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Yokukansankachimpihange increased body weight but not food-incentive motivation in wild-type mice

Takuya Hamaguchi[1], Iku Tsutsui-Kimura[2], Kenji F. Tanaka[2] and Masaru Mimura[1,2]

[1]*Center for Kampo Medicine, Keio University School of Medicine, Tokyo, Japan*
[2]*Department of Neuropsychiatry, Keio University School of Medicine, Tokyo, Japan*

ABSTRACT

Yokukansankachimpihange (YKSCH), a traditional Japanese medicine, is widely used for the amelioration of the behavioral and psychological symptoms of dementia with digestive dysfunction. Regardless of its successful use for digestive dysfunction, the effect of YKSCH on body weight was unknown. Furthermore, if YKSCH increased body weight, it might increase motivation according to Kampo medicine theory. Therefore, we investigated whether YKSCH had the potential to increase body weight and enhance motivation in mice. To address this, C57BL/6J mice were used to evaluate the long-term effect of YKSCH on body weight and food-incentive motivation. As part of the evaluation, we optimized an operant test for use over the long-term. We found that feeding mice YKSCH-containing chow increased body weight, but did not increase their motivation to food reward. We propose that YKSCH may be a good treatment option for preventing decrease in body weight in patients with dementia.

Keywords: Kampo; long-term; operant test; break point; progressive ratio

INTRODUCTION

Yokukansankachimpihange (YKSCH), a Kampo medicine (traditional Japanese medicine), is composed of the nine basic ingredients found in *Yokukansan* (YKS), plus two additional ingredients, *Pericarpium* of *Citrus unshiu* Markovich or *C. reticulata* Blanco (*Chimpi*) and *Tuber* of *Pinellia ternata* Breitenbach (*Hange*). YKS is used for the amelioration of the behavioral and psychological symptoms of dementia (BPSD), particularly for delusions, hallucinations, and agitation or aggression in patients with dementia.[1)]

Although YKSCH is similar to YKS, it is more commonly prescribed for patients whose symptoms include digestive function deficiencies.[2)] Loss of appetite is common in patients with Alzheimer's disease (AD), with an overall prevalence of 34%,[3)] and it causes weight loss over the long term. Interestingly, some studies reported that weight loss might occur before dementia sets in[4,5)] and one review argued the importance of minimizing weight loss.[6)] Although YKSCH has been prescribed to patients with dementia and digestive function deficiencies, it is unclear

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Corresponding author: Takuya Hamaguchi, M.D.

Center for Kampo Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Phone: +81-3-5366-3824, fax: +81-3-5366-3825, e-mail: takuya_hamaguchi@keio.jp

whether YKSCH helps increase body weight. This issue is difficult to evaluate in clinical research. Therefore, we addressed whether YKSCH had the potential to increase body weight in animals.

In Kampo medicine, there are traditional medicine pattern diagnoses, one of which is called a *deficiency pattern*. The *deficiency pattern* indicates that the patient's repairing ability against his/her disease condition is weak or hollow[7] and that the patient lacks stamina and is in a state of depressed physiological function.[8] The *deficiency pattern* includes deficiencies of *yin*, *yang*, *qi*, and *blood*. The *qi deficiency pattern* is characterized by decreased vitality, listlessness, and loss of appetite[2,9] and these symptoms seem to be relevant to a lack of motivation in the prolonged condition of patients with AD. Interestingly, it was reported that *the deficiency pattern* was related to a lower body mass index.[10] From the point of view of Kampo medicine approach, we hypothesized that if YKSCH increased body weight, it might coincide with increased motivation.

To evaluate whether YKSCH increased motivation, we utilized a model organism, a mouse, and used an operant test by which food-incentive motivation was evaluated. The operant test combined with medication is usually conducted for several days or a few weeks in mice. However, a longer-term application of Kampo medicine is empirically required to address the effect in general. Because we planned to conduct the operant test over 20 weeks, we needed to simplify the conventional operant test method and proposed a "long-term operant test method."

