BPB Reports

Report

Browning Effect of Brominated Flame Retardant, TBBP-A, on Undifferentiated Adipocytes

Masahiro Yamasaki,* Shinya Hasegawa, Masahiko Imai, Tetsuya Fukui, and Noriko Takahashi

Department of Health Chemistry, Hoshi University, 2-4-41, Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
Received December 19, 2020; Accepted January 27, 2021

Recent studies have suggested that exposure to brominated flame retardants (BFR) may play a pivotal role in the development of high-fat diet-induced obesity and metabolic disorders in the liver. Ketone bodies produced by β-oxidation are utilized by acetoacetyl-CoA synthetase (AACS), a cytosolic ketone body-utilizing enzyme. Previously, we reported that the gene expression of AACS is upregulated in high-fat diet-induced obesity. Here, we examined the effects of BFR, tetrabromobisphenol A (TBBP-A), on gene expression in adipocyte cell line (ST-13). Treatment of differentiated cells with TBBP-A for 48 h did not have any remarkable effects on lipid accumulation and mRNA expression of AACS, PPAR-γ, SCOT, and FAS, whereas in undifferentiated cells, mRNA expression of for the lipid and ketone body utilizing-factors (AACS, perilipin-1, and FAS) and brown adipose tissue (BAT) related factors (UCPs, PRDM16, CIDEA, and LSD-1) was upregulated. These observations suggest that TBBP-A may dysregulate lipid metabolism in undifferentiated adipocytes during ketone body utilization via AACS.

Key words tetrabromobisphenol A, brominated flame retardants, ketone body, acetoacetyl-CoA synthetase, obesity

INTRODUCTION

Brominated flame retardants (BFRs) are used in furniture, electronic products, and other materials to decrease flammability of these products.[1)] Since BFRs are produced worldwide, they are found in quantifiable levels in wildlife as well as in humans. Recently, environmental issues relating to BFRs have gained immense interest. Moreover, one of the BFRs, hexabromocyclododecane (HBCD) is toxic to liver and other tissues and impairs lipid- and glucose-metabolic homeostasis.[2)] Presently, production and use of HBCD is severely restricted under Stockholm Convention, and therefore, various alternatives are being considered. Tetrabromobisphenol-A (TBBP-A), a common flame retardant, is produced in large volume and is used worldwide.[3,4)] *In vivo* studies have revealed that TBBP-A is less toxic compared to other BFRs, and is found in air, soil, and water, and aquatic organisms.[4,5)] TBBP-A is also found in human milk and serum;[6)] however, due to its low toxicity, TBBP-A has received less attention worldwide. Recently, several studies reported that TBBP-A shows endocrine disruptor activity,[7)] immunotoxicity,[8)] neurotoxicity,[9)] and hepatotoxicity[10)] in animals. Moreover, TBBP-A shares structural similarities with thyroid hormones, which play a major role in lipid metabolism, and has been observed to exert thyromimetic effects on lipid metabolism in rat hepatoma FaO cells.[11)] Since previous studies have reported that HBCD could induce obesity related lipid metabolism disorders, we assume that TBBP-A may also have the ability to disrupt lipid metabolism.

To test whether BFRs contribute to initiation and progression of obesity and related metabolic dysfunction in adipocytes, we used mouse adipocyte cell line (ST-13) and studied the effects of TBBP-A administration on lipid metabolism. Since TBBP-A is not restricted by Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment (RoHS) and is used worldwide, we investigated whether exposure to TBBP-A affects the expressions of genes related to lipid metabolism in differentiated and undifferentiated adipocytes.

MATERIALS AND METHODS

Cell Culture ST-13 cells, the preadipocyte cell line derived from newborn mouse skin, were kindly provided by Dr. Fumio Fukai (Department of Pathophysiology, Science University of Tokyo). ST-13 cells (8.0 x 10^5 cells) were plated in 35 mm dish in DMEM/F-12 (Life Technologies Co., CA, USA) supplemented with 10% newborn calf serum (Life Technologies Co.). After 1 day incubation at 37˚C in an atmosphere containing 5% CO_2, differentiation was induced by addition of MIX-medium supplemented with isobutylmethylxanthine (0.5 mM; Sigma-Aldrich Co., MO, USA), insulin (10 μg/mL; Sigma-Aldrich Co.), dexamethasone (0.25 μM; Nacalai tesque, Kyoto, Japan), and 10% fetal bovine serum (FBS; Life Technologies Co.). After 48 h, the MIX-medium was replaced with DMEM/F-12 containing 10% FBS. This medium was changed every two day. Simultaneously, the undifferentiated cells were cultured. After 4 days, the cells were treated with 0, 0.5, or 1.0 μM/mL TBBP-A (Tokyo Chemical Industry Co., Tokyo, Japan) for 48 h. The living cells were counted after staining with trypan blue (Life Technologies Co.) by the counting chamber (Erma Inc., Tokyo, Japan).

