ORIGINAL

Kamikihito contributes to visceral fat reduction and appetite suppression in PCOS model rats by increasing OTR and leptin expression in visceral fat

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Abstract : Polycystic ovary syndrome (PCOS) is an endocrine disorder that causes infertility as well as obesity. Oxytocin (OT), a neuropeptide involved in appetite and fat metabolism, may be therapeutically beneficial for PCOS. Kamikihito (KKT), a traditional Japanese herbal medicine of Chinese origin, has been shown to up-regulate the expression of OT in the hypothalamus and exert various physiological effects. The present study investigated the anti-obesity effects of KKT in relation to OT using a PCOS model rat. Female rats were implanted with dihydrotestosterone to induce PCOS and divided into a normal food group (NF group) and food containing KKT group (KF group). After surgery, rats were fed normal food for four weeks, followed by food containing 3% KKT for another four weeks. The anti-obesity effects and impact on the ovarian morphology of KKT in PCOS were examined. KKT supplementation reduced food intake, the size of visceral adipocytes, and ovarian weight. The KF group showed increased mRNA expression levels of OT receptors (OTR) and leptin in visceral fat and slightly increased serum levels of OT and leptin. These results suggest the potential of KKT as a therapeutic agent for PCOS, potentially through effects on OT and leptin signaling pathways. J. Med. Invest. 72 : 316-323, August, 2025

Keywords **:** *kamikihito, polycystic ovary syndrome (PCOS), oxytocin, leptin, anti-obesity*

INTRODUCTION

The epidemiology of polycystic ovary syndrome (PCOS), an endocrine disorder, remains unclear ; however, it is often detected in women of reproductive age (1). In addition to the clinical issue of infertility due to ovulation disorders, the risk of obesity and other metabolic abnormalities is increased in patients with PCOS (2, 3). Furthermore, obesity worsens the reproductive and metabolic phenotypes of PCOS, and even though physicians provide lifestyle guidance and other treatments, the dropout rate is high (4, 5). Therefore, the development of a continuable drug with fewer side effects is desired. The latest PCOS model rat was created and reported in our previous study (6). Serum oxytocin (OT) levels were reduced in the PCOS model rat and the peripheral administration of OT attenuated obesity (7, 8).

Oxytocin (OT) is a neuropeptide consisting of 9 amino acids. It is synthesized by the paraventricular nucleus (PVN) and supraoptic nucleus in the hypothalamus. Upon synthesis, OT is released from the posterior lobe of the pituitary gland (9). OT has been shown to contribute to appetite and BW control (10) as well as to fat burning in peripheral tissues (11). We previously reported that obesity was associated with sex hormones and OT and showed a relationship between obesity and decreased endogenous OT in a PCOS model rat, in addition to decreased estrogen in female rats and decreased testosterone in male rats (7, 8, 12-14). The administration of OT was found to reduce food intake (FI) and/or body weight (BW) and promote β-oxidation and lipolysis in the fat tissue of rodents (15, 16), monkeys (17), and humans (18, 19), and these effects were more prominent in obese subjects. As described above, OT has potential as a therapeutic candidate for PCOS (7, 8) ; however, an oral drug has not yet been developed. In addition, since OT is a well-known substance, it is difficult for patents to be newly granted ; therefore, companies are reluctant to devote resources to its development.

Kamikihito (KKT), a traditional Japanese herbal medicine (Kampo) of Chinese origin, comprises 14 medicinal plants. It has been used to treat anemia, insomnia, fatigue, loss of appetite, and mental anxiety and is often prescribed in clinical settings to women going through menopause. In addition to perimenopausal women, KKT was shown to be effective against fatigue in patients receiving anticancer drugs for renal and prostate cancer (20). KKT has also been reported to exert protective effects against behavioral changes in a rat model of depression (21). Furthermore, KKT was found to up-regulate the expression of OT in the rat hypothalamic PVN and acted directly on OT receptors (OTR), which have been reported to exert anti-stress effects (22). Furthermore, it is reported that this action is due to the three herbal medicines (*jujube* seed, *Japanese Angelica* root, and *Ginger* root) contained in KKT that activate OT neurons by activating the OTR in the PVN (23). These findings on KKT, which have recently expanded the scope of its use from administration to perimenopausal women, are of interest, particularly its effects on the central nervous system.

KKT, which activate OT neurons in PVN (22, 23), has been suggested to exert protective effects against obesity in the PCOS model rat with reduced endogenous OT levels. Therefore, the dietetic effects of KKT on PCOS were investigated using the PCOS model rat.

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MATERIAL AND METHODS

Animals and drugs

Female Wistar rats at three weeks of age were obtained from The Jackson Laboratory Japan (Kanagawa, Japan). The rats were fed freely with normal food with trade name MF (Orient Yeast Co., Ltd., Tokyo, Japan) and water. They were kept in an environment where water and food were freely available, room temperature was 24°C, and the light/dark cycle was maintained every 12 hours (lights on at 8:00 am and lights off at 8:00 pm). Surgical procedures and tissue sampling were conducted under anesthesia using sevoflurane, along with a combination of medetomidine, midazolam, and butorphanol (doses : 0.15, 2.00, and 2.50 mg/kg, respectively). The Animal Care and Use Committee of Tokushima University approved all experimental protocols and methods, which adhered to the ARRIVE guidelines (Approval Code : T2022-102. Approval Date : May 11th, 2021).

