Retinoic acid and vitamin D₃ powerfully inhibit *in vitro* leptin secretion by human adipose tissue

C Menendez, M Lage, R Peino, R Baldelli, P Concheiro²,
C Diéguez¹ and F F Casanueva

Department of Medicine, Santiago de Compostela University, Spain

¹Molecular Endocrinology Section and Department of Physiology, Santiago de Compostela University, Spain

²Division of General Surgery, Hospital de Conxo, Complejo Hospitalario Universitario de Santiago, Spain

(Requests for offprints should be addressed to F F Casanueva, PO Box 563, Santiago de Compostela E-15780, Spain; Email: endocrine@usc.es)

Abstract

Leptin, the product of the *ob* gene, is secreted into the circulation by white adipose tissue; its major role being to participate in the regulation of energy homeostasis. Plasma leptin levels are mainly determined by the relative adiposity of the subject; however, the great dispersion of values for any given body mass index and the noteworthy gender-based differences indicate that other factors are operating.

Steroid hormones actively participate in the regulation of leptin secretion; however, non-steroid nuclear hormones have either not been studied or have provided contradictory results. In order to understand the role of hormones of the non-steroid superfamily such as 3,5,3'-tri-iodothyronine (T₃), vitamin D₃ and retinoic acid (RA) in the control of leptin secretion, in the present work doses

of 10⁻⁹, 10⁻⁸ and 10⁻⁷ M of these compounds have been studied on *in vitro* leptin secretion. The organ culture was performed with omental adipose tissue samples from healthy donors (*n*=28).

T₃ was devoid of effect at any dose studied, while an inhibition of leptin secretion was observed with 9-*cis*-RA (slight) and all-*trans*-RA (potent). Interestingly, vitamin D₃ exerted a powerfully inhibitory role at the doses studied, and its action was synergistic with all-*trans*-RA.

In conclusion, *in vitro* leptin secretion by human adipose tissue is negatively controlled by either RA or vitamin D₃. The clinical significance of leptin regulation by this superfamily of nuclear receptors remains to be ascertained.

Journal of Endocrinology (2001) **170**, 425–431

Introduction

Leptin, the product of the *ob* gene expressed mainly by white adipose tissue cells, appears to act as a hormonal signal informing the hypothalamus as to the amount of fat reserves (Casanueva & Diéguez 1999). Furthermore, clear gender-based differences in circulating leptin levels have been shown, these being higher in women than in men with similar amounts of body fat (Casabiell *et al.* 1998). This condition has already been described at birth, when no differences in the adipose tissue reserves are expected (Matsuda *et al.* 1997, Tome *et al.* 1997). Since it has been demonstrated that leptin secretion is influenced by different physiological conditions, such as gestation and gonadal function, the main regulatory mechanisms at adipocyte level should be better clarified. Furthermore, sex steroid hormones have been considered as potential modulators of leptin release. Estradiol acts on human adipose tissue *in vitro* as a releaser of leptin with a potency similar to that of glucocorticoids, and this may well be the background for the gender-based differences in serum leptin concen-

trations (Casabiell *et al.* 1998). Moreover, other sex steroid hormones may contribute to leptin regulation, such as different androgenic steroids, which show a potent inhibitory effect on leptin secretion from adipose tissue of female donors, whereas no effect was observed using testosterone (Piñeiro *et al.* 1999).

All of the previously studied hormones acting on *in vitro* leptin release are steroid hormones that bind to characteristic nuclear receptors (steroid hormone receptors) activating classical transcription factors. However, the participation of another class of nuclear receptors (non-steroid hormone receptors), believed to be bound to the DNA response elements in the absence of ligand, such as retinoic acid (RA), vitamin D₃ and tri-iodothyronine (T₃), in the control of the *ob* gene expression and, consequently, of leptin secretion have not been previously assessed.

