Effects of thyroid hormones on thermogenesis and energy partitioning

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Abstract

Thyroid hormones (TH) are of central importance for thermogenesis, energy homeostasis and metabolism. Here, we will discuss these aspects by focussing on the physiological aspects of TH-dependent regulation in response to cold exposure and fasting, which will be compared to alterations in primary hyperthyroidism and hypothyroidism. In particular, we will summarise current knowledge on regional thyroid hormone status in the central nervous system (CNS) and in peripheral cells. In contrast to hyperthyroidism and hypothyroidism, where parallel changes are observed, local alterations in the CNS differ to peripheral compartments when induced by cold exposure or fasting. Cold exposure is associated with low hypothalamic TH concentrations but increased TH levels in the periphery. Fasting results in a reversed TH pattern. Primary hyperthyroidism and hyperthyroidism disrupt these fine-tuned adaptive mechanisms and both, the hypothalamus and the periphery, will have the same TH status. These important mechanisms need to be considered when discussing thyroid hormone replacement and other therapeutical interventions to modulate TH status.

Introduction

Thyroid hormones (THs) are key in regulating energy homeostasis in both humans and rodents. Maintaining core body temperature is critical for homeothermic species and thermoregulation requires fine-tuned energy partitioning. Here, we will summarise major components of thermoregulation as well as TH-mediated effects on these. We will discuss the contribution of the central nervous system (CNS) as well as peripheral organs and tissues to these processes. Further, we will summarise the effects of (i) cold or heat exposure and (ii) fasting or fed states on metabolic processes. Finally, the disruption of these fine-tuned mechanisms by hyperthyroidism and hypothyroidism will be addressed.

Components of thermoregulation

In homeothermic species, thermal regulation is composed of a number of mechanisms designated to tightly control core body temperature to ensure optimal regulation of metabolic processes. In cooler environments endogenous, activity-dependent heat production of energy demanding metabolic cycles needs to be balanced with heat loss from...
the body surface. Insulation of the body by fur represents an important mechanism to control heat loss (Cannon & Nedergaard 2011, Kasza et al. 2014, 2016, Alexander et al. 2015, Fischer et al. 2016), while the insulating effects of intradermal fat has been questioned at least in rodents (Fischer et al. 2016). Vasocostriction or vasodilatation serves as additional major mechanisms to regulate body temperature via heat loss or conservation by peripheral body parts (Tansey & Johnson 2015). Further, behavioural changes may help to defend core body temperature in heat or cold exposure (Terrien et al. 2011), which is in humans reinforced by the option to change clothing and control the immediate environment by applying heaters or air-conditioning.

Heat production of the body is based on obligatory and facultative thermogenesis. Within the thermoneutral zone (TNZ), obligatory thermogenesis of basal metabolism is sufficient to maintain body temperature. A decrease of ambient temperature below the TNZ activates cold-sensitive thermoreceptors of the skin, reinforces cutaneous vasoconstriction and piloerection to optimise insulation and induces facultative thermogenesis, which consists of shivering and non-shivering thermogenesis.

Shivering thermogenesis is characterised by an activation of involuntary muscle activity to produce heat (Silva 2006), whereas non-shivering (or adaptive) thermogenesis depends predominantly on the activation of brown adipose tissue (BAT) (Cannon & Nedergaard 2004). Non-shivering thermogenesis is mainly initiated by a cutaneous cold-sensation, which via cold-sensitive thermoreceptors activates the sympathetic nervous system (SNS) (Nakamura & Morrison 2008). Subsequently, the SNS stimulates the release of noradrenaline from postganglionic sympathetic fibres and induces β3-adrenergic receptor (ARB3)-mediated pathways that activate lipolysis and the release of free fatty acids (FFA, reviewed in Oelkrug et al. 2015). FFA stimulate mitochondrial energy dissipation by activation of the uncoupling protein 1 (UCP1) in the inner mitochondrial membrane. In the inactivated state, UCP1 is constitutively inhibited by purine nucleoside di- and triphosphates, but the release of FFA overcomes this inhibition (Heaton et al. 1978). Activated UCP1 acts as a proton channel, allowing protons to bypass the proton pathway commonly used by ATP synthase and thereby dissipates the proton motive force as heat instead of ATP; the exact mechanism, however, has not been resolved so far (Crichton et al. 2017). Additionally, UCP1 provokes the activation of the respiratory chain and cellular fatty acid oxidation to compensate for the loss in produced ATP (Ricquier 2017). UCP1 activity is essential for BAT-mediated facultative thermogenesis, which is clearly shown by the thermogenic phenotype of UCP1-ablated mice (Enerback et al. 1997, Golozoubova et al. 2001). Furthermore, UCP1 activity has been shown to be important for maintaining a well-balanced energy homeostasis, as UCP1-knockout mice gain more body weight compared to controls when kept on a high-fat diet at thermoneutral conditions (Feldmann et al. 2009). The effect of non-shivering, facultative thermogenesis is particularly obvious in small rodents which have an unfavourable relation of body surface area to body volume and are thereby extremely sensitive to changes in ambient temperature. In these animals, cold exposure to 4°C increases oxygen consumption by 2- to 4-fold, mainly via an increase in efferent sympathetic activity to BAT and muscle (Lowell & Spiegelman 2000). While it was long believed that BAT was restricted to rodents and new-born humans, the rather recent description of BAT in adult humans has sparked a growing interest in human BAT-thermogenesis and its effects on the human metabolism (Cannon & Nedergaard 2012).

