A New Thiazolidinedione, NC-2100, Which Is a Weak PPAR-γ Activator, Exhibits Potent Antidiabetic Effects and Induces Uncoupling Protein 1 in White Adipose Tissue of KKAY Obese Mice

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Thiazolidinediones (TZDs) reduce insulin resistance in type 2 diabetes by increasing peripheral uptake of glucose, and they bind to and activate the transcriptional factor peroxisome proliferator-activated receptor-γ (PPAR-γ). Studies have suggested that TZD-induced activation of PPAR-γ correlates with antidiabetic action, but the mechanism by which the activated PPAR-γ is involved in reducing insulin resistance is not known. To examine whether activation of PPAR-γ directly correlates with antidiabetic activities, we compared the effects of 4 TZDs (troglitazone, pioglitazone, BRL-49653, and a new derivative, NC-2100) on the activation of PPAR-γ in a reporter assay, transcription of the target genes, adipogenesis, plasma glucose and triglyceride levels, and body weight using obese KKAY mice. There were 10- to 30-fold higher concentrations of NC-2100 required for maximal activation of PPAR-γ in a reporter assay system, and only high concentrations of NC-2100 weakly induced transcription of the PPAR-γ but not PPAR-α target genes in a whole mouse and adipogenesis of cultured 3T3L1 cells, which indicates that NC-2100 is a weak PPAR-γ activator. However, low concentrations of NC-2100 efficiently lowered plasma glucose levels in KKAY obese mice. These results strongly suggest that TZD-induced activation of PPAR-γ does not directly correlate with antidiabetic (glucose-lowering) action. Furthermore, NC-2100 caused the smallest body weight increase of the 4 TZDs, which may be partly explained by the finding that NC-2100 efficiently induces uncoupling protein (UCP)-2 mRNA and significantly induces UCP1 mRNA in white adipose tissue (WAT). NC-2100 induced UCP1 efficiently in mesenteric WAT and less efficiently in subcutaneous WAT, although pioglitazone and troglitazone also slightly induced UCP1 only in mesenteric WAT. These characteristics of NC-2100 should be beneficial for humans with limited amounts of brown adipose tissue.

In this study, we analyzed a novel TZD, NC-2100, that weakly activates PPAR-γ. By comparing the effects of TZDs with various PPAR-γ agonist activities on the plasma glucose levels of KKAY obese mice, we examined whether TZD-induced activation of PPAR-γ directly correlates with adipogenic activities and with antidiabetic action. We also examined possible mechanisms to explain why some but not all TZDs do not promote obesity despite their similar antidiabetic activities because the TZDs had diverse effects on body and fat weights.

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be clinically used in several countries, increases body weight (14, 15). We therefore compared the effects of TZDs on the expression of uncoupling proteins (UCPs) 1, 2, and 3 (16-18) in white adipose tissue (WAT) and brown adipose tissue (BAT). UCPs uncouple proton movement and ATP synthesis at the inner membrane of mitochondria and play a major role in energy expenditure (19). We report herein that NC-2100 and, to a lesser extent, pioglitazone effectively induce BAT-specific UCP1 in WAT of obese KK AY mice.

RESEARCH DESIGN AND METHODS

Drugs. The 2-(p-chlorophenoxy)isobutyric acid ethyl ester (clofibrate) was purchased from Tokyo-Kasei (Tokyo). All of the TZDs were synthesized at Nippon Chemiphar (Misato, Japan). Troglitazone, pioglitazone-HCL (pioglitazone) and NC-2100 were purchased from Tokyo-Kasei (Tokyo). All of the TZDs were synthesized at Nippon Chemiphar (Misato, Japan). Troglitazone, pioglitazone-HCL (pioglitazone) and NC-2100 were purchased from Tokyo-Kasei (Tokyo).

Animals and treatment. Male KKAy/TaJcl obese mice were purchased from Oriental Kobo, Tokyo (13), were kept on a 12-h light-dark cycle, and were provided with food and water ad libitum. The 11-week-old mice were fed either a control powder diet (MF; Oriental Kobo, Tokyo) or a diet containing 0.5% clofibrate, 0.01 or 0.03% pioglitazone, 0.1 or 0.3% troglitazone, or 0.03 or 0.1% NC-2100 for 8 or 14 days, respectively. All of the animals were killed at day 9 to extract total RNA or at day 15 for the determination of fat mass. Food intake was measured every day.

