Non-classic thyroid hormone signalling involved in hepatic lipid metabolism

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Abstract

Thyroid hormones are important modulators of lipid metabolism because the liver is a primary hormonal target. The hypolipidaemic effects of thyroid hormones result from the balance between direct and indirect actions resulting in stimulation of lipid synthesis and lipid oxidation, which favours degradation pathways. Originally, it was believed that thyroid hormone activity was only transduced by alteration of gene transcription mediated by the nuclear receptor thyroid hormone receptors, comprising the classic action of thyroid hormone. However, the discovery of other effects independent of this classic mechanism characterised a new model of thyroid hormone action, the non-classic mechanism that involves other signalling pathways. To date, this mechanism and its relevance have been intensively described. Considering the increasing evidence for non-classic signalling of thyroid hormones and the major influence of these hormones in the regulation of lipid metabolism, we reviewed the role of thyroid hormone in cytosolic signalling cascades, focusing on the regulation of second messengers, and the activity of effector proteins and the implication of these mechanisms on the control of hepatic lipid metabolism.

Key Words
- thyroid hormone receptor
- liver
- lipid metabolism

Thyroid hormone effects on lipid metabolism

The thyroid hormones, triiodothyronine (T₃) and thyroxine (T₄), modulate several physiological processes in organisms and are critical in the growth, development, differentiation and maintenance of metabolic homoeostasis (Oetting & Yen 2007). The ability of thyroid hormones to induce anabolic and catabolic pathways, such as lipogenesis and lipolysis, contributes to thyroid hormone-induced increase in energy expenditure (Pucci et al. 2000), besides several mechanisms involved in this effect, such as sympathetic activity modulation (Silva 2011). Thyroid hormones stimulate lipid synthesis, mobilisation and degradation, although degradation occurs at higher rates, specifically in hyperthyroidism (Muller & Seitz 1984). However, hypothyroidism in patients or in animal models is associated with lipid serum changes characterised by increased cholesterol and, in some cases, increased triglyceride levels (Erem et al. 1999, Pucci et al. 2000).

The liver is the major site for cholesterol and triglyceride metabolism, and thyroid hormones play an important role in hepatic lipid homoeostasis (Malik & Hodgson 2002). Cholesterol is an essential constituent of most biological membranes and it is also a precursor in the synthesis of bile acids, steroid hormones and some vitamins. Normal serum thyroid hormones levels are essential for the maintenance of a sufficient pool of cholesterol to meet the body’s requirements and to
regulate the critical steps of cholesterol synthesis, uptake and metabolism (Repa & Mangelsdorf 2000). Thyroid hormone signalling stimulates 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase and farnesyl pyrophosphate to favour cholesterol synthesis (Ness et al. 1990) and up-regulate the LDL receptor, increasing cholesterol uptake (Shin & Osborne 2003, Lopez et al. 2007). Thyroid hormone stimulates cholesterol 7α-hydroxylase (CYP7A1), enhancing the metabolism of cholesterol into bile acids (Ness et al. 1990). However, in hypothyroid patients, the lack of thyroid hormone leads to increased serum cholesterol caused principally by diminished cholesterol clearance and minor conversion to bile acids, while the opposite phenotype is observed in hyperthyroid patients (Erem et al. 1999).

