Teneligliptin improves metabolic abnormalities in a mouse model of postmenopausal obesity

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Abstract
A decrease in serum estrogen levels in menopause is closely associated with the development of visceral obesity and the onset of type 2 diabetes in women. In the present study, we demonstrated the therapeutic effects of the novel DPP4 inhibitor, teneligliptin, on the features of postmenopausal obesity in mice. In the control group, female C57BL/6 mice were sham-operated and maintained on a standard diet. In the postmenopausal obese group, ovariectomized (OVX) mice were maintained on a high-fat diet, and were referred to as OVX-HF. In the treated group, teneligliptin at 60 mg/kg per day was administrated to OVX-HF, and were referred to as Tene. After a 12-week food challenge, the metabolic phenotypes of these mice were analyzed. Body weight, fat accumulation, and glucose intolerance were greater in OVX-HF than in control, while these abnormalities were markedly improved without alterations in calorie intake in Tene. Teneligliptin effectively ameliorated the characteristics of metabolic abnormalities associated with postmenopausal obesity. Regarding chronic inflammation in visceral adipose tissue, the numbers of F4/80−CD11c−CD206− M1-macrophages in flow cytometry, crown-like structure formation in immunohistochemistry, and proinflammatory cytokine expression were significantly attenuated in Tene. Hepatic steatosis was also markedly improved. Furthermore, decreased energy consumption in the dark and light phases, reduced locomotor activity in the dark phase, and lowered core body temperature in OVX-HF were ameliorated in Tene. Since obesity and reduced energy metabolism are a common physiology of menopause, teneligliptin appears to be beneficial as a treatment for type 2 diabetes in postmenopausal obesity.

Key Words
- dpp4 inhibitor
- adipose tissue macrophage
- energy expenditure
- insulin resistance

Introduction
Obesity is a common postmenopausal feature and is frequently accompanied by a characteristic shift in body fat from subcutaneous depots to a central location (Carr et al. 2003). Android fat deposition is well recognized as a significant risk factor for metabolic syndrome, type 2 diabetes, and cardiovascular disease (Despres 1993). However, lifestyle modifications such as healthful eating and exercise habits are not always applicable to all
patients. Therefore, effective therapies against obesity and diabetes in menopause are important for women’s health.

Estrogen is a cardinal sex hormone that primarily regulates sex functions and reproduction in women, whereas its biological functions are divergent (Gruber et al. 2002, Sherwin 2009, Meyer et al. 2011). Estrogen regulates glucose and lipid metabolism by acting on various target tissues. It has been shown to increase insulin sensitivity and suppress de novo lipogenesis in adipose tissue, the liver and skeletal muscle (Takeda et al. 2003, D’Eon et al. 2005, Nagira et al. 2006, Macotela et al. 2009). It also reportedly enhances cholesterol uptake and suppresses gluconeogenesis in the liver (Bryzgalova et al. 2006). Furthermore, estrogen was shown to attenuate obesity-induced chronic inflammation in adipose tissue by repressing the inflammatory responses of macrophages (Ribas et al. 2011). Previous studies reported that estrogen acted on the CNS, especially on the hypothalamus, suppressing food consumption and increasing energy expenditure, thereby protecting against body weight gain (Xu et al. 2011, Yonezawa et al. 2013). These findings indicated that postmenopausal obesity and insulin resistance are strongly associated with reductions in estrogen levels (Leenen et al. 1994). A meta-analysis previously revealed that estrogen-based hormone replacement therapy reduced abdominal obesity, insulin resistance, and the new onset of diabetes (Salpeter et al. 2006). However, its clinical usage is limited because of the potential side effects of oncogenesis and thrombosis (Rossouw et al. 2002).

