Rapid Publication

Increased Adipose Expression of the Uncoupling Protein-3 Gene by Thiazolidinediones in Wistar Fatty Rats and in Cultured Adipocytes

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Uncoupling protein (UCP) 3 and UCP2, mitochondrial carrier proteins dissipating electrochemical gradient across the mitochondrial inner membrane, have been implicated in the regulation of energy metabolism. The UCP3 gene is expressed abundantly in the skeletal muscle, while the UCP2 gene is detected in the white adipose tissue (WAT) with diffuse localization throughout the body. Uncoupling of electron transport and ATP synthesis has been reported to increase glucose uptake, suggesting that UCP may be involved in glucose metabolism. Thiazolidinediones (TZDs), which are insulin-sensitizing agents for NIDDM, have been reported to increase energy expenditure. To elucidate the pathophysiologic significance of UCP3 and UCP2 in the effect of TZDs on glucose metabolism and energy expenditure, we examined their basal mRNA levels in the WAT, brown adipose tissue (BAT), and skeletal muscle from Wistar fatty rats, a rat model of NIDDM and obesity with leptin receptor defect, and investigated expression of the genes encoding UCP3 and UCP2 in Wistar fatty rats and in Wistar lean rats with 2-week oral administration of 3 mg · kg^{-1} · day^{-1} pioglitazone, a TZD derivative. Basal UCP3 mRNA levels were significantly lower (38 ± 8, 45 ± 13, and 76 ± 6%) in the retroperitoneal WAT, BAT, and skeletal muscle from Wistar fatty rats than in those from Wistar lean rats, while basal UCP2 mRNA levels were significantly higher by 2.1-, 1.8-, and 2.5-fold in the subcutaneous WAT, retroperitoneal WAT, and BAT from Wistar fatty rats, respectively, than in those from Wistar lean rats. In pioglitazone-treated Wistar fatty rats, UCP3 mRNA levels were significantly increased by 2.1-, 2.0-, and 1.6-fold in the epididymal WAT, retroperitoneal WAT, and BAT, respectively, as compared with those in nontreated fatty rats. In pioglitazone-treated lean rats, UCP3 mRNA levels were significantly increased by 1.3-fold in the BAT as compared with those in nontreated lean rats. No significant change of UCP2 mRNA levels was observed in pioglitazone-treated fatty and lean rats. In addition, to examine the direct effect of TZDs on adipocytes, we examined the regulation of UCP3 and UCP2 gene expression using the primary culture of rat mature adipocytes from Sprague-Dawley rats. In rat cultured mature adipocytes, UCP3 mRNA levels were increased in a dose-responsive manner by 10^{-5} to 10^{-4} mol/l pioglitazone, while there was no significant change of UCP2 mRNA levels. These results clearly demonstrate that UCP3 gene expression is upregulated by TZDs in the WAT and BAT in Wistar fatty rats, an obese model with leptin receptor defect, and that adipose UCP3 gene expression is increased in response to TZDs in vitro. The present study suggests the involvement of UCP3 in the effects of TZDs on energy and glucose metabolism. ***Diabetes*** **47:1809–1814, 1998**

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Received for publication 16 June 1998 and accepted in revised form 11 August 1998.

BAT, brown adipose tissue; BSA, bovine serum albumin; PPAR-γ, peroxisome proliferator-activated receptor-γ; PPRE, peroxisome proliferator-activated receptor response element; TZD, thiazolidinedione; UCP, uncoupling protein; WAT, white adipose tissue.

Mitochondrial uncoupling proteins (UCPs) encode proton carriers that uncouple electron transport from ATP synthesis and are implicated in increase of energy expenditure (1). Recently, cDNAs of new members of the mitochondrial carrier family, UCP3 and UCP2, were cloned by several groups, including ours (2–6). While UCP2 gene expression is observed in a wide variety of tissues with abundant levels in the white adipose tissue (WAT), the UCP3 mRNA is detected at high levels in the skeletal muscle, at moderate levels in the brown adipose tissue (BAT), and at low levels in the WAT and heart. The expression of genes for UCP3 and UCP2 has been reported to be regulated by thyroid hormone, glucocorticoid, and food intake (7–12). Increased UCP3 gene expression by leptin has also been described (9)

