Stem Cell Research (2014) 13, 582–591

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REVIEW

# Thyroid hormone action in postnatal heart development

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Received 18 June 2014; received in revised form 30 June 2014; accepted 1 July 2014
Available online 10 July 2014

**Abstract** Thyroid hormone is a critical regulator of cardiac growth and development, both in fetal life and postnatally. Here we review the role of thyroid hormone in postnatal cardiac development, given recent insights into its role in stimulating a burst of cardiomyocyte proliferation in the murine heart in preadolescence; a response required to meet the massive increase in circulatory demand predicated by an almost quadrupling of body weight during a period of about 21 days from birth to adolescence. Importantly, thyroid hormone metabolism is altered by chronic diseases, such as heart failure and ischemic heart disease, as well as in very sick children requiring surgery for congenital heart diseases, which results in low T3 syndrome that impairs cardiovascular function and is associated with a poor prognosis. Therapy with T3 or thyroid hormone analogs has been shown to improve cardiac contractility; however, the mechanism is as yet unknown. Given the postnatal cardiomyocyte mitogenic potential of T3, its ability to enhance cardiac function by promoting cardiomyocyte proliferation warrants further consideration.



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http://dx.doi.org/10.1016/j.scr.2014.07.001

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## Introduction

Thyroid hormone (TH) is a critical regulator of many physiological and developmental processes, following its activation from the stable prohormone (L-thyroxine, T4) to the short-lived active hormone, triiodothyronine (3,5,3′-triiodo-L-thyronine, T3) (Fig. 1). T3 induces amphibian metamorphosis by stimulating the remodeling of specific tissues and organs (Tata, 1993). In mammals, T3 is essential for development, with congenital hypothyroidism resulting in growth retardation, deafness, impaired neurogenesis, and congenital heart malformations (Legrand, 1986; Olivieri et al., 2002). THs also have important effects on oxygen consumption and metabolism. The actions of THs are mediated by the products of two TH receptor (TR) genes, the nuclear proteins, TRα and TRβ, which show differential patterns of expression in development and in adult tissues (Mai et al., 2004). Stimulation of these receptors results in the direct transcriptional activation of a wide range of genes (genomic effects). More recently, non-genomic effects of TH initiated at the cell surface, in the cytoplasm or in mitochondria have also been identified (Davis and Davis, 2002; Davis et al., 2011). Adding further complexity, TH signaling is highly regulated by the expression of cell and tissue-specific TH transporters that concentrate THs in target cells, by the relative expression and distribution of TR isoforms, by interaction of TRs with corepressors and coactivators, by cross-talk with several other signaling pathways, and by the sequence and location of the TH response element. Furthermore, TH signaling is tightly regulated by the activation and catabolism of THs by three selenoenzyme iodothyronine deiodinases: D1, D2 and D3—considerations that have been comprehensively reviewed elsewhere (Zoeller et al., 2007; Kress et al., 2009; Brent, 2012).

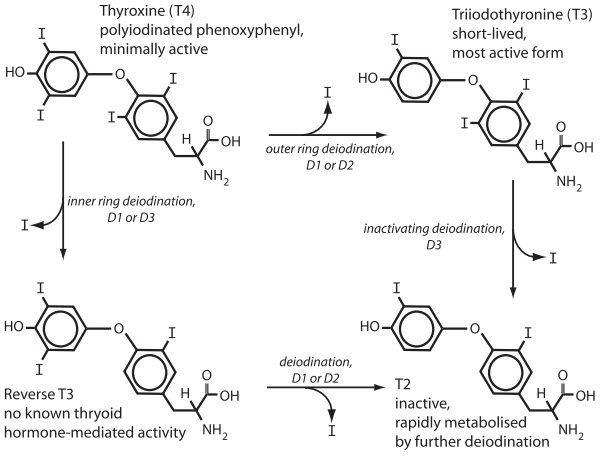


**Figure 1** Deiodinase-mediated metabolism of thyroid hormone. Deiodination of the outer, phenolic ring of T4 catalyzed by type 1 or type 2 iodothyronine deiodinases (D1 or D2, respectively) converts T4 to active T3. Both T4 and T3 are inactivated by deiodination of the inner tyrosyl ring by Type 3 iodothyronine deiodinase (D3) to yield reverse T3 or T2, respectively. D1 can also convert T4 to reverse T3, which is further metabolized to T2 by D1 or D2.

T3 has profound effects on the cardiovascular system with chronic hyperthyroidism in adulthood resulting in a physiological-type of cardiac hypertrophy, characterized by a predominant increase in cardiomyocyte (CM) length rather than width, and by enhanced expression of α-MHC, as well as a marked reduction in systemic vascular resistance accompanied by increased cardiac contractility, systolic hypertension, and increased cardiac output. In contrast, prolonged hypothyroidism results in diastolic hypertension, reduced cardiac output and stroke volume, as well as cardiac dilatation and even overt heart failure. The effects of T3 on the heart are due to the transcriptional regulation of a number of contractile and calcium handling genes (Maillet et al., 2013). These effects have been most widely studied with respect to their role in postnatal heart development, and under the premise that CMs exit the cell cycle and become terminally differentiated soon after birth.

