CMLS, Cell. Mol. Life Sci. 60 (2003) 1311–1321
1420-682X/03/071311-11
DOI 10.1007/s00018-003-2290-x

CMLS Cellular and Molecular Life Sciences

Review

Vitamin A and the regulation of fat reserves

M. L. Bonet, J. Ribot, F. Felipe and A. Palou *

Laboratori de Biologia Molecular, Nutrició i Biotecnologia, Departament de Biologia Fonamental i Ciències de la Salut, Universitat de les Illes Balears, Cra. Valldemossa Km 7.5, 07071 Palma de Mallorca (Spain),
Fax: +34 971 173184, e-mail: andreu.palou@uib.es

Received 18 October 2002; received after revision 10 December 2002; accepted 13 December 2002

Abstract. Beyond their classical nutritional roles, nutrients modify gene expression and function in target cells and, by so doing, affect many fundamental biological processes. An emerging example, which is the focus of this review, is the involvement of vitamin A in the regulation of the level and functioning of body fat reserves. Retinoic acid, the carboxylic acid form of vitamin A, is a transcriptional activator of the genes encoding uncoupling proteins, and results in animals indicate that whole body thermogenic capacity is related to the vitamin A status. Retinoic acid also influences adipocyte differentiation and survival, with high doses inhibiting and low doses promoting adipogenesis of preadipose cells in culture. Moreover, vitamin A status can influence the development and function of adipose tissues in whole animals, with a low vitamin A status favouring increased fat deposition.

Key words. Retinoic acid; uncoupling proteins; thermogenesis; UCP1; UCP2; UCP3; adipogenesis; PPARγ.

Introduction

Regulation of body fat reserves and body weight of mammals is achieved through a very complex and integrated system of which key elements are: (i) control of feeding, basically through appetite modulation; (ii) control of energy efficiency and in particular of adaptive thermogenesis, a process that allows dissipation of part of the energy contained in food as heat, instead of accumulating it as fat; (iii) control of adipose tissue development and metabolism and (iv) the control of nutrient partitioning, i.e. the distribution of nutrients between tissues, which conditions the possibilities of adipose tissue growth (reviewed in [1]). Each of these processes is influenced by a variety of individual genetic traits, which are in turn modified by environmental and psychosocial factors. Among these, dietary factors can greatly condition the development of obesity and its medical complications.

The connection between diet and body homeostasis is becoming increasingly understood with the realization that beyond their classical nutritional roles, nutrients (and also nonnutrient components) in foods can modify gene expression and function in target cells. Through effects on gene expression mainly, vitamin A affects at least two processes related to energy balance: adaptive thermogenesis and the development and metabolism of adipose tissues. Evidence for a physiologically relevant regulatory role of vitamin A in the body weight control system has accumulated and is reviewed here.

Mechanisms of retinoid action

Vitamin A is found in the body in three main forms or vitamers that differ in their oxidation state: the hydroxyl form (retinol), the aldehyde form (retinal) and the carboxylic form (retinoic acid, RA) (fig. 1). Vitamin A vitamers exist in different isomeric forms and are usually found complexed with proteins or, in the case of retinol, esterified to organic acids (mainly fatty acids) forming retinyl esters, which constitute the storage form of the vitamin. The three vitamin A vitamers together with their

* Corresponding author.

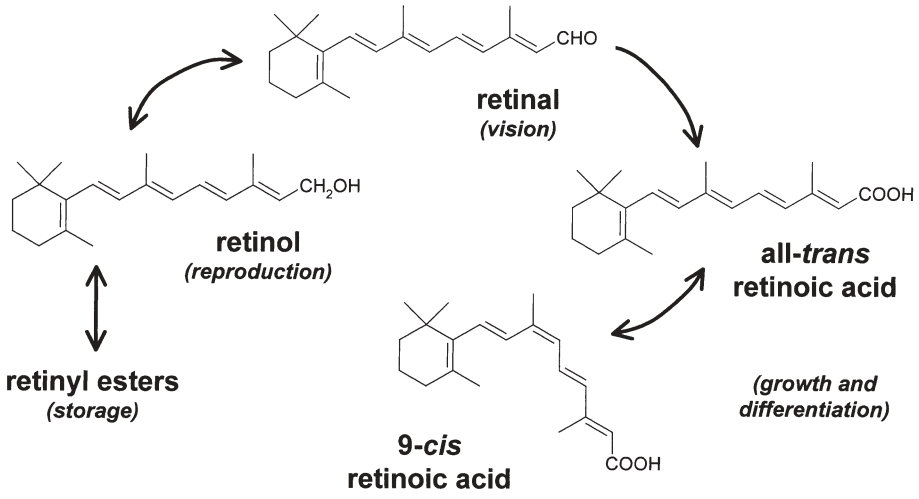

Figure 1. Chemical structures of some biologically active retinoids. Retinol appears to be essential for fertility and may be stored as retinyl esters or converted to the aldehyde form (retinal). The latter is required for the vision process and can be converted into all-trans-retinoic acid (RA) through an irreversible oxidation. all-*trans* RA and its natural isomers are responsible for most of the effects of vitamin A on cell growth and differentiation.

metabolites and synthetic analogues are collectively named retinoids.

Retinoids play a critical role in a variety of essential life processes, including vision, reproduction, hematopoiesis, embryonic development and modulation of the growth and differentiation of a wide variety of mammalian cell types. Other than vision, which requires retinal, the active form of vitamin A in most of these processes is RA [2]. Two biologically active isomers of RA are known, all-*trans* RA and 9-*cis* RA. Isomerization in vivo between the two isomers has been described [3].

Most of the biological effects of RAs involve the activation of ligand-dependent transcription factors of the nuclear hormone receptor superfamily, the retinoid receptors. Two types of these receptors are known: the retinoic acid receptors (RARs), which are responsive to both all-*trans* RA and 9-*cis* RA, and the rexinoid receptors (RXRs), which are responsive to the 9-*cis* RA isomer specifically. Three subtypes of RARs (RARα, RARβ and RARγ) and RXRs (RXRα, RXRβ and RXRγ) have been described in mammalian tissues, which are encoded by different genes and show distinct developmental- and tissue-specific regulated expression [4, 5].

RARs bind as RAR:RXR heterodimer to specific DNA target sequences (RA response elements or RAREs) in promoter/enhancer regions of RA-responsive genes. In a ligand-dependent manner and through protein cofactors (corepressors and coactivators) that it tethers, the RAR:RXR heterodimer interacts with components of the basal transcriptional machinery and/or conditions changes in chromatin condensation, resulting in changes in the frequency of transcription initiation (reviewed in [6]). This ultimately leads to changes in gene expression that mediate biological effects. Activation of RA-target genes upon all-*trans* RA or 9-*cis* RA binding to the RAR moiety is common, but there are also examples of RA-dependent repression (i.e. genes negatively regulated by RA). Moreover, the net effect of RA on the transcription of a given gene can be cell type dependent (see for instance references [7, 8]), probably reflecting differences in chromatin structure and in the collection of receptors and cofactors that are present and their functional state.