At the age of four weeks, female rats were subjected to implantation of a specifically designed silicon tube (provided by As One Co., Ltd., Tokyo, Japan) with precise dimensions. The inner diameter of the tube was 3 mm, the outer diameter was 5 mm, and the filling section was 10 mm. This implantation procedure involved the use of the silicon tube filled with diluted dihydrotestosterone (DHT) to effectively induce the PCOS model (6). This day was designated as Day 0. DHT was dissolved in peanut oil at 20% ethanol concentration to reach 16 mg/mL. The PCOS model rats were studied in two groups, a normal food group (NF group, n = 8) and a group receiving food containing KKT (KF group, n = 8). Body weight (BW) and food intake (FI) were measured until the rats reached 8 weeks of age. Additionally, starting from 3 weeks after surgery (at 7 weeks of age), representative PCOS model rats underwent daily collection of vaginal epithelial smears over a 10-day period to evaluate estrous cyclicity. Vaginal smears were collected by inserting the tip of a glass pipette about 5 mm into the vulva and letting water in and out. Subsequently, water from the collected vaginal smears was gently dropped onto glass slides, air-dried, Giemsa stained, and used to determine the stage of the estrus cycle—proestrus, estrus, metestrus, and diestrus.

Food containing KKT was prepared by a company that produces the normal food used in the present study, as previously described, and had a solid shape for rodents at a concentration of 3% KKT (24). KKT was provided by Tsumura & Co, Tokyo, Japan. Ingredients required to make 5.0 g of dry KKT are *Astragalus* root (3.0g, *Astragalus membranaceus* Bunge), *Bupleurum* root (3.0g, *Bupleurum falcatum* L.), *jujube* seed (3.0g, *Zizyphus jujuba var. spinosa (Bunge) Hu ex H. F. Chow*), *Atractylodes Lancea* rhizome (3.0g, *Atractylodes japonica* Koidz. ex Kitam.), *Ginseng* root (3.0g, *Panax ginseng* C. A. Mey.), *Poria* strain (3.0g, *Pinus densiflora* Siebold & Zucc.), *Longan* aril (3.0g, *Euphoria longana* Lam.), *Polygala* root (2.0g, *Polygala tenuifolia* Willd.), *Gardenia* fruit (2.0g, *Gardenia jasminoides* J. Ellis), *jujube* fruit (2.0g, *Ziziphus jujuba var. inermis* (Bunge) Rehder), *Japanese Angelica* root (2.0g, *Angelica acutiloba* (Siebold & Zucc.) Kitag.), *Glycyrrhiza* root (1.0g, *Glycyrrhiza uralensis* Fisch.), *Ginger* root (1.0g, *Zingiber officinale* Roscoe), *Saussurea* root (1.0g, *Saussurea lappa* (Decne.) C. B. Clarke). These plants were identified on "World Flora Online" (www.worldfloraonline.org) or MPNS (http://mpns.kew.org). After 8 weeks of age, the NF group was fed normal food and the KF group was fed food containing KKT, and their BW and FI were measured until 12 weeks of age. In addition, the ratio of BW gain efficiency, calculated as total weight gain from Days 0 to 28 and from Days 28 to 56 divided by the respective cumulative FI, was measured in both groups.

Tissue sampling and processing

At 12 weeks of age, all rats were euthanized by decapitation, and various tissues were collected, including blood, brain, left ovary, and visceral and subcutaneous fat. The weights of the parametrial and perirenal deposits (visceral fat), inguinal deposit (subcutaneous fat), and the left ovary were recorded. After excision, each fat sample was immediately weighed, and small samples were obtained for further analysis. For histological examination, visceral fat, subcutaneous fat, and the left ovary were fixed in a 4% paraformaldehyde solution. Frozen brain and visceral fat samples were used for central and peripheral mRNA assays, respectively. Whole blood was centrifuged at 3000 rpm and 4°C for a duration of 20 minutes, and serum was collected and stored at -20°C for analysis of oxytocin (OT) and leptin concentrations.

Histological analysis

Fat and the left ovary samples were embedded in paraffin and subjected to hematoxylin and eosin staining. Histological images were captured and analysis was performed using cellSens Standard (Olympus., Co., Tokyo, Japan). About 80-100 adipocytes per sample were selected that maintained their shape, and their average area was calculated. Additionally, the number of abnormal follicles in the left ovary was quantified. Abnormal follicles were identified as those exhibiting enlargement and thinning of the granulosa cell layer.

Biochemical examination

Isolated serums were aliquoted in 300 μL portions. Measurement of oxytocin in serum was performed by ASKA Pharmaceutical Medical Inc. (Kanagawa, Japan), and measurement of leptin was performed by Oriental Yeast, Co. (Tokyo, Japan).

Real-time polymerase chain reaction (PCR)

mRNA expression levels were analyzed in both hypothalamic and fat samples. Extraction of mRNA was performed using the RNeasy Mini Kit (Qiage, Hilden, Germany). Conversion of the resulting mRNA to cDNA was performed using the SuperScript III First-Strand Synthesis System (Invitrogen Co., Life Technologies Japan Ltd., Tokyo, Japan). The cDNA was amplified and measured by PCR using Fast SYBR® green (Invitrogen Co.).

The mRNA expression levels of OT, OT receptors (OTR), leptin receptors (ObRb), neuropeptide Y (NPY), and pro-opiomelanocortin (POMC) in hypothalamus and OTR and leptin in visceral fat were measured. The expression levels were normalized using GAPDH as the reference gene for the hypothalamus and 18S rRNA as the reference gene for visceral fat. The primer sequences and directions used in the present study are summarized in Table 1.

