RESEARCH

Evaluation of safety and anti-obesity effects of DWP16001 in naturally obese dogs

Beomseok Rhee1,2, Rahman Md Mahbubur1, Changfan Jin1,2, Ji-Soo Choi3, Hyun-Woo Lim3, Wan Huh3, Joon Seok Park3, Jumi Han3, Sokho Kim1, Youngwon Lee2 and Jinho Park4*

Abstract

Background: The aim of this study was to investigate the anti-obesity effects of DWP16001, a sodium-glucose cotransporter-2 (SGLT2 inhibitor), in naturally obese dogs. A total of 20 dogs were divided into four equal groups: one obese control (OC group), and three treated groups; DWP0.2 group, DWP0.5 group, and DWP1 group. OC group fed with food for maintenance and treated groups were fed with food for maintenance with 0.2 mg/kg DWP16001, 0.5 mg/kg DWP16001 and 1 mg/kg DWP16001, respectively. The food for maintenance was provided to dogs as 2 RER (Resting energy requirement) in kcal and DWP16001-supplemented food was administered once a day for 8 weeks.

Results: Body condition score, body weight, and fat thickness were significantly reduced (P < 0.05) in the DWP0.2 group compared with the OC group, respectively without affecting the food consumption. At the 10th week the food consumption rate was 101.35 ± 2.56, 166.59 ± 4.72, 98.47 ± 1.44 and 123.15 ± 2.45% compared with initial food consumption rate. Body fat percentage, chest and waist circumference, blood glucose, and insulin were reduced compared to OC group but not significantly different from those of the OC group during experimental period. Serum alanine aminotransferase, alkaline phosphatase, creatine phosphokinase, and creatinine were significantly reduced in DWP0.2 group on 8 weeks. Serum cholesterol and triglycerides were reduced but not significantly. No specific adverse effects were observed throughout the experiment, and hematological parameters were unchanged. The results indicate that DWP16001 was not harmful to the dogs in our study and might have anti-obesity effects in naturally obese dogs.

Conclusions: The above results and discussion suggest that DWP16001 is safe and might have anti-obesity effects in naturally obese dogs.

Keywords: Dogs, DWP16001, Obesity, SGLT2 inhibitor

Background

Obesity contributes to the morbidity of many diseases, including diabetes, dyslipidemia, cardiovascular disease, neurological diseases, gall bladder disease, musculoskeletal disorders, urinary tract and reproductive disorders, and certain types of cancer [1, 2]. Obesity results from higher energy intake than expenditure, which can lead to dysfunction of adipocytes, triggering pathological disorders of multiple organs and systems [2]. Worldwide, obesity and overweight disorder are reported to affect 609 million and 1.9 billion adults, respectively, in each year, representing about 39% of the global population [3].

The large body of literature addressing obesity and its prevention and treatment indicates the importance of obesity in human medical science [3]. Last decade, one health which the collaborative efforts of multiple disciplines working locally, nationally, and globally for people, animals and environment is attracting attention [4].

*Correspondence: jpark@jbnu.ac.kr
4 Department of Veterinary Internal Medicine, College of Veterinary Medicine, Jeonbuk National University, Iksan, Republic of Korea
Full list of author information is available at the end of the article
Many studies and opinions of obesity in companion animal suggested relationship between human obesity and dog obesity [5, 6]. There are major risk factors for obesity, such as feeding habits, physical inactivity, and genetic are similar among humans and dogs [7, 8]. In addition, the lifestyles of companion animal are almost fully owner-dependent; consequently, the attitudes, behaviors, and habits of their owners, such as offering excessive foods and not allowing for optimum exercise, and life events such as sterilization, can contribute to owner-influenced canine obesity [9, 10]. The human-animal bond is a predominant factor in the association between obesity in people and their dogs.

The prevalence of overweight and obesity in dogs is similar to that of humans. The prevalence of canine overweight and obesity in Brazil is 40.5% [10], although similar study in USA reported that overweight and obesity range from 19.7–59.3% in companion dogs [11]. It has been demonstrated that obesity induces similar comorbidities in companion animals, such as metabolic abnormalities, endocrinopathies, orthopedic disorders, cardiorespiratory disease, urogenital disorders, and neoplasia [12]. In the Labrador Retriever, moderate obesity has been reported to reduce life expectancy by almost two years [13]. Therefore, prevention of obesity in canine species should be taken seriously.

The sodium-glucose cotransporter-2 (SGLT2) inhibitor DWP16001 is a new drug for treatment of type 2 diabetes, developed by Daewoong Pharmaceutical Co., Ltd (Seoul, Republic of Korea), and is currently undergoing a Phase 3 clinical trial in Korea (Registration No. NCT04632862) after getting promising outcome at Phase 2 clinical trial (Registration No. NCT04014023) [14]. In the present study, the food of naturally obese dogs was supplemented with DWP16001 to evaluate the anti-obesity effects by assessing body condition score (BCS), chest and waist circumference, hematological parameters, and serum and urinary biochemical profiles.