Thyroid hormones stimulate the hepatic synthesis of fatty acids (FA), as well as esterification to triglycerides, by direct stimulation of the following rate-limiting enzymes: acetyl-CoA carboxylase and FA synthase (FAS; Muller & Seitz 1984). Besides the direct effect in the transcription of these enzymes, thyroid hormones promote a rapid and important increase in Spot14 (S14 (THRSPI)) expression, a protein which is able to interact physically and functionally with the thyroid hormone receptor (TR) to regulate malic enzyme gene expression (Chou et al. 2007). The elevated S14 expression is important to increase lipogenic enzyme expressions induced by T3 (Cunningham et al. 1998). In this way, hyperthyroid patients present approximately a threefold increase in hepatic lipogenesis when compared with euthyroid subjects (Cachefo et al. 2001). Although lipogenesis is increased in experimental models of hyperthyroidism, reduced production of triglycerides and VLDL as well as decreased hepatic intracellular triglyceride content is observed (Keyes et al. 1981, Laker & Mayes 1981). This can, in part, be explained by the increased FA oxidation observed in these models (Keyes et al. 1981). Beyond these mechanisms that counterbalance increased triglyceride synthesis, there are extra-hepatic thyroid hormone action that increase lipid clearance from the circulation and reduce lipid levels (Packard et al. 1993, Prieur et al. 2005). However, in hypothyroidism, concomitant with decreased FA synthesis (Laker & Mayes 1981), decreased FA oxidation is also observed, causing increased VLDL secretion by the liver (Al-Tonsi et al. 2004).

In subclinical hypothyroidism, defined as an increased serum TSH level in the setting of normal free peripheral thyroid hormone concentrations, it is usual to observe lipid disorders, as elevated serum cholesterol (Duntas & Brenta 2012) and lipoprotein disturbances (Sigal et al. 2011), that are normalised by T4 treatment (Sigal et al. 2011, Duntas & Brenta 2012). The mechanism involved in these lipid abnormalities is unclear, but some researchers suggest direct hypercholesterolaemic effect of TSH in the liver, independent of serum thyroid hormone alteration (Tian et al. 2010, Wang et al. 2012, Xu et al. 2012).

**Signalling pathways involved in thyroid hormone action**

Most of the described thyroid hormone effects are mediated by the canonical, or classic, pathway. This mechanism requires the hormone interaction with the nuclear TR, which can act as transcription factors, interacting directly with specific DNA sequences on the promoter of thyroid hormone-responsive genes (TRE), regulating the transcription rate of target genes (Bassett et al. 2003, Lazar 2003, Moeller & Broecker-Preuss 2011). Two distinct genes express the TRβ and TRα isoforms (Harvey & Williams 2002), codifying TRβ1, TRβ2 and TRα1. This classic mechanism accounts for several of the lipid reduction effects induced by thyroid hormones. For example, the hepatic receptor for LDL, responsible for cholesterol uptake from the blood to the liver, is up-regulated by thyroid hormone at the transcriptional level, as indicated by the increase in LDL receptor mRNA expression induced by thyroid hormone (Ness & Lopez 1995) and the presence of two functional TREs on the rat gene promoter (Lopez et al. 2007). The sterol response element binding protein-1c (SREBP-1c (SREBF1)), a transcription factor involved in lipogenesis, also has a functional TRE in its promoter region (Hashimoto et al. 2006b), similar to the enzymes CYP7A1 (Shin et al. 2006) and FAS (Radenne et al. 2008), which are important in cholesterol metabolism and lipogenesis respectively.

TR can form homodimers or interact with other nuclear receptors, such as retinoid X receptor (RXR), generating heterodimers (Forman et al. 1992, Bogazzi et al. 1994). Heterodimerisation leads to more efficient T3-dependent transcriptional activity than TR/TR homo-dimerisation (Oetting & Yen 2007). Beyond physical interaction, TR and other nuclear receptors depend on the same cofactors and corepressors to modulate gene expression and sometimes compete for the same DNA binding site (Liu & Brent 2010). For this reason, changes in T3-TR action can affect RXR pathways and vice versa and can also perturb other nuclear receptor pathways. In lipid metabolism, Hashimoto et al. demonstrated that the Δ337T TRβ mutation diminished the TR-RXR hetero-dimerisation that is normally stimulated by thyroid
hormone. Therefore, RXR becomes available to interact with the liver X receptor (LXR) and it increases LXR activity, intensifying LXR’s hypocholesterolaemic effect (Hashimoto et al. 2006a). Likewise, the P398H mutation in TRα inhibits PPARα from activating target genes, impairing FA oxidation (Liu et al. 2007).