Oil Red O Staining ST-13 cells were fixed with 15% for-

*To whom correspondence should be addressed. e-mail: ymskmshr@hoshi.ac.jp

maldehyde in PBS for 20 min, followed by sequential rinsing with PBS, distilled water, and 60% isopropanol. Thereafter, cells were stained with oil red-O (0.18% in 60% isopropanol; Sigma-Aldrich) at 37˚C for 10 min and then rinsed with 60% isopropanol followed by distilled water. Quantification of stained lipids was performed by eluting the stain off the dish by incubation with 100% isopropanol for 10 min, followed by measuring the absorbance of the elutes (at 510 nm).

Preparation of RNA RNA was prepared from ST-13 cells using ISOGEN (Nippon Gene Co., Tokyo, Japan). The integrity of RNA was confirmed by its electrophoresis on a denaturing agarose gel containing formaldehyde.

RT-PCR Aliquots of total RNA (4 μg) isolated from ST-13 cells were subjected to reverse transcriptase polymerase chain reaction (RT-PCR) to analyze the gene expressions using a set of primers specific for each factor, and visualized using Lumivision imager (AISIN SEIKI Co., Tokyo, Japan). The following primers were used for amplification: forward (tccgcaaccatgtccaagct) and reverse (atcacatgcacagctggatg) for mouse acetoacetyl-CoA synthetase (AACS), forward (cgaagatggcggctctcaaa) and reverse (gatgcttcaagttgaaatct) for mouse succinyl-CoA: 3-oxoacid CoA-transferase (SCOT), forward (ccatggaggaggtggtgata) and reverse (cgtctcgggatctctgctaa) for mouse fatty acid synthase (FAS), forward (atcagtacccgtatttgaga) and reverse (agggactccagccgttcata) for mouse perilipin-1, forward (gaaactctgggagattctcc) and reverse (gatcacatgcagtagctgca) for mouse peroxisome proliferator-activated receptor-γ (PPAR-γ), forward (acagctgtctgtcctacaga) and reverse (gttcattacggaacatctcg) for mouse uncoupling protein-1 (UCP-1), forward (gaccatggcttggacttcagc) and reverse (cttgccttgttcaaaacgga) for mouse UCP-3, forward (cagaggagttctttcagacc) and reverse (taacacggccttgaagcttg) for mouse cell death-inducing DNA fragmentation factor-alpha-like effector A (CIDEA), forward (agcagatctctgaagacttg) and reverse (tcgcatatgaatggcttcac) for mouse PR domain containing 16 (PRDM16), forward (tgagatctagagttgggacc) and reverse (atagagagctccatctggct) for mouse fibroblast growth factor-21 (FGF-21), and forward (atatcacagccgagttcctg) and reverse (tagcagttgtactgccaacg) for mouse lysine-specific demethylase-1 (LSD-1).

Measurement of Total Ketone Bodies The total ketone bodies in culture medium were determined using ketone body assay kit (Ketone Test Sanwa, Sanwa Kagaku Co., Tokyo, Japan).[12]

Statistical Analysis All data are presented as means ± SD and were analyzed using unpaired Student's t-tests. The differences were considered to be statistically significant for p values < 0.05.

RESULTS

Effects of TBBP-A on Lipid Accumulation in Differentiated Adipocytes To investigate the effects of TBBP-A on lipid metabolism in white adipocytes, we examined the cellular lipid accumulation in mature adipocyte cell culture (ST-13 cells) treated with TBBP-A. Treatment with TBBP-A was not affected cell viability (control: 2,423.3 ± 213.1 x 10^3 cells, 0.5 μM TBBP-A: 2,623.3 ± 285.9 x 10^3 cells, 1.0 μM TBBP-A: 2,306.6 ± 148.1 x 10^3 cells). Figure 1A and B illustrates the oil red-O stained cells after 48 h TBBP-A treatment. TBBP-A did not significantly affect lipid accumulation levels. Moreover, mRNA levels of ketone body-utilizing enzyme (AACS and SCOT) were not significantly different in TBBP-A treated cells (Fig. 1C).