**Results**

**Effects of DWP16001 on BCS, body weight, body fat percentage, fat thickness, chest and waist circumference**

The BCS of the DWP 0.2 group was significantly lower ($p < 0.05$) than that in the OC group at 4, 8, and 10 weeks after the start of administration. A trend toward reduction of BCS was observed in the DWP 0.5 group over the entire experimental period. In contrast, the BCS of the DWP 1 group was slightly increased but lower than that in the OC group by the end of the experiment (Fig. 1A). The BW of the DWP 0.2 group was significantly lower ($p < 0.05$) than that of the OC group at 4 weeks after the start of administration until the end of the trial, and the change of BW was lowest among all groups. Similar reductions of BW occurred in the DWP 0.5 group: BW was reduced at 2 and 4 weeks and then slowly increased (Fig. 1B). There was no significant difference in body fat percentage among all the groups, but it was lowest in the DWP 0.2 group (Fig. 1C). Reduced fat thickness was measured in all treated groups, but this change was significant only at 8 and 10 weeks in the DWP 0.2 group (Fig. 1D). At the point of 8 weeks, the chest and waist circumference of all three treated groups were reduced compared to initial, but the reductions were not significant at any time point (Table 1).

**Rate of change in food consumption and resting energy requirement**

At the 10th week the food consumption rate was 101.35 $\pm$ 2.56, 166.59 $\pm$ 4.72, 98.47 $\pm$ 1.44 and 123.15 $\pm$ 2.45% compared with initial food consumption rate. Interestingly, food consumption increased significantly ($p < 0.001$) in the DWP 0.2 and DWP 1 groups compared with the OC group but decreased in the DWP 0.5 group over the 10-week test period (Table 2). Analysis of resting energy requirement (RER, i.e., base level of calories required) at 4 and 8 weeks after the start of administration of DWP16001 showed that the RER of the DWP 0.2 group was significantly lower than that in the OC group ($p < 0.05$; Table 2).

**Effects of DWP16001 on hematological and serum biochemical profiles**

There were no significant differences in hematological findings among the treatment groups over the 10-week test period, except for PLT. The PLT count of the DWP 1 group was significantly lower than that in the OC group ($p < 0.05$; Table 3). Serum TC and TG concentrations in the DWP 0.2 group were lower than those in the OC group. Fasting glucose and serum insulin concentrations of all treated groups were reduced compared with those in the OC group, but only GLU in the DWP 1 group and insulin concentration in the DWP 0.5 group were significantly lower than those in the OC group. Serum TG concentration of treated groups (DWP 0.2, 142.00 $\pm$ 95.55 mg/dL; DWP 0.5, 133.86 $\pm$ 70.19 mg/dL; DWP 1, 282.60 $\pm$ 419.03 mg/dL) was lower than that in the OC group (361.60 $\pm$ 622.34 mg/dL). Furthermore, serum GGT, ALT, ALP, CPK, and CRP concentrations declined significantly in the DWP 0.2 group compared with the OC group. Urinary GLU excretion was greater in treated groups, especially in the DWP 0.5 and DWP 1 group (Table 3). Indeed, the glucose excretion level was increased in time dependent after the start of the
test substance administration in DWP16001 treated group until 8 weeks (data not shown).

**Adverse reactions**

During Week 2 of the test, one dog in the DWP0.2 group developed hematuria, and one dog in the DWP1 group developed conjunctivitis with dry eye, both of which improved without treatment. Compared with other agents, adverse effects of DWP16001 were not observed including diarrhea, flatulence, bloating, abdominal pain, and dyspepsia.

**Discussion**

Treatment of type 2 diabetes and obesity with SGLT2 inhibitors has shown promise, as these drugs reduce hyperglycemia and body weight gain in mice [15]. Human clinical trials also proved that SGLT2 inhibitors reduce body weight about 1.5–2 kg compared to placebo group [16]. SGLT2 is important in renal glucose reabsorption; selective blockade of SGLT2 regulates blood glucose to appropriate levels by promoting urinary excretion of glucose. SGLT2 inhibitors act in an insulin-independent manner and are not associated with pancreatic b-cell
Change rates of waist circumferences (%)

| Weeks | OC     | DWP0.2 | DWP0.5 | DWP1  |
|-------|--------|--------|--------|-------|
| 0     | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| 2     | 99.39 ± 3.13  | 99.20 ± 3.44  | 98.19 ± 4.31  | 94.79 ± 6.67  |
| 4     | 99.64 ± 3.44  | 97.16 ± 2.74  | 97.16 ± 3.23  | 99.81 ± 2.43  |
| 6     | 96.92 ± 5.74  | 95.08 ± 3.96  | 95.60 ± 4.55  | 100.54 ± 4.98 |
| 8     | 96.99 ± 4.44  | 96.08 ± 2.60  | 93.90 ± 3.11  | 99.20 ± 4.49  |
| 10    | 99.52 ± 6.24  | 99.18 ± 3.01  | 96.43 ± 2.76  | 100.24 ± 5.15 |

