TSH receptor activation and body composition

Anna de Lloyd1,*, James Bursell2,*, John W Gregory2, D Aled Rees1 and Marian Ludgate1

1Centre for Endocrine and Diabetes Sciences and 2Department of Child Health, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK
(Correspondence should be addressed to M Ludgate; Email: ludgate@cf.ac.uk)
*(A de Lloyd and J Bursell contributed equally to this work)

Abstract

The impacts of hyper and hypothyroidism on body composition, i.e. the relative quantity and quality of bone, adipose tissue and muscle, have traditionally been attributed uniquely to abnormal levels of free thyroid hormones. The presence of biologically active TSH receptors in bone, fat and muscle, raises the possibility that both thyroid hormones and TSH contribute to the changes in body composition associated with thyroid disease. This review evaluates the evidence for this in terms of the in vitro experimental approaches applied, data from in vivo sources (i.e. mouse models) and patient-based studies.

The TSH receptor and its role in the thyroid

The established biological function of the TSH receptor (TSHR) in the thyroid gland is to regulate synthesis and secretion of thyroid hormones from follicular thyroid cells; it also plays an important role in controlling the growth and development of the thyroid gland (Vassart & Dumont 1992). The TSHR is a G protein-coupled receptor and shares the classic structure of the serpentine receptor family (i.e. seven membrane spanning segments, three extracellular loops, three intracellular loops, an amino terminal ectodomain and an intracellular carboxy terminal). The hormonal binding specificity of the receptor is determined by the ectodomain (or a subunit; Kleinau & Krause 2009) whilst coupling to the G protein is via the serpentine portion.

The TSHR is encoded by ten exons located on chromosome 14 and is coupled mainly to the a subunit of the stimulatory guanine-nucleotide-binding protein. In the thyroid, ligand binding predominantly activates adenylate cyclase with a resultant increase in the intracellular concentration of cAMP. Stimulation of the TSHR via this cAMP second messenger system regulates the transcription of genes central to thyroid hormone synthesis. Recent studies have illustrated the potential heterogeneity of signalling via the TSHR, either as the consequence of coupling to other G proteins (Gq/G11; Kero et al. 2007) or as a result of cascades stimulated by the liberated G protein β/γ subunits (Zaballos et al. 2008). Thus, TSHR activation can up-regulate kinases such as phosphoinositide-3 kinase (Zaballos et al. 2008) and P70S6K (Cass & Meinkoth 1998) and increase concentrations of the second messengers inositol-phosphate (IP) and diacylglycerol.

Chronic stimulation of the TSHR leads to over activation of the cAMP pathway that in turn causes thyroid hyperplasia and hyperthyroidism. This process occurs in Graves’ disease (GD), the commonest cause of hyperthyroidism in which thyroid stimulating antibodies (TSAB) bind the receptor and mimic the action of TSH (Prabhakar et al. 2003). When TSH or TSAB bind to the receptor, they induce a conformational change such that the ectodomain is transformed from a tethered inverse agonist into a full agonist of the receptor’s serpentine portion (Vlaeminck-Guillem et al. 2002). This model can also account for a ligand-independent pathogenetic mechanism of receptor activation; gain-of-function mutations. When these are somatic, they cause thyroid toxic adenoma (Parma et al. 1993) but in the germline, they produce familial non-autoimmune hyperthyroidism (Duprez et al. 1994).

More than 30 point mutations that result in increased constitutive activity in the receptor have been described (Paschke & Ludgate 1997). These mutations are located predominantly in exon 10, which encodes the serpentine portion of the TSHR. All activating mutations induce an increase in cAMP levels in the absence of TSH but retain TSH responsiveness. The phenotype can vary according to the specific germline mutation but also between individuals harbouring the same point mutation (for example the age of onset of hyperthyroidism). This variation is likely to reflect epigenetic and environmental factors in addition to the
inherent biological activity of the particular mutant form (e.g. a minority of mutations will increase both cAMP and IP3 concentrations (Fuhrer et al. 2003). Gain-of-function mutations require only one affected allele to induce hyperthyroidism whereas both receptor alleles must be affected for hypothyroidism to occur (homozygous hyt/hyt mouse (Stein et al. 1994)).