Here, after a brief consideration of TH biology, we revisit the role of TH in postnatal heart development, given recent evidence indicating that the proliferative competence of CMs may be retained until well after the neonatal period, allowing murine CMs to undergo a proliferative burst during preadolescence in response to a T3 surge (Naqvi et al., 2014); that T3 can induce DNA synthesis in terminally differentiated adult CMs (Ledda-Columbano et al., 2006), and that remodeling post-myocardial infarction in mice is associated with local hypothyroidism of spared myocardium due to re-expression of D3, a thyroid-inactivating enzyme normally expressed only in the fetus (Janssen et al., 2013).

## TH biology

### TH production and ontogeny of the hypothalamic–pituitary–thyroid axis

TH is synthesized and secreted by the thyroid gland, a follicular organ that synthesizes TH by iodination of tyrosine residues in the glycoprotein, thyroglobulin. Thyroid stimulating hormone (TSH) secreted by the anterior pituitary in response to thyrotropin releasing hormone (TRH) elaborated by neurons of the hypothalamic paraventricular nucleus activates TSH receptors expressed on the basolateral membrane of thyroid follicular cells. This results in adenylyl cyclase activation and enhanced iodide uptake into the thyroid gland via the sodium/iodide symporter, which in turn is used in the biosynthesis of THs. Both T4 and T3 are synthesized and released from the thyroid gland, are carried in the circulation bound to specific proteins (thyroxine-binding globulin, transthyretin and albumin), and exert a negative feedback on both the release of pituitary TSH as well as on the activity of TRH on hypothalamic neurons.

In the fetus, the hypothalamic–pituitary–thyroid system develops in the absence of maternal influences, since neither the natural iodothyronines, T3, T4 and its inactive metabolite, reverse T3 (rT3), nor TSH, can cross the mammalian placenta. The fetal thyroid gland starts secreting THs from midgestation in humans, and from 3 to 5 days before birth (embryonic days 17.5–18) in rats (Auso et al., 2004; Ahmed et al., 2008; Morreale de Escobar et al., 2004), with maturation of the hypothalamic–pituitary axis being complete by birth in humans but delayed until approximately postnatal day 12–15 (P12–15) in rats and mice (Fisher and Klein, 1981; Howdeshell, 2002). T3, T4 and TSH levels increase markedly within a few hours of birth in humans to levels 2 to 8-fold above those in cord blood (Fisher and Klein, 1981) (Fig. 2). These changes are driven, at least in part, by postnatal cooling of the neonate (Fisher and Odell, 1969), with the increase in T3 likely being compensatory to enhance adaptive thermogenesis (Ribeiro et al., 2001). T3 and T4 levels also increase after birth in mice and rats (Fig. 2), but more gradually with an initial peak at P5–6 and then a more marked rise by P12–15 (Hadj-Sahraoui et al., 2000) to levels 2.5- to 15-fold those observed at the end of gestation (Howdeshell, 2002); the latter rise coincident with maturation of the hypothalamo-hypophyseal portal system and, thus, of the hypothalamic–pituitary–thyroid axis. Presumably, the delayed maturation of this axis in rodents prevents a more rapid perinatal increase of T3, which in humans occurs in response to postnatal cooling. Survival of rodents despite the lack of a rapid increase in T3 levels and, thus, thermogenesis, may perhaps be due to their ability to rapidly activate autophagy, a response that allows the neonatal animal to survive the profound starvation that occurs during the period between birth and the onset of lactation (Kuma et al., 2004). In this regard, it is of interest that fasting suppresses the activity of TRH neurons (Zoeller et al., 2007).

Although target organ responses at the cellular level are initiated by the binding of T3 to the TR, given the complexities of TH signaling, target organ responses cannot be predicted from circulating levels alone, but ultimately depend on the level of TR occupancy. At normal adult serum T3 levels, the contribution from serum T3 alone is approximately 50% occupancy in most tissues. Thus, despite the marked postnatal increases in T3 in the mouse, the initial increase to the level seen at P5–6 would result in only a small fraction of TRs being occupied, and even after the later T3 increase at P12–15, there would at most be 50% TR occupancy. TH action and concentration within cells are relatively independent of circulating hormone levels because TH entry into cells occurs not only by diffusion, but also via active and specific transcellular transport involving members of the monocarboxylate transporter family (MCT8 and MCT10), organic anionic transport proteins (OATPs) and L-type amino acid transporters (Zoeller et al., 2007; Gereben et al., 2008), although only MCT8 and MCT10 appear to be expressed in the heart (Danzi and Klein, 2014). In addition, THs are subject to cellular metabolism by two 5′-deiodinases (D1 and D2) that convert the prohormone T4 to T3 (Gereben et al., 2008) (Fig. 1). Of these, D2 is critical as it resides in the endoplasmic reticular (ER) membrane thereby allowing ready access of cytosolically generated T3 to the nucleus because of the proximity of the ER to the nuclear compartment, and the long residence time (hours) of T3