The purposes of this study were: 1) to determine whether YKSCH had the potential to increase body weight, 2) to propose a "long-term operant test method," and 3) to address whether YKSCH had the potential to increase motivation in mice.

METHODS

Ethical statement

All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Research Committee of Keio University School of Medicine.

Animals

C57BL/6J male mice were purchased at 7 weeks of age (Oriental Yeast Co., ltd., Tokyo, Japan). Mice were maintained at 24°C with a 12-h light/dark cycle. All studies were performed during the light cycle.

Drugs

YKSCH extract powders were provided by Tsumura & Co. (Tokyo, Japan) and a YKSCH-containing chow was made with normal chow (MF, Oriental Yeast Co., ltd.) that contained 2.8% YKSCH (Oriental Yeast Co., Ltd.). The YKSCH extract powders were added to excipients to make YKSCH extract granules, which are marketed as a medicine for patients under the national health insurance in Japan. As a medicine, a single dose is 2.5 g and the daily dose for an adult is 7.5 g of extract granules. The daily dose contains 4.5 g of extract powders of the following mixed crude drugs: *P. ternata* Breitenbach (*Araceae*), Tuber 5.0 g; *Atractylodes lancea* DC or *A. chinensis* Koidzumi (*Compositae*), Rhizoma 4.0 g; *Poria cocos* Wolf, sclerotium 4.0 g; *Cnidium officinale* Makino (*Umbelliferae*), Rhizoma 3.0 g; *Uncaria rhynchophylla* Miquel, *U. sinensis Haviland*, or *U. macrophylla* Wallich (*Rubiaceae*), Uncus 3.0 g; *C. unshiu* Markovich or *C. reticulata* Blanco (*Rutaceae*), Pericarpium 3.0 g; *Angelica acutiloba* Kitagawa or *A. acutiloba* Kitagawa ver. *sugiyamae* Hikino (*Umbelliferae*), Radix 3.0 g; *Bupleurum falcatum* Linne (*Umbelliferae*), Radix 2.0 g; and *Glycyrrhiza uralensis* Fisher or G. *glabra* Linne (*Leguminosae*), Radix 1.5 g.[11]

Body weight measurement study

Twelve mice were fed and housed in groups of three with water available *ad libitum* in each home cage. During the first week (from 7 to 8 weeks of age), all mice were fed normal chow ad libitum. At 8 weeks of age, the mice were divided into two groups. One group received normal chow *ad libitum* (n = 6, 2 cages) and the other received chow containing YKSCH *ad libitum* (n = 6, 2 cages). Every week, the body weight of each mouse and the food consumption of each cage were measured. This experiment continued until the mice reached 28 weeks of age. Body weight and food consumption were compared between the normal chow group and the YKSCH-containing chow group.

Motivation experiment

Operant chamber

Two-lever operant test chambers (Med Associates Inc., St. Albans, VT, USA) enclosed in sound-attenuating compartments were used. The reinforcers were sucrose pellets (20 mg each, dustless precision pellets, Bio-Serv, Frenchtown, NJ, USA), which were delivered into a feeder arranged between the two levers. A Siemens Nixdorf computer programmed in MED-PC (Med Associates Inc.) controlled the experiment and collected data.

Controlled feeding

Mice were fed and housed individually with water available *ad libitum* in each home cage for the first week (from 7 to 8 weeks of age). At 8 weeks of age, the mice were fed normal or YKSCH-containing chow. Different from the ad libitum feeding in the body weight measurement study, the day before every session of the operant training and test, food intake was restricted to the amount of 2.3 g in both groups.

