REVIEW

The mutual dependence between bone and gonads

Gerard Karsenty
Department of Genetics and Development, Columbia University, HHSC 701 West 168th Street, HHSC1602, New York, New York 10032, USA
(Correspondence should be addressed to G Karsenty; Email: gk2172@columbia.edu)

Abstract

It has long been known that sex steroid hormones regulate bone mass accrual. This observation raises the testable hypothesis that bone may in turn regulate the synthesis and secretion of sex steroid hormones in one or both genders. This hypothesis is comprised within a more general hypothesis that bone mass, energy metabolism, and reproduction are regulated coordinately. The identification of osteocalcin as an osteoblast-specific secreted molecule allows us to address this question in molecular terms. This review details how the regulation of male fertility by osteocalcin was unraveled, and how osteocalcin signaling in Leydig cells of the testis occurs. It also discusses the implication of this novel mode of regulation of testosterone synthesis observed in males but not in females.


Introduction

The principle of mutual dependence between organs as an underlying notion of vertebrate physiology has been verified over and over through mouse genetics. It is clear nowadays that there are interconnections between the different organs in regulation of whole organism physiology. The organs need to communicate with each other to keep our body functional. Based on this view of physiology, it was recently demonstrated that bone is not only a recipient of hormonal inputs but also an endocrine organ of major importance. Multiple studies have shown that bone, via an osteoblast-secreted hormone called osteocalcin, promotes β-cell proliferation, insulin secretion, and insulin sensitivity in muscle, liver, and white adipose tissue (Lee et al. 2007, Im et al. 2008, Aonuma et al. 2009, Fernandez-Real et al. 2009, Hwang et al. 2009, Kanazawa et al. 2009, Kindblom et al. 2009, Pittas et al. 2009, Ferron et al. 2010, Rached et al. 2010, Winhofer et al. 2010, Yeap et al. 2010, Levinger et al. 2011).

It has long been recognized that bone mass accrual is profoundly regulated by sex steroid hormones, which are necessary for the bone growth and for the maintenance of skeletal integrity (Khosla et al. 2001, Riggs et al. 2002, Nakamura et al. 2007). The biological importance of this regulation is best exemplified by the fact that gonadal failure triggers bone loss in both genders. Estrogen deficiencies at menopause in women and androgen decrease in elderly men are the major pathogenic factors in the development of osteoporosis (Riggs et al. 1998, 2002, Khosla et al. 2001, Vanderschueren et al. 2004, Khosla & Riggs 2005, Nakamura et al. 2007, Khosla 2010a,b). Considering that endocrine regulation is often subjected to feedback loop mechanisms, the regulation of bone mass accrual by gonads suggests that bone, in its endocrine capacity, may affect the reproductive functions in one or both genders.

This review is a presentation of the unexpected influence the skeletal system exerts on male reproduction.

Sex steroid hormones regulate bone mass accrual


Sex steroid hormones regulate bone growth

Sex steroids play an important role in bone growth and the attainment of peak bone mass. They are, at least in part,

In addition to the sex steroid hormones, several studies have shown that other hormones negatively regulated by estrogen, such as growth hormone (GH) and insulin-like growth factor 1 (IGF1), may further contribute to the development of the skeletal sexual dimorphism (Lupu et al. 2001, Venken et al. 2005, Callewaert et al. 2010a,b). IGF1 levels are higher in males vs females during early puberty (Callewaert et al. 2010b,c), and mice lacking GH receptor (GHR), IGF1, or both show a severe bone growth retardation (Lupu et al. 2001). These evidence strongly suggest that skeletal dimorphism in bone growth can not only be attributed to the differences in sex steroid hormone secretion and action in males and females, but also depends on complex gender- and time-specific interactions between several factors (Callewaert et al. 2010a).

