Thyroid Economy—Regulation, Cell Biology, Thyroid Hormone Metabolism and Action: The Bianco Special Edition: Metabolic Effects of Thyroid Hormones

Thyroid Hormone and Adipocyte Differentiation

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Thyroid hormones act as pleiotropic factors in many tissues during development, by regulating genes involved in differentiation. The adipose tissue, a target of thyroid hormones, is the main place for energy storage and acts as a regulator of energy balance, sending signals to keep metabolic control. Adipogenesis is a complex process that involves proliferation of preadipocytes and its differentiation into mature adipocytes. This process is regulated by several transcription factors (CCAAT/enhancer-binding proteins [C/EBPs], peroxisome proliferator-activated receptors [PPARs]) that act coordinately, activating adipocyte-specific genes that will provide the adipocytic phenotype. Thyroid hormones regulate many of those genes, markers of differentiation of adipocytes, those involved in lipogenesis, lipolysis, and thermogenesis in the brown adipose tissue (BAT). Triiodothyronine (T3) actions are achieved either directly through specific thyroid response elements (TREs), by regulating other key genes as PPARs, or through specific isoforms of the nuclear T3 receptors. The availability of T3 is regulated through the deiodinases D3, D2, and D1. D3 is activated by serum and mitogens during proliferation of preadipocytes, while D2 is linked to the differentiation program of adipocytes, through the C/EBPs that govern its functionality, providing the T3 required for thermogenesis and lipogenesis. The relationship between white adipose tissue (WAT) and BAT and the possible reactivation of WAT by activation of uncoupling protein-1 (UCP1) is discussed.

Introduction

THYROID HORMONE ACTIONS ARE PLEIOTROPIC, involving the regulation of many physiological systems. Their actions are especially important during development. One of the best-studied actions of thyroid hormones is the regulation of the metamorphosis of amphibians (1–4). Thyroid hormones regulate the growth and maturation of many organs and tissues during the fetal and neonatal life (5,6) as well as other specific organs like the cochlea or specific regions of the retina (7,8). Many tissues are regulated by thyroid hormones up to their complete development, including actions on groups of genes involved in the differentiation program. The supply of thyroid hormones is also regulated in a time-specific way and in precise areas of the brain, through the deiodinases D2 and D3, as demonstrated in the human fetal brain (9) or in the cochlea (10). During the adult life, thyroid hormones regulate the metabolism and function of many tissues, such as liver, heart, skin, muscle, or adipose tissue.

The adipose tissue is one of the targets of thyroid hormones. The adipose tissue is specialized in the transport, synthesis, storage, and mobilization of lipids. Its main function is the storage of energy in the form of triglycerides, and it constitutes a reservoir of energy to be used in times of caloric deprivation.

There are two types of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT), which have distinct features. The WAT is considered as a reservoir of lipids and energy and morphologically is characterized by a large lipid droplet that fills the cellular space. WAT is distributed in many different anatomical locations: subcutaneous and visceral (omental) fat, which have different lipolytic sensitivity and response to drugs and hormones and represent a different health risk (insulin resistance and metabolic syndrome) (11). But we can find also fat deposits covering other organs such as the heart, kidney, or the sexual organs (perirenal and perigonadal depots). To which extent this adipose tissue is pure WAT is under discussion because some of these locations are reminiscent of the primitive BAT locations. WAT in

humans is a large and disperse tissue along the body accounting for about 15% of total body weight in control subjects, a percentage that can increase up to 40% in obese patients.

The BAT is a tissue specialized in the adaptation to cold by adaptive or facultative thermogenesis or to rich diets (protection against obesity). BAT is found predominantly in hibernating animals, small rodents, and newborns (12). The main function of BAT is to dissipate energy instead of storing it. The dissipation of energy is accomplished by the BAT-specific protein, uncoupling protein-1 (UCP1), which generates heat by uncoupling the respiratory chain. BAT is found in small, disperse locations in the body, protecting heart, kidneys, aorta, and other organs. It is highly innervated and irrigated. Morphologically BAT is characterized by multilocular lipid droplets and abundant mitochondria, which increase under cold exposure. The capacity of BAT to dissipate energy is considered as a possible tool against obesity in case it could be reactivated.

Proliferation and Differentiation of Adipocytes

The adipose tissue was considered for many years only as a reservoir of lipids, a place for lipid storage and mobilization, but during the last 15 years there has been a burst of information related to adipose tissue, its regulation, its secretory function, the adipocyte-specific genes, and the signaling pathways altered in pathological situations, leading to a better knowledge of this tissue and the main features of its functional unit, the adipocyte.

The research on adipose tissue was hampered for years due to the difficulties inherent to its high lipid content and the lack of suitable cell culture models for its molecular study. The establishment of preadipose cell lines, derived from NIH 3T3 fibroblasts (3T3-L1 and 3T3-F442 cell lines), allowed the study of the differentiation of the adipocyte. The pioneering studies from the groups of Spiegelman and Lane have thrown light on the molecular biology of the adipocyte (13,14). They initially identified a series of lipogenic and glycolytic enzymes that increased several fold during the process of the adipocyte

differentiation, by measuring its activity, protein levels, and mRNAs. Among these markers were the fatty-acid-binding protein aP2 (15), glycerophosphate dehydrogenase (GPD) (13), acetyl-CoA carboxylase (ACC) (16), the stearoyl-CoA desaturase (SCD) (17), the fatty acid synthetase (FAS) (16), the lactic dehydrogenase (LDH), lipoprotein lipase (LPL), malic enzyme (ME), phosphoenolpyruvate carboxykinase (PEPCK), and some new genes as adipsin (18,19) and adipoQ (20), now called adiponectin. Among those proteins some are early markers of adipocyte differentiation and others appear later on, as seen in Figure 1, and as reviewed by Ailhaud *et al.* (21). The C/EBPs transcription factors were early identified by Lane and coworkers as the ones governing the process of adipocyte differentiation (22,23).

The adipocyte is the functional unit of the adipose tissue. This cell, specialized in the storage of lipids, acquires its full capacity after a complex process called adipogenesis, which involves proliferation from preadipocytes or mesenchymal-type cells and a coordinated process of differentiation that confers the adipocyte the full capacity to accomplish its specialized functions.

Proliferation of preadipocytes

The preadipocyte is a mesenchymal-type cell that derives from pluripotent stem cells, which become predetermined to be preadipocyte. The factors that trigger these first steps from pluripotent stem cells into preadipocytes have not been elucidated. Osteoblasts, myoblasts, and adipocytes derive from a common mesenchymal precursor cell (24,25). Mesenchymal stem cells seem to differentiate into adipocytes under PPAR γ 2 activation (26), but the intermediate steps that trigger the activation are far from being identified. Leukemia inhibitory factor (LIF) has been proposed as one of the markers of these initial steps, inducing the adipocytic phenotype together with PPAR γ 2. The preadipocyte factor 1 (Pref-1, also called *Dlk1*) is an imprinted gene found only in preadipocytes and is a potent inhibitor of adipogenesis (27). Pref-1 activates MEK-extracellular signal-regulated kinase (ERK) phosphorylation (28–30) and is a marker of preadipocyte stage (Fig. 1). Certain *HOX* genes display a specific expression in WAT, and the expression of four *HOX* genes appears to discriminate between WAT and BAT (31). A recent study using microarrays has identified Pref-1 as one of the markers of the proliferative stage in brown preadipocytes, while C/EBP δ and Necdin are markers of proliferating brown and white preadipocytes (32). Necdin is a transcriptional regulator that would inhibit the activation of the PPAR γ 1 promoter (33).

