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Review

PPARγ in the control of brown adipocyte differentiation

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Abstract

The effects of fatty acids and retinoic acid (carotene) on brown adipose tissue differentiation are mediated by activation of the transcription factors PPARγ and PPARα in combination with RXR. There is good support for the idea that activated PPARγ promotes adipogenesis also in brown adipose tissue. However, the issue is more complex concerning the full differentiation to the brown adipocyte phenotype, particularly the expression of the brown-fat-specific marker UCP1. The effect of norepinephrine on PPARγ gene expression, at least in-vitro, is negative, PPARγ-ablated brown adipose tissue can express UCP1, and PGC-1α coactivates other transcription factors (including PPARα); thus, the significance of PPARγ for the physiological control of UCP1 gene expression is not settled. However, importantly, the effects of PPAR agonists demonstrate the existence of a pathway for brown adipose tissue recruitment that is not dependent on chronic adrenergic stimulation and may be active in recruitment conditions such as prenatal and prehibernation recruitment. The ability of chronic PPARγ agonist treatment to promote the occurrence of brown-fat features in white adipose tissue-like depots implies a role in anti-obesity treatment, but this will only be effective if the extra thermogenic capacity is activated by adrenergic stimulation.

Keywords: Brown adipose tissue; UCP1; PPARγ

1. Introduction

A decade has now passed since the transcription factor PPARγ was functionally identified [1], in the brown-adipocyte-like cell line HIB-1B, as one of the two components of the transcription-regulating factor "ARF6", the other being RXRα (retinoid X receptor). Immediately prior to this, PPARγ had been genetically identified as a mammalian homologue to PPARγ in *Xenopus* [2,3] and had been demonstrated to be a protein that had the requisite ability to enhance the expression of the adipocyte-characteristic gene aP2, i.e. the adipose tissue-specific form of the fatty acid binding protein [3]. Thus, the involvement of PPARγ in the adipogenic process was established.

However, already in the 1980s, effects on brown adipose tissue and brown adipocytes of what were to become known as PPARγ agonists had been reported, although the mechanism remained undefined. Present day PPARγ agonists were then known as insulin sensitizers (which they, of course, still are), and the treatment of animals with these compounds (i.e. the thiazolidinediones) had remarkable effects also on brown adipose tissue. Thus, treatment of animals with the thiazolidinedione ciglitazone led to effects that can be interpreted to represent the recruitment of brown adipose tissue, i.e. that the tissue acquired an enhanced capacity for nonshivering thermogenesis [4–6].

Particularly, in a notably prescient experiment, Thurlby et al. [6] demonstrated that although chronic ciglitazone treatment led to a large increase in what today would be called UCP1 content in brown adipose tissue, this did not influence the metabolic rate of the animals. Only if the brown adipose tissue in the animals was also acutely stimulated with a β_3-adrenergic agonist was the enhanced capacity of the tissue revealed (Fig. 1). This experiment thus demonstrated that agents that increase UCP1 amount do not necessarily alter metabolism, an insight that still requires repetition.

In these early studies, which were all in-vivo studies, it was not possible to distinguish between direct and indirect

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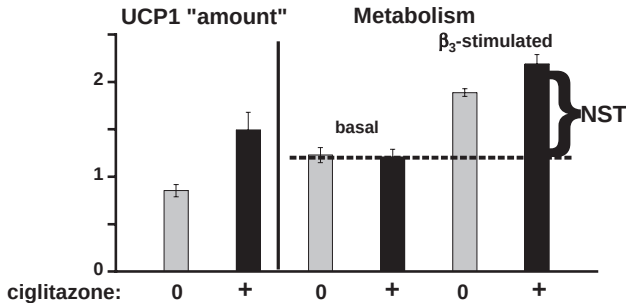

Fig. 1. An early demonstration of the recruiting effect of a PPARγ agonist (here the thiazolidinedione ciglitazone) on brown adipose tissue. The figure is drawn based on data presented by Thurlby et al. [6]. Mice were treated with ciglitazone for 3–4 weeks. The amount of UCP1 was determined by [^{3}H]GDP binding. The basal rate of oxygen consumption and that observed acutely as a response to an injection of a β_3-adrenergic agonist were measured. Note that the thiazolidinedione treatment increased the amount of UCP1 but this did not influence basal metabolism; only when nonshivering thermogenesis (NST) was induced pharmacologically by the β_3-agonist was the increased thermogenic capacity revealed.

effects of the thiazolidinediones. Indeed, the conclusions of the early papers were generally that the effects on brown adipose tissue were in some way secondary to the insulin-sensitizing effect, mediated e.g. via an activation of the sympathetic nervous system. This tenet was fundamentally changed when it was observed that the thiazolidinedione pioglitazone, which recruited brown adipose tissue in-vivo [7], could also dramatically increase the expression of UCP1 in brown adipocytes in culture, alone or in synergy with norepinephrine [7]. The implication since then has been that a direct brown adipocyte target for thiazolidinediones must exist. PPARγ turned out to be this target.

Two comments on terms may be appropriate here. One is that the term PPARγ, peroxisomal proliferator activated receptor-gamma, should be seen as a historical and phylogenic landmark, rather than as an indication of the predominant effect of PPARγ in brown adipose tissue: While peroxisome recruitment is also part of the general recruitment process in brown adipose tissue [8,9], this is only one of the functions that can be ascribed to PPARγ in the tissue.

The other comment concerns brown versus white adipose tissue. In this overview, we only refer to studies of PPARγ performed in brown adipose tissue and do not extrapolate to this tissue from studies performed in white adipose tissue, mainly because brown and white adipose tissues are so close and yet so different that such extrapolation may be misleading. However, a difficulty is that although certain adipose tissue depots are acceptably "brown" or "white" (the interscapular and epididymal depots are generally referred to as such), other depots, such as e.g. the inguinal depot, may — depending on conditions or animal species or strain — be somewhere in between. Indeed, an effect of PPARγ activation may be to promote the brown versus white phenotype, as will be seen below.

2. The recruitment process in brown adipose tissue

The issue we will examine here, based on the literature and our unpublished observations, is to which extent PPARγ is a mediator of the recruitment process in brown adipose tissue, by itself or in combination with other factors. The recruitment process in brown adipose tissue [10] is the combined result of enhanced cell proliferation and enhanced cell differentiation, including the enhanced expression of the brown-fat-specific uncoupling protein-1 UCP1 [11] that endows the tissue with an increased capacity for non-shivering thermogenesis. Acutely, nonshivering thermogenesis is controlled by the sympathetic nervous system through norepinephrine release in the tissue, and most physiologically induced events of recruitment (e.g. as those occurring in cold environments or as an effect of overeating) can be understood as occurring as a consequence of chronic sympathetic stimulation of the tissue. PPARγ could be involved in such recruitment processes and it could also be involved in recruitment processes that are not understandable in this way (as discussed under Perspectives below).