Next, we examined the effect of TBBP-A on preadipocytes. Treatment with TBBP-A was not affected nonstimulated cell viability (control: 2,536.6 ± 100.9 x 10^3 cells, 0.5 μM TBBP-A: 2,893.3 ± 311.0 x 10^3 cells, 1.0 μM TBBP-A: 2,776.6 ± 384.7 x 10^3 cells). After treating with TBBP-A for 48 h, oil red-O staining was not detected in all groups (data not shown). Figure 2 illustrates the effects of TBBP-A treatment on gene expression of ketone body-utilizing enzymes in nonstimulated ST-13 cells. The expression level of AACS mRNA was upregulated by 48 h TBBP-A treatment (Fig. 2A). Previous studies have reported that AACS is overexpressed in lipogenic tissues, such as liver and white adipose tissue, and plays a pivotal role in lipogenesis in adipocyte cell lines.[13,14] Moreover, it has been reported that in subcutaneous white adipose tissue, AACS was upregulated in rats with high-fat diet-induced obesity.[15] In contrast, mRNA levels of SCOT, the ketone body-utilizing enzyme responsible for energy production in mitochondria, were not affected by TBBP-A. Moreover, expression levels of FAS and perilipin-1 mRNAs were increased upon treatment with TBBP-A, whereas PPAR-γ mRNA expression did not reveal any significant difference. Perilipin-1 regulates the lipid storage in adipose tissues,[16] while PPAR-γ is required for adipogenesis.[17] Collectively, TBBP-A affected the gene expressions of lipid metabolism-related factors in preadipocytes, and these effects of TBBP-A might be independent of classical adipogenesis via PPAR-γ mediated pathway.

TBBP-A Increased the Expression of "Beige Adipocyte-Genes" in Undifferentiated Adipo-Cells Two types of adipose tissues, white adipose tissue (WAT) and brown adipose tissue (BAT), play distinct roles in energy metabolism.[18] WAT is involved in energy storage, whereas BAT is involved in thermogenesis. Our observations (Figs. 1 and 2) revealed the possibility that TBBP-A could induce the lipid synthesis from ketone bodies. But lipid accumulation was not observed in undifferentiated cells after TBBP-A treatment. Therefore, we further examined whether lipid consumption in preadipocytes was affected by TBBP-A. Figure 3 illustrates the effects of TBBP-A treatment on gene expressions of BAT specific factors in ST-13 preadipocytes. UCP-1 and UCP-3 expressed in brown adipocytes are related to conversion of stored lipids into body heat.[19] Moreover, UCP-2 is expressed widely and recent studies suggest that UCP-3 may be regulated in a manner similar to UCP-1.[20] Hence, we examined the effects of TBBP-A on UCP-1 and UCP-3 mRNA expression levels. The expression of UCP-1 and UCP-3 mRNA was increased by TBBP-A treatment for 48 h, as shown in Fig. 3A. Moreover, TBBP-A significantly increased the expression levels of PRDM16, LSD-1, and CIDEA genes in a dose-response manner. LSD-1 regulates brown adipocyte metabolism during coexpression of PRDM16,[21] whereas CIDEA inhibits the uncoupling process in BAT.[22] Thus, these factors may act as the molecular markers of induced "browning" of white adipocytes. Due to the increased expression of BAT specific genes, TBBP-A may act as a "browning inducer" in undifferentiated adipocytes. In contrast, the mRNA levels of FGF-21, which is a secreted during ketone body production in liver and BAT, were not affected by TBBP-A treatment.

Next, we examined the effects of TBBP-A on the production of ketone bodies in adipose cell line. The concentration of total ketone bodies was slightly higher in the culture medium of undifferentiated ST-13 cells treated with TBBP-A at con-

