# THEMATIC REVIEW

## Deiodinases: the balance of thyroid hormone

# Local control of thyroid hormone action: role of type 2 deiodinase

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## Abstract

The thyroid gland predominantly secretes the pro-hormone thyroxine ($T_4$) that is converted to the active hormone 3,5,3′-L-triiodothyronine ($T_3$) in target cells. Conversion of $T_4$ to $T_3$ is catalyzed by the type 2 iodothyronine deiodinase enzyme (DIO2), and $T_3$ action in target tissues is determined by DIO2-regulated local availability of $T_3$ to its nuclear receptors, TR$\alpha$ and TR$\beta$. Studies of *Dio2* knockout mice have revealed new and important roles for the enzyme during development and in adulthood in diverse tissues including the cochlea, skeleton, brown fat, pituitary, and hypothalamus. In this review, we discuss the molecular mechanisms by which DIO2 controls intracellular $T_3$ availability and action.

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## Introduction

Thyroid hormones are important homeostatic regulators that act via nuclear thyroid hormone receptors (TRs) in virtually all tissues during development and throughout postnatal life. 3,5,3′,5′-L-tetraiodothyronine (thyroxine, $T_4$) is a pro-hormone that circulates at a high concentration in peripheral blood relative to the active hormone 3,5,3′-L-triiodothyronine ($T_3$). Concentrations of $T_4$ and $T_3$ in target tissues are controlled by metabolism; local conversion of $T_4$ to $T_3$ is catalyzed by the type 2 iodothyronine deiodinase enzyme (DIO2), while the type 3 enzyme (DIO3) prevents activation of $T_4$ and inactivates $T_3$. This pre-receptor control of ligand availability to TRs in target cells is a crucial mechanism that regulates the timing of cellular responses to thyroid hormones in a tissue-specific manner. The physiological importance of this coordinated process has been demonstrated in several organ systems by a series of elegant *in vivo* studies, and in this study, we review recent developments with particular emphasis on the importance of hormone activation mediated by DIO2.

## Thyroid physiology

### The hypothalamic–pituitary–thyroid axis

Circulating thyroid hormone concentrations are maintained in the euthyroid range by a classical negative feedback loop (Fig. 1). Thyrotropin-releasing hormone (TRH) is synthesized in the hypothalamic para-ventricular nucleus (PVN) and stimulates synthesis and release of TSH from thyrotroph cells in the anterior pituitary gland. TSH in turn acts via the TSH receptor (TSHR) in thyroid follicular cells to stimulate cellular growth and the synthesis and release of $T_4$ and $T_3$ into the circulation (Kopp 2001). Thyroid hormone action is mediated in target tissues by TRs, but thyroid hormones also inhibit TRH and TSH synthesis and secretion in the hypothalamus and pituitary to complete a negative feedback loop (Forrest *et al*. 1996*b*, Nikrodhanond *et al*. 2006). This negative feedback loop maintains circulating thyroid hormones and TSH in a physiological inverse relationship that defines the hypothalamic–pituitary–thyroid (HPT) axis set-point (Bassett & Williams 2008).

### Circulating thyroid hormones and uptake into target cells

The thyroid gland predominantly secretes the inactive pro-hormone $T_4$, as well as small amounts of the physiologically

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active thyroid hormone $T_3$. Both $T_4$ and $T_3$ are lipophilic and poorly soluble in water, and over 95% of thyroid hormones are protein bound in the circulation. Thyroxine-binding globulin (TBG), transthyretin, albumin, and several lipoproteins function as the transport proteins for $T_4$ and $T_3$ in plasma. Free thyroid hormone levels are thus dependent upon the concentrations and saturations of these proteins. The unbound free $T_3$ fraction represents 0·3% of the total $T_3$ concentration in plasma, but because of its higher binding affinity for TBG, the free $T_4$ fraction represents just 0·02% of the total plasma $T_4$ concentration. Thus, despite the total $T_4$ concentration being 50-fold greater than total $T_3$, the circulating free $T_4$ concentration is only fourfold higher. $T_4$ is exclusively synthesized and secreted by thyroid follicular cells, whereas the majority of circulating $T_3$ is generated in peripheral tissues by enzymatic removal of a 5′-iodine from $T_4$. Due to their lipophilic nature, thyroid hormones had been likely to enter target cells by a passive process of diffusion (Friesema *et al.* 2005). In fact, the free hormones enter target cells via an energy-dependent, ATP-requiring, stereospecific, and saturable transport mechanism that is mediated by the monocarboxylate transporter 8 (MCT8; Friesema *et al.* 2003, 2006, Dumitrescu *et al.* 2006), MCT10, and other transporter proteins including OATP1c1, a member of the $Na^+$-independent organic anion transporter protein (OATP) family (Jansen *et al.* 2005, Heuer 2007, van der Deure *et al.* 2010; Fig. 2). Transport via MCT8, for example, increases uptake of $T_4$ and $T_3$ by tenfold (Friesema *et al.* 2003).

