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Oxytocin treatment reduced food intake and body fat and ameliorated obesity in ovariectomized female rats

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ABSTRACT

Recent studies have shown that oxytocin reduces food intake and body weight gain and promotes lipolysis in some species, including humans. Interestingly, these effects of oxytocin are more marked in obese individuals. Although the menopausal loss of ovarian function induces increased visceral adiposity and some metabolic disorders, no safe medical interventions for these conditions have been established. In this study, we evaluated the effects of oxytocin on appetite, body weight, and fat mass in ovariectomized rats. Six-day oxytocin treatment attenuated cumulative food intake and body weight gain, and reduced visceral and subcutaneous fat weight and adipocyte cell area in ovariectomized rats. Blood examinations indicated that 6-day oxytocin treatment did not alter renal or hepatic functions. Instead, it might prevent ovariectomy-induced liver damage. In addition, acute oxytocin treatment did not affect body temperature or locomotor activity. These results indicate that oxytocin might be useful for treating or preventing menopause-induced metabolic disorders, without causing any adverse effects.

1. Introduction

Oxytocin, which is a 9-amino acid neuropeptide, is synthesized in two hypothalamic regions, the paraventricular nucleus and supraoptic nucleus (Du Vigneaud et al., 1953). The magnocellular oxytocin neurons project into the posterior pituitary gland and secrete oxytocin into the peripheral circulatory system (Bargman and Scharrer, 1951), whereas the parvocellular oxytocin neurons project and secrete oxytocin into several regions of the central nervous system, such as the arcuate nucleus (Ludwig et al., 2002; Maejima et al., 2014It is well established that oxytocin promotes labor and lactation at the peripheral level in mammalian females (Kiss and Mikkelsen, 2005). In addition, recent studies have shown that oxytocin affects some behavioral and psychological functions, such as trust (Kosfeld et al., 2005; Veening and Olivier, 2013), bonding (Johnson et al., 2016; Numan and Young, 2016), empathy, and social communication (Burkett et al., 2016; Domes et al., 2007), and parental and maternal nurturing (Jin et al., 2007; Naber et al., 2010). It has also been reported that the administration of oxytocin ameliorated some psychiatric diseases, such as autism, depression, and schizophrenia (Aoki et al., 2014; Bradley and Woolley, 2017; Feldman et al., 2016; Wang et al., 2018).

Recently, the effects of centrally secreted oxytocin on metabolic, appetite, and body weight regulation and their underlying mechanisms have been clarified (Altirriba et al., 2015). Oxytocin receptor-deficient mice exhibited late-onset obesity accompanied by increased amounts of abdominal fat and impaired cold-induced thermogenesis (Takayanagi et al., 2008). Similarly, the intracerebroventricular (i.c.v.) injection of an oxytocin antagonist increased food intake in mice (Zhang et al., 2011). These findings indicate that endogenous oxytocin affects metabolic functions and feeding behavior. In addition, the intracerebroventricular, intraperitoneal (i.p.), subcutaneous, or intranasal injection of oxytocin decreases food intake in some species, such as mice (Maejima et al., 2011, 2015, 2017, 2018; Roberts et al., 2017; Zhang and Cai, 2011; Zhang et al., 2011), rats (Arletti et al., 1990; Blevins et al., 2016; Deblon et al., 2011; Morton et al., 2012; Olson et al., 1991; Ong et al., 2015; Rinaman and Rothe, 2002; Roberts et al., 2017), monkeys (Blevins et al., 2015), and humans (Lawson et al., 2015; Thienel et al., 2016). In addition, the injection of oxytocin directly and indirectly promotes lipolysis in adipose tissue and reduces fat mass in these species (Altirriba et al., 2014; Blevins et al., 2015; Blevins et al., 2016; Deblon et al., 2011; Eckertova et al., 2011; Gajdosechova et al., 2014; Maejima et al., 2017; Morton et al., 2012; Plante et al., 2015; Roberts et al., 2017; Yi et al., 2015; Zhang and Cai, 2011; Zhang et al., 2011). More recently, it has been shown that the intranasal

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administration of oxytocin caused reductions in fat mass in obese prairie voles (Seelke et al., 2018). Interestingly, these effects of oxytocin on food intake and fat mass are more marked in diet-induced and genetically obese rodents (Altirriba et al., 2014; Maejima et al., 2017; Plante et al., 2015) and obese humans (Thienel et al., 2016). For example, chronic oxytocin administration caused reductions in body weight and food intake, and improved glucose and fat metabolism and cardiac structural remodeling in diabetic male mice (*db/db*), but not in their non-diabetic littermates (Plante et al., 2015).

The menopausal loss of ovarian function causes increased visceral adiposity and some metabolic disorders, such as diabetes, hyperlipidemia, and cardiovascular disease (Carr et al., 2004; Ferrara et al., 2002; Palmisano et al., 2017; dos Reis et al., 2003; Tchernof and Despres, 2000). It has been demonstrated that hyperphagia and decreased energy expenditure contribute to visceral adiposity and associated diseases in menopausal women (Lovejoy et al., 2008) and ovariectomized rodents (Iwasa et al., 2017a, 2017b; Liang et al., 2002; Meli et al., 2004; Richard, 1986; Rogers et al., 2009; Wade and Gray, 1979). Although estrogen replacement might be an effective treatment for these conditions, the long-term use of this treatment might increase the risk of adverse effects, such as thromboembolism and breast cancer.