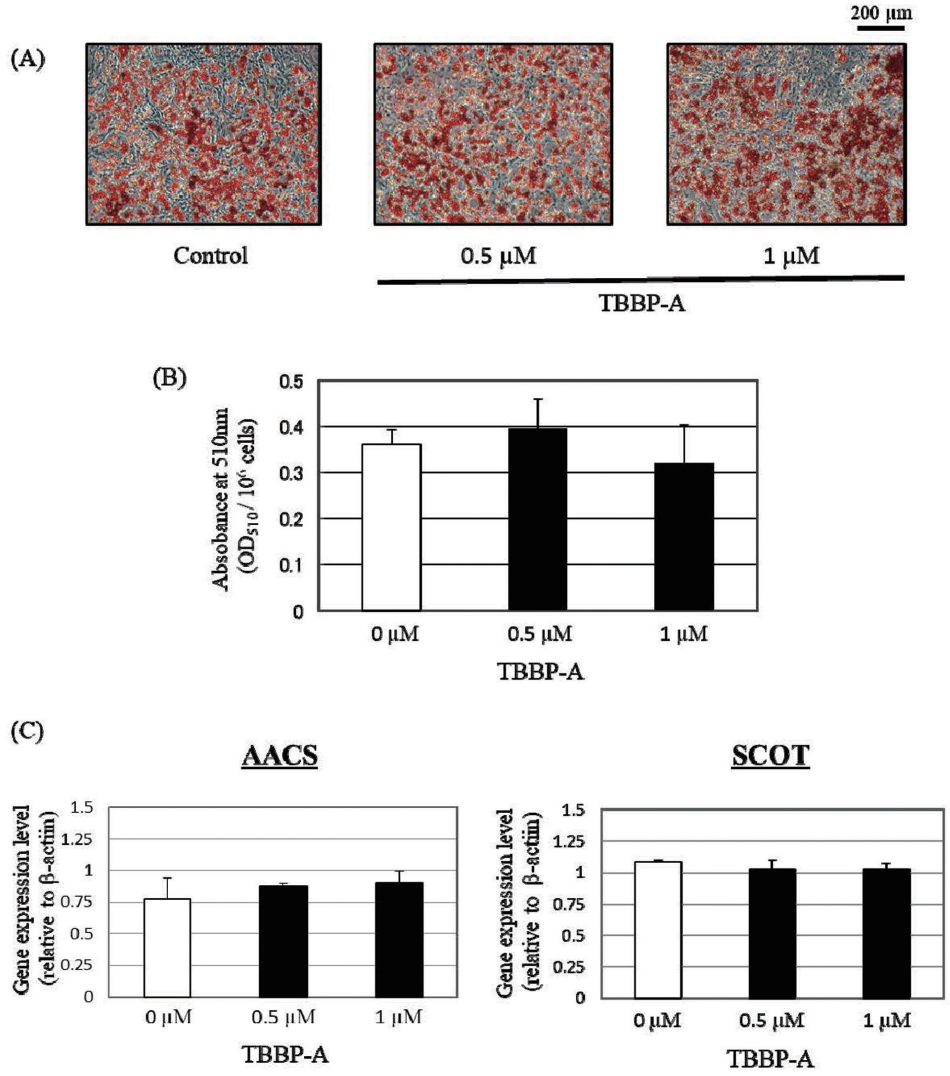

Fig. 1. Effects of TBBP-A on Lipid Accumulation and Ketone Body Metabolism in Differentiated Adipocytes

ST-13 cells were differentiated using MIX-medium and treated with BFRs for 48 h. A: Oil red-O staining was performed to differentiate lipid droplets in cells treated with TBBP-A or PBS (control). B: Quantification of the stained lipid droplets was performed using the eluted Oil red O stain and measuring absorbance at 510 nm. Each bar represents the mean ± SD (n = 4). C: RT-PCR analysis for AACS and SCOT in cells treated with TBBP-A or PBS. The expression level of β-actin was used for standardizing amount of gene expression levels. Each bar represents the mean ± SD (n = 4). *$p < 0.05$ compared with PBS treated cells.

centrations above 1.0 μM compared to that of untreated controls (Fig. 3B). Since ketone bodies are derived from fatty acid oxidation, our results suggest that TBBP-A increased lipid metabolism and promotes the production of ketone bodies via stimulation of BAT specific genes in preadipocytes.

DISCUSSION

Obesity induces an unusual metabolic state involving lipid oxidation and ketone body production.[23] Previously, we reported that gene encoding AACS, which is a ketone body-utilizing enzyme, is overexpressed in mature adipocytes present in the subcutaneous WAT, and is upregulated in high-fat diet-induced obese rats.[15] These results indicate that ketone body utilization for synthesizing lipidic substances is affected by the nutritional states. The present study revealed that expression of AACS and FAS mRNA was increased in ST-13 preadipocytes in response to TBBP-A treatment. TBBP-A possibly induced ketone body utilization for cellular lipid synthesis in undifferentiated preadipocytes, but not in differentiated mature adipocytes. In contrast, since the gene expression level of SCOT, the key enzyme for generating energy from ketone bodies[24] was not altered by TBBP-A, we infer that AACS, and not SCOT, orchestrates the TBBP-A-induced metabolic disorder related to ketone body utilization in adipocytes.