Operant training

We conducted an operant training session 6 times per week. The operant training session was initiated at a fixed ratio (FR)-1 reinforcement schedule, whereby a single active lever press elicited the delivery of a food pellet. A trial was started with the house light turned off and two levers presented. Only one lever was designated as active for triggering the delivery of the food reward; the other lever was designated as inactive. After the food delivery, an 8-s inter-trial interval was added, during which levers were retracted and the house light was switched on, followed by automatic initiation of the next trial. The inter-trial interval allowed time for mice to consume the food pellet. Following a session in which ≥ 50 trials were attained, the session schedule was increased to FR-2, in which two active lever presses triggered the delivery of one food pellet. Similar to the FR-1 to FR-2 transition, following a session in which ≥ 50 trials were attained, the schedule was increased to FR-3 and then to a progressive ratio (PR) reinforcement schedule. For the PR reinforcement schedule, the response ratio schedule was calculated using the following formula: = [5exp (R*0.2)] – 5, where R was the number of food rewards already earned plus 1.[12] The final ratio completed (the number of the active lever presses in the final trial a mouse completed/how long a mouse pressed the active lever with a struggle to get one reward) represented the break point, which was calculated using the following formula: = [5exp ((R – 1)*0.2)] – 5. This was used as an index of food-incentive motivation. Following a session in which ≥ 10 trials were attained and the mean time spent to complete the required number of lever presses was < 10 s, the schedule was changed to the PR-10 reinforcement schedule. The PR-10 reinforcement schedule was the same as the PR reinforcement schedule with one limitation: if the number of active lever presses did not reach the number necessary for food rewards within 10 min of the beginning of the session, the session was terminated. When the

number of rewards earned in a session deviated by ≤ 10% for three consecutive days, learning was considered completed in the operant training session. If the learning was not completed before 15 weeks of age, the mouse was excluded (Figure 1).

Establishment of a "long-term operant test method"

The PR test can evaluate food-incentive motivation, but it is commonly conducted every day until the endpoint. To follow motivation status over the long term, such as over 6 months, with the use of Kampo medicine,[13] a daily test would be laborious and cumbersome. In this study, we determined the minimum number of sessions required over one week that resulted in no dropout owing to the loss of association learning.

Twelve mice were used and continued to be fed normal chow after 8 weeks of age. After the completion of the operant training session 6 times per week, the mice were divided into two groups: 6 mice received the operant test session once a week and 6 mice received it twice a week. The test session with the PR-10 reinforcement schedule was continued for 8 weeks and the break point was evaluated. After we determined whether it was better to use the reinforcement schedule once or twice a week, we used the method with the better result as the "long-term operant test method."

Indices of the operant test

The indices in the PR-10 reinforcement schedule were as follows: the break point was recognized as an index of food-incentive motivation (see the explanation in the section "Operant training" under Motivation experiment under Methods); %Accuracy [active lever press numbers / (active and inactive lever press numbers) × 100] was calculated and considered an index of cognitive function; and the collect reward latency (see Figure 2) was recognized as an index of appetite.

Study of the long-term effects of YKSCH

Sixteen mice were used and at 8 weeks of age, the mice were divided into two groups: a normal chow group (n = 6) and YKSCH-containing chow group (n = 10). The body weight of all mice was measured before every session of the operant training and test. After completion of training, the test session with the "long-term operant test method" was continued until mice were 39 weeks of age. Break point, %Accuracy, collect reward latency, and body weight were evaluated.

Statistical analysis

Two-factor repeated measures ANOVA and Spearman's rank correlation were performed using IBM® SPSS® Statistics version 23 software (IBM Corp., Armonk, NY, USA). In the analysis of two-factor repeated measures ANOVA, a Greenhouse–Geisser correction was used for violations of the sphericity assumption. In the Spearman's rank correlation, Spearman's coefficients were denoted by r_s.