Change rates of waist circumferences (%)

| Weeks | OC     | DWP0.2 | DWP0.5 | DWP1  |
|-------|--------|--------|--------|-------|
| 0     | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| 2     | 101.93 ± 8.89 | 100.53 ± 4.71 | 99.57 ± 7.11  | 95.97 ± 11.67 |
| 4     | 104.05 ± 6.64 | 97.39 ± 12.15 | 98.45 ± 4.94  | 99.18 ± 8.09  |
| 6     | 96.08 ± 7.08  | 98.96 ± 15.47 | 96.30 ± 3.83  | 103.75 ± 10.54|
| 8     | 102.61 ± 6.40 | 98.89 ± 11.98 | 93.84 ± 5.54  | 99.76 ± 12.53 |
| 10    | 103.54 ± 2.93 | 101.98 ± 10.78| 96.00 ± 4.72  | 101.33 ± 14.44|

Data are expressed as Mean ± S.D.

OC Obese normal control, DWP0.2 DWP16001 0.2 mg/kg/day, DWP0.5 DWP16001 0.5 mg/kg/day, DWP1 DWP16001 1 mg/kg/day

* A significant difference at p < 0.05 level compared to the OC

Table 2 Change rates of food consumption and resting energy requirement (%)

| Weeks | OC     | DWP0.2 | DWP0.5 | DWP1  |
|-------|--------|--------|--------|-------|
| 0     | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| 2     | 102.10 ± 1.31 | 126.52 ± 5.84*** | 95.13 ± 1.67*** | 100.80 ± 4.20 |
| 4     | 101.38 ± 1.25 | 146.64 ± 5.37*** | 92.34 ± 2.10*** | 116.02 ± 3.09***|
| 6     | 99.75 ± 1.19  | 153.71 ± 4.11*** | 92.30 ± 1.60*** | 121.84 ± 2.50***|
| 8     | 98.74 ± 1.41  | 168.32 ± 4.80*** | 93.64 ± 1.67*  | 121.88 ± 2.36***|
| 10    | 101.35 ± 2.56 | 166.59 ± 4.72*** | 98.47 ± 1.44  | 123.15 ± 2.45***|

Change rates of resting energy requirement

| Weeks | OC     | DWP0.2 | DWP0.5 | DWP1  |
|-------|--------|--------|--------|-------|
| 0     | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| 2     | 99.51 ± 1.39  | 96.98 ± 2.69  | 98.63 ± 0.72  | 97.75 ± 2.32  |
| 4     | 99.48 ± 1.10  | 93.41 ± 5.79  | 97.93 ± 1.00  | 98.11 ± 2.18  |
| 6     | 98.74 ± 1.32  | 95.21 ± 4.10  | 97.54 ± 2.73  | 98.95 ± 2.07  |
| 8     | 99.04 ± 1.45  | 94.64 ± 3.59*  | 96.92 ± 2.00  | 99.19 ± 4.05  |
| 10    | 99.12 ± 1.77  | 96.15 ± 4.75  | 98.79 ± 2.30  | 99.97 ± 6.15  |

Data are expressed as Mean ± S.D.

OC Obese normal control, DWP0.2 DWP16001 0.2 mg/kg/day, DWP0.2 DWP16001 0.5 mg/kg/day, DWP1 DWP16001 1 mg/kg/day

* A significant difference at p < 0.05 level compared to the OC

** A significant difference at p<0.01 level compared to the OC

*** A significant difference at p<0.001 level compared to the OC

Table 1 Change rates of chest and waist circumferences (%)

Function. Therefore, SGLT2 inhibitors are attracting attention as alternative or combination therapies for type 2 diabetes. Several SGLT2 inhibitors, including empagliflozin, dapagliflozin, canagliflozin, and ipragliflozin, have been approved for type 2 diabetes [15]. Recently, the SGLT2 inhibitor DWP16001 was shown to be distributed to the kidney (target organ) and to exert more selective and sustained SGLT2 inhibition compared to other SGLT-2 inhibitors such as ipragliflozin and dapagliflozin [17]. In this study, we investigated the anti-obesity effects when the dogs are fed food for maintenance plus supplemented DWP16001 and safety of DWP16001 in naturally obese dogs. The results of the study demonstrated that DWP16001 reduces BCS, body weight, body fat percentage, fat thickness, and chest and waist circumference without affecting food consumption. The most effective results were found when food was supplemented with 0.2 mg/kg DWP16001.