Statistical analysis

The results are presented as means ± standard error of the mean (SEM). Student's *t*-test was used for statistical analysis between the two items. A two-way repeated measures analysis of variance (ANOVA) was used for statistical analysis of the changes in BW and FI in the observation period. A significance level of $P < 0.05$ was used to determine statistical significance.

RESULT

Changes in BW and FI before and after the addition of KKT to food

BW was slightly lower in the KF group than in the NF group after the start of KKT administration (Figure 1A). Weekly FI was lower in the KF group than in the NF group, and was

significantly lower for the first week after the addition of KKT (the Student's *t*-test : $p < 0.05$; $df = 14$, $t = 2.703$; Figure 1B). Cumulative FI was significantly lower in the KF group than in the NF group after the addition of KKT (two-way ANOVA : treatment, $F(1, 56) = 11.021$, $p < 0.01$; Day, $F(3, 56) = 1055.568$, $p < 0.001$; interaction, $F(4, 56) = 0.154$, $p = 0.9260$; Figure 2A). There were no significant differences observed in BW gain efficiency rates on Days 0-28 (without KKT) or Days 28-56 (with KKT) between the NF and KF groups (Figure 2B).

Comparison of body fat, ovarian weight, and the reproductive phenotype with or without KKT

No significant differences were noted in visceral, subcutaneous, or total fat weight between the KF and NK groups (Figure 3A). On the other hand, the size of visceral adipocytes was significantly smaller in the KF group than in the NF group (the Student's *t*-test : $p < 0.01$; $df = 14$, $t = 4.356$), whereas that of subcutaneous adipocytes did not significantly differ (Figure 3B, 3C). Ovarian weight was significantly lower in the KF group than in the NF group (the Student's *t*-test : $p < 0.01$; $df = 14$, $t = 3.054$), while the number of abnormal follicles was slightly smaller in the KF group than in the NF group (Figure 4A). The estrus cycle was examined using vaginal smear findings starting at 7 weeks of age to establish whether they exhibited a reproductive phenotype similar to PCOS. The typical normal female rat has an estrus cycle of 4-5 days ; however, representative cases had irregular sexual cycles, reflecting the clinical picture of PCOS (Figure 4B, 4C).

Table 1. Primer sequences and directions

Primer	Sequence	Direction
oxytocin	GAACACCAACGCCATGGCCTGCCC	Sense
	TCGGTGCGGCAGCCATCCGGGCTA	Antisense
oxytocin receptor	CGATTGCTGGGCGGTCTT	Sense
	CCGCCGCTGCCGTCTTGA	Antisense
leptin	GGTCACCGGTTTGGACTTCAT	Sense
	CTGGTCCATCTTGGACAAACTCA	Antisense
ObRb	GCAGCTATGGTCTCACTTCTTTTG	Sense
	GTTCCCTGGGTGCTCTGA	Antisense
NPY	GGGGCTGTGTGGACTGACCCT	Sense
	GATGTAGTGTCGCAGAGCGGAG	Antisense
POMC	CCTCACCACGGAAAGCA	Sense
	TCAAGGGCTGTTCATCTCC	Antisense
GAPDH	ATGGCACAGTCAAGGCTGAGA	Sense
	CGCTCCTGGAAGATGGTGAT	Antisense
18S rRNA	GACGGACCAGAGCGAAAGC	Sense
	AACCTCCGACTTTCGTTCTTGA	Antisense

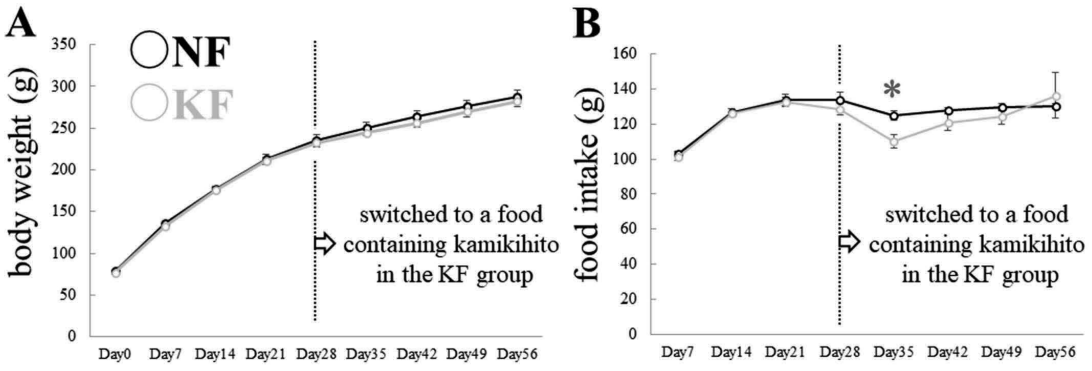

Fig 1. Weekly (A) body weight and (B) food intake in NF and KF groups. Starting on Day 28, the diet was switched to a food containing kamikihito in the KF group. *P <0.05, **P <0.01.

Real-time PCR and serum biochemical analyses of appetite control

There were no significant differences observed in the hypothalamic mRNA expression levels of OT, OTR, ObRb, NPY, or POMC, whereas those of OT was slightly higher in the KF group (Figure 5A). The visceral fat mRNA expression level of OTR was significantly higher in the KF group than in the NF group (the Student's *t*-test : $p < 0.01$; $df = 14$, $t = -3.123$), as was the expression level of leptin (the Student's *t*-test : $p < 0.01$; $df = 14$, $t = -3.652$; Figure 5B). Serum OT and leptin levels were slightly higher in the KF group than in the NF group (Figure 5C).