As noted above, oxytocin has more marked effects in obese individuals. In addition, oxytocin has been used in the clinical setting, such as to induce labor, and no severe adverse effects have been reported so far. In the present study, the effects of oxytocin on body weight, food intake, and fat mass were examined in ovariectomized rats. Hepatic enzyme levels, lipid profiles, and renal function were assessed to evaluate oxytocin's effects, including any adverse effects. The levels of hypothalamic and adipose factors, such as appetite-regulating factors and pro-inflammatory cytokines, were also measured. It was reported that third and fourth ventricular oxytocin administration increased the temperature of brown adipose tissue in diet-induced obese rats (Roberts et al., 2017), and oxytocin receptor-knockout mice failed to maintain thermogenesis during cold exposure (Kasahara et al., 2013). In addition, in male rats it has been shown that the central administration of oxytocin increased core body temperature (Ong et al., 2017), whereas the peripheral administration of oxytocin caused reductions in body temperature and heart rate (Hicks et al., 2014). Furthermore, previous study has suggested that locomotor activity is affected by peripheral administration of oxytocin (Maejima et al., 2015). Thus, the effects of oxytocin on body temperature and locomotor activity were also evaluated in present study. These experiments were conducted to evaluate the utility of oxytocin against menopause-induced metabolic disorders and the adverse effects of oxytocin treatment.

2. Materials and methods

2.1. Animals

Eight-week-old adult female Wistar rats were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan), and housed in a room under controlled light (12 h light, 12 h darkness; lights turned on at 0800 and turned off at 2000) and temperature (24 °C) conditions with free access to food and water. In total, 22 rats were used in this study. The ovariectomy and tissue sampling procedures were carried out under sodium pentobarbital (60–80 mg/kg, i.p.)-induced anesthesia. All animal experiments were conducted in accordance with the ethical standards of the animal care and use committee of the University of Tokushima.

2.2. Effects of the 6-day injection of oxytocin on body weight, food intake, fat weight, adipocyte size, and the levels of central and peripheral factors

Three weeks after the ovariectomy (11 weeks of age), the rats were randomly divided into oxytocin-administered and saline-administered groups ($n = 7$ per group). The rats received i.p. injections of oxytocin (500 μg/day, Peptide Institute, Osaka, Japan) or saline at 2 h before the dark phase for 6 days. The dose of oxytocin was based on the findings of a previous study, which showed that 1600 μg/kg oxytocin was effective at regulating body weight and food intake (Maejima et al., 2011). All of the rats in the oxytocin group were given the same dose of oxytocin. The oxytocin was dissolved in distilled water, and the injected volume did not exceed 0.3 ml. The rats were housed individually in acrylic cages, and the daily changes in their body weights and food intake were measured in the morning (0900–1000). After 6 days, they were killed by decapitation under sevoflurane anesthesia, and the brain, blood, visceral fat (the parametrial, perirenal, and mesenteric deposits), and subcutaneous fat (the inguinal deposit) were collected. The visceral fat (the parametrial, perirenal, and mesenteric deposits were combined) and subcutaneous fat were weighed immediately after being removed. Thereafter, tissue samples (around 300–400 mm^3) of visceral (parametrial) and subcutaneous fat were dissected. Serum was separated by centrifugation and stored at −20 °C, and the tissue samples were stored at −80 °C and/or fixed in 4% paraformaldehyde. The serum samples were used to obtain biochemical measurements, and the frozen tissue samples were used to measure the mRNA expression levels of central and peripheral factors. The fixed tissue samples were used for the histological analysis.

2.3. Effects of the injection of oxytocin on body temperature and locomotor activity

Another set of female rats was ovariectomized, and pre-calibrated temperature-sensitive radiotransmitters (TA11TA-F10; Data Sciences International, New Brighton, MN, USA) were implanted into the peritoneal cavity at 2 weeks after the ovariectomy procedure. After a 1-week recovery period, body temperature and locomotor activity were measured using remote radio-biotelemetry. The rats were divided into oxytocin-administered and saline-administered groups ($n = 4$ per group). After a 24-h preliminary measurement period, oxytocin (500 μg) and saline were i.p. injected at 1 h before the dark phase in the oxytocin group and control group, respectively. After the injection procedure, further measurements were obtained for 24 h. The radiotransmitter signals were recorded every 10 min and directly converted into body temperature and locomotor activity using the DATAQUEST software (Data Sciences).

2.4. Histology

Fixed visceral and subcutaneous fat samples were dehydrated with ethanol and xylene and sliced into sections after being embedded in paraffin. Serial 4-μm-thick sections were stained with hematoxylin and eosin, and histological images were captured using a Zeiss Imager M2 microscope with the AxioVision version 4.8 acquisition software (Zeiss). The mean area of 50 randomly selected adipocytes per specimen was determined using the ImageJ software.

2.5. Biochemical analysis

The serum levels of total protein, albumin (Alb), blood urea nitrogen (BUN), creatinine (CRE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin (T-BIL), total cholesterol, free cholesterol, low-density lipoprotein cholesterol (LDL-CHO), and high-density lipoprotein cholesterol (HDL-CHO) were measured by a commercial laboratory (Oriental Yeast Co., Ltd.).

2.6. Quantitative real-time polymerase chain reaction

Whole hypothalamic explants were dissected from the frozen brains, as described previously (Iwasa et al., 2017a). Briefly, the brain sections

Table 1
Primer sequences, product sizes and annealing temperature.

Primer	Sequence	Annealing T (°C)
NPY forward	GGG GCT GTG TGG ACT GAC CCT	66
NPY reverse	GAT GTA GTG TCG CAG AGC GGA G	
AgRP forward	TGAAGAAGA CAG CAG CAG ACC	
AgRP reverse	AAG GTA CCT GTT GTC CCAAGC	
POMC forward	CCT CAC CAC GGAAAG CA	66
POMC reverse	TCAAGG GCT GTT CAT CTC C	
IL-1β forward	GCT GTG GCA GCT ACC TAT GTC TTG	61
IL-1β reverse	AGG TCG TCATCATCC CAC GAG	
TNF-α forward	AGC CCT GGT ATG AGC CCA TGT	65.5
TNF-α reverse	CCG GAC TCC GTG ATG TCTAAG T	
IL-6 forward	TCCTACCCCAACTTCCAATGCTC	67
IL-6 reverse	TTGGATGGTCTTGGTCCTTAGCC	
OT forward	GAACACCAACGCCATGGCCTGCCC	351
OT reverse	TCGGTGCGGCAGCCATCCGGGCTA	
OTR forward	CGATTGCTGGGCGGTCTT	161
OTR reverse	CCGCCGCTGCCGTCTTGA	
GAPDH forward	ATG GCA CAG TCAAGG CTG AGA	70
GAPDH reverse	CGC TCC TG GAA GAT GGT GAT	