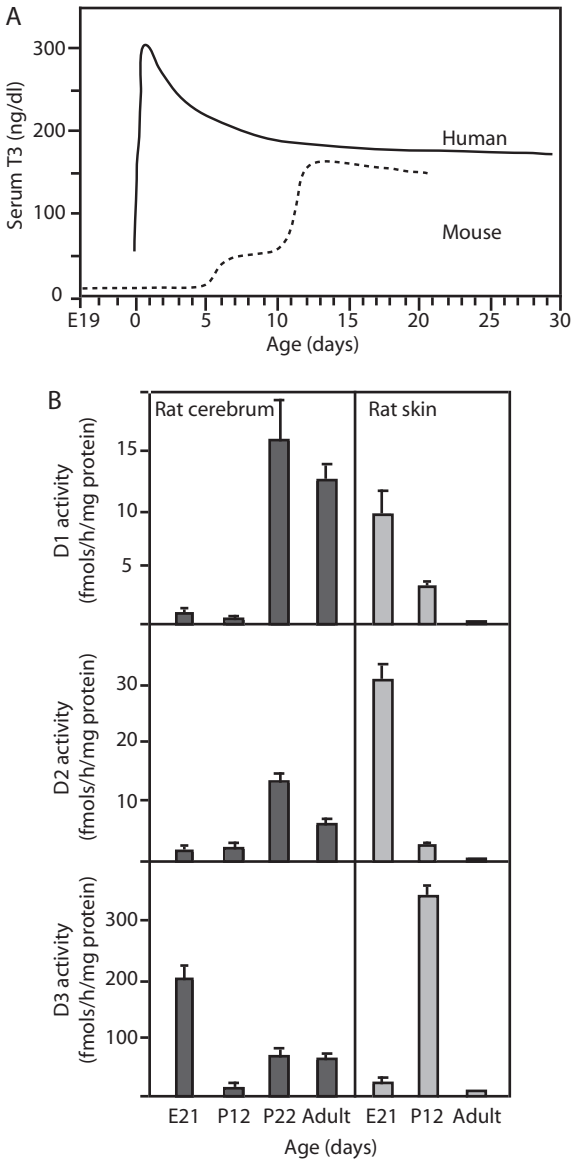


**Figure 2** Ontogeny of serum T3 and iodothyronine deiodinase levels. A, Human and mouse serum T3 levels. (Data reproduced from (Fisher and Klein, 1981; Hernandez et al., 2006; Hadj-Sahraoui et al., 2000)) B, D1, D2 and D3 activities in cerebrum and skin of female rats (Data +/− SE reproduced from (Bates et al., 1999)).

thus generated. In contrast, T3 generated by D1, a plasma membrane-bound enzyme, rapidly (minutes) equilibrates with plasma T3 and contributes little to local intracellular T3 production. A third deiodinase (D3) converts T4 and T3 to the inactive iodothyronines, rT3 and T2, respectively. Expression of these enzymes varies in different tissues and throughout life. There is evidence that rodent CMs metabolize little or no T4 or T3 (Everts et al., 1996; Gereben et al., 2008), consistent with low D1, D2 and D3 activities in the healthy adult heart (Gereben et al., 2008). There are no studies of deiodinase expression during cardiac development, however, in general, D3 expression is high during fetal

life, whereas expression of D1 and D2 increases after birth (Fig. 2). Teleologically, D2 and D3 are thought to provide a homeostatic mechanism for the reciprocal regulation of thyroid activity in response to variations in environmental iodine availability, as well as for controlling cellular TH activity in a manner that is relatively independent of circulating hormone levels (Gereben et al., 2008).

## TH receptors and signaling

The cellular actions of TH may be genomic (initiated within the cell nucleus), non-genomic (at the plasma membrane, including integrin $\alpha v\beta 3$-mediated signaling or effects on membrane ion channels/pumps, in the cytoplasm or at the level of the mitochondria) or overlapping (Davis and Davis, 2002; Davis et al., 2011). Postnatal cardiac development is a highly regulated and programmed process. The role of TH in this process is mainly via nuclear T3-activated TR-mediated transcriptional regulation (Fig. 3). Classically, TH action includes the transport of T3 into the target cell, including CMs, by TH transporters and then the binding of T3 to nuclear TRs that in turn hetero-dimerize with the retinoic acid receptor (RXR). The TR–RXR complex thus formed then binds with high affinity to T3 response elements (TREs; 10–15 bp located near transcriptional start sites of T3-responsive genes) and recruits coactivators, which increase histone acetylation. This leads to an opening of chromatin structure that is permissive for target gene transcription (Lazar, 1993; Brent, 1994; Harvey and Williams, 2002). TH receptors found in the cytosol can also bind T3 and activate the regulatory subunit (p85) of PI3 kinase (PI3K), which in CMs has been shown to phosphorylate Akt and mammalian target of rapamycin (mTOR) to increase protein synthesis (Kenessey and Ojamaa, 2006). Although non-genomic actions of T3 on contractility and membrane ion channels and pumps have been documented, TH-mediated integrin signaling has not been investigated in CMs (Davis and Davis, 2002; Davis et al., 2011).

