Biochemical and Biophysical Research Communications 427 (2012) 299–304

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Ternatin, a cyclic peptide isolated from mushroom, and its derivative suppress hyperglycemia and hepatic fatty acid synthesis in spontaneously diabetic KK-A^y mice

Misato Kobayashi [a], Haruna Kawashima [a], Kumiko Takemori [b], Hiroyuki Ito [c], Atsushi Murai [a], Shun Masuda [d], Kaoru Yamada [d], Daisuke Uemura [d], Fumihiko Horio [a,*]

[a] Department of Applied Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan
[b] Department of Food and Nutrition, Kinki University Faculty of Agriculture, Nara 631-8505, Japan
[c] Department of Biomedical Engineering, Kinki University Faculty of Biology-Oriented Engineering, Wakayama 649-6493, Japan
[d] Department of Chemistry, Kanagawa University, Kanagawa 259-1293, Japan

ARTICLE INFO

Article history:
Received 6 September 2012
Available online 18 September 2012

Keywords:
Ternatin
Cyclic peptide
Anti-diabetic
Blood glucose
Triglyceride
KK-A^y mice

ABSTRACT

(−)-Ternatin is a highly methylated cyclic heptapeptide isolated from mushroom *Coriolus versicolor*. Ternatin has an inhibitory effect on fat accumulation in 3T3-L1 adipocytes. [D-Leu^7]ternatin, a ternatin derivative, also inhibited fat accumulation in 3T3-L1 cells, although the effectiveness of [D-Leu^7]ternatin was lower than that of ternatin. In this study, we investigated the effects of ternatin and [D-Leu^7]ternatin on obesity and type 2 diabetes in KK-A^y mice, an animal model for spontaneously developed type 2 diabetes. We continuously administered ternatin (8.5 or 17 nmol/day) or [D-Leu^7]ternatin (68 nmol/day) to mice via a subcutaneous osmotic pump. Unexpectedly, neither ternatin nor [D-Leu^7]ternatin affected body weight or adipose tissue weight in KK-A^y mice. In contrast, it was demonstrated that both ternatin and [D-Leu^7]ternatin suppress the development of hyperglycemia. In liver, the SREBP-1c mRNA level tended to be lower or significantly decreased in mice treated with ternatin or [D-Leu^7]ternatin, respectively. Moreover, we found that ternatin directly lowered the SREBP-1c mRNA level in Hepa1-6 hepatocyte cells. This study showed that ternatin and [D-Leu^7]ternatin each had a preventive effect on hyperglycemia and a suppressive effect on fatty acid synthesis in KK-A^y mice.

1. Introduction

Type 2 diabetes is the leading cause of chronic kidney disease (diabetic nephropathy), blindness (diabetic retinopathy), and non-traumatic lower-limb amputations (diabetic neuropathy). The incidence of type 2 diabetes is dramatically increasing due to changes in lifestyle such as in diet and activity level [1]. The interaction between impaired insulin secretion and insulin resistance is involved in the development of type 2 diabetes. Obesity is a major risk factor for insulin resistance, type 2 diabetes, dyslipidemia, cardiovascular disease, and fatty liver. A strong link between obesity and insulin resistance has been reported in animals and human studies [2]. Therefore, the amelioration of obesity leads to both the improvement of insulin sensitivity and the prevention of type 2 diabetes.

Various natural products, including crude extracts and isolated compounds, from plants or plant parts such as various berries [3–7], soybean [8,9], tea plant (*Camellia sinensis*) [10,11], and lotus (*Nelumbo nucifera*) [12]), reportedly have physiological effects, such as the inhibition of lipase activity, the suppression of appetite, the stimulation of energy expenditure, the inhibition of adipocyte differentiation, and the regulation of lipid metabolism [13]. Numerous components from natural products can be utilized to safely treat diet-induced obesity and type 2 diabetes.

(−)-Ternatin (ternatin) is a highly methylated cyclic heptapeptide isolated from mushroom *Coriolus versicolor* [14,15]. Although ternatin had been known as an anti-bacterial or anti-microbial compound, its inhibitory effect on fat accumulation was recently shown by using 3T3-L1 adipocytes [14]. In preadipocytes at the early stage of differentiation, ternatin reduced the mRNA levels of CCAAT/enhancer binding protein-α (C/EBP-α and sterol regulatory element binding protein-1c (SREBP-1c), and tended to suppress the peroxisome proliferative activated receptor-γ (PPAR-γ mRNA level [16]. This suppression of preadipocyte differentiation

Abbreviations: ACC, acetyl-CoA carboxylase; C/EBP-α, CCAAT/enhancer binding protein-α; FAS, fatty acid synthase; PPAR-γ, peroxisome proliferative activated receptor-γ; SREBP-1c, sterol regulatory element binding protein-1c.