The DWP16001-treated groups in our study had reduced blood glucose concentration and increased urinary glucose excretion. These results are consistent with those of many previous clinical studies that have reported that orally administered SGLT2 inhibitors reduce body weight and improve hyperglycemia and T2D through induction of urinary glucose excretion [18–20]. Furthermore, SGLT2 inhibitors have been shown to induce reductions in body weight and fat mass in mouse models fed a high-salt and high-fat diet [15, 21]. In obese subjects, elevated serum TC, TG, AST, ALT, CPK, BUN, and CRE have been reported [15, 22] all of which were showed reduction in DWP16001 treated group, although it cannot be definitively concluded due to the difference in the type of food fed, it is thought that the DWP16001 has had some influence. Increasing TC and TG concentrations are indicators of hyperlipidemia [2, 22] which were increased in OC group and lowered by DWP16001 treatment indicating anti-lipidemic effect [23]. Elevated BUN and CRE concentrations are seen in kidney disorders, and increased AST, ALT, and CPK concentrations are indicators of fatty liver which were increased in OC group and lowered by DWP16001. Our findings suggest DWP16001 treatment might have effects on obesity-induced pathological disorders.

Indeed, BCS assessment is widely used to identify obesity in pet animals, with a nine-stage scoring system typically being recommended. Under the nine-stage scoring, dogs with a BCS of 6 or 7 are considered overweight, whereas those with a BCS of 8 or 9 are obese [10, 24]. This study comprised dogs with BCSs of 7 or more, and the results indicated that DWP16001 treatment significantly reduced the BCS after four weeks of administration in the DWP 0.2 group. Similarly, body weight, body fat percentage, fat thickness, and chest and waist circumference were reduced. The reductions of body fat percentage and chest and waist circumference might have been secondary to reduction of fat thickness in this study. Moreover, the decrease in glucose (and hence calories available) induced by DWP16001 might have induced increased lipid mobilization, resulting in reduction of fat mass and body weight [15]. A common side effect of
medication-related weight loss is long-term loss of appetite [25, 26]. Interestingly, food consumption in the DWP 0.2 and DWP1 groups increased significantly despite reductions in BCS and BW but was reduced in the DWP 0.5 group. These findings indicate that the appropriate dose of DWP16001 could reduce body weight gain and obesity without altering feeding behavior. Urinary glucose excretion results in physiological compensatory sugar intake [27]. As a result of this test, no effect on consistent feed intake was observed at all doses, but an increase in feed intake was observed in the DWP1 group might be for compensatory glucose intake whose weight loss effect was confirmed. Based on this result, excellent weight loss effect despite an increase in feed intake in the DWP0.2 group was observed. Likewise, it has been reported that empagliflozin, another SGLT2 inhibitor, increased food intake significantly despite reductions in body weight gain in mice [15, 27].

Thrombocytopenia or platelet dysfunction results in skin and mucosal bleeding, which are associated with hematuria [28]. In the present study, we also analyzed hematuria and thrombocytopenia for the experimental dogs. Platelet count was significantly reduced in the DWP1 group compared with the OC group, and hematuria was found in one dog in the DWP0.2 group, which recovered without treatment. Therefore, hematuria and thrombocytopenia do not appear to be correlated in this study. In the DWP1 group, one dog developed conjunctivitis with dry eye, which improved without treatment. Dry eye is strongly depended on environmental temperature, air flow and moisture contents [29]. The dogs here owned by owner was not possible to provide optimum environmental factors. The owner was suggested to change the environmental condition but it was not confirmed that this factor induced dry eye. Additionally, dry eye is associated with hyperglycemia and hyperlipidemia [30], but these conditions, where present in the study dogs, were not severe. Although SGLT2 inhibitors can induce dry skin, dermatitis, and subcutaneous tissue including rash, eruption, urticaria, erythema, and eczema [31], there have been no reports of dry eye induction. Continuous consumption of anti-obesity drugs can induce other adverse effects, including diarrhea, bloating, abdominal pain, and dyspepsia [26]. While such symptoms were not observed in our study, additional experimental studies are necessary to establish the long-term efficacy and safety of DWP16001.

Table 3 Effects of DWP16001 on blood, serum and urinary biochemistry of obese dogs in 8 weeks