The effects of uncoupling of mitochondrial ATP synthesis on glucose metabolism have been investigated by the incubation of cultured cells with chemical uncouplers (13,14). Glucose uptake was increased by chemical uncouplers in L6 cells and 3T3-L1 cells. The mechanism of increase of glucose uptake is considered to be compensation for decrease of the ATP-to-ADP ratio caused by uncoupling of ATP synthesis. These data suggest the involvement of UCP3 and UCP2 in glucose metabolism through regulation of glucose uptake in the skeletal muscle and adipose tissues.

Thiazolidinediones (TZDs) are insulin-sensitizing agents for NIDDM (15–17). TZDs bind to and activate the peroxisome

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proliferator-activated receptor-γ (PPAR-γ) (18), which modulates the activity of several genes involved in adipocyte differentiation and glucose homeostasis (19,20). So far, the pathophysiologic significance of UCP3 and UCP2 in the effects of TZDs on glucose metabolism remains to be elucidated. Furthermore, TZDs have been reported to increase energy expenditure in rodents (21,22), which may also contribute to improvement of glucose metabolism.

To elucidate the pathophysiologic significance of UCP3 and UCP2 in the effects of TZDs on glucose metabolism, we examined the basal mRNA levels of UCP3 and UCP2 in Wistar fatty rats, a rat model of NIDDM with obesity, and investigated the regulation of their gene expression in Wistar fatty rats and Wistar lean rats by chronic administration of pioglitazone, a TZD derivative. Furthermore, to elucidate the direct effect of TZDs, we also examined the regulation of gene expression of UCP3 and UCP2 by TZDs in vitro. This is the first report of examination of UCP3 mRNA both in vivo and in vitro, and UCP2 mRNA in vivo, in response to TZDs.

RESEARCH DESIGN AND METHODS

Animals and drug administration. For the study, 12-week-old male Wistar fatty (*fa/fa*) rats and their lean (+/?) littermates were used. Rats were housed in a temperature-, humidity-, and light-controlled room with free access to water and food. Pioglitazone (AD4833; Takeda Chemical Industries, Osaka, Japan) suspended in 0.5% methylcellulose solution was orally administered 3 mg · kg^{-1} · day^{-1} to Wistar fatty rats and Wistar lean rats (n = 4, each) by a stomach tube for 14 days (23). Methylcellulose solution was also administered to nontreated Wistar fatty rats and to nontreated Wistar lean rats (n = 4, each). Blood samples were taken from the tail vein on the previous day of the sacrifice for measurement of plasma glucose, triglyceride, and insulin levels.

Total RNA extraction and Northern blot analysis. Wistar fatty rats and Wistar lean rats were killed by decapitation after a blow to the head. Tissues were immediately removed from the rats, frozen in liquid nitrogen, and stored at –80°C until use. Epididymal WAT, subcutaneous WAT, mesenteric WAT, retroperitoneal WAT, interscapular BAT, and gastrocnemius muscle were sampled. Total RNA was extracted using Trizol reagent (Gibco, Grand Island, NY). Filters containing 10 µg of total RNA were used. The density of 18S rRNA stained with ethidium bromide was used to monitor the amount of total RNA in each sample. Northern blot analyses of UCP1, UCP2, and UCP3 mRNAs were performed using rat UCP1, UCP2, and UCP3 cDNA probes as described previously (5).

