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Actions of thyroid hormones in bone

Wpływ hormonów tarczycy na tkankę kostną

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Abstract

Thyroid hormones are required for skeletal development and establishment of peak bone mass. Hypothyroidism in children results in growth retardation with delayed skeletal development, whereas thyrotoxicosis accelerates bone maturation. In adults, T_3 regulates bone turnover and bone mineral density, and normal euthyroid status is essential to maintain optimal bone strength. Population studies indicate that hypothyroidism and hyperthyroidism are both associated with an increased risk of fracture. Nevertheless, the mechanism of T_3 action in bone is incompletely understood. Studies in mutant mice have demonstrated that T_3 action in bone is mediated principally by T_3 receptor α (TRα). T_3 exerts anabolic actions during growth to stimulate peak bone mass acrrual, but has catabolic effects on the adult skeleton that increase bone turnover. Recent studies have also suggested that TSH may have direct actions in bone cells, but such effects are difficult to resolve *in vivo* because thyroid hormone and TSH concentrations are maintained in an inverse relationship by the hypothalamic-pituitary-thyroid axis. Current understanding is based on studies in mice that harbor germline mutations in the genes encoding TRα, TRβ or the TSH receptor and it is not clear whether the skeletal effects of these mutations result from disruption of primary T_3 actions in bone cells or whether they are secondary to systemic effects on other endocrine pathways that regulate skeletal development and bone mass. Tissue-specific disruption of thyroid hormone signalling in bone cells will be required to address this issue. Such studies are likely to identify key components of the T_3 signalling pathway that may represent suitable drug targets for treatment of osteoporosis.
(Pol J Endocrinol 2009; 60 (5): 380–388)

Key words: *thyroid hormones, T_3, T_4, bone turnover, osteoporosis*

Streszczenie

Hormony tarczycy są niezbędne dla prawidłowego rozwoju układu kostnego i uzyskania szczytowej masy kostnej. Niedoczynność tarczycy u dzieci prowadzi do ograniczenia (opóźnienia) tempa wzrostu spowodowanego opóźnieniem rozwoju kości, podczas gdy nadczynność tarczycy przyspiesza dojrzewanie układu kostnego. U dorosłych hormony tarczycy regulują obrót kostny i gęstość mineralną układu kostnego. Stan eutyreozy jest więc istotny dla utrzymania optymalnej jakości układu kostnego. Wyniki badań populacyjnych wykazały, że zarówno niedoczynność, jak i nadczynność tarczycy są związane ze zwiększonym ryzykiem złamań kości. Mimo to mechanizm działania trijodotyroniny (T_3) w kości nie jest do końca poznany. W badaniach zmutowanych myszy wykazano, że działanie T_3 na kość zachodzi poprzez interakcje hormonu z receptorem α (TRα). W okresie młodości i wzrastania T_3 wywiera na kość efekt anaboliczny i odpowiada za szczytowy przyrost kości. W późniejszym okresie życia T_3 wywiera efekt kataboliczny, zwiększając resorpcję wapna i obrót kostny. W ostatnim okresie wyniki niektórych badań mogą wskazywać, że TSH ma bezpośredni wpływ na komórki kości, ale problem ten jest bardzo trudny do ostatecznego ustalenia w warunkach *in vivo*, wobec tego, że stężenie TSH i hormonu tarczycy jest utrzymywane w odwrotnym wzajemnym stosunku (sprzężeniu zwrotnym) przez układ podwzgórze–przysadka–tarczyca. Obecna wiedza w tym zakresie wynika z doświadczeń myszami, u których zmutowano geny kodujące TRα, TRβ i receptor TSH. Jednak nie można kategorycznie ustalić, czy wpływ tych mutacji na szkielet zależy od zmienionej ekspresji T_3 w komórkach kośćca, czy jest następstwem tych mutacji na czynność innych układów endokrynnych, które regulują rozwój szkieletu i masę kostną. Blokowanie sygnału od hormonu tarczycy w poszczególnych tkankach i układach będzie konieczne, aby rozwiązać te niejasności. Przeprowadzenie takich badań pozwoli zidentyfikować kluczowe mechanizmy działania T_3 na komórki kości i określić nowe cele w leczeniu osteoporozy.
(Endokrynol Pol 2009; 60 (5): 380–388)

Słowa kluczowe: *hormony tarczycy, T_3, T_4, obrót kostny, osteoporoza*

Effects of thyroid hormones on the skeleton in humans

Development and growth

Thyroid hormones exert a critical influence on the development of the skeleton. Thyroid hormone deficiency in children results in retarded skeletal development, delayed bone age, and growth arrest accompanied by epiphyseal dysgenesis [1, 2]. By contrast, in childhood thyrotoxicosis there is accelerated skeletal development and growth with advanced bone age. However, short stature may ultimately occur despite advanced skeletal maturation because of premature fusion of the epiphyseal growth plates, leading to early cessation of growth.