Besides forming RAR:RXR heterodimers, RXRs mediate 9-*cis* RA-induced gene expression by interacting as homodimers with a specific subset of RAREs found in the promoter of 9-*cis* RA target genes, and are promiscuous heterodimerization partners for other nuclear hormone receptors, such as the thyroid hormone receptors (TRs), the vitamin D receptors (VDRs) and the peroxisome proliferator activated receptors (PPARs, a lipid-activated family of the nuclear hormone receptor superfamily) (reviewed in [5, 6]). Some RXR-containing heterodimers (such as TR:RXR, VDR:RXR and RAR:RXR) are activated by the partner's ligand but not by an RXR ligand (9-*cis* RA) alone. Other RXR heterodimers, including the PPAR:RXR [9], can be activated by ligands of either partner and are synergistically activated in the presence of both ligands. In addition, 9-*cis* RA may in some instances favour RXR homodimer formation [10], which can lead to unavailability for heterodimer formation with other receptors. Therefore, RXR ligands have the potential to affect the signalling of other pathways.

Another mechanism by which retinoid receptors can affect gene expression is by interfering positively or negatively,

in a ligand-dependent manner, with the activity of other transcription factors, a mechanism known as 'transcriptional cross-talk'. A well-established example is the mutual transrepression between ligand-bound RARs and the transcription factor AP1 (the FOS-JUN heterodimer), which results in inhibition of the ability of both AP1 and the RAR to transactivate their corresponding target genes (reviewed in [11]). AP1 induces the expression of genes involved in cell proliferation in response to growth factors and inflammatory peptides, and transrepresion of AP1 may account, at least in part, for the antiproliferative effects of retinoids reported in many cancer and noncancer cell lines.

Retinoids can affect cellular functions through other mechanisms. For instance, activation of the phosphatidylinositol 3-kinase/Akt signalling pathway by all-*trans* RA, presumably through an extragenomic action of its liganded receptor, was recently found to be essential for neural differentiation of human neuroblastoma cells [12]. Direct effects of retinoids, nonmediated by retinoid receptors, can also be of importance. As an example, a direct interaction of all-*trans* RA with protein kinase C (PKC) resulting in the prevention of PKC activation was demonstrated in vitro [13], suggesting that RA may contribute to fine tuning of PKC activity in the cell. Phosphatidylinositol 3-kinase/Akt and PKC signalling pathways control many cell functions at various levels, including gene expression, and therefore modulation of these pathways by RA can result in specific changes in gene expression and cell metabolism.

Vitamin A and adipose tissues

Vertebrates possess two general types of adipose tissue, brown and white. White adipose tissue (WAT) stores energy in the form of triacylglycerols and releases energy in the form of free fatty acids according to the nutritional needs of the animal, whereas brown adipose tissue (BAT) can use its fat stores to produce heat in a regulated manner (see below). In addition, adipose tissues actively participate in systemic control of energy balance and other physiological functions through the secretion of key signalling molecules, among them various proteins (collectively named adipokines), such as leptin, resistin, adiponectin and tumour necrosis factor [14, 15].

Adipose tissues play an active role in retinoid homeostasis and metabolism (reviewed in [16]): they can take up circulating retinol, store it as esters with fatty acids (retinyl esters), convert it into RA, and mobilize their retinol stores to meet both local and total body demands (fig. 2). The liver is the main organ involved in retinoid storage and metabolism [17], but it has been estimated that in the rat, all adipose depots could account for 15–20% of the total body retinoid stores [18]. Retinol in adipose tissue can be derived from lipoprotein lipase hydrolysis of retinyl esters contained in postprandial chylomicrons [19] or can be taken up from circulating retinol: retinol-binding protein (RBP) complexes. RBP is a small protein expressed mainly, although not exclusively, in the liver that is critical for the mobilization of retinol from tissue storage pools [20]. Interestingly, both WAT and BAT

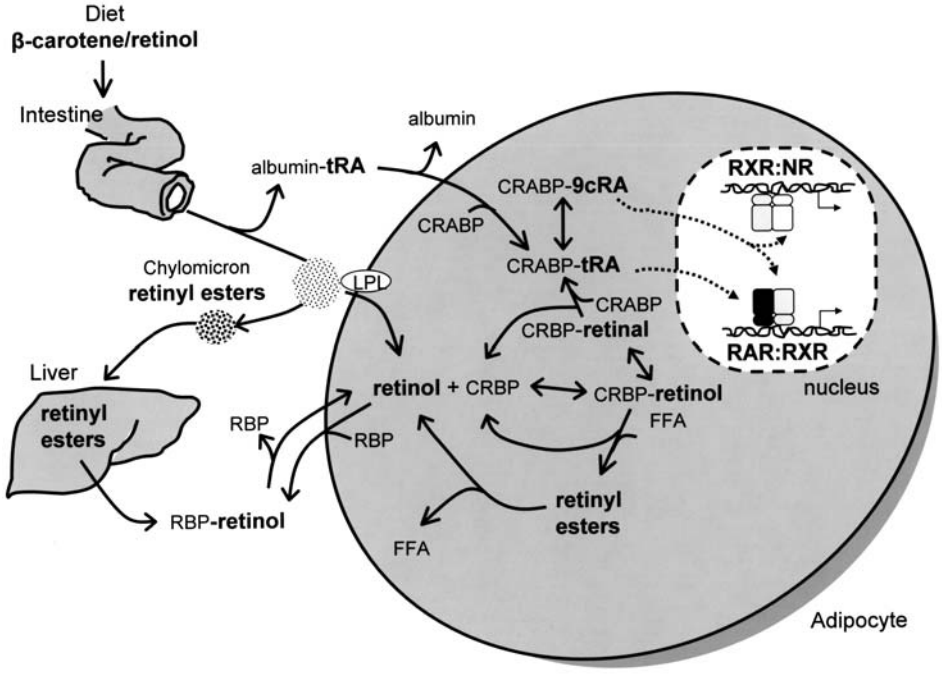

Figure 2. Overview of retinoid metabolism in adipocytes. See text for details. Abbreviations: 9cRA, 9-*cis* retinoid acid; tRA, all-*trans* retinoic acid; CRABP, cellular retinoic acid binding protein; CRBP, cellular retinol binding protein; LPL, lipoprotein lipase; NR, nuclear hormone receptor; RAR, retinoic acid receptor; RXR, rexinoid receptor; RBP, retinol binding protein.

are well equipped for retinol export, since the two tissues express RBP at high levels [18]; indeed, retinol efflux from adipocytes has been demonstrated [21]. Inside the cells, retinol complexes with cellular retinol binding protein (CRBP), which is crucial for solubilizing the retinol in the aqueous cytosol, for protecting the cell from the membranolytic effects of free retinol and for retinol metabolism, as the retinol-CRBP complex appears to be the optimal substrate for either retinol esterification or oxidation to RA [20]. Like RBP, CRBP is highly expressed in adipose tissues [18]. Adipose tissues contain RA at concentrations similar to that found in the liver [22]. This RA is mainly the result of local synthesis [22], presumably from retinol or certain carotenoids by mechanisms similar to those operating in other tissues (reviewed in [23, 24]), but some derives from systemic RA [22], which circulates in blood at low levels bound to albumin.

In addition of being involved in retinoid homeostasis, adipose tissues have long been recognized as potential sites of retinoid action. Retinoid receptors belonging to both the RAR and RXR subfamilies are expressed in WAT and BAT [16, 25–27]. The two adipose tissues show a differential pattern of expression of RAR and RXR subtypes: RARβ and RXRγ messenger RNAs (mRNAs), for instance, are expressed at low levels in WAT depots and differentiated 3T3-L1 white adipocytes [26] and are more abundant in BAT and brown adipocytes differentiated in culture [27, 28]. Interestingly, RAR and RXR mRNAs experience characteristic changes in their relative abundances throughout the process of acquisition of the mature adipocyte phenotype [28, 29]. Furthermore, RA automodulates the steady-state levels of its receptors (both RAR and RXR isoforms), and this seems to be an important component of the response of adipose tissues to RA (see below) [26, 28, 30, 31].