Cell culture and adipocyte differentiation assay. NIH3T3L1 mouse fibroblasts (American Type Culture Collection, Rockville, MD) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 1 mg/ml gentamicin. The cells were seeded and grown to confluence in a 24-well culture plate in a 5% CO2 atmosphere at 37°C. At postconfluence, the medium was changed to DMEM/5% FCS supplemented with 0.5 mmol/l 1-methyl-3-isobutylxanthine, 0.5 mmol/l dexamethasone, and 1 mg/ml gentamicin for 48 h. The medium was then treated with test chemicals in DMEM/5% FCS (23). The activities of glycero1-3-phosphate dehydrogenase (GPDH) in the cells were measured at 9 days postconfluence as described (24).

Transient transfection reporter assay. CV-1 cells were transfected with the (GAL4-USAX-4)T-KLuciferase reporter vector (25), sPG-GAL4-mPPAR-γ-ligand binding domain (LBD) or sPG-GAL4-mPPAR-γ-LBD (26) expression vectors, or pCMX-β-gal-β-galactosidase expression vector by liposomal delivery (1,2-dimyristylxylopropyl-3-dimethyl-hydroxy ethyl ammonium bromide [Gibco BRL, Rockville, MD]). NC-2100 or typical PPAR activators were added 6 h after transfection. At concentrations described in the figure legends. At 10 h after the addition of the compounds, the transfected cells were lysed, and luciferase and β-galactosidase activities were determined. Results were normalized to β-galactosidase activity (26).

RNA preparation and Northern blot analysis. The animals were killed by decapitation. Liver, intestine, lumbar subcutaneous WAT, mesenteric WAT, and interscapular BAT were removed and were quickly frozen in liquid nitrogen. The samples were stored at -80°C until extraction of total RNA. Total RNA was prepared from the liver, intestine, adipose tissues, or the cultured NIH3T3L1 cells by the acid guanidium thiocyanate-phenol-chloroform extraction method (27). Northern blot analysis was carried out essentially as previously described (28). The cDNA probes included peroxisomal hydratase-dehydrogenase (HD) bifunctional enzyme, apolipoprotein(E) (apo(E)), apo(AIV), adipocyte fatty acid binding protein (A-FAB), fatty acid transport protein (FATP), lipoprotein lipase (LPL), and UCP2 and have been previously described (29). The cDNAs for UCP1, UCP3, cytosolic acyl-CoA thioesterase (CEPT-1), very-long-chain acyl-CoA synthetase (VLACS), and ribosomal protein S14 were obtained by cloning polymerase chain reaction (PCR) products of cDNA synthesized from the livers of mice fed with [4-choloro-6-(2,3-xylidino)-2-pyrimidinyl-thio]acetic acid (WY14,643) (Tokyo-Kasei, Tokyo) as previously described (29). The synthetic oligonucleotides used to amplify respective cDNA sequences were 5'-AGACTCTAGAGGCTTATCG (corresponding to nucleotide numbers from 396 to 817 of the published mouse sequence) (33) (GenBank accession number U63419), 5'-GGGAGCGTTCATGTATCGGG for UCP1 (corresponding to nucleotide numbers from 691 to 978 of the published mouse sequence) (30) (GenBank accession number AB01742), 5'-AACCTGGACCCTTTCCTGGGATCA and 5'-GGCTCCCCTCCAAAGGTGATGTTG for CTE-1 (corresponding to nucleotide numbers from 466 to 1165 of the published mouse sequence) (32) (GenBank accession number Y14004), and 5'-CTTACAGGGCAGCTTAAATGCT and 5'-GGGTTCCCCCTCCAAAGGTGATGTTG for CTE-1 (corresponding to nucleotide numbers from 466 to 1165 of the published mouse sequence) (32) (GenBank accession number Y14004) and 5'-CTTACATGTGTACGCTTACG (corresponding to nucleotide numbers from 396 to 817 of the published mouse sequence) (32) (GenBank accession number Y14004). The cDNA for heart muscle-type fatty acid binding protein (H-FAB) was obtained as described (29) using oligo-tailed cDNA, oligo, and a specific primer: 5'-GATGTTAGTGCTGGCTGGTGGTGG and 5'-GGCCTGAGCTTGACTGAAGCTT for UC3 (corresponding to nucleotide numbers from 432 to 931 of the published mouse sequence) (31) (GenBank accession number U63419), 5'-AACCTGGACCCTTTCCTGGGATCA and 5'-GGCTCCCCTCCAAAGGTGATGTTG for CTE-1 (corresponding to nucleotide numbers from 466 to 1165 of the published mouse sequence) (32) (GenBank accession number Y14004), and 5'-CTTACAGGGCAGCTTAAATGCT and 5'-GGGTTCCCCCTCCAAAGGTGATGTTG for CTE-1 (corresponding to nucleotide numbers from 466 to 1165 of the published mouse sequence) (32) (GenBank accession number Y14004) and 5'-CTTACATGTGTACGCTTACG (corresponding to nucleotide numbers from 396 to 817 of the published mouse sequence) (32) (GenBank accession number Y14004). The cDNA for heart muscle-type fatty acid binding protein (H-FAB) was obtained as described (29) using oligo-tailed cDNA, oligo, and a specific primer: 5'-GATGTTAGTGCTGGCTGGTGGTGG and 5'-GGCCTGAGCTTGACTGAAGCTT for UC3 (corresponding to nucleotide numbers from 432 to 931 of the published mouse sequence) (31) (GenBank accession number U63419), 5'-AACCTGGACCCTTTCCTGGGATCA and 5'-GGCTCCCCTCCAAAGGTGATGTTG for CTE-1 (corresponding to nucleotide numbers from 466 to 1165 of the published mouse sequence) (32) (GenBank accession number Y14004), and 5'-CTTACATGTGTACGCTTACG (corresponding to nucleotide numbers from 396 to 817 of the published mouse sequence) (32) (GenBank accession number Y14004). The cDNA for heart muscle-type fatty acid binding protein (H-FAB) was obtained as described (29) using oligo-tailed cDNA, oligo, and a specifi...
RESULTS

