Thyroid Hormone Actions in Cartilage and Bone

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Abstract
Thyroid hormones exert widespread and complex actions in almost all tissues during development, throughout childhood and in adults. The skeleton is an important T3-target tissue that exemplifies these processes, and yet understanding of the specific cellular and molecular mechanisms of T3 action in bone and cartilage remains incomplete. Here, the skeleton is considered as a T3-target tissue. The actions of thyroid hormones during skeletal development and in chondrocytes and growth plate cartilage during post-natal linear growth are outlined. The physiological importance of these actions are discussed in relation to patients with autosomal dominant mutations in genes encoding the thyroid hormone receptors TRα1 and TRβ, and in mice harbouring deletions or mutations of the orthologous genes. The role of thyroid hormones and the control of T3 action in bone turnover and maintenance are also outlined, and T3 action in bone-forming osteoblasts and bone-resorbing osteoclasts discussed. The physiological and functional consequences of T3 action in bone are considered in relation to mutant mouse models and to effects on bone mineral density and fracture susceptibility in humans. Finally, new studies identifying a putative role for thyroid hormone metabolism in articular cartilage maintenance and the pathogenesis of osteoarthritis are considered. The pharmacological context of these new findings is discussed, emphasising the importance of this emerging field of study in thyroid hormone pathophysiology.
regulation of thyrotropin-releasing hormone (TRH) in the hypothalamus and thyroid-stimulating hormone (TSH, thyrotropin) synthesis and secretion in the anterior pituitary [2]. More than 95% of circulating thyroid hormones are bound to plasma proteins and the remaining free hormones (fT4, fT3) represent the biologically available and active fraction.

**Cellular T3 Availability**

fT4 and fT3 enter peripheral target cells via specific cell membrane transporters with differing patterns of tissue distribution. Several thyroid hormone transporters have been identified, including the monocarboxylate transporters 8 and 10 (MCT8, MCT10), the organic acid transporter protein-1c1 (OATP1c1) and the non-specific L-type amino acid transporters 1 and 2 (LAT1, LAT2) [3]. Once inside the cell, thyroid hormones are metabolized further. The type 2 deiodinase (Dio2) catalyses conversion of T4 to T3 by removal of an outer ring iodine thus activating the pro-hormone, whereas the type 3 enzyme (Dio3) inactivates T4 and T3 by removal of a 5-iodine atom [4]. The relative tissue activities of these two enzymes determine the intracellular T3 concentration and provide a pre-receptor control mechanism that regulates availability of the active hormone to the cell nucleus.

**Thyroid Hormone Receptors**

The thyroid hormone receptors TRα1, TRβ1 and TRβ2, bind ligand with high affinity and act as hormone-inducible transcription factors that regulate expression of target genes in response to T3. The TRs are located in the nucleus even in the absence of hormone and, in the case of genes that are positively regulated by T3, these unoccupied TRs interact with co-repressor proteins to repress target gene transcription [5]. After T3 binding, co-repressor is released and co-activator proteins are recruited so that hormone-dependent activation of gene expression is initiated in T3-responsive cells. The TRα1 and TRβ1 isoforms are expressed in almost all tissues, but in different relative concentrations that vary at discrete stages of development and with age [6], whereas TRβ2 expression is restricted to the pituitary and hypothalamus [7]. Overall, pre-receptor control of T3 availability, together with the diversity of TR isoform expression, provides a sophisticated mechanism that coordinates and regulates temporal and spatial responses to thyroid hormones in critical target tissues during development and in adulthood [5, 6, 8]. T3 action is also regulated by tissue-specific differences in expression of co-repressor and co-activator proteins that further modulate TR activity [9–12].

