# The Effects of Rexinoids and Rosiglitazone on Body Weight and Uncoupling Protein Isoform Expression in the Zucker *fa/fa* Rat

Valur Emilsson, Jacqueline O'Dowd, Steven Wang, Yong-Ling Liu, Matthew Sennitt, Richard Heyman, and Michael A. Cawthorne

**Agonists for the retinoid X receptor (RXR), the rexinoids, and the peroxisome proliferator-activated receptor gamma (PPARγ), the thiazolidinediones, are effective in the treatment of insulin resistance in rodent models by enhancing insulin action and improving glycemic control. In the present study, we compared the effects of rexinoids and a thiazolidinedione on body weight and mitochondrial uncoupling protein (UCP) isoform mRNA expression in the obese Zucker *fa/fa* rat. Long-term (2 weeks) oral treatment with the rexinoids LG100268 and LG100324 reduced food intake and body weight gain, whereas rosiglitazone (BRL49653) tended to increase both food intake and weight gain. LG100268 and LG100324 increased brown adipose tissue (BAT) UCP-1 mRNA content by 2.7-fold ($P < .002$) and 3.1-fold ($P < .001$), respectively, while BRL49653 had no effect on BAT UCP-1 mRNA content. Neither the rexinoids nor the thiazolidinedione had any effect on the level of mRNA encoding UCP-2 and the recently described PPARγ coactivator-1 (PGC-1). LG100324 increased UCP-3 mRNA content by 3.6-fold ($P < .0005$) in muscle and 4.3-fold ($P < .0002$) in white adipose tissue (WAT). LG100268 increased UCP-3 mRNA content in WAT by 2-fold ($P < .005$) but was without any effect on muscle UCP-3. BRL49653 increased UCP-3 mRNA content by 2.1-fold ($P < .005$) in muscle and 2.7-fold ($P < .003$) in WAT. Thus, the rexinoids, but not the thiazolidinedione, have an antiobesity action by reducing food intake, and the increase in UCP-1 mRNA content in BAT may reflect a stimulation of BAT UCP-1 activity.**


INSULIN RESISTANCE and pancreatic β-cell dysfunction are characteristic metabolic abnormalities of non–insulin-dependent diabetes mellitus (NIDDM),[1] a condition that is strongly associated with obesity.[2] A new class of antidiabetic compounds that enhance insulin sensitivity have been described that act via the nuclear receptor subfamilies, the peroxisome proliferator-activated receptor gamma (PPARγ) and the retinoid X receptor (RXR). The activated receptors bind DNA as heterodimer partners and regulate genes that are involved in lipid and carbohydrate metabolism.[3,4] PPARγ is the functional receptor for the thiazolidinedione rosiglitazone (BRL49653),[5,6] which has been shown to enhance insulin action in animal models of NIDDM[7-9] and in patients with NIDDM.[10] Agonists for RXR are also found to be effective in treating animal models of insulin resistance and NIDDM.[11,12] In vitro studies have demonstrated that BRL49653 induces adipocyte conversion,[13] and thiazolidinediones have been associated with some weight gain in both animal models[14] and clinical studies.[15]

Nonshivering thermogenesis is a critical component of overall energy balance. Brown adipose tissue (BAT) is the major site of nonshivering thermogenesis in small mammals. The thermogenic capacity of rodents is associated with an increase in mitochondrial content and an increase in the activity of the BAT-specific uncoupling protein-1 (UCP-1), which is a proton leak allowing the uncoupling of oxidative phosphorylation from electron transport.[16] Recently, two homologs of UCP-1, now named UCP-2 and UCP-3, have been identified.[17,18] The UCP-2 and UCP-3 genes have significant homology to the UCP-1 gene and are expressed in BAT, as well as other tissues, including skeletal muscle.[19-21] Furthermore, when transfected into yeast, both UCP-2 and UCP-3 are able to uncouple oxidative phosphorylation from adenosine triphosphate synthesis.[17,20] Although uncoupling activity has not been demonstrated in animal tissues, nevertheless, by analogy to UCP-1 in BAT of rodents, the UCP homologs could play a role in skeletal muscle thermogenesis in humans. Support for this suggestion has come from several studies. Thus, the expression of all UCP mRNA isoforms in BAT is increased by cold exposure.[22] Furthermore, thyroid hormones increase BAT UCP-1 mRNA and muscle UCP-3 mRNA content,[20,23] and $\beta_3$-adrenoceptor agonists increase the transcription of UCP-1 in BAT and UCP homologs in white adipose tissue (WAT) and skeletal muscle.[21] Moreover, it has been demonstrated that in mice strains that either resist obesity (A/J) or develop obesity (C57Bl/6J) on a high-fat diet, the expression of UCP-2 mRNA is increased in WAT of A/J mice but not in C57Bl/6J mice. However, there was no difference in the expression level in BAT or skeletal muscle and no change in UCP-3 mRNA expression.[24] UCP-3 mRNA expression in skeletal muscle is decreased in *fa/fa* rats relative to lean rats,[21] and it is also decreased in patients with NIDDM.[25] In addition, in Pima Indians, the body mass index negatively correlates with UCP-3 mRNA expression in skeletal muscle and there is a positive correlation between UCP-3 mRNA expression and the resting metabolic rate.[26] These findings suggest that under some circumstances both UCP-2 in WAT and UCP-3 in skeletal muscle might be associated with energy metabolism.