**NC-2100 is a weak activator for both PPAR-α and PPAR-γ in vitro but is a weak but specific PPAR-γ activator in vivo.** To examine whether a new TZD, NC-2100, activates PPAR-γ, we first used an in vitro system. The CV-1 cell line was cotransfected with chimeric receptor plasmids that have mouse PPAR-α or PPAR-γ ligand binding domain and the reporter luciferase gene that has the receptor binding site. The effects of NC-2100 on the expression of luciferase were investigated in comparison with typical PPAR-γ activators (troglitazone, pioglitazone, and BRL-49653) and PPAR-α activators (5,8,11,14-eicosatetraynoic acid and Wy14,643). NC-2100 activated PPAR-γ in a dose-dependent manner with a maximal activation nearly equal to those of BRL-49653 and pioglitazone (Fig. 2B). However, NC-2100 was weaker than these TZDs, with a 50% effective concentration (EC₅₀) of 10- to 30-fold greater. Unexpectedly, NC-2100 also activated PPAR-α in a similar manner to PPAR-γ (Fig. 2A). Thus, the in vitro transient transfection reporter assay showed that NC-2100 was a weak activator for both PPAR-α and PPAR-γ.

Compounds that exhibit PPAR-α- or PPAR-γ-activating activities in forced expression systems with CV-1 cells and chimeric genes do not always activate the corresponding receptor in vivo. Therefore, we next examined the in vivo effect of NC-2100 with whole animals and cultured cells. First, KKAy obese mice were fed a diet containing NC-2100 or a control diet containing clofibrate, troglitazone or pioglitazone, and PPAR-γ activators to compare their in vivo effects. After feeding for 8 days, total RNA was isolated from the livers and WAT of mice, and the levels of several mRNAs were examined with Northern blots (Fig. 3). Among the PPAR-α-regulated genes, HD bifunctional enzyme and CTE-1 were activated, whereas apo(AIV) was downregulated in the liver by clofibrate as reported (35) but not by NC-2100 or control TZDs. In contrast, NC-2100 and control TZDs activated transcription of the PPAR-γ target genes, including A-FAB, FATP, and LPL in WAT (29). Compared with 0.1% of NC-2100 or other TZDs, 0.03% NC-2100 was clearly less effective, although 0.1% NC-2100 showed a similar antidiabetic effect. These results obtained with whole animals suggest that NC-2100 does not activate PPAR-α but weakly activates PPAR-γ.