Teneligliptin is clinically a novel class of anti-diabetic drug as a member of the dipeptidyl peptidase 4 (DPP4) inhibitor family in Japan and Korea, and is undergoing clinical trials in Europe and the USA. Teneligliptin increases the levels of active incretin hormones, glucagon-like peptide 1 (GLP1) and glucose-dependent insulinotropic peptide (GIP), by inhibiting the enzymatic activity of DPP4 as an underlying pharmacological mechanism (Demuth et al. 2005). Increases in incretin have been shown to enhance the secretion of insulin and suppress that of glucagon, and also improves glucose metabolism in patients with diabetes. Since incretin has been suggested to exhibit extra-pancreatic effects in human and animal models (Drucker & Nauck. 2006, Holst 2007), additional therapeutic values are predicted in the use of incretin-related drugs. In this regard, GLP1 analogues were previously reported to have weight-reducing effects in type 2 diabetics, whereas DPP4 inhibitors did not (Drucker & Nauck. 2006, Amori et al. 2007). In contrast, a recent study indicated that teneligliptin increased energy expenditure in male mice fed a high-fat diet (HFD) (Fukuda-Tsuru et al. 2014), and vildagliptin, another DPP4 inhibitor, enhanced energy expenditure during intra-duodenal lipid infusion in healthy men (Heruc et al. 2014).

Although teneligliptin is known to improve glucose metabolism in diet-induced obese male mice (Fukuda-Tsuru et al. 2014), its impact on chronic inflammation in adipose tissue has not yet been characterized in detail. Furthermore, its potential benefits in female mice or in women with type 2 diabetes remains unknown. Since reductions in energy consumption represent one of the physiological changes associated with menopause, we hypothesized that the administration of teneligliptin may reverse reduced energy metabolism and improve metabolic phenotypes in menopause. Therefore, we administered teneligliptin to a postmenopausal obese mouse model, and investigated its effects on body composition, systemic glucose metabolism, adipose tissue inflammation, and energy metabolism.

Materials and methods

Chemicals

Teneligliptin hydrogenbromide hydrate (3-[(2S,4S)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)piperazin-1-yl]pyrrolidin-2-yl-carbonyl] thiazolidine hemipentahydrate) was kindly provided by Mitsubishi Tanabe Pharma Corporation (Osaka, Japan).

Animals and experimental design

Female C57BL6/J mice purchased from Japan SLC (Shizuoka, Japan) were divided into three experimental groups: control, postmenopausal obesity (OVX-HF), and teneligliptin-treated postmenopausal obesity (Tene). In the control group, mice were sham-operated at 8 weeks old, and maintained on a regular diet (CE-12; Clea Japan, Tokyo, Japan). In the OVX-HF and Tene groups, mice were ovariectomized (OVX) at 8 weeks old, and maintained on a HFD (HF; 60% fat diet, D12492; Research diets, New Brunswick, NJ, USA) from 9 weeks old. The metabolic phenotypes of these mice were analyzed after a 12-week diet challenge. Adequate gonadectomy in OVX mice was confirmed in each mouse by atrophy of the uterus on dissection. The averaged uterus weights were 78 ± 4 mg in control, 25 ± 7 mg in OVX-HF, and 22 ± 4 mg in Tene. Mice had free access to drinking water with or without teneligliptin (60 mg/kg). The dosage of teneligliptin was
determined according to the previous literature. Although the dosage used in the present study was relatively higher compared to that in clinical use for human, this dosage (60 mg/kg) of teneligliptin is reported to improve glucose metabolism in diet-induced obese male mice without showing toxicity (Fukuda-Tsuru et al. 2014). The concentration of teneligliptin in the water bottle was standardized twice a week based on daily water consumption and weekly-measured mean body weight. Since our previous study showed that the metabolic phenotypes of sham-operated high-fat fed mice or OVX mice fed on a normal diet mice were less obvious than those of OVX-HF (Yonezawa et al. 2012), these two groups were not included in the present study. Mice were housed on a 12 h light:12 h darkness cycle (lights on at 0700 h) in a temperature-controlled colony room (23–25 °C), and provided water ad libitum. All experimental procedures used in this study were approved by the Committee of Animal Experiments at the University of Toyama.

Measurements of serum parameters
Mice were deprived of food overnight and blood samples were collected from the abdominal aorta under anesthesia. After centrifugation at 1500g for 20 min, the supernatants of the blood samples were separated and subjected to measurements. Serum levels of insulin were measured using an ELISA kit (Morinaga, Kanagawa, Japan). Blood glucose levels were measured with a Free Style NIPRO kit (NIPRO, Osaka, Japan). Serum levels of cholesterol and triglycerides were determined with a colorimetric kit (Wako Pure Chemical, Osaka, Japan) (Yonezawa et al. 2012, Wada et al. 2013a). Serum analyses by EIA and ELISA kits were conducted in duplicate. The inter-assay coefficient of variations were <10% in each analysis.