The effects of TH are mediated by the TRs, and both TR$\alpha$ and TR$\beta$ are nuclear proteins. This is in contrast to steroid hormone receptors, which directly regulate transcription, but prior to activation are localized in the cytoplasm (Brent, 2012; Plateroti et al., 2001) (Fig. 3). Thus, TH must not only gain access to the cell, but also to the nucleus, which, as described above, requires coordination between circulating TH levels, hormone uptake and conversion of the prohormone T4 to T3 (Zoeller et al., 2007). Investigations in various TR knock-out mice show that deletion of TR$\alpha$1 results in hypothyroidism and significant cardiac effects including bradycardia, decreased contractile function and decreased contractile protein ($\alpha$-myosin heavy chain) and ion channel mRNA expression (HCN2 pacemaker channel and the transient outward $K^+$ channel, $I_{to}$) (Gloss et al., 2001; Johansson et al., 1998; Wikstrom et al., 1998). Deletion of TR$\beta$ results in hyperthyroidism and tachycardia; this is not associated with contractile dysfunction or changes in $I_{to}$ mRNA expression, but expression of HCN2 and $\beta$-MHC mRNA is increased (Gloss et al., 2001).

The TR$\alpha$ gene encodes a T3-binding splice variant, TR$\alpha$1, and two non-T3-binding splice variants (TR$\alpha$2 and TR$\alpha$3) (Cheng et al., 2010). The TR$\beta$ gene encodes three T3-binding splice variants, TR$\beta$1-3, of which only TR$\beta$1 and TR$\beta$2 are expressed

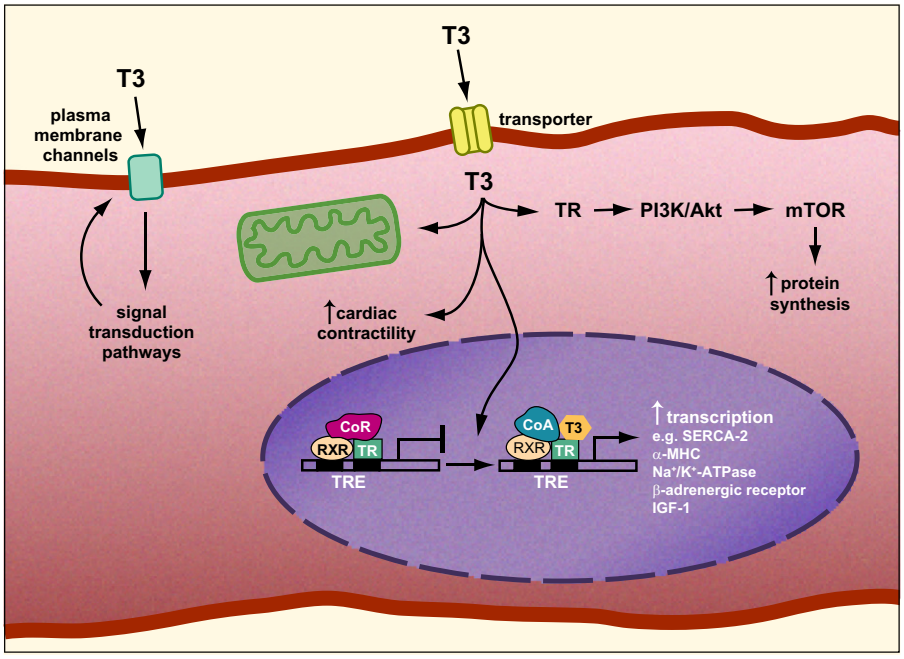


**Figure 3** Thyroid hormone receptors and signaling in CMs. T3, triiodothyronine, enters cells by diffusion or by specific transporters. Binding of T3 to thyroid hormone receptors in the nucleus results in heterodimerisation with the retinoic acid receptor and high-affinity binding of the receptor complex to T3 response elements to regulate transcription of T3-responsive genes (genomic effect). T3 can also directly modulate cell membrane ion channels, bind to cytoplasmic thyroid hormone receptors to increase protein synthesis or mediate contractility (non-genomic effects).

