doi: 10.4183/aeb.2018.287

SHORT CHAIN FATTY ACIDS UPREGULATE ADIPOKINE PRODUCTION IN TYPE 2 DIABETES-DERIVED HUMAN ADIPOCYTES

Y. Naraoka[1,*], T. Yamaguchi[2], A. Hu[2], K. Akimoto[1], H. Kobayashi[1,2]

Juntendo University, Graduate School of Medicine, [1]Department of Hospital Administration, [2]Center for Advanced Kampo Medicine and Clinical Research, Tokyo, Japan

Abstract

Purpose. Short chain fatty acids (SCFAs) play a major regulatory role in adipocyte function and metabolism. The aim of this study was to investigate the effects of SCFAs on adiponectin and leptin expression in adipocytes, and also to determine whether the effects of SCFA treatment in visceral adipocytes obtained from healthy subjects are different relative to the effects in adipocytes from patients with type 2 diabetes.

Materials and Methods. Human pericardiac preadipocytes and human pericardiac preadipocytes type 2 diabetes were differentiated into adipocytes for 21 days in 48-well plates. After differentiation, two kinds of mature adipocytes, human pericardiac adipocytes (HPAd) and human pericardiac adipocytes-type 2 diabetes (HPAd-T2D) were incubated with or without 1 mM of acetic acid (AA), butyrate acid (BA), and propionic acid (PA). After 48 hours of incubation, intracellular lipid accumulation was measured using oil red staining. In addition, mRNA levels of adiponectin, leptin and Peroxisome Proliferator-Activated Receptor γ (PPARγ) were determined by Real-Time PCR system.

Results. In HPAd, SCFA supplementation did not inhibit lipid accumulation. By contrast, both AA ($p<0.01$) and PA ($p<0.01$) significantly inhibited lipid accumulation in HPAd-T2D. Regarding mRNA levels of adiponectin, no significant changes were found in HPAd, while all three types of SCFAs significantly increased ($p<0.05$) adiponectin expression in HPAd-T2D. Leptin mRNA expression levels were significantly increased by treatment with all three types of SCFAs in both HPAd ($p<0.05$) and HPAd-T2D ($p<0.05$).

Conclusion. SCFAs inhibited lipid droplet accumulation and increased mRNA expression of adiponectin and leptin in T2D-derived adipocytes.

Key words: acetic acid, propionic acid, butyric acid, adiponectin, leptin.

INTRODUCTION

Short-chain fatty acids (SCFAs) are saturated fatty acids with 2 to 4 carbons, and include acetic acid (AA), propionic acid (PA) and butyric acid (BA), which are the most abundant SCFAs in the intestine produced as the major end products of gut microbial fermentation of dietary fiber. In the latest decade, there is increasing evidence that SCFA activate cells via G-protein coupled receptors (GPR) such as GPR41 and GPR43 and also regulate the gut immune system (1, 2). The actions of SCFAs are not limited to the gut for they can influence the brain and other organs by modulating the permeability which controls of energy intake or expenditure, body weight, hormones secretion and insulin sensitivity of the host (3). AA is the most abundant SCFA in systemic circulation that can cross the blood-brain barrier to be taken up in the hypothalamus. Results of the uptake lead to a decrease in food intake through appetite suppression (4). PA is a substrate for gluconeogenesis in the liver and constitutes 69% of total body glucose production in mice (5). BA is the major intestinal energy source, and is locally consumed as ~60-70% of the energy needs of isolated colonocytes (6, 7). In obese mice, oral administration of BA reduced body weight, via energy expenditure and fat oxidation (8).

While the white adipose tissue is known for storing lipid droplets, it is also an endocrine organ that secretes various types of proteins called adipokines, including adiponectin, which enhances energy expenditure; leptin, which regulates appetite; and inflammatory cytokines such as TNF-α or IL-6 (9). In particular, the relationship between adipokines and adipocytes has been receiving attention in recent years for its influence on obesity and diabetes.

Adiponectin is specific and the most abundant adipokine in the circulation and exclusively expressed from adipose tissue. Previous studies have suggested that a certain level of serum adiponectin is required to regulate energy homeostasis and glucose and lipid metabolism, and prevent metabolic disease (10). Higher adiponectin levels are reportedly associated with age and body mass index (BMI) in healthy subjects, while

*Correspondence to: Yuna Naraoka, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan, E-mail: ynaraoka@juntendo.ac.jp

lower serum adiponectin levels have been found in subjects with obesity and type 2 diabetes (T2D) (11-13). In addition, maternal obesity caused maternal-fetal metabolism disorders included in low serum adiponectin level in rat (14). On the other hand, an injection of adiponectin in obese mice has been found to enhance insulin sensitivity by decreasing the levels of plasma fatty acids and triglycerides (15).