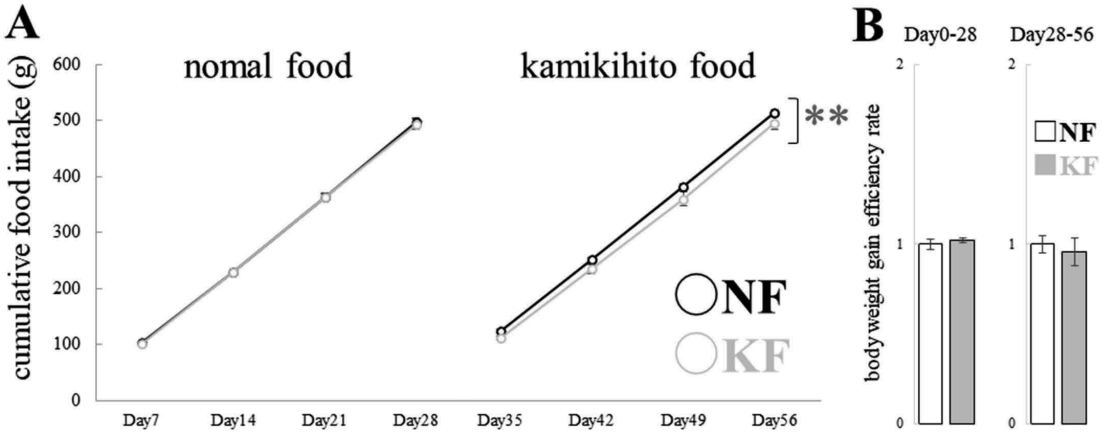

Fig 2. (A) Cumulative food intake and (B) the body weight gain efficiency (the amount of weight gain divided by the amount of food intake) rate from Day 0 to Day 28 (normal food) and from Day 28 to Day 56 (switched to a food containing kamikihito in the KF group) in NF and KF groups. *P <0.05, **P <0.01.

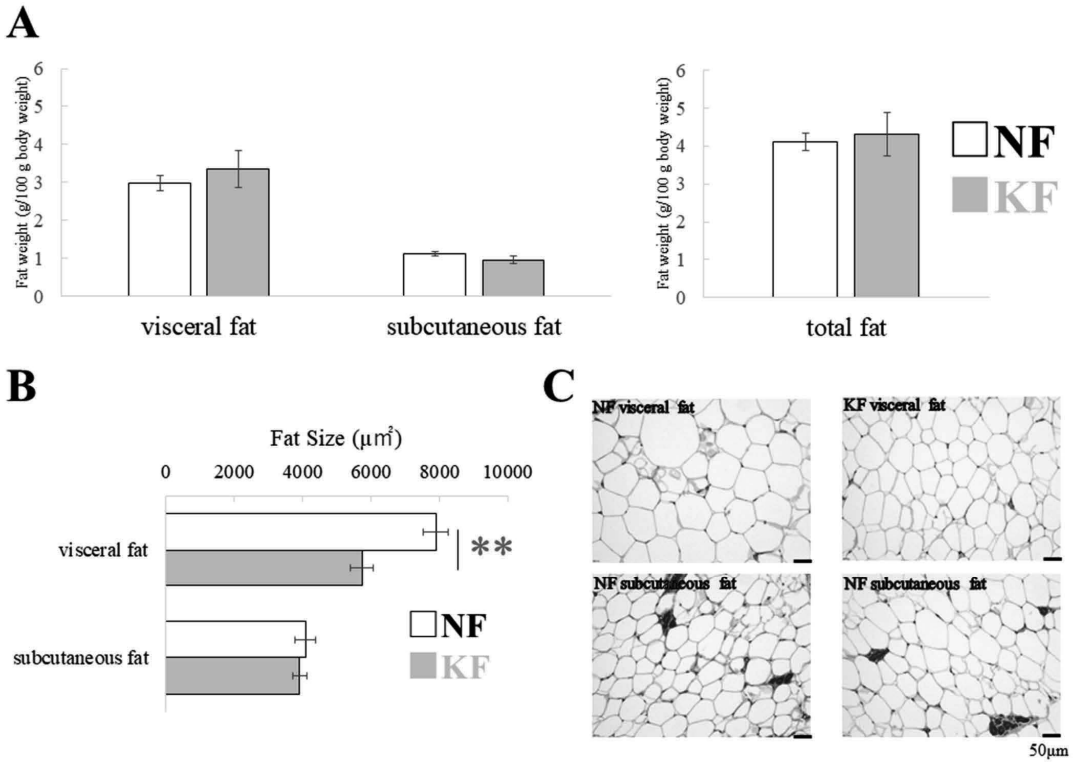

Fig 3. (A) Weights of visceral, subcutaneous, and total fat per 100 g body weight, (B) adipocyte sizes, and (C) morphologies of visceral and subcutaneous fat in NF and KF groups. *P <0.05, **P <0.01.