We will first summarize what is known about the control of the amount of PPARγ in brown adipose tissue, then what is known about the control of its activity, and finally what the effects of PPARγ activation are.

3. What controls PPARγ amount?

3.1. At the level of gene expression

PPARγ distinguishes itself amongst the PPARs as being the one demonstrating the highest expression in adipose tissues, with brown adipose tissue expression being particularly high [12–14].

During the differentiation of brown (pre)adipocytes in-vitro, it is observed (Fig. 2) that PPAR expression is high already in undifferentiated, proliferating brown preadipocytes [15,16] (our unpubl. obs.). As seen, PPARγ increases somewhat until the time of adipocyte conversion. It may be said that the presence of PPARγ precedes the expression of the adipocyte marker aP2 and also the emergence of the ability of the brown adipocytes to respond to norepinephrine with UCP1 gene expression (Fig. 2). In this way, a causative or necessary function of PPARγ in the differentiation process can be formulated. It should, however, be observed that the increase in e.g. UCP1 occurs under conditions of practically stable levels of PPARγ gene expression, implicating that the UCP1 gene expression is not a simple effect of the presence of this protein.

During embryogenesis, PPARγ appears suddenly in brown adipose tissue at embryonic day 18.5 (when compared with day 15.5), and at that point, it is then only found there, not in any other tissue [17] (although it is transiently expressed in the brain earlier during development). This is approximately the time when identifiable brown adipose

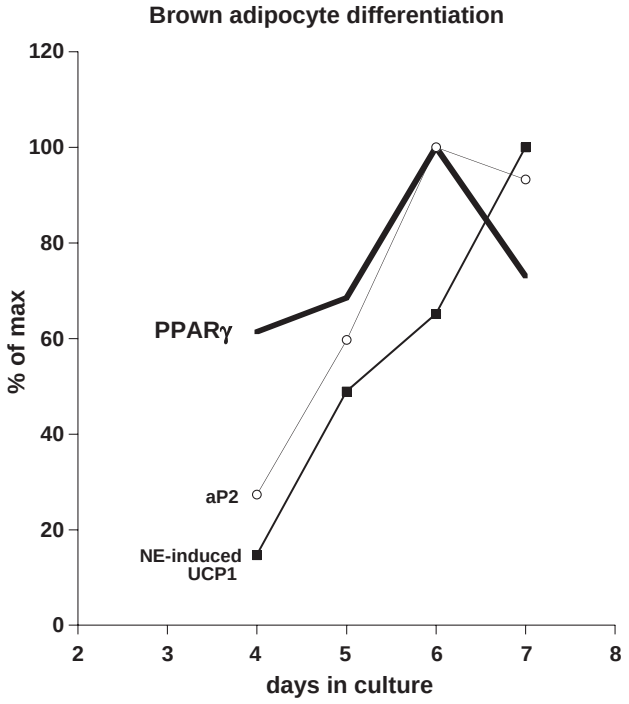

Fig. 2. The expression of PPARγ and some brown adipocyte differentiation markers during the differentiation of primary brown adipocytes in culture. Expression levels were followed by Northern blot studies in primary cultures of brown adipocytes, initiated from stromal-vascular cells on day "0". The UCP1 mRNA values refers to those observed after 2 h of norepinephrine stimulation (without norepinephrine, UCP1 is practically not expressed). Means from 4 independent experiments. Our unpubl. obs.

tissue can be observed in the embryo [18,19] and the expression of PPARγ at this time is well established [20–22].

The expression of PPARγ during adipocyte differentiation is generally thought to be secondary to a self-enforcing positive loop between C/EBPα and PPARγ expression [23]. However, in contrast to what is the case for white adipose tissue, the expression of C/EBPα is not necessary for adipocyte conversion in brown adipose tissue [24]. The expression of PPARγ in the mice that lack C/EBPα has not been directly investigated, and it is therefore not formally possible to know as to whether the differentiation ability of brown versus white adipose tissue is due to C/EBPα not being a necessary regulator of PPARγ in this tissue—or whether the tissue differentiates without PPARγ. However, other observations on PPARγ would tend to favour the interpretation that C/EBPα is not a necessary factor for PPARγ gene expression in brown adipose tissue.

The PPARγ gene can give rise to at least two different mRNAs, PPARγ1 and PPARγ2, which in their turn code for different proteins. These two mRNA species are so close in size that they are difficult to distinguish on e.g. Northern blots. However, using real-time PCR, we have found that 95% of all PPARγ mRNA in primary cultures of brown adipocytes is PPARγ2 [16]. Direct comparisons of the absolute levels of PPARγ1 and PPARγ2 mRNA in brown adipose tissue in-vivo have not as yet been performed.

3.2. The effect of norepinephrine on PPARγ gene expression in brown adipocytes

Since norepinephrine promotes brown adipose tissue recruitment, since PPARγ agonists stimulate brown adipose tissue recruitment both in-vivo and in-vitro, and since PPARγ is especially expressed in brown adipose tissue, all implications would support the notion of norepinephrine being positive for PPARγ gene expression. This is, however, not the case, at least not in cultured brown adipocytes. As early mentioned [25], the effect of norepinephrine in brown adipocytes is the opposite of what is expected: a decrease in PPARγ levels. Norepinephrine leads to a marked (50%) decrease in PPARγ mRNA levels in brown adipocytes. The decrease is transient: The nadir is obtained after about 4 h, and after about 24 h, PPARγ gene expression is back to initial values [16].

The repressive effect of norepinephrine is mediated via β-adrenergic receptors. As the effect of norepinephrine is observed both in undifferentiated preadipocytes (that only express β_1-receptors) and in mature brown adipocytes (that couples via β_3-receptors), it would seem that both β_1- and β_3-receptors can mediate the signal (selective β_3-stimulation as well) [16]. (β_2-Adrenoceptors are not expressed in brown adipocytes [26]).

The further mediation (cf. Fig. 3) is probably via the activation of adenylyl cyclase and an increase in cAMP levels, and subsequent activation of protein kinase A. The subsequent steps have not been identified, but could be expected to involve the phosphorylation of CREB [27].