Meanwhile, leptin has also been implicated in obesity and diabetes. Leptin is specifically synthesized in adipocytes, and is known to regulate appetite by stimulating the hypothalamus or enhance energy metabolism via sympathetic activation (16-17). Additionally, leptin is a potent anorectic hormone that increases fatty oxidation in an AMPK-dependent manner in both the liver and muscle tissues. A previous study has demonstrated that a physiological dose of leptin injection in patients with generalized lipodystrophy led to significant improvements in insulin resistance, serum triglyceride levels, and fatty liver (18).

Both GPR 41 and GPR43 are expressed in human white adipose tissues (19-21), and their activation by SCFAs leads to subsequent adiponectin or leptin induction from adipocytes (22). PA and BA stimulate leptin secretion from both minced mouse adipose tissue and differentiated Ob-Luc cells (23). In addition, BA inhibits lipolysis and increases adiponectin expression in piglets (24), while AA decreases lipid droplet in adipocytes via inhibition of Peroxisome Proliferator-Activated Receptor gamma (PPARγ) in rats and protects against the development of obesity and obesity-linked type 2 diabetes (25).

PPARs are ligand-activated transcription factors of nuclear hormone receptor family. The role of PPARs in modulating lipid, glucose and amino acid metabolism is well established (26-27). PPARγ is abundantly expressed in adipose tissue, and works as a master regulator of adipocyte differentiation and also gene expression of adipokines (28). PPARγ agonists are one of the targets to treat for type 2 diabetes through improving insulin sensitivity (29), which is partially mediated through the expression of adiponectin (30-31).

Most of the studies to date have used animals or adipocytes from healthy subjects, and very few studies have thoroughly investigated the effects of SCFAs in adipocytes, particularly those harvested from T2D patients. Further, there is a paucity of information on the mechanisms of acute effects of SCFAs on white adipocyte differentiation, lipid accumulation, and adipokine production. We hypothesized that the action mechanism of adipokine secretion from T2D-derived adipocytes by SCFA or expression of cell surface receptor may differ from that of healthy adipocytes.

Therefore, the aim of this study was to first investigate the effects of SCFAs on adiponectin and leptin expression in adipocytes, and secondly, to determine the effects of SCFA treatment in healthy visceral adipocytes compared with that of T2-derived adipocytes.

MATERIAL AND METHODS

Cell culture

Human preadipocyte (APPLICATIONS, INC) and human preadipocytes-Type 2 Diabetes (APPLICATIONS, INC) are primary cells derived from human pericardiac adipose tissue. Cells were cultured in preadipocyte growth medium with growth supplement (APPLICATIONS, INC) in collagen-coated microplate (IWAKI) at 37°C and under 5% CO_2. The culture medium was changed every other day until 89-90% confluence. Then adipocytes were differentiated to mature adipocytes by changing the medium to adipocyte differentiation medium (APPLICATIONS, INC) and were used for 21 days after initiation of differentiation. The medium was changed once in 3 days. Then, cells were used for the experiments.

Cells were treated with 1 mM of acetic acid (AA), butyric acid (BA) or propionic acid (PA), or without them in 48- or 6-well micro plate at 37°C, 5% CO_2, for 48h. After the incubated time periods, oil-red O assay was taken to estimate oil accumulation in adipocytes. RNA was extracted to measure adipokine gene expression in adipocytes used for quantitative real-time PCR.

Oil-red O staining

Oil Red O staining was performed in cells that had been washed twice in 250 µL of PBS. The cells were then stained with a filtered, 0.36% solution of Oil Red O in 60% isopropanol for 15 minutes at room temperature. The Oil Red O solution was removed and cells were washed 3 times with water. The Oil Red O stain was eluted with 250 µL of 60% isopropanol with shaking for 30 minutes at room temperature, and absorbance at 570 nM was recorded.

RNA isolation and quantitative real-time PCR

Total RNA was extracted from cells treated with or without SCFA for 48h using RNeasy Plus mini kit according to the manufacturer's instructions (QIAGEN,

Hilden, Garman), and cDNA was synthesized with 100 μg of RNA, oligo (dt) primers, and Super Script III reverse transcriptase (Invitrogen Carlsbad, CA, USA). 100 ng of diluted cDNA samples were used for quantitative real-time PCR (step at 98°C followed by 50 cycles of 10 sec of 98°C, 10 sec of 60°C, and 30 sec at 68°C) in the presence of 10 μmol/L specific forward and reverse primers and KOD cyber qPCR Mix. β-actin served as loading controls. Amplification reactions were performed on a PikoRealTM Real-Time PCR system (Thermo Fisher SCIENTIFIC, MA, USA) using KOD cyber qPCR Mix (TOYOBO, Osaka, Japan). Gene expression was calibrated to that of β-actin. Each sample was analyzed in triplicate. As negative controls, water was used as a template for each reaction. The primer sequences for the RT-qPCR are described in Table1.