Cell culture. Male Sprague-Dawley (SD) rats (180–200 g; Shimizu Breeding Laboratories, Kyoto, Japan) fed ad libitum were used for the primary culture of rat adipocytes. The rats were anesthetized with a gas mixture of 70% CO_2 and 30% O_2 and were killed by decapitation. The epididymal fat pads were removed, minced, and digested with collagenase (type II; Sigma, St. Louis, MO). The preparation of the cells was carried out at 37°C in a Krebs-Ringer bicarbonate–HEPES buffer (pH 7.4) containing 10 mmol/l $NaHCO_3$, 30 mmol/l HEPES, 200 mmol/l adenosine, and 5% (vol/vol) bovine serum albumin (BSA) (fraction V; Sigma) as described previously (24). All incubations were carried out at 37°C in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 5% (vol/vol) BSA, L-glutamine (2 mmol/l), 200 mmol/l adenosine, and 100 mg/dl glucose. Isolated adipose cells were distributed equally into plastic dishes in a final incubation volume of 5 ml (20% cytocrit). The cells were incubated for 24 h with 10^{-6} to 10^{-4} mol/l pioglitazone, 10^{-4} mol/l troglitazone (CS045; Sankyo, Tokyo, Japan), or without either agent. Pioglitazone and troglitazone were dissolved in dimethylsulfoxide (DMSO). All the cells were exposed to a final concentration of 0.1% DMSO. After the incubation, RNA was extracted from the adipocytes as described above.

Analytical method. Data are expressed as means ± SE. The mRNA levels in 10 µg of total RNA of the BAT from nontreated Wistar lean rats were defined as 100 U. Statistical significance of differences between groups was assessed by Student's unpaired t test.

RESULTS

Profiles of rats used in this study. Table 1 summarizes profiles of Wistar fatty rats and their lean controls used in this study. Compared with Wistar lean rats, Wistar fatty rats showed hyperglycemia (352 ± 26 vs. 119 ± 1 mg/ml, P < 0.01), hyperinsulinemia (1095 ± 182 vs. 165 ± 43 µU/ml, P < 0.01), and hypertriglyceridemia (469 ± 77 vs. 54 ± 4 mg/ml, P < 0.01). Pioglitazone was administered orally (3 mg · kg^{-1} · day^{-1}) to the Wistar fatty rats and to their lean controls (n = 4, each) for 2 weeks. Pioglitazone improved hyperglycemia (116 ± 16 vs. 352 ± 26 mg/ml, P < 0.01), hyperinsulinemia (550 ± 101 vs. 1095 ± 182 µU/ml, P < 0.01), and hypertriglyceridemia (87 ± 2 vs. 469 ± 77 mg/ml, P < 0.01) in Wistar fatty rats compared with nontreated Wistar fatty rats. Reduced plasma triglyceride levels were also observed in Wistar lean rats with pioglitazone treatment (43 ± 2 vs. 54 ± 4 mg/ml, P < 0.01) compared with nontreated Wistar lean rats.

Basal UCP3 and UCP2 gene expression in Wistar fatty rats and their lean controls. We investigated basal expression of UCP3 and UCP2 genes in the epididymal WAT, subcutaneous WAT, mesenteric WAT, retroperitoneal WAT, BAT, and skeletal muscle (gastrocnemius muscle) from 14-week-old Wistar fatty rats and their lean controls without pioglitazone treatment. The UCP3 gene expression was decreased to 38 ± 8% in the retroperitoneal WAT (P < 0.01), 45 ± 13% in the BAT (P < 0.01), and 76 ± 6% in the skeletal muscle (P < 0.05) from Wistar fatty rats compared with that in Wistar lean rats, while no significant difference was observed in the epididymal WAT (65 ± 31%), subcutaneous WAT (60 ± 20%), or mesenteric WAT (119 ± 17%) (Fig. 1*A*). UCP2 gene expression was increased to 207 ± 35% in the subcutaneous WAT (P < 0.05), 178 ± 5% in the retroperitoneal WAT (P < 0.01), and 248 ± 29% in the BAT (P < 0.05) from Wistar fatty rats compared with that in Wistar lean rats, while no significant difference was observed in the skeletal muscle (76 ± 7%), epididymal WAT (110 ± 20%), or mesenteric WAT (105 ± 26%) (Fig. 1*B*). UCP1 gene expression was significantly lower by 64 ± 5% in

TABLE 1
Effects of pioglitazone treatment on body weight, epididymal fat weight, and plasma levels of glucose, insulin, and triglyceride in 14-week-old Wistar fatty rats and Wistar lean rats

