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Original Article

Brown Adipose Tissue PPARγ Is Required for the Insulin-Sensitizing Action of Thiazolidinediones

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Brown adipose tissue (BAT) plays a critical role in metabolic homeostasis. BAT dysfunction is associated with the development of obesity through an imbalance between energy expenditure and energy intake. The nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) is the master regulator of adipogenesis. However, the roles of PPARγ and thiazolidinediones (TZDs) in the regulation of BAT metabolism remain unclear. TZDs, which are selective PPARγ activators, improve systemic insulin resistance in animals and humans. In the present study, we generated brown adipocyte-specific PPARγ-deficient mice (BATγKO) to examine the *in vivo* roles of PPARγ and TZDs in BAT metabolism. In electron microscopic examinations, brown adipocyte-specific PPARγ deletion promoted severe whitening of brown fat and morphological alteration of mitochondria. Brown adipocyte-specific PPARγ deletion also reduced mRNA expression of BAT-selective genes. Although there was no difference in energy expenditure between control and BATγKO mice in calorimetry, norepinephrine-induced thermogenesis was impaired in BATγKO mice. Moreover, pioglitazone treatment improved diet-induced insulin resistance in the control mice but not in the BATγKO mice. These findings suggest that BAT PPARγ is necessary for the maintenance of brown adipocyte function and for the insulin-sensitizing action of TZDs.

Key words: PPARγ, brown adipose tissue, thiazolidinediones

Adipose tissue includes two functionally distinct types: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is the major site for the storage of excess energy from food intake as triacylglycerol (TAG). TAG is broken down into free fatty acids and glycerol and is released to supply other tissues. In addition, recent research showed that adipose tissue secretes a variety of bioactive proteins and regulates both systemic insulin sensitivity and energy metabolism. A positive balance between energy intake and energy expenditure results in the development of obesity. Adipose tissue dysfunction in obesity is characterized by adipocyte hypertrophy, fibrosis, and a pro-inflammatory adipose tissue phenotype, which result in ectopic fat deposition and inflammation in visceral adipose tissue [1,2]. Brown adipose tissue (BAT) is thermogenic and contains large amounts of mitochondria to dissipate chemical energy as heat. BAT stimulation activates the release of free fatty acids from TAG and uses uncoupling protein 1 (UCP1) to uncouple the respiratory chain. BAT stimulation also results in heat production [3]. Though it had been thought that active BAT in humans occurs only in newborns,

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positron emission tomography/computer tomography (PET/CT) recently revealed that healthy adult humans possess active BAT also. PET/CT signals in BAT were lower in elderly, obese, and insulin-resistant patients [4]. Recent work has shown that BAT activation or increased BAT mass can improve glucose metabolism [5]. Increasing BAT mass by transplantation rescued insulin resistance induced by a high-fat diet [6, 7]. BAT is now recognized as a potential therapeutic target of obesity and type 2 diabetes.

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear hormone receptor superfamily of ligand-gated transcription factors [8]. PPARγ has been shown to play roles in diverse biological processes, including adipogenesis, and both glucose and lipid metabolism. The antidiabetic thiazolidinedione (TZD) drugs exert their beneficial effects on hyperglycemia and systemic insulin resistance by activating PPARγ in patients with type 2 diabetes [9]. However, there is debate about which cell acts as the primary mediator of the antidiabetic effects of TZDs. We therefore used BAT-specific PPARγ deletion to determine whether PPARγ or TZDs could regulate BAT functions in mice. Here we show that BAT-specific PPARγ deletion in mice results in reduced BAT mass and impaired norepinephrine-induced thermogenesis. Moreover, BAT PPARγ is required for the insulin-sensitizing action of TZD.

Materials and Methods

Materials. Dimethyl sulfoxide and pioglitazone were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Generation of BATγKO mice. *Pparg*$^{flox/flox}$ mice (IMSR_JAX: 004584) and *Ucp1*-Cre mice (IMSR_JAX: 024670) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA), and cohorts were established by mating F1 *Pparg*$^{flox/+}$; *Cre*$^{+}$ mice to littermate *Pparg*$^{flox/+}$; *Cre*$^{-}$ mice. All animals were genotyped before use to confirm that they were either *Pparg*$^{flox/flox}$; *Cre*$^{+}$ mice or *Pparg*$^{flox/flox}$; *Cre*$^{-}$ mice. The mice were maintained under a 14 h light/10 h dark cycle at a constant temperature (22°C) with free access to food and water; they were fed either a standard chow diet (MF; Oriental Yeast, Tokyo) or a high-fat diet (HFD) (D12331; Research Diets, New Brunswick, NJ, USA). Total fat tissue was measured by microcomputed tomography (LaTheta LCT-200; Hitachi-Aloka Medical, Tokyo).

For analysis of *Pparg* mRNA expression in various tissues, 10-week-old male *Pparg*$^{flox/flox}$; *Cre*$^{+}$ mice and *Pparg*$^{flox/flox}$ mice were used. The mice were maintained in a light-controlled room at a constant temperature with free access to food and water; they were fed either the standard chow diet or the high-fat diet. After 16 weeks on the HFD, the mice were administered pioglitazone (15 mg/kg body weight) or dimethyl sulfoxide once daily by oral gavage for 4 weeks.

All animal studies were approved by the Institutional Animal Care and Use Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (approval number: OKU-2013017).

Glucose and insulin tolerance tests. For the oral glucose tolerance test, the mice were fasted for 16 h and then administered glucose (1.0 g/kg body weight) by oral gavage. Blood samples were taken at regular time points (0-120 min), and blood glucose levels were measured with a portable glucose meter. For the insulin tolerance tests, the mice were fasted for 4 h and handled 30 min prior to injection of human regular insulin (2.0 U/kg body weight) intraperitoneally. Blood samples were taken at regular intervals (0-120 min), and blood glucose was measured as described above.