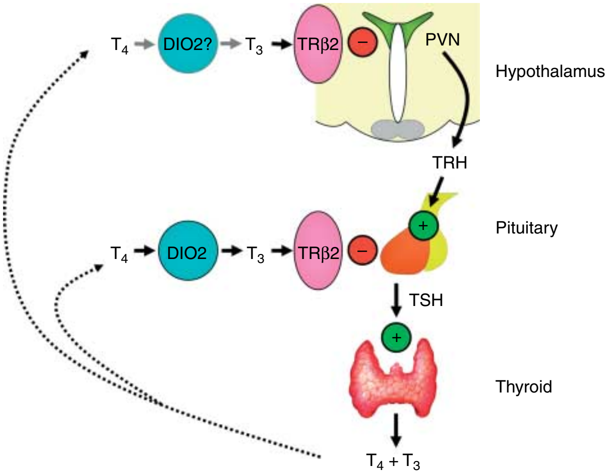

**Figure 1** Negative feedback regulation of the hypothalamic–pituitary–thyroid axis. The role of DIO2 in negative feedback control of the HPT axis occurs predominantly in thyrotrophs of the anterior pituitary gland. PVN, para-ventricular nucleus; TRH, thyrotropin-releasing hormone; DIO2, type 2 deiodinase enzyme; TRβ2, thyroid hormone receptor β2; $T_4$, thyroxine; $T_3$, 3,5,3′-L-triiodothyronine.

## Thyroid hormone metabolism

### *Deiodinase enzymes*

The iodothyronine deiodinases are selenocysteine-containing enzymes that metabolize thyroid hormones to active or inactive products (Bianco *et al.* 2002). The type 1 deiodinase enzyme (DIO1) is rather inefficient with an apparent Michaelis constant ($K_m$) of $10^{-6}$–$10^{-7}$ M and catalyzes removal of inner or outer ring iodine atoms in equimolar proportions to generate $T_3$, reverse $T_3$ ($rT_3$), or 3,3′-diiodothyronine ($T_2$) depending on the substrate. Most of the circulating $T_3$ is derived from conversion of $T_4$ to $T_3$ by the actions of DIO1, which is localized to the plasma membrane and expressed in liver and kidney. Nevertheless, activity of the DIO2 enzyme in skeletal muscle may also contribute to circulating levels of $T_3$, although its role is controversial and may differ between species (Bianco *et al.* 2002, Maia *et al.* 2005, Bianco & Kim 2006, Heemstra *et al.* 2009*b*, Larsen 2009). The DIO2 enzyme is considerably more efficient than DIO1, catalyzing only the removal of an outer ring iodine atom from the pro-hormone $T_4$ with a $K_m$ of $10^{-9}$ M to generate the physiologically active product $T_3$. The major role of DIO2 is to control the intracellular $T_3$ concentration, its availability to the nucleus, and the saturation of the nuclear $T_3$ receptor in target tissues. Moreover, DIO2 is likely to protect tissues from the detrimental effects of hypothyroidism because its low $K_m$ continues to permit the efficient local conversion of $T_4$ to $T_3$. $T_4$ treatment of cells, in which MCT8 and DIO2 are co-expressed, results in increased $T_3$ target gene expression (Friesema *et al.* 2006), indicating that thyroid hormone uptake and metabolism coordinately regulate $T_3$ action. By contrast, the DIO3 enzyme irreversibly inactivates $T_3$, or prevents $T_4$ being activated, by catalyzing removal of an inner ring iodine atom with a $K_m$ of $10^{-9}$ M to generate $T_2$ or $rT_3$ respectively. Thus, inactivating DIO3 prevents thyroid hormone access to specific tissues at critical times and reduces TR saturation (Bianco *et al.* 2002, Bianco & Kim 2006).

### *Control of intracellular $T_3$ availability*

The relative activities of DIO2 and DIO3, which have the same $K_m$ values for substrate, consequently regulate intracellular concentrations of $T_3$ and its availability to the nuclear TR (Bianco *et al.* 2002, Bianco & Kim 2006, St Germain *et al.* 2009). In conjunction with serum-derived $T_3$, DIO2 and DIO3 are important local modulators of thyroid hormone responsiveness *in vivo*. Expression of both enzymes is regulated in a temporo-spatial and tissue-specific manner, resulting in varying levels of $T_3$ action in individual tissues at distinct times during development (Bates *et al.* 1999, St Germain *et al.* 2009). Acting together, DIO2 and DIO3 thus control cellular $T_3$ availability by a mechanism that is largely independent of serum thyroid hormone concentrations (Bianco & Kim 2006).

