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The role of T-bet in obesity: lack of T-bet causes obesity in male mice

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Abstract

The association of T helper (Th) 1 cells with obesity is well documented in both animals and humans. The T-box transcription factor (T-bet) is known as the transcription factor that is responsible for the development of Th1 cells. However, the role of T-bet in obesity has never been elucidated. The present study aimed to investigate the regulatory function of T-bet on obesity in mice. Th1 cytokine levels were decreased, whereas Th2 cytokine level and GATA-3 messenger RNA (mRNA) expression were increased in T-bet knockout (KO) mice. T-bet KO male mice induced obesity as a result of increased body weight and food efficiency despite the fact that they feed a control diet. T-bet KO mice have an increase in weight of white adipose tissue and levels of triacylglyceride and low-density lipoprotein cholesterol. Interestingly, the expression levels of energy expenditure-related genes were decreased in T-bet KO mice. Both T-bet KO male and female mice had impaired glucose tolerance. In white adipose tissue, leptin, the increase in peroxisome proliferator receptor-γ and CAAT/enhancer-binding protein α mRNA expressions in T-bet KO mice was more than that in wild-type mice. Furthermore, we found that the level of interleukin (IL)-6 mRNA expression in white adipose tissue was elevated in T-bet KO mice but not IL-1β and tumor necrosis factor-α. IL-6 mRNA expression was increased in adipocyte fraction and stromal vascular fraction in white adipose tissue of T-bet KO mice. Taken together, our results reveal that T-bet may affect obesity through the regulation of IL-6 expression in adipocytes of white adipose tissue.

Keywords: Obesity; T-bet; Leptin; Interleukin-6; Adipose tissue

1. Introduction

Obesity is a chronic disease of multifactorial origin that develops from the interaction of social, behavioral, psychological, metabolic, cellular and molecular factors [1]. It is the condition under which adipose tissue is increased and can be defined as an increase in body weight that results in excessive fat accumulation [2]. Many studies have reported that overweight and obesity are major causes of comorbidities, cardiovascular diseases, various cancers and other health problems, which can lead to further morbidity and mortality [3].

T helper (Th) lymphocytes differentiate into two distinct subsets, Th1 and Th2, as defined by various functions and cytokine characterizations [4,5]. The functional differences between Th subsets are explained primarily through the activities of the cytokines they secrete. Interferon-γ (IFN-γ) is the characteristic cytokine of Th1 cells, which also produce interleukin (IL)-2 and tumor necrosis factor (TNF)-β. IL-4 is the corresponding characteristic cytokine of Th2 cells, which also secrete IL-5, IL-6, IL-9 and IL-13. The T-box transcription factor (T-bet) was first described as the transcription factor that is responsible for the development of Th1 $CD4^+$ T cells [6]. T-bet induces the production of IFN-γ and regulates the Th1 cell-migratory program by regulating the expression of chemokines and chemokine receptors such as CCL3 and CCL4. By inducing IFN-γ in $CD4^+$ T cells, T-bet regulates inflammation and immunoregulation factors. T-bet expression in Th1 cells contributes to chronic inflammatory disease such as Crohn's disease. Neurath et al. [7] detected the increase of the production of Th1 cytokines, IFN-γ and TNF-β, which is associated with patients with Crohn's disease but not with healthy people. In addition, Th1-mediated mouse models of chronic intestinal inflammation showed higher T-bet expression. Whereas overexpression of T-bet exacerbated Th1-related chronic colitis in mice, transfer of T-bet-deficient T cell fails to induce colitis [7]. In contrast, Th2-mediated chronic inflammation, ulcerative colitis, is related with more production of Th2 cytokines, IL-4 and IL-6. T-bet KO mice are more sensitive to Th2-mediated colitis, and high levels of IL-4 production were shown [8]. These reports suggest that T-bet expression in $CD4^+$ T cells balances Th1 and Th2 responses in the inflammation reaction.

Inflammation is associated with the development of obesity-related metabolic diseases [9]. Especially, adipose tissue is associated

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with chronic inflammation. Inflammation influences adipose tissue functions, leading to the modulation of obesity-induced inflammatory responses [10,11]. Nishimura et al. [12] suggest that obese adipose tissue activates $CD8^+$ T cells; it promotes the recruitment and activation of macrophages in adipose tissue. Furthermore, circulating levels of IL-6, a major proinflammatory factor, are related to body mass index, insulin resistance and intolerance to carbohydrates [13]. However, the physiological mechanisms between obesity and T-bet have not been well defined.

In this work, we report studies on the weight gain of T-bet KO mice compared to wild-type (WT) mice on a control diet or high fat (HF) diet feeding condition. We measured white adipose tissue (WAT) and brown adipose tissue (BAT) weight. Furthermore, we measured the rectal body temperature, energy expenditure-related genes [peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator-1 (PGC-1α), uncoupling protein (UCP) 1 and UCP2)] expressions and glucose tolerance. Also, we investigated the changes of fat accumulation, triacylglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, leptin, adiponectin, PPAR-γ and CAAT/enhancer-binding protein-α (C/EBPα). In addition, we analyzed proinflammatory cytokine messenger RNA (mRNA) expressions to investigate the regulator effects of T-bet on the control diet-fed or HF diet-fed mice.

2. Materials and methods

2.1. Reagents

Murine recombinant IFN-γ/IL-2, biotinylated IFN-γ/IL-2 and anti-murine IFN-γ/IL-2 were purchased from Pharmingen (San Diego, CA, USA). Fetal bovine serum (FBS) was purchased from Gibco BRL (Grand Island, NY, USA). Avidin peroxidase, 2′-azino-bis (3-ethylbenzithiazoline-6-sulphonic acid) tablets substrate (ABTS) and other reagents were purchased from Sigma (St. Louis, MO, USA).