Measurements of hepatic triglycerides
The hepatic triglyceride content was determined using a triglyceride colorimetric kit (Wako Pure Chemical Industries Ltd, Osaka, Japan) after extraction of the lipid fraction from frozen liver specimens by the method of Bligh and Dyer with minor modifications (Wada et al. 2013a).

Glucose and insulin tolerance tests
Glucose and insulin tolerance tests (ITTs) were conducted after 12 weeks of treatment with teneligliptin. In the oral glucose tolerance test (OGTT), mice fasted for 6 h were challenged orally with a glucose solution (2 g/10 ml per kg body weight) and blood glucose levels were measured from the tail vein 0, 15, 30, 60, and 120 min after the glucose challenge. In the ITT, mice fasted for 2 h were injected intraperitoneally with human regular insulin (0.75 U/kg body weight). Blood samples were collected from the tail vein 0, 30, 60, 90, and 120 min after the injection (Wada et al. 2010, Yonezawa et al. 2012).

Analysis of body fat composition by magnetic resonance imaging
Body fat composition was analyzed by magnetic resonance imaging (MRI) under anesthesia 12 weeks after the treatment with teneligliptin, as described previously (Yonezawa et al. 2012). In brief, series of T1-weighted axial slices were analyzed with the software Image J (National Institute of Health, Bethesda, MD, USA).

Histological analysis and immunohistochemistry
Isolated perigonadal adipose tissue and livers were fixed in 10% formaldehyde for 24 h and embedded in paraffin. Six-micro meter-thick sections were stained with hematoxylin-eosin (HE). The size of adipocytes was analyzed, as described previously (Yonezawa et al. 2012, Wada et al. 2013a). In brief, perigonadal fat was measured in 300 cells per mouse by utilizing a digital video analyzer (VH Analyzer VH-H1A5, Keyence, Osaka, Japan). Regarding CD11c immunostaining, paraffin-embedded sections were incubated with a hamster anti-mouse CD11c antibody (dilution, 1:100, 10% in each analysis). Serum analyses by EIA and ELISA kits were conducted in duplicate. The inter-assay coefficient of variations were <10% in each analysis.

Isolation of adipocytes and stromal-vascular fractions and a flow cytometric analysis
The stromal-vascular fraction (SVF) was isolated from perigonadal adipose tissues, and used for flow cytometric analysis, as described previously (Fujisaka et al. 2009). In brief, cells were incubated with anti-mouse CD16/CD32 (BD Biosciences, San Jose, CA, USA) for 10 min and then stained with an anti-mouse CD45 PE-Cy7 antibody (BioLegend), PE hamster anti-mouse CD11c antibody (BD Biosciences), and rat anti-mouse CD206: Alexa Fluor 647 antibody (AbD Serotec) or the matching control isotypes for 30 min at 4 °C. The cells were then rinsed twice and resuspended in Phamingen stain buffer. After incubating with 7-amino-actinomycin D (BD Biosciences), the cells were analyzed using a FACSCanto cell analyzer (BD Biosciences). Data was
analyzed using FlowJo (Tree Star, Ashland, OR, USA). M1 macrophages were identified as F4/80-positive/CD11c-positive/CD206-negative cells.

**Real-time quantitative PCR**

RNA extraction, reverse transcription, and real-time PCR using SYBR green were conducted, as described previously (Wada et al. 2010, Yonezawa et al. 2012). The relative expression of objective mRNAs was calculated as a ratio to that of the 18S ribosomal protein. Primer sequences are listed in Table 1.

**Energy consumption, locomotor activity, blood pressure, and core body temperature**

Oxygen consumption (VO₂), the production of carbon dioxide (VCO₂), energy consumption, the respiratory quotient (RQ), and locomotor activity (counted by an inflated ray sensor system) were measured in metabolic chambers (MK-5000RQ, Muromachi Kikai, Tokyo, Japan) with free access to food and water, as described previously (Yonezawa et al. 2012, Wada T et al. 2013a). Blood pressure and heart rate were measured using a blood pressure monitor for mice and rats (MK-2000ST; Muromachi Kikai, Tokyo, Japan) (Yonezawa et al. 2012). Rectal temperature was monitored using an electronic thermometer (PTC-301, Unique Medical, Tokyo, Japan) under random-fed conditions (Yonezawa et al. 2012, Ichihara et al. 2013).

