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The role of thyroid hormone and brown adipose tissue in energy homoeostasis

Prof. Antonio C Bianco, MD and Elizabeth A McAninch, MD

Division of Endocrinology, Diabetes and Metabolism, University of Miami Miller School of Medicine, Miami, FL, USA

Abstract

The presence of brown adipose tissue (BAT) in adults has become increasingly well defined as a result of functional imaging studies of thermogenically active BAT. Findings from these studies have created a surge of scientific interest in BAT, because it represents a potential therapeutic target for obesity—a condition with profound health consequences and few successful therapies. BAT contributes to overall energy expenditure in small mammals and neonates through adaptive thermogenesis. Thyroid-hormone signalling, particularly through induction of type II deiodinase, has a central role in brown adipogenesis in vitro and BAT development in mouse embryos. Additionally, because of high intracellular expression of type II deiodinase, adult BAT has enhanced thyroid-hormone signalling with several thyroid-hormone-dependent thermogenic pathways, including expression of the genes *Ppargc1a* and *Ucp1*. BAT thermogenesis explains the essential part played by thyroid hormone in energy homoeostasis and adaptation to cold. Stimulation of BAT in adults, specifically through thyroid-hormone-mediated pathways, is a promising therapeutic target for obesity.

Introduction

All living organisms need a steady supply of energy to support constancy of the interior milieu. Energy substrates, most commonly carbohydrates and fatty acids, are obtained from the environment and can be stored to ensure availability in the event of starvation. To preserve energy homoeostasis, supply and use need to be kept in balance. A relative excess of energy supply results from either an increase in energy intake or a decrease in energy use, and is the underlying cause of obesity. The challenge faced by clinicians and scientists is to restore balance in an environment that predisposes people to an energy surplus. Although balance can be restored by restriction of energy intake, increased energy expenditure is also a target. Brown adipose tissue (BAT) provides a physiological model through which energy expenditure can be accelerated rapidly—eg, during the arousal of a hibernating animal. The finding of BAT in adult human beings suggests that identification and targeting of pathways

Correspondence to: Prof Antonio C Bianco, University of Miami Miller School of Medicine, Miami, FL 33136, USA, abianco@deiodinase.org.

Contributors

Each author contributed equally to the Review.

Conflicts of interest

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to accelerate BAT energy expenditure could help to restore energy balance in obese people. In this Review we explore how thyroid-hormone signalling in BAT activates thermogenesis, and examine the relation between BAT and type II deiodinase (D2). D2 is both a key regulator of BAT function and uncoupling protein-1 (Ucp1) induction, and needed for proper development of BAT and differentiation of brown adipocytes. D2-expressing BAT is present in adults, and new findings of its physiological and therapeutic relevance could advance this specialty.

In most vertebrates, cellular oxidation of energy substrates and ATP synthesis are coupled; about 65% of the energy released during the oxidative processes is channelled into ADP phosphorylation and stored as ATP, with the rest released as heat. As the amount of available ATP reduces and the proportion of ADP to ATP increases (eg, to keep up with housekeeping requirements or during muscle contraction), substrate oxidation accelerates and, in coupled mitochondria, new ATP molecules form.¹ However, in rare instances physiological uncoupling of the mitochondria can occur, such that substrate oxidation is accelerated several fold without a parallel increase in the synthesis of ATP, with most energy being lost as heat.^{2,3} One site in which physiological uncoupling typically occurs is in BAT.

By contrast with white adipose tissue, which mainly provides energy storage and release in response to systemic demand, BAT is a specialised tissue that expends energy through heat production. The unique functional roles maintained by the brown and white adipose tissues are also evident in their structural differences—BAT has dense mitochondrial content, is richly innervated by sympathetic fibres, and is highly vascularised, whereas white adipose tissue has few mitochondria and is much less innervated and vascularised. Mitochondrial uncoupling in BAT is mediated by Ucp1, a 32 kDa protein expressed in the inner membrane of the mitochondria, which dissipates the proton gradient of the inner mitochondrial membrane without ADP phosphorylation.⁴ Because of the ability of BAT to expend energy through heat production, it is a promising target for potential obesity therapies.

Exposure to cold prompts the hypothalamus to initiate a shivering response, an important involuntary mechanism to preserve body temperature in adult human beings and other large mammals. However, shivering inevitably causes convective heat loss because of body oscillations, and is therefore a less efficient form of heat production than mitochondrial uncoupling, particularly in small mammals with a high surface-to-mass ratio.⁵ Thus, the role of mitochondrial uncoupling in BAT, the main site of non-shivering thermogenesis, is essential. The hypothalamus also initiates BAT stimulation through activation of the sympathetic nervous system, increasing the release of catecholamines throughout the body, but particularly in BAT. BAT is especially important in mammals that live in cold environments and are thus at risk of developing hypothermia, and in hibernators who periodically need to rapidly elevate their core temperature to arise from hibernation.

In neonatal mammals, including newborn human babies, BAT plays an important part in maintenance of core temperature by non-shivering adaptive thermogenesis.⁶ Until recently, in human beings BAT was thought to be present only in neonates because of their size and sensitivity to cold. The anatomical distribution of BAT in adults has been difficult to elucidate, and thus the physiological contribution of this tissue to thermogenesis was largely

dismissed. Although most rodents maintain gross deposits of BAT throughout their lives, in people BAT reduces in size after infancy and can dissipate altogether, as shown in autopsy series.⁷ Some researchers even speculated that remnant BAT stores went through complete remission in adults, only to be reactivated pathologically in patients with pheochromocytoma.⁸ However, incidental findings from PET imaging studies showed active BAT in adults that responds to cold exposure.^{9–12} These findings have reawakened scientific interest in the topic, and as a result the presence and function of BAT in adults is becoming increasingly well defined. The role of BAT in adults in the basal state is not clear, but evidence suggests that increased BAT activity contributes to increased energy expenditure in human beings exposed to cold.^{13,14}

BAT is intensely innervated by the sympathetic nervous system, and BAT thermogenesis is activated by noradrenaline¹⁵ and thyroid hormone,¹⁶ and inhibited by corticosteroids.^{17,18} Intracellular cyclic AMP accumulates after stimulation of β -adrenergic receptors by noradrenaline and rapidly activates lipolysis and Ucp1-mediated mitochondrial uncoupling. Noradrenaline stimulation also accelerates brown-adipocyte uptake of fatty acids and glucose from the circulation (through both insulin-dependent and insulin-independent transporters) for use as substrates for oxidation. Accordingly, mice which have a triple knockout of the three known β -adrenergic receptors have defective cold-induced and diet-induced BAT thermogenesis,¹⁹ suggesting that β 1-adrenergic receptors are important to signalling.²⁰ At the same time, hypothyroid mammals can develop profound hypothermia and succumb rapidly to cold exposure,²¹ largely because heat production in BAT becomes unresponsive to noradrenaline stimulation in the absence of adequate thyroid hormone.^{16,22} Additionally, BAT promotes diet-induced thermogenesis and might thereby contribute to resistance to obesity.²³ Circulating T₄ can be activated in BAT, because it contains D2.²⁴ This enzyme generates active thyroid hormone within brown adipocytes, triggering thyroid-hormone-dependent thermogenic pathways.^{16,25} By contrast with noradrenaline and thyroid hormone, glucocorticoids inhibit cold-induced and diet-induced expression of *Ucp1*, disrupting thermal homeostasis in mice during cold acclimatisation¹⁷ and diet-induced thermogenesis in BAT during feeding with high-fat diets.¹⁸

D2 is a thioredoxin-fold-containing selenoenzyme that converts the prohormone thyroxine (T₄) into the active metabolite tri-iodothyronine (T₃).^{26,27} *Dio2* is a cyclic-AMP-dependent gene that encodes D2; thus, BAT stimulation by noradrenaline rapidly creates a localised state of thyrotoxicosis within brown adipocytes (figure 1).²⁸ Saturation of thyroid-hormone receptors with D2-generated T₃ in BAT stimulates several T₃-dependent processes, including expression of *Ucp1*, a T₃-dependent gene.^{29,30} These findings show the crucial relation between thyroid hormone and BAT, and have illuminated understanding of their joint contribution to energy homeostasis.