Indirect calorimetry. The metabolic rates of the mice were measured by an O_2/CO_2 metabolism measuring system for small animals (MK-5000RQ; Muromachi Kikai, Tokyo). Locomotor activity was measured by an ACTIMO-100 monitoring system (Shinfactory, Fukuoka, Japan). Mice were housed individually and maintained at 22°C under a 12: 12-h light-dark cycle. Food and water were available ad libitum.

Norepinephrine-induced thermogenesis was measured as described previously [10]. Briefly, the mice were anesthetized with pentobarbital (90 mg/kg, *i.p.*), and indirect calorimetry was performed for 30 min at 33°C to obtain basal respiration. Individual mice were then briefly removed from the calorimetry chamber, injected with norepinephrine (1 mg norepinephrine bitartrate/kg, subcutaneously), and returned to the chamber. Oxygen consumption was then measured for another 60-80 min.

Analysis of gene expression by QPCR. Total RNA was extracted from tissues using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. First-strand

cDNA synthesis was performed using a PrimeScript™ first-strand cDNA Synthesis Kit (Takara Bio, Kusatsu, Japan). Total RNA was converted into first-strand cDNA using oligo(dT) primers as described by the manufacturer. PCR was performed using cDNA synthesized from 0.5 μg total RNA in a StepOne Plus Real-Time PCR system (Thermo Fisher Scientific) with specific primers and SYBR Premix Ex Taq™ (Takara Bio). The relative abundance of mRNAs was standardized using *36B4* mRNA as the invariant control. The primers used are listed in the Table 1.

Serum analysis. After serum was collected from the tail vein, serum AST, ALT, and TG were measured using a Fuji Drychem 7000V (Fujifilm, Tokyo). Concentrations of serum adiponectin (Otsuka Pharmaceutical, Tokyo), non-esterified fatty acids (NEFAs) (Wako Pure Chemical Industries, Osaka, Japan), and insulin (Morinaga Institute of Biological Science, Yokohama, Japan) were measured in duplicate with colorimetric assays.

Histological analysis. For light microscopic analysis, brown adipose tissue, epididymal adipose tissue, and liver tissue were fixed for 12-16 h at room temperature and embedded in paraffin. Hematoxylin and eosin (H&E) staining was performed. For electron microscopic analysis, tissues were prepared as described previously [11].

Statistical analysis. Unpaired two-tailed Student's *t*-test and two-way ANOVA were used. $P < 0.05$ was considered statistically significant.

Results

BAT-specific deletion of PPARγ results in reduced BAT mass. To investigate the physiological role of PPARγ in BAT, we crossed *Pparg*$^{flox/flox}$ mice with mice expressing Cre recombinase in a brown adipocyte-specific manner (Ucp1-Cre), thus generating BAT-specific PPARγ-knockout mice (hereafter referred to as BATγKO mice). BATγKO mice showed a virtually complete absence of PPARγ mRNA in BAT, with intact PPARγ mRNA expression in WAT, skeletal muscle, and other tissue (Fig. 1A). The BATγKO mice were born in a Mendelian ratio, and there were no obvious morphological differences from the control mice (Wild type, Ucp1-Cre only, or *Pparg*$^{flox/flox}$) at a young age (data not shown). The total body weight of the BATγKO mice was not different from that of the control mice

Table 1 PCR primers used to amplify genes of interest in this study

Gene	Primer	Sequence
Pparg	Forward	TTGAGCCCAAGTTCGAGTTTG
	Reverse	CGGTCTCCACACAGAATGATG
Fabp4	Forward	TTCGATGAAATCACCGCAGA
	Reverse	AGGGCCCCGCCATCT
Cidea	Forward	TGACATTCATGGGATTGCAGAC
	Reverse	CATGGTTTGAAACTCGAAAAGGG
Cox8b	Forward	GAACCATGAAGCCAACGACT
	Reverse	GCGAAGTTCACAGTGGTTCC
Dio2	Forward	AATTATGCCTCGGAGAAGACCG
	Reverse	GGCAGTTGCCTAGTGAAAGGT
Elovl3	Forward	GATGGTTCTGGGCACCATCTT
	Reverse	CGTTGTTGTGTGGCATCCTT
Pgc1a	Forward	TATGGAGTGACATAGAGTGTGCT
	Reverse	GTCGCTACACCACTTCAATCC
Ppara	Forward	AACATCGAGTGTCGAATATGTGG
	Reverse	CCGAATAGTTCGCCGAAAGAA
Prdm16	Forward	CCACCAGCGAGGACTTCAC
	Reverse	GGAGGACTCTCGTAGCTCGAA
Ucp1	Forward	GACCTTCCCGCTGGACACT
	Reverse	CCCTAGGACACCTTTATACCTAATGG
Adipoq	Forward	GTTGCAAGCTCTCCTGTTCC
	Reverse	GCTTCTCCAGGCTCTCCTTT
Ccl2	Forward	TAAAAACCTGGATCGGAACCAAA
	Reverse	GCATTAGCTTCAGATTTACGGGT
Emr1	Forward	CTGCACCTGTAAACGAGGCTT
	Reverse	GCAGACTGAGTTAGGACCACAA
Il6	Forward	TAGTCCTTCCTACCCCAATTTCC
	Reverse	TTGGTCCTTAGCCACTCCTTC
Ppard	Forward	TTGAGCCCAAGTTCGAGTTTG
	Reverse	CGGTCTCCACACAGAATGATG
Slc2a4	Forward	GATTCTGCTGCCCTTCTGTC
	Reverse	ATTGGACGCTCTCTCTCCAA
Tnfa	Forward	ACGGCATGGATCTCAAAGAC
	Reverse	AGATAGCAAATCGGCTGACG
Ucp3	Forward	TACCCAACCTTGGCTAGACG
	Reverse	GTCCGAGGAGAGAGCTTGC
Cd36	Forward	GGAGCCATCTTTGAGCCTTC
	Reverse	TGGATCTTTGTAACCCCACAAG
Fasn	Forward	TGAGCACACTGCTGGTGAAC
	Reverse	CAGGTTCGGAATGCTATCCA
Scd1	Forward	ATCGCCCCTACGACAAGAAC
	Reverse	GTTGATGTGCCAGCGGTACT
Cpt1a	Forward	ACAACAACGGCAGAGCAGAG
	Reverse	GGACACCACATAGAGGCAGAAG
Acc1	Forward	ATCCTGCGAACCTGGATTCT
	Reverse	CCCACCAGAGAAACCTCTCC
36B4	Forward	GAGGAATCAGATGAGGATATGGGA
	Reverse	AAGCAGGCTGACTTGGTTGC