RESULTS

To determine whether YKSCH had the potential to increase body weight in mice, we conducted a body weight measurement study with *ad libitum* feeding in mice who received normal (n = 6) or YKSCH-containing (n = 6) chow. The body weight of both groups was approximately the same at 8 weeks of age. Between 8 and 28 weeks of age, body weight increased more in

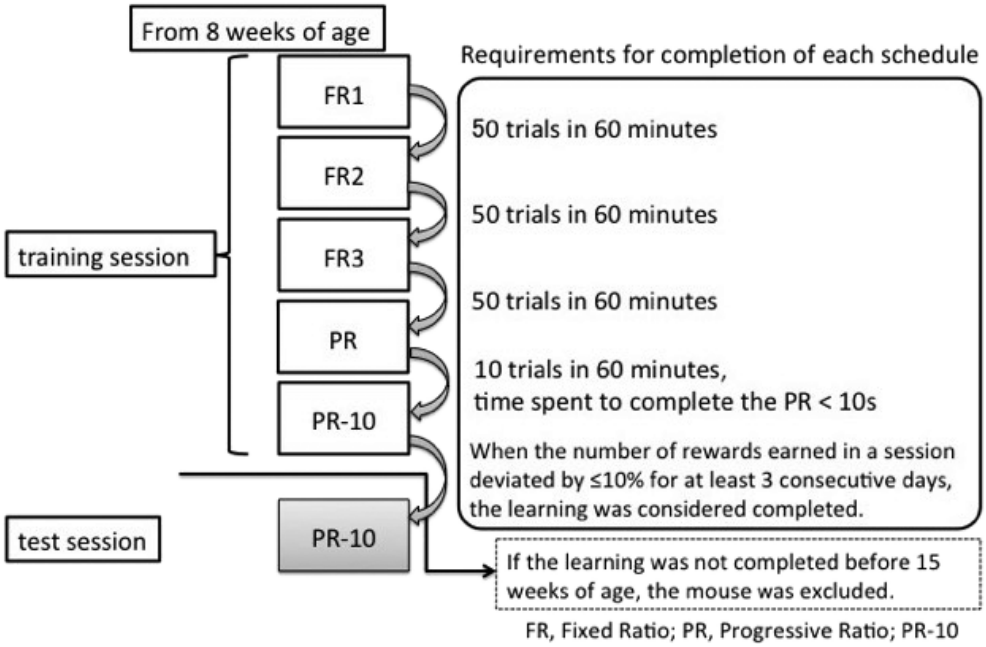

Fig. 1 The schedule of operant training

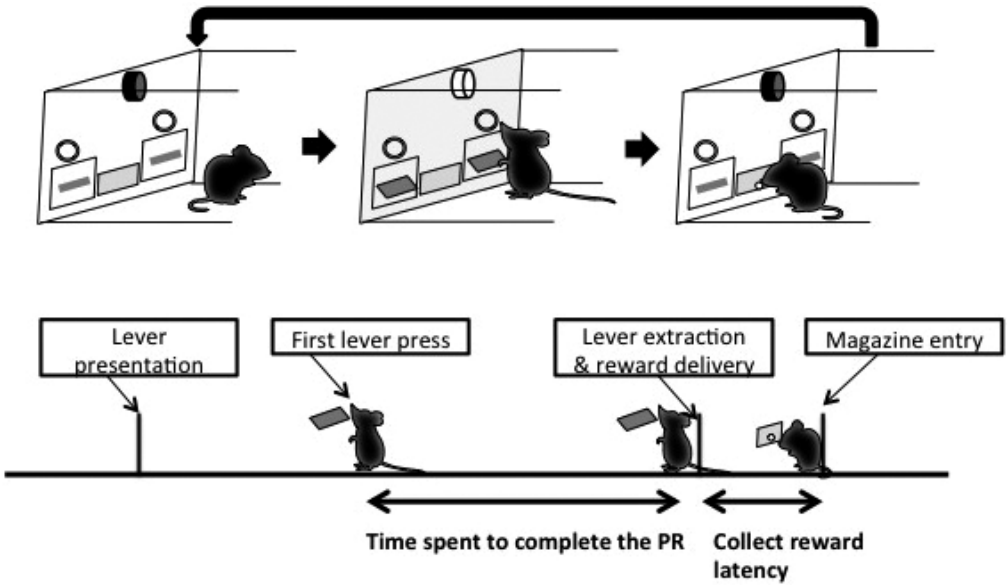

Fig. 2 Illustration of the time spent to complete the PR and the collect reward latency

the group that received YKSCH-containing chow than in the group that received normal chow (week: $F_{1.71,\ 17.07} = 170.27$, $p < 0.001$; group: $F_{1,\ 10} = 8.27$, $p = 0.016$; week × group interaction:

$F_{1.71,\ 17.07}$ = 4.63, p = 0.029, Figure 3).