The worldwide obesity epidemic has made understanding of the interaction between thyroid-hormone metabolism and BAT activity increasingly important. At present, obesity therapy attempts to reduce energy input or increase energy expenditure and to tip the balance towards total body-energy deficit. Because most treatment methods for obesity focus on increased physical activity, which patients find difficult to successfully adhere to, increasing

energy expenditure is a challenging therapeutic target. Therefore, the use of thyroid-hormone signalling in BAT is an important alternative therapeutic target.

D2-mediated thyroid-hormone activation

Thyroid hormone is produced in the thyroid gland, and circulates systemically to reach target tissues. T_4 is secreted in higher quantities by the thyroid gland than is T_3 , but is a less biologically active prohormone. Most T_3 (about 80% in human beings and about 60% in rats³¹) is produced outside of the thyroid parenchyma, and exerts its action by entering target cells and binding to thyroid-hormone receptors that regulate transcription of T_3 -responsive genes. The present understanding of thyroid-hormone signalling is that, in addition to T_3 entering target cells from plasma, thyroid-hormone signalling is modified at the level of the individual cell through selective activation of T_4 (by D2) or inactivation of T_4 and T_3 by type III deiodinase (D3).³¹ D2-containing cells convert T_4 into T_3 that mixes with the incoming T_3 from plasma and eventually binds to thyroid-hormone receptors. Subsequently, T_3 leaks back into the plasma and feeds into the circulating pool of T_3 to preserve systemic thyroid-hormone homeostasis. Because of this mechanism, D2-expressing cells, including mature brown adipocytes, have high intracellular concentrations of T_3 and activation of thyroid-hormone receptors, establishing a strong T_3 -dependent mRNA footprint (the collection of mRNAs expressed in a cell at any given time).³² By contrast, thyroid-hormone signalling is reduced in D3-expressing cells because of inactivation of incoming T_4 and T_3 from plasma.³¹ D3 expression predominates during the early stages of BAT development and brown adipogenesis, diminishing thyroid-hormone signalling in developing brown adipocytes.^{33,34} Mature brown adipocytes and BAT have minimal D3 activity.^{33,34}

Thyroid hormone and energy expenditure

Energy homeostasis and prompt availability of energy substrates (eg, glucose, phosphocreatinine, and ATP) are necessary for preservation of cell and organ functionality, maintenance of body temperature, growth and reproduction, and digestion, absorption, processing, and storing of nutrients. In a resting individual at room temperature, the sum total of the energy used in these processes is known as the basal metabolic rate, and the heat produced as a result of the thermodynamic inefficiency of energy transfer is known as obligatory thermogenesis.

Typically, mammals function at higher metabolic rates than the basal metabolic rate because any activity that disrupts the resting state promotes ATP breakdown. In mammals, the metabolic rate can be increased by many factors—eg, voluntary or involuntary physical activity, transfer to a cold environment, hypercaloric feeding, or fever. The heat derived from an increase in the metabolic rate is known as adaptive thermogenesis, which, as opposed to obligatory thermogenesis, can fluctuate rapidly in response to triggering signals, including catecholamines and thyroid hormone. Adaptive thermogenesis provides an evolutionary advantage that has allowed endothermic animals to live in and dominate almost all environments. BAT is a key site of adaptive thermogenesis in response to cold exposure to preserve core temperature (known as cold-induced non-shivering thermogenesis) or to

dissipate an excess of dietary energy intake that otherwise would be stored as fat (known as diet-induced thermogenesis).

Thyroid hormone accelerates both obligatory and adaptive thermogenesis, but through different mechanisms. In obligatory thermogenesis, thyroid hormone accelerates ATP breakdown through several pathways, with heat production constituting a byproduct of ATP synthesis. This process is exemplified by thyroid-hormone disorders; in severe hypothyroidism energy expenditure can be reduced by half, and in severe hyperthyroidism energy expenditure can be doubled. In BAT, thyroid hormone promotes adaptive thermogenesis by mitochondrial uncoupling through expression of *Ucp1*. To a lesser extent, thyroid hormone enables shivering thermogenesis by changing the contractile properties of skeletal muscle fibres.^{35,36} Thus, thyroid hormone has a multifaceted contribution to obligatory and adaptive thermogenesis.

Thyroid-hormone and noradrenaline signalling pathways are interconnected, and affect each other while contributing to energy homeostasis. During systemic hypothyroidism, sympathetic activity generally increases, which partly compensates for diminished thyroid-hormone signalling.³⁷ The opposite occurs during thyrotoxicosis, in which sympathetic activity tends to be greatly reduced.³⁸ What does this association mean in terms of energy homeostasis? When studied in a comprehensive monitoring system for laboratory animals that included computer-controlled open-circuit calorimetry, systemically hypothyroid mice downregulated genes in thermogenic gene programmes in skeletal muscle but not in BAT, maintaining daily energy expenditure at normal amounts at room temperature (22°C).³⁹ Only at thermoneutrality (30°C) did hypothyroid mice decrease expression of genes in the BAT thermogenic programme and slow the rate of energy expenditure compared with controls. Thus, increased sympathetic activity in BAT compensates for systemic hypothyroidism by activating D2 and elevating local production of T₃, preserving intracellular signalling by thyroid hormone to some extent. Once this compensatory mechanism is suppressed by movement of animals to thermoneutrality, daily energy expenditure is reduced, and animals fall into overt systemic hypothyroidism.³⁹ A similar situation was reported in mice fed a low-iodine diet, which disrupts the thyroid's ability to synthesise and secrete thyroid hormone.⁴⁰ These animals showed a compensatory increase in D2 activity in BAT that prevented local T₃ concentrations from decreasing substantially compared with other tissues, which implies that local T₃ concentration in BAT is maintained by induction of D2 mRNA and activity.