* Corresponding author. Address: Department of Applied Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8601, Japan. Fax: +81 52 789 4075.
E-mail address: horiof@agr.nagoya-u.ac.jp (F. Horio).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.09.045

brought about the reduction of mRNA levels of adipocyte fatty acid binding protein (aP2), lipoprotein lipase, fatty acid synthase (FAS), and acetyl-CoA carboxylase 2 (ACC2), and led to the reduction of cellular lipid accumulation [16]. In the differentiated 3T3-L1 adipocytes, ternatin treatment also reduced triglyceride synthesis, but ternatin's effectiveness in differentiated adipocytes was lower than that observed in the preadipocytes [16].

[D-Leu^7]ternatin, a ternatin derivative, was also demonstrated to inhibit fat accumulation in 3T3-L1 cells, and the effective dose of [D-Leu^7]ternatin was eight times that of ternatin [17]. The cytotoxicity value (IC_{50}) of [D-Leu^7]ternatin was 12-fold higher than that of ternatin [17]. From these results, [D-Leu^7]ternatin's inhibition of fat accumulation was lower than ternatin's, but the low toxicity of [D-Leu^7]ternatin is beneficial. The mechanism underlying this derivative's action is thought to be similar to that of ternatin. In addition, it is unclear whether ternatin and [D-Leu^7]ternatin have an anti-obesity effect or an anti-diabetic effect *in vivo*. From this background, in the present study we investigated whether the administration of ternatin or [D-Leu^7]ternatin suppresses the development of obesity and type 2 diabetes in KK-A^y mice, a model for spontaneously developed type 2 diabetes.

2. Materials and methods

2.1. Animals

Four-week-old male KK-A^y mice (CLEA, Tokyo, Japan) were purchased and acclimatized for 1 week before the experiments began. All mice were maintained at a controlled temperature of 23 ± 3 °C and 55 ± 5% humidity on a 12-h light/dark cycle and allowed free access to water and a standard laboratory diet (CE-2; CLEA). The composition of the diet was as follows: protein, 254 g/kg; fat, 51 g/kg; non-nitrogenous substances, 506 g/kg; crude fiber, 35 g/kg; crude ash, 67 g/kg; energy, 15.2 MJ/kg; sufficient minerals and vitamins to maintain the health of the mice.

2.2. Administration of ternatin or [D-Leu^7] ternatin derivative in KK-A^y mice

Ternatin was synthesized according to the method previously described [14]. [D-Leu^7]ternatin derivative was synthesized by Peptide Institute Inc. (Osaka, Japan). Ternatin was administered to each mouse at a dose of 8.5 or 17 nmol/day via subcutaneous continuous infusion for 4 weeks using an osmotic pump. [D-Leu^7]ternatin derivative was also administered to each mouse at a dose of 68 nmol/day. The dose of the [D-Leu^7]ternatin derivative was calculated from the compound's inhibitory effect on fat accumulation in 3T3-L1 cells [17]. The concentration (EC_{50}) of [D-Leu^7]ternatin derivative showing a 50% effect of the compound's maximum fat accumulation inhibition of this compound was eight times that of ternatin. These cyclic peptides were dissolved in 70% DMSO and loaded into osmotic minipumps (model 1004; Alzet, Cupertino, CA) according to the manufacturer's protocol. Control groups received pumps loaded with vehicle (70% DMSO in sterile water). An osmotic pump was surgically inserted subcutaneously into the back of each mouse.

2.3. Experimental procedure in mouse

Experiment 1: Mice were divided into three groups and subcutaneously administered vehicle (control group, 6 mice), 8.5 nmol/day ternatin (8.5 nmol ternatin group, 7 mice), or 17 nmol/day ternatin (17 nmol ternatin group, 6 mice) via the osmotic pumps. *Experiment 2:* Mice were divided into two groups and administered vehicle (control group, 6 mice) or 68 nmol/day [D-Leu^7]ternatin derivative ([D-Leu^7] group, 6 mice) via the osmotic pumps.

The mice were allowed free access to drinking water and diet (CE-2; CLEA) for 4 weeks. Blood samples were collected from the tail vein once a week to measure the serum glucose concentration. Blood was collected from the tail vein at 10:00 after 1-h diet deprivation. The collected blood was kept at room temperature for 15 min for coagulation. The serum was then obtained from the coagulated blood by centrifugation at 2430*g* for 10 min at 4 °C. The serum was kept at −30 °C prior to use. Serum glucose was measured by the assay kit using the glucose oxidase method, Glucose II-test (Wako Pure Chemical, Osaka, Japan). At the end of the experiment, the mice were killed by decapitation, and serum, liver, and fat pad were collected. The animal care and experimental procedures were approved by the Animal Research Committee of Nagoya University and were conducted according to the Regulations for Animal Experiments at Nagoya University.

2.4. Serum components analysis

Serum triglyceride and cholesterol concentrations were, respectively measured by a Triglyceride-E test (Wako Pure Chemical) and a Cholesterol-E test (Wako Pure Chemical). A commercially available ELISA kit was used to determine the serum concentration of insulin (Morinaga Seikagaku, Kanagawa Japan).

