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New insights into regulation of lipid metabolism by thyroid hormone

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Abstract

Purpose of review—Thyroid hormone (3,3′,5-triiodo-L-thyronine) plays an important role in thermogenesis and maintenance of lipid homeostasis. The present article reviews the evidence that 3,3′,5-triiodo-L-thyronine regulates lipid metabolism via thyroid hormone receptors, focusing particularly on in-vivo findings using genetically engineered mice.

Recent findings—That lipid metabolism is regulated via thyroid hormone receptor isoforms in a tissue-dependent manner was recently uncovered by using knockin mutant mice harboring an identical mutation in the *Thra* gene (*Thra1*^{PV} mouse) or the *Thrb* gene (*Thrb*^{PV} mouse). The mutation in the *Thra* gene dramatically decreases the mass of both white adipose tissue and liver. In contrast, the mutation in the *Thrb* gene markedly increases the mass of liver with an excess depot of lipids, but no significant abnormality is observed in white adipose tissue. Molecular studies show that the expression of lipogenic genes is decreased in white adipose tissue of *Thra1*^{PV} mice, but not in *Thrb*^{PV} mice. Markedly increased lipogenic enzyme expression, and decreased fatty acid beta-oxidation activity contribute to the adipogenic steatosis and lipid accumulation in the liver of *Thrb*^{PV} mice. In contrast, reduced expression of genes critical for lipogenesis mediates decreased liver mass with lipid scarcity in *Thra1*^{PV} mice.

Summary—Studies using *Thra1*^{PV} and *Thrb*^{PV} mice indicate that apo-thyroid hormone receptor-beta and apo-thyroid hormone receptor-alpha-1 mediate distinct deleterious effects on lipid metabolism. Thus, both thyroid hormone receptor isoforms contribute to the pathogenesis of lipid abnormalities in hypothyroidism, but in a target tissue-dependent manner. These studies suggest that thyroid hormone receptor isoform-specific ligands could be designed as therapeutic targets for lipid abnormalities.

Keywords

lipid metabolism; mouse models; mutations; thyroid hormone; thyroid hormone receptors

Introduction

Thyroid hormone (3,3′,5-triiodo-L-thyronine) maintains lipid homeostasis via its effects on gene expression in target organs, including the liver and adipose tissues. Thyroid hormone receptors, members of ligand-dependent transcription factor superfamily, mediate the genomic actions of 3,3′,5-triiodo-L-thyronine [1]. Two thyroid hormone receptor genes (*THRA* and *THRB*) encode 3,3′,5-triiodo-L-thyronine-binding thyroid hormone receptor

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isoforms, α1 (TRα1) and β (TRβ), respectively. TRβ is the major isoform in the liver, kidney, and thyroid [2], whereas TRβ1 is predominantly expressed in the brain and adipose tissue [2,3]. Thyroid hormone receptors bind to specific DNA sequences known as thyroid hormone response elements (TREs) to mediate positive or negative regulation of 3,3′,5-triiodo-L-thyronine target genes. A host of co-regulatory proteins further modulates the gene-regulatory functions of thyroid hormone receptors [4,5•].

The regulation of lipid homeostasis by 3,3′,5-triiodo-L-thyronine is complex, as it involves the coordinated regulation of several target tissues, mainly the adipose tissue, and the liver. There are two types of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). The main functions of WAT are in the transport, synthesis, storage, and mobilization of lipids. An elevated level of 3,3′,5-triiodo-L-thyronine in hyperthyroidism is associated with increased lipolysis and lower body weight. By contrast, a lower level of 3,3′,5-triiodo-L-thyronine in hypothyroidism is associated with cold intolerance and weight gain with reduced lipolysis and cholesterol clearance. Mice devoid of all thyroid hormone receptor isoforms exhibit decreased body temperature and basal metabolic rate, growth retardation, and an increased amount of fat tissue [6,7].

BAT is a tissue specialized in adaptive thermogenesis with the expression of mitochondrial uncoupling protein 1 (UCP1) in responding to cold induction. In contrast to WAT, the main function of BAT is to dissipate energy, not to store it. Therefore, the conversion of WAT to BAT is sought as a possible strategy to treat obesity. In rats fed a high-calorie diet, a TRβ-selective agonist GC-24 confers resistance to diet-induced obesity through the promotion of energy expenditure [8]. In addition, a recent case report [9•] indicates that in a diabetic patient with extreme insulin resistance due to a mutation in the insulin receptor gene, thyroid hormone induces BAT and ameliorates diabetes.