We also evaluated the food consumption of the mice in each cage weekly. Although it appeared that a larger amount of food was consumed by the YKSCH-containing chow group, there was no significant interaction between week and group (week: $F_{1.68,\ 3.36}$ = 12.07, p =0.030; group: $F_{1,\ 2}$ = 15.94, p = 0.057; week × group interaction: $F_{1.68,\ 3.36}$ = 2.25, p = 0.236, Figure 4). The graphical representation of this data indicated that the lines in the two groups were parallel and YKSCH intake did not alter food consumption between 8 and 28 weeks of age. However, the difference in the food consumption between the two groups at 8 weeks of age was 0.21 g per mouse per day on average. It was unclear whether this difference was caused by the intake of YKSCH during the first 7 days. These results indicated that the consumption of YKSCH in the diet resulted in an increase in body weight, but did not change food consumption over 20 weeks.

With respect to the above data, we addressed the rationale of the Kampo medicine approach, that is, that increased body weight may coincide with increased motivation. We first established a "long-term operant test method" that was optimized to follow motivation status over months in mice. We tested whether the PR-10 reinforcement (see methods) schedule once or twice a week worked just after completion of training. One out of 6 mice in the once-a-week group had not pressed any active lever; however, all 6 mice in the twice-a-week group pressed enough active levers to evaluate their food-incentive motivation, indicating that the twice-a-week test schedule worked without any dropout during the transition from the training to the test. All mice with a successful transition sustained their operant conditioning. The twice-a-week PR-10 reinforcement schedule operant test was adopted for use as the "long-term operant test method."

Thereafter, using the "long-term operant test method," we evaluated the long-term effects of YKSCH on motivation. We compared the data from the normal (n = 6) and YKSCH-containing (n = 10) chow groups. In this experiment, food intake was restricted to the amount of 2.3 g in both groups the day before every session of the operant training and test, which was different from the *ad libitum* feeding in the body weight measurement study. The data of indices obtained from the operant training and test sessions are shown in Figure 5.

The result of the study of the long-term effects of YKSCH demonstrated that the break point of both groups gradually increased from 15 to 39 weeks of age (week: $F_{3.84,\ 53.72}$ = 3.22, p = 0.021), but YKSCH did not enhance motivation compared with the control (group: $F_{1,\ 14}$ = 0.02, p = 0.882; week × group interaction: $F_{3.84,\ 53.72}$ = 1.30, p = 0.283, Figure 5A). The sequential line graph of the break point displayed a large gap between the last three sessions of the operant training and the first session of the operant test. This was likely due to the decrease in number of sessions per week from 6 to 2.

The results also demonstrated that the %Accuracy of both groups was stable in a similar manner from 15 to 39 weeks of age (week: $F_{7.39,\ 103.47}$ = 1.38, p = 0.220; group: $F_{1,\ 14}$ = 0.32, p = 0.584; week × group interaction: $F_{7.39,\ 103.47}$ = 0.93, p = 0.494, Figure 5B). The average %Accuracy from 15 to 39 weeks of age was 93.5 ± 0.2% in the normal chow group and 92.2 ± 0.1% in the YKSCH-containing chow group, indicating that mice in both groups well remembered which lever was the active lever.

The results of the collect reward latency of both groups was stable in a similar manner from 15 to 39 weeks of age (week: $F_{6.52,\ 78.29}$ = 1.65, p = 0.140; group: $F_{1,\ 12}$ = 0.76, p = 0.400; week × group interaction: $F_{6.52,\ 78.29}$ = 0.44, p = 0.860, Figure 5C). The average collect reward latency from 15 to 39 weeks of age was 1.25 ± 0.01 s in the normal chow group and 1.45 ± 0.02 s in the YKSCH-containing chow group. This result indicated that the appetite in both groups did not change during the operant test period, supporting the result that indicated that the food-incentive motivation did not change with the administration of YKSCH.