**The Skeleton Is a T3-Target Organ**

MCT10 appears to be the major thyroid hormone-specific transporter expressed in the growth plate, although MCT8 is expressed widely in cartilage-forming chondrocytes, bone-forming osteoblasts and bone-resorbing osteoclasts, whilst expression of the less specific LAT1 and LAT2 transporters has also been identified in skeletal tissue. By contrast, OATP1c1 mRNA is not expressed in bone or cartilage [13–15]. Thyroid hormone metabolism also occurs in skeletal cells [16]. Although Dio1 is not expressed in cartilage and bone [14, 16], the activating enzyme Dio2 is expressed only in osteoblasts [14, 17], whilst the inactivating Dio3 enzyme is present in all skeletal cell lineages, particularly during development and prior to weaning [14, 18]. The T3 receptors, TRα1 and TRβ1, are each expressed in the skeleton and quantitative RT-PCR studies indicate that TRα1 mRNA is expressed at levels at least ten-fold greater than TRβ1 [19, 20]. Both receptor isoforms are present in growth plate chondrocytes, bone marrow stromal cells and bone-forming osteoblasts. However, it is not certain whether TRs are expressed in bone-resorbing osteoclasts or in osteocytes, which communicate environmental, endocrine and paracrine cues and coordinate bone formation and bone resorption [16]. Thus, all the factors required for locally regulated T3 action, including thyroid hormone transporters, metabolizing enzymes and receptors, are present in cartilage and bone indicating the skeleton is a physiological target tissue for thyroid hormone throughout life.

**Cartilage**

**Endochondral Ossification, Bone Formation and Linear Growth**

The formation of long bones and linear growth occur by the process of endochondral ossification [21, 22]. During endochondral ossification, condensations of mesenchyme form a cartilage scaffold that supports bone development (fig. 1). Chondrocyte progenitor cells undergo clonal expansion and proliferate before differentiating into hypertrophic chondrocytes and ultimately undergoing programmed cell death. During this process, a mineralizing cartilage matrix is secreted, which forms the scaffold for vascular invasion and bone deposition. Newly laid down bone is continuously remodelled by bone-resorbing osteoclasts and bone-forming osteoblasts in or-
der to shape the developing long bone in response to local mechanical stresses and paracrine signalling cues. Linear growth subsequently occurs via the same orderly mechanism in specialized epiphyseal growth plates positioned at the proximal and distal extremities of long bones. The process of endochondral ossification continues until early adulthood when the growth plates become quiescent and ultimately fuse, resulting in cessation of linear growth [21, 22]. Bone mineral deposition and accrual of bone mass continue after linear growth has stopped such that peak bone mass is reached by the age of approximately 25 years.

Thyroid Hormone Action in Growth Plate Chondrocytes

TRα1 and TRβ1 are expressed in reserve and proliferating chondrocytes, indicating the epiphyseal growth plate is directly responsive to T3 [23] (fig. 1). T3 stimulates clonal expansion of chondrocyte progenitor cells, but inhibits subsequent cell proliferation whilst promoting hypertrophic chondrocyte differentiation and cell volume expansion [23–26]. Thus, in primary growth plate chondrocyte cultures, T3 stimulates alkaline phosphatase and collagen X expression, and enhances cartilage matrix mineralization [23]. During endochondral ossification the pace of chondrocyte proliferation and differentiation is regulated by several paracrine factors, including insulin-like growth factor-1 (IGF1), Wnt morphogens, bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs), and by a negative feedback loop involving Indian hedgehog (Ihh) and parathyroid hormone-related peptide (PTHrP) [27–29]. The set-point of this feedback loop and the rate of linear growth are sensitive to changes in thyroid status in vivo and regulated by local thyroid hormone metabolism and T3 availability [30, 31]. T3 also regulates expression of growth plate matrix proteoglycans [32] and collagen-degrading enzymes such as aggrecanase-2 (a disintegrin and metalloproteinase with thrombospondin motifs 1, ADAMTS5) and matrix metalloproteinase-13 (MMP13) [33–35]. In summary, thyroid hormone is essential for the coordinated progression of endochondral ossification,
acting to stimulate expression of genes that control chondrocyte maturation and cartilage matrix synthesis, mineralization and degradation.

**Thyroid Hormone Effects on Linear Growth and Skeletal Development**

During childhood and adolescence, hypothyroidism causes growth arrest resulting in epiphyseal dysgenesis and severely delayed bone age [36, 37]. Thyroxine replacement results in ‘catch-up’ growth, although full predicted final height may not be reached, especially when the diagnosis and treatment of hypothyroidism is delayed. Thyrotoxicosis in childhood accelerates linear growth and advances bone age, but ultimately results in persistent short stature due to early fusion of the epiphyses. In severe cases, craniosynostosis results from premature closure of the skull sutures and fontanelles and may be associated with intellectual deficit [38, 39]. Furthermore, in congenital hyperthyroidism resulting from constitutive activating mutations of the TSH receptor gene (TSHR), advanced bone age has been reported, although early thyroidectomy is effective to prevent long-term consequences of hyperthyroidism [40]. These observations indicate extraordinary sensitivity of the developing skeleton to thyroid hormones, and reveal that euthyroid status is essential for normal skeletal development and postnatal growth.