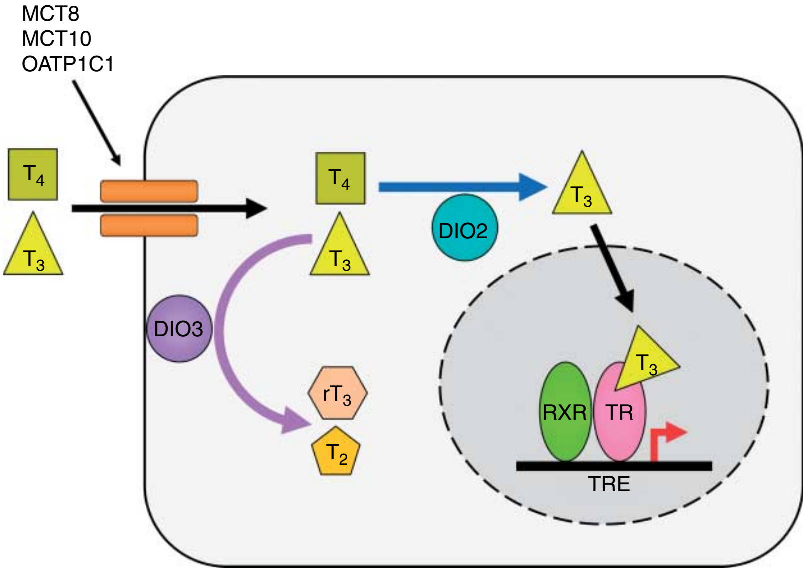

**Figure 2** Regulation of intracellular supplies of $T_3$ to the nucleus of $T_3$ target cells. MCT8 and MCT10, monocarboxylate transporters 8 and 10; OATP1C1, organic acid transporter protein-1C1; DIO2 and DIO3, type 2 and 3 deiodinase enzymes; TR, thyroid hormone receptor, RXR, retinoid X receptor; $T_4$, thyroxine; $T_3$, 3,5,3′-L-triiodothyronine; $rT_3$, 3,3′,5′-triiodothyronine; $T_2$, 3,3′-diiodothyronine.

## Thyroid hormone action

### *Thyroid hormone receptors*

Thyroid hormone actions in target cells are ultimately determined by the availability of $T_3$ to its nuclear receptor (St Germain *et al.* 2009; Fig. 2). TRα and TRβ are members of the steroid/TR superfamily (Sap *et al.* 1986, Weinberger *et al.* 1986). The TRs are ligand-inducible transcription factors that regulate expression of hormone-responsive target genes. In mammals, the *THRA* gene encodes three C-terminal variants of TRα. TRα1 is a functional receptor that binds both DNA and $T_3$, whereas TRα2 and TRα3 fail to bind $T_3$ and act as antagonists *in vitro* (Harvey & Williams 2002). A promoter within intron 7 of mouse *Thra* gives rise to two truncated variants, TRΔα1 and TRΔα2, which act as potent dominant-negative antagonists *in vitro*, although their physiological role is unclear (Chassande *et al.* 1997). The *THRB* gene encodes two N-terminal TRβ variants, TRβ1 and TRβ, both of which act as functional receptors. Two further transcripts, TRβ3 and TRΔβ3, have also been described in the rat, but their physiological role is also uncertain (Williams 2000, Harvey *et al.* 2007). TRα1 and TRβ1 are expressed widely, but their relative concentrations differ during development and in adulthood due to tissue-specific and temporo-spatial regulation (Forrest *et al.* 1990). The current understanding is that most $T_3$ target tissues are either predominantly TRα1 or TRβ1 responsive or lack TR isoform specificity. Expression of TRβ2, however, is markedly 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 (Fig. 1; Abel *et al.* 1999, 2001), while in the cochlea and retina TRβ2 is an important regulator of sensory development (Ng *et al.* 2001, Jones *et al.* 2007).

### *Developmental control of $T_3$ action*

Unliganded TRs compete with $T_3$-bound TRs for DNA response elements. They act as potent transcriptional repressors and have been shown to have critical regulatory roles in the development of a number of key tissues (Hashimoto *et al.* 2001, Chassande 2003, Venero *et al.* 2005, Wallis *et al.* 2008). Unoccupied TRs interact with co-repressor proteins, including nuclear receptor co-repressor (NCoR) and the silencing mediator for retinoid and TR (SMRT), which recruit histone deacetylases and maintain a non-permissive closed chromatin structure to inhibit gene transcription. Ligand-bound TRs, however, interact with steroid receptor co-activator 1 (SRC1) and other related co-activators in a hormone-dependent fashion. These co-activator proteins function as histone acetyl transferases, thereby promoting an open nucleosome structure leading to target gene activation. The contrasting chromatin-modifying effects of liganded and unliganded TRs, thus, greatly enhance the magnitude of the transcriptional response to $T_3$ (Harvey & Williams 2002, Chassande 2003). In addition to the positive stimulatory effects on target gene expression, $T_3$ also mediates transcriptional repression to inhibit the expression of certain key target genes, including TSH. Although such negative regulatory effects are physiologically critical, the responsible underlying molecular mechanisms have not been fully characterized (Cheng *et al.* 2010). Although expression of both TRα1 and TRβ1 is widespread, their relative levels of expression differ between tissues during embryogenesis and in postnatal life. Differential control of TRα1 and TRβ1, therefore, provides another mechanism to regulate tissue-specific $T_3$ responses during development and growth (O'Shea *et al.* 2006). Although the free $T_4$ concentration is approximately fourfold greater than free $T_3$, the TR-binding affinity for $T_3$ is 15-fold higher than its affinity for $T_4$ (Lin *et al.* 1990). Thus, $T_4$ acts as a pro-hormone, which must be metabolized to $T_3$ for the mediation of thyroid hormone actions (Bianco & Kim 2006). Taken together, the temporo-spatial and tissue-specific regulated expression of both the DIO2 and the DIO3 enzymes (Bates *et al.* 1999) and the TRα1 and TRβ1 nuclear receptors (Forrest *et al.* 1990) combine to provide a complex but co-ordinated system for fine control of $T_3$ availability and action in individual cell types during development. DIO3 is expressed in fetal tissues and the utero-placental unit where it acts as a barrier that prevents maternal thyroid hormone access to the developing fetus (Wasco *et al.* 2003).