Cellular responsiveness to RA is conferred through two structurally and pharmacologically distinct families of receptors: the retinoic acid receptors (RAR) and the retinoic X receptors (RXR); the transcriptional activity of RAR and RXR can be reciprocally modulated by direct

interactions between the two proteins. These two receptors have a higher degree of cooperativity in binding to the target DNA. RXR also interacts directly with and enhances the binding of nuclear receptors conferring responsiveness to vitamin D₃ and thyroid hormone T₃. At physiological concentrations, vitamin D₃, thyroid and retinoid receptors appear to require RXR as their exclusive partner for high affinity binding to their response elements (Yu *et al.* 1991, Kliewer *et al.* 1992, Villarroya *et al.* 1999). Adipose tissue have long been recognized as a potential site for the action of RAs, and the two retinoid receptors, RAR and RXR, are both expressed in adipose tissue (Villarroya *et al.* 1999). All-*trans*-RA has been recently described as new regulator of *UCP1* gene and *ob* gene in rat perirenal white adipose tissue (Kumar & Scarpace 1998), but there are presently no data available regarding its action on the *in vitro* leptin secretion by human adipose tissue.

The aim of this study was to assess the effect of non-steroid receptor activation on leptin secretion using an *in vitro* system of human omental adipose tissue culture.

Materials and Methods

Omental adipose tissue was obtained from 28 patients during elective abdominal surgery. The tissue donor group comprised 15 women, aged 61.3 ± 4.1 years, body mass index (BMI) 29.0 ± 2.3 , and 13 men, aged 52.5 ± 3.6 years and BMI 27.4 ± 0.9 . Patients were taking no drugs or antibiotics at the time of abdominal surgery and the presence of malignancy was an exclusion criterion. The study was approved by the Hospital Ethical Committee and each participating subject provided informed consent.

Samples were processed for tissue culture as previously described (Casabiell *et al.* 1998). Briefly, excised adipose tissue was immediately transported to the laboratory in ice cold Krebs–Ringer HEPES (KRH) buffer (NaCl 125 mM, KCl 5 mM, MgSO₄ 1.2 mM, CaCl₂ 2 mM, KH₂PO₄ 1.2 mM, glucose 6 mM, HEPES 25 mM, pH 7.4). After removing vessels and conjunctive tissue, adipose tissue was washed with KRH and cut into small pieces with sharp scissors. Tissue fragments were placed in six-well dishes (300–400 mg adipose tissue/well) containing 2.5 ml DMEM plus 0.5% FCS, supplemented with penicillin (100 U/ml) and streptomycin sulfate (100 µg/ml). After a pre-incubation period of 1 h at 37 °C under a humidified atmosphere of 95% air–5% CO₂, the media were aspirated, and 2.5 ml of fresh medium (with or without stimuli) were dispensed into each well. Culture media were then collected every 24 h and replaced with fresh medium, again with or without stimuli.

In order to evaluate the possible effect of the non-steroidal nuclear receptor superfamily activation on leptin secretion, 3,5,3'-tri-iodothyronine (T₃), 9-*cis*-retinoic acid (9-*cis*-RA), all-*trans*-retinoic acid (all-*trans*-RA) and

1 α ,25-dihydroxycholecalciferol (vitamin D₃) at concentrations of 10^{−9}, 10^{−8} and 10^{−7} M, were tested respect to control samples with the appropriate vehicle. Moreover, since it was shown that the activities of RXR are suppressed when complexed with VDR (vitamin D₃ receptor) and RAR, it was decided to administer the combination of all-*trans*-RA and vitamin D₃ at the same concentration of 10^{−7} M.

For each tested variable (either untreated or treated sample) the leptin value from a given subject sample was the mean of three independently incubated samples. The medium was collected and analyzed every 24 h to obtain the 24 h secretion and the cumulative secretion until 96 h. Samples were stored at −20 °C until leptin assay. Leptin levels were measured by RIA using commercial kits (Human leptin RIA, Linco Research Inc., St Charles, MO, USA). The limit of sensitivity was 0.5 ng/ml, the intraassay coefficient of variation was 8.3%, and the interassay coefficient of variation was 6.2%.

All drugs and reagents were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Statistical analysis

Data are presented as the means $\pm$ s.e. of the increment of absolute value. Leptin secretion is expressed as the total of leptin secreted into the well by a given sample (in ng/ml) with respect to total volume and divided by the amount of fat tissue in grams, i.e. ng leptin/g tissue. A *t*-test for paired data was used to evaluate leptin secretion over control samples. The ANOVA test for repeated measures was used to evaluate leptin secretion during all 96 h with respect to control samples. *P* < 0.05 was considered significant.