The preadipocytes, also called “precursor” cells or mesenchymal-type cells, present in the stroma-vascular fraction of the adipose tissue, even in adult animals, though they are present in smaller number. The identification of these precursor cells has been very important for the research in adipose tissue because it has allowed to establish primary cultures of preadipocytes, which proliferate and differentiate in culture (34). The proliferation of preadipocytes occurs under the stimulation of several growth factors present in serum, mainly those from the family of the fibroblast growth factors (FGFs). The proliferation of brown preadipocytes is highly stimulated under cold exposure and was first studied *in vivo* using tritiated thymidine, establishing the β -adrenergic nature of the process of proliferation (35–37), while insulin

FIG. 1. Different stages of white adipocyte differentiation are shown, from precursor cells to mature adipocytes. The enzymes and proteins expressed at the different stages of differentiation are shown in the right column. Modified from Ailhaud *et al.* (21).

FIG. 2. Proliferation of brown preadipocytes. (A) We show the increases in DNA synthesis in brown preadipocytes using different growth factors (EGF, PDGF, aFGF, and bFGF), alone or combined with Vasopresin (V) and/or norepinephrine (NE). (B) Antimitotic effect of triiodothyronine (T3) on DNA synthesis induced by growth factors, measured as thymidine incorporation (40).

was proposed as a mitotic factor for white adipocytes (38). Studies in primary cultures of brown preadipocytes demonstrated that the $\beta 1$ adrenergic stimulation increases DNA synthesis (39). Our studies showed that norepinephrine (NE), a poor mitogen itself, increases the mitogenic action of serum, growth factors, and the neuropeptide vasopressin (40) (Fig. 2A). Therefore, NE has an important role in brown adipocyte proliferation, besides its role in increasing thermogenesis, specifically UCP1 mRNA expression.

The role of thyroid hormones during the proliferative stage of adipocytes seems to be antimitogenic, as the addition of triiodothyronine (T3) inhibits the mitogenic activity of bFGF and aFGF in brown preadipocytes (41) (Fig. 2B). In fact, D3 activity and mRNA are highly induced by growth factors in primary cultures of brown adipocytes (42,43), suggesting that a reduction of T3 levels is physiologically important under mitogenic stimulation. Large D3 increases (activity and mRNA) are also observed when serum is added to primary cultures of brown adipocytes (44) (Fig. 3). This led us to propose that D3 acts as a marker of proliferation in brown preadipocytes. As opposite, little D2 activity is observed during the proliferative stages, pointing to a differential role of both deiodinases, D3 being present during proliferation and D2 having a role during the differentiation stages.

Proliferation studies using white preadipocytes are scarce, although serum clearly stimulates DNA synthesis and proliferation in white preadipocytes in primary cultures (un-

published results). The identification of the specific growth factors responsible for the proliferation of white preadipocytes requires further investigation. It has been postulated that the proliferation of white preadipocytes requires only T3, insulin, and transferrin in serum-free medium (45,46). However, evidence indicates that the preadipocytes of obese people secrete mitogenic factors that induce a higher proliferation rate than the conditioned media derived from preadipocytes from control subjects (47). Macrophage-secreted factors have also been proposed as mitogens in human preadipocytes (48), but the specific growth factors or adipokines involved have not been identified. FGF10 has been proposed as one of the mitogens for WAT, because the development of WAT in FGF10^{-/-} mouse embryos is greatly impaired due to a decreased proliferative activity of WAT, indicating that FGF10, not C/EBP α , is required for the proliferation of white preadipocytes (49). Adipose tissue is a source of growth factors that could stimulate proliferation, such as insulin growth factor I (IGF-I), IGF-binding proteins, tumor necrosis factor α (TNF- α), angiotensin II, and macrophage colony-stimulating factor (MCSF) (50).

Differentiation of the adipocytes: the role of transcription factors

The process of adipocyte differentiation was first studied as a unique process in preadipose cells lines (3T3-L1 and

FIG. 3. Time-course induction of D3 activity (left) and D3 mRNA (right) using 10% newborn calf serum (NCS) in brown adipocytes (44).

3T3-F442). Differentiation was usually induced by adding dexamethasone and agents that increase cAMP (IBMX). T3 was often included in the "differentiation cocktail." In this way it is difficult to predict if the effects observed are due to the process of differentiation itself or to the action of the hormones added. During the adipose differentiation of 3T3-F442A or 3T3-L1 cells, there is a large increase in the transcription of specific genes and increases in the synthesis of numerous proteins, including the lipogenic enzymes GPD, FAS and ME, and many others as described above (13,16). There is a sequential activation of a whole set of proteins and enzymes that act as differentiation markers, with different timings for their transcriptional increases (Fig. 1). Early markers are LPL or IGF-1, preceded by the families of transcription factors C/EBPs and PPAR γ . They are followed by many lipogenic enzymes and other proteins: GPD, ME, FAS, aP2, Glut4, ACC, and the β -adrenergic receptors, among many others. Later markers of differentiation are leptin, adiponin, PEPCK, the α -2 adrenergic receptors, and others (21). Recently, studies using genomic techniques (microarrays) (51) have shown that the cellular programs associated with adipocyte differentiation are more complex than previously thought as derived from the changes observed *in vitro* in the mentioned cell lines and the changes in gene expression associated with adipocyte development, revealing that adipocyte differentiation *in vivo* and *in vitro* are quite different. Those studies confirmed many genes that had been previously reported to increase during differentiation of cell lines, for example, C/EBP δ , C/EBP β , C/EBP α , aP2, adiponin, adiponectin, LPL, HSL, SCD1, GPD, and so on. Other genes were induced later on, as PEPCK, β 3-AR, PFKI, IGF-II, and so on. But several genes were expressed only in cell lines *in vitro* or only cells derived from tissues *in vivo*. These data suggest that one or more transcriptional programs are activated exclusively *in vivo* to generate the adipocyte phenotype.

Therefore, the differentiation of preadipocytes into adipocytes is achieved by the coordinate activation of transcription of several adipose-specific genes (52). The adipocyte differentiation follows a common adipogenic transcriptional pathway, regulated by the transcription factor families of C/EBPs and PPARs. All the adipogenesis is regulated by the sequential activation of these transcription factors and the nuclear proteins (coactivators or inhibitors) that regulate them.

The C/EBPs are transcription factors of the basic leucine zipper family. Several members of the C/EBP family (C/EBP α , C/EBP β , and C/EBP δ) have tissue-specific expression patterns and recognize a common DNA-binding element. C/EBP α is expressed in brown and white adipose tissues, placenta, and liver. C/EBP α is a master regulator of adipose tissue development. C/EBP α is required for the differentiation of 3T3-L1 preadipocytes, and when overexpressed, is able to trigger the differentiation of 3T3-L1 preadipocytes. It also works as an antimitotic signal inducing proteins associated with growth arrest (GADD45 and p21) (53). The induction of C/EBP α in preadipocytes increases the expression of several adipocyte-specific genes (aP2, Glut4) and the accumulation of triglycerides (53,54).

The C/EBP β and possibly C/EBP δ transcription factors are expressed earlier than C/EBP α in the differentiation program and activate transcriptionally C/EBP α , triggering the process of differentiation, while PPAR γ and C/EBP α induce the dif-

ferentiation from preadipocytes into adipocytes, followed by the adipo-specific gene expression. Most of those genes (SCD1, aP2, S14, PEPCK, and Glut4) have C/EBP-binding domains in their promoters and are activated in a coordinated program during adipogenesis (22), and we cannot forget to include two important ones for the differentiation of brown adipocytes, UCP1 and D2, which we will comment later on. C/EBP β and C/EBP δ increases precede those of C/EBP α . During the development of BAT during fetal life, C/EBP β and C/EBP δ increases also precede C/EBP α expression (55).