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In severe cases, craniosynostosis may occur due to premature closure of the sutures of the skull [3]. Resistance to thyroid hormone is an autosomal dominant condition resulting from dominant negative mutations of the thyroid hormone receptor TRβ. The clinical features of RTH are variable because the functional properties of mutant TRβ proteins differ according to the specific mutation and according to other genetic modifying factors that are currently unknown [4]. Accordingly, diverse skeletal abnormalities have been described in RTH, including short stature, osteoporosis, increased bone density, and various craniofacial and other skeletal malformations [4–6]. The reasons for such diversity are unknown but no prospective analysis has been performed in a series of patients with RTH. Available information is largely derived from case reports and may be confounded by treatment of patients by thyroidectomy or anti-thyroid drugs that could influence skeletal development.

In summary, clinical evidence from children with hypothyroidism, thyrotoxicosis, or RTH indicates that thyroid hormones are essential for bone development. Thyroid hormone deficiency results in developmental delay, whereas thyroid hormone excess accelerates bone formation and growth. Thus, euthyroid status during childhood is essential for normal linear growth and to establish peak bone mass in early adulthood [1].

Bone maintenance

In the adult skeleton, thyroid hormones act as homeostatic regulators that maintain bone mass. In hypothyroidism there is reduced bone turnover affecting both bone resorption and bone formation, and the prolonged bone formation phase leads to an increased mineralization phase [7]. Recent population studies have shown that hypothyroidism is associated with an increased risk of fracture, although the underlying mechanisms resulting in this association are unclear [8–10]. In both pre-menopausal and post-menopausal women and in men, thyrotoxicosis is an established risk factor for osteoporosis [11]. Osteoporosis in thyrotoxicosis results from high bone turnover, with disproportionate increases in bone resorption and bone formation that lead to a loss of approximately 10% of bone mass per remodelling cycle [12]. The accelerated bone loss in established thyrotoxicosis results in low bone mineral density and an increased risk of fracture. Recent studies have investigated whether endogenous subclinical hyperthyroidism (suppressed TSH in the presence of normal circulating T_3 and T_4 levels) or excessive thyroid hormone replacement leading to TSH suppression are associated with reduced bone mineral density and fracture. These studies have produced conflicting results but have been confounded by differences in study design, the inclusion of heterogeneous groups of patients, small numbers of patients, and a lack of clear prospective data [11]. Population studies, however, suggest that subclinical hyperthyroidism may be associated with an increased risk of fracture at the hip and lumbar spine in post-menopausal women [13, 14].

In summary, clinical studies indicate that euthyroid status in adults is essential for normal bone turnover and mineralization and to maintain optimal bone strength [11, 15, 16].

The structure of the skeleton is established during development and growth and determined initially by the acquisition of peak bone mass. Thereafter, skeletal integrity is maintained by the dynamic process of bone turnover (Fig. 1), which continues throughout life and determines the rate of bone loss during adulthood [17]. Thus, bone strength and fracture risk are primarily determined by peak bone mass acquired during growth and the rate of bone loss in adulthood. Euthyroid status is essential for optimal bone mineral deposition during growth, and thyroid hormones regulate the rates of bone resorption and formation, thereby maintaining homeostatic regulation of bone turnover and bone loss during adulthood [1, 11, 18]. Thus, the set-point of the hypothalamic-pituitary-thyroid (HPT) axis, which defines normal euthyroid status within an individual [19, 20], is a key homeostatic regulator of skeletal integrity throughout life that may ultimately determine fracture risk.

Human genetics

In healthy individuals, free T_3, free T_4, and TSH levels fluctuate over a range that is less than 50% of the normal reference range [20]. This variation in thyroid status within an individual is narrower than the broad inter-individual variation seen in the population. Each person has a unique HPT axis set-point that lies within the population reference range, indicating that there is variation in tissue sensitivity to thyroid hormones between normal individuals [19]. Data from the UK Adult Twin Registry estimate the heritability for free T_3 concentration at 23%, free T_4 at 39%, and TSH at 65%, whilst estimates from a Danish twin study were 64%, 65%, and 64%, respectively [21, 22]. A genome wide screen identified eight quantitative trait loci linked to circulating fT_3, fT_4, and TSH levels, indicating that thyroid status is inherited as a complex genetic trait [23]. Similarly, unbiased genome wide association studies and candidate gene approaches have shown that osteoporosis is a polygenic disorder in which many genes and signalling pathways exert small contributions that influence bone size, BMD, and fracture susceptibility [24].