	Wistar lean rats		Wistar fatty rats	
	Nontreated	Pioglitazone	Nontreated	Pioglitazone
Body weight (g)	404 ± 4	439 ± 3*	549 ± 3‡	628 ± 11*
Glucose (mg/dl)	119 ± 1	118 ± 3	352 ± 26‡	116 ± 16*
Triglyceride (mg/dl)	54 ± 4	43 ± 2*	469 ± 77‡	87 ± 2*
Insulin (µU/ml)	165 ± 43	146 ± 17	1,095 ± 182‡	550 ± 101*
Epididymal fat weight (g)	2.9 ± 0.2	3.7 ± 0.3†	7.6 ± 0.2‡	8.5 ± 0.6

Data are means ± SE. n = 4, each. Pioglitazone was administered 3 mg · kg^{-1} · day^{-1} for 2 weeks. *P < 0.01 and †P < 0.05 vs. their nontreated control rats; ‡P < 0.01 vs. nontreated Wistar lean rats.

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the BAT ($P < 0.01$) from Wistar fatty rats compared with that in Wistar lean rats (data not shown).

Regulation of UCP3 and UCP2 gene expression by pioglitazone in vivo. To elucidate the regulation of gene expression of UCP3 and UCP2 by TZDs, we examined UCP3 and UCP2 mRNA levels in the WAT, BAT, and skeletal muscle from Wistar fatty rats and Wistar lean rats with 3 mg · kg^{-1} · day^{-1} oral administration of pioglitazone for 2 weeks. UCP3 mRNA levels were increased to 209 ± 34% in the epididymal WAT ($P < 0.05$), 195 ± 11% in the retroperitoneal WAT ($P < 0.01$), and 159 ± 14% in the BAT ($P < 0.05$) from pioglitazone-treated Wistar fatty rats compared with that in nontreated Wistar fatty rats (Fig. 2*A*). No significant change of UCP3 mRNA levels in the skeletal muscle (92 ± 10%) (data not shown), subcutaneous WAT (137 ± 52%), or mesenteric WAT (97 ± 28%) was observed in treated Wistar fatty rats compared with nontreated Wistar fatty rats (Fig. 2*A*). In pioglitazone-treated Wistar lean rats, UCP3 mRNA levels were significantly increased to 131 ± 7% only in the BAT ($P < 0.05$), compared with those in nontreated Wistar lean rats (Fig. 2*B*). No significant change of UCP2 mRNA levels was observed in all tissues studied in the present study from Wistar fatty rats and Wistar lean rats (Fig. 2*C* and *D*). UCP1 mRNA levels were significantly increased to 179 ± 15% in the BAT from Wistar fatty rats ($P < 0.01$) and to 132 ± 1% in the BAT from Wistar lean rats ($P < 0.01$) compared with those in their nontreated control rats (data not shown).

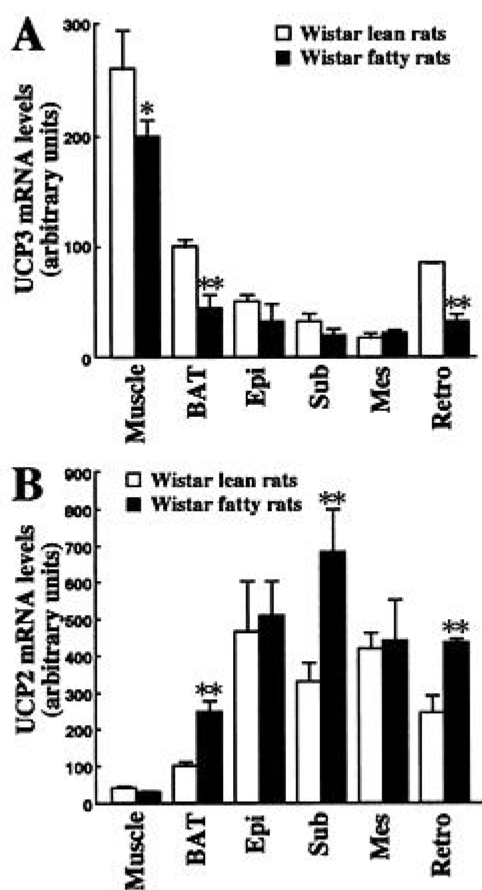

FIG. 1. Basal UCP3 and UCP2 mRNA levels in Wistar fatty rats in comparison with Wistar lean rats at the age of 14 weeks. Ten micrograms of total RNA from the skeletal muscle (Muscle), interscapular BAT (BAT), epididymal WAT (Epi), subcutaneous WAT (Sub), mesenteric WAT (Mes), and retroperitoneal WAT (Retro) were analyzed by Northern blot analyses. *$P < 0.05$; **$P < 0.01$ vs. Wistar lean rats. Data are expressed as means ± SE. $n = 4$. *A*: UCP3 mRNA levels; *B*: UCP2 mRNA levels.