Statistical analysis

Statistical analysis was performed using the SPSS version 23.0 software (IBM). Data are expressed as mean ± SE. Within-group changes of each condition in response to SCFA treatment compared to control was evaluated using the Wilcoxon test. Between-group differences of control samples were evaluated using the Mann-Whitney test.

RESULTS

Accumulation of lipid droplets in adipocytes

In HPAd, PA significantly increased lipid accumulation (57.6±1.48μg/mL) compared with control (51.2±0.74μg/mL) ($p<0.01$; Fig. 1a), but SCFA supplementation did not inhibit lipid accumulation. In HPAd-T2D, AA(43.6±0.81μg/mL) ($p<0.01$; Fig. 1b) and PA (44.7±0.85μg/mL) ($p<0.01$; Fig. 1b) significantly inhibited lipid accumulation compared with control (47.1±0.76μg/mL).

Gene expression of adipokines in adipocytes

To determine whether SCFAs increase adipokine secretion from adipocytes, adiponectin and leptin mRNA levels were quantified in pericardiac adipocytes. As shown in Figure 2, adiponectin mRNA levels were significantly lower in HPAd-T2D (0.01±0.001) ($p<0.01$; Fig. 2a), while leptin mRNA expression was significantly higher in HPAd-T2D (1.46±0.153) compared to HPAd (adiponectin; 1.05±0.167, leptin; 0.25±0.014) ($p<0.01$; Fig. 2b). In terms of adiponectin mRNA levels, no significant changes were found in HPAd (Fig. 3a), while in HPAd-T2D, all three SCFAs (AA; 0.04±0.008, BA; 0.02±0.003, PA; 0.03±0.003) significantly increased adiponectin expression compared to samples without SCFA treatment (0.01±0.001)($p<0.05$; Fig. 3b). Leptin mRNA expression levels were significantly increased by treatment of all SCFA in HPAd (AA; 0.95±0.035, BA; 1.22±0.058, PA; 1.25±0.316) ($p<0.05$; Fig.4a) and HPAd-T2D (AA; 16.91±2.424, BA; 5.57±1.018, PA; 4.51±1.091) ($p<0.05$; Fig. 4b) compared to samples without SCFA treatment (HPAd; 0.25±0.014, HPAd-T2D; 1.46±0.153).

Table 1. Primer sequences for the RT-qPCR

	5'-forward primer-3'	5'-reverse primer-3'
B-actin	CTTCTACAATGAGCTGCGTGTG	GTGAGGATCTTCATGAGGTAGTCAGTC
Adiponectin	AAGGAGATCCAGGTCTTATTGG	ACCTTCAGCCCCGGGTAC
Leptin	TTTGGCCCTATCTTTTCTATGTCC	TGGAGGAGACTGACTGCGTG
PPARγ	GAGCCCAAGTTTGAGTTTGC	CAGGGCTTGTAGCAGGTTGT

