Newly reported roles of thyroid-stimulating hormone and follicle-stimulating hormone in bone remodelling

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Abstract Thyroid-stimulating hormone (TSH) and follicle-stimulating hormone (FSH) have both been recently implicated in bone remodelling. Clinical evidence, as well as data from TSH receptor and thyroid hormone receptor knockout mice, suggest that TSH has a direct effect on skeletal homeostasis, although some data are conflicting. Recently, the exogenous administration of TSH has been shown to positively impact bone in oophorectomised rats. These data, along with their potential implications for the treatment of severe osteoporosis, are discussed.

Résumé L'ATSH et la FSH sont impliquées de façon conjointe dans le remodelage osseux. Il est évident que sur le plan clinique et après analyse des données, la stimulation des récepteurs hormonaux de l'hormone thyroïdienne a un effet direct sur l'homéostasie osseuse. Récemment, il a été montré que l'utilisation d'hormones thyroïdiennes avait également un effet positif sur l'os des rats ovariectomisés. Toutes ces données et leurs conséquences thérapeutiques sont discutées dans les cas d'ostéoporoses sévères.

Introduction

Thyroid-stimulating hormone (TSH) and follicle-stimulating hormone (FSH) are two structurally related glycoprotein

hormones synthesised by the anterior pituitary gland. TSH and FSH consist of a common alpha subunit non-covalently bound to a unique beta subunit, which confers specificity to distinctive glycoprotein hormone receptors [18]. TSH is produced in response to elevated levels of thyroid-releasing hormone secreted by hypothalamic neurons. FSH is secreted in response to gonadotropin-releasing hormone, which is also produced by hypothalamic neurons. The production of both hormones is regulated by a classical negative feedback loop (Fig. 1). Although structurally related, TSH and FSH have distinct physiological roles. TSH is known to stimulate the release of thyroid hormone, induce iodine uptake by the thyroid gland, promote thyroid growth and protect thyroid cells from apoptosis [5]. FSH stimulates the maturation of ovarian follicles and facilitates spermatogenesis [3]. More recently, TSH and FSH have been shown to influence bone remodelling [1, 17].

The role of TSH and FSH in bone remodelling

Abe et al. [1] provided the first evidence that TSH influences skeletal remodelling using TSH receptor (TSHR) knockout mice. In these studies, mice that were TSHR-deficient exhibited severe osteoporosis and supplementation with thyroid extract failed to reverse the decreased bone mineral density (BMD) observed (Fig. 2). TSH was shown to influence both osteoclast formation and survival, as well as osteoblast differentiation. The effects of TSH on bone were shown to be attributed to the modulation of tumour necrosis factor- α (TNF- α) [6, 16]. Subsequently, Sun et al. [17] demonstrated that FSH also played a direct role in regulating bone mass. Neither FSH receptor null mice nor FSH β null mice exhibited hypogonadal bone loss, suggesting that FSH may be required for post-menopausal bone

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Fig. 1 Hypothalamic pituitary axis. For both systems, the hypothalamus secretes releasing hormones that act on the pituitary gland. In response, the pituitary gland releases follicle-stimulating hormone (FSH) or thyroid-stimulating hormone (TSH). FSH and TSH then act at remote sites to induce endocrine effects. Adapted from Alcohol Health & Research World 1998; 22(3):153–164

loss. FSH was shown to directly regulate osteoclastic bone resorption. FSH effects on bone cells appear to be mediated through $TNF-\alpha$ as well [7]. Based upon these data, as well as previous findings, Zaidi proposed an integrative model of skeletal surveillance by the hypothalamus and pituitary gland [19]. In this model, one aspect of regulation is through changes in pituitary hormone levels (i.e. FSH and TSH). The second level of control results from changes in stimulation frequency through the sympathetic nervous system.

Interestingly, a recent publication describing mice that lack either thyroid hormone receptor α ($TR\alpha$) or β ($TR\beta$) suggests that thyroid hormone, and not TSH, plays a direct role in bone remodelling [2]. Mice that lacked $TR\beta$ had elevated levels of thyroid hormones and TSH, while those

that lacked $TR\alpha$ were predominantly euthyroid. The $TR\alpha^{0/0}$ mice displayed an increase in bone mass, while the $TR\beta^{-/-}$ mice were osteoporotic. Bassett et al. [2] discuss this finding, which contrasts with the report by Abe et al. [1], suggesting that the thyroid supplement given to the mice in the previous study was not provided early enough in life. Bassett et al., however, conclude that their findings do not exclude the possibility that TSH does play a role in bone remodelling. The studies by Abe et al. and Bassett et al. represent one approach to determining potential roles of proteins and receptors in bone remodelling, namely, the use of knockout mice. Other approaches, such as evaluating hormone levels within human populations and examining naturally occurring mutations, have also been explored.