Regulation of UCP3 and UCP2 gene expression by TZDs in vitro. To examine whether the increased UCP3 gene expression in the WAT in vivo was mediated via direct effect of TZDs on adipocytes, we investigated the regulation of UCP3 and UCP2 gene expression in vitro, using the primary culture of rat mature adipocytes from SD rats. UCP3 mRNA levels were augmented in a dose-responsive manner: 2.9-fold with 10^{-5} mol/l pioglitazone ($P < 0.05$), 4.2-fold with 10^{-4} mol/l pioglitazone ($P < 0.05$), and 4.1-fold with 10^{-4} mol/l troglitazone ($P < 0.05$), compared with those in control experiments after 24-h incubation (Fig. 3*A*). No significant change of the UCP2 gene expression was observed with 10^{-6} to 10^{-4} mol/l pioglitazone or with 10^{-4} mol/l troglitazone (Fig. 3*B*).

DISCUSSION

This study demonstrates that UCP3 mRNA levels were significantly lower in the retroperitoneal WAT, BAT, and skeletal muscle from Wistar fatty rats compared with those in Wistar lean rats, while UCP2 mRNA levels were significantly higher in the subcutaneous WAT, retroperitoneal WAT, and BAT from Wistar fatty rats than those in Wistar lean rats. These findings suggest that the expression of the UCP3 and UCP2 genes appears to be differentially regulated in Wistar fatty rats, a rat model of NIDDM with obesity. Several reports have described differential regulation of the UCP3 and UCP2 gene expression. We have reported increased expression of the UCP3 gene in skeletal muscle and of the UCP2 gene in WAT from rats fed a high-fat diet (5). In the BAT from C57BL/6J *ob/ob* mice, UCP3 mRNA levels were lower compared with those from their lean controls, while UCP2 mRNA levels were higher than the levels from their lean controls (9). In humans, BMI was positively correlated with UCP2 mRNA levels but not with UCP3 mRNA levels (8). Short-term exposure to cold causes a rise in UCP1 and UCP2 mRNAs, but not in UCP3 mRNA (7,25). The administration of leptin increased UCP3 mRNA levels without significant change of UCP2 mRNA levels in mouse skeletal muscle and BAT (9). Wistar fatty rats are a model with a leptin receptor defect (26). Because leptin is involved in the increase of UCP3 mRNA levels, as described above, the reduced UCP3 gene expression in the WAT, BAT, and skeletal muscle from Wistar fatty rats observed in the present study may be explained by a defect in the leptin receptor system. Wistar fatty rats are also a hereditary obese model. Because UCP2 mRNA levels are correlated with BMI in the WAT in humans, as described above, the increased UCP2 mRNA levels in the WAT from Wistar fatty rats may be related to obesity. Further studies are necessary for the elucidation of the molecular mechanism of differential regulation of UCP3 and UCP2 gene expression.

We demonstrate that the oral administration of 3 mg · kg^{-1} · day^{-1} pioglitazone for 2 weeks significantly increased UCP3 gene expression by 2.1-fold in the epididymal WAT, 1.9-fold in retroperitoneal WAT, and 1.6-fold in the BAT from 14-week-old Wistar fatty rats without significant change of UCP2 mRNA levels. We also demonstrate that pioglitazone increased UCP3 mRNA levels, but not UCP2 mRNA levels, in