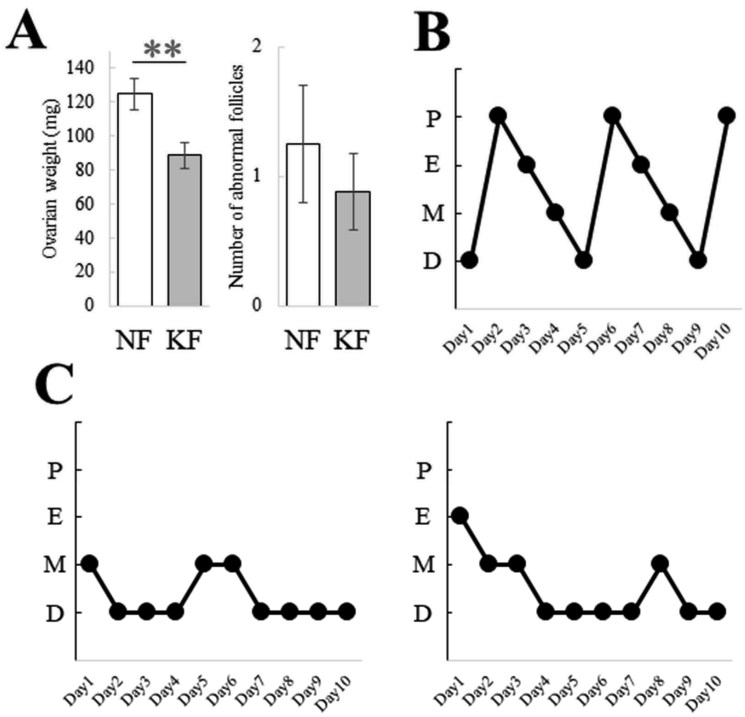

Fig 4. (A) Ovarian weight and number of abnormal follicles in NF and KF groups. (B) A typical estrus cycle of a female rat and (C) representative estrus cycles of PCOS model rats (typical two cases) in the present study over a 10-day period. P = proestrus, E = estrus, M = metestrus, D = diestrus. *P <0.05, **P <0.01.

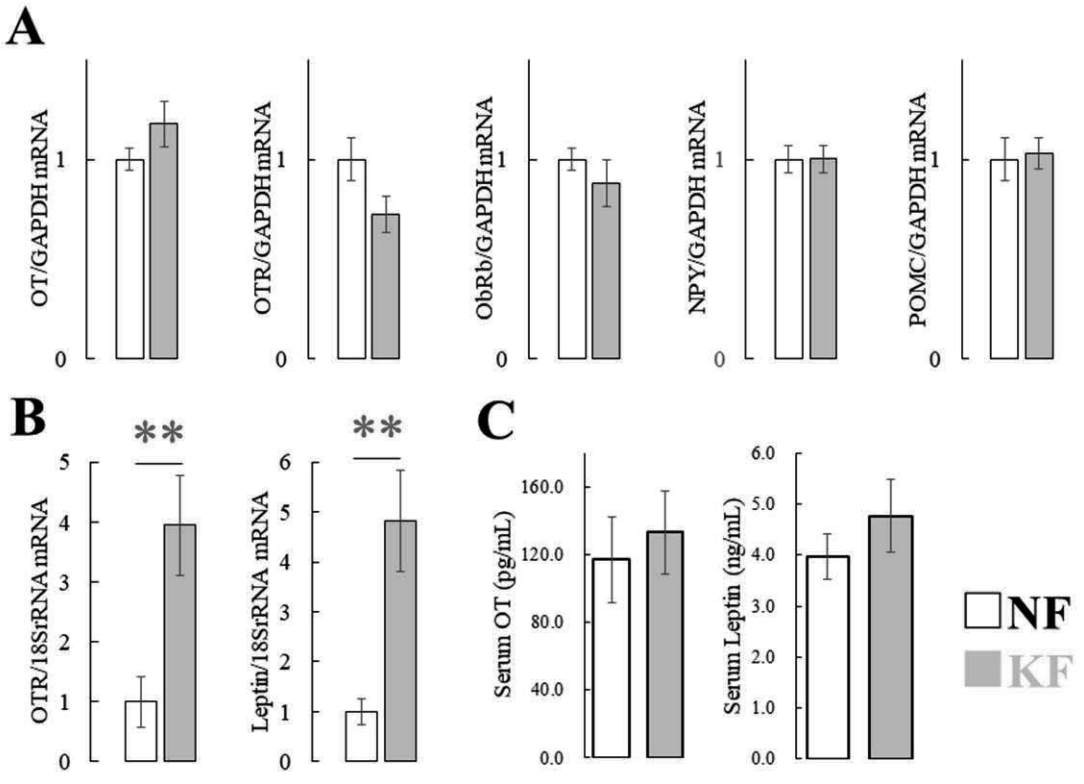

Fig 5. mRNA expression levels of oxytocin (OT), OT receptor (OTR), leptin, leptin receptor (ObRb), neuropeptide Y (NPY), and pro-opiomelanocortin (POMC) in (A) Hypothalamus and (B) visceral fat, and (C) serum levels of OT and leptin in NF and KF groups. *P <0.05, **P <0.01.

DISCUSSION

PCOS is an endocrine disorder with the clinical issue of infertility and an increased risk of obesity that is often observed in women of reproductive age (1-3). The latest PCOS model rat was created in our previous study (6) and we showed that its serum OT level was reduced and also that the administration of OT was expected to attenuate obesity (8). However, this study was limited by the lack of oral OT drugs. In clinical practice, OT is used as an intravenous drug to manage pregnancy, and nasal drops are used to treat autism. When OT is used for anti-obesity prophylaxis, an oral formulation would be more appropriate, but no oral OT regimen has been developed. Therefore, we decided to focus on KKT as an agent that enhances endogenous OT rather than administering OT externally. KKT has been shown to up-regulate the expression of OT, act directly on OTR in the PVN of rats, and decrease psychological stress (22, 23). Therefore, KKT, which increases endogenous OT levels, is expected to attenuate obesity in PCOS model rats with reduced endogenous OT.