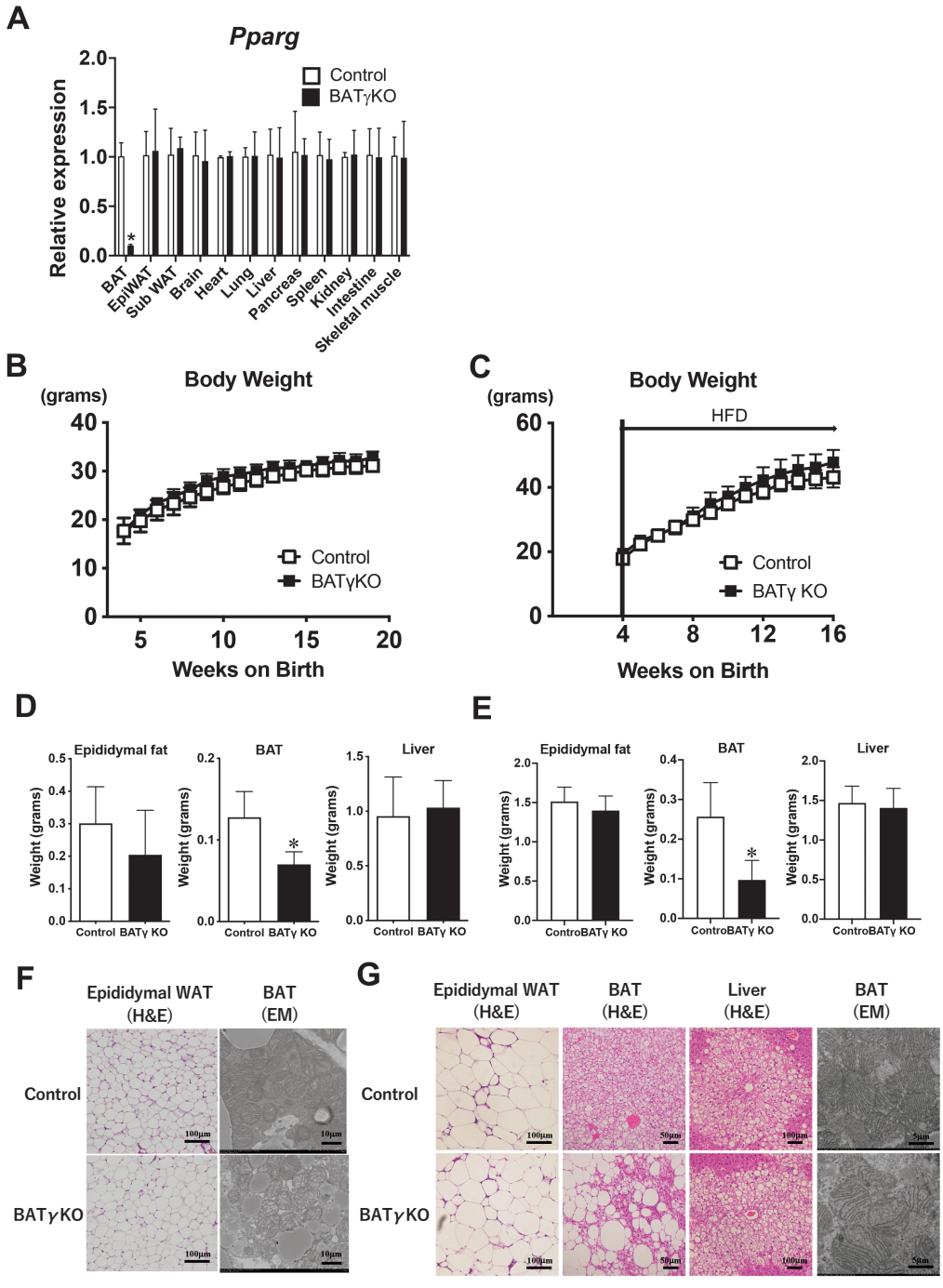

Fig. 1 Characterization of brown adipocyte-specific PPARγ-deficient (BATγKO) mice. **A**, *Pparg* mRNA expression in various tissue of male $pparg^{flox/flox}$ (Control) and BATgKO mice (n = 5); **B**, Body weights of male control and BATγKO mice on a chow diet (n = 10). Results are expressed as mean ± SD; **C**, Body weights of male control and BATγKO mice on a high-fat diet (HFD) (n = 10). Results are expressed as mean ± SD; **D**, Tissue weights of male control and BATγKO mice on a chow diet (n = 4–7). Results are expressed as mean ± SD, $*p < 0.05$; **E**, Tissue weights of male control and BATγKO mice on the HFD (n = 6). Results are expressed as mean ± SD, $*p < 0.05$; **F**, H&E staining analysis of epididymal WAT and electron microscopic analysis of BAT in male control and BATγKO mice fed the chow diet; **G**, H&E staining and electron microscopy of WAT, BAT, and liver in male control and BATγKO mice fed the HFD. The experiments were repeated 2–3 times with different sets of animals.