|                | OC               | DWP0.2          | DWP0.5          | DWP1             |
|----------------|------------------|-----------------|-----------------|------------------|
| WBC (x 10^9/μL)| 7.93 ± 2.83      | 11.12 ± 3.69    | 7.75 ± 2.04     | 10.01 ± 3.35     |
| RBC x 10^12/μL| 6.90 ± 0.99      | 6.92 ± 0.79     | 72.22 ± 0.32    | 65.5 ± 1.08      |
| Hb (g/dL)      | 16.36 ± 1.98     | 16.34 ± 2.68    | 16.40 ± 0.70    | 16.16 ± 2.15     |
| Hct (%)        | 47.94 ± 7.61     | 49.86 ± 7.08    | 50.26 ± 1.94    | 46.76 ± 6.13     |
| Platelet (x 10^9/μL)| 422.60 ± 12.98 | 392.20 ± 11.16  | 307.40 ± 6.02   | 299.10 ± 7.65*   |
| Glu (mg/dL)    | 113.80 ± 10.85   | 107.20 ± 10.85  | 97.77 ± 10.19   | 94.00 ± 13.47    |
| Insulin (ng/mL)| 133.27 ± 16.43   | 126.60 ± 15.45  | 99.82 ± 18.48*  | 110.75 ± 2.91    |
| TC (mg/dL)     | 236.40 ± 69.21   | 183.84 ± 36.01  | 298.00 ± 71.14  | 309.00 ± 129.40  |
| TG (mg/dL)     | 361.60 ± 622.34  | 142.00 ± 95.55  | 133.86 ± 70.19  | 282.60 ± 419.03  |
| TP (mg/dL)     | 6.48 ± 0.66      | 6.78 ± 0.66     | 6.43 ± 0.32     | 6.14 ± 0.54      |
| ALB (g/dL)     | 3.04 ± 0.30      | 3.48 ± 0.42     | 2.95 ± 0.19     | 2.92 ± 0.29      |
| GGT (IU/L)     | 13.20 ± 14.55    | 7.80 ± 1.64     | 9.46 ± 5.37     | 21.60 ± 29.36    |
| ALT (IU/L)     | 50.60 ± 29.33    | 18.84 ± 4.00*   | 36.80 ± 14.43*  | 38.60 ± 19.72    |
| AST (IU/L)     | 44.80 ± 16.21    | 31.00 ± 1.83    | 31.84 ± 12.92   | 110.00 ± 167.92  |
| ALP (IU/L)     | 211.60 ± 199.74  | 84.60 ± 63.96*  | 146.20 ± 121.67 | 186.20 ± 184.59  |
| CPK (IU/L)     | 129.40 ± 35.82   | 111.12 ± 30.97* | 136.50 ± 42.99  | 260.40 ± 126.79  |
| BUN (mg/dL)    | 15.12 ± 6.30     | 18.70 ± 10.29   | 22.25 ± 8.64    | 23.44 ± 13.17    |
| CRE (mg/dL)    | 1.20 ± 0.31      | 0.78 ± 0.21*    | 0.82 ± 0.15     | 0.92 ± 0.36      |
| Urinary GLU (grade) | 0             | 2.6 ± 0.12     | 4               | 4                |

OC Obese control group, DWP0.2 group DWP16001 0.2 mg/kg/day, DWP16001 0.5 mg/kg/day, DWP1 group DWP16001 1 mg/kg/day
Data are expressed as Mean ± S.D
* A significant difference at \( p < 0.05 \), post hoc test following one-way ANOVA versus OC

RBC Red blood cell, Hct Hematocrit, Hb Hemoglobin, WBC White blood cell, PLT Platelets, AST Aspartate aminotransferase, ALT Alanine aminotransferase, ALP Alkaline phosphatase, CPK Creatine phosphokinase, TP Total protein, GLU Glucose, TC Total cholesterol, TG Triglyceride, ALB Albumin, GGT Gamma-glutamyl transferase, BUN Blood urea nitrogen and CRE Creatinine
Conclusions

The above results and discussion suggest that DWP16001 is safe and might have anti-obesity effects in naturally obese dogs. Additionally, the DWP16001 0.2 and 0.5 mg/kg/day groups showed a trend toward reduced body weight, body condition score, basic calorie requirement, chest and waist circumference, body fat percentage, and fat thickness, although it cannot be definitively concluded due to the difference in the type of food fed. In this study, DWP16001 was administered to naturally obese dogs in clinical practice. Thus, the owner and veterinarian evaluating each dog differed, the dogs were of different breeds and ages, and the number of individuals in each group was small. The results are considered to be the effect of administration of the DWP16001; however, further studies are needed to confirm the effects, as we did not find clear dose dependence of effects in our study population.