β-actin beta-actin, PPARγ Peroxisome Proliferator-Activated Receptor gamma

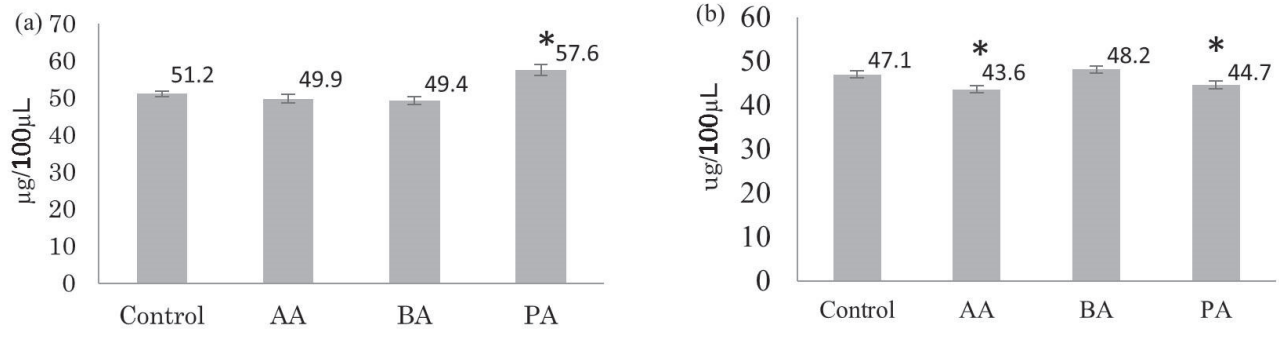

Figure 1. Effects of SCFA against lipid accumulation compared to normal adipocytes (HPAd) and T2D-derived adipocytes (HPAd-T2D). HPAd (a) and HPAd-T2D (b) were treated with or without SCFAs for 48 hours. Results are expressed as mean ± SE. Human adipocytes, HPAd; Human adipocytes-Type 2 diabetes, HPAd-T2D. *$p<0.01$.

Gene expression of PPARγ in adipocytes

To determine whether similar results could be obtained with SCFA treatment, PPARγ mRNA expression levels from adipocytes were measured. In HPAd, AA ($p<0.05$) significantly increased PPARγ expression (0.96±0.045) (Fig.5a), while in HPAd-T2D, all three SCFAs (AA; 0.12±0.001, BA; 0.10±0.004, PA; 0.15±0.012) significantly increased PPARγ expression compared to samples without SCFA treatment (0.04±0.001) (Fig.5b).

DISCUSSION

Our study demonstrated that in T2D-derived adipocytes, treatment with AA and PA significantly inhibited the accumulation of lipid droplets, while in normal adipocytes, AA and BA treatment tended to decrease the size of adipocytes (Fig. 1a).

A previous study reported that AA inhibits the accumulation of lipid droplets (24). Acetate is absorbed by tissues and activated to acetyl-CoA with concomitant formation of adenosine monophosphate (AMP) by the catalytic activity of acetyl-CoA synthetase in cytosol. The elevation in AMP concentration increases the AMP/ATP ratio following the phosphorylation of AMP-activated protein kinase (AMPK) (32). AMPK acts as the key metabolic master switch and regulates a number of enzymes involved in lipid homeostasis (33). Our results showed that it was not only AA but also PA that inhibited accumulation of lipid droplets (Fig. 1b), and that their activity may have depended on the activation of PPARγ. In this study we showed that treatment with AA in normal adipocytes and treatment with all three SCFAs in T2D-derived adipocytes

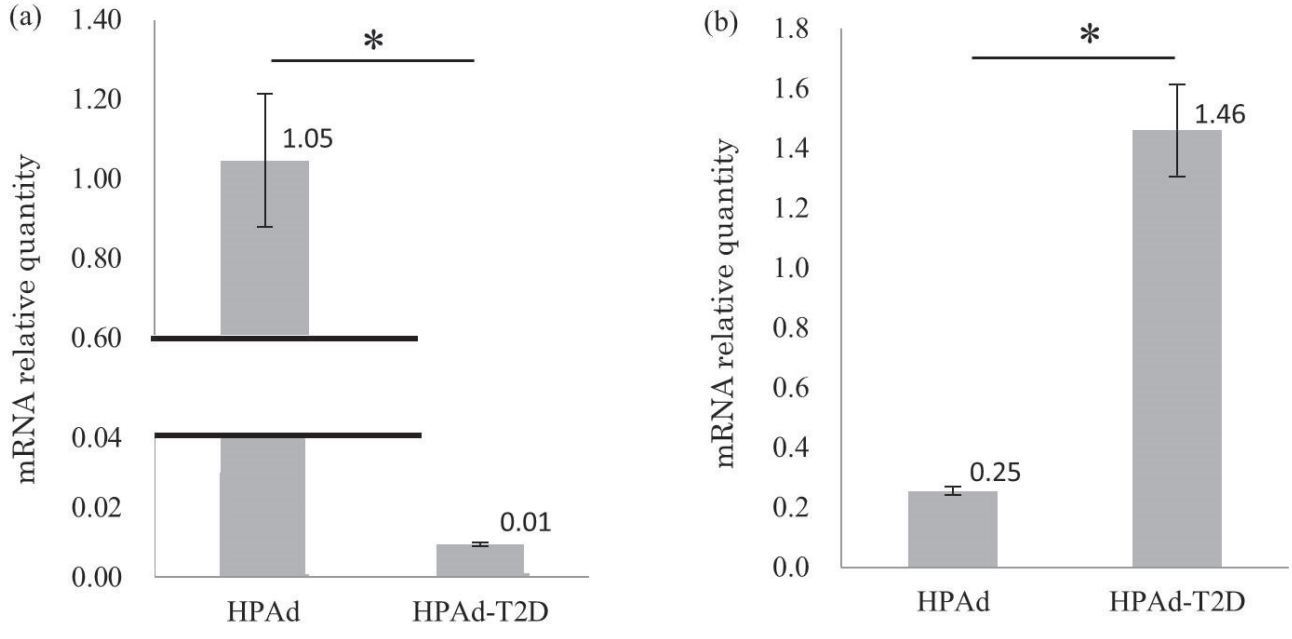

Figure 2. The mRNA expression without SCFA compared to HPAd and HPAd-T2D in control samples analyzed by qPCR. Adiponectin, a; Leptin, b. Results are expressed as mean ± SE. Human adipocytes, HPAd; Human adipocytes-Type 2 diabetes, HPAd-T2D. *p<0.01.