The effect of norepinephrine on PPARγ gene expression occurs with a delay of about 1 h. This in itself indicates that a more complex process than a simple phosphorylation event is involved. Such more time-consuming events could include the synthesis of controlling proteins. In agreement with this, the treatment of brown adipocytes with the protein synthesis inhibitor cycloheximide eliminates the repressive effect of norepinephrine on PPARγ gene expression. However, cycloheximide does more than this: It also increases the basal level of PPARγ gene expression [16]. A possible interpretation could be that a repressor protein is involved in the control of PPARγ expression. This possibility is depicted in Fig. 3: Here a PPARγ transcription repressor protein is suggested to be constantly being synthesized and exerting its inhibitory effect on the PPARγ gene. The inhibition of the synthesis of this repressor (with cycloheximide) relieves the inhibition; norepinephrine could thus act by increasing the synthesis of the repressor.

3.3. Effects of exogenous and endogenous norepinephrine in-vivo

From the above observations in brown adipocyte cultures, it would be expected that norepinephrine stimulation would lead to the repression of PPARγ gene expression also in-vivo. However, as seen in Fig. 4A, we observed that

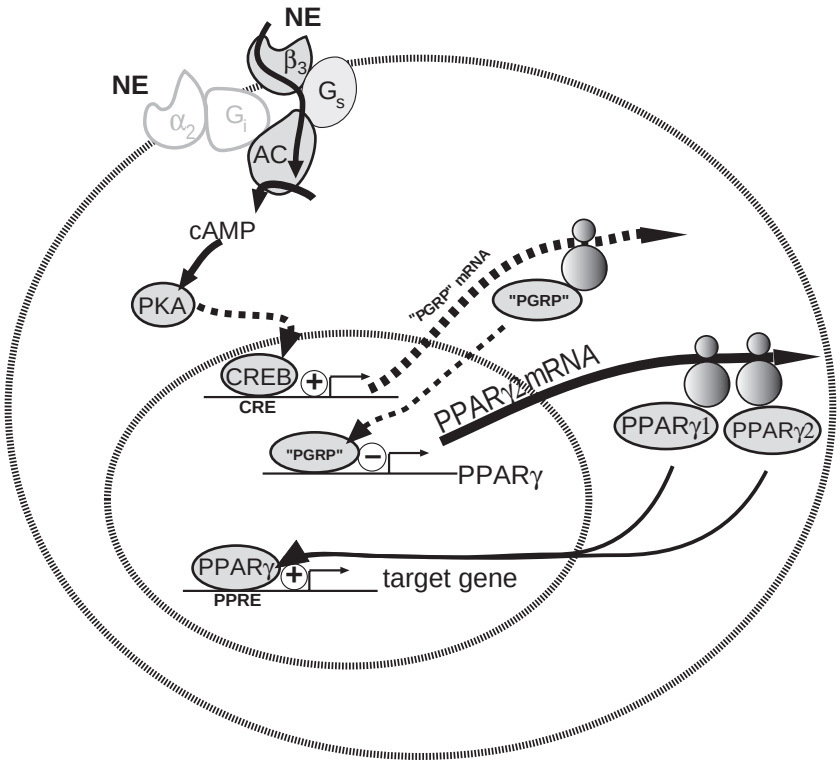

Fig. 3. Tentative scheme for the mediation of the effect of norepinephrine on the expression of PPARγ in brown adipocytes. AC, adenylyl cyclase; PKA, protein kinase A; CREB, cyclic AMP response element binding protein; "PGRP", hypothetical PPARγ repressor protein. Expanded from [16].

norepinephrine injection, if anything, led to an increased PPARγ expression after 3 h (however, no effect of a selective β_3-agonist was observed in [28]).

The issue of the control of PPARγ expression becomes more complicated when the effect of cold exposure is studied. Cold exposure leads to a chronically increased adrenergic stimulation of the cells in the tissue and may thus be considered to represent a continuous endogenous norepinephrine stimulation (although other affectors are also released in the tissue). Again, with short-term cold exposure (3 h), a marked increase in PPARγ mRNA levels were observed in the tissue (Fig. 4B). However, during prolonged exposure to cold, there was no marked induction or repression of PPARγ (Fig. 4C); similarly, there is no postnatal increase in PPARγ mRNA levels, although marked brown adipose tissue recruitment takes place during this period [22]. Thus, it would not seem that an increased expression level of PPARγ is involved in the physiological recruitment process in brown adipose tissue. It may be noted that the cold-exposed mouse data presented here are in contrast to other data obtained with mouse [29,22] and to data from cold-exposed rats, where a transient decrease in PPARγ mRNA levels were seen in the cold [9]. However, a complication in the interpretation of some in-vivo data is that we have indications that a daily rhythm of PPARγ mRNA may occur, both in animals in the warm and in the cold (Fig. 4D). Thus, the effect of short-time cold exposure may not always be distinguishable from daily variations. The tendency to a high level of PPARγ mRNA during the light period (Fig. 1D) may be a reflection of the semi-fasting state of the mice during this time. Indeed, fasting has been reported to increase PPARγ gene expression and protein amounts [30,31] in the tissue.

In conclusion, it is evident from both in-vitro and in-vivo experiments that the levels of PPARγ gene expression in brown adipose tissue are hormonally controlled—but the physiological significance of these alterations is not understood. Concerning PPARγ2 gene expression, it would seem difficult at the moment to reconcile the observations on adrenergic effects in cell culture with the observations in-vivo (a clear parallelism is normally seen, e.g. concerning UCP1 gene expression).

3.4. PPARγ protein synthesis

The alterations in PPARγ mRNA levels should also be reflected in different amounts of PPARγ protein in the tissue. There are, however, only few reports on protein levels in brown adipose tissue. Concerning PPARγ and similar transcription factors, it may also be pointed out that the transcription-regulating activity of these factors is not necessarily a function of their protein levels: Their transcriptional activity is regulated in different ways, particularly by ligand binding, and provided that the protein levels are supersaturating for the binding sites (which is perhaps likely), marginal alterations in protein amount may not result in significant alterations in the effect of PPARγ on gene transcription.