On the other hand, an increase in ambient temperature above the TNZ triggers processes that are also energy consuming. In response to a heat challenge, heat-dependent thermoreceptors of the skin are activated to induce vasodilatation and sweating (Cannon & Nedergaard 2004). These mechanisms increase heat loss via the body surface and thereby allow to decrease body temperature and to maintain it at an optimal level. In addition, the metabolic needs of the body are reduced by inactivity (Tansey & Johnson 2015).

Conclusively both, heat production as well as heat conservation, clearly increase energy expenditure above the needs in thermoneutrality (Cannon & Nedergaard 2004, Silva 2005, Westerterp 2017).

**Thyroid hormones and the regulation of thermogenesis**

The role of THs to directly stimulate energy expenditure has been well described and studied in great detail, whereas its role in the regulation of body temperature has not been completely elucidated as yet. It is widely accepted that THs modulate thermogenesis directly by changing the functionality and transcription rate of UCP1 and obligatory thermogenesis by increasing metabolic cycling or by direct actions on the sodium/potassium and the calcium pump in skeletal muscle (Silva 2006). In addition to these peripheral effects, THs affect sympathetic nervous system activity and interact with the central control of
Central regulation of core body temperature by thyroid hormones

As confirmed in multiple experimental models of hypothyroidism and hyperthyroidism, both in animal models and humans, THs may act directly on key metabolically active organs and thus alter obligatory thermogenesis. This aspect of thermogenesis may also be regulated via the central nervous system, but the main impact of these central regulators is to integrate different external stimuli and to adapt facultative thermogenesis accordingly (Coppola et al. 2007, Herwig et al. 2008, Klieverik et al. 2009b, Lopez et al. 2010). This regulatory circuit respond to changes in external temperature clues sensed e.g. by skin temperature sensors but also directly to alterations in core body temperature via a complex, multilocular central nervous system of temperature sensitive centres interacting with different aspects of thermal regulation.

Within the past years, novel complex interactions within the hypothalamus regarding THs, thermogenesis and energy partitioning, have been revealed mainly in rodents. Here, we will restrict the discussion to centres in the dorsomedial (DMH), the ventromedial (VMH), the preoptic area (POA), and the paraventricular nucleus (PVH) of the hypothalamus because all regions have been shown to interact with THs. Most importantly, the PVH contains hypophysiotropic TSH-releasing hormone (TRH) neurons, which regulate the HPT and sensitively respond to changes in TH status by reducing TRH release into the hypothalamo-hypophyseal portal system. Anatomically, hypophysiotropic TRH neurons are located in a species-specific manner: In rats, they are only located in the medial and periventricular subdivisions at the mid and caudal levels of the PVN, in mice, these neurons can be found only at the mid-level of the PVN (Fekete & Lechan 2014). The TRH-thyroid-stimulating hormone (TSH)-TH feedback loop is mediated by a direct activation of thyroid hormone receptor (TR) isof orm β-dependent signalling to decrease TRH and TSH secretion. Recent observations suggest an important modulating role of tanycytes at the blood–brain barrier to control hypothalamic TH concentrations (Muller-Fielitz et al. 2017). Following cold exposure, TRH neurons are activated and induce an increase in preproTRH processing, release of TRH from the median eminence and a subsequent stimulation of the thyroid with higher circulating TH levels (Nillni 2010). Thus, both TRH/TSH and TH concentrations are increased indicating an alteration of the set-point of the hypothalamic-pituitary-thyroid (HPT) axis following cold stimulation, an observation confirmed in humans (Celi et al. 2010, Joseph-Bravo et al. 2016).

The PVH neurons receive input from other central regions such as the POA (Larsen et al. 1994). The latter respond to peripheral vascular temperature sensors, which detect changes in ambient temperature and/or core body temperature. In addition, these sensors transmit afferent autonomic signals to the brain stem and the POA of the hypothalamus. Direct heating or cooling of the POA results in a modulation of thermogenesis by changing eff erent SNS-signalling through activation of γ-aminobutyric acid (GABA)ergic neurons, which project to the dorsomedial DMH and the VMH (Morrison 2016). Activation as well as inhibition of these neurons powerfully decreased or increased core body temperature by app. 2°C by modulating sympathetic tone to BAT, liver and muscle (Tan et al. 2016, Zhao et al. 2017).

Cooling of the POA has direct effects on the regulation of the HPT. In rats, it induces a shift of the set-point of the TRH-TSH-TH axis and an increased release of THs (Martelli et al. 2014). In contrast, i.c.v. injections of triiodothyronine (T3) with an increased T3 concentration in the POA results in a dose-dependent decrease in TRH and TSH, interestingly accompanied by a drop in core temperature (Moffett et al. 2013). Both observations obtained in a difficult methodological setting and so far, unconfirmed indicate a direct regulatory role of the POA to link core body temperature and thyroid function.

Apart from these TRH-mediated adaptions of the hypothalamic-pituitary-thyroid axis and subsequent changes in TH-regulated thermogenesis, TRH affect thermogenesis of the BAT by activating BAT-innervating neurons in the spinal cord. Cold exposure activates cold-sensitive hypothalamic neurons that express TRH. These neurons subsequently activate neurons in the spinal cord, thereby increasing the sympathetic outflow to BAT (Cabr al et al. 2012). Through both mechanisms TRH may thus mediate thermogenesis, and this explains why TRH-knockout mice are intolerant to cold (Yamada et al. 2003).