Materials and methods

Animal selection, grouping, and monitoring

Naturally obese but otherwise healthy adult dogs, regardless of breed, sex, and altering a dog’s reproductive status, were selected for this clinical trial. The dog owners provided informed consent for their pet to participate in the clinical trial from veterinary hospital. All dogs were fed with their ordinary food from owner and report food type, consumption quantity to veterinary hospital. All dogs were diagnosed with obesity according the findings of a physical examination, including body weight and body conditions score (BCS) of 7 or more, 9 stages in total [32]. Only one dog was analyzed per household to ensure individual feeding. The selected dogs were generally healthy without underlying disease, such as abnormalities of the liver, heart, and/or kidneys. Dogs used for breeding purposes or treated with long-acting steroids, drugs that affect endocrine conditions (such as lipid improvement, cholesterol inhibitors, diabetes drugs), or drugs that affect weight or energy consumption (such as phenobarbital) within 30 days prior to commencement of our trial were excluded. A total of 20 dogs were divided in four groups of 5 animals each randomly: one obese control (OC group), and three treated groups; DWP0.2 group, DWP0.5 group, and DWP1 group. OC group was fed with food for maintenance and three treated groups were fed with food for maintenance plus supplemented with 0.2 mg/kg DWP16001, 0.5 mg/kg DWP16001 and 1 mg/kg DWP16001 in the collagen capsule, respectively. The DWP16001 in the collagen capsule was administered once daily, and the food was also administered once daily for eight weeks. The breeds included in this study were Shetland Sheepdog (5/20), Maltese (3/20), Pompitz (2/20), Bichon Frise (1/20), Border Collie (1/20), Cocker Spaniel (1/20), Japanese Spitz (1/20), Labrador Retriever (1/20), Poodle (1/20), Shih Tzu (1/20), Wire Fox Terrier (1/20), Yorkshire Terrier (1/20), and mixed (1/20). In this study, 10/20 dogs were male (10/10 were neutered), and 10/20 dogs were female (7/10 were spayed). The age range was 3 to 11 years, and the average age of the dogs was 7.15 years. Nine of the 20 dogs were aged ≤ 6 years, while eight were 7–9 years of age, and three were ≥ 10 years of age. These dogs were divided in four groups of 5 animals each randomly for the experiments. The owners were asked to closely monitor their animals and requested to contact the investigators immediately if any abnormalities were found. In addition, each dog was examined by a veterinarian once weekly until the end of the trial.

All animal experiments were conducted in compliance with Animal Experimental Ethics Regulations noticed by the IACUC (Institutional Animal Care and Use Committee) in KNOTUS Co., Ltd. And this was approved by the IACUC in KNOTUS Co., Ltd. (Approval number: KNOTUS IACUC 20-KC-003). We complied with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

Measurement of body weight, chest and waist circumference, and resting energy requirement

Body weight (BW) and chest and waist circumference were measured just before administration of the first meal of DWP16001-supplemented food and once every two weeks thereafter for 10 weeks. The chest and waist circumference were measured at the thickest part of the chest and thinnest part between the chest and the hind legs, respectively, and the measurement was repeated four times to obtain a mean circumference. The resting energy requirement (RER) was calculated using the following equation from previous study [33, 34].

\[
\text{RER in kcal/day} = 70 \times (\text{initial body weights})^{0.75}
\]

Measurement of fat thickness

Fat thickness was assessed by measuring the right angle distance from the T10 spinal process to the skin using radiographs (Titan 2000 X-ray system, COMED Medical Systems Co., Ltd., Seoul, Korea) obtained on the first day of DWP16001 administration and at 4, 8, and 10 weeks thereafter.

Hematology and serum and urine biochemical tests

Hematological testing was performed on the day before the first treatment and then once every two weeks thereafter for 10 weeks. Part of the blood collected on the test day was stored in a CBC tube containing EDTA-2 K, an
anticoagulant, and the red blood cell (RBC) count, hematocrit (Hct), hemoglobin concentration (Hb), white blood cell (WBC) count, and platelet count (PLT) were assessed using an automatic blood analyzer (ADVIA 2120i™, Siemens Healthcare Diagnostics, Vienna, Austria), as described previously [35]. For biochemical analysis, part of the blood collected on the test day was transferred to a vacutainer tube containing a clot activator, left at room temperature for 15–20 min to solidify, and then centrifuged at 3000 rpm to obtain serum. Serum biochemical parameters were assessed using a Hitachi 7180 instrument (Hitachi, Tokyo, Japan) together with the scheduled hematological test [35]. The biochemical parameters assessed were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine phosphokinase (CPK), total bilirubin (TBIL), glucose (GLU), total cholesterol (TC), triglycerides (TG), total protein (TP), albumin (ALB), gamma-glutamyl transpeptidase (GGT), blood urea nitrogen (BUN), creatinine (CRE), inorganic phosphorus (IP), calcium (Ca), and C-reactive protein (CRP). Serum insulin concentration was measured at 2, 4, 6, 8, and 10 weeks with a Quantikine Canine Insulin ELISA Kit (Catalog Number: DINS00, R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s protocol. Urine biochemical parameters were assessed using a urine test strip at 2, 4, 6, and 10 weeks after the start of administration of the DWP16001, while urinary glucose (GLU) excretion was measured using a URiSCAN® Strip (YD Diagnostics CORP., Yongin-Si, Republic of Korea). Strip guided grade (0 – 4) revealed like as 0: Negative, 1: NA, 2: ≥ 250, 3: ≥ 500, 4: ≥ 1,000.

Statistical analysis
Prism 5.03 software (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analysis of the data. The result value compared with 100% was normalized based on the value before treatment with the test substance. Results are expressed as mean ± S.D. Data normality was assumed for the results of this test, and significance was tested between test groups using parametric one-way ANOVA. If significance was recognized, Dunnett’s multiple comparison test was used to post-test was carried out. The level of significance was set at p < 0.05.