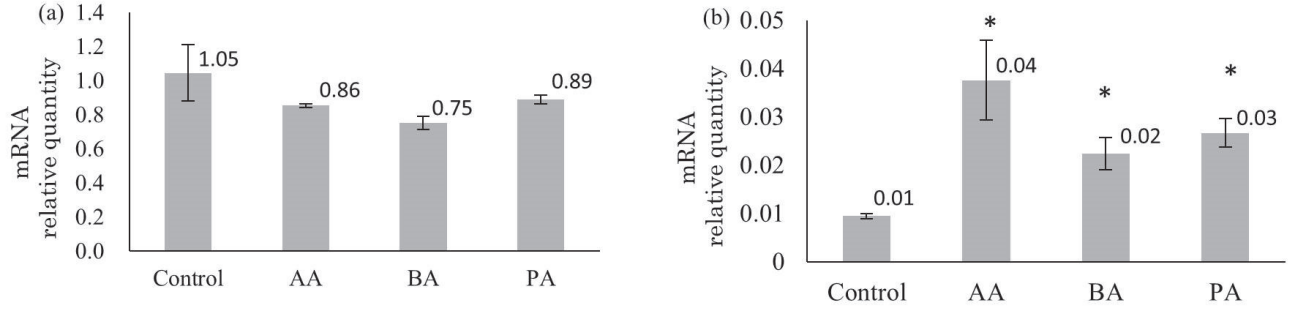

Figure 3. Adiponectin mRNA expression levels with or without SCFA analyzed by qPCR in adipocytes. HPAd (a) and HPAd-T2D (b) were treated with SCFAs for 48 hours and compared to samples without SCFA treatment. Results are expressed as mean ± SE. Human adipocytes, HPAd; Human adipocytes-Type 2 diabetes, HPAd-T2D. *p<0.05.

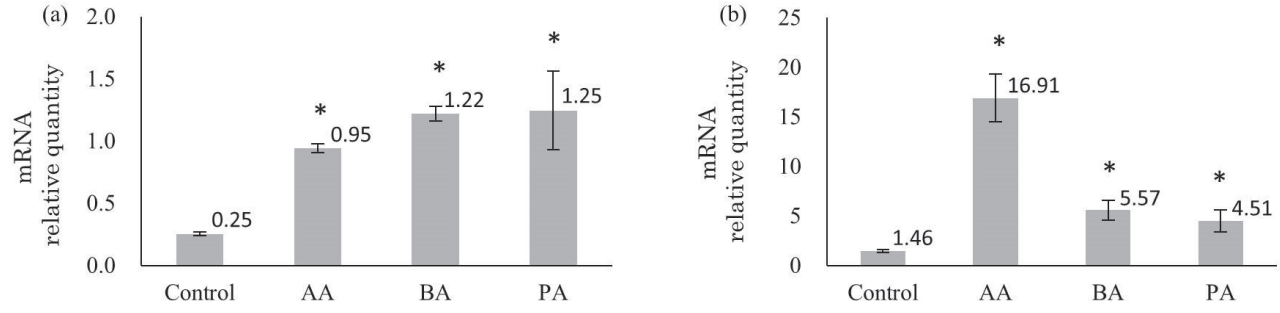

Figure 4. Leptin mRNA expression levels with or without SCFA analyzed by qPCR. HPAd (a) and HPAd-T2D (b) were treated with SCFAs for 48 hours and compared to samples without SCFA treatment. Results are expressed as mean ± SE. Human adipocytes, HPAd; Human adipocytes-Type 2 diabetes, HPAd-T2D. *p<0.05.