Thyroid hormones and energy partitioning

The arcuate nucleus (ARC) is located close to the median eminence and is key in integrating peripheral information
on energy stores, nutritional and metabolic signals due to its anatomical site with a relatively permeable blood–brain barrier (Smith & Ferguson 2014). As discussed in multiple excellent reviews, the ARC is central to the control of food intake and energy expenditure either directly or via close neuronal interaction with other hypothalamic centres such as the PVH, DMH or VMH (Cone 2005, Krashes et al. 2016). NPY/AGRP-positive neurons of the ARC dominate the regulation of energy intake whereas proopiомelanocortin-(POMC) and melanocortin receptor subtype 4- (MC4R) ARC neurons control energy expenditure.

Both are targets of the TH feedback loop. By that they act as a relay centre integrating TH-dependent signalling on energy partitioning and the distribution of metabolic cycling in response to temperature challenges. Changing the nutritional supply expressed by an increase of glucose concentrations within the physiological range induces an increase in TRH expression (Breit et al. 2016). These ex vivo and in vitro experiments indicating a direct nutritional link on the HPT axis fit well with the inhibition of preproTRH (and CRH) in the PVH during fasting, a process again controlled by the ARC. In knockout mice lacking either MC4R or NPY or both, the drop of TRH, TSH and peripheral TH could convincingly be attributed to NPY/AGRP indicating a nutritionally driven link to the central control of the thyroid axis. This is paralleled by a hepatic, MC4R and NPY governed effect on the metabolism of thyroxine (T4) and T3 (Vella et al. 2011). T3 repress MC4R within the PVH and ARC in a dose-dependent manner by specifically binding to TRA and TRB (Decherf et al. 2010). T3 availability in these nuclei is regulated by uptake of T4 (and T3) from the blood into the surrounding microglia where the deiodinase type 2 (DIO2) converts T4 to T3. Both hormones are delivered via a monocarboxylate transporter 8-dependent mechanism to their targets in the ARC, i.e. AGRP-positive neurons. Microglial DIO2 appears to be acutely upregulated during starving, whereas an increased mitochondrial biogenesis is regarded to play an important memory function to regulate food intake following starvation and to compensate for the accompanying weight loss (Coppola et al. 2007).

The described physiological pattern of inhibition or activation of TRH under starvation or abundant energy supply alters the setpoint of the HPT axis and by that modulates the peripheral metabolism. Leptin has a pivotal role in mediating this adaption. Leptin levels drop in response to food deprivation, causing a shift in the HPT axis setpoint via the ARC (previously summarised in detail (Fekete & Lechan 2014)). In the fasted state, local T3 levels also rise in the ARC and the excitability of AGRP neurons increases. Via projections to the PVH, AGRP release suppresses TRH processing and secretion by inhibiting MC4R (Decherf et al. 2010). This mechanism alters the setpoint of the HPT axis with lower TRH and subsequently TSH concentrations for a given circulating TH level. The pattern is in line with observations in hypothyroid rats. When local hypothalamic levels of T3 decreased, AGRP expression decreased in parallel; this could be reversed by acute T3 application (Herwig et al. 2008). It thus fits closely to the above discussed modulation of the setpoint by a cold or hot environment where similar changes in the activity of TRH processing was observed (Fig. 1). Lopez and coworkers further delineated the biochemical cascade of T3 feedback on hypothalamic centres and stressed the importance of the VMH for the feedback of TH. Following acute T3 administration, they identified adenosine monophosphate-activated protein kinase (AMPK) as the major signalling target. T3 decreased AMPK, which increased malonyl-CoA, decreased carnitine-palmitoyltransferase (CPT) alongside with an increased AGRP/NPY expression, which activated the SNS outflow and acutely induced hyperthermia at thermoneutrality (Lopez et al. 2010, Lage et al. 2016).

Recent data indicate that co-receptors of the TH receptors, like retinoid X, farnesoid X and liver X receptor (LXR), play an important role in the regulation of the negative feedback of TH on the hypothalamus, dominantly mediated via TR8 (Ribeiro et al. 2001, Mullur et al. 2014). LXR, activated by cholesterol metabolites and oxysterols, represses TRH synthesis and release. They alter the setpoint of the HPT axis, but at the same time, MC4R pathways are repressed and AGRP is activated, a process which could be counteracted by T3 (Ghaddab-Zroud et al. 2014). These data illustrate that the HPT axis may be activated or inhibited by a central modulation of local hypothalamic TH levels, caused by temperature-dependent or nutritionally derived cues that, in conjunction with a modulation of other factors like the SNS activity, will alter the peripheral regulation of TH-dependent metabolic pathways in target organs like BAT, WAT, liver and muscle.