**Statistical analysis**

Data are expressed as the mean ± S.E.M. P values were determined by a one-way ANOVA with Bonferroni’s correction and P < 0.05 was considered significant.

**Table 1** Primer list

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<td>Rn18s</td>
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**Results**

Teneligliptin attenuated body weight gain, fat accumulation, and serum insulin and triglyceride levels in the mouse model of postmenopausal obesity

We examined the body consumption and metabolic phenotypes of each mouse (Fig. 1). Figure 1A shows the profiles of body weight changes in the experimental period. Body weights of both OVX-HF and Tene were heavier than controls when fed on HFD more than 8 weeks. The increase in body weight observed in OVX-HF was effectively attenuated in Tene. In order to identify the tissue protecting against body weight gain in Tene, we analyzed the body composition of these mice. Representative MRI at the level of the urinary bladder (Fig. 1B) and estimated fat volumes from the diaphragm to the anus are shown (Fig. 1C). Increases in visceral and subcutaneous fat in OVX-HF were significantly attenuated in Tene. The increase observed in the weight of perigonadal fat in OVX-HF on dissection was also significantly decreased in Tene (Fig. 1D). In contrast, no significant differences were noted in lean mass volume between these groups (Fig. 1C). The change in body composition was not caused by different food consumption levels (6.1 ± 2.0 kcal/day in OVX-HF and 6.9 ± 1.3 kcal/day in Tene). Elevated fasted serum insulin levels in OVX-HF were decreased to the control level in Tene. Although serum total cholesterol levels were similarly elevated in OVX-HF and Tene, serum triglyceride levels were lower in Tene than in OVX-HF (Fig. 1E).

Teneligliptin improved glucose intolerance, but did not affect insulin sensitivity in the mouse model of postmenopausal obesity

In order to evaluate the anti-diabetic effects of teneligliptin, OGTT and ITT were conducted in each mouse
group (Fig. 2). No significant differences were observed in blood glucose levels after 6 h of fasting between the three groups. In contrast, serum glucose levels at 30, 60, and 120 min in OGTT were significantly elevated in OVX-HF, but were markedly attenuated in Tene (Fig. 2A). Similar results were obtained in a comparison of the area under the curve (AUC). According to the ITT analysis, blood glucose levels after 2 h of fasting were significantly higher in OVX-HF and Tene than in control. No significant differences were noted in elevated blood glucose levels in ITT between the OVX-HF and Tene groups (Fig. 2B).

**Teneligliptin attenuated chronic inflammation in perigonadal fat**

Teneligliptin effectively prevented glucose intolerance and visceral fat accumulation observed in OVX-HF (Figs 1 and 2). Since visceral fat accumulation in obesity is closely associated with glucose dysregulation, we next examined the perigonadal adipose tissue of each mouse group. In order to investigate macrophage infiltration in adipose tissue, cells in the SVF of perigonadal fat were analyzed by flow cytometry. Elevated numbers of F4/80-positive macrophages and F4/80-positive/CD11c-negative/CD206-negative M1 macrophages in OVX-HF were significantly reduced in Tene (Fig. 3A, B, and C). M1 macrophages in obese adipose tissue are known to form a cluster of macrophages around dead adipocytes, named a crown-like structure (CLS) (Lumeng et al. 2007). Therefore, we conducted a histological analysis of adipose tissue. Figure 3D shows representative immunohistochemistry with the anti-CD11c antibody. Few M1 macrophages were detected and CLS were almost absent in the control group. However, these inflammatory findings were more prominent in the adipose tissue of OVX-HF. Notably, a significant reduction in the formation of
Teneligliptin attenuated hepatic steatosis in the mouse model of postmenopausal obesity

In order to further clarify the therapeutic impact of teneligliptin, we investigated the liver phenotypes of the mice tested. HE staining revealed marked vacuolar degeneration in OVX-HF, indicating fat accumulation in the liver (Fig. 4A). Vacuolar degeneration of the liver was clearly attenuated in Tene. Consistent with histological findings, the increases observed in liver weight and hepatic triglyceride content in OVX-HF were significantly prevented in Tene (Fig. 4B and C). In addition, the mRNA expression of G6pase and Pepck, two rate limiting enzymes of gluconeogenesis, was slightly increased in OVX-HF and decreased in Tene (Fig. 4D). The hepatic expression of Mcp1 and Tnfα also increased slightly in OVX-HF, and returned to control levels in Tene. These results indicated that teneligliptin prevented the development of hepatic steatosis in a mouse model of postmenopausal obesity.