Although there is probably an association between the sympathetic nervous system and thyroid-hormone signalling in most mammals, the very high surface-to-body-mass ratio of mice increases the risk of hypothermia; therefore, mice benefit from such a powerful compensatory mechanism. In larger mammals, including rats, the compensatory increase in the sympathetic nervous system is less intense, and systemic hypothyroidism substantially reduces the basal metabolic rate. A byproduct of the association between the sympathetic nervous system and thyroid-hormone signalling in small rodents is that at room temperature (22°C) systemically hypothyroid mice³⁹ and rats⁴¹ are not overly sensitive to diet-induced obesity (because of BAT activation). Only at thermoneutrality (30°C) did hypothyroid mice become obese when placed on a high-fat diet.³⁹ These findings are in contrast with those of

euthyroid control mice, which gained more bodyweight and fat on high-fat diets at all temperatures with activation of BAT and acceleration of daily energy expenditure, but not activation of the skeletal muscle thermogenic programme.³⁹

Expression of UCP1

Normal systemic thyroid status is essential for cold-induced adaptive thermogenesis.²¹ Hypothyroid animals develop severe hypothermia resulting in death after a few hours of exposure to cold, largely because of impaired BAT thermogenesis.¹⁶ Impaired non-shivering adaptive thermogenesis in hypothyroidism has also been reported in large mammalian neonates; thyroidectomy of the late-gestation ovine fetus resulted in impaired non-shivering thermogenesis, hypothermia, and reduced *Ucp1* abundance in neonatal lambs.⁴²

Systemic thyrotoxicosis accelerates energy expenditure and obligatory thermogenesis, indirectly decreasing thermogenic activity in BAT.⁴³ However, at physiological concentrations, thyroid hormone stimulates expression of the *Ucp1* gene by transcriptional and post-transcriptional mechanisms, as evidenced by analysis of *Ucp1* mRNA isolate from BAT nuclei of cold-exposed hypothyroid rodents,^{16,29} and in freshly isolated brown adipocytes.^{30,44} *Ucp1* responds rapidly to T_3 via two thyroid-hormone-responsive elements, located about 2000 bp upstream of the transcription start point, which synergise with the cyclic-AMP-responsive element that is much closer to the transcription start point.⁴⁵

Brown adipocytes have about 8000 thyroid-hormone receptors per cell, a relatively high concentration that is equivalent to that of hepatocytes.⁴⁶ Thyroid hormone acts in the brown adipocyte through both isoforms of thyroid-hormone receptor (α [Thra] and β [Thrb]), which control distinct pathways. Thra has an essential role in mediation of thyroid-hormone effects on adrenergic sensitivity as shown by impaired adaptive thermogenesis and inducible cyclic-AMP production after administration of the Thrb-selective ligand GC-1 to hypothyroid mice.⁴⁷ Thra-deficient mice (*Thra*^{-/-}) have defective adaptive thermogenesis despite normal concentrations of *Ucp1* in BAT, indicating that T_3 -regulated expression of *Ucp1* is independent of *Thra* status.⁴⁸ Thra-deficient mice are intolerant to cold; they eat more but are leaner than wild types, and are more resistant to diet-induced obesity. Disruption of BAT thermogenesis in *Thra*^{-/-} mice activates an alternative facultative thermogenesis that is more energy demanding and is associated with reduced fuel efficiency, leanness, increased capacity to oxidise fat, and relative resistance to diet-induced obesity compared with Thra-sufficient mice. Muscle lipoprotein lipase (an indicator of lipid oxidative activity) and deiodinases are important to all these processes.⁴⁹ By contrast, mice with the dominant negative *Thrb*^{PV} mutation (a frame-shift mutation that induces resistance to thyroid hormone) have defective thermogenesis with reduced expression of *Ucp1* and heat production during norepinephrine infusion.⁵⁰ Notably, even in white adipose tissue, *Ucp1* expression is induced by T_3 via a mechanism that is dependent on Thrb,⁵¹ which suggests a role for thyroid-hormone signalling in so-called browning of white adipose tissue. Thus, Thra mediates synergism between thyroid hormone signalling and the sympathetic nervous system in BAT, whereas Thrb mediates T_3 regulation of *Ucp1* in BAT; both isoforms are needed for adequate adaptive thermogenesis in BAT.

Gene expression and thermogenesis in BAT are sensitive to thyroid-hormone administration. BAT is unique because D2 expression, which is normally inhibited by T₃ in other tissues,⁵² is increased by exposure to T₃ in vitro or in vivo.⁵³ However, previous attempts to give thyroid hormone to induce weight loss were confounded by tachycardia, bone loss, and muscle catabolism;⁵⁴ thus administration of T₃ should not be considered as a strategy to accelerate adaptive thermogenesis. Because Ucp1 expression is positively regulated through Thrb, the therapeutic potential of a Thrb-selective agonist, GC-24, has been studied in a rat model of diet-induced obesity.^{55,56} Treatment with GC-24 accelerated daily energy expenditure, eliminating the increase in adiposity without promoting cardiac hypertrophy. GC-24 also improved glucose tolerance by increasing insulin sensitivity, and normalised plasma concentrations of triglyceride; plasma concentrations of cholesterol were only partly normalised by GC-24. Of course, this improvement could result from improved Thrb signalling in many tissues, but gene-expression profiles in mouse and rat models suggested that BAT was the main target.^{55,56} Other Thrb-selective agonists have been shown to increase daily energy expenditure and promote weight loss in rodents without adverse effects.⁵⁷

No data from human clinical trials of THRB stimulation have been reported, but both mouse and primate models suggest that Thrb selectively stimulates BAT and promotes adaptive thermogenesis. For example, administration of the Thrb-selective agonist KB-141 to cynomolgus monkeys for only 7 days resulted in statistically significant weight loss in the absence of heart-rate stimulation, signs of illness, or changes in food consumption, compared with before treatment.⁵⁸ Additional studies are needed to confirm these results in humans, but selective activation of Thrb in BAT is a very promising pathway.

Deiodinase regulation of thyroid-hormone signalling

Key transcription factors that are directly or indirectly regulated by T₃, such as peroxisome proliferator-activated receptors, define the genetic programme implicated in white and brown adipogenesis (figure 2). Therefore, by modulation of thyroid-hormone signalling, deiodinases can affect brown adipogenesis.⁵⁹ The involvement of deiodinase expression in brown adipogenesis is supported by the finding that mice with knocked out *Cebpa* (a gene that encodes CCAAT/enhancer binding protein α , a transcription factor expressed during adipogenesis) have blunted expression of *Ucp1* and *Dio2*,⁶⁰ and that D2 is present in human cultured preadipocytes.⁶¹

The development of BAT is a precisely coordinated process under the regulation of local thyroid-hormone signalling as a result of synchronised changes in deiodinase activity. In the mouse embryo, BAT develops between embryonic days 16.5 and 18.5, during which time D2 expression is increased and D3 expression is decreased, thus increasing local thyroid-hormone signalling.³⁵ Targeted disruption of *Dio2* (eg, in *Dio2*-knockout mice) results in metabolically impaired mature brown adipocytes, suggesting that D2 is an important regulator of BAT development. *Dio2*-knockout embryos show impaired expression of key genes of the adipogenic programme (*Fabp4*, *Cidea*, and *Acs15*) and impaired thermogenic identity (ie, decreased expression of genes involved in thermogenesis) of these cells (*Ucp1*, *Ppargc1a*, and *Dio2*). When studied in vitro, *Dio2*-knockout pre adipocytes show delayed

maturation as assessed by lipid accumulation, such that fewer *Dio2*-knockout cells are able to terminally differentiate into brown adipocytes; this effect is reversed with T₃ treatment of the adipocyte cultures.³³ In *Dio2*-knockout embryos, impaired BAT development is associated with decreased expression of *Ucp1* and *Ppargc1a* (as a result of the lack of D2-generated T₃) even in the absence of thermogenic stimulus, which contributes to inadequate BAT thermogenesis.³³