In the present study, we compared the effects of long-term (2 weeks) treatment with the rexinoids LG100268 and LG100324 versus the thiazolidinedione BRL49653 on body weight and UCP isoform mRNA expression in the obese Zucker *fa/fa* rat, an animal model of insulin resistance and morbid obesity. Both rexinoids reduced body weight gain, while treatment with BRL49653 increased body weight. Furthermore, the rexinoids increased BAT UCP-1 mRNA (relative to α-tubulin) while

*From the Clore Laboratory, Buckingham University, Buckingham, UK; and Ligand Pharmaceuticals, San Diego, CA.*

*Submitted January 24, 2000; accepted May 29, 2000.*

*Present address: V.E., Decode Genetics, Reykjavik, Iceland; Y.-L.L., Department of Physiology, St George's Hospital Medical School, London, UK.*

*Address reprint requests to Michael A. Cawthorne, PhD, Clore Laboratory, University of Buckingham, Hunter Street, Buckingham, MK18 1EG, UK.*



*0026-0495/00/4912-0016$10.00/0*

*doi:10.1053/meta.2000.18692*

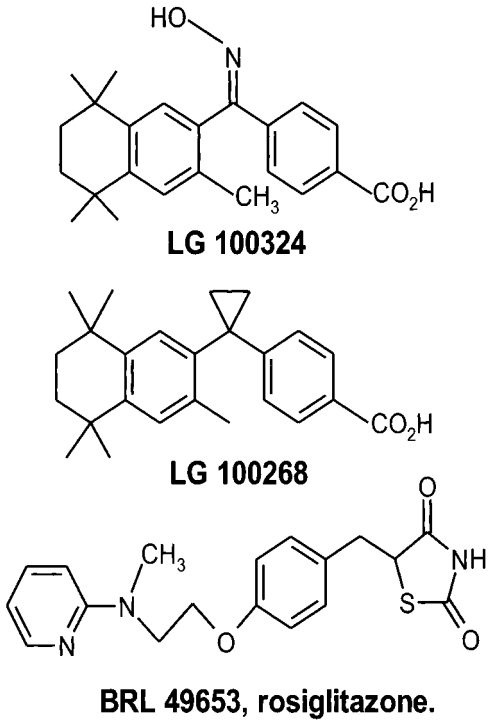


**Fig 1. Structure of the rexinoids LG100268 and LG100324 and the thiazolidinedione BRL49653.**

BRL49653 had no effect on the mRNA levels of BAT UCP-1. Both rexinoids and the thiazolidinedione increased the expression of UCP-3 mRNA (relative to α-tubulin) in skeletal muscle, BAT, and WAT, while these compounds had no effect on mRNA levels of UCP-2 or the recently described PPARγ coactivator-1 (PGC-1).[27] Our results suggest that the rexinoids are more potent than the thiazolidinedione in increasing the tissue mRNA content of UCP isoforms in BAT, and therefore, the antiobesity action of rexinoids might involve the stimulation of BAT thermogenesis and a reduction of food intake.

## MATERIALS AND METHODS

### *Animals and Treatment*

Male obese (*fa/fa*) Zucker rats (St George's Hospital Medical School, London, UK) aged 10 to 11 weeks were fed on a rat and mouse toxicology diet (Bantin and Kingman, Hull, UK) with free access to water. They were maintained at a temperature of 21° ± 1°C on a 12-hour light-dark cycle. The rats were housed singly and dosed orally (single daily dose) with the rexinoids LG100268 (20 mg · kg$^{-1}$) or LG100324 (20 mg · kg$^{-1}$) or the thiazolidinedione BRL49653 (3 mg · kg$^{-1}$) or vehicle for 2 weeks. The structures of these compounds are shown in Fig 1. LG100268, LG100324, and BRL49653 were prepared at Ligand Pharmaceuticals (San Diego, CA). The body weight change and daily food intake were measured during the 2-week treatment period. The animals were then killed 12 hours after the last dose and the tissues (interscapular BAT, epididymal WAT, back muscle, and brain) were dissected, snap-frozen, and stored at −80°C prior to RNA analysis. Changes in food intake and body weight are expressed as the mean ± SEM, and statistical significance between groups was determined using ANOVA followed by Dunnett's test.