Several groups have reported that PPAR-γ ligands promote adipocyte differentiation in various cultured fibroblasts and mesenchymal stem cell line systems (6,7,12). Thus, we next examined whether NC-2100 promotes the terminal differentiation of the preadipocyte cell line NIH3T3L1. As markers of adipocyte differentiation, we measured increases in the activities of GPDH (24) in cells treated with various concentrations of NC-2100 or pioglitazone and troglitazone as control TZDs (Fig. 4). NC-2100 promoted terminal differentiation in a dose-dependent manner, although it was less effective than the control TZDs. BRL-49653 was the strongest inducer of terminal differentiation. It was about 50-fold stronger than troglitazone or pioglitazone. NC-2100 was an ~30-fold weaker inducer of GPDH activities than troglitazone or pioglitazone. NC-2100 was an ~30-fold weaker inducer of GPDH activities than troglitazone or pioglitazone. NC-2100 was an ~30-fold weaker inducer of GPDH activities than troglitazone or pioglitazone.

The concentrations of various TZDs required for adipocyte differentiation, we measured increases in the activities of GPDH (24) in cells treated with various concentrations of NC-2100 or pioglitazone and troglitazone as control TZDs (Fig. 4). NC-2100 promoted terminal differentiation in a dose-dependent manner, although it was less effective than the control TZDs. BRL-49653 was the strongest inducer of terminal differentiation. It was about 50-fold stronger than troglitazone or pioglitazone. NC-2100 was an ~30-fold weaker inducer of GPDH activities than troglitazone or pioglitazone. The concentrations of various TZDs required for adipocyte differentiation of cultured fibroblasts differed significantly, but these differences did not directly correspond to their antidiabetic activities (see below).

Although NC-2100 activated both PPAR-α and PPAR-γ similarly in the in vitro system, all in vivo analyses performed strongly suggest that NC-2100 is a weak but specific PPAR-γ activator, at least in whole animals. **NC-2100 has a strong antidiabetic activity.** TZDs are known to increase the sensitivity of peripheral tissues to insulin via an unclear mechanism, and their ability to activate PPAR-γ alone may not be sufficient. Thus, we next examined the effect of NC-2100 on plasma glucose and triglyceride lev-
els with KKAY obese mice. As shown in Fig. 5, treatment of the obese mice with 0.1% NC-2100 for 1 or 2 weeks significantly lowered both glucose and triglyceride levels to levels comparable with 0.03% pioglitazone treatment and to levels lower than with 0.1% troglitazone treatment. NC-2100 and pioglitazone decreased free fatty acid levels to nearly 50% of the control level, whereas troglitazone had little effect (unpublished data). These data indicate that NC-2100, even at 0.1% has antidiabetic activities comparable with troglitazone and pioglitazone.

NC-2100 has a small effect on body weight and fat weight. In our study, NC-2100 caused the smallest increases in body weight and fat mass (Fig. 6). Pioglitazone induced an ~20% increase in body weight and at least a 2-fold increase in the weight of subcutaneous WAT and BAT over 2 weeks. Troglitazone induced an ~10% increase in body weight with a small effect on fat weight. In contrast, NC-2100 induced at maximum a 5% increase in body weight with no effect on the weight of mesenteric WAT deposits. NC-2100 apparently increased the weight of the subcutaneous WAT deposits more than troglitazone (Fig. 6B), but the difference between NC-2100 and troglitazone was not statistically significant. Similarities regarding the effects of NC-2100 and troglitazone on the weight of the subcutaneous WAT deposits as well as a strong fat weight–increasing activity of pioglitazone were confirmed by measuring the sizes of adipocytes in the fat tissues (Fig. 7). These differences do not simply reflect changes in food intake because pioglitazone and NC-2100 stimulated food intake by 40–50% and 20–30%, respectively, but troglitazone did not. The differences in the effects on fat weights and on the sizes of adipocytes may partly explain a unique feature of NC-2100 that does not induce body weight increase as other TZDs do.