2.5. Hepatic lipids analysis

Frozen livers were homogenized in chloroform/methanol (2:1), and liver lipids were extracted into organic solvents. A portion of this extract was dried, and the triglycerides in this dried material were measured by the Triglyceride-E test. Another portion of the extract was also used to measure total lipid content according to the method of Folch et al. [18].

2.6. RNA preparation and gene expression analysis

Total RNA was extracted from frozen tissues using TRIzol reagent (Invitrogen, Carlsbad, CA). It was then treated with DNase using a TURBO DNA-free kit (Ambion, Austin, TX). cDNA was synthesized using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Gene expression was quantified by real-time PCR using an ABI 7300 real-time PCR system with the Thunderbird qPCR Mix or the Thunderbird SYBR qPCR Mix (Toyobo, Tokyo, Japan). TaqMan primers and probes were used to determine mRNA levels of PPAR-γ (TaqMan Gene Expression Assays, Mm01 184322_m1, Applied Biosystems) and 18S rRNA (Pre-developed TaqMan Assay Reagents, Eukaryotic 18S rRNA, Applied Biosystems). The primers for SYBR Green assay were as follows: ACC sense, 5′-TG ACAGACTGATCGCAGAGAAAG-3′; ACC antisense, 5′-TGGAGA CCCCA CACACA-3′; β-acitn sense, 5′-AGATGACCCAGATCATGTTTGAGA-3′; β-actin antisense, 5′-CACAGCCTGGATGGCTACGT-3′ FAS antisense, 5′-TCAGCCACTTGAGTGTCCTC-3′; SREBP-1c sense, 5′-GGAGC CATG GATTGCACATT-3′; SREBP-1c antisense, 5′-GGCCCGGGAAGTCACT GT-3′; FAS sense, 5′-GGGTTCTAGCCAGCAGAGTC-3′. The level of each mRNA was normalized to that of the corresponding 18S rRNA (KK-A^y mice) or β-actin (Hepa1-6).

2.7. Ternatin treatment in Hepa1-6 cells

Hepa1-6 cells (RBRC-RCB1638 RIKEN BRC Cell Bank, Japan) were grown in high-glucose Dulbecco's modified Eagle medium (DMEM, Wako Pure Chemical) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a 5% CO_2-humidified incubator. To examine the cellular toxicity of ternatin, Hepa1-6 cells were treated with DMSO (vehicle) or

ternatin (1.3, 13, 130 nM, 1.3, and 13 μM, dissolved in DMSO) for 30 h. After this culture, cell viability was assayed by a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan).

To examine ternatin's effect on various mRNA levels, Hepa1-6 cells were seeded on 12-well plates (2×10^5 cells per well) on day 0. On day 1, DMSO or ternatin (1.3 and 13 nM) was added to the culture medium. Forty-eight hours after this addition, cells were harvested and used for measuring various mRNA levels.

2.8. Statistical analysis

All results are expressed as means ± SEM. Phenotypic data were statistically analyzed by one-way ANOVA, and a subsequent Dunnet's test was carried out to compare the means of all groups (experiment 1 and Hepa1-6 cells). In experiment 2, phenotypic data were statistically analyzed by either Student's *t* test or Welch's test. When the variances of each group were equal, mean values were compared using the former test. When the variances of each group were unequal, the significance of differences was determined using the latter test. Values of $P < 0.05$ were considered statistically significant (StatView; SAS Institute, Cary, NC).

3. Results

3.1. Administration of ternatin (8.5 or 17 nmol/day) in KK-A^y mice (experiment 1)

KK-A^y mice were administered vehicle (control) or either 8.5 or 17 nmol/day ternatin. The initial body weight, final body weight, and total food intake did not differ among the three groups (Table 1). At the beginning of the experiment (5 weeks of age), the blood glucose concentration in the control group was 230 ± 9 mg/dl (Fig. 1A). The blood glucose concentrations in the control group were increased dramatically, reaching 533 mg/dl by the end of the experiment. The blood glucose concentrations at 2 and 3 weeks into the experiment were significantly lower in the 8.5 nmol ternatin group than in the control group. At 3 weeks into the experiment, the blood glucose concentration in the 17 nmol ternatin group was also significantly lower than that in the control group. The liver weight (g) tended to be lower in the 17 nmol ternatin group than in the control group (Table 1, $p = 0.084$). Liver triglyceride content and liver total lipids were significantly lower in the 17 nmol ternatin group than in the control group (Fig. 1B and C). The values of liver triglyceride content and liver total lipid content in the 8.5 nmol ternatin group were similar to those in the 17 nmol ternatin group, and the values in the 8.5 nmol ternatin group were not significantly different from those in the control group. The tissue weights of subcutaneous fat, epididymal fat, retroperitoneal fat, and mesenteric fat in the 8.5 nmol or the 17 nmol ternatin group were not different from those in the control group (Table 1). The serum concentrations of insulin and lipids (triglycerides and cholesterol) did not differ between the control and two ternatin groups (Table 1).