Unliganded TRs are key factors that prevent premature cell differentiation and maintain cell proliferation in order to allow organogenesis to proceed in the developing fetus (Plateroti *et al.* 2001, Flamant *et al.* 2002, Chassande 2003). In mammals, the reciprocally regulated decrease in DIO3 activity and increase in DIO2 activity at birth results in a rapid rise in $T_3$ production (Bates *et al.* 1999). Similar changes have been observed during metamorphosis and hatching in amphibians and birds respectively (Huang *et al.* 2001). An increase in DIO2 expression in target tissues together with a decrease in DIO3 expression results in an increased intracellular $T_3$ concentration, binding of $T_3$ to its nuclear receptor, and initiation of cell differentiation (Campos-Barros *et al.* 2000, Sachs *et al.* 2000, Huang *et al.* 2001, Plateroti *et al.* 2001, Flamant *et al.* 2002, Mai *et al.* 2004, Ng *et al.* 2004). Thus, TRs function as developmental switches that are dependent on the activities of the deiodinases and which regulate the onset of $T_3$ target tissue differentiation during embryogenesis. For example, in the developing embryo, DIO2 has been shown to regulate the pace of endochondral ossification and bone formation (Dentice *et al.* 2005), while activity of DIO2 in developing cartilage is regulated by the morphogen Indian hedgehog and the ubiquitin ligase WSB-1 (Dentice *et al.* 2005). In addition, ubiquitin-mediated degradation of DIO2 has been shown to regulate thyroid hormone activation in several other tissues (Bianco & Larsen 2005, Fekete *et al.* 2007, Sagar *et al.* 2007). In this situation, ubiquitin-mediated proteasomal degradation of DIO2 is increased following exposure to substrate ($T_4$), and this mechanism thus represents a rapid and sensitive posttranslation mechanism to control and limit the DIO2 activity and $T_3$ production (Gereben *et al.* 2000, Steinsapir *et al.* 2000, Zavacki *et al.* 2009).

These considerations are important physiologically and in thyroid disease. In thyroid hormone deficiency, DIO2 expression and activity are increased, whereas its expression and activity are reduced in thyrotoxicosis. By contrast, DIO3 expression is regulated in a reciprocal manner at extremes of thyroid dysfunction. Thus, the ratio of DIO2 and DIO3 activity determines homeostatic control of $T_3$ availability to the nuclear receptor even in thyroid disease. In the brain, the DIO2 activity is increased in response to hypothyroidism (Burmeister *et al.* 1997), whereas activity of DIO3 is markedly reduced (Friedrichsen *et al.* 2003). This response is considered to protect the developing brain from changes in circulating thyroid hormones and to mitigate the severe and detrimental effects of hypothyroidism (Calvo *et al.* 1990, Guadano-Ferraz *et al.* 1999, Heuer 2007). Thus, maintenance of thyroid hormone availability in specific brain regions is critically regulated by reciprocal expression of DIO2 and DIO3 (Tu *et al.* 1997, 1999, Bianco *et al.* 2002, Kester *et al.* 2004).

## *Dio2* knockout mice

In order to investigate the function of DIO2 *in vivo*, knockout mice deficient in the enzyme were generated and found to have normal fertility (Schneider *et al.* 2001). *Dio2* knockout (D2KO) mice exhibit isolated hypothyroidism in critical tissues that depend on DIO2-catalyzed $T_4$ to $T_3$ conversion to regulate cellular thyroid status. Thus, D2KO mice have an elevated circulating TSH concentration, are unable to sustain a normal body temperature following cold exposure despite a normal circulating $T_3$ concentration, are deaf, and have brittle bones (de Jesus *et al.* 2001, Schneider *et al.* 2001, Ng *et al.* 2004, Bassett *et al.* 2010), suggesting important physiological roles for DIO2 in the pituitary gland and brain, in brown adipose tissue, in the cochlea, and in the skeleton.