Results

Spontaneous (non-stimulated) leptin secretion by omental adipose tissue into the incubation medium was well maintained in samples obtained from both men and women (*n* = 28) from 0 to 96 h. As there was a progressive decrease in the spontaneous secretion after 96 h, no longer time periods were studied. No signs of tissue damage were observed throughout the incubation period (data not shown). As no gender-based differences were observed in any test, values were pooled.

The addition of T₃, (10^{−9}, 10^{−8}, 10^{−7} M) to the incubation medium (*n* = 8) during the entire period of incubation did not significantly change the leptin secretion rate in adipose tissue during any period studied (Fig. 1).

When the adipose tissue fragments from eight donors were incubated in the presence of 9-*cis*-RA (10^{−9}, 10^{−8}, 10^{−7} M) no significant changes were observed in the secretion of leptin compared with controls, although a small significant inhibition was observed at 48 h by the 10^{−7} M dose (Fig. 2).

Figure 1 Mean $\pm$ S.E. leptin secretion into the incubation medium by human adipose tissue from eight donors after treatment with 10^{-9} , 10^{-8} and 10^{-7} M of T₃ (3,5,3'-tri-iodothyronine). Values are expressed as the increment over control values for cumulative secretion (lines) or for each 24-h incubation period (bars).

The incubation throughout the 96-h period of adipose tissue fragments from nine donors in the presence of all-*trans*-RA showed a clear-cut inhibition in leptin secretion at 48 h, 72 h and 96 h period ($P < 0.05$) at the 10^{-7} M concentration (Fig. 3). Moreover, when data were analyzed by ANOVA test of repeated measures, the dose 10^{-7} M clearly inhibited the leptin secretion over time ($P < 0.005$) (Fig. 3).

A clear-cut inhibition was observed when the adipose tissue fragments from eight donors were incubated for 96 h in the presence of vitamin D₃ at different concentrations (10^{-9} , 10^{-8} , 10^{-7} M); in particular, the spontaneous leptin secretion rate was inhibited at the 72-h and 96-h periods ($P < 0.05$) at the concentration of 10^{-7} M and at the 96-h period ($P < 0.05$) at the concentration of 10^{-8} M (Fig. 4). When data were analyzed by ANOVA test of repeated measures, the dose of 10^{-7} M clearly inhibited the leptin secretion continuously ($P < 0.05$) (Fig. 4).

In order to ascertain the effect of different interactions among the hormones used, the result of the association between all-*trans*-RA and vitamin D₃, both at 10^{-7} M, was analyzed. A significant inhibitory effect on leptin secretion was observed only at 72 h. In particular, analyzing data as increment over control, all-*trans*-RA+vitamin D₃ induced a clear-cut inhibition of leptin secretion with respect to the single administration of both compounds ($P < 0.05$, both), the action being synergistic and not merely additive (Fig. 5).

Discussion

Differential control of gene modulation has become a central point in modern molecular biology. Because of

Figure 2 Mean $\pm$ S.E. leptin secretion into the incubation medium by human adipose tissue from eight donors after treatment with 10^{-9} , 10^{-8} and 10^{-7} M of 9-*cis*-RA. Values are expressed as the increment over control values for cumulative secretion (lines) or for each 24-h incubation period (bars). * $P < 0.05$ vs control secretion.

their lipophilic characteristics, different hormones can cross the lipid bilayer of the cell membrane and interact at the nuclear level. In particular, two different classes of nuclear receptors are present: steroidal- and non-steroidal hormone receptors. The second class binds non-steroid hormones such as vitamin D₃, 9-*cis*-RA, all-*trans*-RA and T₃, among others. The steroid hormone receptors in their unliganded state, are associated with proteins (heat shock proteins, HSP) that prevent their direct interaction with DNA. Ligand binding induces a conformational change in the receptor that releases HSP and enables the receptor to bind its cognate response element and interact with different coactivator proteins. In contrast, the main non-steroid receptors (vitamin D₃, thyroid hormone and retinoids) are not associated with proteins, so they bind directly to their DNA response elements (Mangelsdorf *et al.* 1990, 1995, Kliewer *et al.* 1999); moreover, these receptors can be associated with cofactors regulating the transcription of target genes (Chen & Evans 1995, Hörlein *et al.* 1995, Hortwitz *et al.* 1996). It has been shown that leptin secretion can be modulated by activation of steroid nuclear receptors (Casabiell *et al.* 1998, Piñeiro *et al.* 1999), but no