Vitamin A and the thermogenic system

Some energy is released as heat (thermogenesis) in all bioenergetic transformations; the term 'adaptive thermogenesis' refers to a variety of mechanisms that specifically function to produce heat in a physiologically regulated manner. The best known of these mechanisms is the one operating in BAT. Key to BAT thermogenesis is the activity of the uncoupling protein 1 (UCP1), an inner mitochondrial membrane protein uniquely expressed in differentiated brown adipocytes that is capable of short-circuiting the proton gradient generated by the respiratory chain during nutrient oxidation, thereby uncoupling fuel oxidation from ATP synthesis and generating heat (reviewed in [32]).

In small mammals, where the tissue is abundant, BAT thermogenesis plays a critical role in the adaptation to cold, and appears to play an important role in the control of energy efficiency and body weight as well [1, 32–34]. Considering that BAT is scarce in adult humans, the discovery in 1997 of novel, UCP1-like, putative uncoupling proteins that are expressed in non-BAT tissues, such as UCP2 and UCP3 (reviewed in [35]), led to a renewed interest on UCPs as potential, though not confirmed, players in the regulation of whole-body energy metabolism. Both in rodents and humans, UCP3 is selectively expressed in BAT and skeletal muscle, two tissues that are thought to be major contributors to overall metabolic rate, and UCP2 in a variety of tissues. However, the physiological role(s) of the novel UCPs is still a matter of debate, and it has been suggested that they may be involved as well in downregulation of mitochondrial reactive oxygen species production, downregulation of ATP synthesis and facilitation of the handling of lipids as fuel substrate [36–40].

Adaptive thermogenesis is, by definition, regulated. In the case of BAT thermogenesis, regulation exerted by the sympathetic nervous system (SNS), which densely innervates the tissue, is well established. The noradrenaline released from stimulated SNS terminals promotes brown adipocyte cell proliferation, mitochondriogenesis and UCP1 expression at the transcriptional level, and activates thermogenesis by stimulating lipolysis, rendering free fatty acids that are used as a fuel for thermogenesis and are activators of the UCP1 [41, 42]. Various hormones and nutrients contribute to the regulation of the expression/activity of the UCPs, and vitamin A, in the form of RA, appears to play an important role.

Vitamin A and UCP1 expression

RA is a transcriptional activator of UCP1 gene expression, as demonstrated both in brown adipocyte cell culture systems [43, 44] and whole animals [44–46]. β-carotene and other naturally occurring carotenoids also stimulate UCP1 expression in cultured brown adipocytes, an effect that could be due, at least in part, to their local conversion into RA [47].

In confluent brown adipocytes in primary culture, all-*trans* RA and 9-*cis* RA displayed a similar effectiveness as UCP1 inducers that was comparable to that of noradrenaline, dose-dependent and dependent on the stage of cell differentiation, the effect being restricted to differentiated cells [43, 44]. Corroborating the results obtained in cell systems, it was found that in vivo administration of all-*trans* RA or 9-*cis* RA to mice led to an increase of BAT-specific UCP1 protein and mRNA content that correlated with a significant loss of BAT lipids and BAT weight [44, 46]. An increased BAT UCP1 mRNA level was also reported in rats acutely treated with RA [45], and in rats and mice chronically fed vitamin A-supplemented diets with 40–50-fold the usual dose [48, 49]. Long-term vitamin A-deficient diet feeding, on the other hand, triggered in mice a reduction of BAT thermogenic capacity in terms

of UCP1 mRNA and protein expression levels that correlated with increased BAT weight and BAT adiposity [46]. Considered together, these results indicate that in rodents, thermogenic capacity of BAT is related to the animal's vitamin A status, thus supporting the importance of retinoids as physiological regulators of the BAT thermogenic system. The RA regulatory pathway may modulate changes in BAT thermogenesis associated with ontogeny and cell differentiation that are not attributable to adrenergic regulation.

The retinoid responsiveness of the UCP1 gene is quite well understood at the molecular level. Expression of the UCP1 gene is under the control of two regions upstream of the gene: a proximal regulatory region and a distal enhancer, which are required for hormonal, nutritional and differentiation-dependent regulation [50] (fig. 3). The action of RA on the UCP1 gene occurs through a complex retinoid-responsive region in the distal enhancer. There are at least two cis elements inside this region that may contribute to the RA effects: a noncanonical RARE [51, 52] that binds RAR:RXR heterodimers and a PPAR response element [53] that binds PPAR:RXR heterodimers. RAR:RXR heterodimers can transactivate transcription of sensitive genes upon binding of all-*trans* RA or 9-*cis* RA to the RAR moiety, while PPAR:RXR heterodimers require binding of 9-*cis* RA to the RXR moiety, in addition to a PPAR ligand (likely certain fatty acids or fatty acid derivatives), for maximal transactivating effect on transcription [9]. The involvement of both RAR- and RXR-dependent pathways is indicated by the ability of overexpressed receptors to enhance the responsiveness of the UCP1 gene to retinoids in transfection experiments [28, 43] and by the ability of selective receptor antagonists to suppress RA-induced UCP1 appearance in cell culture systems [31].

Vitamin A and UCP3 expression

Like the UCP1 gene promoter, the UCP3 gene promoter contains a RARE and a PPAR response element [54, 55], and there is compelling evidence that RA can modulate the expression of UCP3 in skeletal muscle. Induction of UCP3 gene transcription by RA was reported in differentiated myotubes in cell culture [55, 56]. In mice, both acute RA treatment and chronic dietary vitamin A supplementation resulted in increased skeletal muscle UCP3 mRNA and protein expression levels [49], while long-term vitamin A-deficient diet feeding leads to reduced levels [F. Felipe, J. Ribot, M. L. Bonet et al., unpublished results].

The upregulating effect of retinoids on UCP3 expression in vivo was found in muscle but not in BAT [46, 49]. Several hypotheses can be suggested to explain this muscle-specific effect of retinoids. First, RA-stimulated UCP3 gene expression could require the interaction of RAR : RXR heterodimers with a muscle-specific transcription factor. In fact, it has been shown in transfection experiments that the RAR : RXR-mediated stimulatory effect of RA on UCP3 gene expression is completely dependent on cotransfection of MyoD [55], a master regulator of muscle cell differentiation that is selectively expressed in cells of the myogenic lineage. Another possibility is that RAR and/or RXR isoforms differentially expressed in muscle and BAT mediate the effect of retinoids on the UCP3 gene. In this context, it is known that of the three known RXR subtypes, RXRγ displays a restricted expression in cells of the myogenic lineage [57].