Sex steroid hormones maintain skeletal integrity

Testosterone and estrogens are also crucial for maintaining bone mass accrual during adulthood in the female and male skeleton. The loss of ovarian function underlies the development of osteoporosis (Riggs et al. 2002, Vanderschueren et al. 2004). As a matter of fact, estrogen deficiency is a major pathogenic factor in the bone loss associated with menopause and the development of osteoporosis in postmenopausal women (Riggs et al. 1998, 2002, Khosla et al. 2001, Khosla & Riggs 2005, Nakamura et al. 2007). After menopause, an imbalance between rates of bone formation and bone resorption, favoring the latter, leads to an accelerated bone loss during the first years after menopause (Garnero et al. 1996, Clarke & Khosla 2010). This rapid bone loss can be prevented by estrogen administration, and characteristically results in an increase in bone mineral density during the first months of treatment (Lindsay et al. 1976, 1980, Stevenson et al. 1990). Additionally, the loss of testicular function also underlies bone loss in men. Although osteoporosis more commonly affects women, the loss of androgens in males following castration or a decrease in androgen levels related to aging has the same dramatic effect on the skeleton (Stepan et al. 1989, Riggs et al. 2002, Vanderschueren et al. 2004, Kaufman & Vermeulen 2005).

The traditional view of the sex steroid hormones presenting androgens and estrogens as a male and female hormones respectively has been reconsidered recently (Callewaert et al. 2010a). A series of evidence shows that estrogens may have a crucial role in the maintenance of bone mass accrual and skeletal homeostasis in elderly men (van den Beld et al. 2000, Bouillon et al. 2004, Mellstrom et al. 2006, Rochira et al. 2007, Araujo et al. 2008). These data suggest that the role of sex steroid hormones in age-related bone loss in women and men is the result of disturbances affecting a complex network regulating bone mass that remains to be further investigated.

Estrogen and androgen mode of action in skeleton

The sex steroid hormones may influence skeletal physiology, at least in part, by acting directly on bone cells via their classical receptors for estrogens or androgens (Noble et al. 1999, Vidal et al. 1999, Bord et al. 2001). However, mice deficient for ERα (zERKO), ERβ (BERKO), double ER (DERKO), and AR (ARKO) did not produce phenotypes similar to those observed in the absence of estrogen or androgen (Couse & Korach 1999, Couse et al. 1999, Oz et al. 2000, Vidal et al. 2000, Lindberg et al. 2001, Riggs et al. 2002, Vanderschueren et al. 2004, Callewaert et al. 2009). This may be explained by the fact that in these models the levels of sex steroid hormones are abnormally high, and/or that these hormones may also have nongenomic modes of action. Androgen and estrogen can transmit antiapoptotic effects on osteoblasts in vitro with a similar efficiency via either AR or ERs, irrespective of whether the ligand is an androgen or an estrogen (Kousteni et al. 2001, 2002, Manolagas et al. 2002). Hence, at the present time the mechanisms mediating estrogen and androgen function in the bone, via ERs and AR, are not fully elucidated.

Estrogens, mainly 17β-estradiol, are essential for the maintenance of the balance between bone formation and bone resorption (Riggs et al. 2002, Vanderschueren et al. 2004). At the cellular level, Estrogens affect the generation, lifespan, and functional activity of osteoclasts (Fig. 1). They decrease osteoclast formation and activity, while increasing osteoclast apoptosis (Fig. 1; Hughes et al. 1996, Imai et al. 2009). The role of estrogen regulation of osteoclasts is less clear, and investigations have produced conflicting results and thus will not be presented here. At the molecular level, Estrogens favor osteoclast apoptosis by decreasing the production of cytokines, such as interleukin 1 (IL1), IL6, tumor necrosis factor a (TNFα), and macrophage colony-stimulating factor (Hughes et al. 1996, Jimi et al. 1996, Pacifici 1996, Manolagas et al. 2002, Riggs et al. 2002, Xing & Boyce 2005). In addition, Estrogens suppress bone resorption by inhibiting the osteoclast activity (Fig. 1). Estrogens enhance the expression of transforming growth factor (TGF)-β and OPG, inhibitors of osteoclast activity, while decreasing the
expression of RANKL, an activator of osteoclast activity (Bodine et al. 1995, Gill et al. 1998, Tau et al. 1998, Michael et al. 2005). Lastly, Estrogens suppress osteoclast formation by upregulating expression of Fas ligand (Fasl), a gene that belongs to the TNF family (Nakamura et al. 2007, Krum et al. 2008). Therefore, estrogens actions are important physiological regulators of bone remodeling during adulthood.