Figure 3 Mean $\pm$ S.E. leptin secretion into the incubation medium by human adipose tissue from nine donors after treatment with 10^{-9} , 10^{-8} and 10^{-7} M of all-*trans*-RA. Values are expressed as the increment over control values for cumulative secretion (lines) or for each 24-h incubation period (bars). * $P < 0.05$ vs control secretion. The inhibition induced by 10^{-7} M was significant across time (ANOVA repeated measures) ($P < 0.005$).

in vitro data is available, and there are controversial *in vivo* data regarding the action and modulation of non-steroidal receptors. Since thyroid hormones and leptin are both involved in the regulation of energy metabolism, the real relationship between them is not completely understood. In particular, clinical studies on the effect of thyroid status on leptin secretion in humans showed different results (Bornstein *et al.* 1997, Corbetta *et al.* 1997, Sreenan *et al.* 1997, Valcavi *et al.* 1997, Pinkney *et al.* 1998, Sesmilo *et al.* 1998). Assessment of leptin levels in rats with a wide range of thyroid status, ranging from overt hypothyroidism to severe hyperthyroidism, indicates that thyroid hormones may exert an inhibitory influence on leptin secretion (Escobar Morreale *et al.* 1997, Masaki *et al.* 1997, Fain *et al.* 1997, Fain & Bahouth 1998). *In vitro* studies on 3T₃-L₁ preadipocytes have shown a clear stimulation induced by T₃ on leptin secretion (Yoshida *et al.* 1997).

As *in vivo* studies do not allow differentiation between spontaneous adipocyte secretion and the stimulatory action of blood-borne and/or exogenous substances, in this work a systematic analysis was undertaken using an *in vitro* human adipose tissue organ culture system. Using this model, the addition of T₃ at the different doses to the tissue culture did not induce modification in leptin secretion rate, throughout the entire 96 h. These results are in line with those reported in the literature where no effects of thyroid hormones were observed in patients with thyroid dysfunction (Sreenan *et al.* 1997).

The mechanisms of retinoic action are unknown; however, they may be broadly similar to those of steroid

Figure 4 Mean $\pm$ S.E. leptin secretion into the incubation medium by human adipose tissue from eight donors after treatment with 10^{-9} , 10^{-8} and 10^{-7} M of vitamin D₃. Values are expressed as the increment over control values for cumulative secretion (lines) or for each 24-h incubation period (bars). * $P < 0.05$ vs control secretion in the same period. The inhibition induced by 10^{-7} M was significant at each 24-h incubation period (ANOVA) ($P < 0.05$).

hormones (Heyman *et al.* 1992, Perlmann & Jansson 1995, Willy *et al.* 1995). Moreover, since leptin synthesis and secretion have been shown to be affected by compounds activating protein kinase C (PKC) and cAMP (Piñeiro *et al.* 1998), the effects of 9-*cis*-RA and all-*trans*-RA may also be related to the direct stimulatory activity on cAMP and PKC activity (Makowske *et al.* 1988). It has been shown that oral administration of all-*trans*-RA to male rats was able to induce a decrease in mRNA leptin levels in *in vivo* perirenal white adipose tissue (Kumar & Scarpace 1998).

The effect of 9-*cis*-RA on leptin secretion rate by adipose tissue culture was characterized by a slight inhibition observed during the 48-h period. Since it was shown that peroxisome proliferator-activated receptor- γ (PPAR γ) activation is followed by an inhibition of leptin secretion and that RXR is a partner of PPAR γ , we can speculate that the slight but significant inhibition of leptin secretion observed after 9-*cis*-RA treatment could be due to an indirect mechanism of PPAR γ /RXR activation. Moreover, the administration of all-*trans*-RA to the adipose tissue culture induced a clear and prolonged inhibition in leptin secretion rate observed over time.