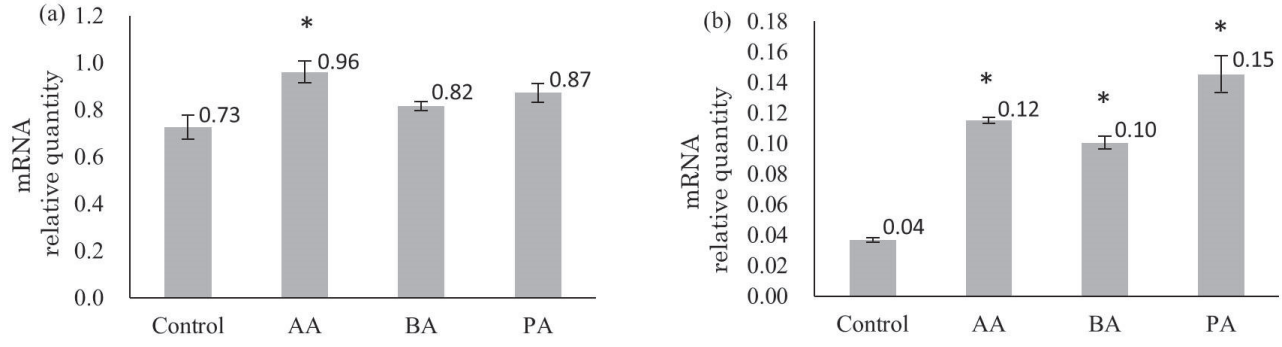

Figure 5. PPARγ mRNA expression levels with or without SCFA analyzed by qPCR. HPAd (a) and HPAd-T2D (b) were treated with SCFAs for 48 hours and compared to samples without SCFA treatment. Results are expressed as mean ± SE. Human adipocytes, HPAd; Human adipocytes-Type 2 diabetes, HPAd-T2D. *p<0.05.

increased mRNA levels of PPARγ (Figs. 5a, 5b). Given that the dysregulation of PPARγ is linked to the development of obesity, T2D, atherosclerosis, and the other disease conditions (28, 34), regulation of PPARγ in normal adipocytes may have not the same results as that of T2D adipocyte. In addition, activated expression of PPARγ by its agonist led to apoptosis of bloated adipocyte and decrease in adipocyte size in obese rats (35). Thus, we speculate that PPARγ dysregulation occurs in T2D adipocytes, and those SCFAs, which act as PPARγ agonists as observed from the upregulation of their mRNA expression, consequently inhibited the accumulation of lipid droplets in adipocytes.

The comparison of mRNA levels of adiponectin and leptin in HPAd with HPAd-T2D showed significantly higher mRNA levels of adiponectin in HPAd, whereas significantly higher mRNA levels of leptin were noted in HPAd-T2D (Fig. 2). These results are in line with the results of a previous study that showed low expression of adiponectin in T2D patients (18), while leptin overexpression was found in obesity including T2D (36).

No significant changes in mRNA expression of adiponectin in HPAd were detected following stimulation by SCFAs (Fig. 3a), while all three SCFAs significantly increased the expression of adiponectin in HPAd-T2D (Fig. 3b). A previous study showed that PPARγ increases adiponectin levels by transactivating adiponectin gene expression (37). Since mRNA expression of adiponectin showed a similar tendency to that of PPARγ in HPAd-T2D in our study (Fig. 3b, Fig. 5b), we speculate that mRNA expression of adiponectin was increased via expression of PPARγ. Adiponectin is known to decrease hepatic lipogenesis or increase β-oxidation through adipoR1 mediated activation of AMPK and peroxisome proliferator-activated receptor alpha (PPARα) (38), and also stimulates fatty acid oxidation of AMPK, p38 AMPK, and PPARα in skeletal muscle cell (39). Taken together, our results suggest that SCFAs may have beneficial effects to improve obesity or T2D.

The mRNA expression of leptin was significantly increased by AA, BA and PA in both HPAd and HPAd-T2D compared to control, which confirms findings from a previous study (Fig. 4) (19, 23).

Both GPR 41 and GPR43 are expressed in human adipose tissues: GPR 41 is primarily activated by PA followed by BA and AA, and GPR 43 is activated

by all three SCFAs at a similar rate (20-21). In HPAd, PA induced the highest mRNA expression of leptin, while AA had the most profound effects in HPAd-T2D. These results may be attributed to the differentiation of receptor expression in both types of adipocytes. Further research is required to clarify the differentiation of receptor expression in HPAd and HPAd-T2D.On the other hand, leptin regulates appetite and energy expenditure by stimulating the hypothalamus in healthy individuals, while in obesity, despite high circulating leptin concentration leptin resistance occurs (36). Therefore it is unclear whether the expression of leptin by SCFA stimulation necessarily plays a role in regulating appetite or energy expenditure in obese or T2D patients.

Our findings suggest SCFA may have positive effects on host physiology.