### *RNA Analysis*

Total RNA was isolated using TriReagent and isopropanol precipitation (Amersham Life Sciences, Amersham, UK). The integrity and loading of the RNA was studied by detection scanning of ribosomal RNA bands (28S and 18S) in agarose gels before and after treatment with RNase-free DNase I (GIBCO-BRL, Paisley, UK). Single-stranded cDNA synthesis was performed from approximately 2 μg total RNA using oligo(dT)15-18 (Invitrogen, Carlsbad, CA) in a first-strand synthesis kit (Amersham Pharmacia Biotech, Little Chalfont, Bucks, UK). mRNA expression was determined by polymerase chain reaction (PCR) using the following PCR primer sequences: 5′-CTCACCTTTGAGCTCCTC-3′ and 5′-CTGATTTGCCTCTGGATG-3′ to amplify a 399-bp fragment of UCP-1 (GenBank M11814), 5′-CGAAGCCTACAAGACCATTG-3′ and 5′-AGCATGGTCAGGGCACAGTG-3′ to amplify a 302-bp fragment of UCP-2 (GenBank AB006613), and 5′-GAGAACCCAGGAGTGCAGAG-3′ and 5′-CTGGCGATGGTTCTGTAGGC-3′ to amplify a 346-bp fragment of UCP-3 (GenBank AF035943). A 310-bp fragment of the rat PGC-1 cDNA from BAT was amplified using the mouse primer DNA sequences 5′-AGGATCAGAACAAACCCTGC-3′ and 5′-TGTTCTCAAATGGGGAACCC-3′ (antisense) corresponding to the nucleotides between 611 and 920 in the known mouse PGC-1 cDNA (GenBank AF049330). The 310-bp fragment was cloned into a pCR-TRAP cloning system (GeneHunter, Nashville, TN) and then identified by sequencing using a ThermoSequenase terminator cycle sequencing kit (Amersham Life Sciences, Amersham, UK). Comparison to the mouse counterpart showed 100% homology between the PGC-1 cDNA fragment (corresponding to the mouse 611 to 920 cDNA; GenBank AF049330). Commercially available primers were used to amplify 540 bp of the housekeeping gene α-tubulin (Clontech UK, Basingstoke, Hants, UK). All PCR fragments amplified were sequenced for identification. Bands were quantified using the AlphaImager 1200 system (Flowgen, Ashby-de-la-Zouch, Leicestershire, UK). Quantitation of mRNA expression was performed by quantitative RT-PCR and ethidium bromide staining as described previously,[28] and the levels of UCP isoforms and PGC-1 were normalized to α-tubulin to provide information on the relative changes of tissue gene expression. Results are expressed as the mean ± SEM, and statistical significance between groups was determined by Student's unpaired *t* test (n = 4).

## RESULTS AND DISCUSSION

Oral administration of 20 mg · kg$^{-1}$ · d$^{-1}$ (single daily dose) of the rexinoids for 2 weeks reduced food intake in Zucker *fa/fa* rats relative to controls, whereas the thiazolidinedione BRL49653 (3 mg · kg$^{-1}$ · d$^{-1}$) increased food intake (Table 1). Consequently, whereas the rexinoids decreased body weight gain significantly ($P < .01$) relative to the controls, BRL49653 showed a trend to increase weight gain. Previous studies have demonstrated that thiazolidinediones such as BRL49653 re-

**Table 1. Effects of the Rexinoids and Thiazolidinedione on Food Intake and Body Weight**

| Treatment | Food Consumption (g/rat/d) | | Body Weight Change (g/rat) | |
|---|---|---|---|---|
| | Week 1 | Week 2 | After Week 1 | After Week 2 |
| Control | 30.0 ± 0.8 | 28.4 ± 0.7 | 19.6 ± 3.3 | 29.4 ± 6.8 |
| LG100268 | 24.3 ± 1.2† | 19.2 ± 0.6† | −2.8 ± 6.0* | −6.8 ± 5.8† |
| LG100324 | 25.2 ± 1.1† | 18.4 ± 0.9† | −3.7 ± 6.9* | −7.5 ± 8.4† |
| BRL49653 | 34.9 ± 0.7† | 31.6 ± 0.7 | 23.1 ± 5.9 | 43.8 ± 5.5 |

NOTE. Results are the mean ± SEM. There were 6 rats in each group.