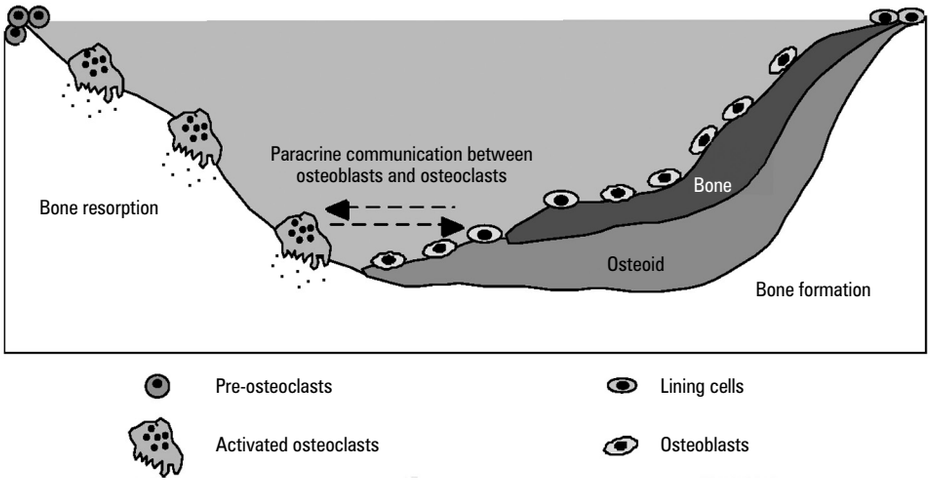

Figure 1. *The bone remodelling cycle. The continual process of bone remodelling is essential for maintenance of bone mass and micro-architecture. Bone remodelling involves osteocytes, osteoclasts, and osteoblasts. Osteocytes form from osteoblasts that have become embedded within mineralized bone. When local skeletal micro-damage occurs or when there is a reduction in mechanical loading, osteocytes respond either by releasing cytokines and chemo-attractants or by undergoing apoptosis. These responses result in local recruitment of osteoclast precursor cells and cause mature osteoclasts to initiate bone resorption. Osteoclasts excavate a resorption cavity over a period of 3–5 weeks until this process is followed by recruitment of osteoblasts. Osteoblasts secrete and mineralize osteoid to replace the resorbed bone over a period of approximately three months. Coupling of osteoclast and osteoblast activities via signalling between the two cell lineages regulates the bone remodelling cycle and results in skeletal homeostasis with preservation of bone strength*

Rycina 1. *Cykl przebudowy kości. Ciągły proces przebudowy kości jest niezbędny do zachowania masy kostnej i prawidłowej mikroarchitektury. W tym procesie uczestniczą osteocyty, osteoklasty i osteoblasty. Osteocyty przekształcają się z osteoblastów, które zostały otoczone zmineralizowaną tkanką kostną. Gdy powstają mikrouszkodzenia szkieletu lub następuje zmniejszenie mechanicznego obciążenia kości, osteocyty uwalniają cytokiny i substancje chemotaktyczne lub ulegają apoptozie. Reakcja osteocytów powoduje miejscową rekrutację prekursorów osteoklastów i stymuluje dojrzałe osteoklasty do resorpcji kości. Osteoklasty drążą jamkę resorpcyjną przez okres 3–5 tygodni, po czym następuje rekrutacja osteoblastów. Rolą tych komórek jest produkcja i mineralizacja osteoidu, który zastępuje kość usuniętą przez osteoklasty w ciągu około 3 miesięcy. Sprzężenie aktywności osteoklastów i osteoblastów poprzez przekazywanie sygnałów między tymi dwiema liniami komórkowymi reguluje cykl przebudowy kości i zapewnia homeostazę tkanki kostnej i zachowanie wytrzymałości kości*

These observations raise the possibility that variations in bone turnover, BMD, and fracture susceptibility in normal individuals may be associated with differences in their HPT axis set-points. Furthermore, genes that establish the HPT axis set-point and thus regulate thyroid status may also influence the acquisition of peak bone mass, skeletal growth, and bone turnover and thereby contribute to the genetic determination of fracture risk. This hypothesis is consistent with observations in other physiological complex traits including BMI, blood pressure, heart rate, atherosclerosis, serum cholesterol, and psychological well-being, in which variations have been associated with small alterations in thyroid function and with polymorphisms in thyroid pathway genes that are themselves associated with altered serum thyroid hormone and TSH concentrations [25]. These new developments in our understanding the physiological regulation of the HPT axis and thyroid hormone action in target tissues have been extended recently to investigation of the skeleton, and these studies suggest common genetic factors may be involved in the determination of thyroid status, bone turnover, and BMD [26, 27].

Future prospective studies investigating the relationships between variations in the HPT axis set-point and genes regulating thyroid hormone transport, metabolism and action with bone mass and fracture risk will need to be well designed and adequately powered. Stringent exclusion criteria will be required to define large populations of individuals that can be followed up prospectively for prolonged periods. Nevertheless, such studies have the potential to individualise fracture risk prediction and inform the choice of preventative therapy [25].