Recently, autophagy of lipid droplets was described as an important additional pathway involved in FA oxidation induced by thyroid hormone (Sinha et al. 2012). In animals with impaired autophagy, the thyroid hormone effect on FA oxidation is abolished. Thyroid hormones increase autophagy in the presence of TR and nuclear receptor corepressor (NCoR), before any significant increase in the levels of hepatic lipases or oxidation enzymes (Sinha et al. 2012). This mechanism seems important to delivering FAs to the mitochondria for β-oxidation.

The TRβ isoform seems to be the most important pathway involved in the thyroid hormone effect on lipid metabolism because selective TRβ agonists have similar effects to T₃ in reducing serum lipids (Johansson et al. 2005) and the TRβ disruption impairs FA oxidation (Araki et al. 2009) even in the presence of TRα overexpression (Gullberg et al. 2000, 2002). However, mice models of TRα signalling disruption have revealed the contribution of this TR isoform to thyroid hormone effects on lipid metabolism (Liu et al. 2007, Jornayvaz et al. 2012). The TRαPV knock-in mouse and the TRα knockout mice showed reduction of lipogenic gene expression in the liver (Araki et al. 2009, Jornayvaz et al. 2012), contrary to TRβ1PV knock-in mouse (Araki et al. 2009), suggesting the distinct regulation by both isoforms on hepatic lipid metabolism and the TRα involvement in hepatic lipogenesis (Fig. 1).

Besides the important involvement of TR, additional mechanisms participate in thyroid hormone effects on lipid metabolism. The lipid-lowering effect of thyroid hormone on FaO rat hepatoma cells, which does not express TR, occurs via non-receptor-mediated mechanisms that seem to involve both a short-term action by stimulation of mitochondrial O₂ consumption and a long-term action by differential transcriptional effects on PPARs (Grasselli et al. 2011). These data reinforce the evidence that thyroid hormone action on hepatic lipid metabolism involves many signalling pathways.

The post-translational modification of a protein, such as phosphorylation, can increase or reduce activity and can affect the binding affinity for other proteins. Previous studies suggest that a general increase in the phosphorylation state of the cell would intensify T₃ action by activating the transcription of target genes (Lin et al. 1992). TR and RXR receptors as well as different coactivators are targets of these phosphorylation events. In particular, TR can be phosphorylated in the cytosol (Glineur et al. 1989) and in the nucleus (Sugawara et al. 1994). Phosphorylation initiates TR heterodimerisation with RXR and can decrease degradation, which in turn would increase transcription (Davis et al. 2000).

Researchers have observed that the classic mechanism of thyroid hormone action can be supplemented by others, initiated outside the nucleus, involving different signalling transduction pathways (Moeller & Broecker-Preuss 2011). These mechanisms are described by some authors as nongenomic action of thyroid hormone (Davis & Davis 2002, Oetting & Yen 2007). However, the term nongenomic may be confusing as thyroid hormone induction of these non-classical signalling pathways may also lead to regulation of gene transcription. Among the non-classical mechanisms of thyroid hormone actions are hormone-induced changes in the phosphorylation pattern of effectors protein (Shih et al. 2004); in modulation of the availability of second messengers (Yamauchi et al. 2008) and in modification of the mRNA stability (Narayan & Towle 1985, Serrano-Nascimento et al. 2010).