in the heart (Schwartz et al., 1994; Jones et al., 2003). Structurally, the DNA binding domains are similar for both TRα and TRβ but they differ most in their amino termini, with TRα having greater potency on TREs than TRβ (Hollenberg et al., 1995). Cardiac TRα1 and TRα2 mRNA expression decreases during postnatal development; in contrast to this change, cardiac TRβ1 and TRβ2 mRNA expression increases (White et al., 2001) such that TRα1 and TRβ1 each accounts for 40% of T3-binding capacity in the adult and TRβ2 for 20% (Schwartz et al., 1994). The impact of these changes is enhanced by the opposite roles of the liganded (holoreceptor) and unliganded (aporeceptor) states of the TRs. In the absence of T3, TH aporeceptors recruit co-repressors and repress the transcription of target genes (Fig. 3). When T3 levels increase after birth, hormone binding results in the holoreceptor exchanging co-repressors for co-activators. This activates transcription of the same target genes that were previously repressed by the aporeceptors (Hu and Lazar, 2000) (Fig. 3). Thus, in the mouse fetus, TRα aporeceptors repress heart rate and TRβ expression, as well as the expression of several genes encoding ion channels involved in cardiac contractile activity. Immediately after birth, the liganded TRα holoreceptor produces an increase in heart rate via increased expression of some of the previously repressed genes. Based on these considerations Mai et al. (2004) propose that TRα acts as a molecular switch to control heart function during early postnatal life.

## Postnatal cardiac development

In response to the rapid increase in body size, between birth and puberty, the murine heart increases in size almost 4-fold, with a commensurate increase in stroke volume (Naqvi et al., 2014). How are these profound changes in cardiovascular hemodynamics and morphology achieved?

### Cardiovascular hemodynamics

Postnatal developmental changes in cardiac contractility are complex. The immature perinatal heart lacks T-tubules, has a low density of diads and gap junctions, small CM size, poorly developed sarcoplasmic reticulum and reduced sensitivity to calcium ions (Vornanen, 1996). During the early postnatal period cardiac contractile force increases commensurate with increased expression and function of ion channels (Wetzel and Klitzner, 1996); a response that continues until adulthood, even after morphological development has already ceased (Capasso et al., 1982; Leblanc et al., 1998). In addition to enhanced ion channel expression and function, increases in contractile performance are due to a number of developmental changes. These include: 1) maturation of excitation–contraction coupling with increased expression of sarcolemmal and reticular $Ca^{2+}$ channels and transporters (Tanaka and Shigenobu, 1989; Wibo et al., 1991); 2) changes in the expression and ion-binding properties of contractile protein isoforms (McAuliffe et al., 1990; Martin et al., 1991); and 3) the development of autonomic innervation of the heart (Robinson, 1996). Early in the postnatal period, enhanced contractility is mainly due to changes in $Ca^{2+}$ transporter activity, contractile protein phosphorylation, or both (Kameyama et al., 1986). Long term, however, expression of these proteins also changes (Ogawa et al., 1992; Maki et al., 1996; Protas and Robinson, 1999).

TH contributes importantly to the maturational enhancement of contractile function via a reciprocal effect on gene transcription. Thus, TH increases the transcription of genes encoding many cardiac proteins that enhance inotropy, including the sarcoplasmic reticular $Ca^{2+}$ATPase-2 (SERCA-2), α-myosin heavy chain (α-MHC), the β1-adrenergic receptor, $Na^+/K^+$-ATPase, cardiac troponin I (CTNI), and voltage-gated potassium channels (v1.5, Kv4.2, Kv4.3) (Klein and Danzi, 2007; Maillet et al., 2013). However, TH also inhibits the expression of genes encoding proteins that in some cases suppress contractile function, such as β-MHC, phospholamban, as well as suppressing the expression of the $Na^+$–$Ca^{2+}$ exchanger (NCX), TRα1, and type V and VI adenylyl cyclases (ACs) (Arsanjani et al., 2011). Not surprisingly, therefore, T3 administration not only stimulates the expression of α-MHC, the high ATPase activity MHC isoform, but also decreases the expression of β-MHC (Morkin, 1993; Lompre et al., 1984; Izumo and Mahdavi, 1988). Conversely, hypothyroidism inhibits α-MHC expression and stimulates β-MHC expression; an effect mediated by miR-208, a cardiac-specific microRNA (van Rooij et al., 2007).

In addition to profound changes in cardiac contractility, postnatal development is associated with an increase in blood volume that is driven by the rapid body growth that occurs during this period, as well as by increases in blood pressure, stroke volume and cardiac output (D'Souza et al., 1995; Jurko, 2004). TH is an important regulator of these hemodynamic alterations (Danzi and Klein, 2012; Biondi et al., 2002). Supporting this are the findings that hyperthyroidism is associated with an increase in blood volume, venous volume return, cardiac output, contractility, heart rate and pulse pressure, and a decrease in systemic vascular resistance (Klein and Ojamaa, 2001). And conversely, hypothyroidism is associated with a decrease in cardiac output, a narrow pulse pressure, and an increase in systemic vascular resistance (Bengel et al., 2003; Danzi and Klein, 2003; Biondi et al., 2002; Kiss et al., 1994). The T3-induced decline in systemic vascular resistance stimulates renin release and sodium reabsorption, resulting in blood volume expansion and an increase in venous return (Resnick and Laragh, 1982). Erythropoietin stimulation also contributes to the rise in blood volume. Heart rate and cardiac output increase significantly (by up to 300%) in the hyperthyroid state versus euthyroid controls. The net effect of these hemodynamic changes is a rise in systolic blood pressure and a widening of pulse pressure (Prisant et al., 2006). The increase in cardiovascular hemodynamics allows for increased blood flow leading to enhanced perfusion to provide for the substrate and oxygen demands of peripheral tissues (Biondi et al., 2002).