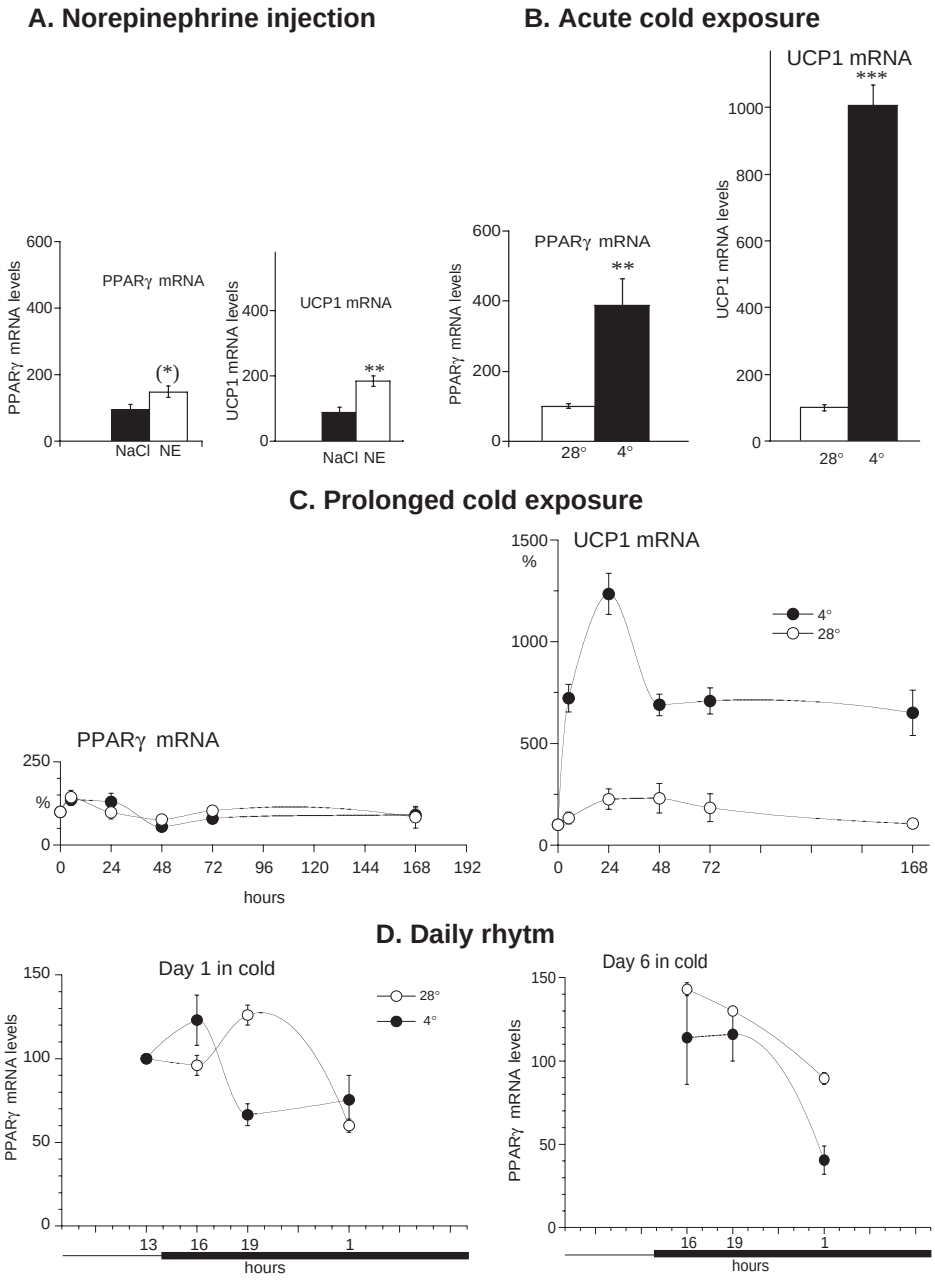

Fig. 4. The effect of norepinephrine injection and cold exposure on the expression of PPARγ and UCP1 in brown adipose tissue. (A) Effect of norepinephrine (NE) injection. Mice preacclimated to 28 °C were injected with 1 mg norepinephrine per kilogram body weight (or with the saline vehicle). Three hours later, the mice were killed and mRNA levels quantified. Means±S.E. from 6 animals; **P*<0.01, (*)*P*<0.10. (B) Effect of short-term cold exposure. Mice preacclimated to 28 °C were transferred to 4 °C or remained at 28 °C. Three hours later, the mice were killed and mRNA levels were quantified. Means±S.E. from 6 animals; ***P*<0.01, (*)*P*<0.10. (C) Effect of long-time cold exposure. (D) Effect of time of day on PPARγ gene expression. Our unpubl. obs.

From the two PPARγ mRNA species, two different proteins, PPARγ1 and PPARγ2, can be formed. Little is known concerning the functional significance of these different protein species. In brown adipocytes, where more than 95% of the total PPARγ mRNA represents PPARγ2, immunoblotting with anti-PPARγ reveals several bands, including the expected PPARγ2, as well as phosphorylated PPARγ2 [16]. However, a band of lower molecular weight of 52 kDa is also observable, corresponding to the molecular weight of PPARγ1. Therefore, we suggest that the PPARγ2 mRNA gives rise to both PPARγ1 and PPARγ2 proteins. This is theoretically feasible, because the PPARγ2 mRNA includes the intact translation initiation site of PPARγ1, and we therefore propose (Fig. 3) that both PPARγ1 and PPARγ2 are formed from the same mRNA, in approximately equal amounts. (There is some experimental support for this, because at least in in-vitro transcription system, both proteins are formed in equal amounts from PPARγ2 mRNA [32]; also observable in the figures in [33]). In the tissue in adult animals, the

dominant protein isoform is PPARγ1 [22] (our unpubl. obs.).

It has been possible to obtain animals with a selective ablation of PPARγ2. In these animals, there is a loss of PPARγ2 protein but not of PPARγ1 protein, i.e. two-thirds of the total PPARγ protein remains. Despite this, there is a reduction in lipid accumulation in brown adipose tissue (and in white) [34], combined with a very modest decrease in adipogenic gene expression in the tissue (UCP1 gene expression has not been studied in brown adipose tissue in these mice).

Although there are no well-established differential roles for PPARγ1 and PPARγ2 with respect to function, we have good indications that there are differences in their stability. When PPARγ2 mRNA levels are reduced in brown adipocytes by norepinephrine stimulation, both the PPARγ1 and the PPARγ2 protein levels are reduced, finally to same extent as the reduction in mRNA level. However, the kinetics are quite different: PPARγ1 protein temporally nearly reflects the decrease in mRNA, whereas the decrease in PPARγ2 protein is clearly delayed [16] (analyses of data in [22] would tend to support this difference). This would indicate a protein half-life of PPARγ1 in the order of perhaps about 30 min, whereas the half-life of PPARγ2 would be around 5 h.