D2-generated T₃ in fully developed BAT

Blocking of BAT D2 with iopanoic acid in vivo,⁶² and in freshly isolated brown adipocytes, decreases adrenergic induction of *Ucp1* gene expression⁴⁴ and lipogenesis,⁶³ suggesting that D2-generated T₃ contributes to thermogenesis in adult BAT. One injection of T₄ to hypothyroid rats is sufficient to restore *Ucp1* expression in BAT, indicating that D2 can rapidly normalise thyroid-hormone signalling in BAT.¹⁶ Findings of subsequent studies of global *Dio2*-knockout mice have suggested that such animals have impaired thermogenic response to cold,⁶⁴ despite having normal plasma concentrations of T₃,⁶⁵ and survive by increased sympathetic activity⁶⁶ and compensatory shivering (a behaviour not typically shown by cold-exposed rodents). More importantly, the 30 min thermal response of interscapular BAT to norepinephrine infusion was diminished in *Dio2*-knockout mice (0.4°C vs 0.9°C for controls), clearly indicating the reduced capacity of BAT in *Dio2* knockouts to generate heat.⁶⁴ Furthermore, freshly isolated brown adipocytes from *Dio2* knockouts have impaired norepinephrine-induced *Ucp1* gene expression.⁶⁴ *Dio2*-knockout mice fed a high-fat diet had reduced diet-induced thermogenesis, resulting in obesity, glucose intolerance, and liver steatosis, compared with wild-type mice.⁶⁷ This finding implicates D2-generated T₃ in execution of both non-shivering and diet-induced BAT thermogenesis. Because of the essential part played by D2 during BAT development and brown adipose differentiation, the metabolic phenotype of the *Dio2*-knockout mouse probably shows both a congenital defect in BAT development and a role for D2-generated T₃ in adult BAT. Importantly, D2 is expressed in other metabolically relevant tissues, such as the medial basal hypothalamus and skeletal muscle—sites that probably contribute to the metabolic phenotype of the *Dio2*-knockout mouse. Substantial amounts of BAT mixed with muscle fibres have been reported, suggesting that some of the metabolic effects of skeletal muscle could in fact be mediated by BAT. For example, patients with resistance to THR_B have increased serum concentrations of thyroid hormone and whole-body energy expenditure, together with increased skeletal-muscle mitochondrial uncoupling.⁶⁸ How much of this effect is mediated by mitochondrial uncoupling in skeletal muscle versus BAT mixed with skeletal muscle is not clear.

D2-mediated thermogenesis in BAT

The adrenergic system and cyclic AMP are key physiological pathways and molecules of BAT activation. Additionally, several other endogenous and exogenous molecules activate BAT via the D2 pathway, including bile acids,⁶⁹ flavonols,⁷⁰ and chemical chaperones.⁷¹ Bile acids activate D2 and *Ucp1*-mediated thermogenesis in BAT via the Gpbar1 pathway; Gpbar1 is a G-protein-coupled receptor that accelerates cyclic-AMP production and thermogenesis in BAT. Dietary supplementation with bile acid promotes Gpbar1-mediated thermogenesis in BAT and protects against diet-induced obesity and insulin resistance. This

effect is lost in *Dio2*-knockout mice, and thus relies upon induction of cyclic-AMP-dependent D2.⁶⁹ Treatment of brown adipocytes and human skeletal myocytes with bile acid increases D2 activity and oxygen consumption, through a process mediated by increased cyclic-AMP production stemming from binding of bile acids to Gpbar1.⁶⁹ The pathway between bile acid, Gpbar1, cyclic AMP, and D2 is a crucial mechanism for fine-tuning of energy homeostasis, and can be targeted to improve metabolic control.⁶⁹ In fact, the Gpbar1-selective agonist INT-777 is efficacious in vivo to increase energy expenditure and reduce adiposity in a mouse model of diet-induced obesity.⁷² Similarly, but through a different molecular pathway, kaempferol and other flavonols stimulate D2 expression through a cyclic-AMP-mediated mechanism in primary cultures of human skeletal myocytes.⁷⁰ This stimulation leads to increased D2-mediated production of T₃, expression of thermogenically relevant genes, and acceleration of oxygen consumption.

D2 is a protein of the endoplasmic reticulum, and its expression is substantially reduced during stress of the endoplasmic reticulum via a mechanism mediated by eukaryotic initiation factor 2 that decreases D2 mRNA translation.⁷³ Conversely, exposure of D2-expressing human cell lines to the chemical chaperones tauroursodeoxycholic acid or 4-phenylbutyric acid increases D2 expression, D2 activity, and T₃ production. In brown adipocytes, these chaperones induced T₃-dependent genes and roughly doubled oxygen consumption, an effect partly lost in *Dio2*-knockout cells.⁷¹ In wild-type mice, but not in *Dio2*-knockout mice, tauroursodeoxycholic acid lowered the respiratory quotient, doubled D2 activity in BAT, and normalised glucose intolerance associated with a high-fat diet.⁷¹

D2 is a centrally important enzyme, linking several metabolic signalling pathways that can be triggered by endogenous and exogenous molecules. D2 activation through these pathways increases localised production of T₃ in BAT, and probably in other metabolically relevant tissues such as skeletal muscle, accelerating consumption of oxygen.

Expression of D2 in human BAT and response to thyroid hormone

The presence of D2 in interscapular depots of BAT in premature and fullterm human neonates is similar to that in rodents in terms of onset of development and peak distribution.⁷⁴ Findings from a PET study¹² in adults of ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) uptake in an anatomical distribution consistent with BAT depots showed that biopsy samples from these tissues had significantly higher concentrations of D2 than did those in corresponding white adipose depots. An analysis of human deep neck fat showed that these samples had a gene expression profile similar to that of classic rodent interscapular BAT.⁷⁵ The presence of D2 in human BAT, and the similarities in gene expression, lends support to the applicability of the findings from rodent models of BAT physiology to human beings.

At least one documented case has shown that systemic thyroid-hormone status in human beings can alter BAT activity, as measured by ¹⁸F-FDG PET.⁷⁶ A patient with thyroid cancer treated with thyroidectomy, radioactive iodine, and suppressive levothyroxine underwent PET and CT scans, which showed ¹⁸F-FDG uptake in a pattern consistent with BAT that was confirmed by CT-guided biopsy. Imaging repeated after levothyroxine withdrawal showed decreased ¹⁸F-FDG uptake in the areas previously identified as active BAT, and this uptake

again increased after resumption of levothyroxine. The transient, systemic hypothyroid state suppressed ^{18}F -FDG uptake in BAT that had been previously active under the systemic hyperthyroid state created by suppressive levothyroxine therapy. This patient also had type-A insulin resistance due to homozygous mutation of the α subunit of the insulin receptor. Her clinical course before the diagnosis of thyroid cancer was consistent with poor glucose control and profound insulin resistance, but changed in the months after her thyroid-cancer therapies because of remarkable improvement of her glycaemic control. Findings from metabolic studies done before and after levothyroxine withdrawal suggested that in the hypothyroid state, she had worsened fasting glucose and concentrations of glycated $\text{HbA}_{1\text{C}}$, reduced resting energy expenditure, and increased respiratory quotient, indicating reduced oxidation of fatty acids. The suppressive levothyroxine therapy might have stimulated her BAT and provided non-insulin-mediated glucose disposal and metabolism, resulting in her substantially improved glucose homeostasis.