Fig. 2 Reduced bone mineral density (BMD) in $TSHR^{-/-}$ and $+/-$ mice. A PIXImus (Lunar) bone densitometer was used to measure the BMD at specified sites. Reprinted from Cell 2003; 115:151–162 with permission from Elsevier

Historically, the decreased BMD observed in overt hyperthyroid patients was attributed to elevated levels of thyroid hormone; a direct role for TSH was not suggested until 2003 [1, 4, 13]. However, thyroid hormone and TSH are inversely related in a physiological setting; therefore, it is difficult to dissociate whether skeletal effects are due to increased thyroid hormone or decreased TSH. In several studies where TSH and thyroid hormone have been evaluated with respect to BMD, the levels of TSH, and not thyroid hormone, are found to correlate with BMD. Kim et al. [8] measured serum TSH, T3 and T4 levels in healthy post-menopausal Korean women, as well as BMD by dual-energy X-ray absorptiometry (DXA). Free T4 concentrations were not correlated with BMD at the lumbar spine or femoral neck, while TSH showed a significant correlation in this population of women with TSH levels within the normal reference range. In a subsequent study of white and black post-menopausal American women, Morris [11] showed that, in this population, there was an association of low-normal serum TSH levels with osteopenia and osteoporosis as well, while free T4 levels were not correlated to BMD. Additional clinical evidence suggests a direct role of TSH in bone. Individuals with TSHR mutations exhibit decreased BMD [12].

The correlation of serum TSH levels with BMD, and the absence of an association of thyroid hormone levels with bone density, is suggestive of a direct effect of TSH on skeletal remodelling. However, as noted above, a direct assignment of cause is confounded by the inverse relationship between thyroid hormone and TSH under most physiological conditions. Mazziotti et al. [10] performed a study in which thyroidectomised patients on a constant dosage of L-T4 were given recombinant human TSH (rhTSH) in order to monitor for thyroid carcinoma metastases. In this setting, thyroid hormone is effectively held constant and the effects of TSH dosing may be evaluated independent of thyroid hormone effects. Interestingly, in post-menopausal women, TSH stimulation resulted in a significant increase in bone alkaline phosphatase and a decrease in the serum levels of C-telopeptides of type-I collagen (CrossLaps), suggesting an effect of TSH on skeletal remodelling independent of thyroid hormone status. Bone alkaline phosphatase effects were more sustained than those observed for serum CrossLaps, suggesting that TSH effects on osteoclasts *in vivo* may be direct, while those on osteoblasts may involve additional factors (i.e. modification of serum calcium or parathyroid hormone (PTH) levels). The effect of TSH on bone biomarkers was only noted in post- and not pre-menopausal women. Bone turnover was higher in the post-menopausal patients, suggesting that TSH may play a more significant role in this context. Martini et al. reported completing a similar study; however, they were unable to detect changes in bone biomarkers [9]. The size of their study groups (10

individuals) and their assay precision (<10%) may have prevented the detection of a significant change.

TSH restores bone mineral density, microarchitecture and strength in the rat OVX model

As discussed above, deletion of the TSHR in mice lead to severe osteoporosis and clinical data suggest a direct role for TSH in bone remodelling, independent of thyroid hormone. In order to evaluate whether the administration of TSH in a post-menopausal setting may positively impact BMD, we examined the effect of exogenous administration of TSH in a rat ovariectomy (OVX) model [15]. Studies were carried out in a “prevention” setting, where rats were dosed immediately following OVX, and in a “restoration” mode, where treatment followed a period of several months after OVX to allow bone loss to occur prior to the administration of TSH. TSH was able to prevent bone loss and restore BMD in this model. The effects observed were both anabolic and anti-resorptive. TSH was able to enhance both trabecular and cortical bone formation, as well as improve the mechanical strength of bone, as compared to the OVX control group (Fig. 3). At the TSH levels that were effective in restoring bone, no significant elevation in T3 and T4 was measured. *In vitro* and *ex vivo* experiments demonstrated that TSH induces alkaline phosphatase and osteocalcin synthesis in osteoblasts and inhibits osteoclast differentiation and osteoclast-mediated resorption. Interestingly, the *in vitro* and *in vivo* effects of TSH demonstrated a bell-shaped dose-response curve. This phenomenon may help explain why Bassett et al. [2] observed osteoporosis in their knockout mouse model when thyroid hormone and TSH levels were high. Our findings further support the idea that TSH plays a direct role in bone remodelling and suggest that TSH may be a potent agent for the treatment of osteoporosis. Currently, the approved therapies for osteoporosis mainly consist of anti-resorptives (i.e. bisphosphonates, hormone replacement therapy, calcitonin), with only one marketed anabolic agent currently approved for clinical use (i.e. Forteo).