(*Pparg*$^{flox/flox}$) on either the chow diet or the HFD (Fig. 1B and 1C), although the BAT weight of the BATγKO mice was significantly smaller than that of the control mice (Fig. 1D and 1E). Histological analysis of epididymal WAT showed no difference in adipocyte morphology between the control and BATγKO mice on either the chow diet or the HFD. The HFD induces a white-like unilocular adipocyte phenotype in BAT. Brown-to-white conversion was seen in H&E staining of the control BAT, while the BAT of the BATγKO mice showed a mixture of larger white-like unilocular adipocytes and scattered brown adipocytes. Electron microscopic analysis showed accumulation of lipid droplets and disruption of the mitochondrial cristae structure in BAT of the BATγKO mice (Fig. 1F and 1G). We measured serum AST, ALT, NEFA, and triglyceride levels under fasting condition, but there were no obvious differences in any of those levels, or in serum adiponectin levels, between the control and BATγKO mice (Fig. 2A). Next, we measured fasting blood glucose and fasting insulin levels between the control and BATγKO mice on the chow diet. The homeostasis model of assessment-insulin resistance (HOMA-R) was calculated using the following formula: fasting insulin (μU/mL) × fasting glucose (mg/dL) / 405. The homeostasis model of assessment-beta cell (HOMA-β) was calculated as follows: 360 × fasting insulin (μU/mL) / (fasting glucose (mg/dL) – 63). No differences in fasting blood glucose, fasting insulin levels, HOMA-R, or HOMA-β were observed between the control and BATγKO mice. These results suggest that the BAT-specific deletion of PPARγ induced severe whitening of BAT without any changes in serum parameters.

BAT-specific deletion of PPARγ does not change energy expenditure. Although BAT-specific deletion of PPARγ showed no effect on body weight in mice fed either the chow diet or the HFD, BAT plays a critical role in the regulation of energy expenditure. We next

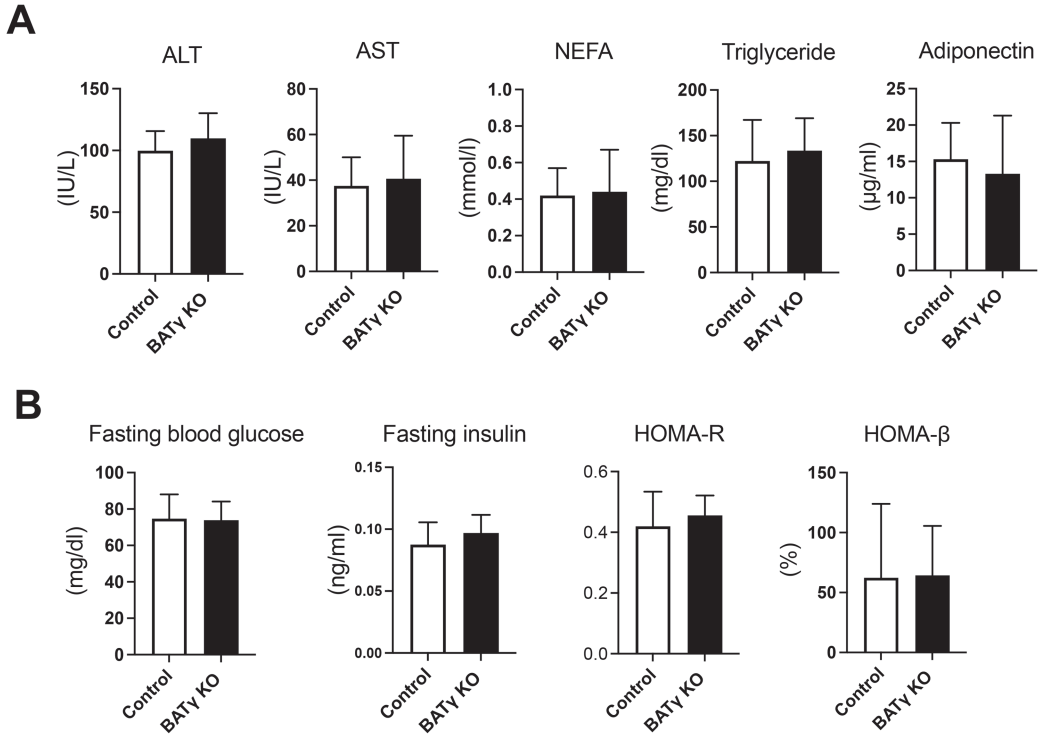

Fig. 2 Serum analysis of brown adipocyte-specific PPARγ-deficient (BATγKO) mice. **A**, Serum parameters of male control and BATγKO mice on the chow diet (n = 10); **B**, Fasting blood glucose and fasting insulin: Tail vein blood was used for glucose and insulin measurement after 16 h fasting during chow diet feeding. The homeostasis model of assessment-insulin resistance (HOMA-R) was calculated using the following formula: fasting insulin (μU/mL) × fasting glucose (mg/dL) / 405. The homeostasis model of assessment-beta cell (HOMA-β) was calculated as follows: 360 × fasting insulin (μU/mL) / (fasting glucose (mg/dL)−63). The experiments were repeated 2–3 times with different sets of animals.

investigated the effect of BAT-specific deletion of PPARγ on physical activity, daily food intake, and energy expenditure. We were unable to detect a difference in food intake or oxygen consumption, but BATγKO mice showed somewhat increased ambulatory activity on both the chow diet and HFD (Fig. 3A and 3B). The body temperature of the BATγKO mice was lower than that of the control mice (Fig. 3C). These results suggest that increased physical activity may have offset the decrease in energy metabolism in BATγKO mice.

BAT-specific deletion of PPARγ abolishes norepinephrine-induced thermogenesis. To investigate energy metabolism in BATγKO mice under conditions not influenced by physical activity, norepinephrine-induced thermogenesis was measured under sedation (Fig. 4A). Similar to the results of the metabolic cage analysis, there was no difference in resting metabolic rate (RMR) between BATγKO and control mice under sedation. However, the response to norepinephrine injection was lower in BATγKO mice on both the chow diet and the HFD (Fig. 4B and 4C). These data suggest that BAT-specific deletion of PPARγ abolished norepinephrine-induced thermogenesis.

BAT-specific deletion of PPARγ enhances the insulin-sensitizing action of thiazolidinediones. Glucose tolerance and insulin sensitivity were similar between BATγKO and control mice fed the chow diet (Fig. 5A and 5B). On the HFD, BATγKO mice demonstrated significantly impaired glucose tolerance, although insulin sensitivity was similar between BATγKO and control mice (Fig. 5C and 5D). To determine whether BAT PPARγ signaling contributes to the insulin-sensitizing action of TZD, we fed mice the HFD to induce obesity and insulin resistance, followed by the HFD supplemented with pioglitazone. Interestingly, the addition of pioglitazone enhanced insulin sensitivity only in control mice, not in BATγKO mice (Fig. 5E). These data suggest that BAT PPARγ signaling is required for the insulin-sensitizing action of TZDs.