Segal et al. (1977) demonstrated that T₃ provokes a rapid increase in 2-deoxyglucose uptake in cultured chick embryo heart cells. This effect was evident within minutes of T₃ addition and was not affected by cycloheximide, a protein synthesis inhibitor, excluding any genomic action (Segal et al. 1977). However, a rapid effect of T₃ on Spot14 expression requires protein synthesis (Jacoby et al. 1987), suggesting that some rapid actions of T₃ could involve the genomic pathway. The involvement of posttranscriptional mechanisms, regulating mRNA stability, seems to justify the rapid effect of T₃ on spot14 mRNA expression, which is not proportional to transcription rate of this gene (Narayan & Towle 1985). This thyroid hormone ability to induce mRNA stability has been described by some authors as a non-classic mechanism involved in rapid changes of mRNA expression (Serrano-Nascimento et al. 2010).

The overall non-classic process is poorly understood, but consistently, intracellular proteins associated with rapid signalling pathways have been observed to participate. Some authors suggest the participation of novel cell surface receptors for thyroid hormone (Davis et al. 2005), whereas others describe involvement of the classic TR in the cytoplasm (Moeller et al. 2006) or TR splicing variants (Hiroi et al. 2006). TR variants are alternatively spliced...
from one of the two genes encoding TR proteins. The TRα-1 splicing variants do not bind to DNA; however, they still interact with T₃. Therefore, some TRα-1 isoforms compete for the hormone with the ‘functional’ receptors and regulate thyroid hormone action. Beyond that, some authors have proposed that splicing variants have specific functions. A TRα1 variant, p28, has been observed at mitochondria (Wrutniak-Cabello et al. 2001), and an interaction with T₃ might directly stimulate oxidative phosphorylation (Oetting & Yen 2007).

The possibility that thyroid hormones interact with membrane surface receptors was reinforced by exposing HeLa and CV-1 cells to free T₄ or agarose-conjugated T₄, which is unable to enter the cell. Both bound and free T₄ resulted in activation of MAPK, also called extracellular signal-regulated kinase (ERK1/2), depending on previous activation of G-protein-coupled receptors (Lin et al. 1999). Studies examining thyroid hormone effects on calcium homoeostasis (Hummerich & Soboll 1989) or glucose uptake (Segal & Ingbar 1990) also suggest the existence of one or more plasma membrane receptors for T₃ or T₄.

Although a specific receptor has not yet been isolated, thyroid hormones can interact with several extracellular matrix proteins, leading to signal transduction events. Specific T₃ binding sites were identified in synaptosomes of the chick embryo that appear to be associated with G proteins (Giguere et al. 1996). Moreover, thyroid hormone can also interact with integrin alpha V beta 3 (αVβ3; Plow et al. 2000), triggering the serine–threonine kinase (MAPK/ERK) pathway. Active phospho-MAPK (pMAPK) translocates to the nucleus, where it can bind to TRβ. This interaction leads to TR phosphorylation, which in turn is important for determining the basal transcription rate of the receptor (Davis et al. 2000) (Fig. 1).

Baumann et al. (2001) described that only 10–15% of TR are in the cytoplasm, characterising them as primarily nuclear receptors. TR interaction with constitutively nuclear proteins, such as RXR and the NCoR, can stabilise TR in the nucleus. However, this does not occur at a steady rate. There are receptors that regularly shuttle from the cytoplasm to the nucleus or vice versa, and the frequency and direction of movement can be modulated by thyroid hormones through nuclear thyroid hormone receptors (TR), which modulate the expression of genes involved in cholesterol and lipid metabolism (bottom left). The non-classic pathway highlights the major target proteins of thyroid hormones in the cytosol: PI3K, Akt, MAPK and PKC, which contribute to the effect of T₃ on SREBP1 expression, and CaMKK and AMPK, which are involved in T₃-induced fatty acid (FA) oxidation. Additionally, T₃-activated MAPK phosphorylates TRβ, increasing its transcriptional activity and thereby also leading to nuclear effects. Full colour version of this figure available via http://dx.doi.org/10.1530/JOE-12-0542.
hormones (Baumann et al. 2001). The presence of TR in the cytoplasm is also defined by its interaction with anchoring cytoplasmic proteins. In pituitary and fibroblasts, a pool of TR remains in the cytoplasm interacting with phosphoinositide-3-kinase (PI3K), and in the presence of T3, this complex dissociates (Storey et al. 2006). Free PI3K stimulates other cytoplasmic proteins, such as Rac-GTPase, the activation of which is essential for the increase in KCNH2 potassium channel permeability induced by thyroid hormones. The new TR–T3 complex can shuttle to the nucleus and participate in the modulation of gene expression (Storey et al. 2006). Therefore, it is important to note that thyroid hormone action initiated by immediate non-classic mechanisms outside the nucleus may culminate in complex nuclear and cellular events (Davis et al. 2008) (Fig. 1). Therefore, thyroid hormone effects on cells may result from a synergism of the non-classic and the classic signalling pathways.