4. Regulation of PPARγ activity

In addition to the possible regulation of PPARγ activity that is simply a reflection of PPARγ amount, the activity is also regulated by other mechanisms. The phosphorylation of PPARγ by MAP kinase leads to reduced activity of the protein [33]. In brown adipocytes, the phosphorylated species of at least PPARγ2 is observable, with about 10% of the protein being in the phosphorylated state and thus likely to be inactive [16]. There are no reports of any physiological or hormonal changes in the degree of phosphorylation in the tissue. PPARγ may be both SUMOylated and ubiquitylated but to date there are no reports on this in brown adipose tissue. Furthermore, PPARγ may be found both in the nucleus and in the cytosol, and the balance between these two locations may be altered during e.g. development [22].

However, the most interesting regulation of PPARγ activity is that exerted by ligands, endogenous or exogenous.

The nature of the endogenous activators is still not established. It is generally accepted that they are (metabolites of) fatty acids, probably polyunsaturated, perhaps with prostaglandins being the best ligands. If this is the case, the synthesis of prostaglandins in the tissue may be a prerequisite for the physiological action of PPARγ but there are presently no studies examining this issue. In any case, the significance of ligand binding is not straightforward. A mutation in the ligand-binding domain (P465L) when present in heterozygotes (homozygotes die) reduces UCP1 gene expression in "minor" brown adipose tissue depots (gonadal and inguinal depots) but not in the major interscapular depot [35]. Based on this, it may be suggested that PPARγ activity is limiting for UCP1 gene expression in more white-fat-like depots, whereas in interscapular brown adipose tissue it is not PPARγ activity that is a limiting factor. As seen below, other observations may support such a view.

Exogenous PPARγ ligands are the thiazolidinediones, of which a series of different types have been used both in-vivo and in-vitro (as well as some non-thiazolidinediones with similar insulin-sensitizing properties), including rosiglitazone (=BRL-49653), ciglitazone, and pioglitazone (and the non-TZD COOH [36]). In addition to their possibly different intrinsic activities and affinities, the possibility exists that they exert to different extents non-nuclear effects, which may or may not be mediated by cytosolically located PPARγ; such effects may include the activation of MAP kinases [37].

4.1. PPARγ and RXR

In the nucleus, PPARγ is always found as a dimer with the retinoid X receptor, RXR. It is thought that it is the combined effect of the activation of these two transcription factors that influences the transcriptional activity of the complex.

The stimulation of brown adipocytes with retinoic acid leads to very marked effects on the expression of the brown adipocyte marker UCP1 [38–41] (Fig. 5). Also carotene

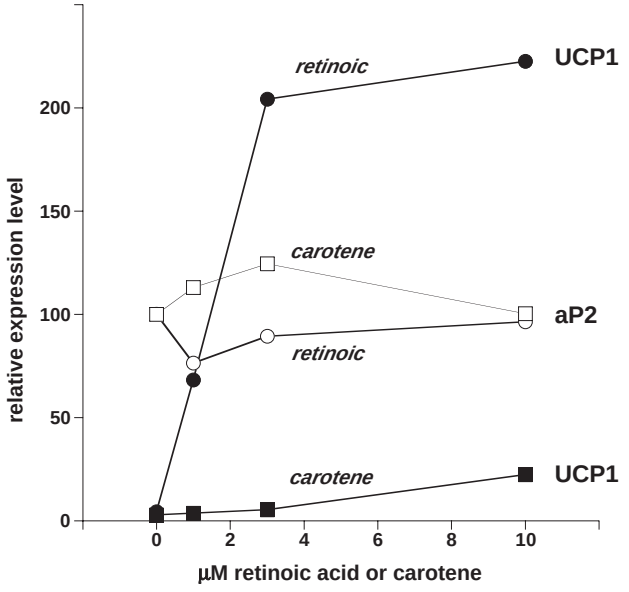

Fig. 5. The effect of retinoic acid and the retinoic acid precursor carotene on the expression of UCP1 and aP2 in brown adipocytes. Brown adipocytes were grown in culture for 5 days and then treated with the indicated concentrations of retinoic acid or β-carotene for 48 h, before the levels of aP2 and UCP1 mRNA were determined. The levels in acutely (2 h) norepinephrine-treated control cells was set to 100%. Our unpubl. obs.

(which is the precursor of retinoic acid) when added directly to brown adipocytes can induce UCP1 gene expression (Fig. 5) and increase UCP1 protein amount [41], although in our hands to a much lesser extent than can retinoic acid itself (or norepinephrine). However, as RXR dimerizes with several transcription factors, including e.g. PPARα, the observed effects are not necessarily due to a reinforcement of the PPARγ effect. Indeed, as seen in Fig. 5, there is almost no effect of retinoic acid or carotene on the expression of the PPARγ target gene aP2, indirectly implying that the retinoic acid effect is not primarily mediated within the framework of the PPARγ/RXR complex.

Studies directly examining the possible interaction between PPARγ activation and RXR activation have not been published, although evidently a synergistic interaction would be expected. However, in a transfected reporter system, such a synergistic effect on UCP1 promoter activity was observed [42].

4.2. PPARγ and PGC-1α

As mentioned above, a PPARγ agonist can induce UCP1 gene expression in brown adipocytes [7] (and others, incl. Fig. 6). When a UCP1 reporter was transfected into the brown-adipocyte-like cell line HIB-1B [20], a PPARγ-agonist-induced response was seen—but not when this reporter was transfected into NIH-3T3 cells [43]. As both cell types expressed PPARγ, the difference in the response implied that HIB-1B contained a further factor that was necessary for the ability of the thiazolidinedione/PPARγ complex to transactivate UCP1 gene expression. With the yeast two-hybrid technique, this extra factor was identified and named PGC-1, i.e. PPARγ cofactor-1 [44]. However, the indications of the original studies, i.e. that PGC-1α was brown-fat-specific, had PPARγ as its only partner, and was specific for inducing UCP1 gene expression, have all proven to be only part of the story concerning PGC-1α. Today, a formulation could be that PGC-1α is essential in the process of activating a more "oxidative" phenotype in different tissues, including the promotion of mitochondriogenesis in these different tissues, and that the mitochondria induced by this pathway exhibit tissue-specific phenotypes, i.e. in brown adipose tissue the phenotype that includes UCP1 expression.