Moreover, we demonstrated that mRNA of BAT specific factors, such as UCPs, PRDM16, LSD-1, and CIDEA, were increased upon TBBP-A treatment. PRDM16 is a transcriptional coregulator overexpressed in BAT and induces expression of mitochondrial and thermogenic genes in brown adipocytes,[25] whereas LSD-1 regulates "browning" of adipocytes.[21] UCPs convert stored lipids into heat and their activity is inhibited by CIDEA in BAT.[22] Surprisingly, our data showed that TBBP-A treatment increased the expression of these factors in ST-13 preadipocytes (Fig. 3). However, PPAR-γ mRNA level was not altered by TBBP-A (Fig. 2). Since PRDM16 stimulates adipogenesis in a PPARγ-dependent manner,[17] our results indicated that the effects of TBBP-A could not be mediated by

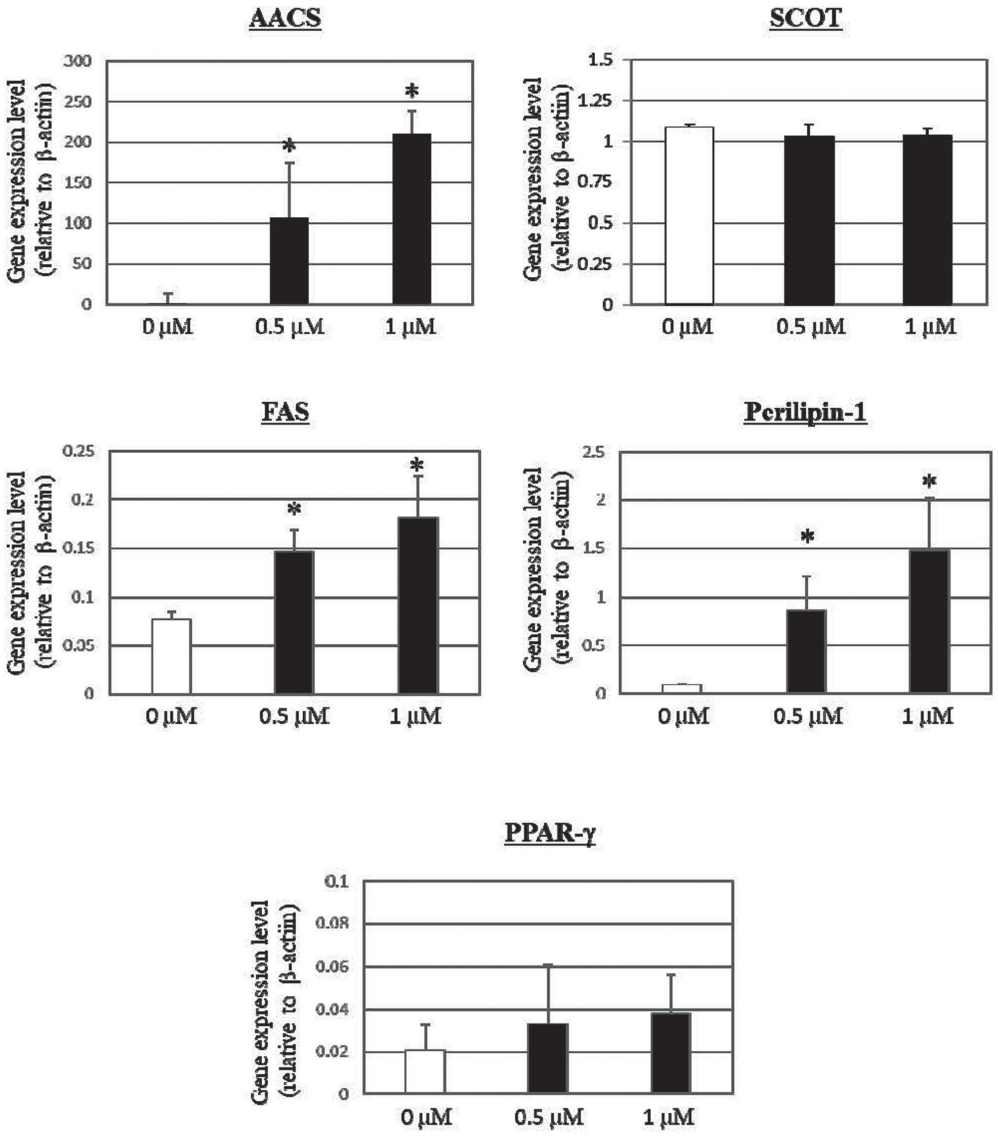

Fig. 2. Effects of TBBP-A on Lipid and Ketone Body Metabolism in Undifferentiated Adipocytes

RT-PCR analysis for AACS and other enzymes in undifferentiated ST-13 cells treated with TBBP-A or PBS. β-actin was used for standardizing amount of gene expression levels. Each bar represents the mean ± SD (n = 4). *$p < 0.05$ compared with PBS treated cells.

normal transcriptional pathway.