The importance of this issue is evident in cardiac physiology (Davis & Davis 2002, Axelband et al. 2011). Nonetheless, many manuscripts on thyroid hormone action in hepatic lipid metabolism or on non-classic thyroid hormone signalling pathway in the liver do not discuss each mechanism and their interconnections. This review aims to summarise recent reports about the non-classic mechanisms that mediate or are potentially involved in thyroid hormone control of lipid homeostasis.

Non-classic pathways of thyroid hormone actions in lipid homeostasis

Evidence of non-classic thyroid hormone signalling in liver is observed by the fact that the administration of a protein kinase inhibitor in embryonic chicken hepatocyte cell culture abolished T3-induced increase in the activity and expression of lipogenic enzymes, such as malic enzyme, FAS and acetyl-CoA carboxylase (Swierczynski et al. 1991).

Among non-classic mechanisms, modulation of second messengers or activation of specific proteins could contribute to thyroid hormone effects on lipid homeostasis. Hereafter, we highlight the main rapid signalling pathways involved in lipid metabolism and those of which are modulated by thyroid hormones.

Influence of thyroid hormones on the PI3K pathway

PI3K can be activated by receptor tyrosine kinase and many other types of cell surface receptors, including some that are G-protein linked (Cantley 2002). Interestingly, rather than proteins, this kinase phosphorylates inositol phospholipids at the third position of the inositol ring, generating specific lipids called phosphoinositides (Falasca & Maffucci 2006). These lipids can trigger numerous intracellular responses ranging from metabolic regulation to cell proliferation and survival (Hirsch et al. 2007). Proteins that interact with these phosphoinositides include 3-phosphoinositide-dependent protein kinase-1 (PDK1), Rac-GTPase, Akt and atypical protein kinase C \( \lambda/\xi \) (PKC\( \lambda/\xi \); Matsumoto et al. 2003, Deberardinis et al. 2006, Storey et al. 2006). Hyperthyroidism in humans or T3 injections in experimental conditions enhance hepatic lipogenesis (Diamant et al. 1972, Laker & Mayes 1981), which is the limiting step in the conversion of acetyl-CoA into long-chain saturated FAs (Wakil et al. 1983) and performed by the hepatic enzyme FAS using the coenzyme NADPH. Initially, the positive effects of thyroid hormones in lipogenesis were partially attributed to induction of FAS mRNA expression (Goodridge et al. 1989), an observation supported when a TRE was identified on the FAS promoter (Radenne et al. 2008). However, because PI3K and ERK1/2 or MAPK inhibitors attenuate the effect of T3 on FAS mRNA, phosphorylation events are involved in the transcriptional regulation of FAS in response to thyroid hormones (Radenne et al. 2008).

The mechanism by which thyroid hormones activate PI3K has been much studied. In various cell lines, TR interact directly with PI3K, specifically with the regulatory cytosolic subunit p85. In the presence of T3, this interaction is perturbed, and unbound PI3K can catalyse the production of phosphoinositides, suggesting recruitment of RAC-GTPase and Akt to the membrane (Storey et al. 2006, Cao et al. 2009). This mechanism has been previously described to promote neuronal survival (Cao et al. 2009) and increase the activity of KCNH2 in the pituitary cell line GH4C1 (Storey et al. 2006), suggesting that it may be associated with reduced cellular excitability and hormone secretion. Although the increased activity of the FAS promoter induced by T3 in hepatocytes was dependent on PI3K (Radenne et al. 2008), the interaction of TR-PI3K has not yet been observed in hepatocytes.