Interestingly, the VMH also modulates a peripheral feedback via leptin and insulin signalling. Insulin sensitivity is markedly increased by selective deletion of activating transcription factor 4 (ATD4) in AGRP neurons. This deletion was paralleled with decreased WAT and increased BAT activity as well as core body temperature of the animals, a pattern also observed in response to cold exposure (Deng et al. 2017). It appears to be mediated by stimulation of phosphatidylinositol-4,5-bisphosphate
3-kinase and subsequent forkhead box O1 (FOXO1) inhibition in the ARC. FOXO1-knockout animals are characterised by increased energy expenditure. Conversely, FOXO1 upregulation increases AGRP expression in ARC, increases food intake and decreases body core temperature as well as BAT activity as measured by UCP1 expression (Hasegawa et al. 2012, Deng et al. 2017). Sirtuin 1 which co-regulates TRs appears to be an important modulator of FOXO-1 (Suh et al. 2013, Cyr et al. 2015). In addition, necdin, which negatively regulates the activity of FOXO1 in the ARC, is regulated by T3. Necdin is downregulated in hyper- and upregulated in hypothyroidism, it may contribute to TH-mediated energy partitioning and to the modulation of the HPT axis, the later by its effects on PVH expression of TRH (Hasegawa et al. 2012).

ATD4 as well as necdin again appear to modulate the setpoint of the HPT and in part of the thermostat. Novel data suggest that other factors important for the feedback regulation may also interact with the setpoint of the thyroid axis.

**Thyroid hormone-mediated effects on adipose tissue**

The major function of WAT is to store energy, which may be mobilised in situations of increased demand such as cold stress, fasting or disease. With the discovery of leptin, the role of WAT as an active player in the control of energy homeostasis has been established (Zhang et al. 1994, Luo & Liu 2016). Mobilisation of substrates is regulated by SNS activity and at the cellular level is mainly mediated through ARB3-induced lipolysis in WAT (Mund & Frishman 2013). Interestingly, in humans, WAT of different anatomical locations is characterised by a different sensitivity to lipolysis, which is greater in visceral than in subcutaneous WAT (Wajchenberg 2000). Depot-specific differences in lipolysis rates can also be seen in mice (Wueest et al. 2012).

As mentioned earlier, BAT contributes significantly to thermogenesis, in a SNS-mediated fashion, for which it uses mainly triglycerides and glucose. These substrates can be provided (i) directly from dietary sources but, especially in the fasted state, are (ii) mobilised from stored energy in WAT and BAT. To facilitate a rapid breakdown of triglycerides for beta oxidation, BAT persists of multilocular cellular lipid droplets and thus provides a high lipid surface (Keipert & Jastroch 2014). Furthermore, it displays a high mitochondrial content of up to 8% to facilitate high-energy turnover rates (Rousset et al. 2004).
Whereas mice and rats possess BAT throughout their entire life span, BAT expression is limited to certain ages in other species, e.g. in lambs and rabbits, it disappears several days to months after birth (reviewed in Oelkrug et al. 2015). In humans, BAT was regarded to be restricted to a short period after birth, but recently has been detected in adults using [18F]-fluorodeoxyglucose positron emission tomography-computed tomography techniques (Nedergaard et al. 2007, Cypess et al. 2009, van Marken Lichtenbelt et al. 2009, Virtanen et al. 2009, Iwen et al. 2017). Remarkably, brown adipocytes are not restricted to the classical BAT depots but can also develop within WAT upon adrenergic stimulation or pharmacological intervention (Keipert & Jastroch 2014, Warner & Mittag 2016). This so-called ‘browning’ of WAT is the subsequent development of beige or brite (‘brown in white’) adipose tissue, has recently been proposed as a major target for the treatment of metabolic disorders in humans (Petrovic et al. 2010, Harms & Seale 2013), as this depots may add to heat production and energy partitioning under certain (patho-) physiological conditions (Cohen & Spiegelman 2015). However, it is still a matter of debate if adult human ‘BAT’ predominantly consists of brown or brown-like (beige) adipocytes and if beige adipocytes can contribute to higher energy turnover rates (Warner & Mittag 2016).

THs also induce ‘browning’ of WAT by direct and indirect effects. In the central nervous system, administration of T3 to the VMH, but not to the ARC, induces ‘browning’ of WAT as shown by increased expression of markers like UCP1, cidea and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (Martinez-Sanchez et al. 2017). Further, the ‘browning’ of WAT by a conjugate of T3 and glucagon which allows to selectively target only glucagon receptor positive tissues including WAT and liver, suggests T3-specific effects on WAT (Finan et al. 2016). TRB agonists (Lin et al. 2015) and hyper- and hypothyroidism in a rodent model also mediated thermogenesis (Weiner et al. 2016, Martinez-Sanchez et al. 2017). In line with these findings, association studies suggest similar direct effects in humans (Martinez-Sanchez et al. 2017).

Interestingly, TSH has been implicated in mediating similar direct effects on WAT. In elegant but currently not independently reproduced experiments, Endo and Kobayashi dissected an isolated lipolytic effect of TSH on WAT in mice bearing a mutation of the TSH receptor (hyt mice). By induction of lipolysis, TSH regulated fuel availability for BAT. It exerted further direct TSH receptor-driven effects on UCP1 expression and thermogenesis in BAT (Endo & Kobayashi 2008, 2012). This suggested role of TSH may provide an additional mechanism to increase FA levels during both, cold exposure and fasting.