The study of the mice with a deletion in the C/EBP α gene (C/EBP α knockout mice) throws some light on the function of this transcription factor. The knockout mice die shortly after birth due to severe hypoglycemia and defective hepatic glycogen storage and gluconeogenesis (56). C/EBP α knockout mice do not have WAT and showed a great reduction in BAT depots and a very low UCP1 mRNA expression. This shows that C/EBP α is essential for the survival of the mice and for the developmental program of liver and adipose tissue. The status of BAT was examined in the C/EBP α knockout mice (57). UCP1 expression was very low; adipogenesis was impaired; the size, number, and function of the mitochondria were reduced. The expression of thyroid hormone receptors (THRs) and PGC1 (the coactivator of PPAR γ) was delayed (57). We found that BAT D2 activity was very low (Fig. 4), together with low T3 levels in BAT; this indicates that C/EBP α is critical to maintain thyroidal status in BAT. Indeed, D2 has C/EBP elements in its proximal promoter. This suggests that D2 plays a crucial role in the differentiation program of fetal BAT and in some way is linked to it for its full functionality, possibly because T3 is absolutely necessary for its function.

Neonatal hypothyroidism decreases C/EBP α and C/EBP β expression in liver, but not in BAT (58), and several TREs have been identified in the C/EBP α promoter (59). In the PEPCK gene, a relationship between C/EBPs and TREs has been described, as in this gene the activation of C/EBPs is required for a functional TRE (60).

Besides the participation of the C/EBPs, adipocyte differentiation is a process regulated also by the PPAR family, specifically by PPAR γ . PPARs belong to the family of nuclear

FIG. 4. D2 activity in fetal (d17 and d18) and neonatal (NB) BAT of CCAAT/enhancer-binding protein (C/EBP) knockout mice and wild-type mice (57). *** refers to $p < 0.05$ vs wild type.

receptors that act as transcription factors regulating changes in gene expression in response to nutritional stimuli and controlling lipid metabolism. PPARs family controls the metabolism of fatty acids, which are natural ligands that activate PPARs, especially arachidonic acid and its metabolites. PPAR family has several members: PPAR α (activated by fibrates and regulates β -oxidation, lipid catabolism, and inflammation), PPAR δ , and PPAR γ , which is quite specific of adipose tissue. PPARs form heterodimers with the X receptor of retinoic acid (RXR), activating the PPAR response elements (PPREs) (DR-1, 6-base pair direct repeats of the sequence RGGTCA spaced by one base) present in the promoter of specific target genes. Most of the genes mentioned above have PPREs in their promoters: aP2, FAS, PEPCCK, LPL, SCD, and so on. Those PPRE-binding sites can bind different isoforms in different tissues, for example, for the LPL gene, the PPAR α isoform in the liver and the PPAR γ isoform in the adipose tissue. There is abundant information that indicates that PPARs play important roles not only in adipogenesis but also in inflammation, atherogenesis, glucose homeostasis, and cancer.

All the adipogenesis is regulated by the activation of the PPAR γ (61), which in turn is regulated by C/EBP α and possibly by C/EBP β (Fig. 5). The ectopical expression of PPAR γ is able to induce fibroblastic cell lines to differentiate into adipocytes under the appropriate stimuli of agonists of PPAR γ (thiazolidinediones) (61).

As the mice with targeted deletion of PPAR γ were lethal, several strategies have been used; the main one was to use the adipose-specific PPAR γ knockout mice. PPAR γ knockout mice presented several alterations with contrasting results. Some studies have showed a reduced fat formation, and protection against obesity and insulin resistance with lipo-

dystrophy (62). The mice with targeted deletion of PPAR γ 2 have insulin resistance, indicating that PPAR γ 2 is necessary for the maintenance of insulin sensitivity (63).

The specific coactivator of PPAR γ , PGC1, was identified in 1998 (64). PGC1 greatly increased under cold exposure in BAT. PGC1 increased the transcriptional activity of PPAR γ and THR on the UCP1 promoter. As the ectopic expression of PGC1 in white adipocytes activated the expression of UCP1 and mitochondrial enzymes of the respiratory chain, PGC1 was considered as the true transcriptional activator of BAT and adaptative thermogenesis, as well as a marker of brown adipocytes (64). Later on, it has been identified as fundamental for several processes like hepatic gluconeogenesis, heart function in which mitochondriogenesis is very important, and inflammation (65–67).

The mice with targeted deletion of PGC1 α present several abnormalities in muscle, hepatic steatosis, increase in body fat, diminished mitochondrial number and respiratory capacity, and abnormal cardiac function (68). The importance of the coactivator of PPAR γ , PGC1, is explained in one of the previous chapters (Chapter 8).

Regulation of gene expression in adipocytes by T3 and its nuclear receptors

T3 regulates adipogenesis and processes related to them as lipogenesis and lipolysis both *in vivo* and in cultured adipocytes (21,69). Indeed, all the isoforms of TRs, TR α 1, TR α 2, and TR β 1, are expressed in white and brown adipocytes and in WAT and BAT, TR α 1 being the predominant TR isoform (70–73). T3 and other hormones regulate the different TR isoforms. Two main approaches have been used to discriminate between the effects of both TR isoforms: a pharmacological approach, using ligands specific of the TR β isoform, and a genetic approach, using knockout and knockin mice.

Selective agonists of the TR β isoform have been used to increase metabolic rate and lower cholesterol, triglycerides, lipoproteinA, and body weight without affecting heart rate (74). The TR β 1 agonist GC-1 was used to stimulate UCP1, with no effect in regulating body temperature, therefore discriminating two isoform-specific actions of T3 in BAT (75).

To identify the specific actions of the α - and β -TR isoforms, several TR knockout and knockin mice were generated (76). Although the first knockout mice studied did not show phenotypic abnormalities concerning adipose tissue metabolism or alterations in energy balance, recent work has identified several phenotypes related to adipose tissue and energy balance, due to point mutations or negative dominant mutations in the TRs. TR α seems to be required for proper thermogenesis (77), while TR β regulates cholesterol metabolism (78). Mice devoid of all TRs have decreased body temperature and basal metabolic rate and are cold intolerant due to insufficient heat production (79). These null TR mice show growth retardation and delayed skeletal maturation together with an increased amount of fat and increases in several adipocyte-specific genes (80). Moreover, certain mutations in the TRs, specifically the P398H mutation in the TR α gene, induce visceral adiposity, hyperleptinemia, a fourfold increase in body fat, increased basal glucose and insulin, and impaired lipolysis in male mice (81). Many genes of the lipogenic and lipolytic pathways were decreased. Adaptative thermogenesis was also reduced. This mutation reduces the

FIG. 5. Transcription factors that regulate the differentiation of adipocytes. The role of the coactivator of PPAR γ , PGC1, is also depicted. Modified from Puigserver and Spiegelman (65).

PPAR α binding to PPRES, interfering with PPAR α signaling (82). Recently, the role of unliganded TRs, acting as aporeceptors and exerting opposite transcriptional effects, has been investigated using a dominant negative mutation (R384C) in the TR α 1 gene that causes a 10-fold reduction in the affinity to T3 (83). These mice were hypermetabolic and had reduced fat depots, hyperphagia, and resistance to diet-induced obesity, together with an induction of the genes involved in glucose handling and lipid mobilization and β -oxidation. The alterations are reversed by increases in T3 levels. Thus, TR α 1 aporeceptor is involved in metabolic homeostasis. Similar results are found in other heterozygous mice with a dominant negative mutation of the TR α 1 (PV/+), in which WAT is reduced, as well as the expression of PPAR γ signaling, affecting adipogenesis (84).