The administration of KKT did not or only slightly affected BW changes. On the other hand, cumulative FI was significantly decreased by the administration of KKT, while BW gain efficiency did not significantly differ in the NF and KF groups. These results suggest that KKT exerted mild anti-obesity effects by decreasing FI in PCOS model rats without affecting nutrient absorption. In the preliminary experiment in the previous study, KKT did not affect BW gain or FI in normal healthy female rats (24). Therefore, the present study showed that KKT does not generally affect FI or BW in normal healthy female rats, but exerts selective therapeutic effects on PCOS, which is associated with obesity.

In the present study, visceral and subcutaneous fat weights did not significantly differ between the NF and KF groups, whereas the size of visceral adipocytes was smaller in latter than in the former. Although there was a discrepancy between fat weight and the size of adipocytes, this appeared to be due to the manual measurement of weight, which has a large error margin, with the evaluation of adipocyte sizes being more accurate, which was also the case in our previous study (14). Regarding the size of adipocytes, KKT may selectively contribute to the combustion of visceral fat among body fat.

Ovarian weight was significantly smaller in the KF group than in the NF group. This result may be related to the abnormal follicle count being slightly lower in the KF group than in the NF group, with no other difference in pathology, and the underlying cause remains unclear. Although a study on changes in the sexual cycle with or without KKT may support this result, the vaginal smear collection procedure itself was not performed on PCOS model rats in the present study because it was considered to be stressful and may have had a negative effect on BW and FI. Therefore, the present study only showed the estrus cycle by a vaginal smear performed on PCOS model rats created under the same conditions, but not used in this study. The relationship between KKT and the estrus cycle needs to be investigated in another experimental system, which has not yet been performed due to the limited amount of feed available ; however, it will be the subject of a future study.

Serum OT levels were expected to be increased by KKT, and only a slight elevation was noted. Similarly, we anticipated increases in the hypothalamic mRNA expression levels of OT and/or OTR by KKT ; however, no significant differences were observed. The oral administration of KKT has been shown to increase c-fos expression in OT neurons in PVN, and KKT has been reported to pass through the blood-brain barrier to the hypothalamus upon oral intake (23). Nevertheless, the reason for the lack of significant differences in the hypothalamic mRNA expression levels of OT and OTR in the present study may be that KKT did not function well due to the unknown pathology of PCOS or that the amount of KKT ingested was insufficient. In addition, it may be difficult to obtain the elevated effect of oxytocin in Kamikichito because serum oxytocin levels are reduced in the PCOS model rats (8). Alternatively, due to technical limitations in the present study, the PVN was not specifically selected when the hypothalamus was excised from the brain, and it is possible that subtle changes in OT and OTR were not detected. On the other hand, the mRNA expression level of OTR in visceral fat was markedly elevated in the KF group. Previous studies reported that OT increased adipose tissue lipolysis and fatty acid β-oxidation (11, 25), and the significant reduction noted in the size of visceral adipocytes with KKT in the present study may have been due to the enhanced fat-burning effects of OT due to an increase in the mRNA expression level of OTR in visceral fat. A previous study reported that jujube fruit, Japanese Angelica root, and ginger root in KKT contributed to the stability of OTR in PVN OT neurons and enhanced the effects of OTR (23). On the other hand, KKT has not been reported to affect OTR of visceral fat. Although the underlying pharmacological mechanisms were not elucidated in the present study, KKT may exert the same effects on OTR of visceral fat as it does on OTR of the PVN. Furthermore, PCOS is often associated with impaired glucose tolerance (3), and is also a chronic inflammatory disease with elevated C-Reactive Protein levels and increased number of leukocytes (26), which is mainly caused by increased production of inflammatory cytokines in visceral fat (27, 28). Although the present study did not evaluate the effects of KKT on glucose tolerance and inflammation, since OTR promotes insulin secretion from pancreatic β-cells (29) and has anti-inflammatory effects (30), the OTR-mediated effects of KKT on glucose tolerance and inflammation should be investigated in future studies. This is because KKT-induced upregulation of OTR mRNA expression in visceral fat may contribute to anti-inflammatory effects and improve insulin resistance.

Leptin is a product of the obese (*ob*) gene, is secreted from adipocytes, is 16 kDa in size, and comprises 167 amino acids (31, 32). It is involved in appetite, weight regulation, fetal growth, inflammatory immune functions, angiogenesis, and fat burning (33, 34). In the appetite center, ObRb is expressed in the PVN and leptin is a molecule upstream of OT (35). Furthermore, leptin increases the transcription of POMC and decreases that of NPY in the hypothalamic arcuate nucleus, leading to appetite suppression (36-38). In the present study, no significant differences were observed in the mRNA expression levels of ObRb, NPY, or POMC in the hypothalamus between the NF and KF groups. On the other hand, the mRNA expression level of leptin in visceral fat markedly increased in the KF group, whereas serum leptin levels were only slightly elevated. These results suggest that KKT exerted mild effects on serum leptin levels, and when used in combination with OT, which also suppresses appetite, KKT contributed to a significant decrease in cumulative FI in the KF group.