The liver is an important 3,3′,5-triiodo-L-thyronine target tissue [10•]. 3,3′,5-Triiodo-L-thyronine increases the expression of several genes involved in hepatic lipogenesis, including fatty acid synthase (*Fas*), hepatic product spot 14, acyl-CoA synthetase 5, fatty acid transporter protein, malic enzyme, and glucose-6-P dehydrogenase [11]. 3,3′,5-Triiodo-L-thyronine also induces genes involved in fatty acid oxidation, such as fatty acid transporter (*Fat*), fatty acid-binding protein, lipoprotein lipase (*Lpl*) [11], and carnitine palmitoyltransferase-1alpha (*Cpt-1*α) [12]. Cpt-1α is a key rate-limiting enzyme in mitochondrial fatty acid oxidation. Many of these metabolic genes (e.g., malic enzyme, *Fas*, and *Cpt-1*α) in the liver are directly regulated by 3,3′,5-triiodo-L-thyronine/thyroid hormone receptor, as the TREs have been reported in promoters of these genes [13].

The accumulation of such evidence clearly demonstrates the important roles of thyroid hormone receptors in maintaining lipid homeostasis. TRβ and TRα1 share high sequence homology in the functional DNA and 3,3′,5-triiodo-L-thyronine-binding domains, but differ greatly in the length and sequences of the amino terminal A/B domains. Studies of mice deficient for either of the two thyroid hormone receptor genes or for both thyroid hormone receptor genes indicate that thyroid hormone receptor isoforms have both redundant roles and specific functions [14]. However, the precise roles of these thyroid hormone receptor isoforms in lipid metabolism have not been fully defined. This article highlights recent advances in thyroid hormone receptor isoform-dependent actions in lipid metabolism learned from genetically engineered mice, particularly from thyroid hormone receptor knockin mutant mice.

Regulation of lipid metabolism by thyroid hormone receptor isoforms *in vivo*

Significant advances in the understanding of thyroid hormone receptor isoform-dependent actions in lipid metabolism came from the use of loss-of-function approach via knockin mutations of the *Thra* or *Thrb* gene in mice. These mice provided valuable information about how thyroid hormone receptor functions in an isoform-dependent and target site-dependent manner.

Distinct regulation of lipid metabolism by thyroid hormone receptor isoforms in white adipose tissue

A wealth of information about how mutations of TRα1 lead to lipid abnormalities came from three *Thra1* knockin mice created by three different groups of investigators. A mutation, known as PV, was targeted to the *Thra* gene locus ($Thra1^{PV}$ mice). The PV mutation was identified in a patient with resistance to thyroid hormone (RTH) [15]. This mutation is a frameshift mutation in the C-terminal 14 amino acids of TRβ, resulting in a complete loss of 3,3′,5-triiodo-L-thyronine-binding activity and transcriptional capacity. The $Thra1^{PV}$ mice express the PV mutation at the corresponding C-terminal region of TRα1 [16]. Homozygous knockin $Thra1^{PV/PV}$ mice die soon after birth and heterozygous $Thra1^{PV/+}$ mice display the striking phenotype of dwarfism [16]. A significant reduction (40%) in total WAT mass (e.g., inguinal, epididymal, and perirenal fat) is persistently observed in male $Thra1^{PV/+}$ mice, up to 1 year of age. But no changes in interscapular BAT mass are apparent. In spite of the fact that $Thra1^{PV/+}$ mice consume significantly more food (~34%) than their wild-type siblings, there is a significant reduction in the serum levels of free fatty acids, total triglycerides, and leptin levels. No changes in glucose or insulin are detected in $Thra1^{PV/+}$ mice, relative to those measurements in their wild-type siblings. Detailed molecular analyses indicate that the impaired adipogenesis in the WAT is mediated by the direct repression in expression of the peroxisome proliferator-activated receptor gamma (*Pparγ*) gene by TRα1PV, leading to reduced expression of lipogenic genes [3].