2.2. Animal experiments

T-bet KO mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). WT mice were purchased from Daehan Biolink (Daejeon, Republic of Korea). All protocols were approved by the institutional animal care and use committee of Kyung Hee University [KHUASP(SE)-11-004]. HF diet (60% kcal) purchased from the Daehan Biolink (Daejeon, Republic of Korea). The animals were maintained under a 12-h light/dark cycle at a constant temperature of 23±2°C. Four groups of mice were fed for 70 days with (1) WT male and female mice feeding the control diet (CJ Feed Co., Ltd.), (2) WT male and female mice feeding the HF diet, (3) T-bet KO male and female mice feeding the control diet and (4) T-bet KO male and female mice feeding the HF diet. Eighty mice were divided into eight groups of 10 mice each. The animals were given free access to food and tap water for 70 days. Body weight was recorded every week. At the end of this period, the animals were fasted overnight. The next day, they were anesthetized with ketamine and rompun (5:3), and then blood samples were collected by cardiac puncture. The epididymal fat pads, BAT and spleen were immediately weighed.

2.3. Glucose tolerance tests (GTT)

For the GTT, overnight fasted mice were administered orally with 1.0 g/kg glucose for T-bet KO and WT groups. Blood was sampled from the retro-orbital plexus before administration and at the indicated times, and glucose was measured using an OneTouch Ultra glucometer (LifeScan, Milpitas, CA, USA).

2.4. Protein extraction in adipose tissue

Adipose tissue was homogenized in lysis buffer [1 mM EDTA, 10 mM Tris (pH 7.5) and 0.25 M sucrose] containing 0.6 mM phenylmethylsulfonylfluoride, 1 mM Na_2VO_3 and 0.27 trypsin inhibitor units of aprotinin/ml (Sigma) and centrifuged (15,000×g, 10 min, 4°C). The interphase was saved (the upper phase contains triglycerides and free fatty acid (FFA)), 1% Triton X-100 was added and the samples were put on a rocking cradle for 1 h at 4°C and then centrifuged (15,000×g, 50 min, 4°C). The supernatant was stored at −70°C until analyzed.

2.5. Tissue digestion and cell fractionation

Epididymal fat pads were isolated from mice by dissection, washed in sterile phosphate-buffered saline (PBS), minced and washed in Krebs–Ringer bicarbonate (KRB) buffer (pH 7.4) containing 4% albumin and 5 mM glucose [14]. The tissues were incubated with collagenase (2 mg/ml; Sigma) on a shaking platform at 37°C for 1 h. Undigested tissue was removed with forceps, and the adipocytes were then separated from other cells by their ability to float upon low-speed (600×g) centrifugation. The medium below the adipocyte fraction (AF) was centrifuged at 1500×g for 10 min to obtain the stromal vascular fraction (SVF), and the resulting pellet was washed three times with warm KRB buffer. Total RNA was extracted from the two fractions.

2.6. Enzyme-linked immunosorbent assay (ELISA)

The ELISA was devised by coating 96-well plates with mouse monoclonal Ab specific to IFN-γ, IL-2 and IL-4. Before subsequent steps in the assay, coated plates were washed with PBS containing 0.05% Tween 20. All reagents used in this assay were incubated for 2 h at 37°C. Recombinant IFN-γ, IL-2 and IL-4 were diluted and used as a standard. Serial dilutions starting from 10 ng/ml were used to establish the standard curve. Serum samples were diluted by diluent buffer (10% FBS in PBS). Assay plates were exposed sequentially to biotinylated mouse IFN-γ, IL-2, and IL-4, avidin peroxidase and ABTS substrate solution containing 30% H_2O_2. The plates were read at 405 nm.

2.7. Blood analysis

Serum was separated immediately after blood sampling by centrifugation at 10,000×g for 20 min. Levels of glucose, triacylglyceride, HDL cholesterol and LDL cholesterol were determined using the colorimetric enzymatic method of Allain et al. [15], with the modifications of Barham and Trinder [16], using an autoanalyzer (Hitachi 747; Hitachi, Japan).

2.8. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis

The mRNA expression levels were determined by RT-PCR. Total RNA was extracted from frozen adipose tissue (100 mg) using a commercially available acid–phenol reagent (QIAZEN). The concentration of total RNA in the final elutes was determined by spectrophotometry. Total RNA (2–4 mg) was heated at 65°C for 10 min and then chilled on ice. Each sample was reverse transcribed to complementary DNA (cDNA) for 90 min at 37°C using cDNA synthesis kit (Amersham Pharmacia, Newark, NJ, USA). PCR was performed with the following primers for mouse — GATA-3 forward, 5′- CTC CTT TTT GCT CTC CTT TTC-3′; reverse, 5′-AAG AGA TGA GGA CTG GAG TG-3′; PGC-1α forward, 5′-CAC GCA GCC CTA TTC ATT GTT CG-3′; reverse, 5′-GCT TCT CGT GCT CTT TGC GGT AT-3′; UCP1 forward, 5′-ATC AGG CAA CAG TGC CAC TG-3′; reverse, 5′-AGC ATA GAA GCC CAA TGA TG-3′; UCP2 forward, 5′-AAC AGT TCT ACA CCA AGG GC-3′; reverse, 5′-AGC ATG GTA AGG GCA CAG TG-3′; leptin forward, 5′-ACG AGC CCT ACC TCA AGA ACT G-3′; reverse, 5′-ATC TGG CAC CCT CAC TCC ATA G-3′; PPAR-γ forward, 5'-GGT GAA ACT CTG GGA GAT TC-3', reverse, 5'-CAA CCA TTG GGT CAG CTC TT-3'; C/EBPα forward, 5'-AGG TGC TGG AGT TGA CCA GT-3', reverse, 5'-CAG CCT AGA GAT CCA GCG AC-3'; adiponectin forward, 5′-AAG GAC AAG GCC GTT CTC T-3, reverse, 5'- TAT GGG TAG TTG CAG TCA GTT GG-3'; IL-1β forward, 5′-AGG CCA CAG GTA TTT TGT CG-3′; reverse, 5′-GCC CAT CCT CTG TGA CTC AT-3′; IL-6 forward, 5'- CGG GAT CCA TGT TCC CTA CTT CAC AA-3', reverse, 5'-CCC AAG CTT CTA CGT TTG CCG AGT AGA-3'; TNF-α forward, 5'-ATG AGC ACA GAA AGC ATG ATC-3', reverse, 5'-TAC AGG CTT GTC ACT CGA ATT-3'; and GAPDH forward, 5'-GGC ATG GAC TGT GGT CAT GA-3'; reverse, 5-TTC ACC ACC ATG GAG AAG GC-3' — to verify if equal amounts of RNA were used for reverse transcription and PCR amplification from different experimental conditions. Products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide.