The body weight results in the study of the long-term effects of YKSCH demonstrated that

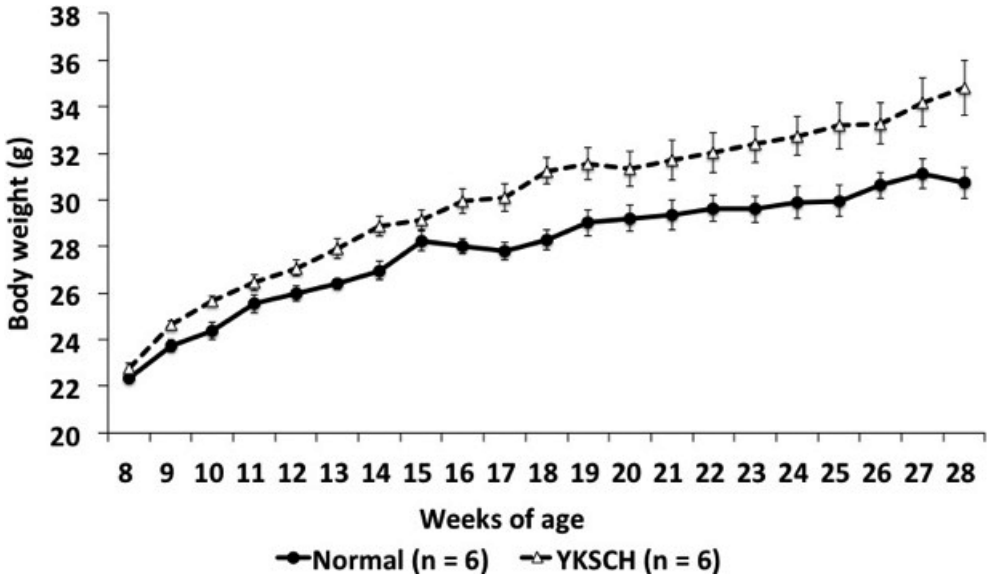

Fig. 3 Results of body weight in the body weight measurement study
The mean and standard error of the body weight are shown.

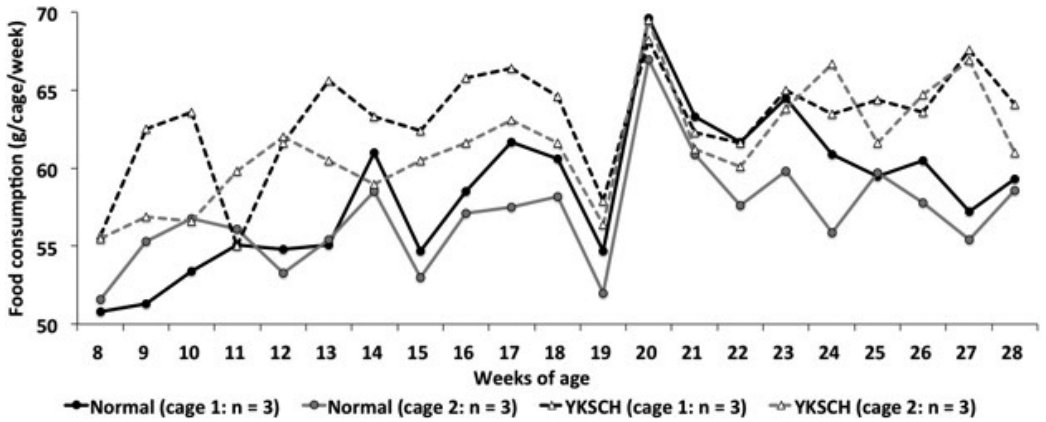

Fig. 4 Results of food consumption in the body weight measurement study
The raw data of each cage are plotted. Mice were fed *ad libitum*.

the increase in body weight of the YKSCH-containing chow group was greater than that of the normal chow group from 15 to 39 weeks of age (week: $F_{2.54,\ 35.55} = 51.21$, $p < 0.001$; group: $F_{1,\ 14} = 6.43$, $p = 0.024$; week × group interaction: $F_{2.54,\ 35.55} = 1.06$, $p = 0.370$, Figure 5D). According to the collect reward latency and body weight data in the study of the long-term effects of YKSCH, YKSCH-containing chow affected the body weight without changing the appetite. These data are consistent with those of the body weight measurement test with *ad libitum* feeding.