Interestingly, RA induction of UCP3 gene expression in cultured myotubes is blocked by insulin [58]. The effect of insulin is likely to be mediated by the sterol regulatory element binding protein-1 c (SREBP-1 c), whose expression is induced by insulin ([58] and references therein), be-

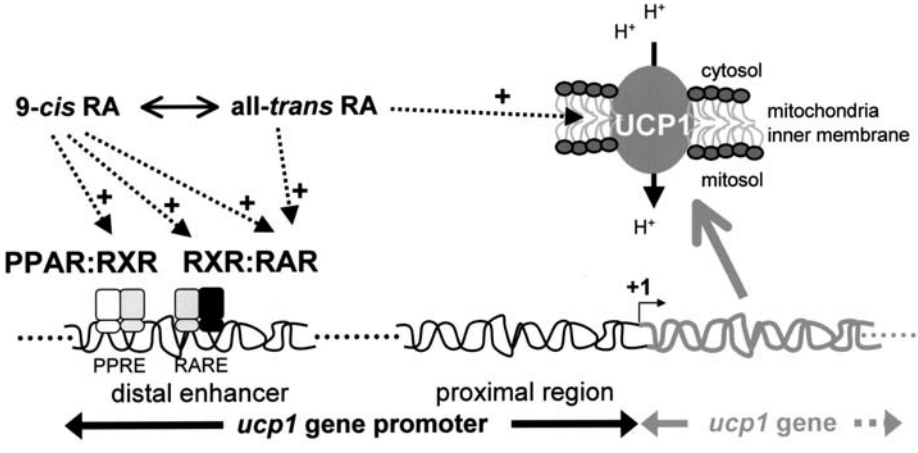

Figure 3. Retinoids and UCP1. Retinoids can stimulate UCP1 gene transcription through effects mediated by both PPAR:RXR and RAR:RXR heterodimers, for which response elements are found in the UCP1 gene promoter. A role for retinoic acid on UCP1 protein activation has also been proposed [62] and is diagrammed. Abbreviations: PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; RA, retinoic acid; RAR, RA receptor; RARE, RA response element; RXR, rexinoid receptor.

cause overexpression of a dominant positive form of SREBP-1c also antagonized the RA induction of UCP3 expression [58]. Negative regulation of muscle UCP3 by insulin is consistent with the idea that the role of UCP3 is linked to the switch from glucose to fat oxidation.

Vitamin A and UCP2 expression

No RARE or PPAR response elements have been described in the promoter of the UCP2 gene of humans or rodents. Moreover, evidence was provided that PPARγ does not bind directly to the mouse UCP2 gene promoter [59]. In spite of that, retinoids [46, 60, 61] and PPAR ligands ([59] and references therein) have been reported to induce UCP2 gene expression in different systems. Focusing on retinoids, 9-*cis* RA was shown to upregulate UCP2 mRNA expression in brown adipocytes in primary culture [60], and all-trans-RA was shown to do the same in L6 myotubes [61]. In keeping with these results in cultured cells, BAT UCP2 mRNA levels were found upregulated after RA treatment of mice and downregulated after chronic vitamin A-deficient diet feeding [46].

Retinoic acid as a modulator of the activity of the UCPs

The possibility exists (reflected in fig. 3) that RA triggers not only increased transcription of the UCP genes but also activation of preexisting UCP molecules, because RA was shown to increase proton transport activity by UCP1 in BAT mitochondria, and by UCP1 and UCP2 ectopically expressed in recombinant yeasts [62]. However, no changes in whole body oxygen consumption were detected in rats following acute RA administration, suggesting that RA may play a more important role in the recruitment of BAT thermogenesis than in the acute activation of thermogenesis [45].

Vitamin A and adipogenesis

RA was recognized as a potent inhibitor of adipocyte differentiation 20 years ago [63], when it was shown that high RA doses (0.1–1 μM), when added to differentiating 3T3-F442A preadipose cells at early stages of the differentiation process, inhibited lipid accumulation and the induction of molecular markers of adipocyte differentiation. RA was also shown to inhibit adipogenesis of brown preadipose cells in culture [44, 64], despite the fact that it induces UCP1 expression in already differentiated brown fat cells [43, 44]. Besides inhibiting adipose conversion, RA (10 μM) has been shown to promote apoptosis of rat preadipocytes (stromal-vascular cells isolated from inguinal adipose tissue) in primary culture [65] and of 3T3-L1 preadipocytes cultured in delipidated serum [29].

Adipogenesis is a complex process that has been extensively studied in committed clonal cell lines (such as 3T3-L1 or 3T3-F442A), where it depends on the cooperative and sequential action of several transcription factors, including various members of the CCAAT-enhancer binding protein (C/EBP) family of b-ZIP transcription factors and a particular subtype of PPARs, PPARγ (reviewed in [66]). Briefly, the regulatory cascade controlling adipogenesis is initiated by the transitory induction of C/EBPβ and C/EBPδ in response to adequate hormonal stimulation. This is followed by the induction of PPARγ, which in turn induces the expression of C/EBPα. PPARγ and C/EBPα activate de novo or enhanced expression of most or all of the adipocyte marker genes. PPARγ acts on transcription as heterodimer with RXR, and of note, expression of RXR isoforms is upregulated during adipogenesis of clonal cell lines [29].

Changes in gene transcription during adipogenesis are tightly coordinated with changes in cell cycle progression. Retinoblastoma protein (RB) plays a key role in this coordination, as first suggested in 1994 [67]. It is known that hypophosphorylation of RB stalls cell cycle progression in G_1 and that phosphorylation of RB signals entry in S phase. The levels of hypophosphorylated RB increase along the adipogenic process [67–69]: this prevents reentry of the cells into the cell cycle and may contribute to strengthen the C/EBPα transcriptional function, because a physical interaction between hypophosphorylated RB and C/EBPα resulting in the potentiation of the transcriptional activity of C/EBPα has been demonstrated in both white and brown adipocyte model systems [67, 69, 70]. Hypophosphorylation of RB requires the presence and activity of cyclin-dependent kinase inhibitors [71]. In 3T3-L1 cells, the shift from dividing preadipocytes to growth-arrested cells is associated with an increase in the expression of two of these inhibitors, p21 and p27 [72]. Interestingly, transcription of the p21 gene is induced by both PPARγ and C/EBPα, thus providing a molecular mechanism coupling growth arrest and adipocyte differentiation [72, 73].

The molecular bases of the RA-inhibitory effect on adipogenesis are multiple (fig. 4). First, RA interferes with the transcriptional activity of C/EBP proteins, so that it blocks the C/EBPβ-mediated induction of downstream genes [74], notably PPARγ. This effect of RA is mediated through activation of RAR, especially RARα [75], but does not depend on binding of liganded RAR to response elements on target genes [74]. Second, RA strongly upregulates RARγ expression in 3T3-L1 preadipocytes, while at the same time it downregulates RXRα expression [26, 30]: this may contribute to the inhibitory effect of RA on adipogenesis by favouring RAR:RXR heterodimer formation over PPARγ:RXR heterodimer formation. Third, addition of RA at the time of adipogenic stimulation lowers the levels of hypophosphorylated RB [76] and favours the maintenance of substantial amounts of hyperphosphorylated RB [68] and thus the retention of the prolifer-