were dissected out via an anterior coronal cut at the posterior border of the mammillary bodies, parasagittal cuts along the hypothalamic fissures, and a dorsal cut 2.5 mm from the ventral surface. Total RNA was isolated from the hypothalamic explants and visceral fat samples using a TRIzol® reagent kit (Invitrogen Co., Carlsbad, CA, USA) and an RNeasy® mini kit (Qiagen GmbH, Hilden, Germany). Then, cDNA was synthesized with oligo (deoxythymidine) primers at 50 °C using the SuperScript III first-strand synthesis system for the real-time polymerase chain reaction (PCR; Invitrogen Co.). The PCR analysis was performed using the StepOnePlus™ real-time PCR system (PE Applied Biosystems, Foster City, CA, USA) and FAST SYBR® green. The mRNA levels of neuropeptide Y (NPY), agouti-related protein (AgRP), proopiomelanocortin (POMC), interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), IL-6, oxytocin, and the oxytocin receptor were quantified. The mRNA expression level of each factor was normalized to that of GAPDH. Dissociation curve analysis was also performed for each gene at the end of the PCR. Each amplicon generated a single peak. Primer sequences, product sizes, and annealing temperatures are shown in Table 1. The PCR conditions were as follows: initial denaturation and enzyme activation were performed at 95 °C for 20 s, followed by 45 cycles of denaturation at 95 °C for 3 s, and annealing and extension for 30 s.

2.7. Statistical analysis

All results are presented as mean ± standard error of the mean (SEM) values. The Student's *t*-test was used for comparisons of body weight and food intake at each time point, and comparisons of subcutaneous/visceral fat weight, mRNA expression, and serological data between the oxytocin and control groups. Two-way repeated-measures analysis of variance (ANOVA) was used for comparisons of the overall changes in body weight and food intake between the oxytocin and control groups. *P*-values of < 0.05 were considered significant. Cohen's d (small effect = 0.2, medium effect = 0.5, large effect = 0.8) and Eta squared (η2) (small effect = 0.06, medium effect = 0.6, large effect = 0.14) values are reported for analyses conducted using the Student's *t*-test and ANOVA, respectively.

3. Results

3.1. Effects of the 6-day injection of oxytocin on body weight and food intake

The 6-day injection of oxytocin significantly affected the body weights of the rats (two-way ANOVA; treatment: $F(1,97) = 16.9$, $P < .001$, $\eta 2 = 0.15$; time: $F(6,97) = 0.36$, $P = .90$; interaction: F

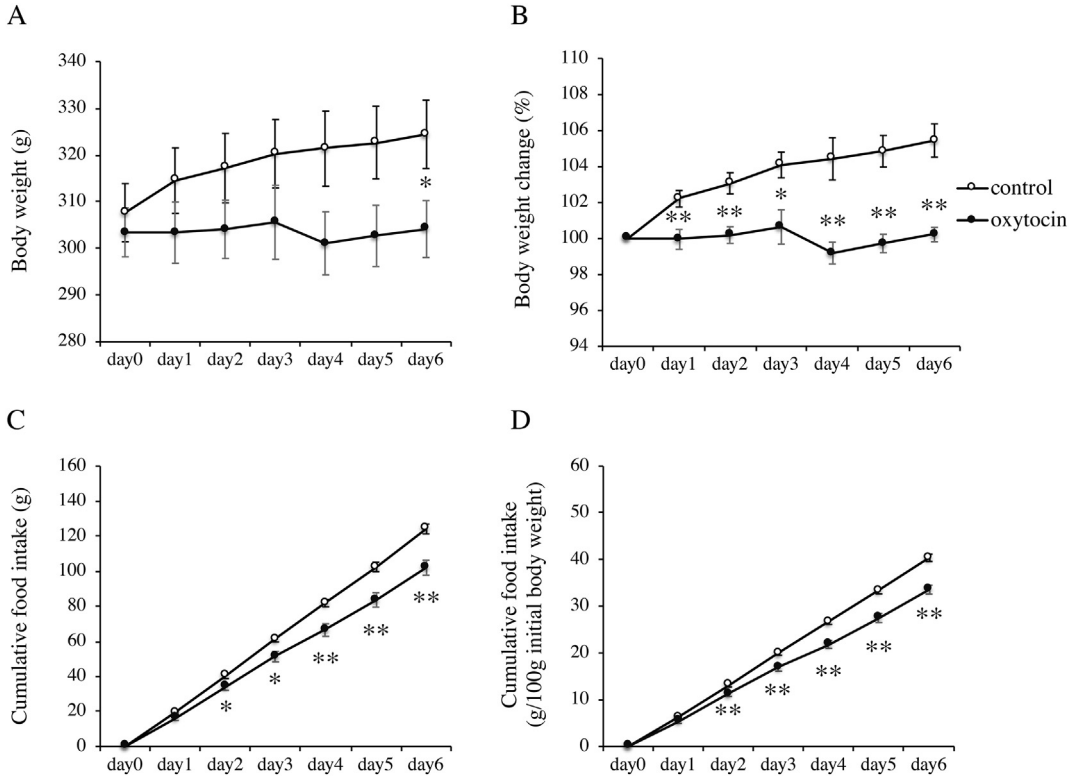

Fig. 1. (A) Body weight, (B) body weight change (% of initial body weight), (C) cumulative food intake, and (D) cumulative food intake relative to body weight in the control and oxytocin groups. Saline was intraperitoneally injected in the control group, and oxytocin (500 μg/day) was intraperitoneally injected in the oxytocin group at 2 h before the dark phase. Data are expressed as mean ± SEM values ($n = 7$ per group); $^{*}P < .05$, $^{**}P < .01$.