Mechanism of thyroid hormone action

Circulating thyroid hormone levels are maintained in the euthyroid range by a negative feedback loop. Thyrotropin releasing hormone (TRH) is synthesized in the hypothalamus and stimulates synthesis and secretion of TSH from the anterior pituitary gland. TSH stimulates growth of thyroid follicular cells and the synthesis and release of thyroid hormones. Thyroid hormones act via thyroid hormone receptors (TRs) in the hypothalamus and pituitary to inhibit TRH and TSH synthesis and secretion. This negative feedback loop maintains circulating thyroid hormones and TSH in an inverse relationship that defines the HPT axis set-point [1].

The thyroid gland secretes the pro-hormone thyroxine (T_4) and a small amount of physiologically active 3,5,3'-L-triiodothyronine (T_3). The majority of circulating T_3 is generated by 5′-deiodination of T_4 in liver and kidney by the type 1 iodothyronine deiodinase enzyme (D1). Circulating free T_4 levels are maintained at 3-4-fold higher concentrations than free T_3. Intra-cellular availability (Fig. 2) of T_4 and T_3 is determined by active uptake of the free hormones by specific cell membrane transporters including monocarboxylate transporter-8 (MCT8), MCT10, and organic acid transporter protein-1c1 (OATP1c1) [28]. Availability of the active hormone T_3 to the nuclear TRs is subsequently controlled by activities of the type 2 and 3 deiodinase enzymes (D2 and D3). D2 converts T_4 to T_3 by catalyzing removal of a 5'-iodine atom, whilst D3 prevents activation of T_4 and inactivates T_3 by removal of a 5-iodine atom to generate the metabolites 3,3',5'-L-triiodothyronine (reverse T_3) and 3,3'-diiodothyronine (T_2), respectively. The relative levels of D2 and D3 therefore regulate the intra-nuclear concentration of T_3 [29]. The *THRA* and *THRB* genes encode three functional TRs: TRα1, TRβ1, and TRβ2, which act as hormone inducible transcription factors that regulate expression of T_3-responsive target genes [30]. TRα1 and TRβ1 are expressed widely, but their relative concentrations differ during development and in adulthood due to tissue-specific and temporo-spatial regulation [31]. Expression of TRβ2, however, is restricted. In the hypothalamus and pituitary, it controls the HPT axis feedback loop by mediating the inhibitory actions of thyroid hormones on TRH and TSH expression [32, 33].

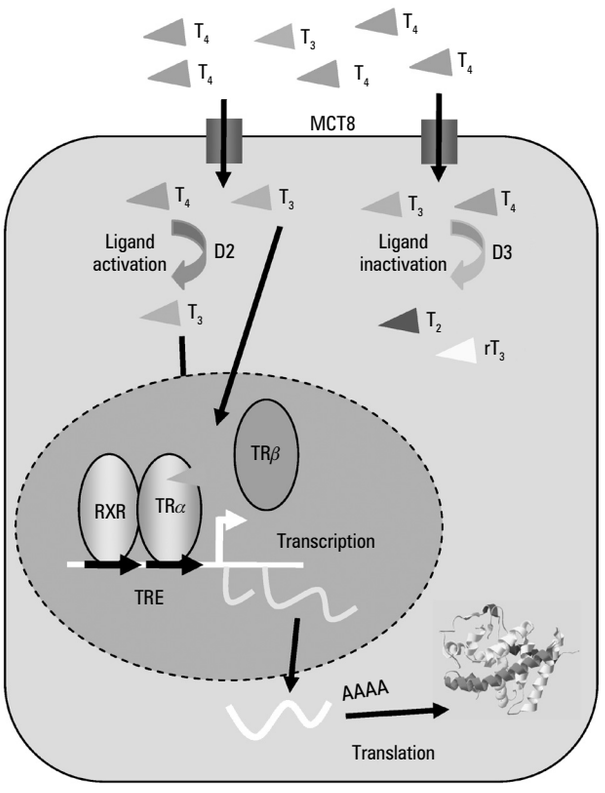

Figure 2. *Mechanism of thyroid hormone action. Levels of circulating free T_4 in serum are 3–4-fold higher than free T_3. T_4 and T_3 enter target cells by active uptake mediated by cell membrane transporters (MCT8). T_3 availability to its nuclear receptors, TRα and TRβ, is controlled by the relative activities of the type 2 and 3 deiodinases (D2 and D3). D2 activates T_4 by converting it to T_3, whereas D3 inactivates both T_4 and T_3. Once inside the nucleus, T_3 binds TRα or TRβ. The hormone bound receptor interacts with retinoid X receptor (RXR) and the TR/RXR heterodimer complex interacts with thyroid hormone response element sequences (TRE) located in the promoter regions of T_3-regulated target genes to control their expression in a hormone-dependent manner*