However, the majorities of recent publications have investigated the effects of SCFAs on one particular tissue or metabolic process and have failed to look at the body system as a whole. Here we propose that SCFAs have a number of metabolic processes which are activated in parallel that affect energy homeostasis and appetite regulation. Furthermore, the site-specific uptake of SCFA across the gut-liver-peripheral tissue axis suggests selectivity in the effect of individual SCFA. It is only by bringing these effects together that the true impact of SCFAs on host energy homeostasis can be explained.

In conclusion, SCFAs inhibited lipid droplet accumulation and increased mRNA expression of adiponectin and leptin in T2D adipocytes. Although some of these mechanisms may involve the activation of PPARγ, particularly with respect to AA, further studies are needed to clarify the role of GPR receptors in adipokine production in adipocytes and phosphorylation of AMPK. Our results may offer insights into drug-free alternatives for the prevention of obesity and treatment of diabetes.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

We are grateful to the Department of Hospital Administration, Juntendo University Graduate School of Medicine for their technical support.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Hirasawa A, Hara T, Katsuma S, Adachi T, Tsujimoto G. Free fatty acid receptors and drug discovery. Biol Pharm Bull. 2008;31(10):1847-1851.
2. Macia L, Thorburn AN, Binge LC, Marino E, Rogers KE, Maslowski KM, Vieira AT, Kranich J, Mackay CR. Microbial influences on epithelial integrity and immune function as a basis for inflammatory diseases. Immunol Rev 2012; 245:164-176.
3. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 2015; 11:577-591.
4. Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, Anastasovska J, Ghourab S, Hankir M, Zhang S, Carling D, Swann JR, Gibson G, Viardot A, Morrison D, Louise Thomas E, Bell JD. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nat Commun. 2014; 5:3611.
5. den Besten G, Lange K, Havinga R, van Dijk TH, Gerding A, van Eunen K, Müller M, Groen AK, Hooiveld GJ, Bakker BM, Reijngoud DJ. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. Am J Physiol Gastrointest Liver Physiol. 2013; 305(12):G900-910.
6. Roediger WE. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut. 1980;21(9):793-798.
7. Ardawi MS, Newsholme EA. Fuel utilization in colonocytes of the rat. Biochem J. 1985;231(3):713-719.
8. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, Bäckhed F, Mithieux G. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell. 2014; 156(1-2):84-96.
9. Babaei A, Zarkesh-Esfahani SH, Bahrami E, Ross RJ. Restricted leptin antagonism as a therapeutic approach to treatment of autoimmune diseases. Hormones (Athens). 2011; 10(1):16-26.
10. Gamberi T, Magherini F, Modesti A, Fiaschi T. Adiponectin Signaling Pathways in Liver Diseases. Biomedicines. 2018; 6(2). pii: E52.
11. Obata Y, Yamada Y, Takahi Y, Baden MY, Saisho K, Tamba S, Yamamoto K, Umeda M, Furubayashi A, Matsuzawa Y. Relationship between serum adiponectin levels and age in healthy subjects and patients with type 2 diabetes. Clin Endocrinol 2013; 79:204-210.
12. Kocot J, Dziemidok P, Kiełczykowska M, Hordyjewska A, Szcześniak G, Musik I. Adipokine Profile in Patients with Type 2 Diabetes Depends on Degree of Obesity. Med Sci Monit. 2017; 23:4995-5004.
13. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 2012; 425(3):560-564.
14. Comandasu DE, Mohora M, Virgolici B, Mehedintu C, Berceanu C, Cirstoiu M, Bratila E. Maternal-Fetal Metabolism Disorders Induced by Maternal Obesity in An Animal Model. Acta Endocrinologica-Bucharest. 1999;257(1):79-83
15.. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001; 7:941-946.
16. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372:425-432.
17. Ahima RS, Flier JS. Leptin. Annu Rev Physiol 2000; 62:413-437.