2.9. Statistical analysis

Results are expressed as the mean±S.E.M. of independent experiments, and statistical analysis was performed using an independent *t* test to determine differences between T-bet KO and WT mice. All statistical analyses were performed using PASW Statistics 18.0 statistical analysis software. A value of $P<.05$ was considered to indicate statistical significance.

3. Results

3.1. Th1/Th2 cytokine phenotypes in T-bet KO mice

In order to confirm the Th1/Th2 cytokine production, we analyzed IFN-γ, IL-2 and IL-4 production on the spleen of T-bet or WT mice. In the HF diet-fed T-bet KO mice, the levels of splenic Th1 cytokines, IFN-γ and IL-2, were significantly decreased by about 57% and 58%, respectively, compared with the HF diet-fed WT mice. Furthermore, IFN-γ and IL-2 levels were significantly decreased in control diet-fed T-bet KO mice compared to the control diet-fed WT mice, whereas IL-4 level was increased in T-bet KO mice compared with the WT mice in the spleen (Fig. 1A). The same phenomenon was observed in adipose tissue (Fig. 1B). IL-4 levels were significantly increased in HF diet-fed

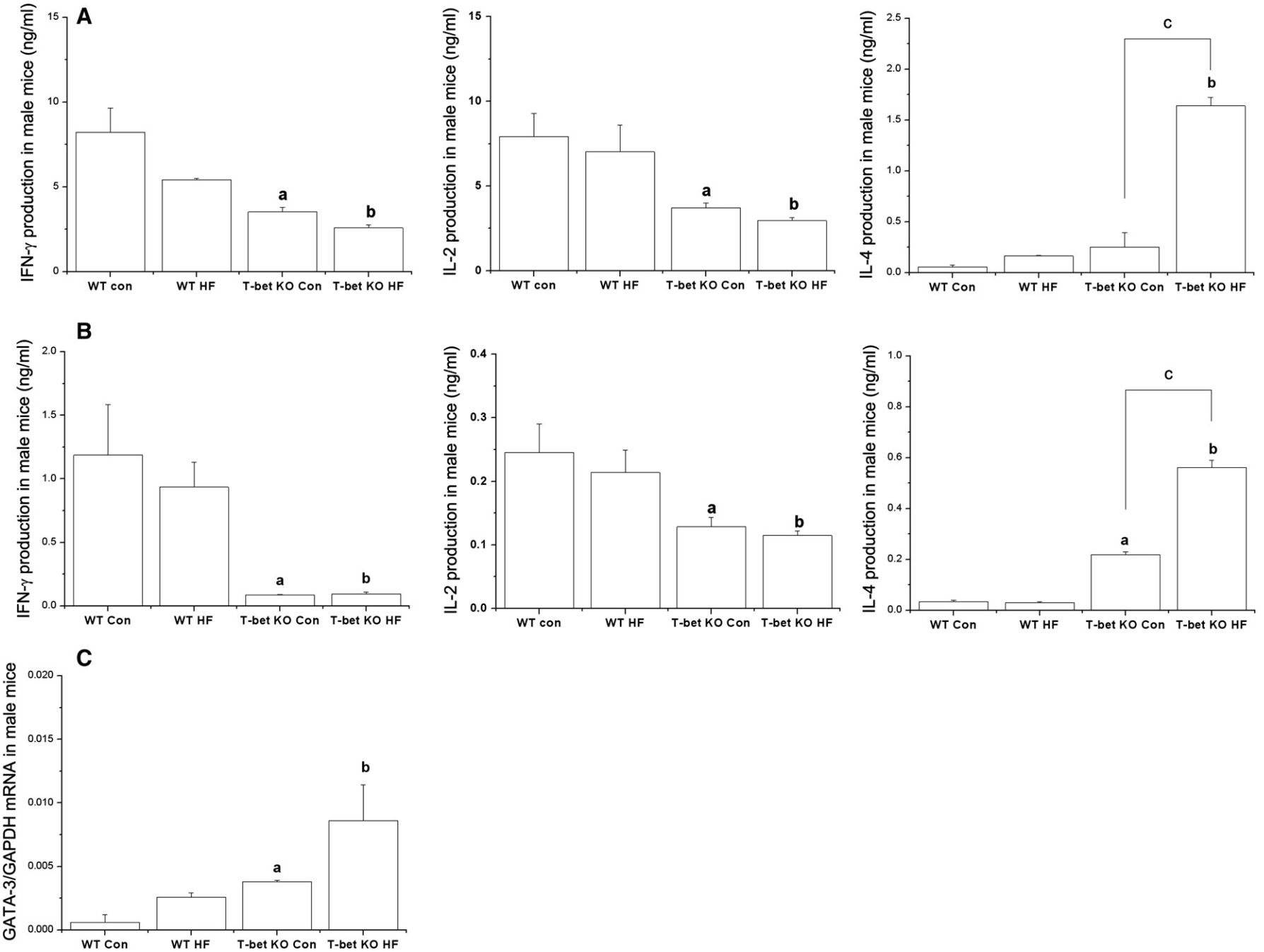

Fig. 1. Th1/Th2 cytokine phenotypes in T-bet KO or WT mice. (A) Spleen. (B and C) Adipose tissue. Values represent mean±S.E.M. $_aP<.05$: significantly different from the control diet-fed WT group. $_bP<.05$: significantly different from the HF diet-fed WT group. $_cP<.05$: significantly different from the control diet-fed T-bet group. Con, control diet; T-bet KO, T-bet KO mice.