It has recently been reported that, during the differentiation of cytotrophoblasts into syncytiotrophoblast, leptin

Figure 5 Mean $\pm$ S.E. leptin secretion into the incubation medium by human adipose tissue from five donors after treatment with 10^{-7} M of all-*trans*-RA, vitamin D₃ and the combination of them. Data are expressed as the increment over control values for each 24-h incubation period; * $P < 0.05$.

secretion rate is stimulated by retinoids, in particular by 9-*cis*-RA (He *et al.* 1995, de la Brousse *et al.* 1996, Jacquemin *et al.* 1996, Guibourdenche *et al.* 2000). This apparent contradiction with our results could be explained by an upregulation of leptin gene expression during the differentiation period of placental cells. In any case, leptin secretion by placenta appears to be controlled in a manner that is different from that found for adipose tissue (García *et al.* 2000).

When vitamin D₃ was administered to adipose tissue culture, a potent inhibition of leptin secretion rate was observed at 96 h for the dose 10^{-8} M ($P < 0.05$) and throughout the whole experiment for the dose of 10^{-7} M. There are no reports available on the action of vitamin D₃ over leptin, either *in vivo* or *in vitro*.

Due to the possible interaction between retinoid and vitamin D₃ receptors, it was decided to analyze the association between all-*trans*-RA+vitamin D₃ at the dose of 10^{-7} , which seems to be the most effective in the inhibition of leptin secretion (Kojima *et al.* 1994, Mangelsdorf & Evans 1995). In particular, analyzing data as absolute increment, when all-*trans*-RA+vitamin D₃ were administered to the adipose tissue culture, a synergistic inhibition of leptin secretion was observed. The capacity of the vitamin D₃ to demonstrate a potent inhibitory effect when administered together with all-*trans*-RA, the effect being synergetic and not merely additive, provide a new possible control mechanism of

leptin secretion as has previously been shown in other cell types for growth hormone (GH) (Bedo *et al.* 1989, García-Villalba *et al.* 1996, Lenoir *et al.* 1996).

Retinoids play a key role in mammalian development and cell differentiation. The effect on leptin secretion herein observed can lead us to speculate as to the possible role over leptin secretion and action during the processes of embryonic development and differentiation since a typical angiogenic activity of leptin was shown.

In conclusion, the present study shows that RA and vitamin D₃ present a direct inhibitory effect on leptin secretion from human adipose tissue culture. The exact mechanisms by which these non-steroidal agents suppress leptin secretion, as well as their role in the *in vivo* regulation of plasma leptin levels, remain to be ascertained.

Acknowledgements

The work was supported by grants from the Fondo de Investigación Sanitaria, the Spanish Ministry of Health, the Xunta de Galicia and by grant 009906153187 from MURST (Rome, Italy) and CISD (Rome, Italy). R.B. is a recipient of a Dottorato di Ricerca course of the University of Rome 'La Sapienza', Faculty of Medicine, granted by the European Commission (EU) Structural Funds.