**NC-2100 induces ectopic expression of UCP1 in WAT.** Our results showing that NC-2100 induced large increases in food intake but not in body weight suggest that energy expenditure was stimulated. UCP1 in BAT contributes to energy expenditure (16). Recent studies have identified 2 additional proteins, UCP2 (17) and UCP3 (18), with sequence homologies to and similar functional properties as UCP1. Therefore, we measured the changes in the levels of the mRNA for UCP1, UCP2, and UCP3 in several tissues of mice treated with NC-2100 and control drugs. Representative Northern blots for UCP1, UCP2, and UCP3 are shown in Fig. 8. UCP2 mRNA levels increased only in subcutaneous WAT in response to clofibrate and TZDs. Among these, NC-2100 was the most effective. In other tissues, including BAT (36), we did not detect significant increases in the mRNA. UCP1 mRNA expression increased in BAT in response to all TZDs as previously reported (37), but the extent of induction was small. Above all, interestingly, UCP1 mRNA was induced both in mesenteric and subcutaneous WAT by NC-2100 in a dose-dependent manner. The levels of mRNA induced by 0.1% NC-2100 were nearly 10% in subcutaneous WAT and 0.5–1.0% in mesenteric WAT of the normal level of UCP1 mRNA in BAT. Pioglitazone and troglitazone also induced UCP1 only in mesenteric WAT, and the levels were quite low. BAT contamination during tissue separation and the conversion of some
white adipocytes to typical brown adipocytes were ruled out by screening for H-FAB, which is exclusive to BAT (38) (Fig. 8B). Another BAT-predominant mRNA for VLACS (Y.F., K.M., unpublished data) that may be a possible fatty acid transporter instead of a peroxisomal acetyl-CoA synthetase (39) also was not induced in these WATs by NC-2100. Pioglitazone and troglitazone caused weak UCP1 mRNA induction in mesenteric WAT and negligible induction in subcutaneous WAT when compared with NC-2100.

Finally, we examined whether NC-2100 induces UCP1 in 3T3L1 cells. NC-2100 and other TZDs, including BRL-49653, did not induce UCP1 mRNA in differentiating or differentiated 3T3L1 cells, although all induced A-FAB (data not shown). Thus, under the conditions in this study, TZDs can induce BAT-specific UCP1 in the WAT of obese mice but not in vitro.

**DISCUSSION**

TZDs such as troglitazone, pioglitazone, and BRL-49653 improve insulin resistance by enhancing insulin action in skeletal muscle, liver, and adipose tissues (3,5) through a mechanism that is not yet fully understood. TZDs are ligands...
for PPAR-\(\gamma\) (6–8), and studies have suggested that TZD-induced activation of PPAR-\(\gamma\) correlates with antidiabetic action (12). A new TZD, NC-2100, weakly activated PPAR-\(\gamma\) (Fig. 2B) and induced the expression of PPAR-\(\gamma\) target genes (Fig. 3) and adipocyte differentiation (Fig. 4). It also exerted PPAR-\(\alpha\)-activating activities only in the transient transfection reporter assay (Fig. 2). All of the compounds that exhibit PPAR-\(\alpha\)- or PPAR-\(\gamma\)-activating activities in this artificial and forced expression system do not activate the corresponding receptor in vivo, and we conclude that NC-2100 is a weak but specific PPAR-\(\gamma\) activator based on the results from in vivo studies. The requirement for high concentrations of NC-2100 to activate PPAR-\(\gamma\) in the transient transfection reporter assay and to induce adipogenesis can be explained by its low affinity to PPAR-\(\gamma\). In addition to the conserved head group that interacts specifically with the receptor and a divergent hydrophobic part that interacts with the large ligand-binding pocket of the receptor in a relatively nonspecific manner as suggested by a recent X-ray structure analysis (40), NC-2100 has a unique nitrogen in the quinoline nucleus (Fig. 1). This basic nitrogen in the center part lies in a \(\alpha\)-helix (41) and may interfere with receptor interaction, thus significantly reducing affinity compared with other TZDs.