Previously, it was reported that ternatin or [D-Leu^7]ternatin derivative decreased the mRNA levels of genes regulating adipogenesis in 3T3-L1 adipocytes [16]. In the present study, the mRNA levels of PPAR-γ and SREBP-1c in the epididymal fat did not differ among these three groups (Fig. 1E). The mRNA levels of FAS or ACC tended to be lower in the 8.5 or 17 nmol ternatin group than in the control group.

In this study, 17 nmol ternatin significantly decreased liver triglyceride content and liver total lipid content (Fig. 1B and C). Therefore, we also measured the mRNA levels of PPAR-γ, SREBP-1c, FAS, and ACC in the liver (Fig. 1D). In the liver, the mRNA level of PPAR-γ, a key regulator of adipogenesis, was not changed among the three experimental groups (Fig. 1D). The SREBP-1c mRNA level in the 8.5 nmol ternatin group tended to be lower than that in the control ($p = 0.06$). However, the mRNA levels of FAS and ACC, which regulate fatty acid synthesis, were not lower in either ternatin group compared to the control group (Fig. 1D).

Table 1
Body composition, food intake, and serum parameters in the control, the 8.5 nmol ternatin group, and the 17.5 nmol ternatin group.

	Control	8.5 nmol ternatin	17 nmol ternatin
Initial body weight (g)	18.9 ± 0.2	18.8 ± 0.3	18.9 ± 0.2
Final body weight (g)	41.4 ± 0.9	41.4 ± 0.6	40.2 ± 1.3
Total food intake (g)	135.1 ± 2.2	130.7 ± 3.7	132.3 ± 4.0
Tissue weight (g)			
Liver	2.67 ± 0.10	2.51 ± 0.08	2.42 ± 0.06
Subcutaneous fat	1.10 ± 0.14	1.02 ± 0.14	1.04 ± 0.10
Epididymal fat	1.33 ± 0.05	1.34 ± 0.05	1.29 ± 0.05
Retroperitoneal fat	0.33 ± 0.02	0.32 ± 0.01	0.35 ± 0.03
Mesenteric fat	0.64 ± 0.03	0.64 ± 0.05	0.59 ± 0.07
Serum insulin (ng/mL)	13.2 ± 3.4	10.1 ± 2.0	10.9 ± 1.4
Serum triglyceride (mg/dL)	292 ± 18	349 ± 20	316 ± 13
Serum cholesterol (mg/dL)	141 ± 4	128 ± 6	141 ± 4

3.2. Administration of [D-Leu^7]ternatin derivative (68 nmol/day) in KK-A^y mice (experiment 2)

The dose of [D-Leu^7]ternatin derivative was eight times that of the 8.5 nmol ternatin group. The final body weight and total food intake were not different between the control and [D-Leu^7] groups (Table 2). Liver weight was significantly lower in the [D-Leu^7] group than in the control group (Table 2). However, the liver triglycerides and total lipid content in the [D-Leu^7] group was not decreased compared to those in the control group (Table 2). Similar to the result in experiment 1, the [D-Leu^7]ternatin derivative had no effect on white adipose tissue weights, such as subcutaneous fat and epididymal fat, or on serum insulin concentration (Table 2). The blood glucose concentration at 3 weeks into the experiment was significantly lower in the [D-Leu^7] group (354 ± 30 mg/dl) than in the control group (466 ± 34 mg/dl, $p = 0.03$) (Fig. 2A).

In experiment 1, we found that the administration of ternatin tended to decrease the hepatic SREBP1c mRNA level (Fig. 1D), but not significantly, and also decreased the FAS mRNA level in epididymal fat (Fig. 1E). Therefore, we also determined the mRNA levels of lipogenic genes in liver and epididymal fat in experiment 2 (Fig. 2B and C). In liver, the SREBP-1c mRNA level was significantly lower in the [D-Leu^7] group than in the control group (Fig. 2B). Unexpectedly, in epididymal fat, the FAS mRNA level was significantly higher in the [D-Leu^7] group than in the control group (Fig. 2C). The [D-Leu^7]ternatin derivative had no effect on PPAR-γ or ACC mRNA levels in both tissues.

3.3. The effect of ternatin in mouse Hepa1-6 cell line

Although data are not shown, the treatment with 1.3 and 13 nM ternatin did not show cytotoxicity in mouse Hepa1-6 cells at all. However, the higher dose of ternatin reduced the number of viable cells (27% reduction at 130 nM; 54% reduction at 1.3 μM; and 63% reduction at 13 μM compared to that of the control (DMSO)). The mRNA levels of SREBP-1c, ACC, and FAS in Hepal-6 cells were measured after 1.3 and 13 nM ternatin treatment (Fig. S1). The mRNA levels of SREBP-1c and ACC were significantly decreased by 13 nM ternatin but not by 1.3 nM ternatin. The treatment with 1.3 or 13 nM ternatin did not affect the FAS mRNA level. Although data are not shown, the cellular triglyceride contents were not changed by 30 h treatment with 13 nM ternatin.