Gene expression in BATγKO mice and control mice. We next examined the expression of metabolism-related genes in BAT, WAT, liver, and skeletal muscle of BATγKO mice and control mice on the chow diet. The mRNA expression levels of *Dio2*, *Pgc1a*, *Ppara*, and *Ucp1* were significantly lower in BAT of the BATγKO mice than in that of the control mice (Fig. 6A). Expression levels of metabolism genes were unchanged in the WAT, liver, and skeletal muscle. Consistent with previous reports [12, 13], pioglitazone administration to control mice fed on the HFD increased brown adipocyte genes, but did not increase brown adipocyte genes of the BATγKO mice fed on the HFD (Fig. 6B). Taken together, these results suggest that BAT PPARγ is necessary for TZD-induced upregulation of brown adipocyte genes in BAT and might regulate the insulin-sensitizing action of TZDs.

Discussion

We have been interested in the role of PPARγ in BAT. In the present study, we critically analyzed BAT PPARγ function by using Cre-lox technology to target PPARγ in a BAT-specific manner. The most striking differences between the control and BATγKO mice were BAT mass and norepinephrine-induced thermogenesis.

PPARγ is essential for both white and brown adipogenesis [9]. PPARγ regulates brown adipogenesis by interacting with many transcription factors such as PPARγ coactivator-1a (PGC-1a), PR domain-containing 16 (PRDM16), and CCAAT-enhancer-binding proteins (C/EBPs) [14]. Thus, we speculate that the impairment of brown adipogenesis was the reason why BATγKO mice showed reduced BAT mass. PGC-1a, which is a master regulator of mitochondrial biogenesis, is highly expressed in BAT and transcriptionally activates the brown fat thermogenic program [15]. The loss of PPARγ downregulates the expression of certain brown adipocyte-specific genes, including Pgc1a, Ucp1, and Dio2; thus, mitochondrial biogenesis and thermogenesis are expected to be impaired in BAT. Contrary to expectations, BATγKO mice failed to show an obese phenotype. We also found no significant metabolic differences in BATγKO mice compared to control mice at the standard temperature of 22°C.

Thermoneutrality is defined as the ambient temperature range in which basal metabolism is sufficient to maintain body temperature. BAT activation or recruitment is not necessary at a thermoneutral temperature [10]. We speculate that the BATγKO mice were not able to use BAT-derived heat production at the standard temperature due to the reduced BAT mass. However, increased physical activity compensated for the impaired BAT function in the BATγKO mice. That may be why we could not see the metabolic difference between the BATγKO and control mice under the standard condition. To our knowledge, there are no reports