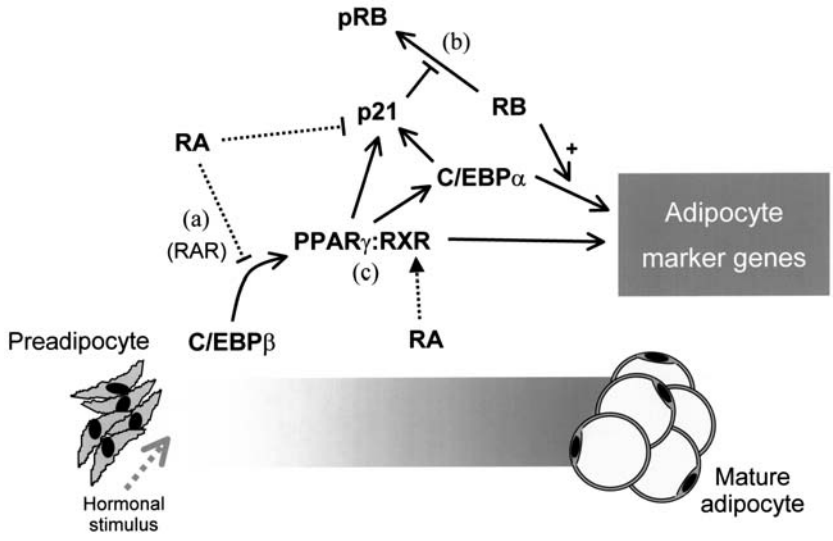

Figure 4. Retinoids in adipocyte differentiation and cell cycle**.** Adipogenesis in response to hormonal stimulus is initiated by the induction of C/EBPβ, which induces the expression of PPARγ, which in turn induces the expression of C/EBPα. PPARγ:RXR heterodimers and C/EBPα activate de novo or enhanced expression of the adipocyte marker genes, and of the cyclin-dependent protein kinase inhibitor p21, favouring RB hypohosphorylation and thus cell cycle exit. In addition, a functional interaction (+) between hypophosphorylated RB and C/EBPα may contribute to adipocyte differentiation. RA interferes with the activity of C/EBPβ in an effect that is mediated through RARs (a); favours hyperphosphorylation of the RB, through downregulation of p21 expression (b); and modulates PPAR:RXR heterodimer formation and activity (c). See text for references and further details. Abbreviations: C/EBP, CCAAT/enhancer binding protein; p21, cyclin-dependent protein kinase inhibitor p21; PPAR, peroxisome proliferator-activated receptor; RB, hypophosphorylated retinoblastoma protein; pRB, hyperphosphorylated retinoblastoma protein; RAR, RA receptor; RXR, rexinoid receptor.

ative capacity, which is incompatible with terminal differentiation. Promotion of RB hyperphosphorylation by RA may be related to the fact that RA elicited a dramatic reduction of the levels of the cyclin-dependent protein kinase inhibitor p21 in 3T3-F442 A cells, as measured by Western blot [76]. Reduction of p21 levels may be secondary to the RA-induced reduction of PPARγ and C/EBPα levels (as stated, these two transcription factors transactivate the p21 gene), but a direct negative effect of RA on p21 gene transcription is also possible, especially considering that the promoter of the p21 gene contains a functional RARE [7].

Contrary to high RA doses, low RA doses (1–10 nM) – close or below the K_d values of RARs – were shown to stimulate adipogenesis of preadipose cells in culture [77]. Moreover, commitment of embryonic stem cells into the adipocyte lineage was found to be dependent on an early, time-defined treatment with all-*trans* RA [78, 79]. The molecular mechanisms by which low concentrations of RA stimulate adipogenesis have not been determined. Low RA doses may be needed to provide enough 9-*cis* RA to ensure the activation of the RXR moiety of the PPARγ:RXR heterodimer (fig. 4). In fact, synthetic specific RXR ligands have been shown to promote adipogenesis, especially in conjunction with PPAR ligands, in keeping with the bifunctionality of the PPAR:RXR heterodimer [80]. It appears that the net effect of retinoids on adipogenesis is the result of a complex balance between RA metabolism and relative RAR and RXR availability in the preadipose cell [16], which in itself is greatly influenced by RA [26, 28, 30, 31].

The studies reviewed in this section suggest that RA influences adipocyte differentiation and survival in cell culture systems. Evidence that RA treatment and vitamin A status influence body adiposity in vivo is reviewed in the next section.

Vitamin A and body fat

Adipose depots of adult animals are made out basically of mature adipocytes but contain a discrete number of preadipocytes that can proliferate and differentiate under appropriate conditions. At any time, adipose tissue mass reflects the number and average volume of adipose cells. Adipocyte volume is determined by the balance between lipogenesis and lipolysis (and thermogenesis, in the case of brown adipocytes). Adipocyte number, on the other hand, is determined by the relative rates of cell acquisition, by preadipocyte replication/differentiation, and of cell loss by apoptosis. There is evidence that in vivo, PPARγ is critical both for adipogenesis and lipogenesis [66]: most PPARγ target genes in adipose tissue are directly implicated in lipogenic pathways.

In adult NMRI male mice, acute RA treatment (100 mg of all-*trans* RA/kg body weight, during the 4 days preceding death) triggered a 12% reduction of body weight that could not be completely accounted for by the observed

changes in energy intake [44, 46, 81], and a strong reduction of body fat content (the combined weight of interscapular BAT, epididymal WAT and inguinal WAT was reduced by 46% in the RA-treated animals, as compared with control animals) [81]. RA-induced reduction of adiposity correlated with downregulation of the expression of transcription factors controlling adipocyte differentiation and metabolism, notably PPARγ, in both WAT and BAT depots [81] and, as discussed in a previous section, with an upregulation of the expression of UCPs in BAT and muscle. Reduction of adiposity after in vivo RA treatment agrees with the inhibitory effect of high RA doses on adipogenesis, its proapoptotic effects on fat cells and its prothermogenic effects, all of which have been demonstrated in cell culture systems (see above). Thus, reduced adipogenesis/lipogenesis and enhanced lipolysis and apoptosis in fat depots, together with enhanced whole body thermogenesis, are all likely to contribute to the reduced adiposity of RA-treated animals.

The effects of long-term dietary vitamin A supplementation on body adiposity and energy balance have been addressed in a few studies. In F-344xBN rats, a 9% decrease of adiposity was reported after dietary vitamin A supplementation (during 8 weeks, with 50-fold the usual dose) that associated with a 31% increase of BAT UCP1 mRNA expression levels [48]. In obesity-prone C57BL/6J mice, chronic dietary vitamin A supplementation (during 18 weeks, with 40-fold the usual dose) was reported to have no impact on body weight or adiposity under a normal fat diet, even though it increased the expression of UCP1 mRNA levels in BAT and of UCP3 mRNA and protein levels in skeletal muscle [49]. Vitamin A supplementation had, nevertheless, some counterbalancing effect on the development of diet-induced obesity in C57BL/6J mice, with a trend to lower body weight gain in the group fed the vitamin A-supplemented high fat diet compared with the one fed the nonsupplemented high-fat diet [49]. This trend was not attributable to differences in energy intake, or to differences in thermogenic capacity (the positive effects of high fat diet and vitamin A supplementation on the expression of UCPs were found in this study to be nonadditive).

The effect of a relative dietary deficit in vitamin A on adipose tissue status has also been addressed. An enhanced expression of PPARγ was found in WAT depots of adult mice chronically fed (10 weeks) a vitamin A-deficient diet (with less than 7% the standard vitamin A content) [81], suggesting that the capability for adipogenesis/lipogenesis increases when the diet is poor in vitamin A. In fact, these vitamin A-deficient diet-fed mice had a markedly increased adiposity (the combined weight of interscapular BAT, epididymal WAT and inguinal WAT was increased by 63% in the deficient diet-fed animals, as compared with the standard diet-fed animals) and a slightly (3%) increased body weight, despite having an energy intake equal to that of standard diet-fed animals [81]. These results agree with the observation that diets poor in dietary fat-soluble vitamins, especially vitamin A and carotenoids, favour adipose tissue formation in sirloin (the so-called bovine marbling) [30]. Of note, there are studies linking a low dietary intake of vitamin A with high incidence of obesity in certain human populations, such as Navajo women [82] and the Havasupai [83]. The hypertrophy of WAT in vitamin A-deficient diet-fed animals may be related to the proadipogenic effect of low RA doses reported in cultured preadipose cells [77].