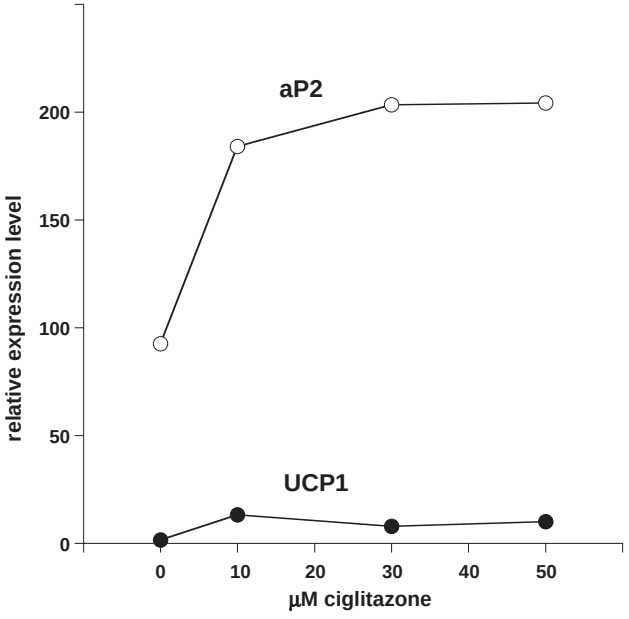

Fig. 6. The effect of the thiazolidinedione ciglitazone on the expression of adipogenic and brown-fat markers in brown adipocytes. Brown adipocytes were grown in culture for 6 days and then treated with the indicated concentrations of ciglitazone for 24 h, before the levels of aP2 and UCP1 mRNA were determined. The levels in acutely (2 h) norepinephrine-treated cells without ciglitazone was set to 100%; the effect of 24 h ciglitazone on UCP1 mRNA is thus <10% of that seen with norepinephrine.

There are clear effects of ablation of the PGC-1α gene on brown adipose tissue [45]: a decrease in mitochondrial fatty acid oxidation and respiratory chain enzymes. However, as PGC-1α is a cofactor for several transcription factors in addition to PPARγ, these effects do not necessarily reflect only the absence of the specific PPARγ/PGC-1α signal.

PGC-1α is well expressed in brown adipocytes and norepinephrine further enhances PGC-1 gene expression (our unpubl. obs). In the animals without PGC-1α in the tissue, cold exposure can no longer increase UCP1 gene expression [45] (but UCP1 is still expressed at a basal level). However, the hypothesis that norepinephrine induces UCP1 gene expression through first inducing PGC-1α which then activates the UCP1 gene is not supported by experiments in which protein synthesis was inhibited when norepinephrine was added to the brown adipocytes. In these cells, the induced level of UCP1 mRNA was equally high, whether protein synthesis was inhibited or not [46]. Thus, an increased PGC-1α protein amount is not required for the mediation of the acute norepinephrine effect on the UCP1 gene.

5. What does PPARγ regulate?

To study the regulatory effects of PPARγ, it is useful to have access to animals that lack PPARγ. However, as global ablation of PPARγ leads to no survival of offspring, more complex gene ablation techniques have had to be used to examine the significance of PPARγ in this way.

5.1. Proliferation or anti-proliferation?

As PPARγ stimulates the acquisition of traits of differentiation, an increased amount of PPARγ may be hypothesized to have anti-proliferative effects. As norepinephrine stimulates proliferation in brown preadipocytes [47], a possible interpretation of the norepinephrine-induced repression of PPARγ gene expression in brown preadipocytes [16] could be that it promotes this proliferative effect of norepinephrine. However, as norepinephrine also represses PPARγ gene expression in mature brown adipocytes [16], such an interpretation of the effect is doubtful.

Instead, it may be noted that chronic treatment of animals with PPARγ agonists leads to an increased cell number in brown adipose tissue depots (e.g. [48]). As PPARγ is expressed in brown preadipocytes, this may be a direct effect but this has not been studied in an in-vitro system. That PPARγ is necessary for the survival of brown adipocytes is deduced from experiments in which PPARγ is selectively ablated from adipocytes, based upon a tamoxifen-induced CRE recombination system. The mature adipocytes then die [49] but are successively replaced with new adipocytes. Thus, PPARγ seems to be essential for mature brown (and white) adipocyte survival.

5.2. Adipogenesis and lipid metabolism

The stimulation of PPARγ in brown adipocytes or in intact animals has adipogenic effects. One example of this is given in Fig. 6. A vast array of genes involved in lipid synthesis and lipid catabolism are induced by chronic treatment with PPARγ agonists. This is e.g. seen as an effect of the PPARγ (non-thiazolidinedione) agonist GW1929 [50]. However, principally the same genes are induced in white adipose tissue [50]. To the extent that PPARγ induces the adipogenic phenotype in brown as well as white adipose tissue, we will not discuss this in detail in the present overview but instead concentrate on the specific brown adipose tissue features, i.e. primarily on UCP1 gene expression.

Although PPARγ agonist can clearly induce adipogenic genes in brown adipose tissue, the physiological function of PPARγ may not be considered demonstrated by such observations. Indeed, in mice that specifically lack PPARγ in adipose tissues (both white and brown), the size of the tissues was remarkably reduced (probably due to poor mature adipocyte survival, as discussed above). However, in the tissue remaining, there was almost no decrease in adipogenic genes in brown adipose tissue [51], indicating at least that PPARγ is not essential for the adipogenic process in brown adipose tissue (again in contrast to the case in white adipose tissue where several adipocyte markers were clearly affected).

5.3. UCP1 gene expression

Concerning the brown adipose tissue, the issue that undoubtedly attracts most interest is the ability of PPARγ agonists (and thus likely of PPARγ) to influence the expression of the brown-fat-specific protein, the uncoupling protein UCP1. The mechanism and the potential metabolic effects of such PPARγ-induced increases in UCP1 amount are reviewed here.

That the effect of PPARγ agonists on UCP1 is directly on the brown adipocytes is seen in Fig. 6. Here, the effect of ciglitazone treatment was rather small (<10% of that seen with norepinephrine) but the effects were based on short-time (1 day) treatment with the PPARγ agonist. However, when brown adipocytes are chronically treated with PPARγ agonists, the result is much more dramatic (Fig. 7). There are some increases in the level of expression of the adipogenic marker aP2 (Fig. 7A)—but much more striking are the effects on the expression of UCP1 (Fig. 7B). As seen, rosiglitazone alone is able to induce UCP1 gene expression to more than double the level induced by norepinephrine, and norepinephrine then loses its stimulatory effect.