Although BAT has a thermogenic role in human neonates,⁷⁷ its importance in healthy adults remains unclear. The fact that human BAT expresses D2 and responds to thyroid hormone supports the translation of findings from rodent models to human beings.

Association between *DIO2* polymorphism and obesity and insulin resistance

Reports of relatively common genetic mutations in the $\beta 3$ -adrenergic-receptor gene (*ADRB3*) and *DIO2* have increased understanding of BAT functionality in adults. A common *DIO2* polymorphism, present in about 15% of the population, is Thr92Ala.⁷⁸ This polymorphism is strongly associated with a phenotype characterised by insulin resistance, but is not associated with severe systemic perturbation in thyroid-hormone concentrations.⁷⁹ The Trp64Arg polymorphism of *ADRB3* generates less cyclic AMP in response to adrenergic agonists than does the wild-type receptor.⁸⁰ Individuals with both the *DIO2* Thr92Ala polymorphism and the *ADRB3* Trp64Arg polymorphism tend to have increased BMI by comparison with individuals without these polymorphisms,⁷⁸ which suggests an interaction between these variants. The *DIO2* Thr92Ala polymorphism is also associated with type 2 diabetes mellitus.⁸¹

Findings from a case-control study⁸² of 1057 patients with type 2 diabetes and 516 controls suggested that the frequencies of *DIO2* Thr92Ala homozygosity were 16.4% for patients with diabetes (n=173) versus 12.0% for controls (n=62), resulting in an adjusted odds ratio of 1.41 (95% CI 1.03–1.94, p=0.03). These data suggest that homozygosity for *DIO2* Thr92Ala polymorphism is associated with increased risk of type 2 diabetes, a conclusion that was supported by findings from a meta-analysis including 11 033 individuals.⁸² A broad range of diseases and disorders have now been associated with the *DIO2* Thr92Ala polymorphism, including intellectual disability, hypertension, osteoarthritis, bipolar disorder, the clinical course of Graves' disease, myocardial remodelling, accelerated bone turnover, and response to lung injury, suggesting a diverse clinical relevance for this locus.⁸³

Within the context of this discussion, the logical assumption is that the *DIO2* Thr92Ala polymorphism results in decreased D2 activity and thus localised tissue deficiency of T_3 and

hypothyroidism, which might have specific implications in BAT. The missense mutation encoded by the Thr92Ala polymorphism is in the D2-coding region, specifically the first aminoacid in an 18-residue instability loop that explains the short half-life of D2.²⁷ Deletion of this loop stabilises D2,⁸⁴ whereas its transfer to other stable proteins shortens their half-life.⁸⁵ Mapping of this mutation to such an important D2 residue lends support to the possibility that it could affect D2 activity or stability, decreasing D2-mediated production of T₃. However, different groups have failed to detect differences in enzyme kinetics (K_m [T₄] and V_{max}) of the Thr92Ala protein when transiently expressed in cultured cells.^{81,86} Findings from a study of tissue samples from individuals with the Thr92Ala polymorphism showed decreased V_{max} in the skeletal muscle and thyroid gland.⁸¹ However, the data have lost relevance because of the subsequent finding that special technical considerations are needed to assay D2 activity in skeletal muscle,^{87,88} which were not considered in the original study.⁸¹ The reported decrease in V_{max} for thyroidal Thr92Ala has not been reproduced under the appropriate assay conditions for D2, but is an interesting research question.

Two studies indirectly support the view that the Thr92Ala mutation of D2 is less catalytically active than the wild-type protein. First, higher doses of levothyroxine were needed to achieve target concentrations of thyroid-stimulating hormone in 191 thyroidectomised patients with the *DIO2* Thr92Ala polymorphism,⁸⁹ and second, the *DIO2* Thr92Ala polymorphism was associated with delayed T₃ secretion in response to thyroid-hormone-receptor stimulation.⁹⁰ Furthermore, in all studies, patients with the *DIO2* Thr92Ala polymorphism had no changes in all other tests of thyroid function,⁸⁶ and thus were systemically euthyroid. Data for the *DIO2* Thr92Ala polymorphism are controversial, with poor reproducibility among different studies,⁸³ which suggests that additional unidentified linkage factors (eg, ethnic background) could be important in the physiological and clinical relevance of the polymorphism.^{91,92} The alterations, if any, in BAT adaptive thermogenesis in these individuals remain to be elucidated.

Conclusions

Thyroid-hormone signalling substantially affects energy homoeostasis and accelerates energy expenditure, partly through activation of BAT by local D2-mediated activation of thyroid hormone. D2-dependent T₃ is necessary for tissue identity and functionality of BAT, and for brown adipogenesis, as shown in animal and cell models. BAT is present and thermogenically active in adults; furthermore, human BAT contains D2 and is known to respond to thyroid-hormone signalling (including mechanisms initiated by THRB). Therefore, available evidence suggests that knowledge from rodent models will probably be applicable in people. The presence of thermogenically active D2-expressing BAT in adults is clinically important; adaptive thermogenesis by BAT represents a potential target for therapeutic interventions to harness the energy expenditure capabilities of BAT, and thus prevent or treat obesity.