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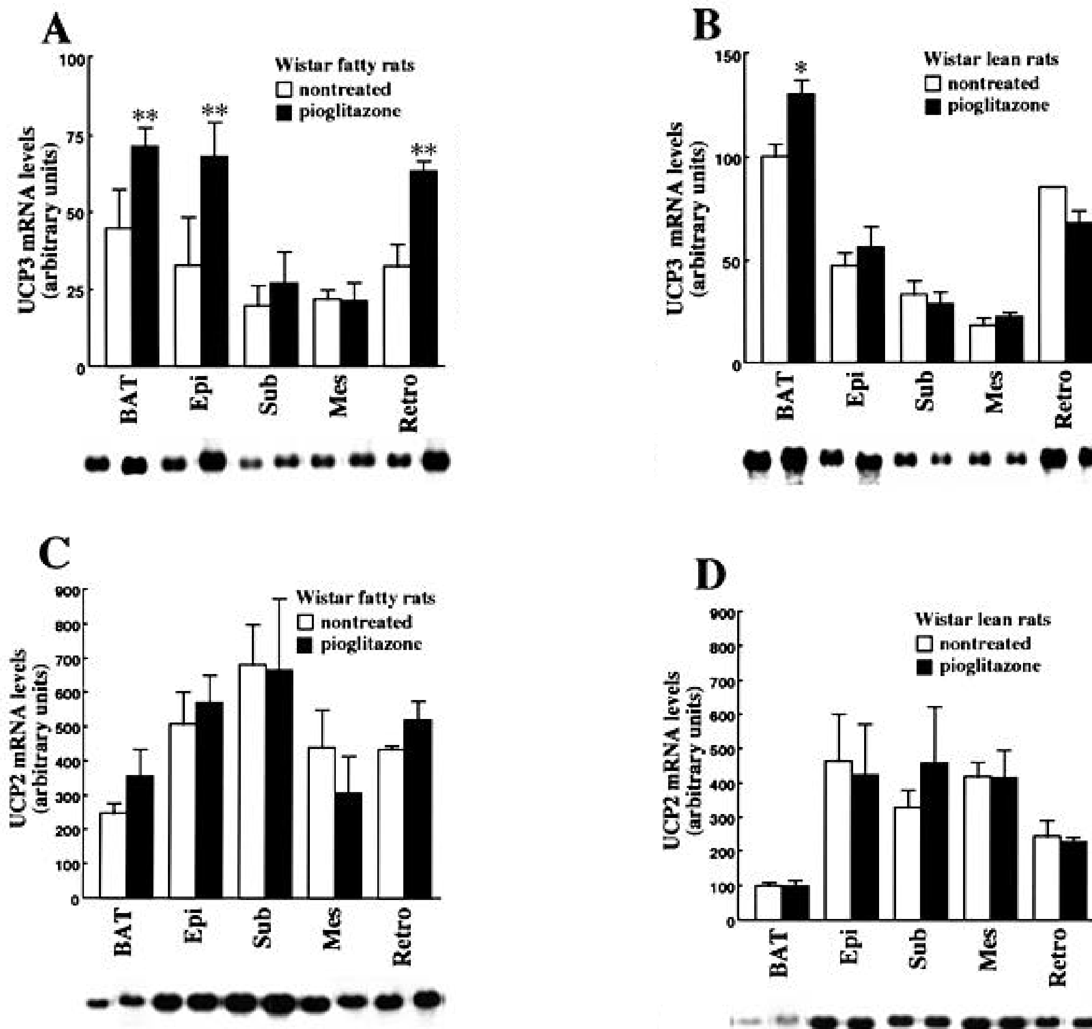

FIG. 2. UCP3 and UCP2 mRNA levels in Wistar fatty rats and Wistar lean rats with or without pioglitazone (3 mg · kg^{-1} · day^{-1}) for 2 weeks. Ten micrograms of total RNA from the interscapular BAT (BAT), epididymal WAT (Epi), subcutaneous WAT (Sub), mesenteric WAT (Mes), and retroperitoneal WAT (Retro) were analyzed by Northern blot analyses. *$P < 0.05$; **$P < 0.01$ compared with their nontreated control rats. Data are expressed as means ± SE. $n = 4$. *A*: UCP3 mRNA levels in nontreated and pioglitazone-treated Wistar fatty rats; *B*: UCP3 mRNA levels in nontreated and pioglitazone-treated Wistar lean rats; *C*: UCP2 mRNA levels in nontreated and pioglitazone-treated Wistar fatty rats; and *D*: UCP2 mRNA levels in nontreated and pioglitazone-treated Wistar lean rats. Representative blots are shown in the lower panels.