*$P < .05$, †$P < .01$ *v* controls.

press *ob* (leptin) gene expression via activation of PPARγ.[29] However, repression of *ob* gene expression is unlikely to be the reason for the increase in food intake in the current experiment, since *fa/fa* Zucker rats contain a missense mutation (Gln → Pro) in the extracellular domain of the leptin receptor generating resistance to the inhibitory action of leptin on food intake.[30]

BAT is the major site of nonshivering thermogenesis in rodents, which is associated in part with an increase in the activity or abundance of the BAT-specific UCP-1.[16] Following the 2-week treatment with the rexinoids LG100268 and LG100324, the level of BAT UCP-1 mRNA increased by 2.7-fold (from 0.26 ± 0.02 to 0.70 ± 0.04, n = 3, $P < .001$) and 3.4-fold (from 0.26 ± 0.02 to 0.89 ± 0.05, n = 3, $P < .001$), respectively. In contrast, following treatment with the thiazolidinedione BRL49653, there was no change in the level of BAT UCP-1 mRNA (Fig 2). None of the treatments affected the weight of the interscapular BAT mass expressed relative to body weight (data not shown). The importance of BAT UCP-1 in maintaining the energy balance, at least in small mammals, is underlined by the findings that most animal models of obesity have impaired BAT thermogenesis and BAT deficiency in rodents results in morbid obesity.[31] Although the antiobesity effect of rexinoids arises from the reduced food intake, the increased UCP-1 mRNA content in BAT could indicate that rexinoids also activate thermogenesis.

Recently, a cold-inducible coactivator of nuclear receptors was identified in BAT, termed PGC-1.[27] Thus, docking of PGC-1 to PPARγ stimulates an apparent conformational change that permits binding of SRC-1 and CBP/P300,[32] resulting in a large increase in transcriptional activity including mitochondrial biogenesis and induction of UCP-2 and nuclear respiratory factors.[33] These findings suggest a role for PGC-1 in nonshivering thermogenesis. In the present study, we used mouse-specific

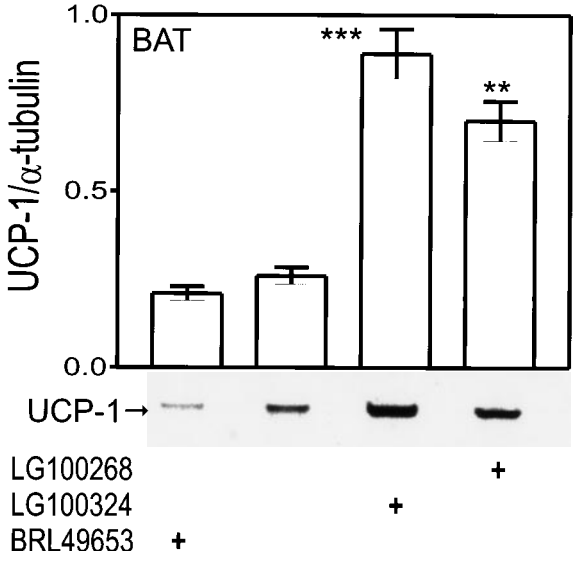


**Fig 2. Effect of 2-week treatment with LG100268, LG100324, and BRL49653 on the level of UCP-1 relative to α-tubulin mRNA in BAT of the Zucker *fa/fa* rat. Results are the mean ± SEM (n = 4) and significance was assessed using Student's unpaired *t* test. ***$P < .001$, **$P < .01$ *v* control vehicle. PCR products of UCP-1 are displayed below the graph.**