18. Ebihara K, Kusakabe T, Hirata M, Masuzaki H, Miyanaga F, Kobayashi N, Tanaka T, Chusho H, Miyazawa T, Hayashi T, Hosoda K, Ogawa Y, DePaoli AM, Fukushima M, Nakao K. Efficacy and safety of leptin-replacement therapy and possible mechanisms of leptin actions in patients with generalized lipodystrophy. J Clin Endocrinol Metab 2007; 92:532-541.
19. Al-Lahham SH, Roelofsen H, Priebe M, Weening D, Dijkstra M, Hoek A, Rezaee F, Venema K, Vonk RJ. Regulation of adipokine production in human adipose tissue by propionic acid. Eur J Clin Invest 2010; 40:401-407.
20. Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, Muir AI, Wigglesworth MJ, Kinghorn I, Fraser NJ, Pike NB, Strum JC, Steplewski KM, Murdock PR, Holder JC, Marshall FH, Szekeres PG, Wilson S, Ignar DM, Foord SM, Wise A, Dowell SJ. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem 2003; 278:11312-11319.
21. Le Poul E, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, Brezillon S, Dupriez V, Vassart G, Van Damme J, Parmentier M, Detheux M. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. J Biol Chem 2003; 278:25481-25489.
22. Zaibi MS, Stocker CJ, O'Dowd J, Davies A, Bellahcene M, Cawthorne MA, Brown AJ, Smith DM, Arch JR. Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. FEBS Lett 2010; 584:2381-2386.
23. Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM, Yanagisawa M. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. Proc Natl Acad Sci 2004; 101:1045-1050.
24. Yan H, Ajuwon KM. Mechanism of Butyrate Stimulation of Triglyceride Storage and Adipokine Expression during Adipogenic Differentiation of Porcine Stromovascular Cells. PLoS One 2015; 10 e0145940.
25. Yamashita H, Maruta H, Jozuka M, Kimura R, Iwabuchi H, Yamato M, Saito T, Fujisawa K, Takahashi Y, Kimoto M, Hiemori M, Tsuji H. Effects of acetate on lipid metabolism in muscles and adipose tissues of type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Biosci Biotechnol Biochem 2009; 73:570-576.
26. Chandra M, Miriyala S, Panchatcharam M. PPARγ and Its Role in Cardiovascular Diseases. PPAR Res. 2017:6404638.
27. Lee TW, Bai KJ, Lee TI, Chao TF, Kao YH, Chen YJ. PPARs modulate cardiac metabolism and mitochondrial function in diabetes. J Biomed Sci. 2017; 24(1):5.
28. Imam MU, Ismail M, Ithnin H, Tubesha Z, Omar AR. Effects of germinated brown rice and its bioactive compounds on the expression of the peroxisome proliferator-activated receptor gamma gene. Nutrients 2013; 5:468-477.
29. Lee CH, Olson P, Evans RM. Minireview: lipid metabolism, metabolic diseases,and peroxisome proliferator-activated receptors. Endocrinology 2003; 144:2201–2207.
30. Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME, Pang Z, Chen AS, Ruderman NB, Chen H, Rossetti L, Scherer PE. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. J Biol Chem 2006; 281: 2654–2660.
31. Kubota N, Terauchi Y, Kubota T, Kumagai H, Itoh S, Satoh H, Yano W, Ogata H, Tokuyama K, Takamoto I, Mineyama T, Ishikawa M, Moroi M, Sugi K, Yamauchi T, Ueki K, Tobe K, Noda T, Nagai R, Kadowaki T. Pioglitazone ameliorates insulin resistance and diabetes by both adiponectin-dependent and-independent pathways. J Biol Chem 2006; 281: 8748–8755.
32. Yamashita H, Fujisawa K, Ito E, Idei S, Kawaguchi N, Kimoto M, Hiemori M, Tsuji H. Improvement of obesity and glucose tolerance by acetate in Type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Biosci Biotechnol Biochem 2007; 71:1236-1243.
33. Srivastava RA, Pinkosky SL, Filippov S, Hanselman JC, Cramer CT, Newton RS. AMP-activated protein kinase: an emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases. J Lipid Res. 2012; 53(12):2490-2514.
34. Lehrke M, Lazar MA. The many faces of PPARgamma. Cell; 123(6):993-999.
35. Okuno A, Tamemoto H, Tobe K, Ueki K, Mori Y, Iwamoto K, Umesono K, Akanuma Y, Fujiwara T, Horikoshi H, Yazaki Y, Kadowaki T. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J Clin Invest 1998; 101:1354-1361.
36. Stern JH, Rutkowski JM, Scherer PE. Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. Cell Metab 2016; 23:770-784.
37. Wu Y, Huang M, Zhao P, Yang X. Vanadyl acetylacetonate upregulates PPARγ and adiponectin expression in differentiated rat adipocytes. J Biol Inorg Chem 2013; 18:623-631.
38. Awazawa M, Ueki K, Inabe K, Yamauchi T, Kaneko K, Okazaki Y, Bardeesy N, Ohnishi S, Nagai R, Kadowaki T. Adiponectin suppresses hepatic SREBP1c expression in an AdipoR1/LKB1/AMPK dependent pathway. Biochem Biophys Res Commun 2009; 382:51-56.
39. Yoon MJ, Lee GY, Chung JJ, Ahn YH, Hong SH, Kim JB. Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. Diabetes 2006; 55:2562-2570.