We observed that TBBP-A increased the concentration of total ketone bodies in culture medium, whereas TBBP-A induced the gene expression of AACS, that is using ketone body for lipid synthesis (Fig. 2). In addition, the lipid accumulation was not detected in undifferentiated cell treated with TBBP-A. Therefore, our results suggest that TBBP-A stimulates the consumption of both lipid and ketone bodies. It is also possible that new fatty acids formed by the abnormal lipid synthesis are metabolized to ketone bodies by β-oxidation in preadipocytes that have undergone browning. Based on these results, we suggest that TBBP-A not only induces the synthesis of lipids from ketone bodies but also the breakdown of lipids to produce new ketone bodies. Moreover, ketone bodies may induce the expression of brown adipocyte related genes. Particularly, Wang *et al*. reported that β-hydroxybutyrate (BHB), one of the ketone bodies, is required for cell differentiation in brown adipocytes *in vivo*.[26] Moreover, they demonstrated that PRDM16-expressing adipose cells secrete ketone body metabolites; thus, it is possible that ketone bodies and brown adipocyte related factors regulate each other in preadipocytes though a positive feedback loop. This feedback may also explain the effects mediated by PPARγ-independent manner as observed in our study in response to TBBP-A treatment.

Collectively, outcomes of the present study reveal that TBBP-A, a widely used BFR, may disrupt lipid metabolism in undifferentiated adipocytes. Moreover, TBBP-A affects both utilization and production of ketone bodies by inducing AACS expression in preadipocytes and BAT specific factors, respectively. Therefore, these findings support the hypothesis that exposure to TBBP-A may anomalously alter the metabolism balance (e.g., between overtaking of high fat-diet and overmaking of ketone body) in obese condition.