the primary culture of rat mature adipocytes in a dose-responsive manner. In addition to pioglitazone, troglitazone, another TZD derivative, also induced UCP3 gene expression in vitro. These results clearly demonstrate that TZDs increase UCP3 gene expression in the adipocytes in vivo and in vitro, while UCP2 gene expression is not induced by TZDs. These findings clearly reveal differential effects of TZDs on UCP3 and UCP2 gene expression.

TZDs exert their biological effects via PPAR-γ (18). The finding that UCP3 gene expression was increased in WAT and BAT, but not in the skeletal muscle, is clearly explained by abundant levels of PPAR-γ mRNA in WAT and BAT and minimal levels of the message in the skeletal muscle (27). UCP1 gene expression has been reported to be upregulated by TZDs via proliferator-activated receptor response element (PPRE) (28–32). The 5′-flanking region of the UCP3 gene may also contain PPRE, which can explain the increase of UCP3 gene expression in the present study. In the present study, UCP2 gene expression was not changed significantly in vivo or in vitro by TZDs. These findings suggest that PPAR-γ is not involved in the regulation of UCP2 gene expression.

In the present study, pioglitazone increased the UCP3 mRNA levels in the epididymal WAT, retroperitoneal WAT, and BAT from Wistar fatty rats, without significant change in the subcutaneous or mesenteric WAT. These findings indicate that adipose expression of the UCP3 gene is augmented in a region-specific manner by TZDs. Further study is necessary for the elucidation of the significance of region-specific induction of the UCP3 gene by TZDs.

It has been demonstrated that UCP3 uncoupled the proton electrochemical gradient across the mitochondrial inner membrane using C_2C_{12} myocytes permanently transfected with human UCP3 cDNA (7). This result indicates that increased expression of the UCP3 gene leads to an increase in energy expenditure. TZDs were reported to increase energy expenditure when administered systemically to rodents

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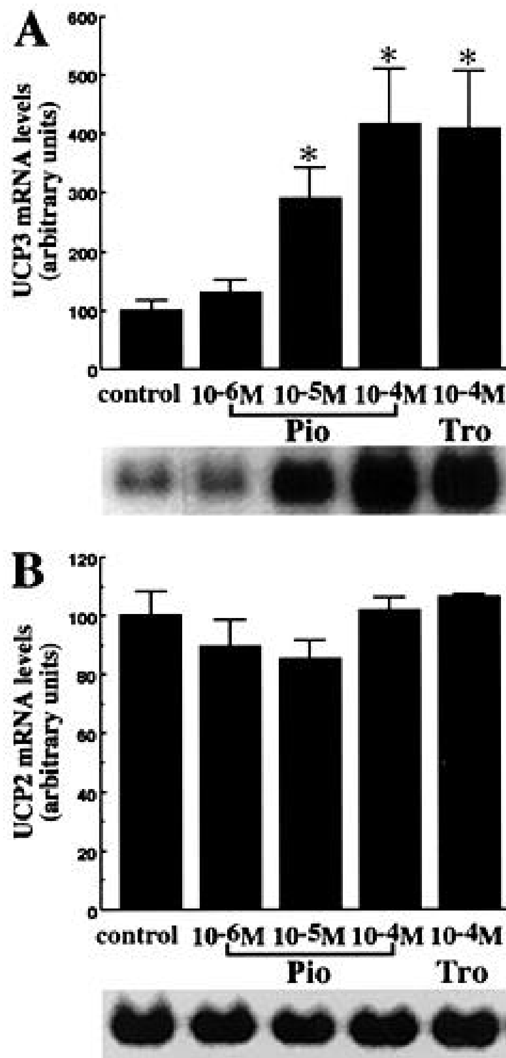

FIG. 3. UCP3 and UCP2 mRNA levels in the primary culture of rat mature adipocytes from SD rats incubated with or without thiazolidinediones. Cells were incubated with pioglitazone or troglitazone or without either agent. All cells were exposed to 0.1% DMSO. Ten micrograms of total RNA was analyzed by Northern blot analysis. Data are expressed as means ± SE. n = 3. *P < 0.05 compared with control experiments. *A*: UCP3 mRNA levels; *B*: UCP2 mRNA levels. Representative blots are shown in the lower panels.