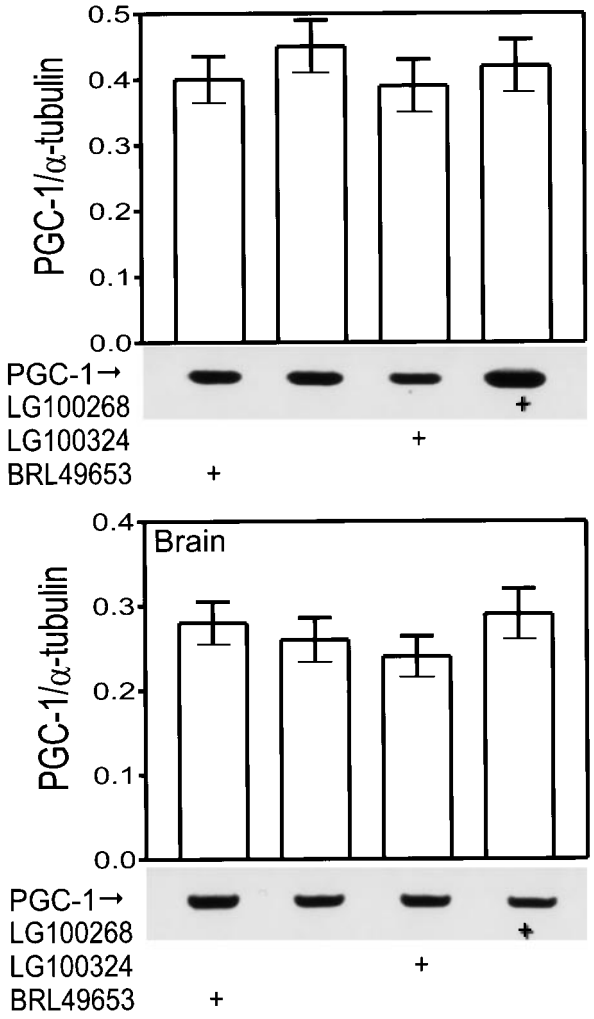


**Fig 3. Effects of 2-week treatment with LG100268, LG100324, and BRL49653 on the level of PGC-1 relative to α-tubulin mRNA in the BAT and brain of the Zucker *fa/fa* rat. Results are the mean ± SEM (n = 4). PCR products of PGC-1 are displayed below the appropriate graph.**

primers to amplify a 310-bp cDNA fragment of rat PGC-1, demonstrating 100% homology with the mouse cDNA counterpart. Using RT-PCR, PGC-1 was detected readily in the BAT and brain of the Zucker *fa/fa* rat, but detection was weak in skeletal muscle and WAT, which agrees well with the previous report.[27] Following the long-term treatment with rexinoids or thiazolidinedione, there was no change in PGC-1 mRNA levels in the BAT or brain of the Zucker *fa/fa* rat (Fig 3). Similarly, we found that treatment with these compounds had no effect on UCP-2 mRNA levels in the different tissues studied (Fig 4). Thus, neither PGC-1 nor UCP-2 genes seem to be targeted by the rexinoids or the thiazolidinedione, at least in Zucker *fa/fa* rats. On the other hand, two recent reports show that RXR and PPARγ agonists can stimulate expression of UCP-2 mRNA in isolated adipocytes from normal rats in vitro.[34,35] The discrepancy also may be related to the different paradigms created by short-term versus long-term (present study) treatment, as well as the in vitro versus in vivo (present study) mode of treatment.