**Syndromes of Resistance to Thyroid Hormone**

Resistance to thyroid hormone (RTH) is an autosomal dominant condition caused by mutations of THRB that result in expression of dominant-negative TRβ proteins. The syndrome is characterized by mildly or moderately increased thyroid hormone concentrations and an inappropriate normal or elevated TSH due to impaired negative feedback control of the HPT axis. Patients with RTH display variable skeletal phenotypes that are confounded by the effects of treatment and the expression of heterogeneous TRβ mutations, which have variable functional properties and activities [41]. Two reports recently described the first individuals with an RTH syndrome resulting from heterozygous mutations of THRA resulting in expression of dominant-negative TRα1 proteins [42, 43]. Subjects have normal levels of TSH but free and total T4 levels lie within or just below the normal range and free and total T3 levels are within or just above the normal range, leading to a markedly reduced T4:T3 ratio. Patients display a phenotype reminiscent of the features of hypothyroidism that include delayed growth with persistent short stature, impaired tooth eruption and patent fontanelles with thickening of the skull vault. These features are consistent with retarded intramembranous and endochondral ossification and demonstrate a critical role for TRα1 in the human skeleton [42, 43]. Mutations in the selenocysteine insertion sequence binding protein 2 gene (SECISBP2) cause a complex multisystem disorder that includes thyroid dysfunction and RTH, which result from abnormal thyroid hormone metabolism due to reduced deiodinase enzyme activity [44, 45]. Affected individuals have growth retardation and delayed bone age who respond to treatment with T3 [46], further demonstrating the requirement for thyroid hormones during growth and skeletal development.

The recent studies in individuals with THRA mutations are entirely consistent with conclusions from studies of mice with mutations or deletions affecting the Thra and Thrb genes [20] (fig. 2). T3 action in bone is mediated principally by TRα1, which is expressed at much higher levels than TRβ in the skeleton [19, 20]. Mice harbouring knockout or dominant-negative point mutations of Thra are euthyroid but display a skeletal phenotype characteristic of juvenile hypothyroidism that includes impaired intramembranous and endochondral ossification with reduced bone mineral deposition during skeletal development and delayed growth [20, 47–51]. Mice with knockout or dominant-negative point mutations of Thrb have disrupted negative feedback regulation of the HPT axis and RTH, but display a skeletal phenotype consistent with the effects of systemic hyperthyroidism on bone. Thus, juvenile TRβ mutant mice have advanced ossification with increased bone mineral deposition but display short stature due to accelerated growth plate maturation [20, 48, 49, 51]. The contrasting phenotypes in mice with Thra and Thrb mutations demonstrate that TRα1 is the major mediator of T3 action in the skeleton. In TRα1 mutant mice skeletal features of hypothyroidism result directly from impaired T3 action in bone and cartilage, whereas the consequences of Thrb mutations are indirect because the elevated thyroid hormones result in an increased skeletal response to T3 that is mediated by the wild-type TRα1 protein expressed in bone [52].

**Bone**

**Bone Remodelling Cycle**

The skeleton undergoes continuous remodelling in response to mechanical stress and injury at multiple sites throughout the skeleton in order to maintain structural...
integrity and strength \cite{53, 54} (fig. 3). The cyclical process of bone turnover and repair is initiated by osteocytes. These cells are embedded within calcified bone and communicate via an elaborate network of dendritic processes. Osteocytes respond to changes in mechanical loading or micro-fracture by undergoing apoptosis with release of cytokines and growth factors that attract osteoclasts to sites of micro-damage. Osteoclasts resorb areas of damaged bone and communicate with osteoblasts, which are then attracted by various growth factors and by degraded matrix proteins released during bone resorption. Osteoblasts subsequently synthesize, secrete and mineralize osteoid to lay down new bone. Completion of the formation phase of the bone remodelling cycle by osteoblasts

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results in the repair of defective bone. Overall, the balanced coupling of bone resorption to bone formation is essential to maintain the architecture, mineralization and strength of bone [53, 54].