The PI3K–MAPK pathway is classically involved in the insulin signalling pathway, through which insulin activates hepatic lipid synthesis (Magan et al. 1997, Kim et al. 1998, Matsumoto et al. 2003). Together, insulin and T3 induce increased FAS mRNA expression and FAS activity to a higher level than either hormone individually (Radenne et al. 2008), suggesting that thyroid hormone action...
synergises with the insulin signalling cascade to activate anabolic pathways.

Similar to T3 modulation of FAS expression, T3 regulation of the sterol regulatory element binding protein-1c (SREBP-1c) in the liver involves different pathways. This transcription factor activates a set of genes, including FAS and acetyl-CoA carboxylase, resulting in the control of lipid synthesis in the liver (Magaina et al. 1997, Kim et al. 1998). Although the genomic effect of T3 is the down-regulation of hepatic mouse SREBP1 expression (Hashimoto et al. 2006b) and the up-regulation of hepatic human SREBP1 (Kawai et al. 2004), two other pathways classically associated with insulin signalling have also been associated with T3 regulation of human SREBP1. Recently, T3-induced SREBP1 expression in the human hepatocarcinoma cell line (Hep G) was attenuated by Akt or ERK inhibition (Gnoni et al. 2012). However, although the T3 effect on ERK activation persisted with the use of agarose-conjugated T3, Akt activation by T3 was abolished, suggesting that T3 interacts with a membrane surface receptor, such as integrin αvβ3, to activate ERK (Gnoni et al. 2012). Therefore, this report describes two independent non-classic pathways of thyroid hormones regulating SREBP1 in response to T3 using the integrin αvβ3 for the MAPK/ERK pathway and, most likely, the TR–PI3K complex for the PI3K/Akt pathway. Considering the essential role of Akt- and ERK-activated signalling pathways in SREBP1 expression, the activation of these enzymes may contribute to the increased expression of many lipogenic enzymes induced by T3.

Furthermore, there is evidence that PI3K/Akt activation may also be involved in the effect of T3 on FA oxidation. PI3K/Akt activation differentially controls expression of the enzyme carnitine palmitoyltransferase 1 (CPT1), the key enzyme of FA oxidation, in hematopoietic cells (Deberardinis et al. 2006) and in skeletal muscle cells (de Lange et al. 2008). In hematopoietic cells, Akt activation induced by IL3 down-regulates CPT1 (Deberardinis et al. 2006), whereas Akt activation induced by T3 in skeletal muscle cells up-regulates CPT1, modulating FA oxidation (de Lange et al. 2008). The disparate effects of Akt activation on CPT1 expression may be a cell-type-specific response, although in hepatocytes, more investigation of the Akt effect on CPT1 expression and on other oxidative enzymes is necessary. Although data are currently scarce, the PI3K pathway is a potential candidate that contributes to thyroid hormone regulatory actions on hepatic lipid metabolism.

Influence of thyroid hormone on the adenyl cyclase – cAMP – PKA pathway

In the presence of a ligand, membrane surface Gs protein-coupled receptors (GPCR) replace GDP with GTP, leading to activation of adenyl cyclase with consequent increased levels of cAMP. cAMP effects are mediated by cAMP-dependent protein kinase (PKA), which phosphorylates target proteins (Tasken & Aandahl 2004).