Rycina 2. *Mechanizm działania hormonów tarczycy. Stężenie krążącej wolnej T_4 w surowicy jest 3–4-krotnie wyższe niż wolnej T_3. Zarówno T_4, jak i T_3 dostają się do komórek poprzez mechanizm aktywnego wychwytu, w którym pośredniczą transportery błonowe (MCT8). Dostępność T_3 dla jej receptorów jądrowych, TRα i TRβ, zależy od względnej aktywności dejodynazy 2 i 3 (D2 i D3). Dejodynaza 2 aktywuje T_4 poprzez przekształcenie jej do T_3, natomiast D3 inaktywuje zarówno T_4, jak i T_3. Po wniknięciu do jądra komórkowego T_3 wiąże się z TRα lub TRβ. Receptor związany z hormonem wchodzi w interakcję z receptorem retinoidowym (RXR), a następnie heterodimer TR/RXR oddziałuje z regulatorową sekwencją odpowiedzi na hormony tarczycy (TRE,* thyroid hormone response*) zlokalizowaną w regionie promotorowym odpowiednich genów regulatorowych i wpływa na ich ekspresję w zależności od stężenia hormonów*

Thyroid hormone action in bone

In the skeleton, TRα1 and TRβ1 are expressed in growth plate chondrocytes, bone marrow stromal cells, and bone-forming osteoblasts, but it is not certain whether they are present in bone-resorbing osteoclasts [18, 34–36]. In the growth plate, T_3 inhibits cell proliferation and stimulates differentiation of hypertrophic chondrocytes to regulate endochondral ossification and linear growth [35]. These regulatory effects of T_3 on the rate of growth plate chondrocyte differentiation occur via

TRα1 and involve interactions with key signalling pathways that control chondrocyte differentiation, including the Indian hedgehog/parathyroid hormone-related peptide feedback loop and the growth hormone/ /insulin-like growth factor-1 (GH/IGF-1) and fibroblast growth factor receptor-3 (FGFR3) signalling pathways [37, 38]. Studies of bone marrow stromal cells indicate that T_3 acts via complex cytokine and growth factor signalling pathways, which are known to regulate communication between osteoblast and osteoclast cell lineages within the bone marrow microenvironment. T_3 also regulates osteoblast differentiation and function via TRα1 and by interacting with FGFR1 signalling [39]. The predominant consequences of hypothyroidism and hyperthyroidism on the skeleton, however, result from effects on osteoclast activity and bone resorption. Despite this, it is not clear whether T_3 exerts direct actions in osteoclasts or whether the effects on bone resorption result from secondary responses of osteoclasts to the actions of T_3 in chondrocytes, bone marrow stromal cells, or osteoblasts. An important challenge in the future will be to characterize the cellular actions of thyroid hormones in bone in detail

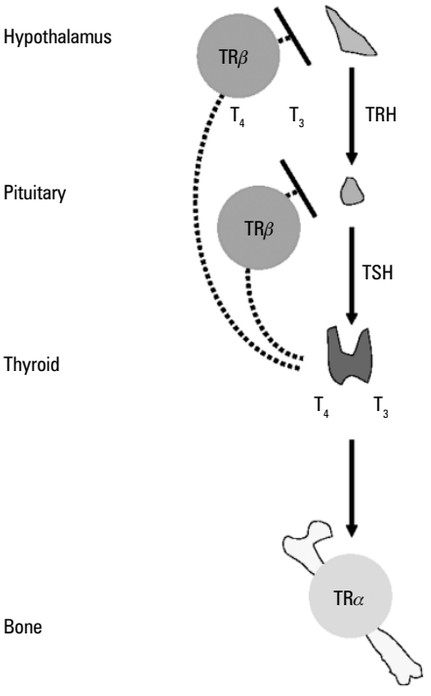

Figure 3. *The hypothalamic-pituitary-thyroid-bone axis. TRH is synthesized in the hypothalamus and stimulates secretion of TSH from the anterior pituitary. TSH stimulates the synthesis and release of thyroid hormones. Thyroid hormones act in bone via TRα to regulate growth and bone turnover. Thyroid hormones also act via TRβ expressed in hypothalamus and pituitary to inhibit TRH and TSH synthesis and secretion*

Rycina 3. *Oś podwzgórze–przysadka–tarczyca–kość. Produkowany w podwzgórzu TRH pobudza sekrecję TSH z przedniego płata przysadki. Z kolei TSH stymuluje syntezę i uwalnianie hormonów tarczycy. Hormony te działają na kość poprzez receptory TRα i regulują wzrost kości i obrót kostny. Hormony tarczycy hamują ponadto syntezę i wydzielanie TRH i TSH za pośrednictwem receptorów TRβ zlokalizowanych w podwzgórzu i przysadce*