### *Role of DIO2 in regulation of the HPT axis*

Analysis of the HPT axis in D2KO mice demonstrated a two- to threefold increase in the circulating TSH concentration, a 27–40% increase in the $T_4$ level accompanied by reduced clearance of $T_4$ from plasma, but a normal $T_3$ (Schneider *et al.* 2001, Christoffolete *et al.* 2007, Galton *et al.* 2007). Serum TSH was suppressed following treatment with $T_3$ but not in response to $T_4$, indicating that D2KO mice have pituitary resistance to feedback regulation by $T_4$ (Schneider *et al.* 2001). Surprisingly, *TRH* mRNA levels in the PVN were not increased in D2KO mice despite their elevated TSH level, suggesting that resistance to $T_4$ suppression results mainly from *Dio2* deficiency in the pituitary rather than the hypothalamus (Rosene *et al.* 2010). Thus, DIO2 is essential for regulation of the HPT axis and enables the pituitary to respond to changes in the circulating $T_4$ level (Fig. 1). In addition, it is important to note that the inactivating DIO3 enzyme is also a key regulator of the HPT axis. Analysis of DIO3-deficient (D3KO) mice indicates that DIO3 plays a key role in the development of the HPT axis set-point. Neonatal D3KO mutants have elevated circulating thyroid hormone concentrations due to impaired $T_3$ clearance, whereas central hypothyroidism is evident after 2 weeks of age and results from defective TRH and TSH responsiveness in the pituitary and thyroid (Hernandez *et al.* 2006, 2007). New-born D3KO mice display tissue thyrotoxicosis in the brain despite low circulating $T_4$ levels. However, they also have increased DIO2 activity as a result of the low circulating $T_4$ levels, and this is reflected by an increase in the cellular $T_3$ concentration (Hernandez *et al.* 2006). These observations demonstrate a vital role for DIO3 in the determination of thyroid status in peripheral target tissues and indicate the close inverse relationship between DIO2 and DIO3 during maturation of the HPT axis.

### *Role of DIO2 in the brain*

In the brain, DIO2 is expressed in glial cells, third ventricle tanycytes, astrocytes, and some sensory neurons including nuclei within the trigeminal and auditory pathways (Guadano-Ferraz *et al.* 1997, 1999, Tu *et al.* 1997). As discussed earlier, studies in D2KO mice indicated that DIO2 is required for local $T_3$ generation in the pituitary and essential for normal control of the HPT axis (Schneider *et al.* 2001). In addition, neonatal D2KO mice have a 25–50% reduction in tissue $T_3$ concentrations throughout the brain, which is similar to that seen in hypothyroid wild-type littermate mice. The reduced tissue $T_3$ concentration in neonatal D2KO mice does not result from increased $T_3$ degradation, as activity of the inactivating DIO3 enzyme is not altered in any brain regions (Galton *et al.* 2007). Thus, deficiency of DIO2 results in reduced local $T_3$ generation throughout the developing brain. Nevertheless, expressions of the $T_3$-responsive genes *Hairless*, *TrkB*, *Rc3*, and *Srg1* are less susceptible to change in D2KO mice compared with the altered expression observed in thyroid-deficient mice (Galton *et al.* 2007). Thus, despite the markedly increased expression of DIO2 in the newborn brain (Bates *et al.* 1999), D2KO mice have a mild neurological phenotype in comparison with the severe consequences of systemic hypothyroidism. Although $T_3$ availability in neurons is dependent on the DIO2 activity in adjacent glial cells, these surprising data reveal that compensatory mechanisms can mitigate the neurological damage resulting from DIO2 deficiency and also show that alternative sources of $T_3$ can access the brain during development (Galton *et al.* 2007). These compensatory sources are not likely to involve increased transport mediated by MCT8 because the levels of MCT8 expression in the brain are similar in euthyroid, hypothyroid, and D2KO mice (Galton *et al.* 2007). Although D2 expression peaks at important times during development of the brain and is required to generate adequate intracellular concentrations of $T_3$ throughout the brain, it seems that the consequences of DIO2 deficiency during central nervous system development are mitigated by other sources of $T_3$ such as the cerebrospinal fluid (CSF) or serum (Galton *et al.* 2007).

The physiological efficiency of these compensatory sources of $T_3$ was demonstrated following assessment of neurobehavioral function, which revealed only minimal differences between D2KO and wild-type mice following testing of reflexes, locomotion and agility, learning and memory, olfaction, anxiety, and exploration (Galton *et al.* 2007). Interestingly, a recent study was performed in which $T_3$ target gene expression in cerebral cortex was compared between hypothyroid wild-type D2KO and MCT8KO mice (Morte *et al.* 2010). The aim was to investigate whether the source of tissue $T_3$ in brain via local $T_3$ generation (disrupted in D2KO mice) or via transport across the blood–brain barrier (disrupted in MCT8KO mice) elicited differing target gene responses. Little effect on $T_3$ target gene response was seen in MCT8KO mice because a compensatory increase in DIO2 expression was identified which was proposed to mitigate local $T_3$ deficiency. By contrast, in D2KO mice, there was increased expression of $T_3$ target genes normally inhibited by $T_3$, but no effect was seen on genes that are normally positively regulated by $T_3$. In hypothyroid wild-type mice, however, expression of both negatively and positively regulated $T_3$ target genes was affected (Morte *et al.* 2010). Taken together, these intriguing observations suggest that the source of $T_3$ in the brain (locally generated $T_3$ versus $T_3$ transported from serum and CSF) may influence the $T_3$ response elicited.