In both, WAT and BAT, the cellular concentration of T3 can be modified, as deiodinase 1 (DIO1) and DIO2 are expressed in WAT and DIO2 in BAT (Calvo & Obregon 2011). Studies in general or fat specific DIO2-knockout animals (Christoffolete et al. 2004, Castillo et al. 2011, Fonseca et al. 2014) support an important role of intracellular T3 in BAT for fatty acid oxidation and lipogenesis. The latter may impair thermogenesis, a defect that could be overcome by an increased lipolysis through compensatory stimulating SNS input. T3 activates UCP1 in concert with the systemic nervous system and fatty acids (Reitman et al. 1999, Lanni et al. 2003, Sell et al. 2004), and these effects are mediated by stimulation of adiponutrin, a triacylglycerol lipase, which hydrolyses triacylglycerol to fatty acids in WAT and BAT (Obregon 2014). In contrast, lipoprotein lipase (LPL) is downregulated by T3.

The different isoforms of thyroid hormone receptors TRA1, TRA2 and TRB1 are detectable in WAT and BAT. TRB expression in BAT is closely linked to UCP1 expression, while adrenergic responsiveness of thermogenesis is TRA dependent (Ribeiro et al. 2001). A loss of TRA1 function, at least with a TRA P398H mutation, induces a predominantly increase in visceral body fat. This is associated with a reduction in adaptive thermogenesis (Liu et al. 2003, 2007) along with an impaired lipolysis, insulin resistance and hyperleptinemia (Bianco & Silva 1988, Hernandez & Obregon 1996).

**Thyroid hormone-mediated effects on the liver**

TH act on hepatic lipid partitioning via low-density lipoprotein (LDL)-receptor (LDLR)-dependent and -independent pathways. Studies in mice suggest that a wide variety of known TH transporters are expressed in the liver. Hyperthyroidism significantly increased monocarboxylate transporter 10 (MCT10) and large amino acid transporter-1 (LAT1) expression (Engels et al. 2015) as well as DIO1. Interestingly, DIO1 has recently been shown to be greatly downregulated in insulin receptor-knockout animals, and this is paralleled by reduced apolipoprotein A1 (Apoa1) gene expression as well lowered high-density lipoprotein (HDL) cholesterol levels (Liu et al. 2016). This regulation of DIO1 and its impact on HDL cholesterol thus reflects pathophysiological changes observed in the metabolic syndrome. It appears to be mediated via a specific thyroid hormone response element and does not
reflect general hepatic hypothyroidism as total hepatic T3 levels remained constant. Further, DIO2-knockout animals are insulin resistant with clearly increased hepatic triglycerides under high-fat diet (Marsili et al. 2011) fitting to observations in humans with a polymorphism of DIO2 (Mentuccia et al. 2002, Dora et al. 2010).

The partitioning of energy from WAT to the liver is achieved by a TH-dependent action on specific transporters such as fatty acid transporter proteins, liver fatty acid (FA)-binding proteins and FA translocase in hepatocytes. Hyperthyroidism increases and hypothyroidism decreases this triglyceride-derived FA uptake into the liver (Klieverik et al. 2009a). TH stimulates the known key lipogenic transcription factors sterol regulatory element-binding protein-1c (Hashimoto et al. 2006), LXR (Hashimoto et al. 2007) and carbohydrate-responsive element-binding protein (Hashimoto et al. 2009) and stimulate transcription of central lipogenic enzymes such as acetyl-CoA carboxylase, malic enzyme, fatty acid synthase, and Spot14 (Liu & Brent 2010). Resistance to TH induced by TRB mutations have been studied in the model of PV/PV mice (Cheng et al. 2010, Zhu & Cheng 2010). They demonstrated markedly increased hepatic lipid accumulation, whereas silencing of TRA resulted in decreased expression of lipogenic genes. This suggests a fine-tuning of lipid partitioning via TR-specific pathways (Fozzatti et al. 2011). Another important mechanism for hepatic lipid regulation is oxidation of FA controlled by TH derived from WAT but as well be released from triglyceride stores within the liver via hepatic lipases. Prolonged exposure to TH increases FA oxidation (Oppenheimer et al. 1991) through hepatic lipase (Nozaki et al. 1992). An even more important mechanism than this stimulation of hepatic beta-oxidation of FFA by TH is an increase of lipophagy under TH (Sinha et al. 2012, Liu & Czaja 2013). Inhibition of this autophagic process in vitro significantly influences ketogenesis indicating impairment of beta-oxidation via a TR-associated process (Sinha et al. 2012, 2014). THs further affect CPT1 and AMPK in the liver just as described for hypothalamic centres. In addition, recent data suggest a role of TSH via TSH receptor-mediated pathways on AMPK activity (Zhang et al. 2015b). There are additional CPT1-dependent effects on glycolysis, which is positively controlled by TH. As shown for the hypothalamic regulation, CPT1 and pyruvate dehydrogenase lipoamide kinase isozyme 4 important for energy sensing are modulated via a direct interaction of T3 with sirtuin1 (Thakran et al. 2013).