Lastly, we analyzed whether there was any relationship between the break point and body weight at 39 weeks of age. There was no correlation in the normal chow group ($r_s = 0.082$, $p = 0.799$, Figure 6A) or the YKSCH-containing chow group ($r_s = –0.068$, $p = 0.776$, Figure 6B),

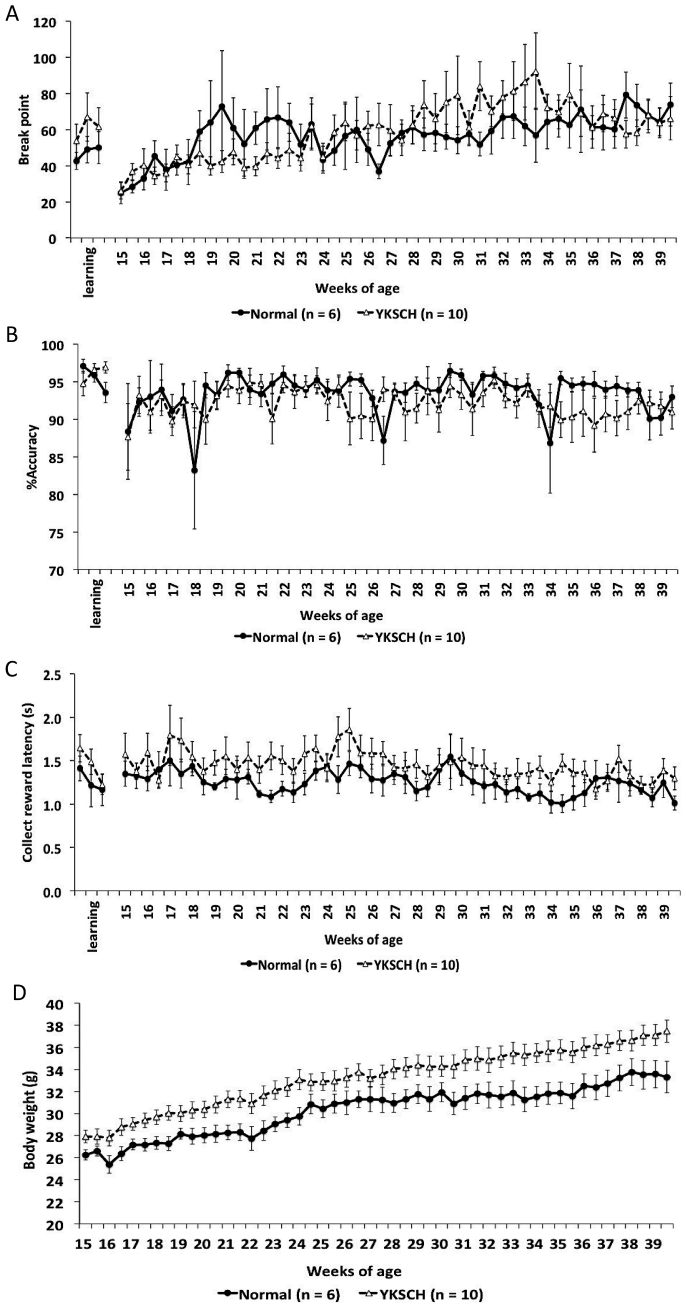

Fig. 5 Results of break point, %Accuracy, collect reward latency, and body weight in the study of the long-term effects of YKSCH

The "long-term operant test method" was conducted from 15 to 39 weeks of age. Two test data were plotted every week. The mean and standard error of the body weight are shown. A) The break point is recognized as an index of food-incentive motivation. B) %Accuracy [active lever press numbers / (active and inactive lever press numbers) × 100] is considered an index of cognitive function. C) The collect reward latency is recognized as an index of appetite. D) Mice were restricted to the amount of 2.3 g of food twice a week.