### *Role of DIO2 in brown adipose tissue and adaptive thermogenesis*

D2KO mice exposed to cold are unable to maintain their body temperature despite the presence of a normal circulating $T_3$ concentration. The mild hypothermia following cold exposure results from impaired energy expenditure in brown adipose tissue that is mitigated by a compensatory shivering response associated with acute weight loss (de Jesus *et al.* 2001). Isolated brown adipocytes from D2KO mice fail to respond normally to adenylyl cyclase activators or noradrenaline resulting in impaired cAMP, oxygen consumption, and mitochondrial uncoupling protein 1 mRNA responses to adrenergic stimulation. These defects are similar to observations in hypothyroidism but are not seen in brown adipocytes obtained from D2KO mice treated with $T_3$ (de Jesus *et al.* 2001). The findings indicate that the cAMP-dependent DIO2 enzyme is required for adrenergic responsiveness and adaptive thermogenesis in brown adipocytes. Further studies, however, revealed a large compensatory increase in brown fat sympathetic stimulation that bypasses the reduced adrenergic responsiveness of D2KO brown adipocytes. The increased sympathetic tone in brown fat induced a marked lipolytic response, which depletes fatty acid stores and results in the defective adaptive thermogenesis and hypothermia observed in D2KO mice (Christoffolete *et al.* 2004). Subsequent studies also showed that bile acids activate DIO2 in brown fat via a cAMP-dependent mechanism involving the G-protein-coupled receptor TGR5, thus identifying a new role for DIO2 in diet-induced thermogenesis as bile acids were also shown to protect mice from diet-induced obesity (Watanabe *et al.* 2006). Accordingly, D2KO mice have greater susceptibility to diet-induced obesity that may result in part from impaired brown adipose tissue development during embryogenesis (Hall *et al.* 2010) as well as impaired diet-induced thermogenesis.

### *Role of DIO2 in muscle*

Muscle is an important $T_3$ target tissue, and euthyroidism is required for its efficient function and regeneration. Recent studies in mice have demonstrated that *Dio2* mRNA and activity are expressed in skeletal muscle. Type I slow-twitch fibers displayed fivefold greater Dio2 activity than type II fast-twitch fibers, and hypothyroidism resulted in a threefold induction of activity without changes in mRNA levels (Marsili *et al.* 2010). MyoD is a master regulator of myogenic differentiation and muscle regeneration, and new studies have established that Dio2-mediated generation of $T_3$ is essential for efficient transcription of MyoD (Dentice *et al.* 2010). Furthermore, the Dio2 activity is present in muscle stem cells and increases during myogenic differentiation. Accordingly, in D2KO mice, myocytes exhibit a hypothyroid phenotype despite normal circulating $T_3$ levels, the expression of $T_3$-responsive genes including MyoD is markedly reduced, and muscle regeneration is delayed following injury. In primary myoblasts, a forkhead box transcription factor, FoxO3, has been shown to induce *Dio2* expression and mediate the surge in Dio2 activity necessary to increase the local intracellular $T_3$ concentration and thereby ensures normal muscle formation and regeneration (Dentice *et al.* 2010). Thus, *Dio2* is essential for skeletal muscle development, function, and repair.

### *Role of DIO2 in the cochlea*

In the cochlea, DIO2 is expressed in periosteal connective tissue surrounding the internal sensory tissues, with enzyme activity peaking at postnatal day P7, a few days prior to the onset of hearing around P14. TR expression, however, is localized to the cochlea sensory epithelium, suggesting that periosteal DIO2 provides a temporo-spatially regulated paracrine supply of $T_3$ to the sensory epithelium that is necessary for correct timing of cochlea development and maturation (Campos-Barros *et al.* 2000). This hypothesis was supported by findings in D2KO mice, which exhibit delayed differentiation of the auditory sensory epithelium and delayed cochlea development with abnormal formation of the tectorial membrane. The resulting deafness in D2KO mice is similar to that seen in systemic hypothyroidism or in TRβ knockout mice (Forrest *et al.* 1996*a*, Rusch *et al.* 1998, 2001) but occurs despite circulating levels of thyroid hormones that are normally permissive for development of hearing. Treatment of D2KO mice with $T_3$ ameliorated the phenotype, indicating that DIO2-dependent local generation of $T_3$ in the surrounding bony labyrinth is essential for development of the cochlea and subsequent auditory function (Ng *et al.* 2004). In this case, the activating DIO2 enzyme functions as a local paracrine amplifier of $T_3$ action to regulate sensory development. More recently, DIO3 was also found to be expressed in the cochlea, and D3KO mice were shown to be deaf and have advanced cochlear maturation (Ng *et al.* 2009), indicating that DIO3 normally protects the cochlea from premature $T_3$-induced differentiation. Thus, development of the cochlea and the onset of normal auditory function require tightly controlled and correctly timed availability of $T_3$ that is achieved by co-ordinated reciprocal alterations in the expression and activities of DIO2 and DIO3.