Interestingly, RA has been shown to inhibit leptin production and secretion by adipose tissues in organ culture [84] and after in vivo administration [45, 46]. Less leptin usually means more hunger and less energy expenditure; considering the lipolytic effects of acute RA doses, the inhibitory effect of RA on leptin production and secretion may be part of a regulatory feedback loop to avoid fat depletion. Dietary vitamin A supplementation also inhibited adipose tissue leptin production and secretion in rodents, to an extent that largely exceeded the small (if any) reduction of fat stores it elicited [48, 49]. The latter result indicates that the inhibitory effect of retinoids on leptin expression is not merely secondary to a reduction of fat content and could be a direct effect.

Concluding remarks

In rodents, vitamin A status plays a role in modulating BAT and WAT function and development, with potential impact on body adiposity and body weight. Acute treatment with RA causes a reduction of adiposity that correlates with a depressed adipogenic/lipogenic potential of adipose tissues (with depressed PPARγ levels) and an increased thermogenic potential in BAT (with increased expression of UCP1 and UCP2) and muscle (with increased expression of UCP3). Chronic dietary vitamin A supplementation also increases thermogenic potential in BAT and muscle, and appears to confer some resistance to the development of obesity under high fat diet. A poor vitamin A status, on the other hand, favours an increment of adiposity that correlates with an increased WAT adipogenic potential and a depressed thermogenic potential. Knowledge of nutrients with both thermogenic and antiadipogenic properties could be useful in designing diets to help control body fat content and body weight.

Acknowledgements. This work was supported by the Spanish Government (Dirección General de Investigación, BFI2000-0988-C06-01, and Ministerio de Sanidad y Consumo, FIS 01/1379) and the European Union (COST Action 918, and QLRT-2001–00183 2000/C324). Francisco Felipe is the recipient of a doctoral fellowship from the Spanish Government (Ministerio de Educación, Cultura y Deportes).

1 Palou A., Serra F., Bonet M. L. and Pico C. (2000) Obesity: molecular bases of a multifactorial problem. Eur. J. Nutr. **39:** 127–144
2 Gudas L. J., Sporn M. B. and Roberts A. B. (1994) Cellular biology and biochemistry of the retinoids. In: The Retinoids. Biology, Chemistry and Medicine, 2nd edn, pp. 443–520, Sporn M. B., Roberts A. B.and Goodman D. S. (eds), Raven Press, New York
3 Kojima R., Fujimori T., Kiyota N., Toriya Y., Fukuda T., Ohashi T. et al. (1994) In vivo isomerization of retinoic acids. Rapid isomer exchange and gene expression. J. Biol. Chem. **269:** 32700–32707
4 Mangelsdorf D. J., Umesono K. and Evans R. M. (1994) The retinoid receptors. In: The retinoids. Biology, Chemistry and medicine, 2nd edn, pp. 319–349, Sporn M. B., Roberts A. B. and Goodman D. S. (eds), Raven Press, New York
5 Chambon P. (1996) A decade of molecular biology of retinoic acid receptors. FASEB J. **10:** 940–954
6 Aranda A. and Pascual A. (2001) Nuclear hormone receptors and gene expression. Physiol. Rev. **81:** 1269–1304
7 Liu M., Iavarone A. and Freedman L. P. (1996) Transcriptional activation of the human p21(WAF1/CIP1) gene by retinoic acid receptor. Correlation with retinoid induction of U937 cell differentiation. J. Biol. Chem. **271:** 31723–31728
8 Nabeyrat E., Corroyer S., Epaud R., Besnard V., Cazals V. and Clement A. (2000) Retinoic acid-induced proliferation of lung alveolar epithelial cells is linked to p21(CIP1) downregulation. Am. J. Physiol. Lung. Cell. Mol. Physiol. **278:** L42–50
9 Mukherjee R., Davies P. J., Crombie D. L., Bischoff E. D., Cesario R. M., Jow L. et al. (1997) Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. Nature **386:** 407–410
10 Zhang X. K., Lehmann J., Hoffmann B., Dawson M. I., Cameron J., Graupner G. et al. (1992) Homodimer formation of retinoid X receptor induced by 9-cis retinoic acid. Nature **358:** 587–591
11 Altucci L. and Gronemeyer H. (2001) The promise of retinoids to fight against cancer. Nat. Rev. Cancer **1:** 181–193
12 Lopez-Carballo G., Moreno L., Masia S., Perez P. and Barettino D. (2002) Activation of the phosphatidylinositol 3-kinase/Akt signaling pathway by retinoic acid is required for neural differentiation of SH-SY5Y human neuroblastoma cells. J. Biol. Chem. **277:** 25297–25304
13 Radominska-Pandya A., Chen G., Czernik P. J., Little J. M., Samokyszyn V. M., Carter C. A. et al. (2000) Direct interaction of all-trans-retinoic acid with protein kinase C (PKC). Implications for PKC signaling and cancer therapy. J. Biol. Chem. **275:** 22324–22330
14 Trayhurn P. and Beattie J. H. (2001) Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. Proc. Nutr. Soc. **60:** 329–339
15 Ailhaud G. (2000) Adipose tissue as an endocrine organ. Int. J. Obes. Relat. Metab. Disord. **24 Suppl 2:** S1–3
16 Villarroya F., Giralt M. and Iglesias R. (1999) Retinoids and adipose tissue: Metabolism, cell differentiation and gene expression. Int. J. Obes. Relat. Metab. Disord. **23:** 1–6
17 Blomhoff R., Helgerud P., Rasmussen M., Berg T. and Norum K. R. (1982) In vivo uptake of chylomicron [^{3}H]retinyl ester by rat liver: evidence for retinol transfer from parenchymal to nonparenchymal cells. Proc. Natl. Acad. Sci. USA **79:** 7326–7330
18 Tsutsumi C., Okuno M., Tannous L., Piantedosi R., Allan M., Goodman D. S. et al. (1992) Retinoids and retinoid-binding protein expression in rat adipocytes. J. Biol. Chem. **267:** 1805–1810
19 Blaner W. S., Obunike J. C., Kurlandsky S. B., al-Haideri M., Piantedosi R., Deckelbaum R. J. et al. (1994) Lipoprotein lipase hydrolysis of retinyl ester. Possible implications for retinoid uptake by cells. J. Biol. Chem. **269:** 16559–16565
20 Noy N. (2000) Retinoid-binding proteins: mediators of retinoid action. Biochem. J. **348:** 481–495
21 Wei S., Lai K., Patel S., Piantedosi R., Shen H., Colantuoni V. et al. (1997) Retinyl ester hydrolysis and retinol efflux from BFC-1 beta adipocytes. J. Biol. Chem. **272:** 14159–14165
22 Kurlandsky S. B., Gamble M. V., Ramakrishnan R. and Blaner W. S. (1995) Plasma delivery of retinoic acid to tissues in the rat. J. Biol. Chem. **270:** 17850–17857
23 Blaner W. S. and Olson J. A. (1994) Retinol and retinoic metabolism. In: The Retinoids. Biology, Chemistry and Medicine, 2nd edn, pp. 229–255, Sporn M. B., Roberts A. B. and Goodman D. S. (eds), Raven Press, New York
24 Napoli J. L. (1996) Retinoic acid biosynthesis and metabolism. FASEB J. **10:** 993–1001
25 Haq R. and Chytil F. (1991) Expression of nuclear retinoic acid receptors in rat adipose tissue. Biochem. Biophys. Res. Commun. **176:** 1539–1544
26 Kamei Y., Kawada T., Kazuki R. and Sugimoto E. (1993) Retinoic acid receptor gamma 2 gene expression is up-regulated by retinoic acid in 3T3-L1 preadipocytes. Biochem. J. **293:** 807–812
27 Villarroya F. (1998) Differential effects of retinoic acid on white and brown adipose tissue: An unexpected role for vitamin A derivates on energy balance. Ann. N. Y. Acad. Sci. **839:** 190–195
28 Alvarez R., Checa M. L., Brun S., Viñas O., Mampel T., Iglesias R. et al. (2000) Both retinoic-acid-receptor and retinoid-X-receptor-dependent signaling pathways mediate the induction of the brown-adipose-tissue-uncoupling-protein-1 gene by retinoids. Biochem. J. **345:** 91–97
29 Chawla A. and Lazar M. A. (1994) Peroxisome proliferator and retinoid signaling pathways co-regulate preadipocyte phenotype and survival. Proc. Natl. Acad. Sci. USA **91:** 1786–1790
30 Kawada T., Kamei Y. and Sugimoto E. (1996) The possibility of active form of vitamin A and D as supressors on adipocyte development via ligand-dependent transcriptional regulators. Int. J. Obes. Relat. Metab. Disord. **20:** S52-S57
31 Bonet M. L., Puigserver P., Serra F., Ribot J., Vazquez F., Pico C. et al. (1997) Retinoic acid modulates retinoid X receptor alpha and retinoic acid receptor alpha levels of cultured brown adipocytes. FEBS Lett. **406:** 196–200.
32 Palou A., Picó C., Bonet M. L. and Oliver P. (1998) The uncoupling protein, thermogenin. Int. J. Biochem. Cell. Biol. **30:** 7–11
33 Lowell B. B. and Spiegelman B. M. (2000) Towards a molecular understanding of adaptive thermogenesis. Nature **404:** 652–660
34 Spiegelman B. M. and Flier J. S. (2001) Obesity and the regulation of energy balance. Cell **104:** 531–543
35 Ricquier D. and Bouillaud F. (2000) The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem. J. **345 Pt 2:** 161–179
36 Arsenijevic D., Onuma H., Pecqueur C., Raimbault S., Manning B. S., Miroux B. et al. (2000) Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nat. Genet. **26:** 435–439
37 Boss O., Hagen T. and Lowell B. B. (2000) Uncoupling proteins 2 and 3: potential regulators of mitochondrial energy metabolism. Diabetes **49:** 143–156
38 Vidal-Puig A. J., Grujic D., Zhang C. Y., Hagen T., Boss O., Ido Y. et al. (2000) Energy metabolism in uncoupling protein 3 gene knockout mice. J. Biol. Chem. **275:** 16258–16266
39 Nedergaard J., Golozoubova V., Matthias A., Asadi A., Jacobsson A. and Cannon B. (2001) UCP1: the only protein able to mediate adaptative non-shivering thermogenesis and metabolic inefficiency. Biochim. Biophys. Acta. **1504:** 82–106
40 Garcia-Martinez C., Sibille B., Solanes G., Darimont C., Mace K., Villarroya F. et al. (2001) Overexpression of UCP3 in cultured human muscle lowers mitochondrial membrane potential, raises ATP/ADP ratio and favors fatty acid vs. glucose oxidation. FASEB J. **15:** 2033–2035