A number of families have now been identified that are affected by loss-of-function TSHR mutations (Sunthorntheprakul et al. 1995). These families have been found to exhibit varying degrees of TSH resistance (as is reflected in the thyroid function test results; extent of the TSH increase and/or thyroid hormone deficiency), which correlates with the clinical phenotype. Individuals with partial resistance to TSH usually retain some TSHR function (Alberti et al. 2002, Jordan et al. 2003), whereas those with complete resistance to TSH do not. TSH responsiveness ultimately dictates the extent of the hypothyroidism and the degree of thyroid gland hypoplasia incurred.

**TSHR expression in tissues other than the thyroid**

In recent years TSHRs have been identified in a number of tissues including brain, testes, kidney, heart, bone, thymus, lymphocytes, adipose tissue and fibroblasts (reviewed in Davies et al. 2002). This suggests that the TSHR may have a wider functional role than is traditionally recognised.

Apart from the earliest studies, which investigated a function for TSH outside the thyroid, much of the recent evidence has been obtained using the PCR. There are several inherent problems with PCR: its sensitivity allows generation of a product even when the target mRNA is present at very low levels (care is therefore required to avoid amplifying from genomic DNA). Also, the presence of a transcript product does not necessarily signify functional protein and finally as PCR is often applied to whole tissues, which are heterogeneous in nature, expression cannot be attributed to a specific cell type. These considerations aside, if the TSHR is expressed at significant levels in tissues outside the thyroid then individuals bearing germline TSHR mutations would be expected to reveal its functional significance; examples will be given in the relevant sub-sections below.

**TSHR and adipose tissue**

Adipose tissue is clearly altered in the hypo- or hyperthyroid state. Weight gain occurs in hypothyroidism whereas thyrotoxic patients display weight loss attributed to increased lipolysis consequent to thyroid hormone-induced upregulation of adrenergic receptors (Haluzik et al. 2003). A role for TSH in this process was first suggested by Rodbell (1964) who reported that TSH triggers lipolysis in rat epididymal fat – however this mechanism would decrease fat mass and thus not accord with the typical hypothyroid phenotype. Winand & Kohn (1972) described high-affinity TSH binding sites in guinea pig orbital tissue (predominantly fat) leading to a TSH-induced increase in cAMP, which is consistent with its lipolytic action. The application of molecular techniques has confirmed the presence of TSHR in rodent fat, including white and brown adipose tissues (BAT), implicating a role for TSH in regulating thermogenesis (Roselli-Rehfuss et al. 1992). The extensive experiments of Haraguchi et al. (1996a,b) revealed that the TSHR is expressed (at levels similar to those found in the rat thyroid) during lineage-specific differentiation of preadipocyte precursors into mature fat cells. Very recent studies reported that TSHR deficient hyt/hyt mice became hypothermic in cold conditions despite thyroxine (T4) administration. Transfection of TSHR into the BAT of these mice resulted in a marked improvement in core temperature, leading to the conclusion that both a functional TSHR and adequate free thyroid hormones were required for normal temperature regulation (Endo & Kobayashi 2008).

It is unclear to what extent these findings can be extrapolated to human subjects where BAT makes up 5% of the body weight of neonates (Lean et al. 1986) and declines further in adults. However, the use of fluorodeoxyglucose-positron emission tomography has revealed several functional BAT depots in adult humans (Nedergaard et al. 2007). These findings have recently been confirmed using cold-induced glucose uptake in human subjects, which identified BAT depots in the head and neck regions (Virtanen et al. 2009).

As in rodents, the presence of a functioning TSHR in human fat tissue was initially suggested by experiments performed on neonatal adipocytes in which TSH and TSAB were shown to mediate lipolysis in vitro (Marcus et al. 1988, Janson et al. 1995). By a few weeks of age adipocyte sensitivity and responsiveness to TSH declined markedly and catecholamines became the primary mediator of lipolysis (as is the case in adults). In keeping with this finding there is known to be a surge in TSH levels occurring in term infants in the first days of life (a 50-fold physiological rise in TSH levels; Oddie et al. 1978). This is believed to drive lipolysis and generate free fatty acids that serve as the primary energy substrate in the first days of life before breast feeding has become established.