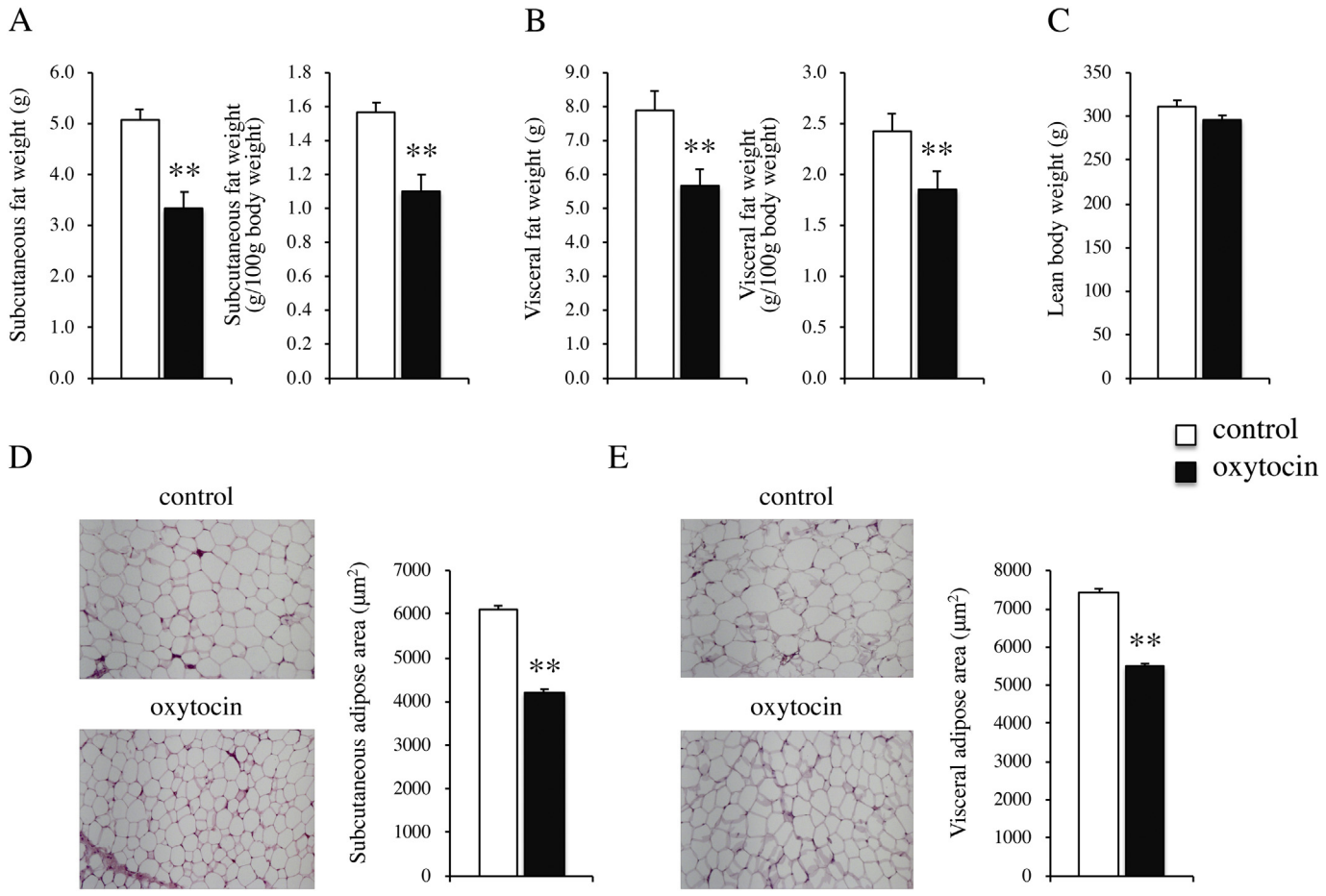

Fig. 2. (A) Subcutaneous fat weight, (B) visceral fat weight, (C) lean body weight and (D) representative photomicrographs and adipocyte area of subcutaneous fat and (E) visceral fat in the control and oxytocin groups. Data are expressed as mean ± SEM values ($n = 7$ per group); $^{**}P < .01$.

(6,97) = 0.37, $P = .89$), and on day 6 the mean body weight of the oxytocin group was significantly lower than that of the control group (Student's *t*-test; $P < .05$, d = 1.14) (Fig. 1A). Similarly, the 6-day injection of oxytocin significantly affected the body weight changes that occurred during the study period (two-way ANOVA; treatment: F (1,97) = 292.4, $P < .001$, η2 = 0.41; time: F (6,97) = 3.97, $P < .01$; interaction: F (6,97) = 4.25, $P = .01$), and the body weight changes observed from day 1 to day 6 were significantly lower in the oxytocin group than in the control group (Student's *t*-test; $P < .05$, d = 1.69–2.79) (Fig. 1B).

The 6-day injection of oxytocin significantly affected cumulative food intake (two-way ANOVA; treatment: F (1,97) = 66.0, $P < .001$, η2 = 0.02; time: F (6,97) = 536.7, $P < .01$; interaction: F (6,97) = 5.59, $P < .01$), and the body weight changes seen from day 1 to day 6 were significantly lower in the oxytocin group than in the control group (Student's *t*-test; $P < .05$, d = 0.91–2.05) (Fig. 1C). Similarly, the 6-day injection of oxytocin significantly affected cumulative food intake relative to body weight (two-way ANOVA; treatment: F (1,97) = 110.7, $P < .001$; time: F (6,97) = 1012.1, $P < .01$; interaction: F (6,97) = 9.40, $P < .01$, η2 = 0.02), and cumulative food intake relative to body weight observed from day 1 to day 6 were significantly lower in the oxytocin group than in the control group (Student's *t*-test; $P < .05$, d = 1.81–3.01) (Fig. 1D).