Many of the genes involved in the differentiation program of adipocytes are regulated by T3. The list of genes includes GPD, ME, PEPCK, S14 (85), FAS (86), GLUT4, and LPL, among many others (87–89). Many of those genes are directly regulated by T3 through the TREs present in their promoters (90–92). Some enzymes such as α -glycerophosphate dehydrogenase (α -GPDH) and ME have been used extensively to check the thyroidal status in experimental animals (usually in liver). Many groups accomplished the study of the T3 actions at the promoter level, identifying the functional TREs and its interactions with other members of the family of nuclear receptors, such as PPARs, retinoic acid, or with insulin response element (IRE) and cAMP response elements (CREs). Usually there is a strong interaction among all these elements and the coactivators that regulate them, as have been described in several genes as UCP1, ME, ACC, and others. We studied the regulation of ME and S14 by T3 in brown preadipocytes in culture (93–95). ME is a lipogenic enzyme that plays a key role in differentiation, and S14, a T3-responding gene and abundant in lipogenic tissues, is now considered a possible transcription factor in lipogenesis. These two genes increase during differentiation of preadipocytes, and its natural progression increases in the presence of by T3. The action of T3 is both transcriptional and stabilizing the mRNA, and it is synergic with the action of insulin. The effect of NE and retinoic acid was also examined (94). A detail study of the effect of T3 on lipid synthesis is presented in the next chapter.

T3 actions on UCP1 and thermogenesis: role of D2

When working with 3T3-L1 preadipocytes (white), the differentiation of adipocytes is measured by its lipogenic capacity and the capacity for lipid accumulation, measuring the increase in lipid droplets and increased gene expression in lipogenic enzymes. But in BAT, the differentiation process involves in addition the acquisition of the full thermogenic capacity, as measured by the full expression of UCP1 and the activation of the process of mitochondriogenesis.

As described above, the function of BAT is to generate heat when the demands increase under cold exposure. The generation of extra heat is accomplished by the mitochondrial UCP1 that uncouples the oxidative phosphorylation. BAT is activated by the sympathetic nervous system (SNS), via the NE released from the nerve endings (96). The binding of NE to the adrenergic receptors and the activation of adenylate cyclase increases cAMP, that activate lipolysis, increasing free

FIG. 6. Expression of uncoupling protein (UCP1) in fetal and neonatal BAT obtained from control and hypothyroid fetus and litters. Hypothyroidism was induced using methylmercapto-imidazol (MMI) in the drinking water (100).

fatty acids (FFA) which activates UCP1 (12). The thermogenic capacity of BAT is determined by the amount of UCP1. UCP1 is transcriptionally activated shortly after NE or cold exposure (97,98). It has been demonstrated that T3 amplifies the adrenergic stimulation of UCP1 (98,99). Moreover, in thermoneutral conditions, like during the intrauterine life, T3 is required for the expression of UCP1 mRNA, and euthyroidism is required during the first postnatal days for the increases in UCP1 mRNA (Fig. 6) (100,101). In cultures of rat brown adipocytes, it is clear that T3 is an absolute requirement for UCP1 adrenergic increases, participating in the stabilization of mRNA transcripts (102). T3 itself is also able to induce the transcription of UCP1 in fetal rat brown adipocyte in primary culture (103).

The study of the UCP1 promoter provided evidence for the presence of CREs (104–106) in the proximal promoter, and an enhancer element was identified that contained several TREs (92,107), some retinoic acid response elements (RAREs) (108,109), and a PPRES (110). There is clear cross-talk among these nuclear receptors and its coactivators for its binding to the UCP1 promoter. In addition, there are negative regulators of the expression of UCP1: serum, mitotic signals involving the activation of c-jun (106), and insulin. Other hormonal treatments (glucocorticoids or sexual hormones) can also modulate UCP1 expression.

Recently, we have examined the role of Triac in cultured brown adipocytes (111). Triac, which binds better than T3 to the TR β isoform, is 10–50-fold more potent than T3 in increasing the adrenergic induction of UCP1 and D2, as well as LPL mRNA. The role of Triac was studied in rats (112). Triac was again more potent than T3 (in terms of doses and concentrations) in the stimulation of UCP1, LPL, and leptin, and at low doses, induced ectopic UCP1 expression in WAT (112).

The adrenergic input also increases D2 deiodinase in BAT (113), causing a marked increase in T3 in BAT, suggesting that T3 plays an important role in this process. It was also demonstrated that the intracellular conversion of thyroxine to T3 was required for the full thermogenic function of BAT (114). This is also true for the adrenergic stimulation of D2 (115,116), which does not take place unless T3 is present. It is evident that D2 participates in the formation of BAT, as evidenced in the experiments described above in C/EBP α knockout mice

(57), where UCP1 expression and D2 activity are blunted, as well as other markers of mitochondriogenesis. D2 has also been implicated in the process of lipogenesis under adrenergic stimuli (89). The analysis of the D2 knockout mice reveals that there is a hyperadrenergic stimulation, which compensates for the lack of T3 production in BAT. Lipogenesis is unable to provide the high FFA levels required during cold exposure, resulting in an impaired adaptative thermogenesis (117).

The importance of deiodinases in the differentiation of white adipocytes is still poorly studied. It is evident that there is a role in lipogenesis and in the expression of genes involved in the differentiation program. In addition D1 is found in WAT (118), but its precise role has not been studied. To what extent the role of D2 and D1 is different from that studied in brown adipocytes remains to be seen.

The distinction between white and brown adipocytes: reinduction of WAT into BAT

There are important questions on the relationships between white and brown adipocytes. In the early 1980s, comparative studies using primary cultures of brown and white adipocytes (34) established that precursor cells from epididymal fat (pure WAT) and from interscapular BAT differentiate into white and brown adipocytes, respectively, with different phenotypes, characteristics, and regulation. The work done for more than 20 years using these primary cultures confirms the hypothesis that precursor cells are already committed to become brown or white adipocytes. The question whether BAT and WAT derive from the same or different preadipocyte precursor cells is still under discussion. It is not known if the undetermined mesenchymal stem cells retain some myogenic or chondrogenic potential, as many reports have proposed. Recently, Timmons *et al.* have analyzed this problem using microarrays to study both preadipocytes in culture (32). They have found a myogenic signature in brown preadipocytes, not found in white adipocytes, in which they have found a transcription factor, Tcf21, which suppresses myogenesis. They have found genes only expressed in brown adipocytes or only in white adipocytes, as well as markers of differentiation and proliferation for white and brown preadipocytes and genes implicated in human obesity (32).

There is a growing interest in this field as brown adipocytes are associated to increased energy expenditure and the conversion of white adipocytes into brown adipocytes is sought as a strategy to fight obesity. Indeed under extreme cold exposure, a reactivation of BAT adipocytes in inguinal WAT is observed, and this type of WAT was called convertible adipose tissue due to its capacity to revert to BAT (119). Many attempts have been made in this sense, and there are an increased number of experimental models in which a reactivation of WAT into BAT is observed. The increase in UCP1 expression is the golden rule to assess such a conversion of WAT into BAT. This fact has been observed using some drugs and also in mice with targeted deletion of a certain genes. Beta 3 adrenergic agonists are able to induce UCP1 in muscle, and ectopic BAT present in muscle provides a mechanism against weight gain (120). Brown adipocytes are also found in WAT (121–123). The same effect is observed using models of hyperleptinemia that depletes fat stores in rats (124,125). Tungstate has a similar effect with reactivation of energy metabolism (126), and we observed that Triac at low doses induce UCP1

expression in inguinal WAT in rats (112). In mice with targeted deletion of the corepressor RIP140 (127), UCP1 expression is increased and the mice are lean and resistant to diet-induced obesity.