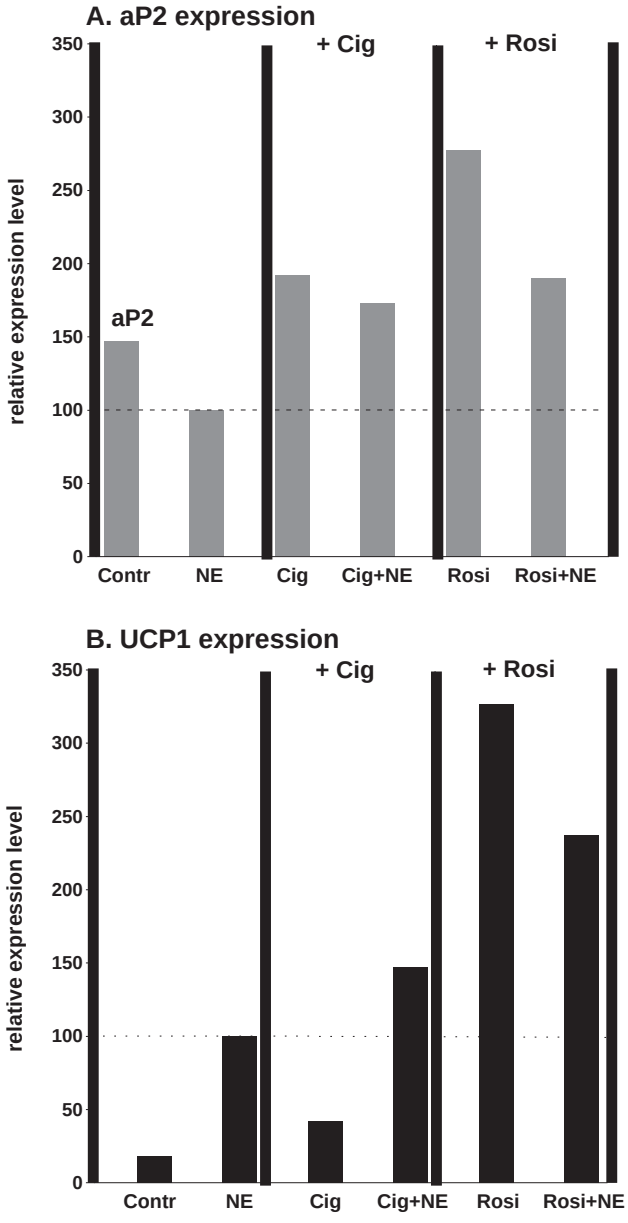

Fig. 7. The effect of chronic treatment of brown adipocytes with PPARγ agonists. Primary cell cultures from brown adipose tissue were cultured in the presence of ciglitazone or rosiglitazone for 7 days (i.e. through both the proliferative and the differentiation states) and then treated with norepinephrine (NE) for 2 h. aP2 and UCP1 mRNA levels were then determined by Northern blots; the levels observed in the norepinephrine-treated control cells were set to 100%. Typical result from 4 independent experiments. Our unpubl. obs.

This type of experiment indicates that PPARγ agonists not only promote differentiation and thus makes UCP1 gene expression possible (as e.g. discussed by [52]) but that they by themselves can induce UCP1 to the full extent.

The ability of the UCP1 gene to respond to PPARγ agonists resides in the distal complex enhancer of the gene. This complex includes a PPRE (PPAR response element) which, in mouse, is localized at −2458 to −2485, with similar locations in the rat and human genomes. The gene structure of UCP1 is summarized in Fig. 8, with special emphasis on the action of PPARs.

The PPRE response element can bind either PPARγ or PPARα [53], and also both PPARγ and PPARα agonists can induce UCP1 gene expression. Based on the general concept of PPARα being a transcription factor for lipid catabolism, it would indeed be more to be expected if it was mainly through PPARα that UCP1 gene expression was induced. PPARα is also only found in brown and not in white adipose tissue [14,13], and it is expressed first when the brown adipocytes are maturing [15]. It has been discussed whether the early studies in HIB cells indicating PPARγ as a main regulator of UCP1 have been influenced by the fact that HIB cells do not express PPARα. However, it is uncertain whether PPARα is found to a reasonable extent in cultures of brown adipocytes. A very low expression of PPARα in cultured brown adipocytes has been observed both at the mRNA level [13] and the protein level [16], whereas Valmaseda et al. [15] have observed high levels. Further, the UCP1-inducing effect of PPARα agonists is enforced by PGC-1α (in a model system) [53], and acute effects of injection of PPARα agonists seem larger than that of injection of PPARγ agonist [54].

In indirect support of the idea that PPARγ may not be as essential for UCP1 gene expression as has generally been envisaged is the observation that animals with no PPARγ expression in brown adipose tissue exhibit no decrease in UCP1 gene expression in the remaining brown adipocytes [51]. Thus, at least in such a system, PPARγ is not essential for UCP1 gene expression, undoubtedly a surprising result considering the experimental studies underlying the identification of PGC-1α. Of course, the ability of exogenous PPARγ agonists to induce UCP1 gene expression does not necessarily reveal anything about the mechanisms normally controlling UCP1 levels in man or mouse.

Concerning UCP1 induction, it must be stressed that an increase in UCP1 protein amount does not necessarily reflect or lead to increased UCP1 activity. UCP1 is not "leaky", and no increased rate of metabolism is e.g. observed in brown adipocytes due to the presence of UCP1 as such [55]; the effect of UCP1 is only revealed when the cells are stimulated with norepinephrine. Also in animals, a high expression of UCP1 can be induced by PPARγ agonist treatment without this having any metabolic effect as such [6,36]. Only after adrenergic stimulation is the increased thermogenic activity revealed [6,36] (Fig. 1).