UCP-3 mRNA is expressed most abundantly in skeletal

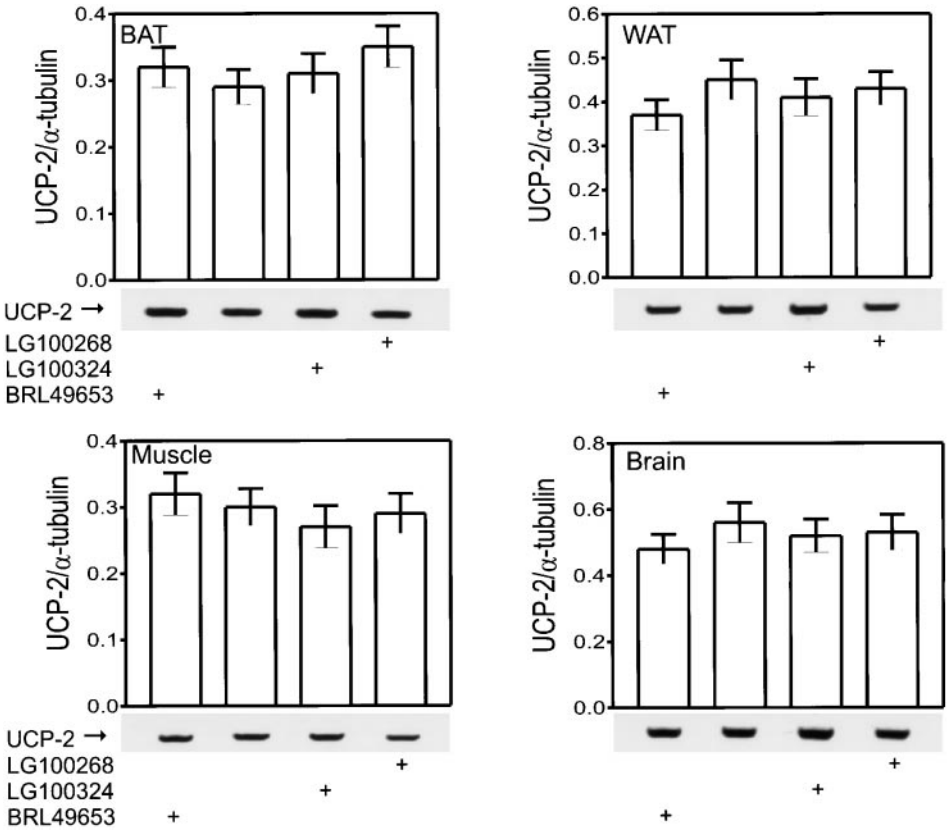


**Fig 4. Effect of 2-week treatment with the rexinoids LG100268 and LG100324 and the thiazolidinedione BRL49653 on the level of UCP-2 relative to α-tubulin mRNA in BAT, WAT, muscle, and brain of the Zucker *fa/fa* rat. Results are the mean ± SEM (n = 4). PCR products of UCP-2 are displayed below the appropriate graph.**

muscle and is also detected readily in BAT and WAT, but not in the brain[21] (and the present study). Treatment with the thiazolidinedione resulted in an increased BAT UCP-3 mRNA content of 2.2-fold (from 0.16 ± 0.01 to 0.35 ± 0.03, n = 3, $P < .002$), increased WAT UCP-3 mRNA content of 2.1-fold (from 0.18 ± 0.02 to 0.42 ± 0.03, n = 3, $P < .002$), and increased skeletal muscle UCP-3 mRNA content of 2-fold (from 0.35 ± 0.04 to 0.73 ± 0.06, n = 3, $P < .002$) (Fig 5). These findings support a recent study by Matsuda et al[36] on the effect of the thiazolidinedione pioglitazone on UCP-3 mRNA in fat. However, this compound did not affect UCP-3 expression in skeletal muscle. These investigators raised the possibility that the increase in UCP-3 mRNA expression may be an attempt to counteract the weight gain associated with the increased food intake. Interestingly, it has been shown previously that the thiazolidinedione insulin-sensitizer ciglitazone does not itself increase thermogenesis but increases the potential for thermogenesis.[37] Alternatively, the increased UCP-3 mRNA expression may be a consequence of the insulin-sensitizer effect of thiazolidinediones and may be related to the increased rate of metabolism in these tissues.

The rexinoids LG100268 and LG100324 also increased UCP-3 mRNA levels in BAT and WAT, with LG100324 being more efficacious. Thus, LG100268 increased BAT UCP-3 mRNA content by 1.9-fold (from 0.16 ± 0.01 to 0.3 ± 0.02, n = 3, $P < .002$) and WAT UCP-3 mRNA content by 2-fold (from 0.18 ± 0.02 to 0.37 ± 0.04, n = 3, $P < .002$), while LG100324 increased BAT UCP-3 mRNA content by 2.8-fold

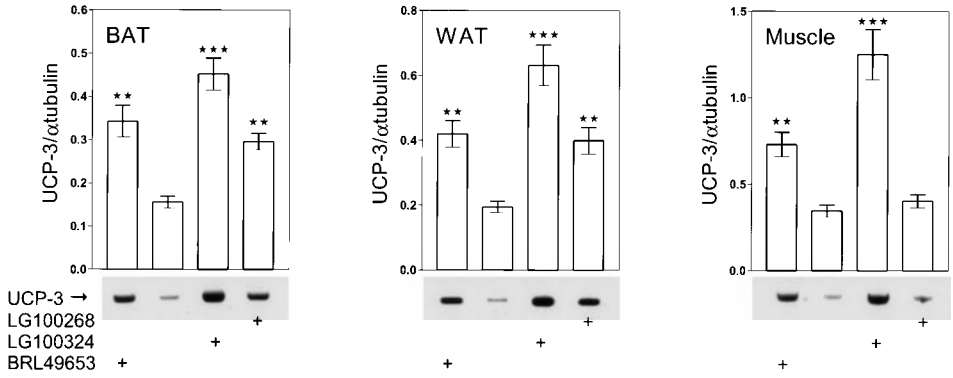


**Fig 5. Effect of 2-week treatment with the rexinoids LG100268 and LG100324 and the thiazolidinedione BRL49653 on the level of UCP-3 relative to α-tubulin mRNA in BAT, WAT, and muscle of the Zucker *fa/fa* rat. Results are the mean ± SEM (n = 4) and significance was assessed using Student's unpaired *t* test. **$P < .02$, ***$P < .002$ (WAT), $P < .003$ (muscle), and $P < .005$ (BAT) *v* control vehicle. PCR products of UCP-3 are displayed below the appropriate graph.**

(from 0.16 ± 0.01 to 0.45 ± 0.04, n = 3, $P < .001$) and WAT UCP-3 mRNA content by 3.4-fold (from 0.18 ± 0.02 to 0.62 ± 0.05, n = 3, $P < .001$). LG100324, but not LG100268, increased UCP-3 mRNA content markedly in skeletal muscle by 3.5-fold (from 0.35 ± 0.04 to 1.24 ± 0.11, n = 3, $P < .002$) (Fig 5).