### *Role of DIO2 in the skeleton*

It is well known that hypothyroidism causes delayed bone formation and linear growth retardation. Possible roles for DIO1 and DIO2 in the skeleton were first studied in the context of growth. A minor and transient impairment of weight gain was initially reported in male D2KO mice, although linear growth was not determined (Schneider *et al.* 2001). Weight gain and growth, however, were normal in D1KO- and in DIO1-deficient C3H/HeJ mice and in combined C3H/HeJ D2KO mutants with DIO1 and DIO2 deficiency (Berry *et al.* 1993, Schoenmakers *et al.* 1993, Schneider *et al.* 2006, Christoffolete *et al.* 2007). We and others showed that DIO1 is not expressed in bone and cartilage (LeBron *et al.* 1989, Dreher *et al.* 1998, Gouveia *et al.* 2005, Williams *et al.* 2008), indicating that DIO1 does not directly influence $T_3$ action in bone.

Nevertheless, important roles for DIO2 during skeletogenesis and in adult bone are emerging. The DIO2 activity was demonstrated in the perichondrium surrounding the embryonic chick growth plate where its activity is regulated by the skeletal morphogen SHH (Dentice *et al.* 2005). SHH is secreted by perichondrial cells and acts in growth plate chondrocytes to stimulate ubiquitin-mediated degradation of DIO2. The resulting modulation of thyroid hormone signaling in the growth plate is accompanied by increased PTHrP signaling, which is also seen in hypothyroidism (Stevens *et al.* 2000) and which regulates the pace of chondrocyte differentiation during early skeletogenesis (Dentice *et al.* 2005). Analysis of developing bone from mice at embryonic days E14·5–E18·5 revealed the presence of DIO2 activity (Capelo *et al.* 2008), suggesting that a similar regulatory role for DIO2 in cartilage during early skeletogenesis may occur in the mouse as well as the developing chick (Dentice *et al.* 2005).

There have been conflicting reports regarding the expression and activity of DIO2 in whole bone tissue extracts and in skeletal cells (LeBron *et al.* 1989, Bohme *et al.* 1992, Ballock & Reddi 1994, Dreher *et al.* 1998, Wakita *et al.* 1998, Gouveia *et al.* 2005, Morimura *et al.* 2005, Capelo *et al.* 2008). Using a sensitive and highly specific HPLC-based assay, we demonstrated that specific DIO2 activity is restricted to differentiated osteoblasts but is undetectable in chondrocytes and osteoclasts (Williams *et al.* 2008). The significance of this finding was investigated in D2KO mice (Bassett *et al.* 2010).

There were no differences in linear growth and bone formation between D2KO and wild-type mice, although adult D2KO mice had brittle bones with impaired resistance to fracture. The phenotype was due to reduced osteoblastic bone formation without impairment of osteoclastic bone resorption, which caused a reduced rate of mineral apposition and prolongation of the formation phase of the bone remodeling cycle, thus facilitating an increase in secondary mineralization that resulted in a generalized increase in bone mineralization density (Bassett *et al.* 2010). The $T_3$ target gene analysis demonstrated cellular $T_3$ deficiency restricted to osteoblasts, indicating that maintenance of adult bone mineralization and optimal bone strength requires local DIO2-mediated production of $T_3$ in osteoblasts (Bassett *et al.* 2010).

These findings suggest that the restricted expression of DIO2 in adult bone is necessary to maintain a higher intracellular $T_3$ concentration in osteoblasts relative to other skeletal cells. As in other tissues, the DIO2 activity in osteoblasts is increased in hypothyroidism and inhibited in hyperthyroidism (Gouveia *et al.* 2005). Thus, DIO2 acts to buffer the effects of altered serum thyroid hormone levels on the skeleton; the adverse effects of $T_3$ deficiency on bone mineralization may be mitigated by increased DIO2 activity in osteoblasts, while inhibition of DIO2 activity in hyperthyroidism limits the detrimental effects of thyroid hormone excess (Bassett *et al.* 2010). This hypothesis suggests that optimal bone mineralization and strength are maintained over the physiological range of systemic thyroid hormone concentrations by the regulated activity of DIO2 in osteoblasts. Escape from this local feedback mechanism in osteoblasts may account in part for the increased susceptibility to fracture observed in hypothyroidism and thyrotoxicosis (Vestergaard & Mosekilde 2002, Vestergaard *et al.* 2005), suggesting the possibility of DIO2 as a therapeutic target for the treatment of osteoporosis.

A recent human population study has also suggested that DIO2 may influence susceptibility to osteoarthritis. A genome-wide linkage analysis identified an association between the *DIO2* polymorphism rs225014 and the generalized symptomatic osteoarthritis (Meulenbelt *et al.* 2008), although the association was not replicated in a subsequent association study and meta-analysis (Kerkhof *et al.* 2010). Nevertheless, a recent meta-analysis has also identified a possible role for DIO3 in osteoarthritis susceptibility (Meulenbelt *et al.* 2010). Taken together, these new and preliminary findings suggest that deiodinase-regulated $T_3$ availability in chondrocytes may play an important role in the regulation of cartilage renewal and repair.