References

1. Bianco AC, Maia AL, da Silva WS, Christoffolete MA. Adaptive activation of thyroid hormone and energy expenditure. *Biosci Rep*. 2005; 25:191–208. [PubMed: 16283553]
2. Nedergaard J, Cannon B. The changed metabolic world with human brown adipose tissue: therapeutic visions. *Cell Metab*. 2010; 11:268–72. [PubMed: 20374959]
3. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev*. 2004; 84:277–359. [PubMed: 14715917]
4. Nicholls DG. The physiological regulation of uncoupling proteins. *Biochim Biophys Acta*. 2006; 1757:459–66. [PubMed: 16725104]
5. Bruck, K. Neonatal thermal regulation. In: Polin, RA.; Fox, WW., editors. *Fetal and neonatal physiology*. Philadelphia, PA: WB Saunders; 1998. p. 676-702.
6. Tiraby C, Tavernier G, Lefort C, et al. Acquisition of brown fat cell features by human white adipocytes. *J Biol Chem*. 2003; 278:33370–76. [PubMed: 12807871]
7. Heaton JM. The distribution of brown adipose tissue in the human. *J Anat*. 1972; 112:35–39. [PubMed: 5086212]
8. Ricquier D, Nechad M, Mory G. Ultrastructural and biochemical characterization of human brown adipose tissue in pheochromocytoma. *J Clin Endocrinol Metab*. 1982; 54:803–07. [PubMed: 7061689]
9. Celi FS. Brown adipose tissue—when it pays to be inefficient. *N Engl J Med*. 2009; 360:1553–56. [PubMed: 19357412]
10. Cypess AM, Lehman S, Williams G, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med*. 2009; 360:1509–17. [PubMed: 19357406]
11. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med*. 2009; 360:1500–08. [PubMed: 19357405]
12. Virtanen KA, Lidell ME, Orava J, et al. Functional brown adipose tissue in healthy adults. *N Engl J Med*. 2009; 360:1518–25. [PubMed: 19357407]
13. Celi FS, Brychta RJ, Linderman JD, et al. Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults. *Eur J Endocrinol*. 2010; 163:863–72. [PubMed: 20826525]
14. Yoneshiro T, Aita S, Matsushita M, et al. Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity (Silver Spring)*. 2011; 19:13–16. [PubMed: 20448535]
15. Heim T, Hull D. The effect of propranolol on the calorogenic response in brown adipose tissue of new-born rabbits to catecholamines, glucagon, corticotrophin and cold exposure. *J Physiol*. 1966; 187:271–83. [PubMed: 4291385]
16. Bianco AC, Silva JE. Intracellular conversion of thyroxine to triiodothyronine is required for the optimal thermogenic function of brown adipose tissue. *J Clin Invest*. 1987; 79:295–300. [PubMed: 3793928]
17. Moriscot A, Rabelo R, Bianco AC. Corticosterone inhibits uncoupling protein gene expression in brown adipose tissue. *Am J Physiol*. 1993; 265:E81–87. [PubMed: 8338156]
18. Poggioli R, Ueta CB, Drigo RA, Castillo M, Fonseca TL, Bianco AC. Dexamethasone reduces energy expenditure and increases susceptibility to diet-induced obesity in mice. *Obesity (Silver Spring)*. 2013; 9:E415–20. [PubMed: 23408649]
19. Bachman ES, Dhillon H, Zhang CY, et al. β AR signaling required for diet-induced thermogenesis and obesity resistance. *Science*. 2002; 297:843–45. [PubMed: 12161655]
20. Ueta CB, Fernandes GW, Capelo L, et al. $\beta(1)$ Adrenergic receptor is key to cold- and diet-induced thermogenesis in mice. *J Endocrinol*. 2012; 14:359–65. [PubMed: 22728333]
21. Sellers EA, You SS. Role of the thyroid in metabolic responses to a cold environment. *Am J Physiol*. 1950; 163:81–91. [PubMed: 14771277]
22. Ribeiro MO, Lebrun FL, Christoffolete MA, et al. Evidence of UCP1-independent regulation of norepinephrine-induced thermogenesis in brown fat. *Am J Physiol Endocrinol Metab*. 2000; 279:E314–22. [PubMed: 10913031]

23. Rothwell NJ, Stock MJ. A role for brown adipose tissue in diet-induced thermogenesis. *Obes Res.* 1997; 5:650–56. [PubMed: 9449154]
24. Leonard JL, Mellen SA, Larsen PR. Thyroxine 5'-deiodinase activity in brown adipose tissue. *Endocrinology.* 1983; 112:1153–55. [PubMed: 6822208]
25. Bianco AC, Silva JE. Optimal response of key enzymes and uncoupling protein to cold in BAT depends on local T3 generation. *Am J Physiol.* 1987; 253(3 Pt 1):E255–63. [PubMed: 3631256]
26. Croteau W, Davey JC, Galton VA, St Germain DL. Cloning of the mammalian type II iodothyronine deiodinase. A selenoprotein differentially expressed and regulated in human and rat brain and other tissues. *J Clin Invest.* 1996; 98:405–17. [PubMed: 8755651]
27. Callebaut I, Curcio-Morelli C, Mornon JP, et al. The iodothyronine selenodeiodinases are thioredoxin-fold family proteins containing a glycoside hydrolase clan GH-A-like structure. *J Biol Chem.* 2003; 278:36887–96. [PubMed: 12847093]
28. Bianco AC, Silva JE. Cold exposure rapidly induces virtual saturation of brown adipose tissue nuclear T3 receptors. *Am J Physiol.* 1988; 255(4 Pt 1):E496–503. [PubMed: 3177636]
29. Bianco AC, Sheng XY, Silva JE. Triiodothyronine amplifies norepinephrine stimulation of uncoupling protein gene transcription by a mechanism not requiring protein synthesis. *J Biol Chem.* 1988; 263:18168–75. [PubMed: 3192531]
30. Rehnmark S, Bianco AC, Kieffer JD, Silva JE. Transcriptional and posttranscriptional mechanisms in uncoupling protein mRNA response to cold. *Am J Physiol.* 1992; 262(1 Pt 1):E58–67. [PubMed: 1733251]
31. Gereben B, Zavacki AM, Ribich S, et al. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr Rev.* 2008; 29:898–938. [PubMed: 18815314]
32. Bianco AC, Silva JE. Nuclear 3,5,3'-triiodothyronine (T3) in brown adipose tissue: receptor occupancy and sources of T3 as determined by *in vivo* techniques. *Endocrinology.* 1987; 120:55–62. [PubMed: 3780570]
33. Hall JA, Ribich S, Christoffolete MA, et al. Absence of thyroid hormone activation during development underlies a permanent defect in adaptive thermogenesis. *Endocrinology.* 2010; 151:4573–82. [PubMed: 20660060]
34. Hernandez A, Garcia B, Obregon MJ. Gene expression from the imprinted Dio3 locus is associated with cell proliferation of cultured brown adipocytes. *Endocrinology.* 2007; 148:3968–76. [PubMed: 17510246]
35. Nunes MT, Bianco AC, Migala A, Agostini B, Hasselbach W. Thyroxine induced transformation in sarcoplasmic reticulum of rabbit soleus and psoas muscles. *Z Naturforsch C.* 1985; 40:726–34. [PubMed: 2934902]
36. Simonides WS, van Hardeveld C. Thyroid hormone as a determinant of metabolic and contractile phenotype of skeletal muscle. *Thyroid.* 2008; 18:205–16. [PubMed: 18279021]
37. Young JB, Saville E, Landsberg L. Effect of thyroid state on norepinephrine (NE) turnover in rat brown adipose tissue (BAT): potential importance of the pituitary. *Clin Res.* 1982; 32:407. abstr.
38. Silva JE. Thermogenesis and the sympathoadrenal system in thyrotoxicosis. In: Braverman, LE., editor. *Werner & Ingbar's the thyroid: a fundamental and clinical text.* 9. Philadelphia, PA: Lippincott Williams & Wilkins; 2005. p. 307-620.
39. Ueta CB, Olivares EL, Bianco AC. Responsiveness to thyroid hormone and to ambient temperature underlies differences between brown adipose tissue and skeletal muscle thermogenesis in a mouse model of diet-induced obesity. *Endocrinology.* 2011; 152:3571–81. [PubMed: 21771890]
40. Lavado-Autric R, Calvo RM, de Mena RM, de Escobar GM, Obregon MJ. Deiodinase activities in thyroids and tissues of iodine-deficient female rats. *Endocrinology.* 2013; 154:529–36. [PubMed: 23142811]
41. Curcio C, Lopes AM, Ribeiro MO, et al. Development of compensatory thermogenesis in response to overfeeding in hypothyroid rats. *Endocrinology.* 1999; 140:3438–43. [PubMed: 10433198]
42. Schermer SJ, Bird JA, Lomax MA, Shepherd DA, Symonds ME. Effect of fetal thyroidectomy on brown adipose tissue and thermoregulation in newborn lambs. *Reprod Fertil Dev.* 1996; 8:995–1002. [PubMed: 8896035]
43. Triandafillou J, Gwilliam C, Himms-Hagen J. Role of thyroid hormone in cold-induced changes in rat brown adipose tissue mitochondria. *Can J Biochem.* 1982; 60:530–37. [PubMed: 7104831]