Androgens favor periosteal bone formation in men, and maintain trabecular bone mass and integrity (Fig. 1). At the cellular level, testosterone increases the lifespan of osteoblasts (Fig. 1) by inhibiting IL6 production (Jilka et al. 1992, Manolagas et al. 2002). Furthermore, androgens stimulate the proliferation of osteoblast progenitors and the differentiation of mature osteoblasts (Fig. 1; Kasperk et al. 1989, Kousteni et al. 2001, 2002, Manolagas et al. 2002). At the molecular level, some evidence indicates that androgens favor osteoblast proliferation and differentiation (Fig. 1) by increasing TGF-β mRNA, as well as promoting responsiveness to fibroblast growth factor and IGF2 (Bodine et al. 1995, Gill et al. 1998, Riggs et al. 2002). Androgens may also decrease osteoclast formation and bone resorption (Fig. 1) by increasing the production of OPG by osteoblasts (Michael et al. 2005). The net result of these testosterone functions leads to an accrual in bone formation (Fig. 1; Seeman 2001).

The skeleton regulates gonadal functions in males

The view of bone merely as an assembly of inert calcified tubes characterized only by its scaffolding properties has proven to be wrong. As a matter of fact, the skeleton has emerged in recent years as an endocrine organ of major importance. Multiple studies have shown that osteocalcin, an osteoblast-specific secreted hormone, promotes β-cell proliferation, insulin secretion, and insulin sensitivity in muscle, liver, and white adipose tissue (Lee et al. 2007, Ferron et al. 2010, Fulzele et al. 2010). Remarkably, it has also been shown that another gene expressed in osteoblasts, Esp (Ptnr), encoding a tyrosine phosphatase called OST-PTP (osteotesticular protein tyrosine phosphatase) exerts metabolic functions opposite to those of osteocalcin (Lee et al. 2007). Genetic and biochemical evidence put OST-PTP as an upstream negative regulator of osteocalcin bioactivity in osteoblasts (Lee et al. 2007, Ferron et al. 2008, 2010, Hinoi et al. 2008, Fulzele et al. 2010). Indeed, Esp−/− mice had a metabolic phenotype that is the mirror image of that of Osteocalcin−/− mice (Lee et al. 2007, Ferron et al. 2008).

The hormonal functions of osteocalcin as a bone-derived molecule have raised a number of questions of biological and clinical relevance. The most pressing one was to determine if the skeleton has physiological functions in addition to those exerted on energy metabolism and glucose homeostasis. As mentioned above, that menopause favors bone loss is well established (Riggs et al. 1998, 2002, Khosla et al. 2001, Vanderschueren et al. 2004, Khosla & Riggs 2005, Nakamura et al. 2007, Khosla 2010a, b). What this medical observation means biologically, as discussed previously, is that gonads regulate bone physiology through the secretion of sex steroid hormones. According to the general principle of feedback control, the regulation of bone mass accrual by gonads implies that bone may affect the reproductive functions in one or both genders. Verifying this hypothesis was of great conceptual importance as it would further enhance the emerging importance of bone as an endocrine organ.

The first evidence supporting this hypothesis came from ex vivo cell assays. Indeed, these studies demonstrated that a factor secreted by osteoblasts, but not by other cells of mesodermal origin, could markedly increase testosterone production in testis explants and primary Leydig cells (Oury et al. 2011). The effect of osteoblast supernatants was limited to males; osteoblasts did not stimulate testosterone or estrogens secretion in females. Recently, this novel and important role of osteoblasts has been verified in vivo. Using DTAo8 mice, an osteoblast-less mouse model, ablation of osteoblasts in adult mice profoundly affects circulating testosterone levels (Yoshikawa et al. 2011).