As early as Nakamura et al. (1983) identified that thyroid hormones increased the activity of type I PKA in the rat liver. This is also true in other cell types because T3 promotes an increase in the phosphorylation state of PKA, activating this protein (Shih et al. 2004). Thyroid hormone-induced PKA activation appears important for TR transcriptional activity because PKA can trigger the activation of the MAPK pathway, which can lead to phosphorylation of TRβ1. This structural change leads to TRβ1 dissociation from the corepressor-silencing mediator of retinoid and thyroid hormone receptors (SMRT), increasing TR transcriptional activity (Davis et al. 2000). These observations may explain how PKA activators generally potentiate thyroid hormone effects (Leitman et al. 1996, Lin et al. 1996a).

The relevance of PKA signalling to hepatic lipid metabolism is highlighted by animal models with higher PKA activity, which protects against diet-induced fatty liver (Cummings et al. 1996). In primary human hepatocytes, active PKA, induced by glucagon or cAMP, phosphorylates hepatocyte nuclear factor 4α (HNF4α), which loses affinity for the CYP7A1 promoter region and consequently does not stimulate transcription (Song & Chiang 2006). Additionally, PKA phosphorylates specific sites on hepatic SREBP1, which greatly suppresses transactivation of this protein and leads to inhibition of SREBP-mediated lipogenesis in humans (Lu & Shyy 2006) and murine hepatocytes (Yamamoto et al. 2007). In rat hepatocytes, PKA directly phosphorylates LXRα protein and inhibits signalling, resulting in the suppression of SREBP1c transcription both in vitro and in vivo (Yamamoto et al. 2007), highlighting the influence of PKA on SREBP1c expression and on the activity of nuclear receptors, such as LXR and TR. Because T3 enhances the expression of SREBP1 (Shin & Osborne 2003) and PKA reduces SREBP1 activity (Lu & Shyy 2006), therefore, a role for PKA as a mediator of thyroid hormone action might be controversial. It was also observed that increasing cAMP levels suppress T3-induced increase in SREBP1 abundance in chicken embryo hepatocytes (Zhang et al. 2003). Nevertheless, this suggests that the net effect will depend on the...

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-12-0542
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set of factors present in the cellular environment, leading to varying degrees of PKA activation.

**Influence of thyroid hormones on the phospholipase C–inositol triphosphate, DAG – calcium and PKC pathway**

When a G-protein-coupled receptor is activated, it triggers a conformational change that activates the Gq protein, stimulating phospholipase C-β (PLC-β), which converts phosphatidylinositol 3,4,5-trisphosphate (PIP3) to inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 molecules trigger calcium release from endoplasmic reticulum stores, intensifying the activation of PKC induced by DAG (Berridge 1993).

The importance of calcium in regulating biological processes has been appreciated for over 120 years (Gaspers & Thomas 2005). Periodic fluctuations or spikes in cytosolic free calcium are a widespread signalling mechanism utilised by extracellular stimuli to exert control over cell physiology in both excitable and non-excitable tissues (Williamson & Monck 1990). Calcium ions can alter some proteins’ function by direct interaction, as in some intra-mitochondrial dehydrogenases (McCormack et al. 1990), or indirectly, through calcium-binding proteins, such as calmodulin (Williamson & Monck 1990). Furthermore, calcium can also influence transcriptional regulators and alter gene expression (Gaspers & Thomas 2005).

Calcium was one of the first second messengers discovered to be regulated by thyroid hormone. For instance, hepatic mitochondria from thyroidectomised rats accumulate calcium upon addition of T₃ (Herd 1978). Segal & Ingbar (1984) also reported a transient increase in cytosolic free calcium in rat thymocytes exposed to T₃. Additionally, livers from fasting rats incubated with T₃ showed an increase in cytosolic calcium and, simultaneously, activation of respiration and gluconeogenesis. These effects were abolished when calcium levels in the extracellular medium were low, indicating the dependence of T₃ action on extracellular calcium (Hummerich & Soboll 1989). Previously, thyroid status was demonstrated to be positively correlated with the mRNA level of the ryanodine channel in the cardiac muscle, where this receptor is mainly regarded as the principal site of calcium release from the sarcoplasmic reticulum, controlling contraction (Dillmann 2010). Pierobon et al. (2006) suggested the existence of a novel ryanodine receptor in rat hepatocytes that would modulate oscillations of the cytosolic calcium pool, but as far as we know, there is no evidence that thyroid hormones regulate this receptor in the liver.