Teneligliptin increased locomotor activity in the dark phase and enhanced energy expenditure in postmenopausal obese mice

Estrogen plays a crucial role in energy homeostasis, and reduced physical activity and energy expenditure are considered the mechanisms underlying obesity in menopause (Rogers et al. 2009, Yonezawa et al. 2012). Since body weight and fat accumulation were less in Tene than in OVX-HF, we examined energy metabolism in these mice. Oxygen consumption (VO₂) and the production of carbon dioxide (VCO₂) in the dark and light phases were markedly reduced in OVX-HF. These parameters of energy expenditure in both phases were significantly greater in Tene than in OVX-HF (Fig. 5A, B, and C). In addition, the decrease observed in spontaneous locomotor activity in the dark phase in OVX-HF was significantly increased in Tene. Furthermore, lowered core body temperature in OVX-HF was ameliorated in Tene (Fig. 5D). Similarly, the expression of Pgc1α and Prdm16 in brown adipose tissue (BAT) was significantly decreased in OVX-HF. On the other hand, the expression of prdm16, Pgc1α, Cidea, and Ucp1 in BAT was not altered by the treatment with teneligliptin (Fig. 5E).

Discussion

DPP4 inhibitors represent a relatively new class of antidiabetic drugs that exert their biological effects by
preventing the DPP4-mediated degradation of GLP1 and GIP, which are incretin hormones. In the present study, we demonstrated the significant effectiveness of teneligliptin in a mouse model of postmenopausal obesity. Teneligliptin ameliorated decreased locomotor activity and energy expenditure, attenuated body fat gain, and suppressed chronic inflammation in visceral adipose tissue. These results strongly suggest that teneligliptin is beneficial for the treatment of type 2 diabetes in postmenopausal obesity.

The number of postmenopausal women is increasing in most developed countries due to an extended healthy life. Although menopause is a natural age-related matter, the postmenopausal metabolic environment varies, and is strongly associated with the onset of illnesses. Cardiovascular disease, osteoporosis, and cognitive decline become more prevalent in menopause (Resnick et al. 1997, Carr 2003). Furthermore, obesity, dyslipidemia, and diabetes are important postmenopausal aspects. Estrogen is an ovarian hormone that declines in menopause, thereby affecting energy, glucose, and lipid metabolism. A previous study reported that estrogen-based hormone replacement effectively improved glucose and lipid metabolism in menopausal women (Salpeter et al. 2006).

Estrogen is a cardinal sex hormone that regulates primary and secondary sexual characteristics, the menstrual cycle, and gestation (Gruber et al. 2002). It also plays a pivotal role in the regulation of multiple metabolic...
Teneligliptin improves postmenopausal obesity

A SAMESHIMA, T WADA and others

Effects of teneligliptin administration on hepatic steatosis and gene expression related to gluconeogenesis in a mouse model of postmenopausal obesity. Representative histological photomicrographs of liver sections in hematoxylin-eosin (HE) staining (A), weight of the liver (B), hepatic triglyceride content (C), and mRNA expression in the liver (D and E) in each mouse are shown. Scale bar = 100 μm. Values are the mean ± S.E.M. (n = 5–7). †P < 0.05 and ††P < 0.01 significantly different from the control; †P < 0.05 and †††P < 0.01 significant difference between the OVX-HF and Tene groups.