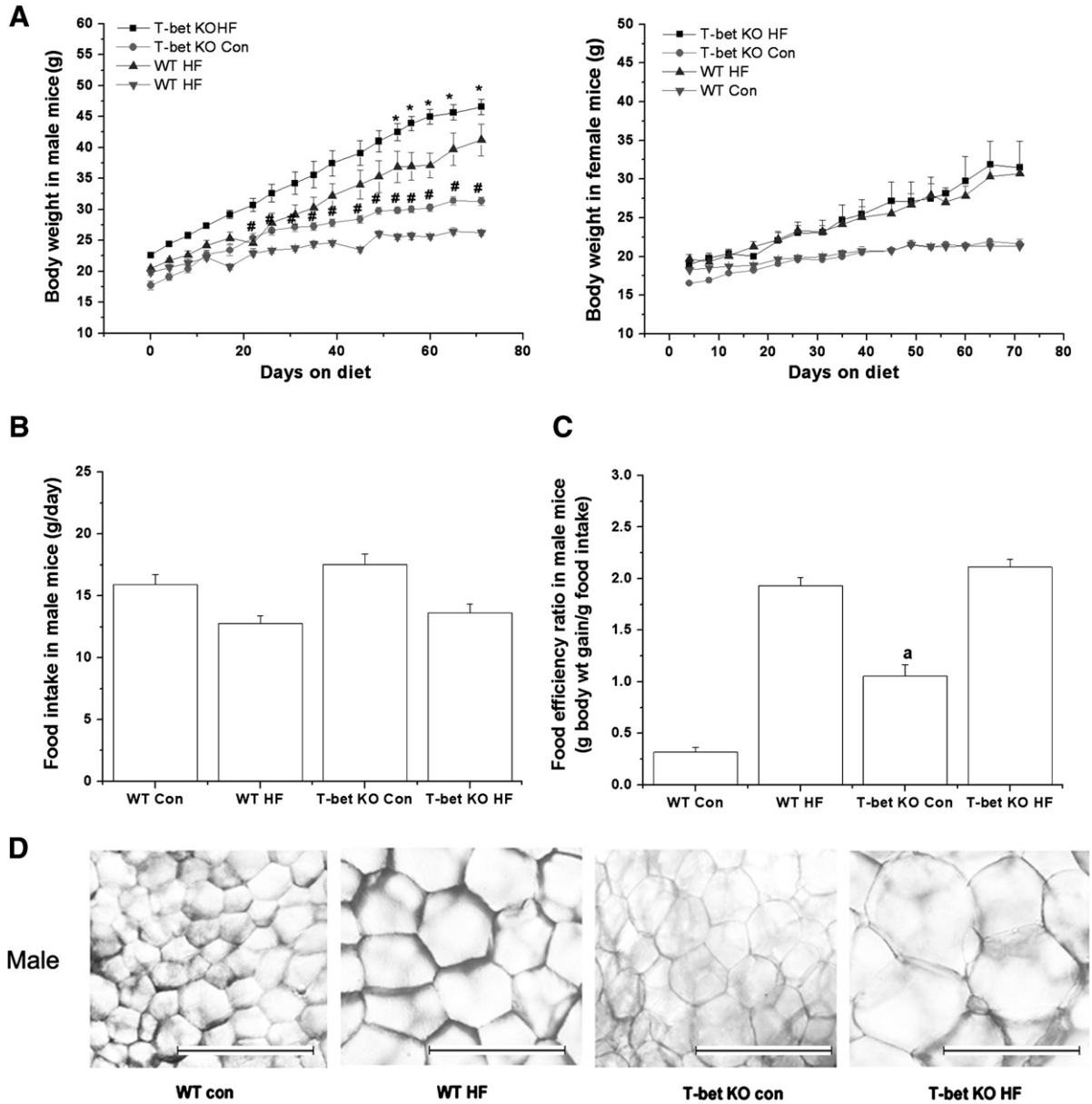

Fig. 2. T-bet regulated body weight on HF diet-induced weight increase. (A) Effects of T-bet deficiency on body weight in the mice fed the HF diet and control diet for 10 weeks. Values represent mean±S.E.M. *P<.05: significantly different from the HF diet-fed WT group. #P<.05: significantly different from the control diet-fed WT group. (B) Amount of food intake. (C) Food efficiency ratio. The values are weight gain divided by food intake. (D) Adipose tissue histology. [a]P<.05: significantly different from the control diet-fed WT group. Con, control diet; T-bet KO, T-bet knockout mice. Original magnification, 200×; scale bar, 100 μm.

T-bet KO mice compared with the control diet-fed T-bet KO mice (Fig. 1A and B). The level of GATA-3 mRNA expression was significantly increased in T-bet KO mice (Fig. 1C).