The thiazolidinediones are insulin sensitizers and also promote adipocyte differentiation,[3] which suggests that such compounds might induce weight gain. These effects of thiazolidinediones are believed to be mediated by activation of the nuclear receptor PPARγ. PPARγ forms a heterodimer with RXR, and RXR ligands such as LG100268 and LG100324 also have insulin-sensitizer properties and induce adipogenesis.[38] Despite these apparent similarities, it is clear that the thiazolidinediones and rexinoids have dissimilar effects on food intake and body weight. The nature of the mechanism of these effects on food intake is not known, but cannot be mediated by leptin in this animal model. The reduction in food intake is almost certainly a major cause of the difference in weight gain induced by rexinoids, but it is possible that there is also a thermogenic mechanism. Pair-feeding studies are needed to address this issue. Nevertheless, there are clear differences between the effects of rexinoids and thiazolidinediones on the selective regulation of the expression of UCP mRNA isoforms. Thus, the rexinoids, but not the thiazolidinedione, increase the tissue content of UCP-1 mRNA in BAT, although there was no change in PGC-1 mRNA. The rexinoid LG100324 was the most potent in stimulating UCP-3 mRNA expression in BAT, WAT, and skeletal muscle. In conclusion, the rexinoids, but not the thiazolidinediones, have an antiobesity action by reducing food intake and may also stimulate thermogenesis in BAT.

## ACKNOWLEDGMENT

The authors thank Liu Zhang at Ligand Retinoid Therapeutics for the synthesis of BRL49653, LG100268, and LG100324. We also thank David Hislop at the Clore Laboratory for technical assistance, and Julie Cakebread for secretarial assistance in the preparation of the manuscript.

## REFERENCES

1. Lillioja S, Mott DM, Spraul M, et al: Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med 329:1988-1992, 1993

2. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM. A balanced overview. Diabetes Care 15:318-368, 1992

3. Tontonoz P, Graves RA, Budavari AI, et al: Adipocyte-specific transcription factor ARF6 is a heterodimeric complex of two nuclear hormone receptors, PPAR gamma and RXR alpha. Nucleic Acids Res 25:5628-5634, 1994

4. Spiegelman BM: Peroxisome proliferator-activated receptor gamma: A key regulator of adipogenesis and systemic insulin sensitivity. Eur J Med Res 2:457-464, 1997

5. Lehmann JM, Moore LB, Smith-Oliver TA, et al: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem 270:12953-12956, 1995

6. Forman BM, Tontonoz P, Chen J, et al: 15-Deoxy-$\Delta^{12,14}$-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell 83:803-812, 1995

7. Oakes ND, Kennedy CJ, Jenkins AB, et al: A new antidiabetic agent, BRL49653, reduces lipid availability and improves insulin action and glucoregulation in the rat. Diabetes 43:1203-1210, 1994

8. Young PW, Cawthorne MA, Coyle PJ, et al: Repeat treatment of obese mice with BRL49653, a new potent insulin sensitizer, enhances insulin action in white adipocytes. Association with increased insulin binding and cell-surface GLUT4 as measured by photoaffinity labeling. Diabetes 44:1087-1092, 1995

9. Buckingham RE, Al-Barazanji KA, Toseland CD, et al: Peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, protects against nephropathy and pancreatic islet abnormalities in Zucker fatty rats. Diabetes 47:1326-1334, 1998

10. Balfour JA, Plosker GL: Rosiglitazone. Drugs 57:921-930, 1999

11. Mukherjee R, Strasser J, Jow L, et al: RXR agonists activate PPARalpha-inducible genes, lower triglycerides, and raise HDL levels in vivo. Arterioscler Thromb Vasc Biol 18:272-276, 1998

12. Lenhard JM, Lancaster ME, Paulik MA, et al: The RXR agonist LG100268 causes hepatomegaly, improves glycaemic control and decreases cardiovascular risk and cachexia in diabetic mice suffering from pancreatic beta-cell dysfunction. Diabetologia 42:545-554, 1999

13. Ibrahimi A, Teboul L, Gaillard D, et al: Evidence for a common mechanism of action for fatty acids and thiazolidinedione antidiabetic agents on gene expression in preadipose cells. Mol Pharmacol 46:1070-1076, 1994