Figure 4

DPP4 is widely distributed in human organs and tissues, and is a serine protease belonging to a subgroup of prolyl oligopeptidases that specifically cleave off N-terminal dipeptides from proteins having proline or alanine at amino acid position 2 (Mentlein 1999). In this regard, teneligliptin lowers blood glucose levels mainly by the enhancement of insulin secretion and the suppression of glucagon secretion via GLP1 and GIP (Demuth et al. 2005). In addition, incretin is known to possess extra-pancreatic effects including weight reduction (Drucker & Nauck, 2006, Holst 2007). Therefore, teneligliptin-induced favorable metabolic consequences such as attenuation of chronic inflammation in the adipose tissue and hepatic steatosis appear to be due to the reduction of visceral fat via the action of incretin hormones in postmenopausal obesity. However, it is possible that pathways in women. Estrogen is considered to exert its biological functions through estrogen receptors (ER). Previous studies with the genetic ablation of ERα, ERβ, and GPR30, a protein-coupled receptor for estrogen, demonstrated the predominant role of ERα in the regulation of glucose and lipid metabolism (Heine et al. 2000, Mårtensson et al. 2009, Handgraaf et al. 2013b). Recent studies indicated that the effects of estrogen in the CNS also controlled energy homeostasis (Clegg et al. 2006). We previously classified the central vs peripheral role of estrogen in the regulation of glucose and energy metabolism (Yonezawa et al. 2012). A disruption in ERα in steroidogenic factor 1 (Sf1) neurons at the ventromedial hypothalamus led to impaired glucose tolerance, reduced energy expenditure, and abdominal obesity, whereas proopiomelanocortin (POMC) neuron-specific ERα knockout mice developed hyperphagia (Xu et al. 2011). Thus, beneficial metabolic effects are greatly attenuated in menopause by the decline of estrogen. Based on these findings, our OVX-HF model appeared to be useful for investigating metabolic changes with menopause because the typical phenotypes of postmenopausal obesity, including glucose intolerance, dyslipidemia, reductions in locomotor activity and energy expenditure were observed in these mice.

Effects of teneligliptin administration on energy metabolism in perigonal fat in a mouse model of postmenopausal obesity. Energy expenditure was measured using a metabolic chamber after 10 weeks of the teneligliptin administration. O2 consumption (A), CO2 production (VCO2) (B), and spontaneous locomotor activity (C) in the dark phase and light phase were analyzed. (D) Core body temperature was measured. (E) The mRNA expression of each gene in the brown adipose tissue was determined by real-time PCR. Values are the mean ± S.E.M. (n = 7–10). †P < 0.05 and ††P < 0.01 significantly different from the control; †P < 0.05 and ††P < 0.01 significant difference between the OVX-HF and Tene groups.

Figure 5
unclarified DPP4 substrates directly affect the factors involved in the worsening of metabolic phenotypes in postmenopausal obesity, since a number of functional peptides are also cleaved by DPP4 (Mentlein et al. 1999). They might affect chronic inflammations in the adipose tissues via modulation of functions in immune cells. Nevertheless, orexigenic peptides known as DPP4 substrates including neuropeptide Y (NPY) might not be involved in the underlying mechanisms of teneligliptin, since food consumption was not different between the OVX-HF and Tene groups.

Recently, DPP4 has been shown to be secreted from adipose tissue in a tissue-volume-dependent manner, and serum DPP4 concentrations were positively correlated with an increased BMI, fat volume, and insulin concentration in humans (Lamers et al. 2011). Because visceral fat accumulation is a notable phenotype of postmenopause, we measured the expression of DPP4 in the perigonadal fat of the mice. However, the expression levels were not different among the control, OVX-HF, and Tene groups (Supplemental Figure 1, see section on supplementary data given at the end of this article). Since the secretion of DPP4 was more prominent in patients with metabolic syndrome than simple obese subjects (Lamers et al. 2011), other metabolic factors might influence the secretion.

The clinical features of the therapeutic outcomes caused by DPP4 inhibitors and GLP1 agonists have been reported. Body weight reductions were only detected in a meta-analysis with the use of a GLP1 agonist (Drucker & Nauck. 2006, Amori et al. 2007). Nevertheless, reductions in body weight have been reported with the administration of DPP4 inhibitors in some clinical studies (Arjona et al. 2013, Ishikawa et al. 2014). In an animal study, a systemic DPP4 deficiency resulted in resistance to body weight gain, reduced body fat and hepatic fat contents, and increased energy expenditure when mice were fed a HFD (Conarello et al. 2003). These findings were consistent with the phenotypes observed in Tene in the present study. In contrast, the administration of des-fluorositagliptin did not affect energy metabolism, whereas body weight gain and lipid accumulation were attenuated in male mice fed a HFD (Lamont & Drucker. 2008, Shimasaki et al. 2013). On the other hand, a recent study indicated that teneligliptin increased energy expenditure in male mice fed a HFD (Fukuda-Tsuru et al. 2014). Since reductions in physical activity and energy expenditure are the hallmarks of postmenopausal obesity, the increases in locomotor activity and energy metabolism observed in Tene appeared to be beneficial for the treatment of type 2 diabetes with postmenopausal obesity. The increase noted in energy expenditure in Tene was not related to the thermogenic function of BAT because the expression of Prdm16, Pgc1a, Cidea, and Ucp1 in BAT were not altered. This result indicated that muscle may be the tissue responsible for increased energy expenditure in Tene (Fig. 5C). The expression of Ucp3 in the soleus muscle was previously shown to increase in male mice fed a HFD and treated with teneligliptin (Fukuda-Tsuru et al. 2014). Further studies are needed in order to elucidate the mechanisms underlying increases in energy expenditure with the administration of teneligliptin.