3.2. Effects of the 6-day injection of oxytocin on fat weight and adipocyte size

The weight of subcutaneous fat was significantly lower in the oxytocin group than in the control group (Student's *t*-test; $P < .05$, d = 1.55). Similarly, the relative weight of subcutaneous fat was significantly lower in the oxytocin group than in the control group (Student's t-test; $P < .01$, d = 2.09) (Fig. 2A). The weight of visceral fat was significantly lower in the oxytocin group than in the control group (Student's *t*-test; $P < .001$, d = 2.41). Similarly, the relative weight of visceral fat was significantly lower in the oxytocin group than in the control group (Student's *t*-test; $P < .05$, d = 1.26) (Fig. 2B). Lean body weight (calculated by subtracting body fat weight from total body weight) did not differ between the oxytocin and control groups (Fig. 2C).

The subcutaneous adipocyte area was significantly lower in the oxytocin group than in the control group (Student's *t*-test; $P < .001$, d = 1.26) (Fig. 2D). Similarly, the area of visceral adipocytes was significantly lower in the oxytocin group than in the control group (Student's *t*-test; $P < .001$, d = 1.28) (Fig. 2E).

3.3. Effects of the 6-day injection of oxytocin on the levels of central and peripheral factors

The oxytocin group exhibited significantly lower serum AST (Student's *t*-test; $P < .01$, d = 1.94), ALT (Student's t-test; $P < .05$, d = 1.29), and lactate dehydrogenase (LDH) (Student's t-test; $P < .001$, d = 2.68) levels than the control group (Fig. 3). In addition, the serum triglyceride level was significantly lower in the oxytocin group than in the control group (Student's t-test; $P < .05$, d = 1.51) (Fig. 3). The serum HDL-CHO level of the oxytocin group was significantly lower than that of the control group (Student's t-test; $P < .05$, d = 1.50), whereas the LDL-CHO/HDL-CHO ratio did not differ between the oxytocin and control groups (Fig. 3). The serum

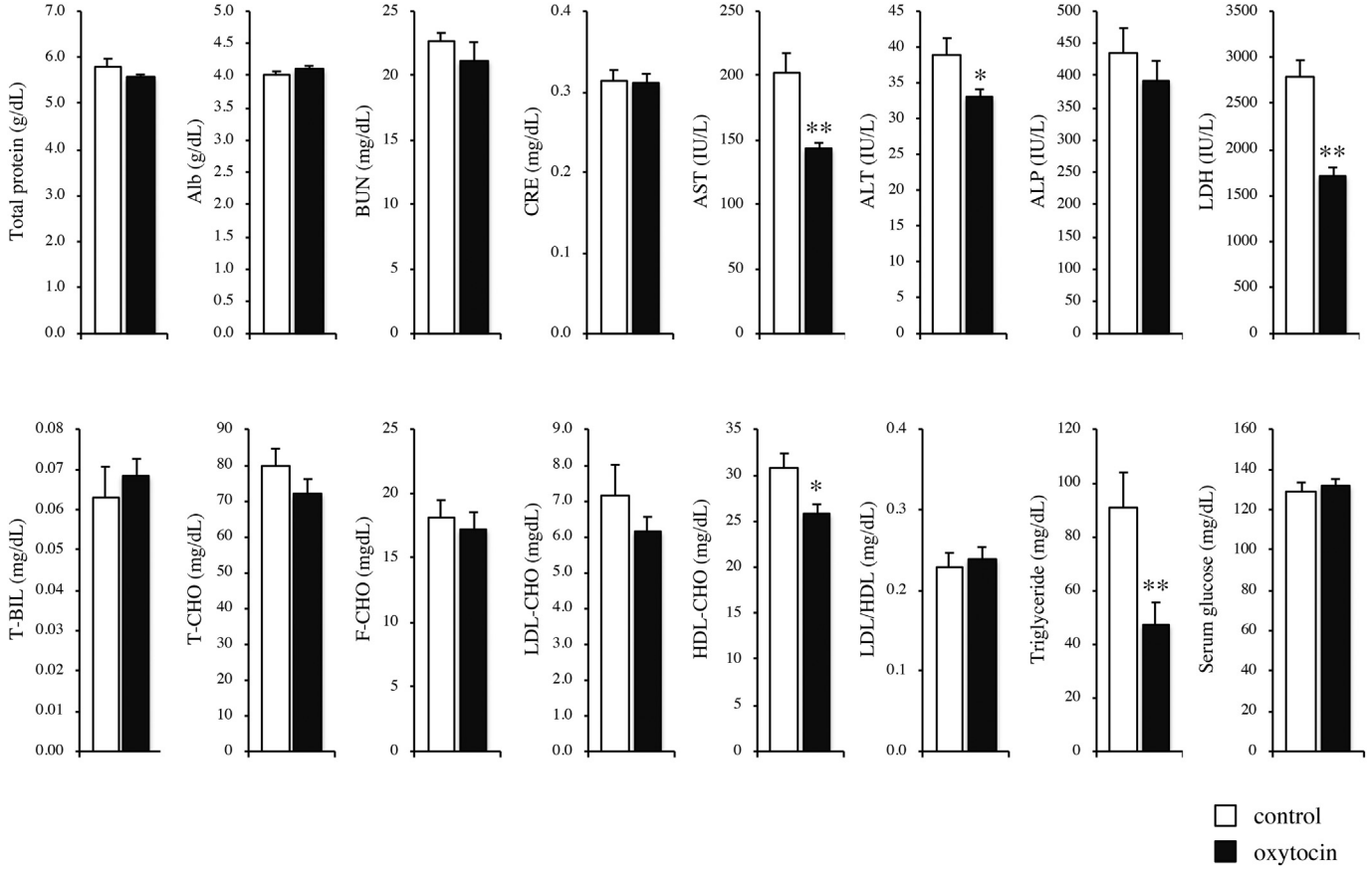

Fig. 3. Blood biochemistry analysis of total protein, albumin (Alb), blood urea nitrogen (BUN), creatinine (CRE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T-BIL), total cholesterol (T-CHO), free cholesterol (F-CHO), low-density lipoprotein cholesterol (LDL-CHO), high-density lipoprotein cholesterol (HDL-CHO), the LDL-CHO to HDL-CHO ratio (LDL/HDL), triglycerides, and serum glucose in the control and oxytocin groups. Data are expressed as mean ± SEM values ($n = 7$ per group); $^{*}P < .05$, $^{**}P < .01$.

levels of other factors did not differ between the oxytocin and control groups.

