evidence that suboptimal vitamin K could have an adverse effect on BMD and increase the risk of bone fracture needs to be supported by RCT to evaluate the protective role of vitamin K in prevention of bone loss.[23]

OSTEOCALCIN AS A HORMONE

In 2007, the research conducted by Karsenty and Ducy reported that the osteocalcin knockout mice were obese, had elevated glucose and lipid concentrations, and reduced insulin levels and glucose tolerance.[24] It is well known that osteoblasts respond to numerous hormonal signals and secrete factors that affect other cell populations both within bone and in other organ systems. The hormonal form of osteocalcin is uncarboxylated and the carboxylated form is inactive.

Osteocalcin is produced specifically by osteoblasts and has several features of a hormone. It is a cell-specific molecule, synthesized as a prepromolecule, and is secreted to the general circulation.[25,26] Because osteocalcin is specifically expressed in osteoblasts, elevated osteocalcin is associated with both high bone formation and high bone turnover. The finding that plasma osteocalcin decreases slightly after feeding suggest that it might be subjected to metabolic regulation.[27]

Lee et al. showed that osteocalcin, a protein originating from osteoblasts, affects adiposity and glucose homeostasis in mice, suggesting that the skeleton, through an endocrine mechanism, influences energy metabolism. Osteocalcin-deficient mice displayed

obesity, hyperglycemia, glucose intolerance, and insulin resistance. In humans, the relationship between osteoblast-derived endocrine factors and parameters reflecting energy metabolism is unknown.[28]

Additionally, an osteotesticular protein tyrosine phosphatase (OST-PTP) has been found to be involved in the activation of osteocalcin. Although it was initially understood that OST-PTP was involved in the regulation of the enzymes of the vitamin K cycle. Rather, OST-PTP dephosphorylates the insulin receptor in osteoblasts, leading to inhibition of insulin signaling.

In another study, the Karsenty group also showed that insulin signaling in osteoblasts limits production of osteoprotegrin, an inhibitor of osteoclast maturation.[29]

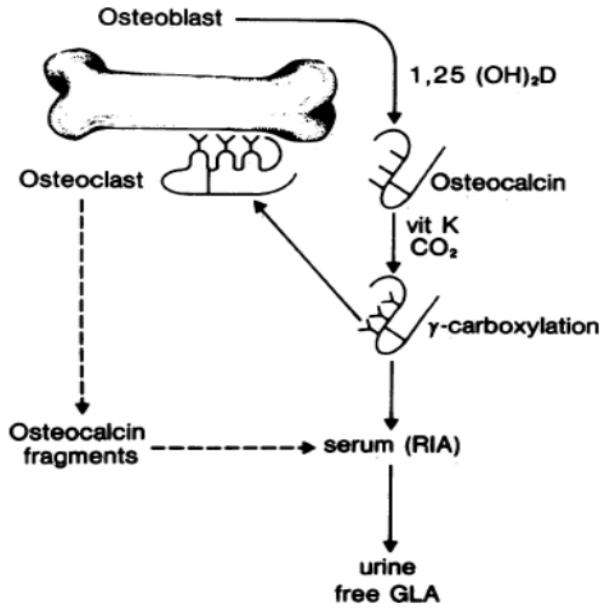

Figure 1: Proposed metabolism of osteocalcin.[40]

This facilitates osteoclast bone resorption, producing an acid environment which induces decarboxylation, and thus activates intact osteocalcin. Initially, a small amount of intact osteocalcin is released during acidification followed by enzymatically produced fragments of the protein. These observations are consistent with clinical studies that show urinary osteocalcin fragments are associated with bone resorption.[30]

High fat mass has been reported to protect against osteoporosis, but recent studies have challenged that view, showing that, after controlling for mechanical loading and lean mass, fat mass might even be inversely correlated with BMD.[31,32]