41 Himms-Hagen J. (1991) Neural control of brown adipose tissue thermogenesis, hypertrophy and atrophy. Front. Neuroendocrinol. **12:** 38–93

42 Cannon B., Jacobsson A., Rehnmark S. and Nedergaard J. (1996) Signal transduction in brown adipose tissue recruitment: noradrenaline and beyond. Int. J. Obes. Relat. Metab. Disord. **20 Suppl. 3:** S36–42

43 Alvarez R., De Andrés J., Yubero P., Viñas O., Mampel T., Iglesias R. et al. (1995) A novel regulatory pathway of brown fat thermogenesis. Retinoic acid is a transcriptional activator of mitochondrial uncoupling protein gene. J. Biol. Chem. **270:** 5666–5673

44 Puigserver P., Vázquez F., Bonet M. L., Picó C. and Palou A. (1996) In vitro and in vivo induction of brown adipocyte uncoupling protein (thermogenin) by retinoic acid. Biochem. J. **317:** 827–833

45 Kumar M. V. and Scarpace P. J. (1998) Differential effects of retinoic acid on uncoupling protein-1 and leptin gene expression. J. Endocrinol. **157:** 237–243

46 Bonet M. L., Oliver J., Picó C., Felipe F., Ribot J., Cinti S. et al. (2000) Opposite effects of vitamin A deficient diet-feeding and retinoic acid treatment on brown adipose tissue UCP1, UCP2 and leptin expression. J. Endocrinol. **166:** 511–517

47 Serra F., Bonet M. L., Puigserver P., Oliver J. and Palou A. (1999) Stimulation of uncoupling protein 1 expression in brown adipocytes by naturally occurring carotenoids. Int. J. Obes. Relat. Metab. Disord. **23:** 650–655

48 Kumar M. V., Sunvold G. D. and Scarpace P. J. (1999) Dietary vitamin A supplementation in rats: suppression of leptin and induction of UCP1 mRNA. J. Lipid. Res. **40:** 824–829

49 Felipe F., Bonet M. L., Ribot J. and Palou A. (2003) Up-regulation of muscle uncoupling protein 3 gene expression in mice following high fat diet, dietary vitamin A supplementation and acute retinoic acid-treatment. Int. J. Obes. Relat. Metab. Disord. **27:** 60–69

50 Cassard-Doulcier A. M., Larose M., Matamala J. C., Champigny O., Bouillaud F. and Ricquier D. (1994) In vitro interaction between nuclear protein and uncoupling protein gene promoter reveal several putative transactivating factors including Ets1, retinoid X receptor, thyroid hormone receptor and a CCAAT box-binding protein. J. Biol. Chem. **269:** 24335–24342

51 Larose M., Cassard-Doulcier A. M., Fleury C., Serra F., Champigny O., Bouillaud F. et al. (1996) Essential cis-acting elements in rat uncoupling protein gene are in an enhancer containing a complex retinoic acid response domain. J. Biol. Chem. **271:** 31533–31542

52 Rabelo R., Reyes C., Schifman A. and Silva E. (1996) A complex retinoic acid response element in the uncoupling protein gene defines a novel role for retinoids in thermogenesis. Endocrinology **137:** 3488–3496

53 Sears I. B., MacGinnitie M. A., Kovacs L. G. and Graves R. A. (1996) Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor gamma. Mol. Cell. Biol. **16:** 3410–3419

54 Acin A., Rodriguez M., Rique H., Canet E., Boutin J. A. and Galizzi J. P. (1999) Cloning and characterization of the 5′ flanking region of the human uncoupling protein 3 (UCP3) gene. Biochem. Biophys. Res. Commun. **258:** 278–283