We previously examined the effects of estrogen replacement in the same mouse model of postmenopausal obesity (Yonezawa et al. 2012). The favorable effects of teneligliptin on energy metabolism appeared to mimic the effects of estrogen replacement because teneligliptin partly reversed the phenotypes of postmenopausal obesity in OVX-HF. Teneligliptin attenuated lipid accumulation and chronic inflammation in the adipose tissue of OVX-HF (Fig. 3). The results obtained with the administration of teneligliptin were consistent with those in OVX-HF treated with estrogen replacement (Yonezawa et al. 2012). However, the impact of these treatments on adipocyte size and numbers differed. Estrogen has been shown to suppress the expression of lipogenic enzymes, such as Ppargc and Fas, as well as enzymes related to lipid uptake, including Lpl (D’Eon et al. 2005), while teneligliptin suppressed the expression of Lpl, but did not affect that of Ppargc or Fas in OVX-HF. On the other hand, a previous study with myeloid-specific ERα knockout mice demonstrated that estrogen attenuated chronic inflammation by directly inhibiting inflammatory responses by macrophages (Ribas et al. 2011). In the present study, teneligliptin effectively attenuated chronic inflammation in the adipose tissue. Furthermore, the treatment with estrogen attenuated hepatic steatosis in OVX mice, whereas this effect was absent in hepatocyte-specific ERα knockout mice (Zhu et al. 2013), indicating the directly preventive effect of estrogen on the development of fatty liver. Teneligliptin also exhibited similar beneficial effects against fatty liver to those of estrogen in the present study (Fig. 4). These effects on the adipose tissue and liver did not appear to be related to the change of the estrogen receptors, since expressions of ERα, ERβ, and GPR30 did not differ among experimental groups (Supplemental Figure 2, see section on supplementary data given at the end of this article). The beneficial effects of DPP4 inhibitors on lipid accumulation in adipose tissue and the liver have also been observed in obese
male mice (Lamont & Drucker, 2008, Shirakawa et al. 2011, Shimasaki et al. 2013, Aroor et al. 2015). Therefore, although the underlying mechanisms of teneligliptin and estrogen differ, they appear to elicit similar beneficial effects on impaired energy metabolism in OVX mice fed a HFD.

Since estrogen has favorable effects on bone metabolism, osteoporosis is a causal disorder postmenopause (Cauley et al. 2003). No significant differences were observed in the weight of the femur between OVX-HF and Tene (data not shown). Therefore, teneligliptin does not appear to markedly affect bone metabolism in OVX mice. On the other hand, a recent study reported that the beneficial effects of sitagliptin on bone mineral density and trabecular architecture in HFD-fed female mice was diminished by ovariectomy (Kyle et al. 2011). In any case, further studies are needed to clarify the impact of DPP4 inhibitors on bone metabolism postmenopause.

In summary, OVX-HF displayed the features of postmenopausal obesity, such as body fat gain, glucose intolerance, dyslipidemia, hepatic steatosis, and reduced energy expenditure. Teneligliptin effectively ameliorated these metabolic abnormalities in the mouse model tested, possibly by mimicking effects against reduced estrogen levels. Teneligliptin increased locomotor activity and energy expenditure, attenuated lipid accumulation in adipose tissue and the liver, inhibited chronic inflammation in adipose tissue, and improved glucose and lipid metabolism. These results indicate that teneligliptin is a suitable anti-diabetic drug for the treatment of type 2 diabetes with postmenopausal obesity.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-15-0239.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
A S performed experiments and reviewed the manuscript. T W derived the hypothesis, performed experiments, and wrote the manuscript. T I, A K, and K S performed experiments. R Y, Y I, M S, and S S reviewed the manuscript and contributed to discussions. H T and T S reviewed and wrote the manuscript.

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