It was recently shown that the osteoblast-derived protein osteocalcin regulates fat mass and glucose homeostasis in mice, suggesting that affected plasma osteocalcin might be involved in the development of obesity and diabetes. Besides being a strong negative independent predictor of plasma glucose, plasma osteocalcin was also a negative predictor of fat mass and trunk fat but not lean mass.[33]

CLINICAL STUDIES

In recent years, the relationship between diabetes and bone mass has received considerable scientific attention. Human studies to date suggest an association between circulating osteocalcin and insulin sensitivity. Studies are thus far unable to define the form of osteocalcin responsible, because they have been primarily cross-sectional, and have not measured different forms of osteocalcin (ucOC, carboxylated osteocalcin, and total osteocalcin).[34-36]

Studies were also not designed in order to explain the impact of carboxylation of osteocalcin on insulin sensitivity.[37] On the contrary, some evidence suggests that vitamin K may have a beneficial effect on fasting measures of insulin resistance. Higher vitamin K intake was associated with reduced insulin resistance and risk of type 2 diabetes.[38]

A fraction of OC enters the blood, where it can be detected, and circulating OC has been widely measured to assess bone turnover.[39] In addition to the newly synthesized OC derived from osteoblasts, the circulating OC pool probably also includes molecules derived from the resorption process when OC embedded in the bone matrix is released.[40]

The main route of circulating OC catabolism is renal filtration and degradation, and the immunoreactive OC is present in urine. Urine OC (U-OC) is a heterogeneous pool of various OC fragments that reflect potentially diverse degradation cascades and that consist mainly of the middle portion of the molecule truncated at both the amino and carboxy terminal. Infact, unfragmented OC has not been found in urine.[41,42]

Till now, it is unclear whether OC fragments are generated by proteolysis of intact OC in blood, are formed during biosynthesis, or whether some fragments are derived directly from bone resorption.

URINARY OSTEOCALCIN (U-OC)

Circulating intact and large fragments of OC are degraded in circulation or peripheral organs, whereas smaller OC fragments are more resistant to degradation and accumulate in urine.[43] Urine, therefore potentially proved to be a better source for OC fragments of resorptive origin. The problems related to the instability of S-OC after sampling is likely to be less severe for U-OC, which is presumably an end-product of fragmentation.[44]

In patients with renal osteodystrophy, the increased osteocalcin levels have been shown to reflect decreased

renal clearance and increased bone formation. When bone is resorbed, osteoclasts liberate osteocalcin fragments. With normal renal function, osteocalcin and these fragments are rapidly cleared by the kidney and contribute to the urine free Gla. In renal failure, these fragments accumulate and are detectable.[45] U-OC can be a new marker of bone turnover despite such limitations as the gradual age related renal impairment and the tendency to show higher pre-analytical variability than serum assays. Urine as a sample material is, however, intriguing because it traditionally has been used for measurement of resorption markers and not markers of osteoblastic origin.[46]

CONCLUSION

Despite many *in vitro* and *in vivo* studies using animal models, osteocalcin's specific function remains controversial. The observation that the most of healthy human have circulating ucOC, raises the question regarding the optimal proportion of osteocalcin that needs to be γ-carboxylated for human health. Recently, a paradigm was proposed in which an endocrine loop, mediated by osteocalcin, links the osteoblast to the islet and the adipocyte.

Based on *in vivo* and *in vitro* studies using a rodent model, it was demonstrated that the uncarboxylated form of osteocalcin is the active form. The mechanism proposed is inconsistent with the current understanding about the kinetics of osteocalcin decarboxylation and the cellular activity of the osteoclast, thus more research work is required to fully understand the role of osteocalcin in the regulation of glucose metabolism. The uncertainty regarding its function has also been a hurdle to the interpretation of changes in the uncarboxylated form used in observational and clinical trials. All the scientific facts and diagnostic researches led us to conclude that OC is useful in the diagnosis and follow-up of high turnover osteoporosis, therefore it can be considered as an important marker of bone turnover.

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