In more recent studies, Crisp et al. (1997) applied northern blot analysis to demonstrate TSHR transcripts in several human adult fat depots including abdominal and orbital sites. This work confirmed earlier PCR based studies, which had indicated increased TSHR expression in orbital fat (Feliciello et al. 1993) from patients with Graves’ ophthalmopathy (GO). In this orbital condition, there is expansion of the orbital contents which results in proptosis (Ludgate & Baker 2002). Subsequent reports from several (but not all) groups revealed that, as in rodents, TSHR expression is upregulated during adipogenesis and the higher level of receptor transcripts in GO orbital fat is the result of this process (Valyasevi et al. 1999, Starkey et al. 2003). However this phenomenon is not unique to orbital fat but occurs in any fat depot undergoing differentiation and is accompanied by increased cAMP in response to TSH activation (Crisp et al. 2000). In contrast to
rodents, the expression level of TSHR in human adipose tissues is always less than in the thyroid. There have been suggestions that receptor abundance might influence downstream signalling and if true, this could explain the identification of non-cAMP mediated TSH signal transduction (e.g. involving the p70S6 kinase, Bell et al. 2000).

Since TSHR expression is highest during adipogenesis several groups have investigated a role for TSHR activation in the process. The findings of these groups have differed and depended upon the preadipocyte source. Zhang et al. (2006) introduced activating mutant TSHRs into human preadipocytes from normal orbit and GO tissue. TSHR activation stimulated the early stages of adipogenesis but inhibited the terminal stages of differentiation. The absence of lipid-filled droplets despite increased cellular lipid content suggested that either lipolysis had been upregulated, or that the cytoskeletal changes necessary for droplet formation had been inhibited. Lipoprotein lipase transcripts remained at the limit of detection in cells expressing activating mutant TSHR, favouring the latter mechanism. The results agree with earlier reports of cAMP elevation rapidly reducing a suppressor of adipogenesis, Wnt, thereby promoting differentiation (Bennett et al. 2002). In contrast, prolonged elevation of cAMP by pharmacological agents reduced accumulation of lipids in the murine 3T3L1 preadipocyte cell line, by decreasing key lipogenic enzymes rather than increasing lipolysis (Spiegelman & Green 1981). Studies performed in our laboratory demonstrated that Gsα signalling (increases cAMP) impedes FOXO1 phosphorylation and inhibits PPARG transcription and the alternative promoter usage required to generate PPARG2, the fat-specific transcription factor necessary for adipogenesis (Zhang et al. 2009). However, in mouse embryonic stem cells TSH stimulated adipogenesis (Lu & Lin 2008).

Orbital fat is distinct from other adipose tissue and has been found to have features in common with BAT. This is exemplified by mice that overexpress adiponectin, which resulted in expansion of both the orbital and the intra-scapular brown-fat pad (Combs et al. 2004). There have been reports of infants with activating TSHR mutations being born with proptosis (De Roux et al. 1996). Although controversial, this may be the result of increased orbital adipogenesis occurring as a consequence of the activating receptor mutation (a similar phenomenon to that occurring in GO; Sorisky et al. 1996). However, in the absence of appropriate magnetic resonance imaging scans to confirm fat expansion in these children, this remains a hypothesis.

Preadipocytes resemble fibroblasts and are mesenchymal stem cells (MSC) that are present in fat tissue and its stroma. MSC are able to differentiate along several distinct lineages including adipocyte, osteoblast, chondrocyte and myocyte, all of which are central contributors to body composition (Schaffler & Buchler 2007). The differences in the impact on adipogenesis observed between embryonic stem cells and the murine cell line suggest a role for TSHR activation in lineage commitment, rather than terminal differentiation per se (Fig. 1).

Most receptor activation is due to circulating TSH, whose levels decrease following food restriction, although this can be prevented by leptin administration (Seoane et al. 2000), implying a role for adipose tissue itself in regulating the hypothalamic/pituitary/thyroid axis (Bluher & Mantzoros 2004). Although the effects are clear cut in rodent studies, the situation in humans is far from clear with contrasting results being reported even when seeking an association between serum TSH and body mass index (BMI; Manji et al. 2006, Nymes et al. 2006).