Acknowledgements
The authors gratefully acknowledge the veterinarians for their contribution of cases. We thank all dog owners for their assistance and willingness to take part in this study.

Authors’ contributions
Beomseok designed the clinical trial, assisted in case management, data analysis, and editing the manuscript. Jinho, Sokho wrote the majority of the manuscript. RAHMAN, Changfan assisted in manuscript editing. Ji-Soo, Hyun-Woo, Wan, Joon Seok, Jumi and Youngwon entered patients into the clinical trial and assisted in case management. All authors read and approved the final manuscript.

Authors’ information
Not applicable.

Funding
There is no external funding for this study.

Availability of data and materials
The datasets supporting the conclusions of this article are included within the article.

Declarations

Ethics approval and consent to participate
This study was conducted in compliance with the Clinical trial management guidelines for veterinary drugs, etc. (No. 2015–29) noticed by the Animal and Plant Quarantine Agency in Republic of Korea. Also all animal experiments were conducted in compliance with Animal Experimental Ethics Regulations noticed by the IACUC (Institutional Animal Care and Use Committee) in KNOTUS Co., Ltd. And this was approved by the IACUC in KNOTUS Co., Ltd. (Approval number: KNOTUS IACUC 20-KC-003). We complied with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The dog owners provided informed consent for their pet to participate in the clinical trial from veterinary hospital. All activities described in the present study that involved animals were performed by a veterinarian as part of routine clinical veterinary procedures and with the written consent of the animal’s owner.

Consent for publication
Consent from the owners was obtained.

Competing interests
The authors declare that they have no competing interests.

Author details
1KNOTUS Co., Ltd., Research Center, Incheon, Republic of Korea. 2Department of Veterinary Medical Imaging, College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea. 3Daewoong Pharmaceutical Co., Ltd., Yongin, Republic of Korea. 4Department of Veterinary Internal Medicine, College of Veterinary Medicine, Jeonbuk National University, Iksan, Republic of Korea.

Received: 30 August 2021 Accepted: 26 May 2022
Published online: 22 June 2022

References
1. Früh SM. Obesity. J Am Assoc Nurse Pract. 2017;29:S3–14.
2. Rahaman MM, Kim MJ, Kim JH, Kim SH, Go HK, Kweon MH, Kim DH. Desalted Salicornia europaea powder and its active constituent, trans-ferulic acid, exert anti-obesity effects by suppressing adipogenic-related factors. Pharm Biol. 2018;56:183–91.
3. Chooi YC, Ding C, Magkios F. The epidemiology of obesity. Metab Clin Exp. 2019;92:6–10.
4. Robinette C, Saffran L, Ruple A, Deem SL. Zoos and public health: a partnership on the One Health frontier. One Health. 2017;3:1–4.
5. Bland IM, Guthrie-Jones A, Taylor RD, Hill J. Dog obesity: veterinary practices’ and owners’ opinions on cause and management. Prev Vet Med. 2010;94:310–5.
6. Kipperman B, German A. The responsibility of veterinarians to address companion animal obesity. Animals. 2018;8:143.
7. German AJ, Ryan VH, German AC, Wood IS, Trayhurn P. Obesity, its associated disorders and the role of inflammatory adipokines in companion animals. Vet J. 2010;185:4–9.
8. McMillan FD. Stress-induced and emotional eating in animals: a review of the experimental evidence and implications for companion animal obesity. J Vet Behav. 2018;3:83–85.

9.onsdy Juliana T, Kata V, Vanda Katalin J, Peter P. Factors affecting canine obesity seem to be independent of the economic status of the country—a survey on Hungarian companion dogs. Animals. 2020;10:1267.

10. Porsani MYH, Teixeira FA, Oliveira VV, Pedrinelli V, Dias RA, German AJ, Brunetto MA. Prevalence of canine obesity in the city of Sao Paulo, Brazil. Sci Rep. 2020;10:14082.

11. Chandler M, Cunningham S, Lund EM, Khanna C, Naramore R, Patel A, Day MJ. Obesity and associated comorbidities in people and companion animals: a one health perspective. J Comp Pathol. 2017;156:296–309.

12. German AJ. The growing problem of obesity in dogs and cats. J Nutr. 2006;136:1940S-1946S.

13. Kealy RD, Lawler DF, Ballam JM, Mantz SL, Biery DN, Greeley EH, Lust G, Segre M, Smith GK, Stowe HD. Effects of diet restriction on life span and age-related changes in dogs. J Am Vet Med Assoc. 2002;220:1315–20.

14. Kim JH, Kim DK, Choi WG, Ji HY, Choi JS, Song IS, Lee S, Lee HS. In Vitro Metabolism of DWP16001, a Novel Sodium-Glucose Cotransporter 2 Inhibitor, in Human and Animal Hepatocytes, Pharmacuetics. 2020;12:865.