Apart from direct action of TH on LDLR-dependent pathways, there are additional T3 effects on lipid partitioning independent of LDLR as both, T3 and the TRB agonist GC1, effectively lower serum cholesterol in LDLR knockout mice by stimulation of Cyp7A1 (Lin et al. 2012). Expression of Cyp7A1 induces metabolism of cholesterol to bile acids (BA) and stimulates their excretion (Bonde et al. 2014). BA acts through a specific receptor, TGR5, expressed in the small intestine but also in BAT, skeletal and cardiac muscle, liver, pancreas and the thyroid (Duboc et al. 2014). BA-activated TGR5 stimulates intracellular cAMP formation in BAT, activates DIO2 most likely through an interaction with tauroursodeoxycholic acid (Watanabe et al. 2006, da-Silva et al. 2011), and this induces thermogenesis in BAT. On the basis of association studies, it seems likely that this applies as well to humans (Ockenga et al. 2012), but this was only confirmed in normal-weight but not in obese subjects (Brufau et al. 2010). BA and TSH levels are negatively associated (Patti et al. 2009, Ockenga et al. 2012, Song et al. 2016). There is a putative negative feedback of BA on the pituitary via pituitary TGR5 (Doignon et al. 2011, Ockenga et al. 2012), but TSH may directly suppress BA formation and secretion via SREBP-2/hepatocyte nuclear factor 4 alpha/CYP7A1 signalling (Song et al. 2015). Hyperthyroidism, most likely via TRB dependent activation, reduces proprotein convertase subtilisin/kexin type 9 (PCSK9) in circulation, lipoprotein cholesterol, apolipoprotein B and APOA1 as well as lipoprotein (a) but has no effect on cholesterol synthesis (Bonde et al. 2014).

By interaction of the transcription factors TRB, retinoid X receptor and peroxisome proliferator-activated receptor (PPAR) alpha, T3 apparently stimulates fibroblast growth factor 21 (FGF21). These findings are exciting as in animal models, FGF21 has recently been shown to modulate hedonic eating behaviour and suppress the intake of sweet food (Talukdar et al. 2016, von Holstein-Rathlou et al. 2016). However, investigations in FGF21 knockout animals suggest that TH and FGF21 are independently regulated and may replace each other in certain critical metabolic situations (Domouzoglou et al. 2014, Zhang et al. 2015a). This fits to data in humans in whom no change in FGF21 was observed in hyperthyroidism or TRB agonist treatment (Bonde et al. 2014).

**Thyroid hormone-mediated effects on glucose metabolism via pancreatic beta-cells and myocytes**

The effects of TH on glucose metabolism have been recently reviewed in several extensive reviews including some of our group (Duntas et al. 2011, Iwen et al. 2013).
The hypothalamic changes associated with fasting and thus low glucose availability or high energy intake have been discussed above in the chapter related to the action of TH in energy partitioning.

Therefore, we will only shortly summarise some of the key data of TH action in primary hyperthyroidism or hypothyroidism and discuss the most recent studies published in more detail. Oral glucose tolerance testing in hyperthyroidism and hypothyroidism both show an increased cumulative glucose and insulin response indicating significant TH dose-dependent insulin resistance (Mittrou et al. 2010). This was surprising as other data on the expression of glucose transporters suggest parallel tissue-specific upregulation of glucose transporter (GLUT) 1, 3, 4 and 5 by TH with decreased levels in hypo- and increased in hyperthyroidism. While in hypothyroidism insulin resistance rests directly on decreased transport of glucose to the respective tissue along with a decreased clearance of insulin, the development of insulin resistance in thyrotoxicosis is unclear. It has been discussed that it depends on a downregulation of hepatic glycogen synthesis and increased glycogenolysis under thyrotoxic conditions. In contrast, in a recently published rat model, glucose tolerance improved by 15% with an improvement in insulin resistance index by 34% when the animals were rendered hyperthyroid for a prolonged period of time (Vazquez-Anaya et al. 2017). These discrepant results were explained by increased transport of TH to the muscle and by an upregulation of the TH transporter MCT10 and the intramuscular DIO2. Thus, the availability of T3 and of glucose in the muscle seems to be fine-tuned by TH. Upregulation of sirtuin1 and UCP2 suggest a stimulation of glucose turn-over (Vazquez-Anaya et al. 2017). In addition, AMPK activity in skeletal muscle is upregulated in hyperthyroid states (Park et al. 2002, Winder et al. 2003, Branvold et al. 2008, Irrcher et al. 2008).

The data on direct effects of TH on the beta-cell mass and on insulin secretion and secretion are even more controversial. In the rat beta-cell line RIN5F, beta-cell proliferation appears to be stimulated via TRA-dependent signalling and the release of insulin is increased in vitro and in vivo (Furuya et al. 2010, Harris et al. 2017). In contrast, ex vivo studies following treatment with high doses of T3 suggest an increased apoptosis of beta-cells fitting to studies on the effects of experimental hypothyroidism during the last weeks of ovine gestation where a stimulated beta-cell mass and hyperinsulinemia was found (Jorns et al. 2010, Harris et al. 2017). With the known effects of hypothyroidism on insulin resistance, the latter findings could be explained by an indirect effect of insulin resistance on the beta-cell. This might as well explain a stunning effect of TH replacement in a single patient with an insulin receptor mutation and severe insulin resistance. Increasing the dose of thyroxine resulted in a dramatic improvement in insulin sensitivity along with the reappearance of active BAT (Skarulis et al. 2010). It may also explain the increase in GLUT4 expression and improvement of insulin resistance in type 2 diabetic patients when exposed to cold (Hanssen et al. 2015).

**Thyroid hormone-mediated effects on peripheral blood flow**

It is beyond the scope of this review to discuss in detail the role of TH on cardiovascular regulation and the heart. Their important role through TRA on the heart rate, on positive inotropy and on heart hypertrophy has recently been reviewed in great detail (Klein & Danzi 2016).