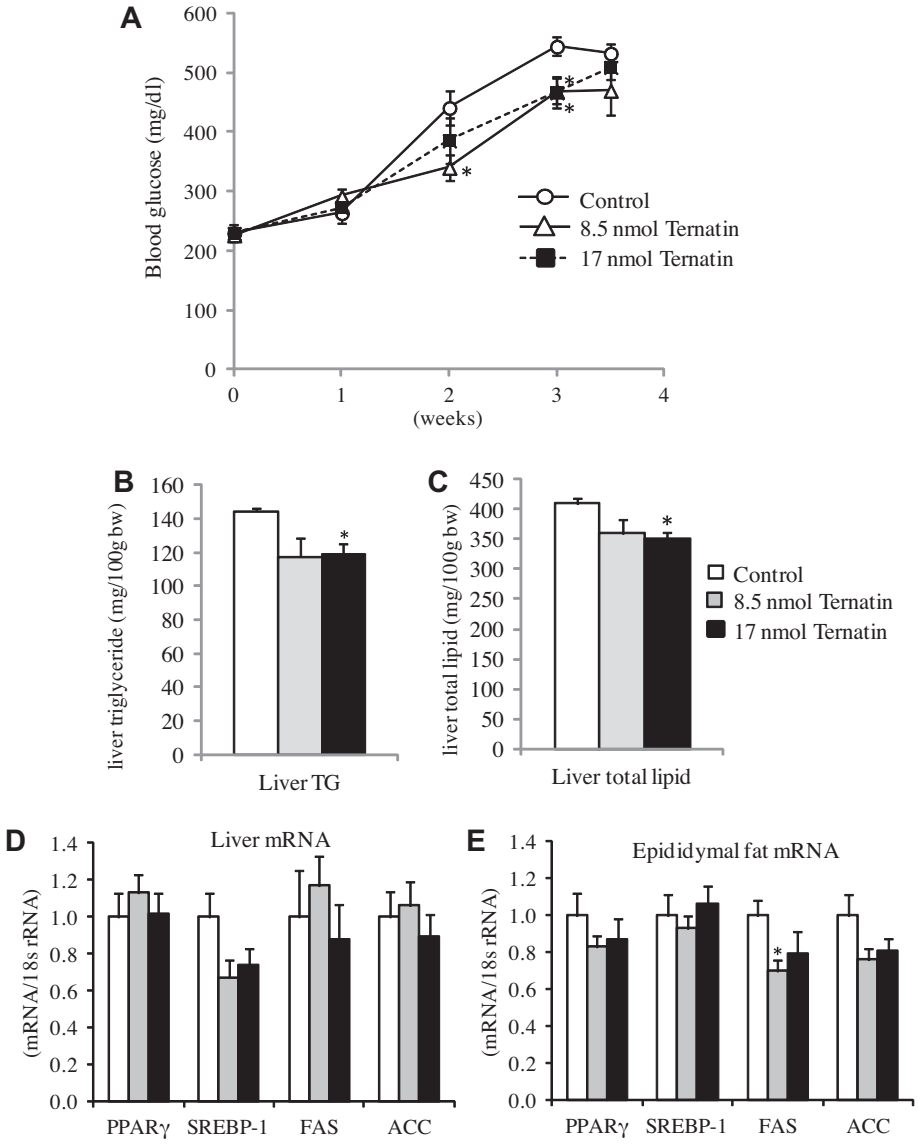

Fig. 1. The blood glucose concentrations, liver lipids, and gene expression levels in KK-A^y mice administered DMSO (control, $n = 6$) or ternatin (8.5 nmol ($n = 7$) and 17 nmol ($n = 6$), experiment 1). (A) Blood glucose concentrations in the control, 8.5 nmol ternatin, and 17 nmol ternatin groups. (B) Liver triglyceride content and liver total lipid content in the three groups at 4 weeks into the experiment. The liver mRNA levels (D) and the epididymal fat mRNA levels (E) of PPAR-γ, SREBP-1c, FAS, and ACC. The mRNA level of the control group was set as 1, and the relative mRNA levels of the ternatin groups were expressed as fold changes with respect to the control. The data are expressed as mean ± SEM. $^*P < 0.05$ vs control group.