(21,22). Taken together with these findings, the present study indicates that the increased energy expenditure induced by TZDs may be partly explained by the increase of UCP3 gene expression in the WAT and BAT. Previous experiments with chemical uncouplers indicated that uncoupling of electron transport and ATP synthesis increases glucose uptake in 3T3-L1 adipocytes (13,14). Therefore, this increased UCP3 gene expression may lead to increase of glucose uptake. TZDs have been reported to increase glucose uptake in 3T3-L1 adipocytes (33–36). Increased UCP3 gene expression may be involved in the increase of glucose uptake by TZDs. It is, thus, tempting to speculate that this increased UCP3 gene expression may be involved in the improvement of glucose metabolism by TZDs.

In the present study, UCP3 gene expression was not significantly changed in WAT or skeletal muscle in Wistar lean rats in response to 3 mg · kg^{-1} · day^{-1} pioglitazone for 2 weeks, although UCP3 mRNA levels were increased (131 ± 7%) significantly in BAT (P < 0.05). In another series of experiments, we observed 1.5-fold, 4.5-fold, and 1.6-fold increase of the UCP3 gene expression in the epididymal WAT (P < 0.01), mesenteric WAT (P < 0.01), and retroperitoneal WAT (P < 0.05), respectively, in 12-week-old Wistar lean rats (n = 4) by administration of 10 mg · kg^{-1} · day^{-1} of pioglitazone for 2 weeks (data not shown). Therefore, a higher dose of TZDs was necessary for the increase of UCP3 gene expression in Wistar lean rats compared with that in Wistar fatty rats, which may be related to the fact that plasma glucose and insulin levels are reduced much more markedly by TZDs in Wistar fatty rats than in Wistar lean rats (37).

In conclusion, we demonstrate that basal UCP3 mRNA levels were lower in WAT, BAT, and skeletal muscle in Wistar fatty rats compared with that in Wistar lean rats, while basal UCP2 mRNA levels were higher in the WAT and BAT. We also demonstrate that TZDs increased the UCP3 gene expression in the adipocytes both in vivo and in vitro, without significant change of UCP2 mRNA levels. Because UCPs have been implicated in energy and glucose metabolism, the present study proposes involvements of UCP3 in some aspects of TZD action.

ACKNOWLEDGMENTS

This work was supported in part by research grants from the Japanese Ministry of Education, Science and Culture; the Japanese Ministry of Health and Welfare; the Yamanouchi Foundation for Research on Metabolic Disorders; the Uehara Memorial Foundation; the Naito Foundation; the Cell Science Research Foundation; the Ono Medical Research Foundation; Otsuka Pharmaceutical, Tokushima, Japan; the Japan Diabetes Foundation; and the Tanabe Medical Frontier Conference. K.D. is the recipient of the Research Fellowships grant of the Japan Society for the Promotion of the Science for Young Scientists.

We acknowledge Drs. T. Tanaka, M. Shintani, and Y. Fukunaga for help and K. Hiramatsu for excellent secretarial assistance.

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Author Queries (please see Q in margin and underlined text)

Q1:“recently discovered” OK for “new members of”? If not, please reword for clarity.
Q2: OK to delete “1x” here and in results, as in abstract?
Q2a: “either” OK for “these” here?
Q3: Rewording of sentence beginning “All the cells...” OK? If not, please reword for clarity.
Q4: “was decreased to...” OK here? If not, please reword for clarity.
Q5: “increased to...” OK?
Q6:“in addition to” OK for “as well as”?
Q7: “another” meant here?>

Refs. 16, 23, 37: Correct that “Drug Res” expands to “Drug Research”? If not, please provide expansion. Is (I) a supplement number?

Ref. 19: Please confirm that *Cell* is the correct journal (changed from *J Biol Chem*)

Ref. 21: Please confirm that 51 is the correct volume number (changed from 57).>

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