In the present study, the direct effects of the administration of KKT on the hypothalamus, the appetite center, in PCOS model rats remain unclear. On the other hand, there was a significant increase in the mRNA expression levels of OTR and leptin in visceral fat. These results suggest that KKT directly exerted fat-burning effects on visceral fat through OT and OTR in the PCOS model rat. Alternatively, KKT may have reduced visceral fat by decreasing food intake in PCOS model rats, contributing to increased mRNA expression of OTR. In other words, KKT may contribute to OTR stability not only in the hypothalamus but also in peripheral tissue. Furthermore, KKT may have indirect appetite suppression effects on the hypothalamus through

leptin. As described above, there are currently no oral OT drugs ; in the PCOS model rat, KKT has potential as an oral drug related to OT because of its anti-obesity effects through OT and leptin.

However, as a limitation of the study, this experiment was conducted with KKT mixed in the food, which is different from the oral administration method in actual clinical practice. To approximate the real clinical situation, KKT could be administered orally several times a day, but it would not be possible to avoid the effects of stress caused by frequent handling, which would result in decreased body weight and food intake. Therefore, the method used in the present study may be a limitation for research on items that are sensitive to the effects of stress, as in the present study.

CONFLICT OF INTEREST (COI) DISCLOSURE

KKT was provided by Tsumura & Co, Tokyo, Japan.

REFERENCES

1. Ding T, Hardiman PJ, Petersen I, Wang FF, Qu F, Baio G : The prevalence of polycystic ovary syndrome in reproductive-aged women of different ethnicity : A systematic review and meta-analysis. Oncotarget 8 : 96351-96358, 2017
2. Ehrmann DA : Polycystic ovary syndrome. N Engl J Med 352 : 1223-1236, 2005
3. Moran LJ, Misso ML, Wild RA, Norman RJ : Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome : A systematic review and meta-analysis. Hum Reprod Update 16 : 347-363, 2010
4. Ladson G, Dodson WC, Sweet SD, Archibong AE, Kunselman AR, Demers LM, Willianms NI, Coney P, Legro RS : The effects of metformin with lifestyle therapy in polycystic ovary syndrome : A randomized double-blind study. Fertil Steril 95 : 1059-1066, 2011
5. Bates GW, Legro RS : Longterm management of polycystic ovarian syndrome (PCOS). Mol Cell Endocrinol 373 : 91-97, 2013
6. Kamada S, Yamamoto Y, Aoki H, Tamura K, Takeda A, Minato S, Masaki R, Yanagihara R, Hayashi N, Yano Y, Imaizumi J, Kagawa T, Yoshida A, Kawakita T, Irahara M, Iwasa T : A novel PCOS rat model and an evaluation of its reproductive, metabolic, and behavioral phenotype. Reprod Med Biol 21 : e12416, 2021
7. Iwasa T, Matsuzaki T, Mayila Y, Kawakita T, Yanagihara R, Irahara M : The effects of chronic oxytocin administration on body weight and food intake in DHT-induced PCOS model rats. Gynecol Endocrinol 36 : 55-60, 2020
8. Yamamoto S, Noguchi H, Takeda A, Arakaki R, Uchishiba M, Imaizumi J, Minato S, Kamada S, Kagawa T, Yoshida A, Kawakita T, Yamamoto Y, Yoshida K, Kon M, Shinohara N, Iwasa T : Changes in endogenous oxytocin levels and the effects of exogenous oxytocin administration on body weight changes and food intake in polycystic ovary syndrome model rats. International Journal of Molecular Sciences. Int J Mol Sci 23 : 8207, 2022
9. Du Vigneaud DV, Ressler C, Trippett S : The sequence of amino acids in oxytocin, with a proposal for the structure of oxytocin. J Biol Chem 205 : 949-957, 1953
10. Altirriba J, Poher AL, Rohner-Jeanrenaud F : Chronic oxytocin administration as a treatment against impaired leptin signaling or leptin resistance in obesity. Front Endocrinol 6 : 119, 2015
11. Fukushima A, Kataoka N, Nakamura K : An oxytocinergic neural pathway that stimulates thermogenic and cardiac sympathetic outflow. Cell Rep 40 : 111380, 2022
12. Iwasa T, Matsuzaki T, Mayila Y, Yanagihara R, Yamamoto Y, Kawakita T, Kuwahara A, Irahara M : Oxytocin treatment reduced food intake and body fat and ameliorated obesity in ovariectomized female rats. Neuropeptides 75 : 49-57, 2019
13. Tokui T, Kawakita T, Yanagihara R, Kamada S, Minato S, Takeda A, Imaizumi J, Yamamoto Y, Yoshida K, Kato T, Irahara M, Iwasa T : Effects of gonadal status and the estrogen milieu on hypothalamic oxytocin gene expression and serum oxytocin levels in female rats. Horm Behav 133 : 105005, 2021
14. Yamamoto S, Arakaki R, Noguchi H, Takeda A, Uchishiba M, Kamada S, Mineda A, Kon M, Shinohara N, Iwasa T : New discoveries on the interaction between testosterone and oxytocin in male rats – Testosterone-mediated effects of oxytocin in the prevention of obesity. Physiol and Behav 266 : 114199, 2023
15. Maejima Y, Aoyama M, Sakamoto K, Jojima T, Aso Y, Takasu K, Takenoshita S, Shimomura K : Impact of sex, fatdistribution and initial body weight on oxytocin's body weight regulation. Sci Rep 7 : 8599, 2017
16. Maejima Y, Yokota S, Nishimori K, Shimomura K : The anorexigenic neural pathways of oxytocin and their clinical implication. Neuroendocrinology 107 : 91-104, 2018
17. Blevins JE, Graham JL, Morton GJ, Bales KL, Schwartz MW, Baskin DG, Havel PJ : Chronic oxytocin administration inhibits food intake, increases energy expenditure, and produces weight loss in fructose-fed obese rhesus monkeys. Am J Physiol Regul Integr Comp Physiol 308 : R431-R438, 2015
18. Lawson EA, Marengi DA, DeSanti RL, Holmes TM, Schoenfeld DA, Tolley CJ : Oxytocin reduces caloric intake in men. Obesity 23 : 950-956, 2015
19. Thienel M, Fritsche A, Heinrichs M, Peter A, Ewers M, Lehnert H, Born J, Hallschmid M : Oxytocin's inhibitory effect on food intake is stronger in obese than normal-weight men. Int J Obes (Lond) 40 : 1707-1714, 2016
20. Tamada S, Ebisu K, Yasuda S, Kato M, Ninomiya N, Yamasaki T, Iguchi T, Nakatani T, Watanabe Y : Kamikihito improves cancer-related fatigue by restoring balance between the sympathetic and parasympathetic nervous systems. Prostate Int 6 : 55-60, 2018
21. Adachi N, Sakhri FZ, Ikemoto H, Ohashi Y, Kato M, Inoue T, Hisamitsu T, Sunagawa M : Kamikihito rescued depressive-like behaviors and hippocampus neurogenesis in chronic restraint stress rats. J Tradit Complement Med 12 : 172-179, 2022
22. Tsukada M, Ikemoto H, Lee XP, Takaki T, Tsuchiya N, Mizuno K, Inoue T, Tsunokawa Y, Okumo T, Matsuyama T, Sunagawa M : Kamikihito, a traditional Japanese Kampo medicine, increases the secretion of oxytocin in rats with acute stress. J Ethnopharmacol 276 : 114218, 2021
23. Maejima Y, Horita S, Yokota S, Ono T, Proks P, Yoshida-Komiya H, Ueta Y, Nishimori K, Misaka S, Shimomura K : Identification of oxytocin receptor activating chemical components from traditional Japanese medicines. J Food Drug Anal 29 : 653-675, 2021
24. Maruyama T, Shimizu M, Ikeda N, Baba K, Yoshimura M, Ueta Y : Expression of oxytocin in hypothalamus and reduction of nociceptive stress following administration of Kamikihi-to in female rats. Front Pharmacol 13 : 961135, 2022
25. Deblon N, Veyrat-Durebex C, Bourgoin L, Caillon A, Bussier AL, Petrosino S, Piscitelli F, Legros JJ, Geenen V, Foti M,