Similar to $Thra1^{PV/+}$ mice, the $Thra1^{R384C}$ knockin mice exhibit a lean phenotype with reduction in white fat mass and decreased leptin levels [17]. However, in contrast to $Thra1^{PV/+}$ mice, $Thra1^{R384C}$ mice display a reduction in interscapular BAT mass (33%). The mice are hypermetabolic and resistant to diet-induced obesity. Increased lipid mobilization and beta-oxidation occur in adipose tissues. Gene expression profiling reveals strong induction of genes involved in lipolysis, lipogenesis, and glucose handling. In epididymal WAT, the expression of acetyl CoA-carboxylase (*Acc1*), *Fas,* glucose transporter type 4 *(Glut4), Pgc1*α, and peroxisome proliferator-activated receptor alpha (*Ppar*α) genes is elevated. However, unlike findings in $Thra1^{PV/+}$ mice, the expression of the *Pparγ* gene is unchanged.

In contrast to the lean phenotype exhibited by $Thra1^{PV/+}$ and $Thra1^{R384C}$ mice, $Thra1^{P398H}$ knockin mice have increased body fat accumulation and elevated serum levels of leptin, glucose, and insulin [18]. The sensitivity to catecholamine-induced lipolysis in adipocytes is significantly reduced at both the receptor and postreceptor levels. Excess WAT is associated with impaired insulin action and elevated serum glucose levels. These abnormalities are markedly distinct from those displayed by $Thra1^{PV/+}$ mice and $Thra1^{R384C}$ mice, in spite of the fact that the mutation sites are all located in the hormone-binding domain.

The molecular basis underlying the different phenotypic manifestations in three *Thra1* knockin mice is not clear. The different phenotypes could reflect their differences in the degree of the loss of 3,3′,5-triiodo-L-thyronine binding and the potency of dominant

negative activity of TRα1 mutants. TRα1PV completely loses 3,3′,5-triiodo-L-thyronine binding and exhibits potent dominant-negative activity [16]. In contrast, TRα1P398H and TRα1R384C only partially lose 3,3′,5-triiodo-L-thyronine-binding activity [18,19]. As the C-terminus of TRα1 contains the domain essential for the interaction with co-repressors or co-activators, the different phenotypes could also reflect the differential interaction of TRα1 mutants with these regulatory proteins. However, in spite of the contrasting abnormalities manifested among these three *Thra1* knockin mice, it is clear that TRα1 plays a critical role in regulation of lipid homeostasis in WAT.

The $Thrb^{PV}$ mouse was created to study the molecular basis of RTH [20]. It harbors the same dominant-negative PV mutation, as that in $Thra1^{PV}$ mice but in the *Thrb* gene locus [20]. The $Thrb^{PV}$ mice faithfully reproduce human RTH with dysregulation of the pituitary–thyroid axis and reduced sensitivity to thyroid hormone in other 3,3′,5-triiodo-L-thyronine target tissues [20]. The fact that $Thrb^{PV}$ and $Thra1^{PV}$ mice exhibit strikingly distinct phenotypes in fertility, survival, and the regulation at the pituitary–thyroid axis [16,20] suggests the regulation of lipid metabolism in WAT by mutant thyroid hormone receptor isoforms could differ. Indeed, unlike the abnormalities in WAT found in $Thra1^{PV}$ mice, no changes in mass and morphology of WAT are apparent in $Thrb^{PV}$ mice [21••]. This contrasting phenotype in these two knockin mice suggests that TRα1 and TRβ have distinct regulatory functions in WAT.

Regulation of brown adipose tissue adaptive thermogenesis by thyroid hormone receptor is isoform-dependent

Heat produced in response to lowering temperature is referred to as adaptive thermogenesis. In small mammals, BAT is the primary site to generate heat owing to its expression of mitochondrial UCP1 protein. In BAT, coordinated actions of adrenergic signaling, UCP1, and 3,3′,5-triiodo-L-thyronine are required for effective adaptive thermogenesis. After exposure to cold, local 3,3′,5-triiodo-L-thyronine concentration in BAT increases [22,23] to coordinate with the sympathetic nervous system and to amplify adrenergic signaling [24] and to increase the expression of UCP1, leading to increased heat production.