Conclusion

In summary, adipogenesis is a complex process that involves activation of transcription of many genes and enzymes, in a cascade of events regulated by transcription factors that rule the process of differentiation (C/EBPs, PPARs, and PGC1). T3 regulates many of the enzymes involved in this process, either directly or through the interaction with other coactivators, such as PPARs. The deiodinases play a role by providing the T3 necessary for this process or limiting its levels. D3 is stimulated by proliferation, while D2 plays a crucial role in the development of the tissue, in thermogenesis and lipogenesis. The reactivation of WAT depots into depots containing BAT adipocytes could be achieved by some drugs and is observed in some models.

References

1. Tata JR 2006 Amphibian metamorphosis as a model for the developmental actions of thyroid hormone. *Mol Cell Endocrinol* **246**:10–20.
2. Brown DD 2005 The role of deiodinases in amphibian metamorphosis. *Thyroid* **15**:815–821.
3. Brown DD, Wang Z, Furlow JD, Kanamori A, Schwartzman RA, Remo BF, Pinder A 1996 The thyroid hormone-induced tail resorption program during *Xenopus laevis* metamorphosis. *Proc Natl Acad Sci USA* **93**:1924–1929.
4. Becker KB, Stephens KC, Davey JC, Schneider MJ, Galton VA 1997 The type 2 and type 3 iodothyronine deiodinases play important roles in coordinating development in *Rana catesbeiana* tadpoles. *Endocrinology* **138**:2989–2997.
5. Bernal J 2002 Action of thyroid hormone in brain. *J Endocrinol Invest* **25**:268–288.
6. Morreale de Escobar G, Obregon MJ, Escobar del Rey F 2004 Role of thyroid hormone during early brain development. *Eur J Endocrinol* **151 Suppl 3**:U25–U37.
7. Forrest D, Erway LC, Ng L, Altschuler R, Curran T 1996 Thyroid hormone receptor beta is essential for development of auditory function. *Nat Genet* **13**:354–357.
8. Roberts MR, Srinivas M, Forrest D, Morreale de Escobar G, Reh TA 2006 Making the gradient: thyroid hormone regulates cone opsin expression in the developing mouse retina. *Proc Natl Acad Sci USA* **103**:6218–6223.
9. Kester MH, Martinez de Mena R, Obregon MJ, Marinkovic D, Howatson A, Visser TJ, Hume R, Morreale de Escobar G 2004 Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. *J Clin Endocrinol Metab* **89**:3117–3128.
10. Ng L, Goodyear RJ, Woods CA, Schneider MJ, Diamond E, Richardson GP, Kelley MW, Germain DL, Galton VA, Forrest D 2004 Hearing loss and retarded cochlear development in mice lacking type 2 iodothyronine deiodinase. *Proc Natl Acad Sci USA* **101**:3474–3479.
11. Wajchenberg BL 2000 Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* **21**:697–738.
12. Cannon B, Nedergaard J 2004 Brown adipose tissue: function and physiological significance. *Physiol Rev* **84**:277–359.

13. Spiegelman BM, Frank M, Green H 1983 Molecular cloning of mRNA from 3T3 adipocytes. Regulation of mRNA content for glycerophosphate dehydrogenase and other differentiation-dependent proteins during adipocyte development. *J Biol Chem* **258**:10083–10089.
14. Lin FT, Lane MD 1994 CCAAT/enhancer binding protein alpha is sufficient to initiate the 3T3-L1 adipocyte differentiation program. *Proc Natl Acad Sci USA* **91**:8757–8761.
15. Bernlohr DA, Angus CW, Lane MD, Bolanowski MA, Kelly TJ, Jr. 1984 Expression of specific mRNAs during adipose differentiation: identification of an mRNA encoding a homologue of myelin P2 protein. *Proc Natl Acad Sci USA* **81**:5468–5472.
16. Mackall JC, Student AK, Polakis SE, Lane MD 1976 Induction of lipogenesis during differentiation in a "preadipocyte" cell line. *J Biol Chem* **251**:6462–6464.
17. Ntambi JM, Buhrow SA, Kaestner KH, Christy RJ, Sibley E, Kelly TJ, Jr., Lane MD 1988 Differentiation-induced gene expression in 3T3-L1 preadipocytes. Characterization of a differentially expressed gene encoding stearyl-CoA desaturase. *J Biol Chem* **263**:17291–17300.
18. Cook KS, Min HY, Johnson D, Chaplinsky RJ, Flier JS, Hunt CR, Spiegelman BM 1987 Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. *Science* **237**:402–405.
19. Flier JS, Lowell B, Napolitano A, Usher P, Rosen B, Cook KS, Spiegelman B 1989 Adipsin: regulation and dysregulation in obesity and other metabolic states. *Recent Prog Horm Res* **45**:567–580.
20. Hu E, Liang P, Spiegelman BM 1996 AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* **271**:10697–10703.
21. Ailhaud G, Grimaldi P, Negrel R 1992 Cellular and molecular aspects of adipose tissue development. *Annu Rev Nutr* **12**:207–233.
22. Christy RJ, Yang VW, Ntambi JM, Geiman DE, Landschulz WH, Friedman AD, Nakabeppu Y, Kelly TJ, Lane MD 1989 Differentiation-induced gene expression in 3T3-L1 preadipocytes: CCAAT/enhancer binding protein interacts with and activates the promoters of two adipocyte-specific genes. *Genes Dev* **3**:1323–1335.
23. Christy RJ, Kaestner KH, Geiman DE, Lane MD 1991 CCAAT/enhancer binding protein gene promoter: binding of nuclear factors during differentiation of 3T3-L1 preadipocytes. *Proc Natl Acad Sci USA* **88**:2593–2597.
24. Cornelius P, MacDougald OA, Lane MD 1994 Regulation of adipocyte development. *Annu Rev Nutr* **14**:99–129.
25. Falconi D, Oizumi K, Aubin JE 2007 Leukemia inhibitory factor influences the fate choice of mesenchymal progenitor cells. *Stem Cells* **25**:305–312.
26. Chen TH, Chen WM, Hsu KH, Kuo CD, Hung SC 2007 Sodium butyrate activates ERK to regulate differentiation of mesenchymal stem cells. *Biochem Biophys Res Commun* **355**:913–918.
27. Swick AG, Lane MD 1992 Identification of a transcriptional repressor down-regulated during preadipocyte differentiation. *Proc Natl Acad Sci USA* **89**:7895–7899.
28. Smas CM, Sul HS 1993 Pref-1, a protein containing EGF-like repeats, inhibits adipocyte differentiation. *Cell* **73**:725–734.
29. Moon YS, Smas CM, Lee K, Villena JA, Kim KH, Yun EJ, Sul HS 2002 Mice lacking paternally expressed Pref-1/Dlk1 display growth retardation and accelerated adiposity. *Mol Cell Biol* **22**:5585–5592.
30. Kim KA, Kim JH, Wang Y, Sul HS 2007 Pref-1 (Preadipocyte Factor 1) activates the MEK/extracellular signal-regulated kinase pathway to inhibit adipocyte differentiation. *Mol Cell Biol* **27**:2294–2308.
31. Cantile M, Procino A, D'Armiento M, Cindolo L, Cillo C 2003 HOX gene network is involved in the transcriptional regulation of *in vivo* human adipogenesis. *J Cell Physiol* **194**:225–236.
32. Timmons JA, Wennmalm K, Larsson O, Walden TB, Lassmann T, Petrovic N, Hamilton DL, Gimeno RE, Wahlestedt C, Baar K, Nedergaard J, Cannon B 2007 Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc Natl Acad Sci USA* **104**:4401–4406.
33. MacDougald OA, Burant CF 2005 Fickle factor foils fat fate. *Nat Cell Biol* **7**:543–545.
34. Nchad M, Kuusela P, Carneheim C, Bjrntorp P, Nedergaard J, Cannon B 1983 Development of brown fat cells in monolayer culture. I. Morphological and biochemical distinction from white fat cells in culture. *Exp Cell Res* **149**:105–118.
35. Bukowiecki LJ, Gelon A, Collet AJ 1986 Proliferation and differentiation of brown adipocytes from interstitial cells during cold acclimation. *Am J Physiol* **250**:C880–C887.
36. Gelon A, Collet AJ, Guay G, Bukowiecki LJ 1988 Beta-adrenergic stimulation of brown adipocyte proliferation. *Am J Physiol* **254**:C175–C182.
37. Rehnmark S, Nedergaard J 1989 DNA synthesis in mouse brown adipose tissue is under beta-adrenergic control. *Exp Cell Res* **180**:574–579.
38. Gelon A, Collet AJ, Guay G, Bukowiecki LJ 1989 Insulin stimulates *in vivo* cell proliferation in white adipose tissue. *Am J Physiol* **256**:C190–C196.
39. Bronnikov G, Houstek J, Nedergaard J 1992 Beta-adrenergic, cAMP-mediated stimulation of proliferation of brown fat cells in primary culture. Mediation via beta 1 but not via beta 3 adrenoreceptors. *J Biol Chem* **267**:2006–2013.
40. Garcia B, Obregon MJ 1997 Norepinephrine potentiates the mitogenic effect of growth factors in quiescent brown preadipocytes: relationship with uncoupling protein messenger ribonucleic acid expression. *Endocrinology* **138**:4227–4233.
41. Garcia B, Obregon MJ 2002 Growth factor regulation of uncoupling protein-1 mRNA expression in brown adipocytes. *Am J Physiol Cell Physiol* **282**:C105–C112.
42. Hernandez A, Obregon MJ 1995 Presence of growth factors-induced type III iodothyronine 5- deiodinase in cultured rat brown adipocytes. *Endocrinology* **136**:4543–4550.
43. Hernandez A, St. Germain DL, Obregon MJ 1998 Transcriptional activation of type III inner ring deiodinase by growth factors in cultured rat brown adipocytes. *Endocrinology* **139**:634–639.
44. Hernandez A, Garcia B, Obregon MJ 2007 Gene expression from the imprinted Dio3 locus is associated with cell proliferation of cultured brown adipocytes. *Endocrinology* **148**:3968–3976.
45. Deslex S, Negrel R, Ailhaud G 1987 Development of a chemically defined serum-free medium for differentiation of rat adipose precursor cells. *Exp Cell Res* **168**:15–30.
46. Deslex S, Negrel R, Vannier C, Etienne J, Ailhaud G 1987 Differentiation of human adipocyte precursors in a chemically defined serum-free medium. *Int J Obes* **11**:19–27.
47. Lau DC, Roncari DA, Hollenberg CH 1987 Release of mitogenic factors by cultured preadipocytes from massively obese human subjects. *J Clin Invest* **79**:632–636.