Thyroid hormone receptor regulation of the HPT axis and the skeleton

Studies of the relative levels of expression of TRs in various tissues have shown that TRβ expression predominates in hypothalamus and pituitary [31–33, 40] (Fig. 3). Accordingly, and in keeping with the causative role for dominant-negative mutations of TRβ in the human syndrome of RTH [4], detailed analyses of transgenic and TR knockout mice have revealed that TRβ regulates the sensitivity of the hypothalamus and pituitary to negative feedback inhibition by thyroid hormones [41]. These studies demonstrate that TRβ controls the HPT axis set-point thereby determining the levels of circulating thyroid hormones. By contrast, TRα is expressed at substantially higher levels than TRβ in bone, suggesting the skeleton may be a predominantly TRα-responsive T_3 target tissue. These findings suggested that mutation or deletion of the *THRA* and *THRB* genes would have differing consequences to the skeleton and would be informative in elucidating the physiological relationship between the central and peripheral actions of thyroid hormones [42, 43] (Fig. 4).

TSH receptor expression in bone

Investigation of the relationship between thyroid hormone actions centrally and in bone was complicated, however, by the finding that osteoblasts and osteoclasts express the TSH receptor (TSHR) [44]. Analysis of congenitally hypothyroid TSHR knockout mice treated with thyroid extract revealed a phenotype of high bone turnover osteoporosis in animals at approximately 6–7 weeks of age. Because of these findings, TSH was proposed as a negative regulator of bone turnover, and it was suggested that bone loss in TSHR knockout mice was a direct consequence of TSH deficiency. Although this raises important and provocative questions regarding the precise role of the HPT axis in skeletal homeostasis, the findings in TSHR knockout mice are confounded because these mice require thyroid hormone supplementation for survival after weaning at 3–4 weeks of age [45]. In mice, T_4 and T_3 levels rise rapidly to reach their physiological peak at 2 weeks of age, and growth velocity is maximal at this time. Since TSHR knockout mice were supplemented with thyroid extract from weaning when already growth retarded, they

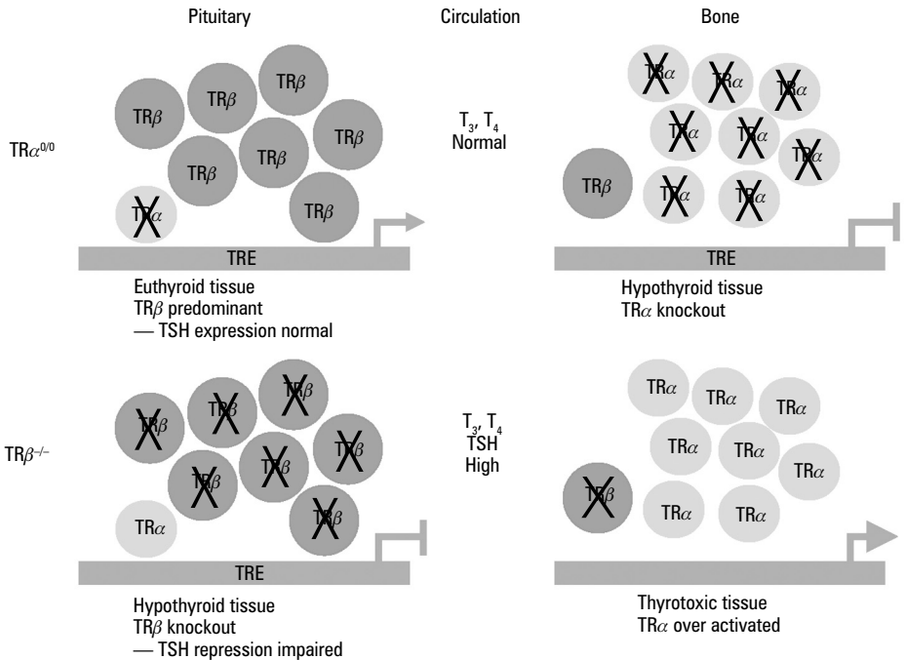

Figure 4. *The relationship between TRα and TRβ expression in pituitary and bone determines the skeletal phenotype of TR knockout mice TRα$^{0/0}$ and TRβ$^{-/-}$. TRβ is predominantly expressed in pituitary whereas TRα is the main TR expressed in bone. Deletion of TRα does not alter pituitary responsiveness to T_3, so physiological negative feedback inhibition of TSH by thyroid hormones continues normally. Thus, TRα$^{0/0}$ mice are euthyroid. By contrast, when TRβ is deleted, negative feedback inhibition of TSH by thyroid hormones is disrupted leading to elevated levels of TSH, T_3, and T_4 in TRβ$^{-/-}$ mice. The effects of these mutations in bone result from loss of skeletal TR expression in TRα$^{0/0}$ mice (skeletal hypothyroidism) or from elevated circulating thyroid hormones in TRβ$^{-/-}$ mice (skeletal hyperthyroidism)*