44. Bianco AC, Kieffer JD, Silva JE. Adenosine 3',5'-monophosphate and thyroid hormone control of uncoupling protein messenger ribonucleic acid in freshly dispersed brown adipocytes. *Endocrinology*. 1992; 130:2625–33. [PubMed: 1374009]
45. Rabelo R, Schifman A, Rubio A, Sheng X, Silva JE. Delineation of thyroid hormone-responsive sequences within a critical enhancer in the rat uncoupling protein gene. *Endocrinology*. 1995; 136:1003–13. [PubMed: 7867554]
46. Bianco AC, Silva JE. Nuclear 3,5,3'-triiodothyronine (T3) in brown adipose tissue: receptor occupancy and sources of T3 as determined by in vivo techniques. *Endocrinology*. 1987; 120:55–62. [PubMed: 3780570]
47. Ribeiro MO, Carvalho SD, Schultz JJ, et al. Thyroid hormone-sympathetic interaction and adaptive thermogenesis are thyroid hormone receptor isoform-specific. *J Clin Invest*. 2001; 108:97–105. [PubMed: 11435461]
48. Marri H, Schifman A, Stepanyan Z, et al. Temperature homeostasis in transgenic mice lacking thyroid hormone receptor-alpha gene products. *Endocrinology*. 2005; 146:2872–84. [PubMed: 15845618]
49. Pelletier P, Gauthier K, Sideleva O, Samarut J, Silva JE. Mice lacking the thyroid hormone receptor-alpha gene spend more energy in thermogenesis, burn more fat, and are less sensitive to high-fat diet-induced obesity. *Endocrinology*. 2008; 149:6471–86. [PubMed: 18719022]
50. Ribeiro MO, Bianco SD, Kaneshige M, et al. Expression of uncoupling protein 1 in mouse brown adipose tissue is thyroid hormone receptor-beta isoform specific and required for adaptive thermogenesis. *Endocrinology*. 2010; 151:432–40. [PubMed: 19906816]
51. Lee JY, Takahashi N, Yasubuchi M, et al. Triiodothyronine induces UCP-1 expression and mitochondrial biogenesis in human adipocytes. *Am J Physiol Cell Physiol*. 2012; 302:C463–72. [PubMed: 22075692]
52. Leonard JL, Kaplan MM, Visser TJ, Silva JE, Larsen PR. Cerebral cortex responds rapidly to thyroid hormones. *Science*. 1981; 214:571–73. [PubMed: 7291997]
53. Hernandez A, Obregon MJ. Triiodothyronine amplifies the adrenergic stimulation of uncoupling protein expression in rat brown adipocytes. *Am J Physiol Endocrinol Metab*. 2000; 278:E769–77. [PubMed: 10780931]
54. Baxter JD, Webb P. Thyroid hormone mimetics: potential applications in atherosclerosis, obesity and type 2 diabetes. *Nat Rev Drug Discov*. 2009; 8:308–20. [PubMed: 19337272]
55. Castillo M, Freitas BC, Rosene ML, et al. Impaired metabolic effects of a thyroid hormone receptor beta-selective agonist in a mouse model of diet-induced obesity. *Thyroid*. 2010; 20:545–53. [PubMed: 20406106]
56. Villicev CM, Freitas FR, Aoki MS, et al. Thyroid hormone receptor beta-specific agonist GC-1 increases energy expenditure and prevents fat-mass accumulation in rats. *J Endocrinol*. 2007; 193:21–29. [PubMed: 17400799]
57. Bryzgalova G, Effendic S, Khan A, et al. Anti-obesity, anti-diabetic, and lipid lowering effects of the thyroid receptor beta subtype selective agonist KB-141. *J Steroid Biochem Mol Biol*. 2008; 111:262–67. [PubMed: 18621127]
58. Grover GJ, Mellstrom K, Ye L, et al. Selective thyroid hormone receptor-beta activation: a strategy for reduction of weight, cholesterol, and lipoprotein (a) with reduced cardiovascular liability. *Proc Natl Acad Sci USA*. 2003; 100:10067–72. [PubMed: 12888625]
59. Obregon MJ. Thyroid hormone and adipocyte differentiation. *Thyroid*. 2008; 18:185–95. [PubMed: 18279019]
60. Carmona MC, Iglesias R, Obregon MJ, Darlington GJ, Villarroya F, Giralt M. Mitochondrial biogenesis and thyroid status maturation in brown fat require CCAAT/enhancer-binding protein alpha. *J Biol Chem*. 2002; 277:21489–98. [PubMed: 11940593]
61. Nomura E, Toyoda N, Harada A, et al. Type 2 iodothyronine deiodinase is expressed in human preadipocytes. *Thyroid*. 2011; 21:305–10. [PubMed: 21323585]
62. Reiter RJ, Klaus S, Ebbinghaus C, et al. Inhibition of 5'-deiodination of thyroxine suppresses the cold-induced increase in brown adipose tissue messenger ribonucleic acid for mitochondrial uncoupling protein without influencing lipoprotein lipase activity. *Endocrinology*. 1990; 126:2550–54. [PubMed: 2328697]