TH-mediated effects on vasoconstriction and vasodilatation have recently been addressed in rodents. In animals with an impaired function of TRA both, vasoconstriction following phenylephrine and vasodilatation following acetylcholine application, is impaired. Thus, thermoregulation via the tail of the animal is no longer operative with profound consequences for cardiovascular function and thermogenesis (Warner et al. 2013). Overall, this aspect of thermoregulation has not been studied in great detail. Additional effects of TH-mediated vasoconstriction or vasodilatation for thermoregulation in mice and humans need to be addressed in future studies.

**Summary of physiological responses**

Considering the mechanisms discussed earlier, a clear role of TH emerged to optimise metabolic adaptation to changes in environment such as exposure to cold or to low energy availability. As schematically shown in Fig. 1A and B, these conditions are characterised by central hypothyroidism as in response to cold exposure and central hyperthyroidism as in fasting.

Under exposure to low temperatures, heat-sensitive neurons in the POA are inactivated, which in turn causes an increase in GABAergic neuronal inputs to the DMH and subsequently to an activation of an efferent sympathetic drive to temperature-sensitive vessels, WAT, BAT, liver and muscle. Whether cold-sensitive neurons are also involved is currently unclear. In parallel, this cold-induced activation via afferent sympathetic neurons
induces an increase in TRH and TSH and a shift of the HPT axis towards higher TH levels. They activate sympathetic efferences to temperature-sensitive vessels resulting in vasoconstriction. Elevated TSH levels allow to optimise the flux of substrates to metabolic organs. High TSH levels may directly induce lipolysis. This effect fuels brown adipocytes with adequate substrates to produce heat under efferent SNS stimulation. There might be an additional effect on T3-dependent browning of WAT. The stimulation of the HPT axis, causing increases in TSH and subsequently TH levels, together with a rise in the SNS activity increases glucose production. It may be supported by positive effects of TH on insulin synthesis and release, but this effect is controversial and not well studied under the fine-tuning conditions of cold exposure. Similarly, the slight increase in TH associated with the change in the setpoint of the HPT axis stimulates the expression of glucose transporters in target tissue like the liver and muscle and thus provides the substrate to endure long-standing or more extensive cold environment by shivering.

The opposite is true for low energy availability as in fasting (Fig. 1B). Here, the negative feedback of leptin on DIO2 is reduced to produce a central state of hyperthyroidism, which leads via inhibition of PVH-derived TRH to a decrease in TSH and a shift in the HPT axis towards a lower set point. This process is greatly supported by an increase in hunger-stimulating peptides like AGRP. AGRP further blocks MC4R and decreases TRH. It is paralleled by a decreased action on melanocortin-derived stimulation of efferent SNS pathways to the periphery via hypothalamic nuclei as the DMH. Whether the slight increase of central TH is uniform and sufficient to counteract this silencing of SNS activity via an activation of the T3-dependent VMH-SNS pathway is not well investigated but appears unlikely. Similarly, the T3 effect on the anterior hypothalamic area (AHA) on blood pressure is likely to be small but not studied under these conditions. WAT, BAT, liver and muscle will under these conditions not be activated to produce FA and glucose. Both pathways will greatly save energy and reduce resting energy expenditure. The lack of TH drive in parallel with low sympathetic activation of peripheral vessels will dissipate heat and in turn stimulate the feeling of cold due to a drop in in core body temperature. Afferent cold-sensitive sympathetic pathways will then be activated when core body temperature drops below a critical threshold and by activation of the POA-DMH-efferent SNS pathway maintain core temperature at a metabolically optimal level.

Summary of pathophysiological changes

Primary hyperthyroidism and hypothyroidism clearly contrast to these conditions as the fine-tuning of central and peripheral TH action is lost under these conditions (Fig. 2A and B).

In primary hypothyroidism, the lack of TH will override the central adaptation and induce by disinhibition TRH release and pituitary TSH secretion. High TSH levels will stimulate lipolysis from WAT and BAT, but the silenced efferent sympathetic drive to the BAT will not allow to adequately consume the fuel. Glucose uptake in the liver and muscle is reduced due to the lack of TH-dependent stimulation of glucose transporter expression, which may cause increased circulating glucose levels. With a reduction of pancreatic insulin synthesis and secretion this will result in insulin resistance. The silencing of metabolic pathways under these conditions may in turn cause a decrease in core body temperature which can no longer be counteracted via TH release from the thyroid gland but only via an afferent sympathetic signalling via POA and DMH to induce efferent sympathetic stimulation. This feedback may kick in early as low TH levels along with a missing sympathetic drive to vascular regulation appear not to allow an adequate early vascular counter-regulation.

Finally, in primary hyperthyroidism (Fig. 2B) high central TH levels downregulate TRH synthesis and release. The subsequent pronounced decrease in TSH secretion no longer contributes to lipolysis, but there appears to be a direct peripheral lipolytic drive through the AMPK-malonyl-CPT1 pathway to provide triglycerides. In contrast, local increase of TH levels in the VMH oppose these AMPK-malonyl-CPT1 driven effects via activation of sympathetic efferences to the liver and BAT. In BAT, they ignite heat production, which is augmented by T3-dependent conversion of WAT to beige fat. In addition, it stimulates hepatic glucose production. The positive action on metabolism also includes cardiovascular system. Via direct central stimulation of parvalbuminergic neurons of the AHA blood pressure is increased. TH directly induces positive chronotropic and inotropic effects on the heart via activation of TRA and blood pressure is also increased by vasoconstriction through increased TH action and sympathetic drive. All these mechanisms will rapidly facilitate an increase in core body temperature. By means of negative feedback heat sensitive neurons in the POA will be activated and decrease via GABAergic projections to the DMH and VMH sympathetic outflow. As TH directly stimulates insulin synthesis and secretion a
variable degree of insulin resistance may result depending on the degree of action on beta cell, glucose production and local regulation. Fine-tuning of peripheral regulation is further governed by many factors including leptin modulating GLUT expression and deiodinases.