### Cardiac morphology

Preload is the hemodynamic force exerted on the ventricular wall during filling and, thus, is directly responsible for ventricular end-diastolic wall stress or tension *sensu strictu*. It contributes greatly to the determination of ventricular end-diastolic volume and modulates myocardial performance significantly. That is, it governs the extent and velocity of wall

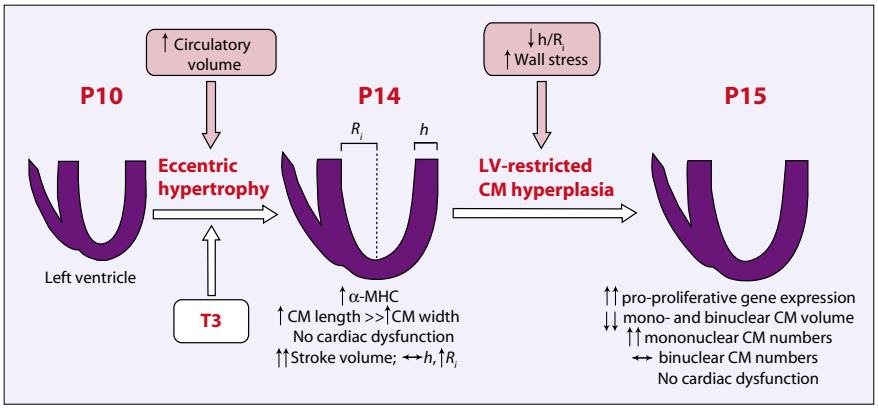


**Figure 4** A model for maturational heart growth during early preadolescence. Maturational heart growth during early preadolescence (P10 to P14) is characterized by eccentric hypertrophy where the increase in LV chamber dimension is not matched by a corresponding increase in LV wall thickness (*h*). This leads to a decrease in LV $h/R_i$ ratio ($R_i$ is the internal LV chamber radius), which is expected to increase LV wall stress due to considerations of the Law of Laplace. The change in heart shape is produced by a T3 surge soon after P10, which results in a prominent increase in CM length with minimal change in CM width, and an increase in $\alpha$-MHC by P14. This is followed by proliferation of mono- and bi−nuclear CMs between P14 and P15, which increased CM numbers by about 40%, thereby establishing the final CM population number during preadolescence.
Adapted from Naqvi et al. (2014).

shortening. Thus, preload plays a major role in regulating stroke volume via the Frank−Starling mechanism. Recently, we found in the mouse that a growth spurt that almost quadruples body weight (and hence circulatory volume) between P10 and P35 is associated with a commensurate 3.5-fold increase in stroke volume (Naqvi et al., 2014). This profound maturational adaptation leads to left ventricular (LV) chamber remodeling, which is characterized by an 86% increase in LV end diastolic dimension (LVEDD) that results in a 4.6-fold increase in LV volume at diastole without a significant change in LV free wall thickness at diastole (FWd or h) (Fig. 4). These changes in ventricular morphology produced a 52% decrease in the LV *h*/*Ri* ratio (where *Ri* is the internal LV chamber radius), consistent with eccentric hypertrophy, and maintained LV weight-to-stroke volume ratio (1.76:1 at P10 versus 1.78:1 at P35). Also, between P10 and P35, LVEDD length-to-diameter ratio decreased by 40% indicating an increase in LV sphericity. At the cellular level, CM length increased 1.7-fold between P10 and P35, with little change in CM width. Detailed analysis of the morphological changes in heart size during this postnatal period from P10 to P35, revealed three distinct growth phases: the first and third between P10 and P14, and P18 and P35, respectively, involve a physiological-type of hypertrophic growth with no change in the number of CMs, whereas an intervening period between P11 and P18 involves heart growth due to CM hyperplasia that results from proliferation of existing CMs, rather than to the maturation of cardiac stem cells (Fig. 4) (Naqvi et al., 2014). Importantly, the adaptive LV remodeling of postnatal maturational growth represents a distinct form of physiological hypertrophy that differs from the pathological hypertrophy associated with the LV volume-overload of mitral regurgitation, where cardiac performance continuously degrades over time (Schiros et al., 2013), and from the physiological hypertrophy associated with endurance exercise, where the increases in LV chamber volume are accommodated by elliptical remodeling of the heart (Schiros et al., 2013) that limits the increase in LVEDD and, thus, in LV end-diastolic wall stress.