**Thyroid Hormone Action in Osteoblasts**

Studies of T3 action in osteoblasts are contradictory, but overall thyroid hormones stimulate osteoblast activity. T3 stimulates synthesis and post-translational modification of type I collagen [55], induces expression of alkaline phosphatase [56] and regulates synthesis and secretion of the bone matrix proteins osteopontin and osteocalcin [55–57]. T3 also promotes bone matrix remodelling by stimulating expression of matrix metalloproteinases-9 and -13 [35]. Furthermore, thyroid hormones regulate key pathways involved in osteoblast proliferation and differentiation. Thus, T3 induces IGF-1 transcription [58] and stimulates expression of its regulatory binding proteins IGF1BP-2 and IGF1BP-4 [59]. Moreover, T3 induces FGFR1 expression and FGF-induced MAPK signalling in osteoblasts [60], and also regulates activity of the Wnt signalling pathway [61]. Together, these studies indicate T3 regulates osteoblast differentiation and function by complex mechanisms involving numerous paracrine and autocrine factors.

In addition, T3 actions in osteoblasts may indirectly influence the activity of osteoclasts by regulating osteoprotegerin (OPG) [62]. OPG is a decoy receptor that inhibits receptor activator of nuclear factor-κB ligand (RANKL) mediated activation of osteoclastogenesis [63]. However, the involvement of T3 in regulation of the OPG/RANKL pathway is controversial as other studies suggest effects of T3 on osteoclastogenesis may be independent of RANKL [64, 65]. Thus, although thyrotoxicosis results in increased osteoclast numbers and activity leading to increased bone resorption in vivo, it is not clear whether T3 acts directly in osteoclasts or whether these responses are secondary to direct actions of T3 in osteoblasts.

**Thyroid Hormone Action in Osteoclasts**

Osteoclasts express both TRα1 and TRβ1 mRNAs but it is not known whether functional receptors are present because available TR antibodies lack the sensitivity required to detect endogenous proteins reliably. Furthermore, studies using mixed primary cultures containing osteoclast lineage cells and bone marrow stromal cells were inconclusive and contradictory [66–70]. Nevertheless, treatment of immortalized osteoblasts or primary bone marrow stromal cells resulted in increased expression of RANKL, interleukin-6, interleukin-8 and prostaglandin E₂, consistent with a likely indirect effect of thyroid hormones on osteoclast function [68, 70].
Thyroid Hormone Effects on Bone Turnover and Maintenance

The bone remodelling cycle (fig. 3) is regulated by thyroid hormones. In hypothyroidism there is reduced bone turnover with impaired bone resorption and formation phases. The resulting increase in duration of the bone remodelling cycle leads to a prolonged period of secondary mineralization [71, 72]. Conversely, in thyrotoxicosis, high bone turnover osteoporosis is due to shortening of the remodelling cycle with uncoupling of the activities of osteoclast and osteoblasts that results in a loss of about 10% of mineralized bone per cycle [72]. Consistent with histomorphometry data, population studies have demonstrated that hypothyroidism is associated with a two- to three-fold increased risk of fracture [73, 74], whilst thyrotoxicosis is an established cause of osteoporosis and fragility fracture [74, 75]. Additional studies also show that thyroid hormone replacement, sub-clinical hyperthyroidism and even variation of thyroid status within the normal reference range are inversely correlated with BMD and may be associated with an increased risk of fracture, particularly in post-menopausal women [75–82]. Taken together, all these studies, and many other clinical investigations, reveal that the adult skeleton is sensitive to small but prolonged periods of altered thyroid status, especially in post-menopausal women.

Studies in adult mice with deletion or dominant-negative mutations of the Thra and Thrb genes are consistent with these clinical data and further elucidate understanding of the cellular mechanisms underlying T3 action in bone (fig. 2). Thus, despite systemic euthyroidism, adult TRα knockout and mutant mice display a phenotype of osteosclerosis with increased bone volume and mineralization accompanied by a net reduction in osteoclastic bone resorption [47, 48]. These findings further support the view that impaired T3 action in skeletal cells results from deletion or mutation of TRα1. By contrast, adult TRβ mutant mice exhibit osteoporosis with increased osteoclastic bone resorption due to the effects of thyroid hormone excess in TRα1 expressing skeletal cells [47, 48, 51, 52]. Studies of mice with deletion of the Dio2 gene further demonstrate a critical requirement for T3 in osteoblasts [17]. Dio2 knockout mice have increased bone mineralization density and brittle bones due to impaired osteoblast activity. Histomorphometry data revealed a reduced bone formation rate with prolongation of the bone remodelling cycle and a longer period of secondary mineralization. Thus, the type 2 deiodinase enzyme is essential to maintain intracellular supplies of T3 in osteoblasts and plays a critical role to maintain optimal bone mineralization and strength [17]. Taken together, all these studies underscore the important role of thyroid hormones in regulating adult bone maintenance.