3.2. T-bet regulated body weight on the control diet-fed or HF diet-induced weight increase

To investigate whether T-bet deficiency affects obesity-related phenotypes, we fed T-bet KO mice the control diet or HF diet, and we measured body weight, food intake and body efficiency ratio compared with WT mice. All mice fed the HF diet increased significantly more weight than those fed the control diet (P<.05). Interestingly, the body weight of T-bet KO male mice significantly increased compared with WT male mice (P<.05). The body weight of the control diet-fed WT mice or T-bet mice was increased by 6.45 ±0.49 and 13.64±0.67 g. The HF diet-fed T-bet KO group gained 23.95±1.08 g of weight, and the HF diet-fed WT group gained 20.75±2.40 g, after 10 weeks (Fig. 2A). However, the body weight of HF diet-fed T-bet KO female mice did not significantly change compared with the HF diet-fed WT mice. Hence, our next experiments were performed without T-bet KO female group. Total food intake of the control diet-fed or HF diet-fed T-bet KO mice was not significantly different from that of WT mice (Fig. 2B). The food efficiency ratio was calculated by dividing the body weight gain by food intake. The food efficiency ratio of control diet-fed T-bet KO mice significantly increased compared with the control diet-fed WT mice. However, there was little difference in food efficiency ratio between HF diet-fed T-bet KO mice and HF diet-fed WT mice (Fig. 2C). The size distribution of white adipocytes in T-bet KO mice is enlarged compared to WT mice (Fig. 2D). Then, we analyzed the weight of adipose tissues in T-bet KO mice. The weight of epididymal WAT in T-bet KO mice was

Table 1
The weight of adipose tissues in HFD-fed mice

	WAT (g)		BAT (g)	
	Male	Female	Male	Female
Con-fed WT group	0.36±0.08	0.20±0.02	0.12±0.10	0.06±0.00
HFD-fed WT group	4.47±0.10	3.36±0.38	0.28±0.03	0.09±0.01
Con-fed T-bet KO group	0.66±0.08[a]	0.43±0.06[a]	0.11±0.03	0.11±0.00[a]
HFD-fed T-bet KO group	5.36±0.22[b]	3.66±0.48	0.19±0.04[b]	0.11±0.00[b]

Values are means±S.E.M. HFD, high-fat diet; Con: control diet; T-bet KO: T-bet KO mice.
[a] $P<.05$ vs. control diet-fed WT group.
[b] $P<.05$ vs. HFD-fed WT group.

significantly increased by the control diet or HF diet feeding compared with WT mice, whereas the weights of BAT in the HF diet-fed T-bet KO group decreased compared to HF diet-fed WT group (Table 1).

3.3. Body temperature, energy expenditure-related genes expressions and glucose tolerance in WT and T-bet KO mice

To perform metabolic analysis, we measured the rectal body temperature, energy expenditure-related genes (PGC-1α, UCP1 and UCP2) and glucose tolerance. Body temperature was significantly increased in T-bet KO mice (Fig. 3A). However, the mRNA expressions of PGC-1α, UCP1 and UCP2 were decreased by T-bet deficiency (Fig. 3B). Fig. 3C shows the oral GTT in WT and T-bet KO mice under the same conditions. T-bet KO mice induced severe glucose intolerance and elevated fasting glucose level compared to WT mice (Fig. 3C and D).

3.4. T-bet KO mice up-regulated the HF diet-induced lipid level

To clarify the mechanism of T-bet deficiency in obese mice, we analyzed the serum lipid parameters in mice after the diet-fed period. Serum glucose and HDL cholesterol levels did not show significant differences between T-bet KO mice and WT mice. In WT mice fed the HF diet, serum triacylglyceride levels increased by 28.9% compared with the control diet-fed WT mice. In control diet-fed T-bet KO mice and HF diet-fed T-bet KO mice, the triacylglyceride level was increased by 77.5% and 89.3%, respectively, compared with the WT groups. The HF diet feeding increased the serum LDL cholesterol by 4.3-fold compared with the control diet group. In control diet-fed T-bet KO group and HF diet-fed T-bet KO group, the LDL cholesterol level increased by 2.6-and 1.5-fold, respectively, compared with the HF diet-fed WT mice (Fig. 4).

3.5. Obesity-related parameter and proinflammatory cytokine mRNA expression in T-bet KO mice

To investigate the obesity-related function of T-bet expression in obese mice, we performed RT-PCR. As shown Fig. 5A and B, the increased level of leptin mRNA by obesity was enhanced in the control diet-fed or HF diet-fed T-bet KO mice. Moreover, the increase in PPAR-γ and C/EBPα mRNA expression in T-bet KO mice was also more than that in WT mice. However, adiponectin mRNA expression level did not show significant differences between T-bet KO mice and WT mice. To define the obesity-related inflammatory response in T-bet KO mice, we analyzed the proinflammatory cytokine mRNA expression in T-bet KO mice. The HF diet induced the