## Conserved and pivotal role of DIO2 in the control of seasonal reproduction

A series of recent elegant studies, initially in the Japanese quail (*Coturnix japonica*; Yoshimura *et al.* 2003) but also in mammals, have identified a major role for DIO2 in the seasonal control of reproduction. Seasonal time measurement is achieved by sensing of the changing photoperiod in temperate zones. Regulatory sensing of the changing photoperiod and the subsequent gonadal response are localized to the medio-basal hypothalamus (MBH). In subtraction hybridization studies in the Japanese quail, DIO2 expression was found to be induced by light and the MBH tissue $T_3$ concentration was increased tenfold following long-day exposure compared with short-day exposure (Yoshimura *et al.* 2003). Furthermore, i.c.v. infusion of $T_3$, like exposure to long-day conditions, stimulated gonadal growth while infusion of long-day-exposed quails with iopanoic acid (a DIO2 inhibitor) prevented testicular growth. These findings demonstrated that DIO2-mediated local conversion of $T_4$ to $T_3$ in the MBH in response to light is a key pathway mediating the photoperiodic seasonal reproduction response (Yoshimura *et al.* 2003). Further studies revealed that the photoperiod response is triggered by light-induced expression of TSH in the pars tuberalis, which subsequently stimulates DIO2 expression in ependymal cells of the MBH via a TSHR-mediated pathway coupled to cAMP that results in light-induced LH secretion (Nakao *et al.* 2008). Additional studies have also revealed that reciprocal changes in DIO2 and DIO3 expression are induced in the MBH in response to changes in the photoperiod (Yasuo *et al.* 2005), and thus coordinated regulation of DIO2 and DIO3 expression has the capacity to mediate sensitive and rapid responses to changes in the photoperiod, thereby highlighting the importance of local control of tissue $T_3$ availability in the MBH for seasonal reproduction. Nevertheless, the precise downstream molecular consequences of increased $T_3$ production in the MBH still remain to be elucidated. A melatonin-responsive photoperiod response system in various mammals has also been shown to involve TSH and DIO2 (Watanabe *et al.* 2004, Revel *et al.* 2006, Yasuo *et al.* 2006, 2007, Hanon *et al.* 2008, Nakao *et al.* 2008, Ono *et al.* 2008), suggesting that seasonal reproduction in mammals and birds is regulated by similar conserved pathways that lie downstream of the initial light or melatonin photoperiod stimulus (Yoshimura 2010).

## Conclusions

In recent years, the importance of controlled intracellular availability of $T_3$ in target tissues has been appreciated. The vital roles played by DIO2 in development, during the establishment of the HPT axis and in specific tissues including the pituitary gland, brain, brown adipose tissue, cochlea, and bone, have been documented in considerable detail. Yet, much remains and exciting and important discoveries are inevitable. For example, recent studies are identifying new roles for DIO2 in the heart (Wang *et al.* 2010), in skeletal muscle (Grozovsky *et al.* 2009), during inflammation (Kwakkel *et al.* 2009), and in the pituitary in response to specific drug challenges (Rosene *et al.* 2010). Given the breadth of expression of DIO2 and its response to cellular stress (Gereben *et al.* 2008), it is likely that the functional repertoire for DIO2 will expand. The immediate challenges will be to identify these new roles and to determine whether functions ascribed to DIO2 from animal studies and genetic manipulation have physiological or pathological importance in man. Of importance in this context, a common Thr92Ala polymorphism has been identified in *DIO2* (Peeters *et al.* 2003). Although *in vitro* biochemical studies indicated no difference in the enzymatic properties of the DIO2 Thr and Ala variants (Peeters *et al.* 2003, Canani *et al.* 2005), thyroid and skeletal muscle tissue extracts from Ala/Ala individuals displayed reduced DIO2 activities (Canani *et al.* 2005). The mechanism responsible for reduced tissue activity is not known but may result from linkage disequilibrium between the Thr92Ala polymorphism and a second functional variant elsewhere (Canani *et al.* 2005). Nevertheless, in addition to osteoarthritis (Meulenbelt *et al.* 2008, Kerkhof *et al.* 2010), the Thr92Ala polymorphism has also been associated with variation in the HPT axis (Peeters *et al.* 2005, Butler *et al.* 2010), altered bone turnover (Heemstra *et al.* 2010), variable and contradictory effects on cognitive parameters, and the response to thyroid hormone replacement (Appelhof *et al.* 2005, Torlontano *et al.* 2008, Heemstra *et al.* 2009*a*, Panicker *et al.* 2009), as well as having an inconsistent relationship to hypertension, insulin resistance, and the metabolic syndrome (Mentuccia *et al.* 2002, 2005, Canani *et al.* 2005, 2007, Grarup *et al.* 2007, Gumieniak & Williams 2007, Gumieniak *et al.* 2007, Peeters *et al.* 2007, van der Deure *et al.* 2009, Dora *et al.* 2010, Estivalet *et al.* 2010).

Ultimately, an important challenge will be to exploit DIO2 as a drug target to manipulate tissue thyroid status, perhaps in the treatment of metabolic disorders including obesity or skeletal disorders such as osteoporosis and osteoarthritis.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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