Earlier studies [25,26] reported decreased body temperature in all models of TRα1-deficient mice, suggesting a regulatory role of TRα1 in thermogenesis. Subsequent studies revealed intricate regulation of cold-induced adaptive BAT thermogenesis by thyroid hormone receptor isoforms. Mice rendered hypothyroid, when treated with 3,3′,5-triiodo-L-thyronine or GC-1, a TRα-specific ligand, restored the UCP1 level to that of euthyroid state. In responding to cold exposure, GC-1-treated mice had impairment in adaptive thermogenesis by failing to reach the same temperature level as the 3,3′,5-triiodo-L-thyronine-treated mice. Furthermore, isolated brown adipocytes treated with GC-1 had decreased cAMP production in responding to adrenergic stimulation compared with those treated with 3,3′,5-triiodo-L-thyronine [27]. These findings indicate that activation of UCP1 by 3,3′,5-triiodo-L-thyronine and augmentation of adrenergic responsiveness are mediated by different thyroid hormone receptor isoforms. TRβ can elicit the activated expression of UCP1 in BAT. However, for adrenergic stimulation, participation of TRβ is not sufficient for full augmentation of 3,3′,5-triiodo-L-thyronine responses, but requires the actions mediated by TRα1.

The requirement of TRα1 in mediating 3,3′,5-triiodo-L-thyronine-augmented catecholamine response in adaptive thermogenesis is further supported by studies using *Thra1* knockin mutant mice. At the basal level, the core temperature of $Thra1^{P398H}$ mice is 0.5°C lower than that of wild-type mice [18], indicating that a functional TRα1 is required to maintain temperature homeostasis. In responding to cold exposure, $Thra1^{P398H}$ mice are defective in adaptive thermogenesis. However, there are no differences in the expression levels of UCP1 between $Thra1^{P398H}$ mice and wild-type mice. These findings further strengthen the

conclusion that in BAT, TRβ regulates the expression of UCP1, whereas TRα1 mediates the 3,3′,5-triiodo-L-thyronine-augmented adrenergic responses in adaptive thermogenesis.

The participation of TRβ in adaptive thermogenesis via regulation of UCP1 expression is further confirmed by recent studies using *Thrb*^{PV} mice [28••]. *Thrb*^{PV} mice exhibit elevated serum thyroid hormone. When they are rendered euthyroid by antithyroid drugs, the norepinephrine-induced thermogenic response of BAT is decreased in both *Thrb*$^{PV/+}$ and *Thrb*$^{PV/PV}$ mice, with concurrent reduced expression of the *Ucp1* gene. Furthermore, both cAMP and glycerol production in response to adrenergic stimulation is decreased in brown adipocytes isolated from *Thrb*^{PV} mice [28••]. Thus, adaptive thermogenesis is regulated by thyroid hormone receptor isoforms acting on different targets in the same tissue.

Contrasting regulation of lipid metabolism by thyroid hormone receptor isoforms in the liver

The major thyroid hormone receptor isoform in the liver is TRβ [2]. Thus, it is not surprising to find abnormalities in the liver of *Thrb*$^{PV/PV}$ mice, namely an enlarged liver that has excess lipid accumulation. The mutation of the *Thrb* gene leads to activated PPARγ signaling and decreased fatty acid beta-oxidation activity that contribute to the adipogenic steatosis and lipid accumulation in the liver of the *Thrb*$^{PV/PV}$ mice [21••]. Interestingly, the liver size of *Thra1*$^{PV/+}$ mice is significantly smaller than that of wild-type mice with a paucity of lipids. Decreased expression of lipogenic enzymes and PPARγ is found in the liver of *Thra1*$^{PV/+}$ mice [3,21••]. These observations indicate that the regulation of lipid metabolism in the liver is thyroid hormone receptor isoform-dependent. As TRα1 is a minor thyroid hormone receptor isoform in the liver, the prominent phenotype exhibited in *Thra1*$^{PV/+}$ mice suggests that TRα1PV may act not only via dominant negative mode of action but also via gain of function. An extensive analysis of 3,3′,5-triiodo-L-thyronine-responsive genes has been reported in the liver of *Thra1*^{P398H} mutant mice [18]. Consistent with that found in the liver of *Thra1*$^{PV/+}$ mice, decreased expression of lipogenic enzymes is evident in the liver of *Thra1*^{P398H} mutant mice [18].