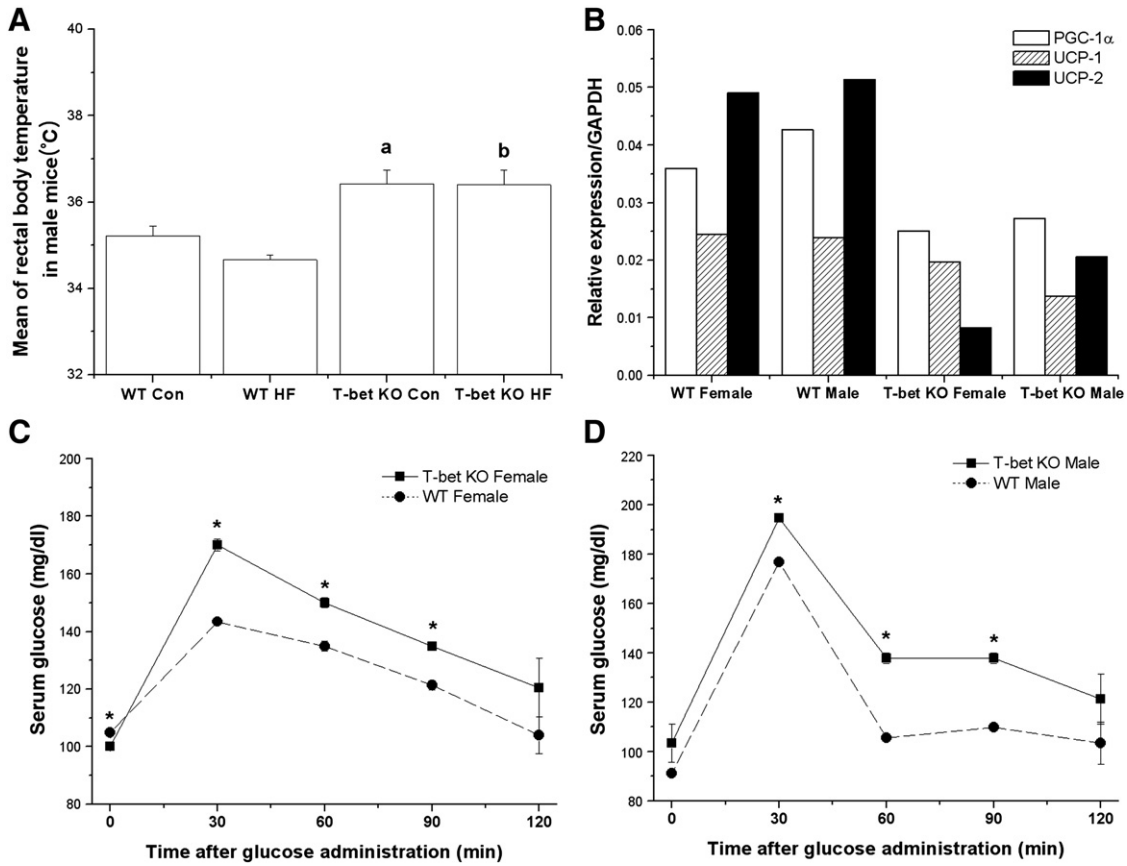

Fig. 3. Body temperature (A), energy expenditure-related gene (B) and glucose tolerance (C and D) in WT and T-bet KO mice. [a]$P<.05$: significantly different from the control diet-fed WT group. [b]$P<.05$: significantly different from the HF diet-fed WT group. *$P<.05$: significantly different from the WT mice. Con, control diet; T-bet KO, T-bet KO mice.

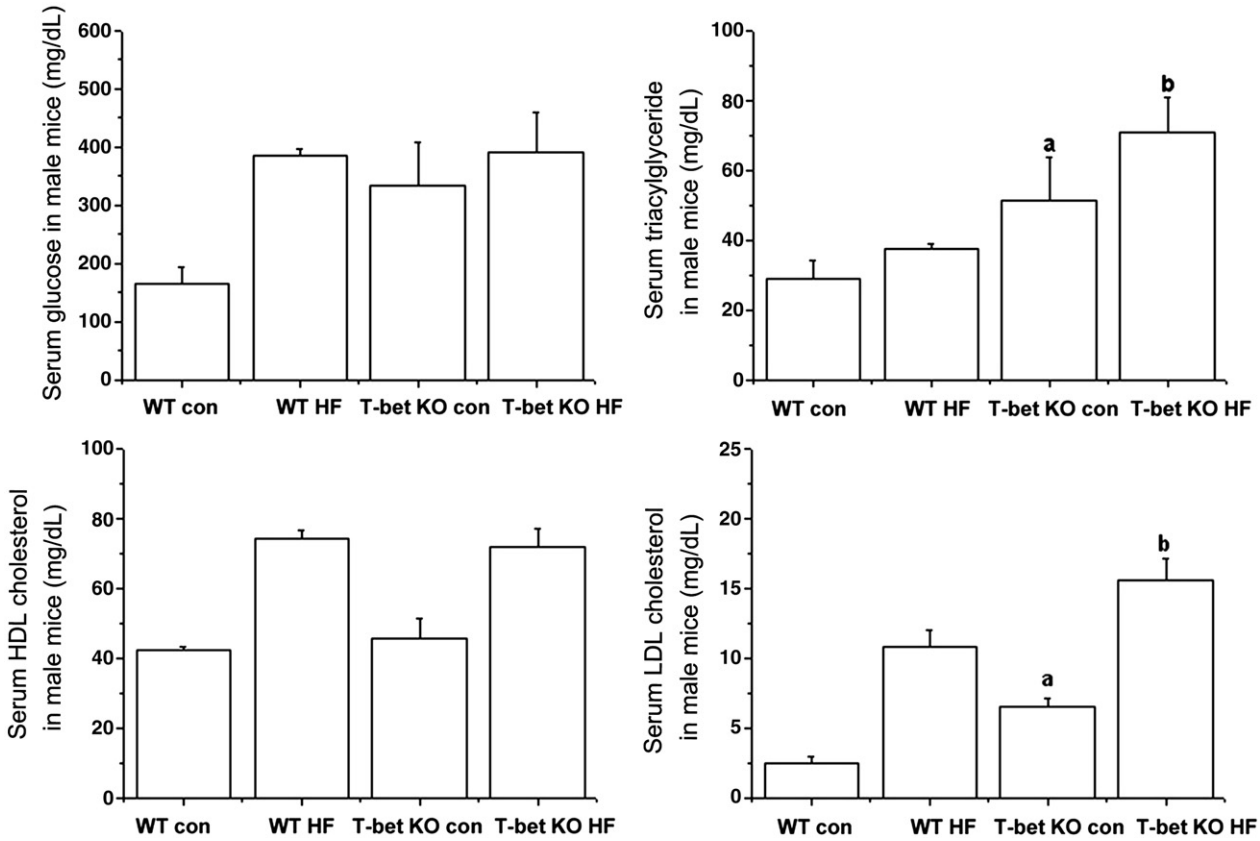

Fig. 4. Effects of T-bet deficiency on the HF diet-induced lipid level. Values represent mean±S.E.M. [a]$P<.05$: significantly different from the control diet-fed WT group. [b]$P<.05$: significantly different from the HF diet-fed WT group. Con, control diet; T-bet KO, T-bet KO mice.

increase in the mRNA levels of IL-1β, IL-6 and TNF-α by 13-, 32-, and 23-fold compared with the control diet group. Taking the HF diet-fed WT mice group values as 100%, IL-6 mRNA expression of the HF diet-fed T-bet KO group had mRNA expression level of 438%, respectively (Fig. 5C and D). Interestingly, IL-6 mRNA expression of the control diet-fed T-bet KO mice was increased to compare the level of WT mice. However, IL-1β and TNF-α mRNA expression level did not show a significant difference between T-bet KO and WT groups in the control diet-fed or HF diet-fed mice. Furthermore, IL-6 mRNA expression of AF and SVF in WAT was increased by T-bet deficiency (Fig. 5E).