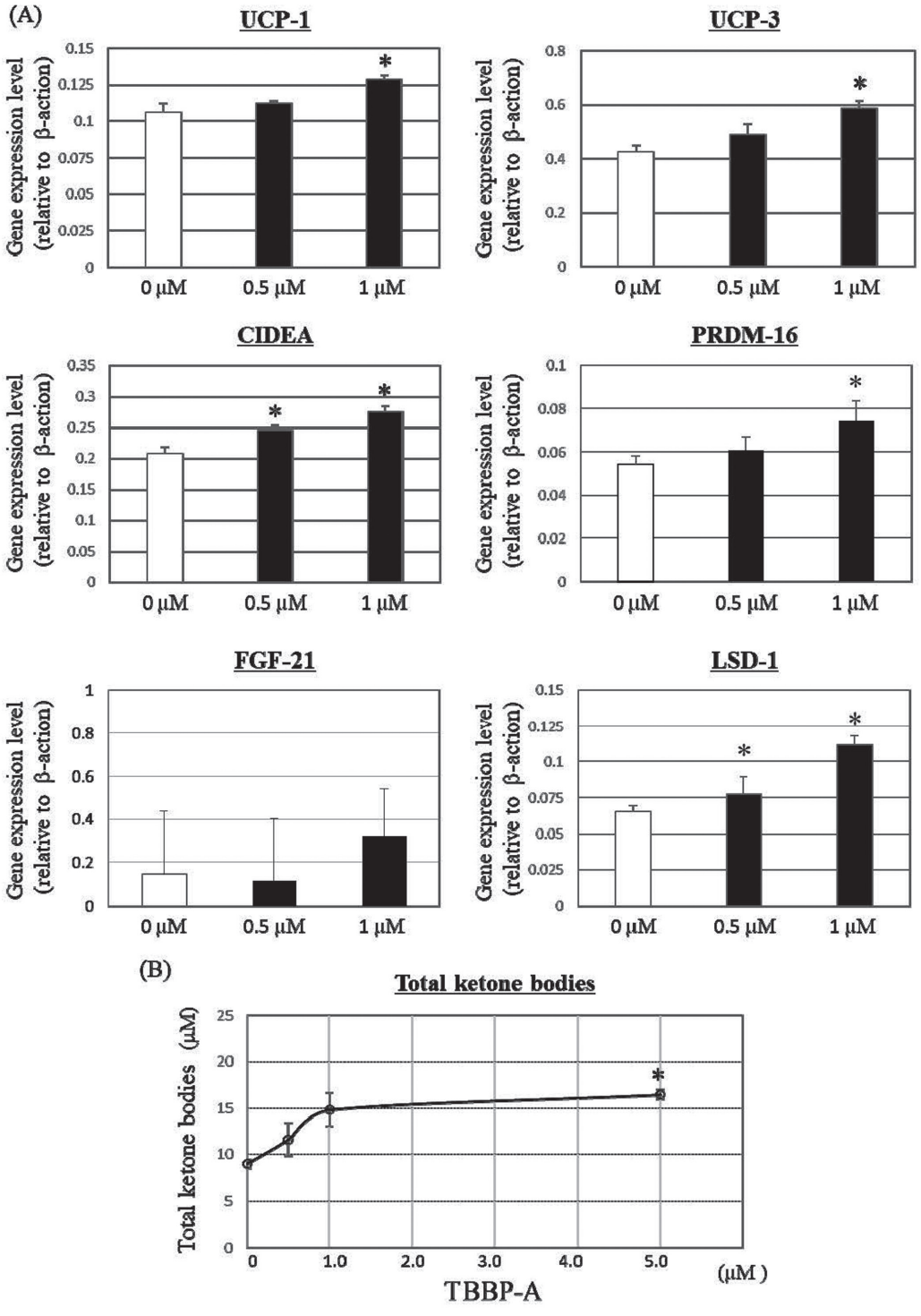

Fig. 3. Effects of TBBP-A on Gene Expressions of BAT Specific Factors in Undifferentiated Adipocytes

A: RT-PCR analysis for BAT specific factors in undifferentiated ST-13 cells treated with TBBP-A or PBS (0 μM). β-actin was used for standardizing amount of gene expression levels. B: The concentration of total ketone bodies (μM) in culture medium of cells treated with TBBP-A for 48 h. Each bar represents the mean ± SD (n = 4). *$p < 0.05$ compared with 0 μM TBBP-A.

Acknowledgment This work was supported by the Japan Society for the Promotion of Science (JSPS), Grants-in-Aid for Scientific Research (KAKENHI) Grant Number JP15K08048, and the science research promotion fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan. We are grateful to Dr. Fumio Fukai, Department of Pathophysiology, Science University of Tokyo, for his important contributions to the experiments of ST-13 cells.

Conflict of interest The authors declare no conflict of interest.