The utility of TSH as an osteoporosis treatment may be limited by the potential thyroid induction that could result if the hormone were administered to euthyroid patients at sufficiently high levels. Preclinical data in the rat OVX model suggest that the doses required for an anabolic effect in bone may be lower than those required for stimulation of the thyroid [15]. In humans, the lowest dose of rhTSH (Thyrogen) that has been administered to normal, euthyroid individuals is 100 µg [14]. At this level, rhTSH induced a significant increase in T3, T4 and thyroglobulin, which persisted for at least four days after injection. In the study

Fig. 3 Micro computed tomography (CT) measurement of distal femurs in rats treated with rat TSH at doses from 0.01 to 0.3 μg for 4 months and 7 months following rat oophrectomy (OVX). **a** Micro CT images of horizontal sections from distal femurs. **b** All doses of TSH increased the trabecular bone volume (BV/TV), trabecular number (Tr.Nm.), trabecular thickness (Tr.Th.) and decreased the trabecular separation (Tr.Sp.), trabecular pattern formation (Tr.pf) and structural model index (SMI) (0-plate to 3-rod). Data are mean $\pm$ SEM. *Significantly different from group OVX (ANOVA/Dunnett test, $P < 0.05$). $n = 12$ for all groups. Reproduced from J Bone Miner Res 2007 (in press) with permission from the American Society for Bone and Mineral Research (ASBMR)

b

	SHAM	OVX	OVX + TSH 0.01 μg	OVX + TSH 0.1 μg	OVX + TSH 0.3 μg
BV/TV (%)	43.54 $\pm$ 12.24*	14.56 $\pm$ 3.52	21.02 $\pm$ 3.45*	23.60 $\pm$ 3.58*	27.49 $\pm$ 8.06*
Tr.Nm (1/mm)	3.39 $\pm$ 0.79	1.09 $\pm$ 0.25	1.37 $\pm$ 0.25	1.48 $\pm$ 0.17*	1.67 $\pm$ 0.37*
Tr.Th (mm)	0.13 $\pm$ 0.01	0.12 $\pm$ 0.01	0.15 $\pm$ 0.01*	0.16 $\pm$ 0.01*	0.16 $\pm$ 0.02*
Tr.Sp (mm)	0.23 $\pm$ 0.11*	0.68 $\pm$ 0.18	0.73 $\pm$ 0.13	0.69 $\pm$ 0.11	0.60 $\pm$ 0.07
Tb.Pf (1/U)	-0.01 $\pm$ 4.15*	9.19 $\pm$ 2.56	5.48 $\pm$ 1.86*	4.65 $\pm$ 0.95*	3.72 $\pm$ 4.23*
SMI	0.47 $\pm$ 0.63*	2.07 $\pm$ 0.31	1.61 $\pm$ 0.31	1.48 $\pm$ 0.12*	1.25 $\pm$ 0.72*
Cr. Th (mm)	0.48 $\pm$ 0.04*	0.44 $\pm$ 0.05	0.46 $\pm$ 0.62	0.51 $\pm$ 0.04*	0.49 $\pm$ 0.39

by Mazziotti et al. [10], 0.9 mg of rhTSH lead to changes in bone biomarkers in post-menopausal, thyroidectomised women. It is unclear from these two studies whether there will be an intermediate dose that yields an effect on bone but no effect on thyroid.

Conclusion

Thyroid-stimulating hormone (TSH) and follicle-stimulating hormone (FSH) are pituitary glycoprotein hormones with well-characterised roles in thyroid homeostasis and gametogenesis, respectively. Interestingly, both have recently been implicated in skeletal remodelling. In a rat model of osteoporosis, TSH positively influenced bone strength and quality at dose levels lower than those required to elicit a thyroid response. Demonstrating both anabolic and anti-

resorptive effects, TSH may represent a promising candidate for the treatment of advanced disease osteoporosis. Obviously, a potential barrier to the use of TSH as a therapy in humans is the possibility of unwanted thyroid stimulation in elderly patients, which may result in associated cardiac complications. Additional studies will be required to determine whether TSH may be administered at low enough dose levels to yield an effect in bone but with no significant impact on the thyroid function. The only anabolic agent that is currently approved for the treatment of osteoporosis is an active peptide of parathyroid hormone (PTH) (Forteo), another naturally occurring hormone produced by the pituitary. As noted above, recent data suggest that the antagonism of FSH may yield a potential anti-resorptive therapy; however, even less is known about the role of FSH in bone remodelling. The newly emerging information about both of these hormones is very thought-provoking and sug-

gests that the modulation of these hormones may be useful in the treatment of advanced osteoporosis.

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