Wahli W, Marzo VD, Rohner-Jeanrenaud F : Mechanisms of the anti-obesity effects of oxytocin in diet-induced obese rats. PLoS ONE 6 : e25565, 2011
26. Gonzalez F : Inflammation in Polycystic Ovary Syndrome : Underpinning of Insulin Resistance and Ovarian Dysfunction. Steroids 77 : 300-305, 2012
27. Escobar-Morreale HF : Polycystic Ovary Syndrome : Definition, Aetiology, Diagnosis and Treatment. Nat Rev Endocrinol 14 : 270-284, 2018
28. Hu C, Pang B, Ma Z, Yi H : Immunophenotypic Profiles in Polycystic Ovary Syndrome. Mediat Inflamm 2020 : 1-10, 2020
29. Maejima Y, Rita RS, Santoso P, Aoyama M, Hiraoka Y, Nishimori K, Gantulga D, Shimomura K : Nasal oxytocin administration reduces food intake without affecting locomotor activity and glycemia with c-Fos induction in limited brain areas. Neuroendocrinology 101 : 35-44, 2015
30. Szeto A, Cecati M, Ahmed R, McCabe PM, Mendez AJ : Oxytocin reduces adipose tissue inflammation in obese mice. Lipids Health Dis 19 : 188, 2020
31. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM : Positional cloning of the mouse obese gene and its human homologue. Nature 372 : 425-432, 1994
32. Munzberg H, Morrison CD : Structure, production and signaling of leptin. Metabolism 64 : 13-23, 2015
33. Saladin R, De Vos P, Guerre-Millo M, Leturque A, Girard J, Staels B, Auwerx J : Transient increase in obese gene expression after food intake or insulin administration. Nature 377 : 527-529, 1995
34. Farr OM, Gavrieli A, Mantzoros CS : Leptin applications in 2015 : what have we learned about leptin and obesity? Curr Opin Endocrinol Diabetes Obes 22 : 353-359, 2015
35. Hakansson ML, Brown H, Ghilardi N, Skoda RC, Meister B : Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J Neurosci 18 : 559-572, 1998
36. Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JK, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, Mackellar W, Rosteck Jr PR, Schoner B, Smith D, Tinsley FC, Zhang X, Heiman M : The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 377 : 530-532, 1995
37. Li MD : Leptin and beyond : an odyssey to the central control of body weight. Yale J Biol Med 84 : 1-7, 2011
38. Diano S : New aspects of melanocortin signaling : a role for PRCP in α-MSH degradation. Front Neuroendocrinol 32 : 70-83, 2011