Articular Cartilage

It is clear that thyroid hormones have essential roles to regulate bone development, growth and maintenance throughout life, but new studies also indicate the potential importance of thyroid hormones in articular cartilage lining the joint surfaces. Genome-wide association and population cohort studies have identified both Dio2 and Dio3 as disease susceptibility loci in osteoarthritis [83–85], and a Dio2 polymorphism has been associated with hip geometry, an independent risk factor for osteoarthritis [86]. In support of a potential role for thyroid hormones in the regulation of articular cartilage maintenance, increased expression of Dio2 protein has been documented in samples of osteoarthritic cartilage [87]. Furthermore, a phase III clinical trial investigating the use of eprotirome, a TRβ-selective agonist, for treatment of hypercholesterolaemia [88] was terminated due to preclinical toxicology studies that identified dose-related articular cartilage damage in dogs treated for 12 months. As a consequence of this finding, the pharmaceutical company involved is not pursuing further work with eprotirome and the programme has been closed. Together, these studies identify an important and likely role for thyroid hormones in the pathogenesis of osteoarthritis and demonstrate the critical importance of a detailed understanding of T3 action in bone and cartilage. Future opportunities for the use of pharmacological modulators of T3 action in the prevention and treatment of chronic disease may be limited in the light of these recent findings and further progress will require full investigation of the long-term consequences to the skeleton and joints.

Conclusions

Studies of the effects of thyroid hormones in cartilage and bone represent a novel and growing field in our understanding of the broader pathophysiological consequences of thyroid hormone action. Although considerable advances have been made in recent years, there are important gaps in our knowledge and a number of specific areas require investigation.
The actions of thyroid hormones in chondrocytes and osteoblasts have been characterized in detail, yet the principal effect of thyroid hormone excess on the adult skeleton is to stimulate osteoclast-mediated bone resorption. It is not known whether T3 exerts direct actions in osteoclasts or whether osteoclast responses are secondary to the actions of T3 in other cells such as osteoblasts, bone marrow stromal cells or macrophages. A major effect of thyroid hormone excess is uncoupling of the resorption and formation phases of the bone remodelling cycle, resulting in a net loss of bone and osteoporosis. This response has been well known for over a century, but the underlying cellular and molecular mechanisms have not been investigated. The osteocyte represents a key candidate T3-target cell as it orchestrates coupling between osteoblasts and osteoclasts to regulate bone turnover, but its possible T3 responsiveness has not yet been investigated. One key area for future study will be to investigate cell lineage-specific actions of thyroid hormones in the skeleton in vivo, perhaps by adopting tissue-specific gene-targeting approaches using cre-lox recombination technology.

The recent and exciting identification of patients with mutations of the THRA gene has provided convincing support for the proposed major role for TRα1 in the skeleton. Yet the patients raise additional questions that will require new studies. Firstly, it is not clear whether treatment of children with supra-physiological doses of thyroid hormones will improve growth or skeletal development in affected individuals or whether other therapeutic approaches such as additional treatment with growth hormone, IFG-1 or even histone-modifying agents will be beneficial. Studies in mouse models will be invaluable to inform future treatment options in humans. Secondly, there is almost no information about the effect of TRα1 mutations on the adult skeleton in humans and little detail is known in mutant mouse models. These are obvious areas for further study that will provide translational benefit to patients in the future.

Finally, identification of a potential role for thyroid hormone signalling in the pathogenesis of osteoarthritis has opened a new field that is sure to attract intense study in the future. The unfortunate cartilage toxicity of eprostilome will also require further investigation to determine whether it is due to drug-specific toxicity or whether it represents a class effect for synthetic TR agonists.

These examples clearly demonstrate the importance and diversity of thyroid hormone action in cartilage and bone. It is a field that is certain to expand and provide new and exciting discoveries in the years ahead.

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