The mRNA expression levels of hypothalamic appetite-regulating factors, pro-inflammatory cytokines, oxytocin, and the oxytocin receptor did not differ between the oxytocin and control groups (Fig. 4). In addition, the mRNA expression levels of pro-inflammatory cytokines in subcutaneous and visceral fat did not differ between the oxytocin and control groups (Fig. 5).

3.4. Effects of the injection of oxytocin on body temperature and locomotor activity

Body temperature and locomotor activity exhibited circadian rhythms during the measurement period. The injection of oxytocin did not affect 24-h body temperature or locomotor activity (Fig. 6A, B).

4. Discussion

Recently, some studies have shown that the administration of oxytocin causes reductions in body weight, appetite, and fat mass in many species, including humans (Maejima et al., 2018), and these effects of oxytocin were more marked in obese individuals (Altirriba et al., 2014; Blevins et al., 2016; Maejima et al., 2017; Roberts et al., 2017; Seelke et al., 2018; Thienel et al., 2016). These findings indicate that oxytocin has advantages for treating obesity and that oxytocin could be used to treat and prevent metabolic disorders. The menopausal or surgical loss of ovarian function are a risk factor for visceral adiposity and some metabolic disorders (Carr et al., 2004; Ferrara et al., 2002; Iwasa et al., 2017a, 2017b; Liang et al., 2002; Meli et al., 2004; Palmisano et al., 2017; dos Reis et al., 2003; Richard, 1986; Rogers et al., 2009; Tchernof and Despres, 2000; Wade and Gray, 1979).

In this study, we showed that the 6-day injection of oxytocin caused marked reductions in body weight gain and food intake in ovariectomized rats. In addition, we found that the 6-day administration of oxytocin caused reductions in visceral and subcutaneous fat weight and adipocyte size. It has been reported that the injection of oxytocin directly and indirectly promotes lipolysis in adipose tissue and reduces fat mass in many species. Oxytocin receptors are expressed in adipose tissue (Gajdosechova et al., 2014; Yi et al., 2015), and oxytocin increased the expression of lipid metabolism-related genes and lipolysis in 3T3-L1 adipocytes and incubated fat pads in rat studies (Deblon et al., 2011; Yi et al., 2015). Thus, the reductions in body fat observed in the present study might have been induced by both the anorectic and lipolytic actions of oxytocin. On the other hand, the administration of oxytocin did not affect lean body weight in the present study, indicating that the observed reduction in body weight might be attributed to lipolysis in adipose tissue, but not to a reduction in other tissues such as muscle or bone. As sarcopenia causes functional impairments and physical disability in menopausal women (Messier et al., 2011), the administration of oxytocin, which causes reductions in fat mass, but not muscle mass, might help to prevent metabolic-related diseases in such women. Recently, it has been revealed that estradiol increases central oxytocin mRNA expression and that the anorectic effects of estrogen are mediated by an oxytocinergic pathway (Sloan et al., 2018). Thus, oxytocin supplementation might be a useful physiological treatment for menopausal women with decreased estrogen levels.

In the current study, the 6-day administration of oxytocin caused reductions in serum AST, ALT, and LDH levels. However, it did not