Yamauchi et al. (2008) elegantly demonstrated with a fluorescent dye technique that T₃ increased calcium intracellular levels within a few seconds, which was essential for the increase in FA oxidation induced by thyroid hormones. Administration of T₃ to HeLa cells promoted a rapid increase in cytosolic calcium, which activated the calcium/calmodulin-dependent protein kinase (CaMKKβ). CaMKKβ, in turn, phosphorylates AMPK-activated protein kinase (AMPK), a serine/threonine kinase that acts as an energy sensor in the cell. This is the limiting step in the inactivation of acetyl-CoA carboxylase, leading to reduction of malonyl CoA content that permits CPT1 activation, favouring lipid oxidation. The T₃ effect on AMPK activation and acetyl-CoA carboxylase expression is attenuated in the presence of a calcium chelator, abolishing T₃-induced FA oxidation (Yamauchi et al. 2008). Because T₃ effects on FA oxidation are dependent on cytosolic calcium increase, this second messenger emerges as an important rapid mechanism for T₃ action on lipid metabolism.

The activation of a Gq-coupled receptor promotes increased intracellular calcium concentration and DAG levels. The latter is another second messenger that is rapidly regulated by thyroid hormones. Kavok et al. (2001) demonstrated that 1-thyroxin administration promoted a biphasic increase in intracellular DAG content in isolated hepatocytes and in liver slices minutes after T₄ application. Using specific inhibitors of PLC and phospholipase D, the authors proposed that these proteins play a role in the thyroid hormone-induced increase in cytosolic DAG. DAG activates PKC, which plays a central role in signal transduction by phosphorylating an array of substrates on serine/threonine residues, including cell surface receptors, enzymes, contractile proteins, transcription factors and other kinases (Idris et al. 2001). Lin et al. (1996b) demonstrated that thyroid hormone can potentiate interferon-gamma-induced antiviral states in a PKC-dependent manner. In Hep G cells, T₃ causes a time-dependent PKC-α cytosol-to-membrane translocation after 5 min and up to 10 min of treatment, and this activation attenuates the stimulatory effect of T₃ on SREBP1 expression (Gnoni et al. 2012). Similarly, thyroid hormone application to cultured myoblasts promoted PKC translocation from the cytosol to the membrane in a PLC-dependent manner (D’Arezzo et al. 2004).
**Conclusion**

The net thyroid hormone effect on lipid metabolism is primarily caused through the classic mechanism, based on changes of the transcription of important lipogenic and lipolytic enzymes by interaction with nuclear TR. Presently, it is known that non-classic signalling pathways also contribute to thyroid hormone metabolic actions, even though the physiological context is still unclear. These mechanisms have been intensively studied, and many proteins involved with the intracellular pathways activated by different membrane receptors contribute to thyroid hormone effects. In this report, we summarised the main second messengers and signalling proteins influenced by T3, including calcium, PI3K, Akt and MAPK, the activation of which culminates in modulation of hepatic lipid metabolism. We also highlight other proteins that participate in these pathways that should be investigated as potential thyroid hormone targets on lipid metabolism. These intracellular pathways not only induce rapid actions in the cell but also increase the expression of transcription factors that can contribute to the classical nuclear thyroid hormone mechanism of action. Therefore, the non-classic signalling pathways of thyroid hormone action are not occasionally activated; on the contrary, they emerge as important accessory mechanisms in thyroid hormone action.

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Received in final form 30 November 2012
Accepted 7 January 2013
Accepted Preprint published online 7 January 2013