Rycina 4. *Zależność między ekspresją TRα i TRβ w przysadce i tkance kostnej determinuje fenotyp szkieletu u zmutowanych myszy TRα$^{0/0}$ i TRβ$^{-/-}$. Głównym miejscem ekspresji receptora TRβ jest przysadka, natomiast TRα — kość. Delecja TRα nie powoduje zmiany wrażliwości przysadki na T_3, a zatem fizjologiczny mechanizm zwrotnego hamowania syntezy TSH przez hormony tarczycy nie ulega zaburzeniu. Co za tym idzie, u myszy TRα$^{0/0}$ występuje eutyreoza. Z kolei w przypadku delecji TRβ następuje zaburzenie ujemnego sprzężenia zwrotnego między stężeniem hormonów tarczycy i TSH, co prowadzi do zwiększenia stężeń TSH, T_3 i T_4 u myszy TRβ$^{-/-}$. Mutacje te wpływają na metabolizm kostny: u myszy TRα$^{0/0}$ brak ekspresji receptora TR w komórkach kości powoduje hipotyroidyzm kostny, a u myszy TRβ$^{-/-}$ dochodzi do wzrostu stężenia krążących hormonów tarczycy i w rezultacie do hipertyroidyzmu*

were actually grossly hypothyroid at the critical stage of thyroid hormone-dependent bone development. Thus, the phenotype in TSHR knockout mice may reflect the effects of severe hypothyroidism followed by "catch-up" growth and accelerated bone turnover in response to delayed thyroid hormone replacement [1]. Furthermore, the susceptibility of patients with Graves' disease to osteoporosis and fracture is not consistent with the hypothesis that TSH negatively regulates bone turnover, because the presence of TSHR stimulating antibodies would be predicted to protect patients from osteoporosis if TSH is a direct negative regulator of bone turnover. In addition, two boys treated with thyroid hormone replacement from birth because of congenital hypothyroidism due to isolated TSH deficiency had normal bone mineral density [46].

Overall, these studies suggest that the skeletal effects of abnormal thyroid status are due primarily to effects of thyroid hormone deficiency or excess, although direct effects of TSH cannot be excluded as a contributing factor. Unfortunately, resolution of this issue is problematic. The relative effects of T_3 and TSH in bone cannot be differentiated readily because the HPT axis maintains thyroid hormones and TSH in a physiological reciprocal relationship because of negative feedback inhibition of TSH by thyroid hormones [1]. Nevertheless, studies in mutant mice have enabled this difficulty to be overcome *in vivo*.

Skeletal phenotypes in two contrasting models of congenital hypothyroidism

Hyt/hyt mice harbour a Pro556Leu mutation in the TSHR. The mutant receptor does not bind TSH and is non-functional, resulting in hypoplasia of the thyroid gland and severe congenital hypothyroidism characterized by a 2000-fold increase in TSH levels in the presence of barely detectable levels of thyroid hormones [47]. Pax8 knockout mice lack an essential transcription factor required for thyroid follicular cell development. They have also congenital hypothyroidism characterized by a 2000-fold elevation of TSH and undetectable thyroid hormone levels, but they possess fully functional TSH receptors [48]. Thus, both hyt/hyt and Pax8 knockout mice have grossly elevated TSH levels, but in hyt/hyt mice the TSHR is non-functional whereas in Pax8 mice it is normal. We reasoned, therefore, that if TSH exerts a physiologically important role during skeletal development and growth then these mice would display opposite skeletal phenotypes [49]. However, both mutants had similar skeletal abnormalities consisting of growth retardation, delayed endochondral ossification, and reduced cortical bone deposition accompanied by retention of calcified cartilage in trabecular bone with impaired remodelling and reduced bone mineralization. These features are typical of the skeletal abnormalities seen in hypothyroidism and occur despite the divergence in TSH signalling in hyt/hyt and Pax8 mice, thus demonstrating that during bone development and growth the effects of thyroid hormone deficiency are independent of TSHR activity [49]. These findings indicate the effects of the HPT axis in bone are mediated principally by thyroid hormones.