To examine whether the potencies of the compounds to activate PPAR-\(\gamma\) correlate with their antidiabetic activities (12), we compared 4 TZDs in vivo and in cultured differentiated cell systems and found that the suggested correlation was not entirely confirmed. First, we found that the concentrations of each TZD required for adipocyte differentiation of NIH3T3L1 cells were quite different (Fig. 4). Ectopic expression of PPAR-\(\gamma\) alone induced differentiation (41), and the concentrations of several PPAR-\(\gamma\) ligands required to activate the receptor are reported to be in good agreement with those required to induce differentiation of preadipose cells to adipocytes (25). However, doses of NC-2100 (0.1%) similar to other TZDs had similar or better antidiabetic effects in obese mice (Fig. 5). Our preliminary examination on the half-lives and bioavailabilities of these TZDs in murine bodies suggests that the differences in these factors alone cannot explain the discrepancies between adipogenesis induction and antidiabetic effects. We hypothesize that the ability to activate PPAR-\(\gamma\) is necessary but not sufficient for antidiabetic activity of TZDs, and TZDs have other in vivo targets that are crucial for full antidiabetic action. Recent studies showing that TZDs have various effects may strengthen our claim (42,43). More recently, another new TZD, MCC-555, was reported to be a potent antidiabetic compound with low activities to induce adipocyte differentiation and a low affinity for PPAR-\(\gamma\) (44), although whether MCC-555 induces UCP1 in WAT is not known.

The 4 TZDs also exerted different effects on food intake, fat mass increases, plasma fatty acid levels, and target gene activation, which further suggests that TZDs interact with more than just PPAR-\(\gamma\). However, in contrast with the strict ligand specificity of the nuclear hormone receptors, PPARs bind and are activated by structurally diverse natural and artificial compounds, and binding of ligands with diverse structures may cause various conformational changes in the receptor, thus leading to various transcriptional complex formations that contain different factors such as coactivators. Thus, the repertoire of the target genes of 1 type of PPAR may depend on the structures of the ligands. Some artificial ligands (drugs) may limit the target genes or cause an exaggerated response of 1 gene.

Among the different effects that TZDs exert, induction of UCP1 in WAT by NC-2100 is noteworthy (Fig. 8). UCP1 is known to be expressed only in BAT. The mouse gene has a
functional PPAR-γ response element in the promoter region, and the expression is strictly regulated in a differentiation-dependent manner (45). The possibility that treatment with NC-2100 induces differentiation of brown adipocytes in WAT (46) is unlikely because we detected UCP1 but not other BAT-specific or -predominant mRNA for H-FAB (38) and a possible fatty acid transporter, FATP2 or VLACS (39) (Y.F., K.M., unpublished data). Furthermore, the amounts of mRNA for PPAR-γ coactivator or PPAR-γ coactivator 1 (47) were too low to be detected by our Northern blot analysis.
from the WAT of NC-2100 fed mice (data not shown). Instead, some perturbation or interaction between PPAR-γ and other BAT-specific transcriptional factors may be induced by NC-2100 in vivo but not in the model cell system we used. To our knowledge, the first report showed that a β3-adrenergic agonist induced similar ectopic expression of UCP1 mRNA in WAT and muscle with the reverse transcription (RT)-PCR method (48), but another report was published showing that the mRNA that responded to hormones and β3-adrenergic agonists was UCP3 when using Northern blots (18). Detection of UCP3 mRNA by Northern blot does not exclude the possibility that UCP1 mRNA was also induced in WAT by hormones and β3-adrenergic agonists, but more quantitative analysis than RT-PCR will be necessary to confirm their interesting finding because we isolated UCP1 cDNA (confirmed by sequencing) with RT-PCR using poly(A) RNA from the mouse liver as described in \textit{RESEARCH DESIGN AND METHODS}.

TZDs have been reported to induce UCP2 in rodent BAT (37), although we could not detect a significant increase of the mRNA in BAT. Kelly et al. (49) recently reported that the effects of TZDs on gene expression of UCP1, UCP2, and UCP3 in BAT were variable depending on genetic background, doses of compounds, and the period of treatment. In any event, this induction may not have a significant effect in humans because the contribution of BAT to energy expenditure is very small in the human body. Effects of TZDs on WAT will be more important. We found that UCP2 mRNA levels increased in subcutaneous WAT in response to TZDs, especially in response to troglitazone and NC-2100 (Fig. 8). Effective induction of UCP2 by these TZDs but not so much by pioglitazone corresponded with lesser potencies of these TZDs required to increase the weight of subcutaneous fat deposits (Fig. 6). Moreover, induction by NC-2100 of UCP1 mRNA in WAT is particularly interesting because forced ectopic expression of UCP1 in WAT of obese A/HeJ mice using an A-FAB gene promoter prevented the development of obesity (50). However, UCP1 induction in WAT may not fully explain the low weight gain with NC-2100 because pioglitazone, which strongly promotes obesity (Fig. 6), weakly induces UCP1 mRNA in WAT.

\textbf{REFERENCES}