48. Lacasa D, Taleb S, Keophiphath M, Miranville A, Clement K 2007 Macrophage-secreted factors impair human adipogenesis: involvement of proinflammatory state in preadipocytes. *Endocrinology* **148**:868–877.
49. Asaki T, Konishi M, Miyake A, Kato S, Tomizawa M, Itoh N 2004 Roles of fibroblast growth factor 10 (Fgf10) in adipogenesis *in vivo*. *Mol Cell Endocrinol* **218**:119–128.
50. Hausman DB, DiGirolamo M, Bartness TJ, Hausman GJ, Martin RJ 2001 The biology of white adipocyte proliferation. *Obes Rev* **2**:239–254.
51. Soukas A, Socci ND, Saatkamp BD, Novelli S, Friedman JM 2001 Distinct transcriptional profiles of adipogenesis *in vivo* and *in vitro*. *J Biol Chem* **276**:34167–34174.
52. Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM 2000 Transcriptional regulation of adipogenesis. *Genes Dev* **14**:1293–1307.
53. Mandrup S, Lane MD 1997 Regulating adipogenesis. *J Biol Chem* **272**:5367–5370.
54. Lin FT, Lane MD 1992 Antisense CCAAT/enhancer-binding protein RNA suppresses coordinate gene expression and triglyceride accumulation during differentiation of 3T3-L1 preadipocytes. *Genes Dev* **6**:533–544.
55. Manchado C, Yubero P, Vinas O, Iglesias R, Villarroya F, Mampel T, Giralt M 1994 CCAAT/enhancer-binding proteins alpha and beta in brown adipose tissue: evidence for a tissue-specific pattern of expression during development. *Biochem J* **302** (Pt 3):695–700.
56. Linhart HG, Ishimura-Oka K, DeMayo F, Kibe T, Repka D, Poindexter B, Bick RJ, Darlington GJ 2001 C/EBPalpha is required for differentiation of white, but not brown, adipose tissue. *Proc Natl Acad Sci USA* **98**:12532–12537.
57. Carmona MC, Iglesias R, Obregon MJ, Darlington GJ, Villarroya F, Giralt M 2002 Mitochondrial biogenesis and thyroid status maturation in brown fat require CCAAT/enhancer-binding protein alpha. *J Biol Chem* **277**:21489–21498.
58. Menendez-Hurtado A, Vega-Nunez E, Santos A, Perez-Castillo A 1997 Regulation by thyroid hormone and retinoic acid of the CCAAT/enhancer binding protein alpha and beta genes during liver development. *Biochem Biophys Res Commun* **234**:605–610.
59. Menendez-Hurtado A, Santos A, Perez-Castillo A 2000 Characterization of the promoter region of the rat CCAAT/enhancer-binding protein alpha gene and regulation by thyroid hormone in rat immortalized brown adipocytes. *Endocrinology* **141**:4164–4170.
60. Park EA, Song S, Olive M, Roesler WJ 1997 CCAAT-enhancer-binding protein alpha (C/EBP alpha) is required for the thyroid hormone but not the retinoic acid induction of phosphoenolpyruvate carboxykinase (PEPCK) gene transcription. *Biochem J* **322** (Pt 1):343–349.
61. Tontonoz P, Hu E, Spiegelman BM 1995 Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor gamma. *Curr Opin Genet Dev* **5**:571–576.
62. Jones JR, Barrick C, Kim KA, Lindner J, Blondeau B, Fujimoto Y, Shiota M, Kesterson RA, Kahn BB, Magnuson MA 2005 Deletion of PPARGgamma in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. *Proc Natl Acad Sci USA* **102**:6207–6212.
63. Medina-Gomez G, Virtue S, Lelliott C, Boiani R, Campbell M, Christodoulides C, Perrin C, Jimenez-Linan M, Blount M, Dixon J, Zahn D, Thresher RR, Aparicio S, Carlton M, Colledge WH, Kettunen MI, Seppanen-Laakso T, Sethi JK, O'Rahilly S, Brindle K, Cinti S, Oresic M, Burcelin R, Vidal-Puig A 2005 The link between nutritional status and insulin sensitivity is dependent on the adipocyte-specific peroxisome proliferator-activated receptor-[gamma]2 isoform. *Diabetes* **54**:1706–1716.
64. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM 1998 A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **92**:829–839.
65. Puigserver P, Spiegelman BM 2003 Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* **24**:78–90.
66. Uldry M, Yang W, St-Pierre J, Lin J, Seale P, Spiegelman BM 2006 Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. *Cell Metab* **3**:333–341.
67. Handschin C, Spiegelman BM 2006 Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. *Endocr Rev* **27**:728–735.
68. Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, Courtois M, Wozniak DF, Sambandam N, Bernal-Mizrachi C, Chen Z, Holloszy JO, Medeiros DM, Schmidt RE, Saffitz JE, Abel ED, Semenkovich CF, Kelly DP 2005 PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. *PLoS Biol* **3**:e101.
69. Oppenheimer JH, Schwartz HL, Lane JT, Thompson MP 1991 Functional relationship of thyroid hormone-induced lipogenesis, lipolysis, and thermogenesis in the rat. *J Clin Invest* **87**:125–132.
70. Hernandez A, Obregon MJ 1996 Presence and mRNA expression of T3 receptors in differentiating rat brown adipocytes. *Mol Cell Endocrinol* **121**:37–46.
71. Teboul M, Torresani J 1993 Analysis of c-erb A RNA expression in the triiodothyronine-sensitive Ob 17 preadipocyte cell line. *J Recept Res* **13**:815–828.
72. Tuca A, Giralt M, Villarroya F, Vinas O, Mampel T, Iglesias R 1993 Ontogeny of thyroid hormone receptors and c-erbA expression during brown adipose tissue development: evidence of fetal acquisition of the mature thyroid status. *Endocrinology* **132**:1913–1920.
73. Bianco AC, Silva JE 1988 Cold exposure rapidly induces virtual saturation of brown adipose tissue nuclear T3 receptors. *Am J Physiol* **255**:E496–E503.
74. Grover GJ, Mellstrom K, Ye L, Malm J, Li YL, Bladh LG, Sleph PG, Smith MA, George R, Vennstrom B, Mookhtiar K, Horvath R, Speelman J, Egan D, Baxter JD 2003 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* **100**:10067–10072.
75. 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. *J Clin Invest* **108**:97–105.
76. Forrest D, Vennstrom B 2000 Functions of thyroid hormone receptors in mice. *Thyroid* **10**:41–52.
77. Wikstrom L, Johansson C, Salto C, Barlow C, Campos Barros A, Baas F, Forrest D, Thoren P, Vennstrom B 1998 Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha 1. *EMBO J* **17**:455–461.
78. Gullberg H, Rudling M, Salto C, Forrest D, Angelin B, Vennstrom B 2002 Requirement for thyroid hormone