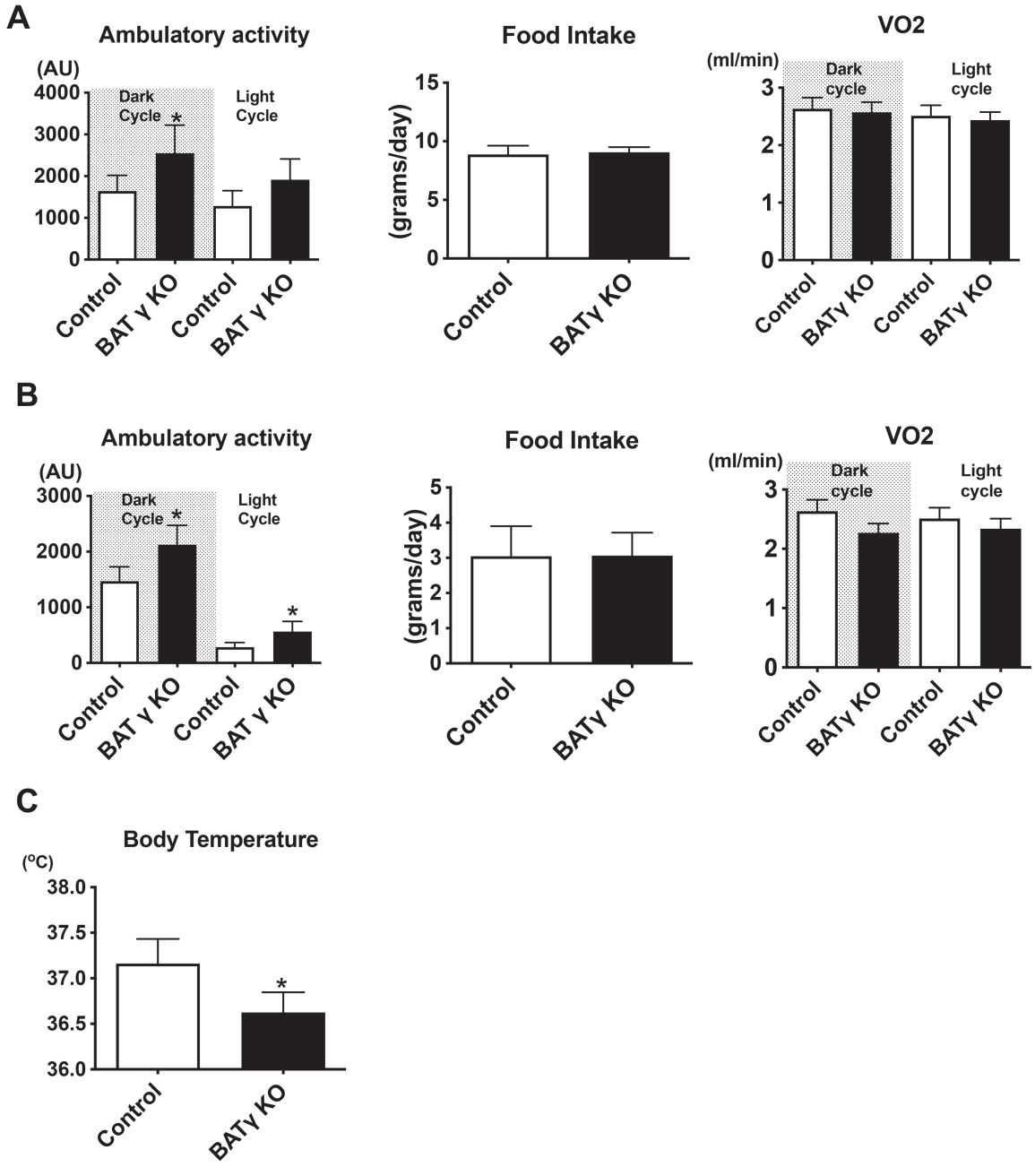

Fig. 3 Effects of brown adipocyte-specific PPARγ deletion on metabolic rates. **A**, Ambulatory activity, food intake, and metabolic rate of male control and BATγKO mice on the chow diet (n=5). Results are expressed as mean ± SD, $*p<0.05$; **B**, Ambulatory activity, food intake and metabolic rate of male control and BATγKO mice on the HFD (n=5). Results are expressed as mean ± SD, $*p<0.05$; **C**, Body temperature of male control and BATγKO mice on the HFD (n=5). The experiments were repeated 2 times with different sets of animals.

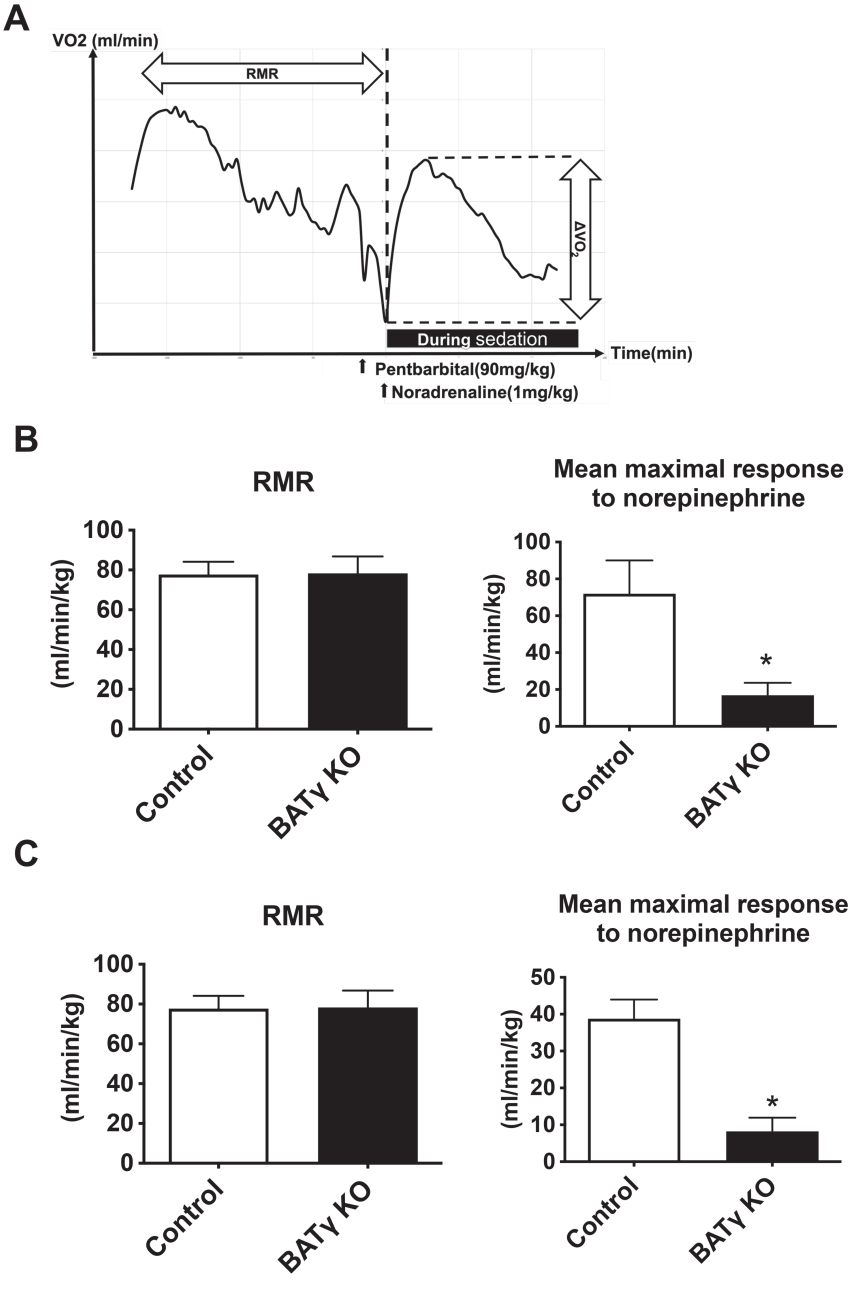

Fig. 4 Effects of brown adipocyte-specific PPARγ deletion on norepinephrine-induced thermogenesis. **A**, Protocol for the comparison of norepinephrine-induced thermogenesis between control and BATγKO mice; **B**, Resting metabolic rate (RMR) and norepinephrine-induced oxygen consumption on the chow diet (n=5). Results are expressed as mean ± SD, $^*p<0.05$; **C**, RMR and norepinephrine-induced oxygen consumption on the HFD (n=5). Results are expressed as mean ± SD, $^*p<0.05$. The experiments were repeated 2 times with different sets of animals.