Molecular basis of thyroid hormone action in bone

In order to investigate the roles of TRα and TRβ in skeletal development and homeostasis *in vivo*, mice harbouring mutations or deletions of the *Thr*α and *Thr*β genes were characterized. Deletion or mutation of TRα does not affect circulating thyroid hormone or TSH levels, and mutant mice are consequently euthyroid. Nevertheless, TRα mutants display transient growth retardation, delayed endochondral ossification, and reduced bone mineral deposition during growth. In adults, impaired bone remodelling with reduced bone resorption results in a marked increase in bone mass, leading to a phenotype of osteosclerosis, in which bone mineralization may be normal or increased depending on the TRα mutation. Thus, mutation of TRα disrupts T_3 action in bone cells in which TRβ is predominantly expressed, resulting in phenotype that recapitulates the skeletal effects of hypothyroidism [50–53]. By contrast, mutation or deletion of TRβ disrupts the HPT axis, leading to resistance to thyroid hormone characterized by elevated levels of thyroid hormones and TSH. TRβ mutants display accelerated endochondral ossification and increased bone mineral deposition during development but have short stature due to premature quiescence of the growth plates. In adult TRβ mutant or knockout mice, increased bone turnover with increased osteoclastic bone resorption results in osteoporosis characterized by reduced bone mass and low bone mineralization density. Thus, elevated levels of circulating thyroid hormones in TRβ mutant mice activate the remaining intact TRα expressed in bone, resulting in phenotype that is characteristic of the effects of hyperthyroidism in bone [43, 51–53]. Accordingly, analysis of T_3 target gene expression in TR mutant mice by in situ hybridization has revealed increased expression in TRβ mutants with elevated thyroid hormone levels but reduced expression in TRα mutants despite their circulating euthyroid status [37, 39, 53]. Overall, these findings demonstrate that thyroid hormones exert anabolic growth promoting actions during skeletal development but exert catabolic responses resulting in bone loss in adults. The differing patterns of expression of TRα and TRβ in the hypothalamus and pituitary and the skeleton indicates that the effects of T_3 in developing and adult bone are mediated by TRα [43].

Are the actions of thyroid hormones in bone direct?

Studies of mice harbouring germline mutations or deletions of the *Thr*α or *Thr*β genes provide compelling evidence of a key role for TRα in bone, but they cannot distinguish whether skeletal defects result from the systemic consequences of TR disruption or from local actions of T_3 in skeletal cells. Thus, thyroid hormones regulate activities of numerous signalling pathways that influence the skeleton including the GH/IGF-1 and sex steroid axes as well as various growth factors and cytokines that regulate bone cell differentiation and function. Thus, it is possible that skeletal responses to thyroid hormones could be largely secondary to direct effects of thyroid hormones in non-skeletal cells, or they could result predominantly from the direct actions of thyroid hormones in bone cells that could include chondrocytes, osteoblasts, osteocytes, and osteoclasts.

In recent studies we investigated expression of the thyroid hormone transporter MCT8 and the deiodinase enzymes in bone cells [54]. MCT8 mRNA was present in all bone cell types, and levels of expression did not differ with cell differentiation. By contrast, the D1 enzyme was not expressed in any bone cell lineage and

activity of the D2 enzyme was restricted to mature differentiated osteoblasts, whilst activity of D3 was present in growth plate chondrocytes prior to weaning but levels declined thereafter and only low levels of D3 were detected in chondrocytes in older animals. Low levels of D3 activity were also detected in osteoblasts and osteoclasts at all stages of maturation [54]. These findings support the likelihood that thyroid hormones have important direct effects in skeletal cells as the activities of D2 and D3 control T_3 availability to the nuclear TR. The differing patterns of expression of D2 and D3 suggest that control of T_3 availability in chondrocytes during skeletal development may be dependent on metabolic clearance by D3, whereas T_3 availability to osteoblasts may be determined by metabolic activation by D2. In order to test these hypotheses and investigate the role of thyroid hormone supply to skeletal cells it will be necessary to characterize the skeletal consequences of deletion of the *Dio2* and *Dio3* genes encoding D2 and D3. Further refinement of our understanding of the molecular and cellular basis for T_3 action in bone will require cell-specific disruption of thyroid hormone signalling in bone cells using cre-lox gene targeting techniques in which expression of Cre recombinase in chondrocytes, osteoblasts, osteocytes, or osteoclasts will enable the TRs, deiodinases, and thyroid hormone transporters to be selectively deleted in individual bone cell lineages. Such approaches will identify which components of the thyroid hormone signalling pathway are responsible for the actions of T_3 in specific bone cell types. These experiments will provide important and definitive characterization of the molecular mechanism of thyroid hormone action in bone and will identify novel drug targets for manipulation of peak bone mass acquisition and bone mineralization.

Summary

- Thyroid hormones are required for growth and establishment of peak bone mass
- Thyroid hormones are required to maintain optimal bone strength
- The set-point of the HPT axis may determine fracture risk
- Hypothyroidism and hyperthyroidism are both associated with increased fracture risk
- Mild degrees of thyroid hormone excess, characterized by suppressed levels of TSH, are associated with increased fracture risk in post-menopausal women
- T_3-action in bone is mediated by TRα
- T_3 has anabolic actions in bone during growth, but exerts catabolic effects on the adult skeleton
- TSH may also act on bone cells, but independent opposing effects of T_3 and TSH are difficult to resolve because circulating thyroid hormone and TSH levels are maintained in an inverse relationship by the HPT axis
- The cellular basis of T_3 action in bone is incompletely understood, and tissue-specific gene targeting will be required to characterize this *in vivo*

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