Both figures provide a highly simplified scheme to explain some of the energy flux associated with an alteration in TH status and its impact on thermogenesis. They illustrate the complexity of the regulation and the large number of further components influencing it. Most importantly, it has been shown that modulation of the activity of temperature sensitive neurons in the POA dramatically alters thermoneutrality and by that the setpoint of the feedback. Energy stores such as subcutaneous fat depots and particularly intradermal fat storage may have profound influence on the insulation process. The schematic model further did not specifically address alterations in food intake via feedback on hedonic and obligatory signal pathways in appetite regulation. Most important, almost all of these data are obtained from animal models which may not reflect the situation in humans. Despite these drawbacks the model may help to better evaluate the current knowledge on TH dependent alterations induced in humans on thermogenesis.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References
Breit A, Wicht K, Boekhoff I, Glas E, Lauffer L, Muckter H & Gudermann T 2016 Glucose enhances basal or melanocortin-induced
cAMP-response element activity in hypothalamic cells. Molecular Endocrinology 30 748–762. (https://doi.org/10.1210/me.2016-1001)


Castillo M, Hall JA, Correa-Medina M, Ueta C, Kang HW, Cohen DE & Bianco AC 2011 Disruption of thyroid hormone activation in type 2 deinolide knockout mice causes obesity with glucose intolerance and liver steatosis only at thermoneutrality. Diabetes 60 1082–1089. (https://doi.org/10.2337/db10-0758)


Decherf S, Seugnet I, Koudisti S, Lopez-Juarez A, Clerget-Froidevaux MS & Demeneix BA 2010 Thyroid hormone exerts negative feedback on hypothalamic type 4 melanocortin receptor expression. PNAS 107 4471–4476.


Fonseca TL, Wernick-De-Castro JP, Castillo M, Bocco BM, Fernandes GW, McAninch EA, Ignacio DL, Moises CC, Ferreira AR,


Larsen PJ, Hay-Schmidt A & Mikkelsen JD 1994 Efferent connections from the lateral hypothalamic region and the lateral preoptic area to the hypothalamic paraventricular nucleus of the rat. Journal of Comparative Neurology 324 299–319. (https://doi.org/10.1002/cne.903420211)


Liu YY & Brent GA 2010 Thyroid hormone crosstalk with nuclear receptor signaling in metabolic regulation. Trends in Endocrinology and Metabolism 21 166–173. (https://doi.org/10.1016/j.tem.2009.11.004)


Liu YY, Schulz JJ & Brent GA 2003 A thyroid hormone receptor alpha gene mutation (P998H) is associated with viscerl adiposity and impaired catecholamine-stimulated lipolysis in mice. Journal of Biological Chemistry 278 38913–38920. (https://doi.org/10.1074/jbc.M306120200)


Martelli D, Luppi M, Cerri M, Tupeone D, Mastrotto M, Perez E, Zamboni G & Amici R 2014 The direct cooling of the preoptic-hypothalamic area elicits the release of thyroid stimulating hormone during wakefulness but not during REM sleep. PLoS ONE 9 e87793. (https://doi.org/10.1371/journal.pone.0087793)


Nillni EA 2010 Regulation of the hypothalamic thyrotropin releasing hormone (TRH) neuron by neuronal and peripheral inputs. Frontiers in Neuroendocrinology 31 134–156. (https://doi.org/10.1016/j.yfrne.2010.01.001)


Reitman ML, He Y & Gong DW 1999 Thyroid hormone and other regulators of uncoupling proteins. International Journal of Obesity and Related Metabolic Disorders 23 (Supplement 6) S56–S59. (https://doi.org/10.1038/sj.ijo.0800948)

Ribeiro MO, Carvalho SD, Schultz JJ, Chiellini G, Scanlan TS, Bianco AC & Brent GA 2001 Thyroid hormone–sympathetic interaction and adaptive thermogenesis are thyroid hormone receptor isoform–specific. Journal of Clinical Investigation 108 97–105. (https://doi.org/10.1172/JCI200112584)


stimulates hepatic lipid catabolism via activation of autophagy. Journal of Clinical Investigation 122 2428–2438. (https://doi.org/10.1172/JCI60580)
Suh JH, Sieglaff DH, Zhang A, Xia X, Cvoro A, Wimmier GE & Webb P 2013 SIRT1 is a direct coactivator of thyroid hormone receptor beta1 with gene-specific actions. PLoS ONE 8 e70097. (https://doi.org/10.1371/journal.pone.0070097)
Zhu X & Cheng SY 2010 New insights into regulation of lipid metabolism by thyroid hormone. Current Opinion in Endocrinology, Diabetes and Obesity 17 408–413. (https://doi.org/10.1097/MED.0b013e32833d6d46)

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