Involvement of TH in this adaptive preadolescent growth of the heart was suggested by marked CM elongation as well as by an increase in the ratio of $\alpha$- to $\beta$-MHC mRNA expression (Fig. 4) (Naqvi et al., 2014), findings consistent with a T3-mediated effect, since neither physiological nor pathological cardiac hypertrophy cause large changes in the $\alpha$- to $\beta$-MHC mRNA ratio, but T3 excess does (Haddad et al., 2008). This is important as increased expression of $\alpha$-MHC, the fast ATPase activity MHC isoform, correlates directly with overall cardiac performance (Krenz and Robbins, 2004). Further, we showed that a surge in circulating T3 levels (5.6-fold increase) precedes the period of CM hyperplasia, and that blockade of T3 biosynthesis with the goitrogen, propylthiouracil (PTU), prevents the increase in $\alpha$- to $\beta$-MHC ratio and in heart weight, as well as the increase in the number of CMs observed during the period of maturational growth between P10 and P18. Given that the surge in circulating T3 levels precedes the CM hyperplastic response by several days, it is unclear if T3-induced CM proliferation is due to a direct or indirect effect. Direct involvement of T3 in CM hyperplasia was suggested by a T3-stimulated increase in DNA synthesis *in vitro* (Naqvi et al., 2014). In support of an indirect effect, we showed that PTU treatment inhibits a developmental increase in cardiac IGF-1 protein and mRNA expression, and cardiac IGF-1 receptor and Akt phosphorylation at P15. The associated abrupt nuclear localization of Akt in CMs at P15 is important because nuclear overexpression of Akt causes CM proliferation (Rota et al., 2005). These findings are supported by an earlier report that showed a pharmacologic effect of T3 administration on cardiac IGF-1 mRNA expression (Kupfer and Rubin, 1992). The effect of T3 on CM proliferation could possibly also involve a morphological change in CM dimensions. T3 prominently increases CM length in cell culture (Pantos et al., 2007). In some

eukaryotic cells, cell division is triggered by progressive cellular elongation that disinhibits a kinase cascade involved in cell cycle reentry (Moseley and Nurse, 2010). Others have found that in fetal rat and sheep, hemodynamic load, which is expected to increase wall stress, is required for a TH-induced increase in ventricular mass (Torres and Tucker, 1993; Segar et al., 2013). We found that postnatal developmental changes in heart morphology favor an uncompensated increase in LV wall stress. Wall stress is a major impetus for cell division in fetal and neonatal CMs *in vivo* (Saiki et al., 1997; Sedmera et al., 2003). Mathematical modeling suggests that this stress is greater in subendocardium than in subepicardium (Mirsky, 1973). This could, in part explain why we found that CM hyperplasia in P15 hearts is more evident in the longitudinal subendocardial than in the circumferential subepicardial cardiac myofibers. All of these considerations suggest that the CM hyperplastic response during maturational heart growth may involve interplay between endocrine, paracrine, morphologic and hemodynamic factors.

Our contention that T3 triggers the postnatal CM hyperplastic response during maturational heart growth is not always, at first glance, consistent with earlier reports. For example, Chattergoon et al. (2012) evaluated the effects on *fetal* CM maturation of T3 given to sheep by infusion on gestational days 125–130 (term ~145 days). These studies suggested that T3 augments CM maturation, as evidenced by increases in cell width, binucleation and expression of the cell cycle inhibitor, p21, as well as by reductions in cyclin D1 and in cell proliferation. Increased expression of phospho-mTOR, ANP and SERCA2a also suggested that T3 promoted CM hypertrophy. However, a hallmark of T3-induced hypertrophy is CM enlargement due mainly to cell elongation, not to an increase in width; the latter being observed with pathological hypertrophy. Similarly, SERCA2a expression falls with pathological hypertrophy. Thus, the findings of Chattergoon et al., suggest that the CM responses observed are complex with elements of both T3-induced and pathological hypertrophy. Given that T3 administration was 'ectopic' in their study, that is, it was given at a time when circulating T3 levels are normally very low, the relevance of these findings for physiological postnatal CM maturation remains unclear. In addition, given that the hemodynamic load on the heart is low during gestation, it is perhaps not surprising that Chattergoon et al. did not observe a hyperplastic effect of T3 on fetal CMs. Of interest in this regard, Yang et al. (2014) reported that T3 drives maturation of CMs derived from human induced pluripotent stem cells (hiPSC-CM); again a situation where T3 is acting on CMs in the absence of a hemodynamic load. Thus, much work is still required to understand the context under which T3 functions as a mitogen in the preadolescent heart.