14. Eldershaw TP, Rattigan S, Cawthorne MA, et al: Treatment with the thiazolidinedione (BRL49653) decreases insulin resistance in obese Zucker hindlimb. Horm Metab Res 27:169-172, 1995

15. Gorson DM: Significant weight gain with rezulin therapy. Arch Intern Med 159:99, 1999

16. Brooks SL, Rothwell NJ, Stock MJ, et al: Increased proton conductance pathway in brown adipose tissue mitochondria of rats exhibiting diet-induced thermogenesis. Nature 286:274-276, 1980

17. Fleury C, Neverova M, Collins S, et al: Uncoupling protein-2: A novel gene linked to obesity and hyperinsulinemia. Nat Genet 13:269-272, 1997

18. Boss O, Samec S, Paoloni-Giacobino A, et al: Uncoupling protein-3: A new member of the mitochondrial carrier family with tissue-specific expression. FEBS Lett 408:39-42, 1997

19. Gong DW, He Y, Karas M, et al: Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, β3-adrenergic agonists, and leptin. J Biol Chem 272:24129-24132, 1997

20. Vidal-Puig A, Solanes G, Grujic D, et al: UPC3: An uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. Biochem Biophys Res Commun 235:79-82, 1997

21. Emilsson V, Summers RJ, Hamilton S, et al: The effects of the β3-adrenoceptor agonist BRL 35135 on UCP isoform mRNA expression. Biochem Biophys Res Commun 252:450-454, 1998

22. Boss O, Samec S, Kuhne F, et al: Uncoupling protein-3 expression in rodent skeletal muscle is modulated by food intake but not by changes in environmental temperature. J Biol Chem 273:5-8, 1998

23. Silva JE, Rabelo R: Regulation of the uncoupling protein gene expression. Eur J Endocrinol 136:251-264, 1997

24. Surwit RS, Wang S, Petro AE, et al: Diet-induced changes in uncoupling proteins in obesity-prone and obesity-resistant strains of mice. Proc Natl Acad Sci USA 95:4061-4065, 1998

25. Krook A, Digby J, O'Rahilly S, et al: Uncoupling protein 3 is reduced in skeletal muscle of NIDDM patients. Diabetes 47:1528-1531, 1998

26. Schrauwen P, Xia J, Bogardus C, et al: Skeletal muscle uncoupling protein 3 expression is a determinant of energy expenditure in Pima Indians. Diabetes 48:146-149, 1999

27. Puigserver P, Wu Z, Park CW, et al: A cold-inducible coactivator

of nuclear receptors linked to adaptive thermogenesis. Cell 92:829-839, 1998

28. Morton NM, Emilsson V, Liu Y-L, et al: Leptin action in intestinal cells. J Biol Chem 273:26194-26201, 1998

29. De Vos P, Lefebvre AM, Miller SG, et al: Thiazolidinediones repress ob gene expression in rodents via activation of peroxisome proliferator-activated receptor gamma. J Clin Invest 98:1004-1009, 1996

30. Chua SC Jr, Chung WK, Wu-Peng S, et al: Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 271:994-996, 1996

31. Hamann A, Flier JS, Lowell BB: Decreased brown fat markedly enhances susceptibility to diet-induced obesity, diabetes, and hyperlipidemia. Endocrinology 137:21-29, 1996

32. Puigserver P, Adelmant G, Wu Z, et al: Activation of PPAR gamma coactivator-1 through transcriptional factor docking. Science 286:1368-1371, 1999

33. Wu Z, Puigserver P, Andersson U, et al: Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98:115-124, 1999

34. Aubert J, Champigny O, Saint-Marc P, et al: Up-regulation of UCP-2 gene expression by PPAR agonists in preadipose and adipose cells. Biochem Biophys Res Commun 238:606-611, 1997

35. Carmona MC, Valmaseda A, Iglesias R, et al: 9-Cis retinoic acid induces the expression of the uncoupling protein-2 gene in brown adipocytes. FEBS Lett 441:447-450, 1998

36. Matsuda J, Hosoda K, Itoh H, et al: Increased adipose expression of the uncoupling protein-3 gene by thiazolidinediones in Wistar fatty rats and in cultured adipocytes. Diabetes 47:1809-1814, 1998

37. Thurlby PL, Wilson S, Arch JRS: Ciglitazone is not itself thermogenic but increases the potential for thermogenesis in lean mice. Biosci Rep 7:573-577, 1987

38. Canan Koch SS, Dardashti LJ, Cesario RM, et al: Synthesis of retinoid X receptor-specific ligands that are potent inducers of adipogenesis in 3T-L1 cells. J Med Chem 42:742-750, 1999