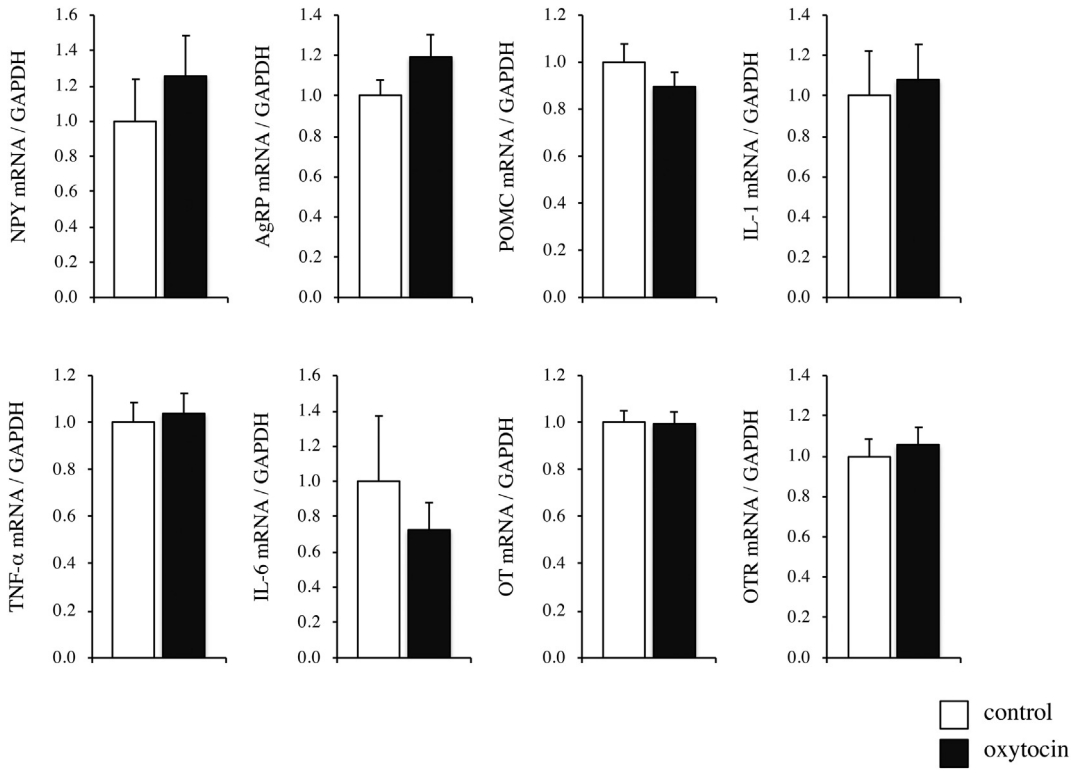

Fig. 4. Hypothalamic mRNA expression levels of neuropeptide Y (NPY), agouti-related protein (AgRP), proopiomelanocortin (POMC), interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), oxytocin (OT), and the oxytocin receptor (OTR) in the control and oxytocin groups. The mRNA expression levels of each gene are normalized to those of GAPDH, and the values for the control rats were defined as 1.0. Data are expressed as mean ± SEM values (n = 7 per group).

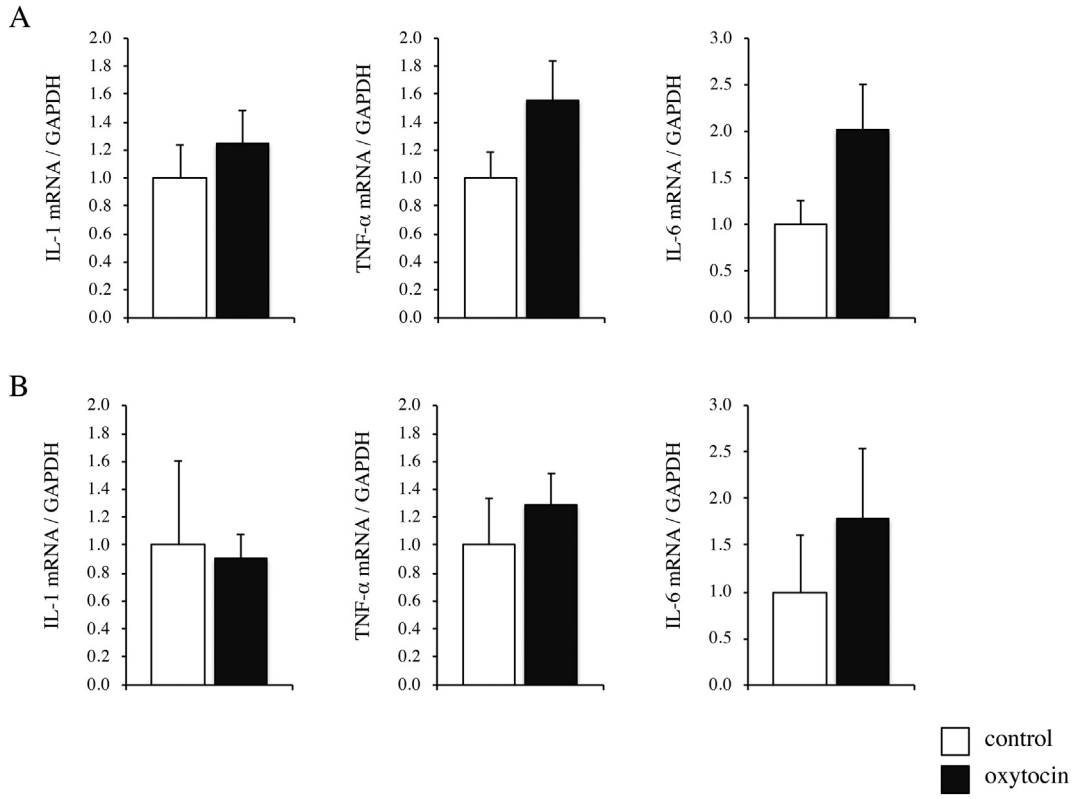

Fig. 5. The mRNA expression levels of interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) in (A) subcutaneous fat and (B) visceral fat. The mRNA expression levels are normalized to those of GAPDH, and the values for the control rats were defined as 1.0. Data are expressed as mean ± SEM values (n = 7 per group).

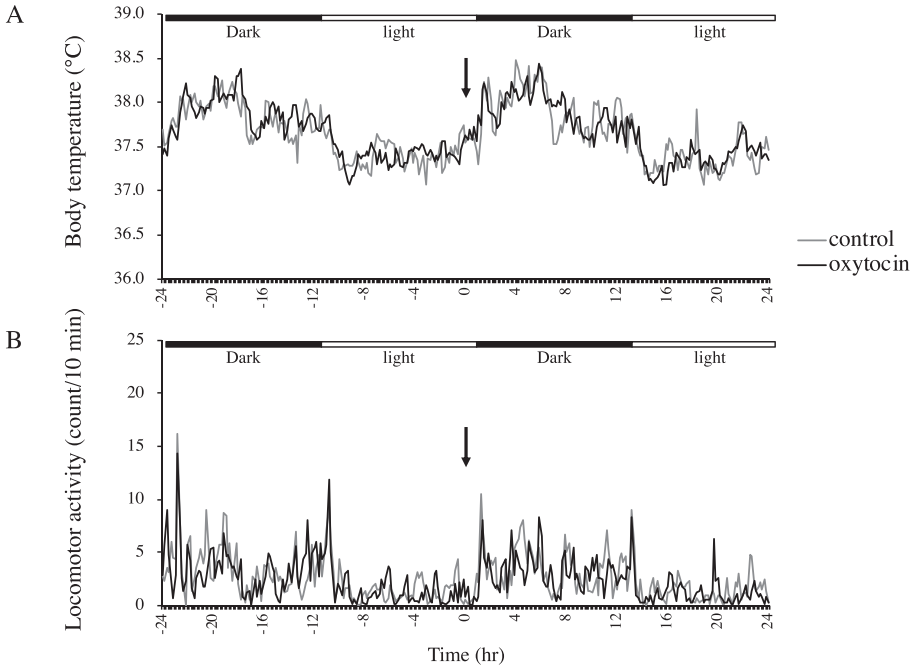

Fig. 6. (A) Body temperature and (B) locomotor activity before and after the administration of oxytocin in the control and oxytocin groups. Saline was intraperitoneally injected in the control group, and oxytocin (500 μg/day) was intraperitoneally injected in the oxytocin group at 1 h before the dark phase. The arrow indicates the time at which each substance was administered. Data are expressed as mean values (n = 4 per group).