- receptor beta in T3 regulation of cholesterol metabolism in mice. *Mol Endocrinol* **16**:1767–1777.
79. Golozoubova V, Gullberg H, Matthias A, Cannon B, Vennstrom B, Nedergaard J 2004 Depressed thermogenesis but competent brown adipose tissue recruitment in mice devoid of all hormone-binding thyroid hormone receptors. *Mol Endocrinol* **18**:384–401.
 80. Kindblom JM, Gevers EF, Skrtic SM, Lindberg MK, Gothe S, Tornell J, Vennstrom B, Ohlsson C 2005 Increased adipogenesis in bone marrow but decreased bone mineral density in mice devoid of thyroid hormone receptors. *Bone* **36**:607–616.
 81. Liu YY, Schultz JJ, Brent GA 2003 A thyroid hormone receptor alpha gene mutation (P398H) is associated with visceral adiposity and impaired catecholamine-stimulated lipolysis in mice. *J Biol Chem* **278**:38913–38920.
 82. Liu YY, Heymann RS, Moatamed F, Schultz JJ, Sobel D, Brent GA 2007 A mutant thyroid hormone receptor alpha antagonizes peroxisome proliferator-activated receptor alpha signaling *in vivo* and impairs fatty acid oxidation. *Endocrinology* **148**:1206–1217.
 83. Sjogren M, Alkemade A, Mittag J, Nordstrom K, Katz A, Rozell B, Westerblad H, Arner A, Vennstrom B 2007 Hypermetabolism in mice caused by the central action of an unliganded thyroid hormone receptor alpha1. *EMBO J* **26**:4535–4545.
 84. Ying H, Araki O, Furuya F, Kato Y, Cheng SY 2007 Impaired adipogenesis caused by a mutated thyroid hormone alpha1 receptor. *Mol Cell Biol* **27**:2359–2371.
 85. Kinlaw WB, Church JL, Harmon J, Mariash CN 1995 Direct evidence for a role of the “spot 14” protein in the regulation of lipid synthesis. *J Biol Chem* **270**:16615–16618.
 86. Moustaid N, Sul HS 1991 Regulation of expression of the fatty acid synthase gene in 3T3-L1 cells by differentiation and triiodothyronine. *J Biol Chem* **266**:18550–18554.
 87. Mariash CN, Kaiser FE, Schwartz HL, Towle HC, Oppenheimer JH 1980 Synergism of thyroid hormone and high carbohydrate diet in the induction of lipogenic enzymes in the rat. Mechanisms and implications. *J Clin Invest* **65**:1126–1134.
 88. Blennemann B, Leahy P, Kim TS, Freake HC 1995 Tissue-specific regulation of lipogenic mRNAs by thyroid hormone. *Mol Cell Endocrinol* **110**:1–8.
 89. Bianco AC, Carvalho SD, Carvalho CR, Rabelo R, Moriscot AS 1998 Thyroxine 5'-deiodination mediates norepinephrine-induced lipogenesis in dispersed brown adipocytes. *Endocrinology* **139**:571–578.
 90. Petty KJ, Desvergne B, Mitsuhashi T, Nikodem VM 1990 Identification of a thyroid hormone response element in the malic enzyme gene. *J Biol Chem* **265**:7395–7400.
 91. Giralt M, Park EA, Gurney AL, Liu JS, Hakimi P, Hanson RW 1991 Identification of a thyroid hormone response element in the phosphoenolpyruvate carboxykinase (GTP) gene. Evidence for synergistic interaction between thyroid hormone and cAMP cis-regulatory elements. *J Biol Chem* **266**:21991–21996.
 92. Rabelo R, Schifman A, Rubio A, Sheng X, Silva JE 1995 Delineation of thyroid hormone-responsive sequences within a critical enhancer in the rat uncoupling protein gene. *Endocrinology* **136**:1003–1013.
 93. Garcia-Jimenez C, Hernandez A, Obregon MJ, Santisteban P 1993 Malic enzyme gene expression in differentiating brown adipocytes: regulation by insulin and triiodothyronine. *Endocrinology* **132**:1537–1543.
 94. Hernandez A, Garcia-Jimenez C, Santisteban P, Obregon MJ 1993 Regulation of malic-enzyme-gene expression by cAMP and retinoic acid in differentiating brown adipocytes. *Eur J Biochem* **215**:285–290.
 95. Perez-Castillo A, Hernandez A, Pipaon C, Santos A, Obregon MJ 1993 Multiple regulation of S14 gene expression during brown fat differentiation. *Endocrinology* **133**:545–552.
 96. Ricquier D, Bouillaud F, Toumelin P, Mory G, Bazin R, Arch J, Penicaud L 1986 Expression of uncoupling protein messenger RNA in thermogenic or weakly thermogenic brown adipose tissue: evidence for a rapid β -adrenoreceptor-mediated and transcriptionally regulated step during activation of thermogenesis. *J Biol Chem* **261**:13905–13910.
 97. Bouillaud F, Ricquier D, Mory G, Thibault J 1984 Increased level of mRNA for the uncoupling protein in brown adipose tissue of rats during thermogenesis induced by cold exposure or norepinephrine infusion. *J Biol Chem* **259**:11583–11586.
 98. Bianco AC, Sheng X, Silva JE 1988 Triiodothyronine amplifies norepinephrine stimulation of uncoupling protein gene transcription by a mechanism not requiring protein synthesis. *J Biol Chem* **263**:18168–18175.
 99. Bianco AC, Kieffer JD, Silva JE 1992 Adenosine 3',5'-monophosphate and thyroid hormone control of uncoupling protein messenger ribonucleic acid in freshly dispersed brown adipocytes. *Endocrinology* **130**:2625–2633.
 100. Obregon MJ, Pitamber R, Jacobsson A, Nedergaard J, Cannon B 1987 Euthyroid status is essential for the perinatal increase in thermogenin mRNA in brown adipose tissue of rat pups. *Biochem Biophys Res Commun* **148**:9–14.
 101. Giralt M, Martin I, Iglesias R, Vinas O, Villarroya F, Mampel T 1990 Ontogeny and perinatal modulation of gene expression in rat brown adipose tissue. Unaltered iodothyronine 5'-deiodinase activity is necessary for the response to environmental temperature at birth. *Eur J Biochem* **193**:297–302.
 102. Hernandez A, Obregon MJ 2000 Triiodothyronine amplifies the adrenergic stimulation of uncoupling protein expression in rat brown adipocytes. *Am J Physiol Endocrinol Metab* **278**:E769–E777.
 103. Guerra C, Roncero C, Porras A, Fernández M, Benito M 1996 Triiodothyronine induces the transcription of the uncoupling protein gene and stabilizes its mRNA in fetal rat brown adipocyte primary cultures. *J Biol Chem* **271**:2076–2081.
 104. Kozak UC, Kopecky J, Teisinger J, Enerback S, Boyer B, Kozak LP 1994 An upstream enhancer regulating brown-fat-specific expression of the mitochondrial uncoupling protein gene. *Mol Cell Biol* **14**:59–67.
 105. Rim JS, Kozak LP 2002 Regulatory motifs for CREB-binding protein and Nfe2l2 transcription factors in the upstream enhancer of the mitochondrial uncoupling protein 1 gene. *J Biol Chem* **277**:34589–34600.
 106. Yubero P, Barbera MJ, Alvarez R, Vinas O, Mampel T, Iglesias R, Villarroya F, Giralt M 1998 Dominant negative regulation by c-Jun of transcription of the uncoupling protein-1 gene through a proximal cAMP-regulatory element: a mechanism for repressing basal and norepinephrine-induced expression of the gene before brown adipocyte differentiation. *Mol Endocrinol* **12**:1023–1037.
 107. Cassard-Doulcier AM, Larose M, Matamala JC, Champigny O, Bouillaud F, Ricquier D 1994 *In vitro* interactions between nuclear proteins and uncoupling protein gene promoter reveal several putative transactivating factors including