**TSHR and muscle**

Thyroid disease is known to affect muscle function. Hyperthyroidism can lead to skeletal muscle weakness, wasting, cardiac myopathy and heart failure. The extra-ocular muscles are affected in GD where inflammation causes hypertrophy and eventually fibrosis, which may result in restricted motility. Hypothyroid patients also suffer muscular involvement including myalgia, weakness and exercise intolerance. It is thus appropriate to investigate whether the TSHR plays a role in the pathogenesis of these muscle disorders.

The presence of TSHR in the extraocular muscles of GD patients has been established using high-stringency PCR and in situ hybridisation techniques (Busuttin & Frauman 2001), which failed to detect TSHR mRNA in either the abdominal or cardiac muscle of these patients. These results differ in their findings from other studies using immunohistochemistry that failed to demonstrate TSHR expression in the extraocular muscles of GO patients (Spitzweg et al. 1997). In general, the evidence for TSHR in extraocular muscle is less abundant than the data demonstrating TSHR in adipose tissue. The selective expression of TSHR in extraocular muscle reported by Kloprogge et al. (2005) contrasts with the more widespread nature of TSHR expression in various GD fat depots (Starkey et al. 2003). The extraocular muscle enlargement in GO has previously been considered to be the result of inflammation and oedema rather than hyperplasia or hypertrophy of the myocytes themselves (Hufnagel et al. 1987).
Contradictory results have also been reported for the presence of the TSHR in cardiomyocytes. Drvota et al. (1995) used northern blots and in vitro cultures to demonstrate a functional TSHR in heart muscle. However, these conclusions were refuted by Busuttil & Frauman (2001) who suggested ‘that certain confounding positive tissues, rather than myocardial tissue itself, may have contributed to the positive results’. This suggestion certainly fits with the report of Spitzweg et al. (1997) who applied immunohistochemistry to show that the TSHR was expressed on perimysial fibroblasts within extracellular muscle, but not in extracellular muscle fibres themselves. Boschi et al. (2005) obtained similar results, concluding that in GO orbits TSHR was expressed on the fibroblast-like cells positioned between myocytes, but not on the myocytes.

Taken together it is likely that the expression of TSHR in muscles from various locations is due to fibroblast-like cells existing within the muscles, which are likely to represent MSC. Co-localisation experiments using antibodies to MSC surface markers (e.g. CD34, Sca1 and the TSHR) are required to confirm or refute this hypothesis.

**TSHR and bone**

Mammalian skeletal remodelling occurs as old bone is resorbed by the action of osteoclasts and is replaced by new bone deposited by osteoblasts. Any disturbance or uncoupling in the equilibrium between these two dynamic processes will lead to either excessive and disordered bone formation or a higher rate of unresorbed bone loss. Thyroid disease is known to affect this balance; hyperthyroidism is associated with a reduction in bone mass and osteoporosis (Greenspan & Greenspan 1999) and even a history of previous hyperthyroidism increases the risk of bone disease associated with thyroid dysfunction (Williams 2009). This postulate is supported by data from mice lacking a functional TSHR and type 2 iodothyronine deiodinase (D2) in human osteoblasts (NHOst) and osteosarcoma (SaOS-2) cell lines. D2 (which converts T4 to the biologically active T3) is regulated by the TSH-receptor/cAMP pathway (TSHR mRNA was demonstrated in the cell lines by PCR). Thus, it seems that TSH may influence bone remodelling directly, as well as indirectly through its effect on D2 and the T3 levels.

The argument for a direct role of TSH in bone metabolism and skeletal remodelling was strengthened by experiments performed on mice homozygous and heterozygous for a functional TSHR and body composition (Achilles et al. 2010). TSHR−/− mice were hypothyroid with normal T3, T4 and TSH levels, grew and reproduced normally although with a significantly reduced BMD. Thyroid hormone supplementation improved body weight but not bone mass, bone length or BMD. Heterozygotes +/− mice, which had normal T3, T4 and TSH levels, grew and reproduced normally although with a significantly reduced BMD. In heterozygotes and homozygous TSHR−/−, mice the reduced BMD was the consequence of increased bone turnover. Ex vivo bone marrow cultures showed a two-fold increase in osteoclast formation in cells from TSHR+/− and −/− mice as well as evidence of upregulated osteoblast differentiation. Experiments demonstrating TSH inhibition of osteoclast formation and survival, as well as independent inhibition of osteoblast differentiation, further demonstrated a possible role of TSH in bone metabolism and were consistent with TSHR expression being maximal during the early and mid phases of osteoblast and osteoclast formation.