References

- Bedo G, Santisteban P & Aranda A 1989 Retinoic acid regulates growth hormone gene expression. *Nature* **339** 231–234.
- Bornstein SR, Torpy DJ, Chrousos GP, Licinio J & Engelmann L 1997 Leptin levels are elevated despite low thyroid hormone levels in the 'euthyroid sick' syndrome [letter]. *Journal of Clinical Endocrinology and Metabolism* **82** 4278–4279.
- Casabiell X, Pineiro V, Peino R, Lage M, Camina J, Gallego R, Vallejo LG, Dieguez C & Casanueva FF 1998 Gender differences in both spontaneous and stimulated leptin secretion by human omental adipose tissue *in vitro*: dexamethasone and estradiol stimulate leptin release in women, but not in men. *Journal of Clinical Endocrinology and Metabolism* **83** 2149–2155.
- Casanueva FF & Diéguez C 1999 Neuroendocrine regulation and actions of leptin. *Frontiers in Neuroendocrinology* **20** 317–363.
- Chen JD & Evans RM 1995 A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* **377** 454–457.
- Corbetta S, Englaro P, Giambona S, Persani L, Blum WF & Beck Peccoz P 1997 Lack of effects of circulating thyroid hormone levels on serum leptin concentrations. *European Journal of Endocrinology* **137** 659–663.
- de la Brousse F, Shan B & Chen J 1996 Identification of the promoter of the mouse obese gene. *PNAS* **93** 4096–4101.
- Escobar Morreale HF, Escobar del Rey F & Morreale de Escobar G 1997 Thyroid hormones influence serum leptin concentrations in the rat. *Endocrinology* **138** 4485–4488.
- Fain JN & Bahouth SW 1998 Effect of tri-iodothyronine on leptin release and leptin mRNA accumulation in rat adipose tissue. *Biochemical Journal* **332** 361–366.
- Fain JN, Coronel EC, Beauchamp MJ & Bahouth SW 1997 Expression of leptin and beta 3-adrenergic receptors in rat adipose tissue in altered thyroid states. *Biochemical Journal* **322** 145–150.
- García MC, Casanueva FF, Diéguez C & Señarís RM 2000 Gestational profile of leptin messenger ribonucleic acid (mRNA) content in the placenta and adipose tissue in the rat, and regulation of the mRNA levels of the leptin receptor subtypes in the hypothalamus during pregnancy and lactation. *Biology of Reproduction* **62** 698–703.
- García-Villalba P, Jimenez-Lara AM & Aranda A 1996 Vitamin D interferes with transactivation of the growth hormone gene by thyroid hormone and retinoic acid. *Molecular and Cellular Biology* **16** 318–327.
- Guibourdenche J, Tarrade A, Laurendeau I, Rochette-Egly C, Chambon P, Vidaud M & Evain-Brion D 2000 Retinoids stimulate leptin synthesis and secretion in human syncytiotrophoblast. *Journal of Clinical Endocrinology and Metabolism* **85** 2550–2555.
- He Y, Chen H, Quon M & Reitman M 1995 The mouse obese gene. Genomic organization, promoter activity, and activation by CCAA/enhancer-binding protein a. *Journal of Biological Chemistry* **270** 28887–28891.
- Heyman RA, Mangelsdorf DJ, Dyck JA, Stein RB, Eichele G, Evans RM & Thaller C 1992 9-*cis* retinoic acid is a high affinity ligand for the retinoid X receptor. *Cell* **68** 397–406.
- Hörlein AJ, Näär AM, Heinzl T, Torchia J, Gloss B, Kurakawa R, Ryan A, Kamei Y, Söderström M, Gass CK & Rosenfeld MG 1995 Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377** 397–404.
- Horvitz KB, Jackson TA, Bain DL, Richer JK, Takimoto GS & Tung L 1996 Nuclear receptor coactivators and corepressors. *Molecular Endocrinology* **10** 1167–1177.
- Jacquemin P, Alsat E & Oury C 1996 The enhancers of the human placental lactogen B, A and L genes: progressive activation during *in vitro* trophoblast differentiation and importance of the DF-3 element in determining their respective activities. *DNA Cell Biology* **15** 845–854.
- Kliwer SA, Umesono K, Mangelsdorf DJ & Evans RM 1992 Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D₃ signalling. *Nature* **355** 446–449.
- Kliwer SA, Lehmann JM & Willson TM 1999 Orphan nuclear receptors: shifting endocrinology into reverse. *Science* **284** 757–760.
- Kojima R, Fujimori T, Kiyota N, Toriya Y, Fukuda T, Ohashi T, Sato T, Yoshizawa Y, Takeyama K, Mano H, Masushige S & Kato S 1994 *In vivo* isomerization of retinoic acids. *Journal of Biological Chemistry* **269** 32 700–32 707.