Osteocalcin regulates testosterone biosynthesis

Since osteocalcin has an important role in the regulation of energy metabolism and glucose homeostasis, it was hypothesized that it may also affect testosterone production. Several experiments have confirmed this hypothesis in vitro and ex vivo. First, using the same co-culture assays described above, the supernatants of wild-type (WT) but not those of Osteocalcin−/− osteoblasts increased testosterone production in testis explants and primary Leydig cells cultures (Oury et al. 2011). Secondly, treating primary Leydig cells with an increasing amount of undercarboxylated osteocalcin, the active form of the hormone, resulted in a dose-dependent increase in
testosterone secretion (Oury et al. 2011). Thirdly, injection of osteocalcin in WT mice significantly increased circulating levels of testosterone (Oury et al. 2011). Lastly, administration of osteocalcin in mice lacking osteoblasts fully restored testosterone to normal serum levels (Yoshikawa et al. 2011).

A role for osteocalcin in the regulation of testosterone biosynthesis was also demonstrated in vivo (Lee et al. 2007). Osteocalcin$^{-/-}$ mice showed a severe decrease in testis and epididymal weights, as well as sperm count. These features are associated with the low circulating testosterone levels observed in Osteocalcin$^{-/-}$ mice (Oury et al. 2011). Perhaps more surprisingly, serum luteinizing hormone (LH) levels (the major regulator of testosterone production) were higher in Osteocalcin$^{-/-}$ compared to WT male mice. These data suggest a compensatory mechanism is functioning that is insufficient to rescue testosterone production in Osteocalcin$^{-/-}$ mice (Oury et al. 2011). In contrast, analyses of Osteocalcin$^{-/-}$ female mice did not show any abnormalities in ovarian, cycling, or in circulating levels of the sex steroid hormones (Oury et al. 2011). These observations suggest that the role of osteocalcin on testosterone production is male specific, and that it acts through a receptor expressed in testis but not in ovaries.

Regulation of sex steroid secretion is subject to feedback inhibition. Indeed, the aromatization of testosterone to estradiol is a large contributing factor to feedback inhibition of testosterone secretion (Schnorr et al. 2001). Accordingly, aromatase inhibition in males results in rather large increases in LH and follicle-stimulating hormone, causing subsequent elevations in testosterone (Leder et al. 2004, T’Sjoen et al. 2005). Interestingly, Osteocalcin-deficient male mice also have significantly increased levels of circulating estradiol leading to a disturbance in testosterone/estradiol ratio (Oury et al. 2011). This observation suggests that osteocalcin may be involved in one of the major mechanisms of feedback inhibition of testosterone secretion. This hypothesis needs to be further investigated.

In summary, these experiments established that osteocalcin is a bone-derived hormone favoring fertility in male mice by promoting testosterone production in Leydig cells (Fig. 2). In other words, it verified that, in at least one gender, there is an endocrine regulation of reproduction by the skeleton (Fig. 2). It also suggests that there may be differences between males and females in the regulation of this function.

**Figure 2** Bone via osteocalcin, an osteoblast-derived hormone, regulates testosterone production in testis. Following its binding to a GPRC6A expressed on Leydig cells of the testes, osteocalcin promotes in a cAMP-response element binding protein (CREB)-dependent manner testosterone production by testis. CREB binds to the promoter regions and activates the expression of several genes encoding for the enzymes that are necessary for testosterone biosynthesis, such as STAR, CYP11A, 3β-HSD, and CYP17. Steroidogenic acute regulatory protein (StAR) is crucial for transport of cholesterol to mitochondria where biosynthesis of steroids is initiated. CYP11A encodes the cholesterol side-chain cleavage enzyme (P450scc) that catalyzes the first and rate-limiting step, which converts cholesterol to pregnenolone. 3β-HSD and CYP17 encode two enzymes required during the conversion of pregnenolone to testosterone. Testosterone is a sex steroid hormone required for many aspects of testicular functions, such as germ cell survival and spermatogenesis.
Osteocalcin acts on Leydig cells through its receptor GPRC6A