4. Discussion

In the present study, we report that T-bet KO mice gained more weight than WT mice when challenged for 10 weeks with the control diet or HF diet. Unexpectedly, this body weight increase was not observed in T-bet KO female mice compared to WT mice. We did not define yet why T-bet KO female mice did not show the difference between the control diet-fed and HF diet-fed group. Further study is needed to clarify this mechanism. Although we demonstrated that HF diet-fed T-bet KO mice did not significantly show the food efficiency ratio compared to the HF diet-fed WT mice, control diet-fed T-bet KO mice significantly increases the food efficiency ratio compared to the WT mice. Furthermore, the size of white adipocytes in T-bet KO mice is larger than that in WT mice. To define whether T-bet deficiency was related to growth factor, we confirmed the level of growth hormone (GH) of serum on T-bet KO mice. However, the level of GH was not changed by the control diet or HF diet feeding in T-bet KO mice (data not shown).

Mammals have two types of adipose tissue that control whole-body energy metabolism, WAT and BAT. These adipose tissues have opposite functions in energy metabolism. WAT is for energy storage and BAT is for cold- and diet-induced thermogenesis [17]. Therefore, the fact that the weights of the BATs in the HF diet-fed T-bet KO group were less decreased than the HF diet-fed WT group is worthy of notice. Furthermore, BAT and skeletal muscle are the two major organs involved in adaptive thermogenesis [18]. We observed a decrease in mRNA expressions of energy expenditure-related genes such as PGC-1α, UCP1 and UCP2 in the skeletal muscle of T-bet KO mice compared with the WT mice. Therefore, these findings show that T-bet deficiency may lead to obesity.

To clarify the mechanism of metabolic system in T-bet deficiency mice, we performed the GTT. Both male and female T-bet KO mice have impaired glucose tolerance compared with the WT mice, reflected in higher glucose after glucose oral administration. The rectal body temperature was significantly increased in T-bet KO mice compared with WT mice. These findings indicated that the altered glucose tolerance in T-bet KO mice could be involved in the development of obesity in both sexes. However, our study showed that obesity was only induced in T-bet KO male mice. Therefore, further studies will be needed to clarify the mechanism of metabolic system in T-bet KO mice.

Next, we are investigating the effects of T-bet deficiency on obesity in obese mice and examining obesity-related factors such as changes of triacylglyceride, HDL cholesterol, LDH cholesterol, Th1/Th2 cytokine in the spleen and WAT, adipokine mRNA expression and proinflammatory cytokine mRNA expression.

HF diet-induced obesity is widely used as a metabolic syndrome model such as insulin resistance and obesity [19]. Obesity was defined