- Kumar MV & Scarpace PJ 1998 Differential effects of the retinoic acid on uncoupling protein-1 and leptin gene expression. *Journal of Endocrinology* **157** 237–243.
- Lenoir C, Dace A, Martin C, Bonne J, Teboul M, Planells R & Torresani J 1996 Calcitriol down-modulates the 3,5,3'-triiodothyronine (T₃) receptors and affects, in a biphasic manner, the T₃-dependent adipose differentiation of Ob 17 preadipocytes. *Endocrinology* **137** 4268–4276.
- Makowske M, Ballester R, Cayre Y & Rosen OM 1988 Immunochemical evidence that three protein kinase C isozymes increase in abundance during HL-60 differentiation induced by dimethyl sulfoxide and retinoic acid. *Journal of Biological Chemistry* **263** 3402–3410.
- Mangelsdorf DJ & Evans RM 1995 The RXR heterodimers and orphan receptors. *Cell* **83** 841–850.
- Mangelsdorf DJ, Ong ES, Dyck JA & Evans RM 1990 Nuclear receptor that identifies a novel retinoic acid response pathway. *Nature* **345** 224–229.
- Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Manuel M, Chambon P & Evans RM 1995 The nuclear receptor superfamily: the second decade. *Cell* **83** 835–839.
- Masaki T, Yoshimatsu H, Kakuma T, Hidaka S, Kurokawa M & Sakata T 1997 Enhanced expression of uncoupling protein 2 gene in rat white adipose tissue and skeletal muscle following chronic treatment with thyroid hormone. *FEBS Letters* **418** 323–326.
- Matsuda J, Yokota I, Iida M, Murakami T, Naito E, Ito M, Shima K & Kuroda Y 1997 Serum leptin concentration in cord blood: relationship to birth weight and gender. *Journal of Clinical Endocrinology and Metabolism* **82** 1642–1644.
- Perlmann T & Jansson L 1995 A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and NURR1. *Genes & Development* **9** 769–782.
- Pinkney JH, Goodrick SJ, Katz J, Johnson AB, Lightman SL, Coppack SW & Mohamed-Ali V 1998 Leptin and the pituitary–thyroid axis: a comparative study in lean, obese, hypothyroid and hyperthyroid subjects. *Clinical Endocrinology* **49** 583–588.
- Piñero V, Casabiell X, Peinó R, García-Vallejo L, Dieguez C & Casanueva FF 1998 PMA inhibits both spontaneous and glucocorticoid-mediated leptin secretion by human omental adipose tissue explants *in vitro*. *Biochemical and Biophysical Research Communications* **252** 345–347.
- Piñero V, Casabiell X, Peinó R, Lage M, Camiña JP, Menéndez C, Baltar J, Dieguez C & Casanueva FF 1999 Dihydrotestosterone, stanozolol, androstenedione and dehydroepiandrosterone sulphate inhibit leptin secretion in female but not in male samples of omental adipose tissue *in vitro*: lack of effect of testosterone. *Journal of Endocrinology* **160** 425–432.
- Sesnilo G, Casamitjana R, Halperin I, Gomis R & Vilardell E 1998 Role of thyroid hormones on serum leptin levels. *European Journal of Endocrinology* **139** 428–430.
- Sreenan S, Caro JF & Refetoff S 1997 Thyroid dysfunction is not associated with alterations in serum leptin levels. *Thyroid* **7** 407–409.
- Tome MA, Lage M, Camina JP, García Mayor RV, Dieguez C & Casanueva FF 1997 Sex-based differences in serum leptin concentrations from umbilical cord blood at delivery. *European Journal of Endocrinology* **137** 655–658.

- Valcavi R, Zini M, Peino R, Casanueva FF & Dieguez C 1997 Influence of thyroid status on serum immunoreactive leptin levels. *Journal of Clinical Endocrinology and Metabolism* **82** 1632–1634.
- Villarroya F, Giral M & Iglesias R 1999 Retinoids and adipose tissue: metabolism, cell differentiation and gene expression. *International Journal of Obesity* **23** 1–6.
- Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA & Mangelsdorf DJ 1995 LXR, a nuclear receptor that defines a distinct retinoid response pathway. *Gene Development* **9** 1033–1045.
- Yoshida T, Monkawa T, Hayashi M & Saruta T 1997 Regulation of expression of leptin mRNA and secretion of leptin by thyroid hormone in 3T₃-L1 adipocytes. *Biochemical and Biophysical Research Communications* **232** 822–826.
- Yu VC, Delsert C, Andersen B, Holloway JM, Devary OV, Näär AM, Kim SY, Boutin J-M, Glass CK & Rosenfeld MG 1991 RXRb: a coregulator that enhances binding of retinoic acid, thyroid hormone and vitamin D receptors to their cognate response elements. *Cell* **67** 1251–1266.

Received 15 January 2001

Accepted 12 April 2001