This work of Abe et al. provides strong evidence for a fundamental role for TSHR in skeletal modelling. A 50% reduction in TSHR expression produced osteoporosis and focal vertebral body osteosclerosis despite normal thyroid function. This group argue that their data support the hypothesis that bone disease associated with thyroid dysfunction may be due to altered TSH levels rather than changes in serum thyroid hormone levels per se (Sun et al. 2006). Interestingly, our own unit has reported brothers with congenital hypothyroidism, homozygous for W546X (Jordan et al. 2003; i.e. the human equivalent of the TSHR−/− mice). One of the boys has developed a benign osteoblastoma on his radius, and we speculate that this mirrors the focal sclerosis seen in the TSHR−/− mice (M Ludgate & J W Gregory, unpublished observation).
Other authors (Galliford et al. 2005) however have suggested that the reduction in BMD in TSHR heterozygote +/− and homozygous −/− mice despite thyroid hormone supplementation may not reflect a role of TSHR but rather a delay in ossification and growth impairment secondary to antenatal hypothyroidism (or the period of hypothyroidism prior to receiving thyroid replacement at 3 weeks of age). This period of foetal hypothyroidism in TSHR −/− mice may have resulted in an increased sensitivity to thyroid hormone as is described in mice with complete deficiency of thyroid hormone receptor that were then given thyroid replacement (Macchia et al. 2001). Thus, the changes noted following supplementation at weaning might reflect a disproportionately large response to T3 and T4 rather than effects of TSHR deficiency.

Subsequent studies have also cast doubt upon the putative role of the TSHR in bone metabolism. Studies using hyt/hyt mice (elevated TSH and inactive TSHRs) that have a point mutation in the Tshr gene and Pax 8 −/− mice (grossly elevated TSH levels with normal TSHR function and thyroid agensis) suggest that the skeletal phenotype of both sets of hypothyroid mice was independent of TSH levels (Bassett et al. 2008). Both groups of mice demonstrated a significant reduction in bone length and micro-mineralisation and a significant increase in growth plate total width, reserve and proliferative zones.

Using mice that lacked thyroid receptor TRα or TRβ isoforms, Bassett et al. (2007), demonstrated that bone loss in thyrotoxic mice was a result of raised circulating free T3 levels and independent of circulating TSH concentrations. Adult TRα null mice had osteosclerosis despite normal levels of TSH and thyroid hormones, whereas TRβ null mice had osteoporosis despite elevated TSH and thyroid hormones. The authors postulated that the changes in bone morphology were mediated principally by the thyroid hormone receptor.

Murphy et al. (2006) investigated the effects of TSH and monoclonal TSAB on mouse and hOBs and osteoclasts. Treatment with TSH or TSAB failed to produce evidence of TSHR activated intracellular signalling as measured by cAMP levels. These authors also used recombinant thyrostimulin, a hormone having high affinity for the TSHR (the product of genes for α-2 and β-5 glycoprotein hormone subunits, Nakabayashi et al. 2002), again without effect. These data question whether the TSHR is involved in bone remodelling and, if it is implicated, the signalling pathway in operation in the absence of a cAMP response.

The role of TSH and the TSHR in bone metabolism however is strengthened by data from human subjects with a TSHR Asp727Glu polymorphism. In vitro studies demonstrate an increased cAMP response to TSH but in vivo expression of this polymorphism is associated with reduced circulating TSH levels despite T4 and T3 within the normal range. Individuals heterozygous for TSHR Asp727Glu were found to have increased femoral neck BMD and bone mineral content (Van Der Deure et al. 2008). These data suggest that the polymorphism is associated with a relative TSHR gain-of-function, which has itself affected bone turnover.