15. Xu L, Nagata N, Nagashimada M, Zhuge F, Ni Y, Chen G, Mayoux E, Kaneko S, Ota T. SGLT2 inhibition by empagliflozin promotes fat utilization and browning and attenuates inflammation and insulin resistance by polarizing M2 macrophages in diet-induced obese mice. EBioMedicine. 2017;20:137–49.

16. Pereira MJ, Eriksson JW. Emerging role of SGLT-2 inhibitors for the treatment of obesity. Drugs. 2019;79:219–30.

17. Choi MK, Nam SJ, Ji HY, Park MJ, Choi JS, Song IS. Comparative pharmacokinetics and pharmacodynamics of a novel sodium-glucose cotransporter 2 inhibitor, DWP16001, with dapagliflozin and ipragliflozin, Pharmacuetics. 2020;12:268.

18. Ferrarini E, Seman L, Seewaldt-Becker E, Hantel S, Pinnetti S, Woerle HJ. A Phase IIb, randomized, placebo-controlled study of the SGLT2 inhibitor empagliflozin in patients with type 2 diabetes. Diabetes Obes Metab. 2013;15:721–8.

19. Polidori D, Mari A, Ferrarini E. Canagliflozin, a sodium glucose co-transporter 2 inhibitor, improves model-based indices of beta cell function in patients with type 2 diabetes. Diabetes. 2014;79:891–901.

20. Rosenwasser RE, Rosenwasser JN, Sutton D, Choksi R, Epstein B. Tofogliflozin: a highly selective SGLT2 inhibitor for the treatment of type 2 diabetes. Drugs of today. 2014;50:739–45.

21. Devenny JJ, Godonis HE, Harvey SJ, Rooney S, Gallo MJ, Pellemounter MA. Weight loss induced by chronic dapagliflozin treatment is attenuated by compensatory hyperphagia in diet-induced obese (DIO) rats. Obesity. 2012;20:1645–52.

22. Rahman MM, Kwon HS, Kim MJ, Go HK, Oak MH, Kim DH. Melatonin supplementation plus exercise behavior ameliorate insulin resistance, hypertension and fatigue in a rat model of type 2 diabetes mellitus. Biomed Pharmacother. 2017;92:606–14.

23. Szekeres Z, Toth K, Szabados E. The effects of SGLT2 inhibitors on lipid metabolism. Metabolites. 2021;11:87.

24. Laflamme DP, Kuhlman G, Lawler DF. Evaluation of weight loss protocols for dogs. J Am Anim Hosp Assoc. 1997;33:1–11.

25. Derosa G, Maffioli P. Anti-obesity drugs: a review about their effects and future. Dis Model Mech. 2012;5:621–6.

26. Tantawy AA, Elsherif NHK, Kenny MA, Aboulfotouh KA, Hassan AE, Kabil ME. Silent bleeding in children and adolescents with immune thrombocytopenia: relation to laboratory parameters and health related quality of life. J Thromb Thrombolysis. 2020;50:258–66.

27. Van Setten G, Labelotuelle M, Baudouin C, Rolando M. Evidence of seasonality and effects of psychometry in dry eye disease. Acta Ophthalmol. 2016;94:499–506.

28. Tang YL, Cheng YL, Ren YP, Yu XN, Shentu XC. Metabolic syndrome risk factors and dry eye syndrome: a meta-analysis. Int J Ophthalmol. 2016;9:1038–45.

29. Sakaeda T, Kobuchi S, Yoshioka R, Haruna M, Takahata N, Ito Y, Sugano A, Fukuwaka K, Hayase T, Hayakawa T, Nakayama H, Takaoka Y, Tsuchin M. Susceptibility to serious skin and subcutaneous tissue disorders and skin tissue distribution of sodium-dependent glucose co-transporter type 2 (SGLT2) inhibitors. Int J Med Sci. 2018;15:937–43.

30. Chun JL, Bang HT, Ji SY, Jeong JY, Kim M, Kim B, Lee YK, Reddy KE, Kim KH. A simple method to evaluate body condition score to maintain the optimal body weight in dogs. J Anim Sci Technol. 2019;61:366–70.

31. Brooks D, Churchill J, Fein K, Linder D, Michel KE, Tudor K, Ward E, Witzel A. 2014 AHA weight management guidelines for dogs and cats. J Am Anim Hosp Assoc. 2014;50:1–11.

32. Kleiber M. The fire of life: an introduction to animal energetics. John Wiley and Sons Inc. 1961;22474.

33. Choi GC, Rahman MM, Kim H, Kim S, Jeong IS. Management of sternal dislocation with and without surgery in cats: owner-assessed long-term follow-up of two clinical cases. J Vet Med Sci. 2018;80:1001–6.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.