4. Discussion

It has been reported that ternatin or its derivative [D-Leu^7]ternatin suppressed the expression of lipogenic genes and inhibited fat accumulation in cultured adipocytes [14,16,17]. These data imply that ternatin or its derivative [D-Leu^7]ternatin may be a valuable drug for the treatment of obesity. Therefore, it is necessary to demonstrate the *in vivo* effect of ternatin or its derivative. The primary aim of this study is to investigate the anti-obesity effect of ternatin or its derivative [D-Leu^7]ternatin in an obese animal model. To investigate this effect, in the present study we selected continuous subcutaneous administration of ternatin by an osmotic pump, because we had no data on the absorption rate of ternatin or its derivative from intestine. We estimated that a dose of 8.5 or 17 nmol/day ternatin brought about its effective concentration in blood, which inhibits fat accumulation in 3T3-L1 cells [16]. Unexpectedly, in mice, ternatin or its derivative did not show any anti-obesity effect such as decreased adipose tissue weight (Tables 1 and 2). On the other hand, fortunately, we demonstrated for the first time that ternatin or its derivative had a suppressive effect on the development of

Table 2
Body composition, food intake, and serum parameters in the control and the 68 nmol [D-Leu^7]ternatin groups.

	Control	[D-Leu^7]
Final body weight (g)	39.6 ± 0.5	39.7 ± 0.7
Total food intake (g)	140.5 ± 2.8	136.6 ± 2.8
Tissue weight (g)		
Liver	2.50 ± 0.04	2.28 ± 0.04*
Subcutaneous fat	1.28 ± 0.10	1.30 ± 0.10
Epididymal fat	1.29 ± 0.04	1.25 ± 0.06
Serum insulin (ng/mL)	13.6 ± 2.6	9.9 ± 1.5
Serum triglyceride (mg/dL)	346 ± 29	312 ± 23
Serum cholesterol (mg/dL)	115 ± 7	119 ± 8
Liver TG (mg/100 g bw)	61.7 ± 6.7	78.4 ± 3.9
Liver total lipids (mg/100 g bw)	237 ± 22	251 ± 16

* $P < 0.05$ vs control group.

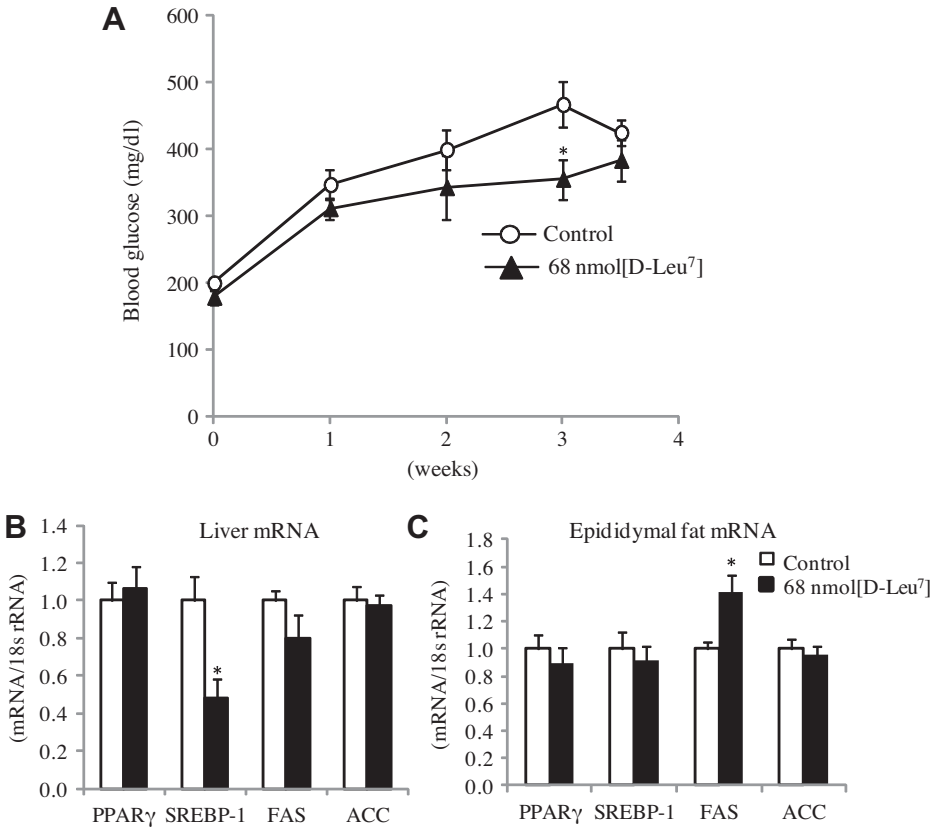

Fig. 2. Blood glucose concentrations and gene expression levels in KK-A^y mice administered DMSO (control, $n = 7$) or 68 nmol [D-Leu^7]ternatin ($n = 8$) (experiment 2).(A) Blood glucose concentrations in the control or the 68 nmol [D-Leu^7]ternatin group. The liver mRNA levels (B) and the epididymal fat mRNA levels (C) of PPAR-γ, SREBP-1c, FAS, and ACC. The mRNA level of the control group was set as 1, and the relative mRNA levels of the [D-Leu^7]ternatin group were expressed as fold changes with respect to the control. The data are expressed as mean ± SEM. *$P < 0.05$ vs control group.

hyperglycemia (Figs. 1A and 2A). In addition, we found that ternatin suppressed the expression of SREBP-1c, which accelerates fatty acid synthesis, in liver and hepatocytes, and that this effect of ternatin might be due to its direct action (Fig. S1). This result suggested that ternatin suppresses the accumulation of liver lipids in mice.