## TH and the heart: potential therapeutic implications

TH replacement has been trialed in the operative setting in an attempt to minimize morbidity and to increase survival of children undergoing complex congenital heart disease surgery (Chowdhury et al., 2001). T3 therapy in these extremely sick patients is aimed at improving cardiac function and optimizing postoperative hemodynamics that may result from observed changes in TH metabolism, which decreases circulating T3 levels, as well as reducing TR-expression (particularly TR$\alpha$) (Dillmann, 2010). TH levels also fall acutely in humans following myocardial infarction and replacement therapy with either T3 or the synthetic TH analog, 3,5-diiodothyropropionic acid (DITPA), which exerts a positive inotropic effect without the potential adverse effects of iatrogenic hyperthyroidism (e.g., tachyarrhythmias, weight loss, anxiety), has been used to limit post-infarct pathological remodeling in preclinical studies (Zheng et al., 2004; Spooner et al., 1999; Mahaffey et al., 1995), as well as in clinical trials of patients with heart failure (Morkin et al., 2002; Goldman et al., 2009). Again in these studies, the rationale for using TH or analogs is largely to enhance cardiac contractility of the uninjured or failing myocardium in situations where TH metabolism is impaired and T3 levels are low (Gerdes and Iervasi, 2010). Where TH metabolism is unaltered, T3 would have to be given with caution as it could potentially deleteriously increase cardiac work and myocardial $O_2$ consumption, although DITPA, with its lack of adverse cardiac effects, should be tolerated.

Although adult CMs have very limited proliferative capacity, it has been suggested that a small population of these cells in regions adjacent to a myocardial infarct, retain the ability to divide (Kajstura et al., 1998; Beltrami et al., 2001). Thus, enhancing their proliferation may augment repair in response to myocardial injury. To this end, Ledda-Columbano et al. (2006) treated rats with T3 for up to 7 days. This resulted in CM cell cycle reentry, as demonstrated by increased expression of cyclin D1, as well as by increased CM cyclin A (a specific S phase marker) and proliferating cell nuclear antigen (PCNA) labeling; increased bromodeoxyuridine incorporation into CM DNA, and increased labeling by the mitotic marker, phospho-histone-3. These findings are consistent with the studies of Soonpaa et al. (1997), who showed that CM-targeted cyclin D1 overexpression from birth promotes CM DNA synthesis in mice and results in infarct regression. Tane et al. (2014) have recently shown, however, that induction of cyclin D1 expression in adult murine CMs results in cell cycle re-entry of >40% of CMs, but that the cell cycle is arrested in mitosis. Thus, cell cycle reentry is not necessarily synonymous with cell proliferation, so it is unfortunate that Ledda-Columbano et al. (2006) did not quantitate CM numbers in T3-treated animals.

Apart from the direct effects of the loss of pump function that follows the massive loss of CMs and subsequent necrosis and fibrosis associated with myocardial infarction, impaired contractility also results in the functional and structural remodeling of spared myocardium. In preclinical models of infarction, DITPA has been shown to attenuate this pathological post-infarct remodeling, although by a mechanism that may involve induction of angiogenesis and arteriolar growth, as well as by limiting the inflammatory response (Zheng et al., 2004; Abohashem-Aly et al., 2011). Of note in this regard, Janssen et al. (2013) have recently reported that post-infarct remodeling is associated with a marked (6-fold) increase in the expression of D3 in spared myocardium. It would be of interest, therefore, to determine if therapy with T3 or DITPA, might also improve remodeling by

an independent mechanism, involving rescue of the relative hypothyroid myocardium resulting from increased D3 expression and, thus, augmentation of CM proliferation.

## Summary and future perspective

During postnatal cardiac development, TH mediates direct transcriptional activation of genes involved in cardiac contractility, regulates hemodynamic changes such as stroke volume, blood volume, heart rate and blood pressure that are associated with the rapid increase in body size during this period, and it initiates a brief burst of subendocardial CM proliferation in preadolescence that involves activation of the IGF-1/IGF-1R/Akt pathway. Although further studies are required to delineate the regulatory mechanisms that govern the complex spatial and temporal actions of TH in the cardiovascular system, insights gained from such studies will prove invaluable in the development of effective myocardial regenerative therapies for the treatment of inherited and acquired heart diseases.

## Acknowledgments

We gratefully acknowledge support from NIH (HL079040; to AH); John and Mary Brock Translational Research Fund grant (to AH); Regenerative Engineering and Medicine (REM) center at Emory and Georgia Tech grant (to AH, NN); Emory and Children's Center for Cardiovascular Biology pilot grant (to AH, NN); American Heart Association Scientist Development grant (13SD16460006; to NN); National Health and Medical Research Council of Australia grants (573732 & 1012190; to RMG, SEI, ML); Heart Foundation of Australia grant (G10S 5148; to RMG, ML, SEI); RT Hall Estate grant (to RMG, ML, SEI); and an Australian Research Council Stem Cells Australia, Special Initiative in Stem Cell Science grant (SR110001002; to RMG).

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