- Ets1, retinoid X receptor, thyroid hormone receptor, and a CACCC box-binding protein. *J Biol Chem* **269**:24335–24342.
108. Alvarez R, de Andres J, Yubero P, Vinas O, Mampel T, Iglesias R, Giral M, Villarroja F 1995 A novel regulatory pathway of brown fat thermogenesis. Retinoic acid is a transcriptional activator of the mitochondrial uncoupling protein gene. *J Biol Chem* **270**:5666–5673.
 109. Rabelo R, Reyes C, Schiffman A, Silva JE 1996 A complex retinoic acid response element in the uncoupling protein gene defines a novel role for retinoids in thermogenesis. *Endocrinology* **137**:3488–3496.
 110. Teruel T, Clapham JC, Smith SA 1999 PPAR α activation by Wy 14643 induces transactivation of the rat UCP-1 promoter without increasing UCP-1 mRNA levels and attenuates PPAR γ -mediated increases in UCP-1 mRNA levels induced by rosiglitazone in fetal rat brown adipocytes. *Biochem Biophys Res Commun* **264**:311–315.
 111. Medina-Gomez G, Hernandez A, Calvo RM, Martin E, Obregon MJ 2003 Potent thermogenic action of triiodothyroacetic acid in brown adipocytes. *Cell Mol Life Sci* **60**:1957–1967.
 112. Medina-Gomez G, Calvo RM, Obregon MJ 2008 Specific role of triiodothyroacetic acid at low doses in rat adipose tissue. *Am J Physiol Endocrinol Metab* (submitted).
 113. Silva JE, Larsen PR 1983 Adrenergic activation of triiodothyronine production in brown adipose tissue. *Nature* **305**:712–713.
 114. Bianco AC, Silva JE 1987 Intracellular conversion of thyroxine to triiodothyronine is required for the optimal thermogenic function of brown adipose tissue. *J Clin Invest* **79**:295–300.
 115. Hernandez A, Obregon MJ 1996 T3 potentiates the adrenergic stimulation of type II 5'-deiodinase activity in cultured rat brown adipocytes. *Am J Physiol* **271**:E15–E23.
 116. Martinez-deMena R, Hernandez A, Obregon MJ 2002 Triiodothyronine is required for the stimulation of type II 5'-deiodinase mRNA in rat brown adipocytes. *Am J Physiol Endocrinol Metab* **282**:E1119–E1127.
 117. Christoffolete MA, Linardi CC, de Jesus L, Ebina KN, Carvalho SD, Ribeiro MO, Rabelo R, Curcio C, Martins L, Kimura ET, Bianco AC 2004 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* **53**:577–584.
 118. Leonard JL, Mellen SA, Larsen PR 1983 Thyroxine 5'-deiodinase activity in brown adipose tissue. *Endocrinology* **112**:1153–1155.
 119. Loncar D 1991 Convertible adipose tissue in mice. *Cell Tissue Res* **266**:149–161.
 120. Almind K, Manieri M, Sivitz WI, Cinti S, Kahn CR 2007 Ectopic brown adipose tissue in muscle provides a mechanism for differences in risk of metabolic syndrome in mice. *Proc Natl Acad Sci USA* **104**:2366–2371.
 121. Guerra C, Koza RA, Yamashita H, Walsh K, Kozak LP 1998 Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J Clin Invest* **102**:412–420.
 122. Xue B, Coulter A, Rim JS, Koza RA, Kozak LP 2005 Transcriptional synergy and the regulation of Ucp1 during brown adipocyte induction in white fat depots. *Mol Cell Biol* **25**:8311–8322.
 123. Xue B, Rim JS, Hogan JC, Coulter AA, Koza RA, Kozak LP 2007 Genetic variability affects the development of brown adipocytes in white fat but not in interscapular brown fat. *J Lipid Res* **48**:41–51.
 124. Commins SP, Watson PM, Padgett MA, Dudley A, Argypoulos G, Gettys TW 1999 Induction of uncoupling protein expression in brown and white adipose tissue by leptin. *Endocrinology* **140**:292–300.
 125. Orci L, Cook WS, Ravazzola M, Wang MY, Park BH, Montesano R, Unger RH 2004 Rapid transformation of white adipocytes into fat-oxidizing machines. *Proc Natl Acad Sci USA* **101**:2058–2063.
 126. Claret M, Corominola H, Canals I, Saura J, Barcelo-Batllo S, Guinovart JJ, Gomis R 2005 Tungstate decreases weight gain and adiposity in obese rats through increased thermogenesis and lipid oxidation. *Endocrinology* **146**:4362–4369.
 127. Leonardsson G, Steel JH, Christian M, Pocock V, Milligan S, Bell J, So PW, Medina-Gomez G, Vidal-Puig A, White R, Parker MG 2004 Nuclear receptor corepressor RIP140 regulates fat accumulation. *Proc Natl Acad Sci USA* **101**:8437–8442.

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