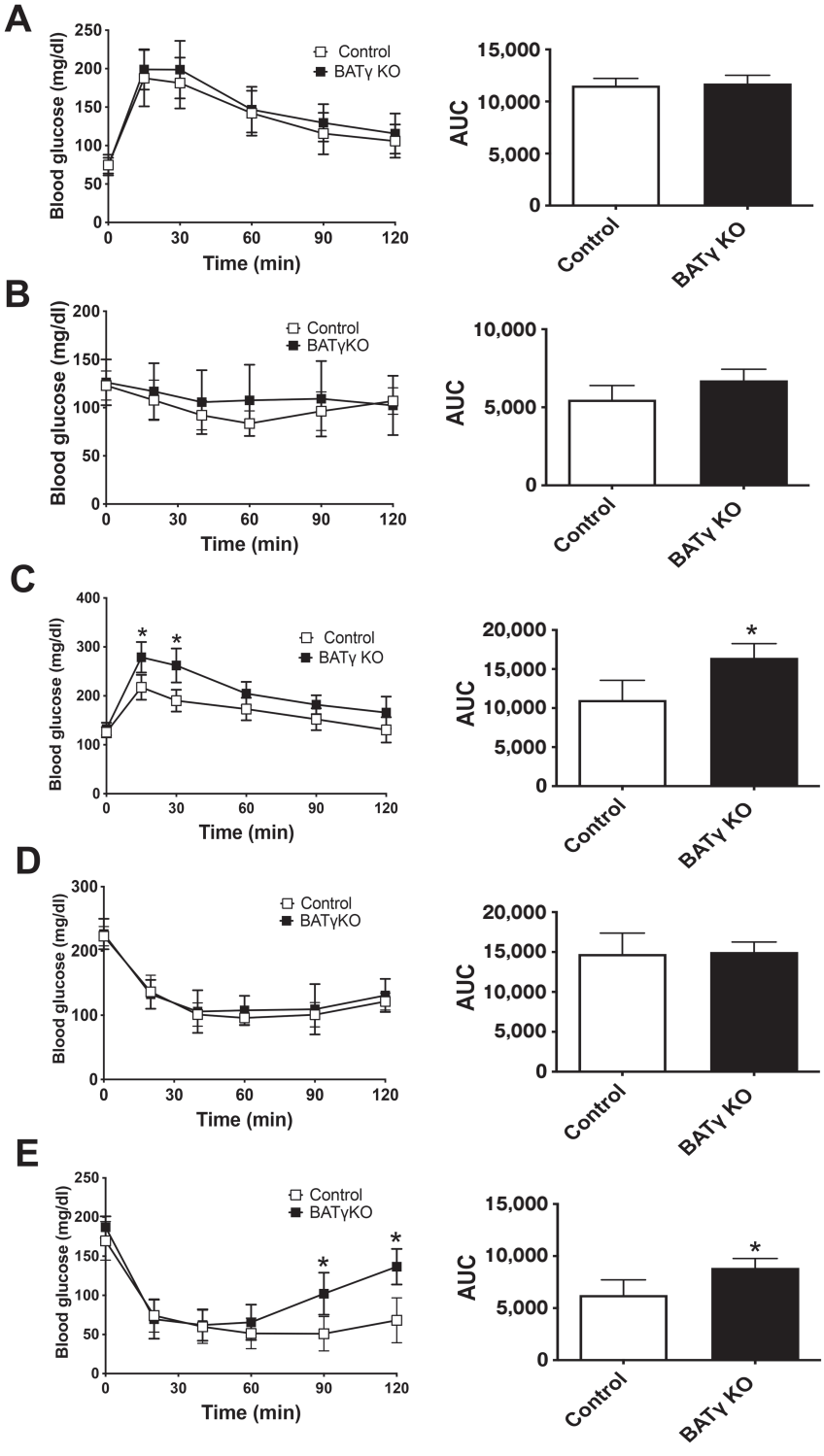

Fig. 5 Effects of brown adipocyte-specific PPARγ deletion on glucose metabolism and the insulin-sensitizing action of pioglitazone. **A**, Glucose tolerance test on the chow diet. Blood glucose in 20-week-old male control and BATγKO mice fed the chow diet was measured at the indicated times after oral gavage of glucose (n = 10). Results are expressed as mean ± SD; **B**, Insulin tolerance test on the chow diet. Blood glucose in 20-week-old male control and BATγKO mice fed the chow diet was measured at the indicated times after intraperitoneal injection with a bolus of insulin (n = 10). Results are expressed as mean ± SD; **C**, Glucose tolerance test on the HFD. Blood glucose in 20-week-old male control and BATγKO mice fed the HFD was measured at the indicated times after oral gavage of glucose (n = 10). Results are expressed as mean ± SD; **D**, Insulin tolerance test on the HFD. Blood glucose in 20-week-old male control and BATγKO mice fed the HFD was measured at the indicated times after intraperitoneal injection with a bolus of insulin (n = 10). Results are expressed as mean ± SD; **E**, Insulin tolerance test on the HFD after pioglitazone treatment. Blood glucose in male control and BATγ KO mice fed the HFD was measured at the indicated times after intraperitoneal injection with a bolus of insulin (n = 10). Results are expressed as mean ± SD, $*p < 0.05$. The experiments were repeated 2 times with different sets of animals.