Differential regulation of adipogenesis by thyroid hormone receptor isoforms in 3T3-L1 cells

Understanding of thyroid hormone receptor isoform-dependent action on the differentiation and maturation of preadipocytes is facilitated by the use of 3T3-L1 cells. The 3T3-L1 cell line has long been used as a model to identify genes critical in the differentiation process [29]. 3T3-L1 cells stably expressing either TRα1PV (L1-α1PV cells) or TRβ1PV (L1-β1PV cells) were generated, and clones with equal amounts of TRα1PV or TRβ1PV proteins in the respective cells were used in the studies [30••]. In control cells, 3,3′,5-triiodo-L-thyronine induces a 2.5-fold increase in adipogenesis of 3T3-L1 cells, evidenced by increased lipid droplets. This increase was mediated by 3,3′,5-triiodo-L-thyronine-induced expression of *Pparγ* and CCAAT-enhancer-binding protein alpha (*C/ebpα*) genes at both the mRNA and protein levels. In L1-α1PV cells or L1-β1PV cells, adipogenesis is reduced 94 or 54%, respectively, indicative of differential inhibitory activity of mutant thyroid hormone receptor isoforms. Concordantly, the expression of *Pparγ* and *C/ebpα* at the mRNA and protein levels is more repressed in L1-α1PV cells than in L1-β1PV cells. In addition, the expression of PPARγ downstream target genes involved in fatty acid synthesis – the *Lpl* and *aP2* involved in adipogenesis – is more inhibited by TRα1PV than by TRβ1PV. Chromatin immunoprecipitation assays showed that TRα1PV is more avidly recruited than TRβ1PV to the promoter to preferentially block the expression of the *C/ebpα* gene [30••]. These results indicate that impaired adipogenesis by mutant thyroid hormone receptor is isoform-dependent. Thus, thyroid hormone receptors not only modulate lipid

storage and mobilization but also directly regulate the differentiation and maturation of adipocytes in an isoform-dependent manner.

Conclusion

The availability of powerful genetically engineered mice has allowed the elucidation of how 3,3′,5-triiodo-L-thyronine via thyroid hormone receptors regulates lipid metabolism and energy homeostasis *in vivo*. Early work using mice deficient in a single subtype thyroid hormone receptor or both thyroid hormone receptors indicated that thyroid hormone receptor isoforms mediate different regulatory roles in lipid metabolism and thermoregulation. The use of thyroid hormone receptor knockin mutant mice not only supports this notion but also significantly advances our understanding of the actions of each thyroid hormone receptor isoform in thermoregulation, as well as maintaining lipid homeostasis. A clear picture has emerged that TRα1 is critical in thermoregulation via regulation at the level of local, via central regulation, or both [17], whereas TRβ seems to be more involved in the regulation of lipid metabolic pathways [31]. A notable example is its role in the regulation of lipogenic and lipolytic enzymes in the liver [21••]. The use of isoform knockin mice that have an identical mutation (*Thra1*^{PV} and *Thrb*^{PV} mice) further reveals that the thyroid hormone receptor isoform action is also target site-dependent, as TRα1 plays a significant role in adipogenesis and maintaining mature adipocyte functions, whereas TRβ is critical in maintaining the lipid metabolic functions of the liver.

In spite of recent progress, it is important to further expand our understanding in the roles of thyroid hormone receptors in the regulation of thermogenesis, especially in the signaling via central regulation. An emerging idea is to explore the possibility of conversion of white adipocytes to brown adipocytes as a strategy to treat obesity. In this regard, further elucidation of 3,3′,5-triiodo-L-thyronine/thyroid hormone receptor actions in BAT differentiation and biology is certainly necessary. Accumulated evidence shows that synthetic thyroid hormone analogs hold promise as lipid-lowering agents [32•]. The finding that regulation of lipid metabolism is thyroid hormone receptor isoform-dependent and target site-dependent opens possibilities that thyroid hormone receptor isoform-specific thyroid hormone analogs can be explored for therapeutic intervention in lipid abnormalities. More recently, studies of RTH patients revealed a critical role of skeletal muscle in contributing to resting energy expenditure, presumably mediated by TRα1 [33••]. The contribution of thyroid hormone receptor isoforms in the regulation of key genes responsible for modulating energy expenditure in skeletal muscles could be elucidated using genetically engineered mice discussed in the present review.

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We regret any reference omissions due to length limitation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 486).

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