In this study, ternatin administration showed an anti-diabetic effect but not an anti-obesity effect. However, it was reported that the oral administration of ternatin in C57BL/6 mice reduced subcutaneous and visceral fat weights [19]. The dosage of ternatin (5 mg/kg body weight/day) in C57BL/6 mice [19] was calculated as about 170 nmol/day. This dosage of ternatin in the previous C57BL/6 study was about 10–20 times that administered in this study. We speculate that this discrepancy in ternatin's effect between the present study and the previous study was caused by the differences in both the dose and manner of administration.

Ternatin or [D-Leu^7]ternatin inhibited the fat accumulation in 3T3-L1 adipocytes [16,20]. It was speculated that this inhibitory effect was provided by the reduction in the expression of lipogenic genes, such as PPAR-γ, SREBP-1c, FAS, and ACC, and by the increase in lipolysis [16]. In this study, ternatin administration tended to reduce the FAS mRNA level in epididymal fat (Fig. 1E). However, ternatin treatment in KK-A^y mice did not change several adipose tissue weights or epididymal fat mRNA levels of PPAR-γ and SREBP-1c (Fig. 1E and Table 1). In a previous report, in 3T3-L1 preadipocytes, 130 nM ternatin in medium decreased lipogenic gene expression and intracellular lipid content [16]. In differentiated 3T3-L1 adipocytes, 1300 nM ternatin but not 130 nM ternatin in the medium lowered PPAR-γ and SREBP-1c mRNA levels and reduced the intracellular lipid content [16]. The effective dose of ternatin (1300 nM) in differentiated 3T3-L1 adipocytes was much higher than its effective dose in preadipocytes [17]. As mouse adipose tissue is composed mostly of differentiated adipocytes, we suppose from the present results that ternatin hardly inhibits fat accumulation in adipose tissue of KK-A^y mice.

In rat primary hepatocytes, ternatin suppressed the triglyceride synthesis and enhanced fatty acid oxidation [16]. We also measured the mRNA levels of lipogenic genes in liver and found that the SREBP-1c mRNA level tended to be decreased in the ternatin groups (Fig. 1D). Consistent with this reduction of SREBP-1c mRNA level in liver, the present study demonstrated for the first time that ternatin administration reduced liver triglyceride content and liver total lipid content (Fig. 1B and C).

Similar to the results with ternatin, [D-Leu^7]ternatin did not suppress either body weight gain or fat accumulations in subcutaneous and visceral fat (Table 2). In contrast, we demonstrated that not only ternatin but also its [D-Leu^7]ternatin derivative had an anti-diabetic effect in KK-A^y mice (Fig. 2A). In liver, [D-Leu^7]ternatin treatment also significantly decreased the SREBP-1c mRNA level (Fig. 2B). However, liver triglyceride content and liver total lipids were not decreased in mice administered [D-Leu^7]ternatin (Table 2). At present, we cannot explain the reason why [D-Leu^7]ternatin did not reduce liver triglyceride content. These results showed that the administration of ternatin or [D-Leu^7]ternatin consistently suppressed the hepatic expression of the SREBP-1c gene in KK-A^y mice. Insulin, an activator of SREBPs, is known to increase the expression of the SREBP-1c gene and proteolytic processing of SREBP proteins [21]. Therefore, we tried to clarify whether or not ternatin directly suppresses the expression of the SREBP-1c gene in a murine

hepatocyte cell line, Hepa1-6. Consequently, we detected decreased SREBP-1c and ACC mRNA levels by ternatin treatment (13 nM) (Fig. S1). These results suggested that the hepatic expression of the SREBP-1c gene was directly suppressed by ternatin, not by the change in serum insulin concentration in KK-A^y mice. In addition, the reduction in serum insulin concentration in mice administered ternatin and [D-Leu^7]ternatin might partially contribute to the decrease in the hepatic SREBP-1c mRNA level.

In conclusion, we determined the effects of ternatin and of [D-Leu^7]ternatin on obesity and type 2 diabetes in spontaneously diabetic/obese KK-A^y mice. Ternatin and [D-Leu^7]ternatin, at the dose adopted in this study, did not affect either adipose tissue weights or lipogenic genes (such as PPAR-γ and SREBP-1c) expression in adipose tissue. We revealed for the first time that ternatin and [D-Leu^7]ternatin partially suppress hyperglycemia in KK-A^y mice. Interestingly, it was suggested that ternatin directly decreases hepatic TG synthesis by reducing the expression of the SREBP-1c gene. Ternatin and its derivative might improve hepatic TG metabolism, leading to the amelioration of type 2 diabetes.

Acknowledgments

This work was supported by an Adaptable and Seamless Technology transfer Program through target-driven R&D (A-STEP) (No.AS231Z03808E) from Japan Science and Technology Agency, and by a grant from The Towa foundation for food research (to F. Horio).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.09.045.