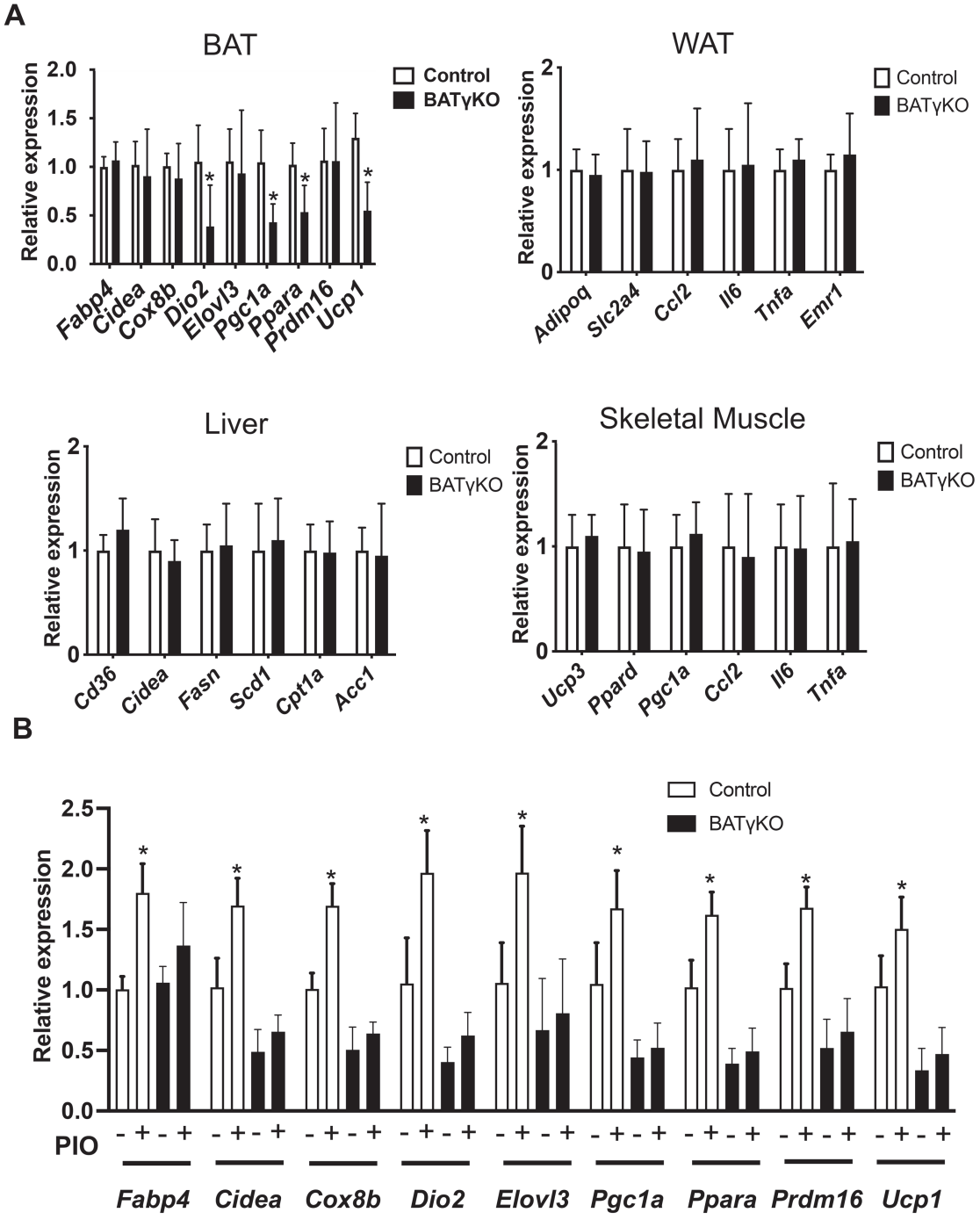

Fig. 6 Effects of brown adipocyte-specific PPARγ deletion on gene expression in BAT. **A**, Transcription levels of the indicated genes analyzed by quantitative RT-PCR on RNA isolated from BAT, WAT, liver, and skeletal muscle of male control and BATγKO mice on the chow diet (n = 10). Results are expressed as mean ± SD, $^{*}p < 0.05$; **B**, Expression levels of brown adipocyte genes in BAT of male control and BATγKO mice on the HFD in the presence and absence of pioglitazone treatment (n = 10). Results are expressed as mean ± SD, $^{*}p < 0.05$. The experiments were repeated 3 times with different sets of animals.

of mouse models in which HFD increases physical activity. It is possible that dysfunction of BAT and deletion of PPARγ in brown adipocytes are involved in this phenomenon, but future studies are needed to resolve this issue.

TZDs were reported as insulin-sensitizing drugs in the early 1980s, though their mechanisms of action were not known until the mid-1990s. The insulin-sensitizing action of TZDs is mediated through PPARγ, and TZDs improve glucose homeostasis and insulin resistance in type 2 diabetes. The mechanisms by which TZDs improve systemic insulin resistance include altering body composition, decreasing intramyocellular and intrahepatocellular fat, decreasing circulating free fatty acid levels, and decreasing circulating proinflammatory cytokines, as well as activating the transcription of genes that regulate adipogenesis, glucose, and lipid metabolism [16]. Because PPARγ is most highly expressed in WAT, the insulin-sensitizing action of TZDs is thought to directly affect WAT. However, PPARγ is expressed in BAT and non-adipose tissue. Two studies reported that TZDs improve systemic insulin resistance in lipodystrophic mice [17,18], indicating that TZDs have another pharmacological target and contribute to the insulin-sensitizing action of TZDs in non-adipose tissue. Several studies have used non-adipose tissue-specific knockouts of PPARγ. Hevener AL *et al.* reported that skeletal muscle-specific PPARγ-knockout mice developed insulin resistance in old age, and the skeletal muscles of these mice were unresponsive to TZD treatment [19]. Gavrilova O *et al.* reported that liver-specific PPARγ-knockout mice showed increased adiposity, hyperlipidemia, and insulin resistance, and these mice remained responsive to TZD treatment [20]. In one study, β cell-specific PPARγ-knockout mice showed alteration in β-cell mass without a change in glucose homeostasis [21]. In another study, the loss of PPARγ in the whole pancreas resulted in hyperglycemia with impaired glucose-stimulated insulin secretion [22]. Two independent groups have reported that PPARγ action in the central nervous system contributes to TZD-induced weight gain through increased leptin sensitivity [23,24]. In addition, several studies revealed that PPARγ plays critical roles in various immune cells, macrophages [25,26], and dendritic cells [27,28]. Taken together, these data suggest that the insulin-sensitizing action of TZDs works through not only white adipocytes but also various cell types, including brown adipocytes.

In summary, our data suggest that BAT PPARγ enhances the insulin-sensitizing action of TZDs in mice on a HFD. BAT-specific PPARγ deletion in mice results in reduced BAT mass and impaired norepinephrine-induced thermogenesis. Moreover, BAT PPARγ deletion fails to enhance systemic insulin sensitivity in pioglitazone-treated mice on a HFD. PPARγ appears to play critical roles in the development of BAT and glucose metabolism. BAT might be a key target of insulin-sensitizing action in TZD-treated patients with type 2 diabetes.

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