Recent work by Sampath et al. (2007) in ovariectomised rats demonstrated that TSH administration increased BMD, trabecular bone volume, trabecular thickness, cortical thickness and enhanced mechanical strength, compared to controls. These changes were mediated by inhibition of osteoclast differentiation and also by activation of osteoblast differentiation. In an extension of this work, Sun et al. (2008) reported that TSH therapy administered as infrequently as once per fortnight displays powerful anti-resorptive action in the bones of ovariectomised rodents.

Effects of TSHR dysfunction on body composition

The potential wider systemic effect of TSHR dysfunction upon body composition rather than just focal bone or adipose changes has been raised by the findings of Vaidya et al. (2004). They assessed the prevalence of premature birth and low birth weight in families with non-autoimmune hypothyroidism and hyperthyroidism secondary to loss-of-function and gain-of-function TSHR mutations respectively. They found that individuals with activating mutations were more likely to be born prematurely (80% compared to 6% of children with inactivating TSHR mutations) and that they were significantly smaller (2338 g compared with 3470 g (P=0.004)). This finding of lower birth weights was not considered to be explained solely by the earlier gestation as they were also smaller as determined by centiles for birth weight (28-4% compared with 49-1%). However, any effect of possible foetal thyrotoxicosis upon premature delivery and foetal birth weight was not determined in this study.

As mentioned earlier, serum TSH levels have been shown to be positively associated with BMI (Nyrnes et al. 2006). However, this was not the finding of others including Peeters et al. (2007); who had assessed a population of euthyroid elderly men. Instead, they reported an association between TSH levels and serum leptin levels within this cohort. This finding again contrasts with the work of Miyakawa et al. (1999), who found that free T4 and TSH did not correlate with serum leptin levels in patients with thyroid disease (GD and Hashimoto’s thyroiditis). Percentage fat mass assessed by bioelectrical impedance was lower in GD patients and higher in hypothyroid patients compared with controls thus reiterating the changes in body composition that are associated with thyroid disease. However, percentage fat mass was not directly related to thyroid hormone or TSH levels.

The hypothesis that activation of the TSHR affects body composition would predict that individuals with germline mutations in the TSHR might demonstrate differences in parameters of body composition such as height, weight, BMI, BMD and body fat. Baker et al. (2004) have reported detailed body composition data using dual energy X-ray
absorptiometry and skin fold measurements in three individuals rendered biochemically euthyroid after treatment for hyperthyroidism, which had been caused by three different gain-of-function mutations. The data demonstrated reduced body fat content in these three children compared with that seen in other unaffected family members. Unfortunately a number of potential confounding factors may have contributed to the changes in BMI and body composition observed in these cases (i.e. nutritional, social and psychological factors, changes associated with age group and the wide variance seen within the normal population) therefore deciphering the contribution made by altered TSHR activity per se is very difficult. Much larger observational studies are required to draw more meaningful conclusions; however, these data lend support to the hypothesis that changes in TSHR activity may influence adipose tissue, muscle and bone sufficiently to affect body composition.

Conclusions

The role of the TSHR in controlling thyroid function is well established and more recent data have confirmed the presence of the TSHR in tissues relevant to body composition. Furthermore, modifications in TSHR activity have been demonstrated to be associated with changes in bone and adipose tissue metabolism (at all stages from neonate to old age) in agreement with the well-characterised phenotype that is the consequence of thyroid dysfunction. The inverse relationship that exists between adipogenesis and osteogenesis is supported by the association of fatty bone marrow and low BMD (Meunier et al. 1971). This suggests that TSHR activation may play a role in determining MSC lineage commitment and therefore influence body composition directly and independently of thyroid hormone level. The signalling cascades responsible may be distinct from the cAMP pathway that predominates in the thyroid. This merits further investigation as thyroid dysfunction is common and affected individuals may be at risk of complications as a consequence of TSAB in GD or altered TSH in subclinical or clinical thyroid disease.

Declaration of interest

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

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Reference


Journal of Endocrinology (2010) 204, 13–20


www.endocrinology-journals.org


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Received in final form 28 August 2009
Accepted 16 September 2009
Made available online as an Accepted Preprint 16 September 2009