The phylogenically close relatives to UCP1, UCP2 and UCP3 are also expressed in brown adipose tissue. Their function there, as anywhere else, is not presently known (although a series of hypotheses have been forwarded concerning these proteins [56]). UCP3 is of special interest as it shows brown-versus-white adipose tissue specificity (UCP2 is also expressed in white adipose tissue and will thus not be discussed here). UCP3 is induced by rosiglitazone in fetal brown adipocytes [57], and this may perhaps

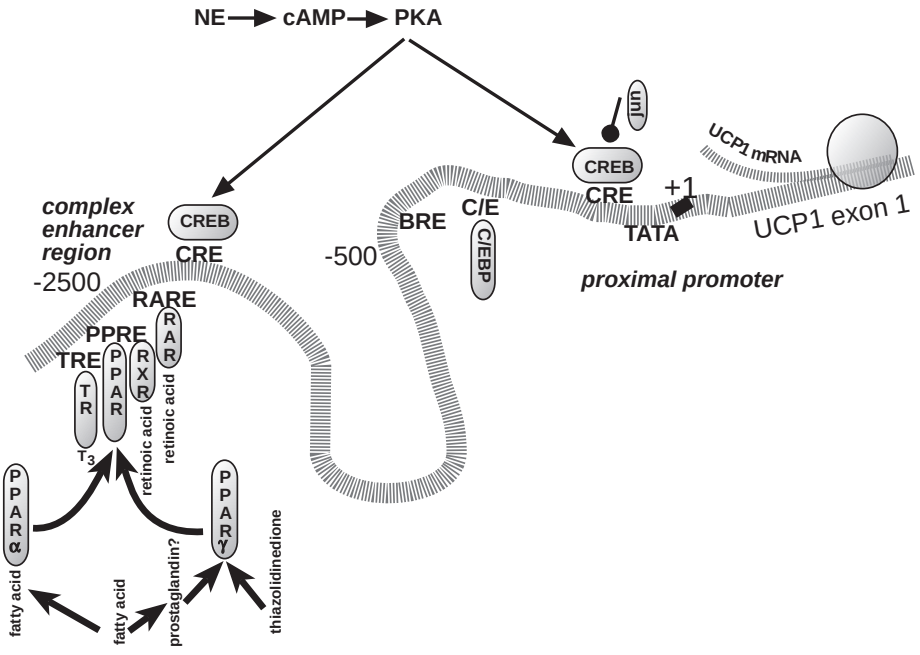

Fig. 8. PPARγ as one of the agents involved in the control of UCP1 gene expression. Adapted after [10].

be seen as a general promotion of brown adipocyte differentiation.

5.4. Induction of UCP1 in apparently "white" adipose tissue

The implication of several of the studies mentioned above may perhaps be that the significance of PPARγ in brown adipose tissue may be more restricted than generally assumed. However, the ability of PPARγ agonists to cause an apparent transformation of white-to-brown adipose tissue is striking ("apparent" because it is not known whether it is "genuine" white adipocytes that transform into brown adipocytes or whether "genuine" brown preadipocytes are stimulated to develop within the "white" depots). Thus, UCP1 gene expression can be induced with PPARγ agonists in both mesenteric and subcutaneous fat [58] (whereas the UCP1 level in interscapular brown adipose tissue is not affected) (it may be noted that a combined PPARγ and PPARα agonist was better than more clean PPARγ agonists [58]). This type of white-to-brown transformation is also observed after PPARγ-agonist treatment in dogs and rats [59]. The possibility that what is seen is not a transformation but a rather a promotion of brown precursors is supported by the observation that UCP1 is induced by a PPARγ agonist in rabbit "white" adipocytes only if they were isolated from previous "brown" depots but not if isolated from white [60].

6. Perspectives

6.1. Physiological significance of the PPARγ recruitment process in brown adipose tissue

Concerning most cases of recruitment of brown adipose tissue, the tenet is that it is the increased chronic sympathetic stimulation, via norepinephrine release, that not only controls acute thermogenesis but also controls the recruitment process [10]. However, there are at least two physiological contexts where this pathway would seem to be counter-intuitive: prenatal and prehibernation recruitment.

In many animals, including mice and rats, brown adipose tissue recruitment occurs primarily after birth and is a consequence of exposure to cold [61]. However, in precocial newborns, normally exemplified by the guinea pig, brown adipose tissue is recruited already at birth (human infants may also belong to this group). It would seem unlikely that brown adipose tissue recruitment in these species is due to an intrauterine activation of the tissue. It is definitely not cold in the uterus, so normal cold-induced recruitment activation cannot take place. Also, chronic sympathetic stimulation would produce further heat, adding to the heat burden of the mother. Thus, during prenatal recruitment, the adrenergic pathway is contra-indicated, and the PPARγ/RXR pathway could therefore be the one that is functionally responsible for prenatal recruitment. There is as yet no direct evidence for this, and even if this were the case, the regulation of the provision of endogenous PPARγ/RXR ligands poses new challenging questions.

The process of preparation for hibernation also represents a recruitment process where the adrenergic pathway would be contra-indicated. During the time when brown fat is recruited, i.e. in late summer, the animal is also interested in gaining as much fat reserves as possible. If brown adipose tissue was "over" active during this period, the price for the possession of an adequate amount of brown adipose tissue would be a reduced amount of energy reserves. Thus, also here, a PPARγ/RXR pathway to recruitment could be the one that explains the paradox. However, there is presently no evidence for the involvement of the PPARγ/RXR pathway, and also here we would need to establish a mechanism that leads to the production of the endogenous activator of PPARγ/RXR under these conditions.

6.2. Making humans slim

The observations that treatment with PPARγ agonists can induce UCP1 gene expression in adipose areas that are normally "white" immediately lends itself to ideas of transforming human adipose depots storing lipid to depots combusting lipid. Indeed, the transfection of human white adipocytes with PGC-1α makes them even more responsive to PPARγ agonists with respect to the induction of UCP1 [62]. The problem is that treatment of humans with PPARγ agonists in reality has the opposite effect: They make people (and mice) more obese, due to their stimulatory effect on the differentiation of (white) adipose cells.

The answer to this problem may be to combine the differentiation-promoting effect of thiazolidinediones with an activation of the extra UCP1 then formed, through adrenergic stimulation. Indeed, this was exactly what was observed by Arch and collaborators in 1987 (Fig. 1). That this can lead to leanness rather than obesity is demonstrated by the mouse studies of Sell et al. [36]. Thus, thiazolidinediones in themselves induced obesity, and a β_3-agonist in itself had only a small effect—but the combined effects of these agents was leanness in animals, without reduction of food intake. Indeed, this is what would be expected of a good slimming agent. However, to our knowledge, no investigations on such treatments are being performed on humans. One reason could be that β_3-agonists have not as such been successful in humans (just as they only have small effects in mice)—but an additional reason could be that the pharmaceutical incentive for this is minor, as the agents to be used are principally already there and are already patented.

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