55 Solanes G., Pedraza N., Iglesias R., Giralt M. and Villarroya F. (2000) The human uncoupling protein-3 gene promoter requires MyoD and is induced by retinoic acid in muscle cells. FASEB J. **14:** 2141–2143

56 Nagase I., Yoshida S., Canas X., Irie Y., Kimura K., Yoshida T. et al. (1999) Upregulation of uncoupling protein 3 by thyroid hormone, peroxisome proliferator-activated receptor ligands and 9-cis retinoic acid in L6 myotubes. FEBS Lett. **461:** 319–322

57 Dolle P., Fraulob V., Kastner P. and Chambon P. (1994) Developmental expression of murine retinoid X receptor (RXR) genes. Mech. Dev. **45:** 91–104

58 Guillet-Deniau I., Mieulet V., Le Lay S., Achouri Y., Carre D., Girard J. et al. (2002) Sterol regulatory element binding protein-1 c expression and action in rat muscles: insulin-like effects on the control of glycolytic and lipogenic enzymes and UCP3 gene expression. Diabetes **51:** 1722–1728

59 Medvedev A. V., Snedden S. K., Raimbault S., Ricquier D. and Collins S. (2001) Transcriptional regulation of the mouse uncoupling protein-2 gene. Double E-box motif is required for peroxisome proliferator-activated receptor-gamma-dependent activation. J. Biol. Chem. **276:** 10817–10823

60 Carmona M. C., Valmaseda A., Iglesias R., Mampel T., Vinas O., Giralt M. et al. (1998) 9-cis retinoic acid induces the expression of the uncoupling protein-2 gene in brown adipocytes. FEBS Lett. **441:** 447–450

61 Hatakeyama Y. and Scarpace P. J. (2001) Transcriptional regulation of uncoupling protein-2 gene expression in L6 myotubes. Int. J. Obes. Relat. Metab. Disord. **25:** 1619–1624

62 Rial E., González-Barroso M., Fleury C., Iturrizaga S., Sanchis D., Jiménez-Jimenéz J. et al. (1999) Retinoids activate proton transport by the uncoupling proteins UCP-1 and UCP-2. EMBO J. **18:** 5827–5833

63 Kuri-Harcuch W. (1982) Differentiation of 3T3 F442 A cells into adipocytes is inhibited by retinoic acid. Differentiation **23:** 164–169

64 Hernández A., García-Jiménez C., Santisteban P. and Obregon M. J. (1993) Regulation of malic-enzyme-gene expression by cAMP and retinoic acid in differentiating brown adipocytes. Eur. J. Biochem. **215:** 285–290

65 Kim H. S., Hausman D. B., Compton M. M., Dean R. G., Martin R. J., Hausman G. J. et al. (2000) Induction of apoptosis by all-trans-retinoic acid and C2-ceramide treatment in rat stromal-vascular cultures. Biochem. Biophys. Res. Commun. **270:** 76–80

66 Rosen E. D., Walkey C. J., Puigserver P. and Spiegelman B. M. (2000) Transcriptional regulation of adipogenesis. Genes Dev. **14:** 1293–1307

67 Puigserver P., Nadal-Ginard B. and Palou A. (1994) Expression and interaction of C/EBPalpha adipogenic transcription factor and retinoblastoma protein in adipocytes during differentiation. Int. J. Obes. Relat. hetab. Disord. **18 Suppl. 2:** 113

68 Shao D. and Lazar M. A. (1997) Peroxisome proliferator activated receptor γ, CCAAT/enhancer-binding protein α, and cell cycle status regulate the commitment to adipocyte differentiation. J. Biol. Chem. **272:** 21473–21478

69 Puigserver P., Ribot J., Serra F., Gianotti M., Bonet M. L., Nadal-Ginard B. et al. (1998) Involvement of the retinoblastoma protein in brown and white adipocyte cell differentiation: functional and physical association with the adipogenic transcription factor C/EBPα. Eur. J. Cell. Biol. **77:** 117–123

70 Chen P., Riley D. J., Chen Y. and Lee W. (1996) Retinoblastoma protein positively regulates terminal adipocyte differentiation through direct interaction with C/EBPs. Genes Dev. **10:** 2794–2804

71 Mittnacht S. (1998) Control of pRB phosphorylation. Curr. Opin. Genet. Dev. **8:** 21–27

72 Morrison R. F. and Farmer S. R. (1999) Role of PPARγ in regulating a cascade expression of cyclin-dependent kinase inhibitors, p18(INK4 c) and p21 (Waf1/Cip1), during adipogenesis. J. Biol. Chem. **274:** 17088–17097

73 Timchenko N. A., Wilde M., Nakanishi M., Smith J. R. and Darlington G. J. (1996) CCAAT/enhancer-binding protein α (C/EBPα) inhibits cell proliferation through the p21 (WAF-1/CIP-1/SDI-1) protein. Genes Dev. **10:** 804–815

74 Schwarz E. J., Reginato M. J., Shao D., Krakow S. L. and Lazar M. A. (1997) Retinoic acid blocks adipogenesis by inhibiting C/EBPβ-mediated transcription. Mol. Cell. Biol. **17:** 1552–1561

75 Kamei Y., Kawada T., Mizukami J. and Sugimoto E. (1994) The prevention of adipose differentiation of 3T3-L1 cells caused by retinoic acid is elicited through retinoic acid receptor alpha. Life Sci. **55:** PL307–312
76 Ribot J., Serra F. and Palou A. (2002) Retinoic acid decreases the expression and phosphorylation state of the retinoblastoma protein in adipocyte terminal differentiation. Int. J. Obes. Relat. Metab. Disord. **26 Suppl. 1:** S65
77 Safonova I., Darimont C., Armi E. Z., Grimaldi P., Reichert U., Schroot B. et al. (1994) Retinoids are positive effectors of adipose cell differentiation. Mol. Cell. Endocrinol. **104:** 201–211
78 Bost F., Caron L., Marchetti I., Dani C., Le Marchand-Brustel Y. and Binetruy B. (2002) Retinoic acid activation of the ERK pathway is required for embryonic stem cell commitment into the adipocyte lineage. Biochem. J. **361:** 621–627
79 Dani C., Smith A. G., Dessolin S., Leroy P., Staccini L., Villageois P. et al. (1997) Differentiation of embryonic stem cells into adipocytes in vitro. J. Cell. Sci. **110:** 1279–1285
80 Tontonoz P., Singer S., Forman B. M., Sarraf P., Fletcher J. A., Fletcher C. D. et al. (1997) Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor gamma and the retinoid X receptor. Proc. Natl. Acad. Sci. USA **94:** 237–241
81 Ribot J., Felipe F., Bonet M. L. and Palou A. (2001) Changes of adiposity in response to vitamin A status correlate with changes of PPAR gamma 2 expression. Obes. Res. **9:** 500–509
82 Wolfe W. S. and Sanjur D. (1988) Contemporary diet and body weight of Navajo women receiving food assistance: an ethnographic and nutritional investigation. J. Am. Diet. Assoc. **88:** 822–827
83 Vaughan L. A., Benyshek D. C. and Martin J. F. (1997) Food acquisition habits, nutrient intakes, and anthropometric data of Havasupai adults. J. Am. Diet. Assoc. **97:** 1275–1282
84 Menendez C., Lage M., Peino R., Baldelli R., Concheiro P., Dieguez C. et al. (2001) Retinoic acid and vitamin D(3) powerfully inhibit in vitro leptin secretion by human adipose tissue. J. Endocrinol. **170:** 425–431