REFERENCES

1) de Wit C. An overview of brominated flame retardants in the environment. *Chemosphere*, **46**, 583–624 (2002).
2) Yanagisawa R, Koike E, Win-Shwe TT, Yamamoto M, Takano H. Impaired lipid and glucose homeostasis in hexabromocyclododecane-exposed mice fed a high-fat diet. *Environ. Health Perspect.*, **122**, 277–283 (2014).
3) Dagani MJ, Barda HJ, Benya TJ, Sanders DC. Bromine Compounds. *Ullmann's Encyclopedia of Industrial Chemistry (7th)*. Wiley-VCH. (2011)
4) Lilienthal H, Verwer CM, van der Ven LT, Piersma AH, Vos JG. Exposure to tetrabromobisphenol A (TBBPA) in Wistar rats: neurobehavioral effects in offspring from a one-generation reproduction study. *Toxicology*, **246**, 45–54 (2008).
5) Yang S, Wang S, Liu H, Yan Z. Tetrabromobisphenol A: tissue distribution in fish, and seasonal variation in water and sediment of Lake Chaohu, China. *Environ. Sci. Pollut. Res. Int.*, **19**, 4090–4096 (2012).
6) Carignan CC, Abdallah MA, Wu N, Heiger-Bernays W, McClean MD, Harrad S, Webster TF. Predictors of tetrabromobisphenol-A (TBBP-A) and hexabromocyclododecanes (HBCD) in milk from Boston mothers. *Environ. Sci. Technol.*, **46**, 12146–12153 (2012).
7) Shaw SD, Blum A, Weber R, Kannan K, Rich D, Lucas D, Koshland C, Dobraca D, Hanson S, Birnbaum L. Halogenated flame retardants: do the fire safety benefits justify the risks? *Rev. Environ. Health*, **25**, 261–305 (2010).
8) Kibakaya EC, Stephen K, Whalen MM. Tetrabromobisphenol A has immunosuppressive effects on human natural killer cells. *J. Immunotoxicol.*, **6**, 285–292 (2009).
9) Nakajima A, Saigusa D, Tetsu N, Yamakuni T, Tomioka Y, Hishinuma T. Neurobehavioral effects of tetrabromobisphenol A, a brominated flame retardant, in mice. *Toxicol. Lett.*, **189**, 78–83 (2009).
10) Kling P, Förlin L. Proteomic studies in zebrafish liver cells exposed to the brominated flame retardants HBCD and TBBPA. *Ecotoxicol. Environ. Saf.*, **72**, 1985–1993 (2009).
11) Grasselli E, Cortese K, Fabbri R, Smerilli A, Vergani L, Voci A, Gallo G, Canesi L. Thyromimetic actions of tetrabromobisphenol A (TBBPA) in steatotic FaO rat hepatoma cells. *Chemosphere*, **112**, 511–518 (2014).
12) Harano Y, Kosugi K, Hyosu T, Uno S, Ichikawa Y, Shigeta Y. Sensitive and simplified method for the differential determination of serum levels of ketone bodies. *Clin. Chim. Acta*, **134**, 327–336 (1983).
13) Hasegawa S, Ikeda Y, Yamasaki M, Fukui T. The role of acetoacetyl-CoA synthetase, a ketone body-utilizing enzyme, in 3T3-L1 adipocyte differentiation. *Biol. Pharm. Bull.*, **35**, 1980–1985 (2012).
14) Yamasaki M, Hasegawa S, Suzuki H, Hidai K, Saitoh Y, Fukui T. Acetoacetyl-CoA synthetase gene is abundant in rat adipose, and related with fatty acid synthesis in mature adipocytes. *Biochem. Biophys. Res. Commun.*, **335**, 215–219 (2005).
15) Yamasaki M, Hasegawa S, Kitani T, Hidai K, Fukui T. Differential effects of obesity on acetoacetyl-CoA synthetase gene in rat adipose tissues. *Eur. J. Lipid Sci. Technol.*, **109**, 617–622 (2007).
16) Sztalryd C, Brasaemle DL. The perilipin family of lipid droplet proteins: gatekeepers of intracellular lipolysis. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids*, **1862** (10 Pt B), 1221–1232 (2017).
17) Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, Spiegelman BM, Mortensen RM. PPAR gamma is required for the differentiation of adipose tissue *in vivo* and *in vitro*. *Mol. Cell*, **4**, 611–617 (1999).
18) Marlatt KL, Ravussin E. Brown adipose tissue: an update on recent findings. *Curr. Obes. Rep.*, **6**, 389–396 (2017).
19) Vidal-Puig A, Solanes G, Grujic D, Flier JS, Lowell BB. UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem. Biophys. Res. Commun.*, **235**, 79–82 (1997).
20) Gong DW, He Y, Karas M, Reitman M. Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. *J. Biol. Chem.*, **272**, 24129–24132 (1997).
21) Zeng X, Jedrychowski MP, Chen Y, Serag S, Lavery GG, Gygi SP, Spiegelman BM. Lysine-specific demethylase 1 promotes brown adipose tissue thermogenesis via repressing glucocorticoid activation. *Genes Dev.*, **30**, 1822–1836 (2016).
22) Gummesson A, Jernås M, Svensson PA, Larsson I, Glad CA, Schéle E, Gripeteg L, Sjöholm K, Lystig TC, Sjöström L, Carlsson B, Fagerberg B, Carlsson LMS. Relations of adipose tissue CIDEA gene expression to basal metabolic rate, energy restriction, and obesity: population-based and dietary intervention studies. *J. Clin. Endocrinol. Metab.*, **92**, 4759–4765 (2007).
23) Bailey CJ. On the physiology and biochemistry of obesity. *Sci. Prog.*, **65**, 365–393 (1978).
24) Mitchell GA, Kassovska-Bratinova S, Boukaftane Y, Robert MF, Wang SP, Ashmarina L, Lambert M, Lapierre P, Potier E. Medical aspects of ketone body metabolism. *Clin. Invest. Med.*, **18**, 193–216 (1995).
25) Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D, Spiegelman BM. Transcriptional control of brown fat determination by PRDM16. *Cell Metab.*, **6**, 38–54 (2007).
26) Wang W, Ishibashi J, Trefely S, Shao M, Cowan AJ, Sakers A, Lim HW, O'Connor S, Doan MT, Cohen P, Baur JA, King MT, Veech RL, Won KJ, Rabinowitz JD, Snyder NW, Gupta RK, Seale PA. PRDM16-driven metabolic signal from adipocytes regulates precursor cell fate. *Cell Metab.*, **30**, 174–189 (2019).