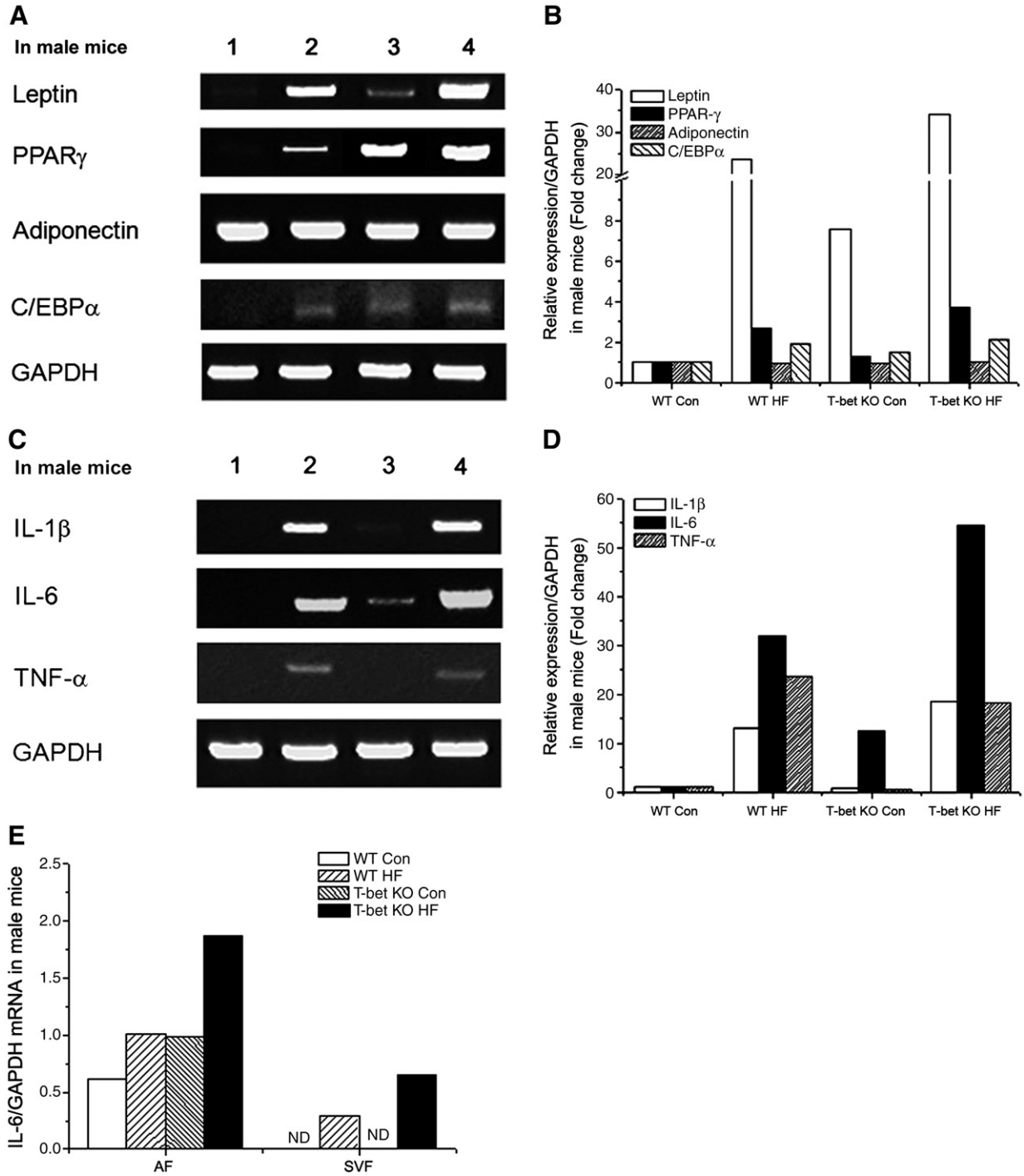

Fig. 5. Obesity-related parameter and inflammatory cytokine mRNA expression in HF diet-fed T-bet KO mice. (A) Regulatory functions of T-bet on obesity-related phenotypes in mice. (B) The mRNA levels were quantitated by densitometry. (C) mRNA expression of inflammatory cytokines on WAT in mice. (D) The mRNA levels were quantitated by densitometry. (E) IL-6 mRNA expressions in AF and SVF. 1: control diet-fed WT mice; 2: HF diet-fed WT mice; 3: control diet-fed T-bet KO mice; 4: HF diet-fed T-bet KO mice. Values represent mean ±S.E.M. Con, control diet; T-bet KO, T-bet KO mice; AF, AF in WAT; SVF, SVF in WAT.

as an excessive accumulation of triacylglyceride in WAT that is the result of excessive energy intake compared to energy usage. The level of LDL in serum is a prime determinant of the risk of symptomatic vascular disease and coronary artery disease [1]. Therefore, T-bet function for regulating the concentration of serum LDL in obese mice is an important point of this study. In this study, the serum triacylglyceride level of the control diet-fed or HF diet-fed T-bet KO mice significantly increased compared with WT mice. Furthermore, the LDL cholesterol level of the T-bet KO group was increased significantly compared with WT mice. These results mean that T-bet deficiency can aggravate diet-induced obesity.

Leptin is a hormone secreted mainly by adipocytes that regulates food intake and energy expenditure. An increase in leptin levels is associated with adiposity, which means overfeeding and adiposity [20]. Treatment of recombinant of human leptin reverses hyperphagia, obesity and impaired T-cell-mediated immunity associated with congenital leptin deficiency [21]. PPAR-γ and C/EBPα are well-known major transcription factors of adipogenesis and activate the

expression of adipocyte marker genes [22]. Adiponectin expression and secretion are needed to differentiate adipocytes [23] and act as a regulatory factor on energy homeostasis, glucose and lipid metabolism, and anti-inflammatory response [1]. In this study, the increase of leptin mRNA expression level by diet-induced obesity was enhanced by T-bet deficiency condition. Moreover, PPAR-γ and C/EBPα mRNA expression also increased in HF diet-fed T-bet KO mice. However, adiponectin mRNA expression level did not change the differences. These results may suggest that T-bet regulates obesity-related phenotypes.

Obesity is associated with increased production of proinflammatory cytokines such as IL-6 and TNF-α, which contribute to chronic, low-grade inflammation [24,25]. Activated WAT increases the synthesis of proinflammatory cytokines such as IL-6, IL-1, IL-8, TNF-α and IL-18, while regulatory cytokines such as IL-10 are decreased [26]. Especially, the elevated IL-6 levels in adipocytes have an effect on adipose tissue-specific gene expression, triacylglyceride release, lipoprotein lipase down-regulation, insulin sensitivity and so on [27]. T-bet deficiency causes a defect in the development of the Th1 subset, with a significant reduction of IFN-γ, but a significant increase in IL-4 production [28]. As shown in Fig. 1, T-bet deficiency reduced the Th1 response by shifting the Th1/Th2 balance away from a Th1 phenotype. According to this hypothesis, Th1/Th2 imbalance induced the Th2 cytokine such as IL-6. We thus want to study the influence of T-bet on IL-6-mediated obesity. To clarify the mechanism of T-bet function in obese mice, we determined mRNA expression levels of inflammatory markers. IL-6 mRNA expression of T-bet KO group increased by HF diet-induced obesity compared with the HF diet-fed WT group. Also, IL-6 mRNA expression of the control diet-fed T-bet KO mice was increased compared with WT mice. IL-6 mRNA expression of AF and SVF was also increased in T-bet KO mice.

In summary, our results indicate that T-bet KO male mice results in greater weight gain, size of white adipocytes, serum triacylglyceride and LDL cholesterol, and T-bet KO mice have decreased BAT. In addition, the mRNA expressions of PGC-1α, UCP1 and UCP2 were decreased in T-bet KO mice. Furthermore, both T-bet KO male and female mice had impaired glucose tolerance. Also, the obesity-related phenotypes such as leptin, PPAR-γ and C/EBPα were increased in T-bet KO mice, and the proinflammatory cytokines mRNA expressions were increased in the HF diet-fed T-bet KO mice. Especially, IL-6 mRNA expression was increased by T-bet deficiency. Taken together as a whole, our data suggest that T-bet could be an important target in terms of regulation of obesity.

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