Once the demonstration of the existence of an osteocalcin/-testosterone axis was made in vivo, the most important question has been to know its mechanisms of action in testis. In a first attempt to address this question, it was shown that osteocalcin enhances the production cAMP in the β-cell of the pancreas and in the Leydig cell of the testis. This observation suggested that the, or at least an, osteocalcin receptor would be a G protein-coupled receptor (GPCR) linked to adenylate cyclase. One GPCR, GPRC6A, was a particularly good candidate to be an osteocalcin receptor since Dr Quarles’s group has shown that its inactivation in mice results in metabolic and reproduction phenotypes similar to those seen in Osteocalcin−/− mice (Pi et al. 2008). The inactivation of Gprc6a in mice leads to an increase in adiposity, decrease in muscle mass, and low circulating testosterone levels associated with elevated estradiol serum levels in male mice (Pi et al. 2008, Oury et al. 2011). Furthermore, it was proposed that GPRC6A was a calcium-sensing receptor that functioned better in the presence of osteocalcin (Pi et al. 2008). In testing this hypothesis in mice, it was first shown that osteocalcin binds to WT but not to Gprc6a-deficient Leydig cells. Secondly, osteocalcin did not stimulate cAMP production in Gprc6a-deficient Leydig cells (Oury et al. 2011). Finally, analyzing the role of osteocalcin in regulation of energy metabolism demonstrated that GPRC6A mediates responses to osteocalcin in β-cells in vitro, and pancreas in vivo (Pi et al. 2011). According to the studies of three different groups, Gprc6a is expressed in brain, skeletal muscle, heart, lung, spleen, kidney, liver, fat, and pancreatic β-cells (Wellendorph & Brauner-Osborne 2004, Kuang et al. 2005, Pi et al. 2005, 2008). It is known that osteocalcin can stimulate insulin secretion in β-cells and promote insulin sensitivity in peripheral tissues including liver, fat, and muscle (Lee et al. 2007, Ferron et al. 2008). Whether osteocalcin acts on the other tissues expressing Gprc6a remains to be determined. Interestingly, GPRC6A is not expressed in ovaries (Oury et al. 2011). This restricted pattern of expression in gonads may explain why osteocalcin can increase testosterone production from testis, but not from ovaries. The investigation of the role of Gprc6a in regulation of bone mass accrual has generated contradicting findings (Pi et al. 2008, 2010, Wellendorph et al. 2009), and thus this aspect of Gprc6a function will not be developed further in this review.

Investigating the downstream cascade mediated by GPRC6A as an osteocalcin receptor led to the identification of cAMP response element binding protein (CREB) as a transcriptional effector of osteocalcin regulation of testosterone biosynthesis (Fig. 2; Oury et al. 2011). The activation of CREB by osteocalcin signaling favors the expression of key enzymes of testosterone biosynthetic pathway in Leydig cells, such as STAR, CYP11A (CYP11A1), CYP17 (CYP17A1), and 3β-HSD (Fig. 2; Oury et al. 2011). Interestingly, the inactivation of osteocalcin in mice does not affect the expression of the aromatase gene, Cyp19a, responsible for estrogen synthesis (Oury et al. 2011).

Conclusion

The unraveling of this novel endocrine role of the skeleton indicates that no organ is left out of the integrating processes required to allow vertebrates to fulfill their physiological functions. This is consistent with the idea that organisms are integrated entities fulfilling their specific functions, and not as isolated group of distinct cell types. When this concept was applied to the skeleton it has yielded surprising results. But, are these findings really surprising? As a matter of fact, if one considers the unique features of bone and the energetic cost that the perpetual cycle of destruction/formation that characterizing bone modeling and remodeling entails, one might find them not so surprising. Rather it serves as a reminder that physiology has to be studied in the context of evolution, the original purpose of physiological functions being to allow survival, in that case bone growth and ambulation, in difficult conditions and not under the light of degenerative diseases that did not exist when these functions emerged.

This foray in bone physiology is evidence that asking why a given physiological function was created may lead to further discovery of interorgan connections. This in turn may allow a better understanding of the pathogenesis of degenerative diseases, and possibly the design of adapted therapies for these diseases. This is the ultimate purpose of an integrated approach physiology illustrated in this review.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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