affect the levels of T-BIL, total protein, Alb, BUN, or CRE. These results indicate that the 6-day administration of oxytocin might not disturb hepatic or renal function. Furthermore, the administration of oxytocin did not affect body temperature or locomotor activity, indicating that it did not induce a febrile response or behavioral abnormalities. However, previous studies have shown that in male rats the central administration of oxytocin increased core body temperature, whereas the peripheral administration of oxytocin reduced it (Hicks et al., 2014; Ong et al., 2017). In addition, the peripheral administration of oxytocin caused reductions in locomotor activity in male mice (Maejima et al., 2015). Regarding the specific reasons for the discrepancies between the findings of these previous studies and the present study, differences in species, age, experimental conditions (e.g., whether ovariectomy was performed), and/or sex might have contributed to these differences. Interestingly, a previous study showed that the effects of oxytocin on body temperature are dose-dependent (Hicks et al., 2014). Thus, the dose of oxytocin used in the present study might not have been sufficient to affect body temperature or locomotor activity in ovariectomized rats. Anyway, oxytocin does not seem to have any obvious adverse effects and so might be safe to use in the clinical setting. It has been reported that increases in AST and ALT levels accompanied by fatty liver are induced in ovariectomized rats (Liao et al., 2015). Our results indicate that the 6-day administration of oxytocin also prevents such adverse effects of ovariectomy. In addition, the administration of oxytocin caused reductions in serum triglyceride levels, but did not affect LDL cholesterol levels, in the current study. It has been reported that serum triglyceride and LDL cholesterol levels are increased in ovariectomized rats (Han and Wang, 2017). Thus, our results indicate that oxytocin administration might partially improve ovariectomy-induced hyperlipidemia.

It has been suggested that oxytocin neurons and POMC neurons reciprocally regulate each other in the hypothalamus; i.e., the anorectic effects of oxytocin are partially mediated by POMC and vice versa (Maejima et al., 2018). In addition, it has been reported that NPY/AgRP neurons project into oxytocin neurons and suppress their neuronal activity in the hypothalamus (Maejima et al., 2018). Therefore, we evaluated the effects of the 6-day administration of oxytocin on the hypothalamic mRNA expression levels of NPY/AgRP and POMC in the present study; however, oxytocin did not alter their expression levels. A previous study also reported that oxytocin administration did not affect the hypothalamic levels of orexigenic or anorexigenic factors in wild-type or leptin-deficient (*ob/ob*) mice (supplementary data of Altirriba et al., 2014). Perhaps the protein levels, but not the mRNA levels, of these factors are affected by oxytocin treatment. Alternatively, some other factors might be involved in the anorectic effects of oxytocin in ovariectomized or postmenopausal conditions. Interestingly, it has been reported that peripherally administered oxytocin stimulates the release of endogenous oxytocin within the central nervous system through the vagal afferent nerves, and it has been suggested that endogenous oxytocin might mainly affect metabolic functions and feeding behavior (Iwasaki et al., 2015; Morton et al., 2012; Zhang et al., 2011). Although the hypothalamic oxytocin mRNA expression level was not affected by the administration of oxytocin in the present study, it is possible that the release of endogenous oxytocin was increased in the oxytocin-treated rats. Further examinations, including evaluations of the peptide levels of central oxytocin, are needed to test this hypothesis.

It has been reported that inflammation in adipose tissue is induced in ovariectomized obese animals (Rogers et al., 2009) and that the hypothalamic inflammation observed in obese animals disturbs the regulation of body weight and food intake. In addition, administering oxytocin for 2 weeks led to reductions in TNF-α expression in the adipose tissue of diet-induced mice (Garrido-Urbani et al., 2018). Therefore, we measured the mRNA expression levels of pro-inflammatory cytokines in adipose tissue and the hypothalamus (Carvalheira et al., 2003; De Souza et al., 2005; Thaler et al., 2013). As a result, it was demonstrated that the 6-day administration of oxytocin did not affect the expression levels of these cytokines, indicating that the effects of oxytocin observed in the present study were not induced by the attenuation of tissue inflammation.

This study was limited by the fact that only ovariectomized rats were used for the experiments, and so we could not compare the effects of oxytocin between ovariectomized and ovarian-intact rats. However, we speculate that the effects of oxytocin might be more marked in ovariectomized rats because, as noted above, the effects of oxytocin on metabolic function are increased in obese individuals. In addition, because oxytocin was only administered for 6 days in this study, the effects and safety of longer-term oxytocin treatment have not been clarified.

5. Conclusion

In conclusion, the 6-day administration of oxytocin caused reductions in body weight gain, food intake, visceral and subcutaneous fat weight, and adipocyte size in ovariectomized rats. Blood examinations indicated that the 6-day administration of oxytocin did not cause alterations in renal or hepatic functions. Instead, oxytocin might prevent ovariectomy-induced liver damage. Physiological examinations also indicated that the administration of oxytocin did not affect thermogenesis or locomotor activity. This is the first study to show the effects of oxytocin on metabolic and feeding functions in ovariectomized individuals. Our results indicate that oxytocin could be used to treat or prevent menopause-induced metabolic disorders, as it does not seem to have any adverse effects, at least in the short term. In addition, the neuroendocrinological mechanisms by which oxytocin reduces food intake and attenuates body weight gain could not be clarified in this study.

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Conflict of interests

The authors declare that they have no conflict of interest.

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