References

[1] J.A. Yanovski, S.Z. Yanovski, Recent advances in basic obesity research, JAMA 282 (1999) 1504–1506.
[2] S.E. Kahn, R.L. Hull, K.M. Utzschneider, Mechanisms linking obesity to insulin resistance and type 2 diabetes, Nature 444 (2006) 840–846.
[3] M. Takikawa, S. Inoue, F. Horio, T. Tsuda, Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice, J. Nutr. 140 (2010) 527–533.
[4] A.S. Attele, Y.P. Zhou, J.T. Xie, J.A. Wu, L. Zhang, L. Dey, W. Pugh, P.A. Rue, K.S. Polonsky, C.S. Yuan, Antidiabetic effects of Panax ginseng berry extract and the identification of an effective component, Diabetes 51 (2002) 1851–1858.
[5] L. Dey, J.T. Xie, A. Wang, J. Wu, S.A. Maleckar, C.S. Yuan, Anti-hyperglycemic effects of ginseng: comparison between root and berry, Phytomedicine 10 (2003) 600–605.
[6] J.T. Xie, C.Z. Wang, M. Ni, J.A. Wu, S.R. Mehendale, H.H. Aung, A. Foo, C.S. Yuan, American ginseng berry juice intake reduces blood glucose and body weight in ob/ob mice, J. Food Sci. 72 (2007) S590–594.
[7] C. Morimoto, Y. Satoh, M. Hara, S. Inoue, T. Tsujita, H. Okuda, Anti-obese action of raspberry ketone, Life Sci. 77 (2005) 194–204.
[8] H. Xu, G.T. Barnes, Q. Yang, G. Tan, D. Yang, C.J. Chou, J. Sole, A. Nichols, J.S. Ross, L.A. Tartaglia, H. Chen, Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance, J. Clin. Invest. 112 (2003) 1821–1830.
[9] C.R. Cederroth, S. Nef, Soy, phytoestrogens and metabolism: a review, Mol. Cell. Endocrinol. 304 (2009) 30–42.
[10] F. Thielecke, M. Boschmann, The potential role of green tea catechins in the prevention of the metabolic syndrome – a review, Phytochemistry 70 (2009) 11–24.
[11] K.A. Grove, J.D. Lambert, Laboratory, epidemiological, and human intervention studies show that tea (*Camellia sinensis*) may be useful in the prevention of obesity, J. Nutr. 140 (2010) 446–453.
[12] E. Ohkoshi, H. Miyazaki, K. Shindo, H. Watanabe, A. Yoshida, H. Yajima, Constituents from the leaves of *Nelumbo nucifera* stimulate lipolysis in the white adipose tissue of mice, Planta Med. 73 (2007) 1255–1259.
[13] J.W. Yun, Possible anti-obesity therapeutics from nature – a review, Phytochemistry 71 (2010) 1625–1641.
[14] K. Shimokawa, I. Mashima, A. Asai, K. Yamada, M. Kita, D. Uemura, (−)-Ternatin, a highly *N*-methylated cyclic heptapeptide that inhibits fat accumulation: strauctures and synthesis, Tetrahedron Lett. 47 (2006) 4445–4448.
[15] K. Shimokawa, I. Mashima, A. Asai, T. Ohno, K. Yamada, M. Kita, D. Uemura, Biological activity, structural features, and synthetic studies of (−)-ternatin, a potent fat-accumulation inhibitor of 3T3-L1 adipocytes, Chem. Asian J. 3 (2008) 438–446.
[16] M. Ito, J. Ito, H. Kitazawa, K. Shimamura, T. Fukami, S. Tokita, K. Shimokawa, K. Yamada, A. Kanatani, D. Uemura, (−)-Ternatin inhibits adipogenesis and lipid metabolism in 3T3-L1 cells, Peptides 30 (2009) 1074–1081.
[17] K. Shimokawa, Y. Iwase, K. Yamada, D. Uemura, Synthesis and inhibitory effect on fat accumulation of (−)-ternatin derivatives modified in the beta-OH-D-Leu(7) moiety, Org. Biomol. Chem. 6 (2008) 58–60.
[18] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509.
[19] K. Shimokawa, K. Yamada, M. Kita, D. Uemura, Convergent synthesis and in vivo inhibitory effect on fat accumulation of (−)-ternatin, a highly *N*-methylated cyclic peptide, Bioorg. Med. Chem. Lett. 17 (2007) 4447–4449.
[20] K. Shimokawa, R. Miwa, K. Yamada, D. Uemura, Importance of the backbone conformation of (−)-ternatin in its fat-accumulation inhibitory activity against 3T3-L1 adipocytes, Org. Biomol. Chem. 7 (2009) 777–784.
[21] M.S. Brown, J.L. Goldstein, Selective versus total insulin resistance: a pathogenic paradox, Cell Metab. 7 (2008) 95–96.