63. Bianco AC, Carvalho SD, Carvalho CR, Rabelo R, Moriscot AS. Thyroxine 5'-deiodination mediates norepinephrine-induced lipogenesis in dispersed brown adipocytes. *Endocrinology*. 1998; 139:571–78. [PubMed: 9449627]
64. de Jesus LA, Carvalho SD, Ribeiro MO, et al. The type 2 iodothyronine deiodinase is essential for adaptive thermogenesis in brown adipose tissue. *J Clin Invest*. 2001; 108:1379–85. [PubMed: 11696583]
65. Schneider MJ, Fiering SN, Pallud SE, Parlow AF, St Germain DL, Galton VA. Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. *Mol Endocrinol*. 2001; 15:2137–48. [PubMed: 11731615]
66. Christoffolete MA, Linardi CC, de Jesus L, et al. Mice with targeted disruption of the Dio2 gene have cold-induced overexpression of the uncoupling protein 1 gene but fail to increase brown adipose tissue lipogenesis and adaptive thermogenesis. *Diabetes*. 2004; 53:577–84. [PubMed: 14988240]
67. Castillo M, Hall JA, Correa-Medina M, et al. Disruption of thyroid hormone activation in type 2 deiodinase knockout mice causes obesity with glucose intolerance and liver steatosis only at thermoneutrality. *Diabetes*. 2011; 60:1082–89. [PubMed: 21335378]
68. Mitchell CS, Savage DB, Dufour S, et al. Resistance to thyroid hormone is associated with raised energy expenditure, muscle mitochondrial uncoupling, and hyperphagia. *J Clin Invest*. 2010; 120:1345–54. [PubMed: 20237409]
69. Watanabe M, Houten SM, Matakci C, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature*. 2006; 439:4848–49.
70. da-Silva WS, Harney JW, Kim BW, et al. The small polyphenolic molecule kaempferol increases cellular energy expenditure and thyroid hormone activation. *Diabetes*. 2007; 56:767–76. [PubMed: 17327447]
71. da-Silva WS, Ribich S, Arrojo e Drigo R, Castillo M, Patti ME, Bianco AC. The chemical chaperones tauroursodeoxycholic and 4-phenylbutyric acid accelerate thyroid hormone activation and energy expenditure. *FEBS Lett*. 2011; 585:539–44. [PubMed: 21237159]
72. Thomas C, Gioiello A, Noriega L, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab*. 2009; 10:167–77. [PubMed: 19723493]
73. Arrojo e Drigo R, Fonseca TL, Castillo M, et al. Endoplasmic reticulum stress decreases intracellular thyroid hormone activation via an eIF2a-mediated decrease in type 2 deiodinase synthesis. *Mol Endocrinol*. 2011; 25:2065–75. [PubMed: 22053000]
74. Houstek J, Vizek K, Pavelka S, et al. Type II iodothyronine 5'-deiodinase and uncoupling protein in brown adipose tissue of human newborns. *J Clin Endocrinol Metab*. 1993; 77:382–87. [PubMed: 8393883]
75. Cypess AM, White AP, Vernochet C, et al. Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. *Nat Med*. 2013; 19:635–39. [PubMed: 23603815]
76. Skarulis MC, Celi FS, Mueller E, et al. Thyroid hormone induced brown adipose tissue and amelioration of diabetes in a patient with extreme insulin resistance. *J Clin Endocrinol Metab*. 2010; 95:256–62. [PubMed: 19897683]
77. Lean ME. Brown adipose tissue in humans. *Proc Nutr Soc*. 1989; 48:243–56. [PubMed: 2678120]
78. Mentuccia D, Proietti-Pannunzi L, Tanner K, et al. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. *Diabetes*. 2002; 51:880–83. [PubMed: 11872697]
79. Peeters RP, van der Deure WM, Visser TJ. Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. *Eur J Endocrinol*. 2006; 155:655–62. [PubMed: 17062880]
80. Kimura K, Sasaki N, Asano A, et al. Mutated human beta3-adrenergic receptor (Trp64Arg) lowers the response to beta3-adrenergic agonists in transfected 3T3-L1 preadipocytes. *Horm Metab Res*. 2000; 32:91–96. [PubMed: 10786926]

81. Canani LH, Capp C, Dora JM, et al. The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab.* 2005; 90:3472–78. [PubMed: 15797963]
82. Dora JM, Machado WE, Rheinheimer J, Crispim D, Maia AL. Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes: case-control study and meta-analysis. *Eur J Endocrinol.* 2010; 163:427–34. [PubMed: 20566590]
83. Bianco AC, Casula S. Thyroid hormone replacement therapy: three ‘simple’ questions, complex answers. *Eur Thyroid J.* 2012; 1:88–98. [PubMed: 24783002]
84. Dentice M, Bandyopadhyay A, Gereben B, et al. The Hedgehog-inducible ubiquitin ligase subunit WSB-1 modulates thyroid hormone activation and PTHrP secretion in the developing growth plate. *Nat Cell Biol.* 2005; 7:698–705. [PubMed: 15965468]
85. Zeold A, Pormuller L, Dentice M, et al. Metabolic instability of type 2 deiodinase is transferable to stable proteins independently of subcellular localization. *J Biol Chem.* 2006; 281:31538–43. [PubMed: 16928685]
86. Peeters RP, van Toor H, Klootwijk W, et al. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *J Clin Endocrinol Metab.* 2003; 88:2880–88. [PubMed: 12788902]
87. Ramadan W, Marsili A, Huang S, Larsen PR, Silva JE. Type-2 iodothyronine 5' deiodinase in skeletal muscle of C57BL/6 mice. I. Identity, subcellular localization, and characterization. *Endocrinology.* 2011; 152:3082–92. [PubMed: 21628384]
88. Larsen PR. Type 2 iodothyronine deiodinase in human skeletal muscle: new insights into its physiological role and regulation. *J Clin Endocrinol Metab.* 2009; 94:1893–95. [PubMed: 19494166]
89. Torlontano M, Durante C, Torrente I, et al. Type 2 deiodinase polymorphism (Thr92ala) predicts l-thyroxine dose to achieve target Tsh levels in thyroidectomized patients. *J Clin Endocrinol Metab.* 2007; 93:910–13. [PubMed: 18073314]
90. Butler PW, Smith SM, Linderman JD, et al. The Thr92Ala 5' type 2 deiodinase gene polymorphism is associated with a delayed triiodothyronine secretion in response to the thyrotropin-releasing hormone-stimulation test: a pharmacogenomic study. *Thyroid.* 2011; 20:1407–12. [PubMed: 21054208]
91. Mentuccia D, Thomas MJ, Coppotelli G, et al. The Thr92Ala deiodinase type 2 (DIO2) variant is not associated with type 2 diabetes or indices of insulin resistance in the old order of Amish. *Thyroid.* 2005; 15:1223–27. [PubMed: 16356084]
92. Nair S, Muller YL, Ortega E, Kobes S, Bogardus C, Baier LJ. Association analyses of variants in the DIO2 gene with early-onset type 2 diabetes mellitus in Pima Indians. *Thyroid.* 2012; 22:80–87. [PubMed: 22142372]

Search strategy and selection criteria

We searched the Medline database up to June 6, 2013, with the search term “brown adipose tissue” in combination with “thyroid” or “deiodinase”. Emphasis was given to publications in the past 5 years, but we did not exclude seminal older publications. We also considered articles from the reference lists of publications identified by this search strategy.

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Figure 1. D2 activity generates T₃ in brown adipocytes and increases nuclear T₃ and thyroid-hormone-receptor saturation

D2=type II deiodinase. T₄=thyroxine. T₃=tri-iodothyronine. Reproduced from Bianco and colleagues.²⁸

Figure 2. Proposed model of positive feedback involving expression of *Dio2*, *Pparg1a*, and *Ucp1* during BAT development

Schematic representation of the proposed role of D2 and D2-generated T₃ in the development of brown adipocytes. D3, which decreases thyroid-hormone signalling, is highest in the undeveloped brown preadipocyte. As the brown preadipocyte matures, D2, increases expression of *Pparg1a* by enhancing thyroid-hormone signalling, which coactivates thyroid-hormone receptors, leading to increased expression of *Ucp1*. *Dio2* is also upregulated by increased T₃ signalling. These D2-dependent pathways provide the mature brown adipocyte with its full thermogenic identity. BAT=brown adipose tissue. D2=type II deiodinase. T₃=tri-iodothyronine. D3=type III deiodinase. Adapted from Hall and colleagues.³³