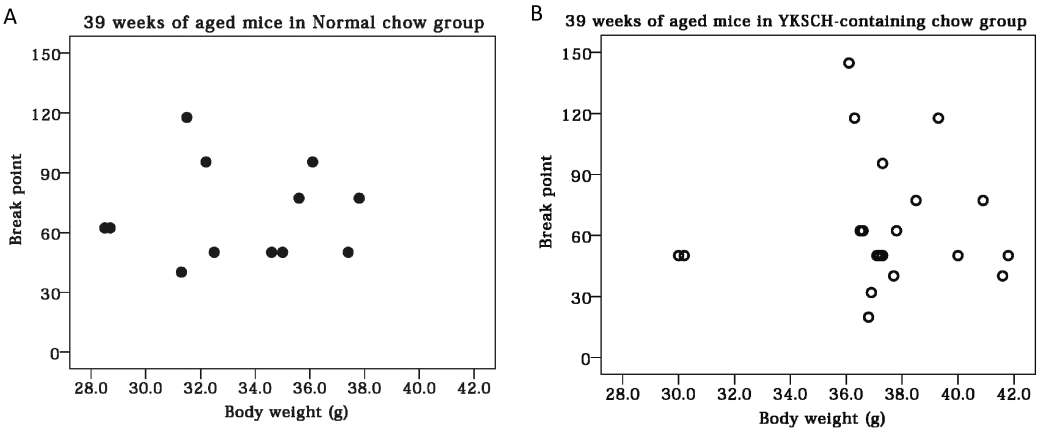

Fig. 6 Correlation between body weight and break point in the study of the long-term effects of YKSCH
The data of the two sessions at 39 weeks of age are plotted.

indicating that the body weight was not a factor that affected motivation in this study.

DISCUSSION

Our results indicated that feeding mice YKSCH-containing chow increased body weight more than feeding mice normal chow, but motivation did not increase in those fed YKSCH-containing chow compared to those fed normal chow. The twice-a-week PR-10 reinforcement schedule operant test enabled us to assess the long-term effect of Kampo medicine on motivation.

In the body weight measurement study, the effect of YKSCH on body weight showed significant week × group interaction. We used YKSCH at a concentration of 2.8% (w/w) (approximately 84 mg/mouse/day), which was similar to the concentration used by Mizoguchi *et al.*, who used 3% (w/w) YKS.[14] Tamano *et al.* used YKSCH at a dose of approximately 0.3 g/kg body weight daily (approximately 9.6 mg/mouse/day) and administered it to zinc-deficient mice from 4 to 6 weeks of age. No difference in body weight was found between the normal and YKSCH groups of zinc-deficient mice.[15] However, the daily dose was less than that in our study and the treatment period was only 2 weeks, which may explain the contrasting result.

The effect of YKSCH on food consumption was unclear in our study and could have been interpreted in two ways. In the first, because of the difference in food consumption between the two groups at 8 weeks of age (0.21 g/mouse/day on average), YKSCH might have increased food consumption during the first 7 days. However, if YKSCH increased food consumption, this might have been related to ghrelin, which is a growth-hormone-releasing acylated peptide.[16] Over 14 days of treatment in mice with a similar Kampo medicine, *rikkunshito* (RKT), which is composed of eight basic ingredients (five of which are also included in YKSCH), plasma acylated ghrelin significantly increased, but food consumption did not increase.[17] Therefore, YKSCH treatment during the first 7 days of our body weight measurement study was unlikely to have increased food consumption at 8 weeks of age in the YKSCH group.

In the second, because of the parallel lines of food consumption from 8 to 28 weeks of age in the two groups, YKSCH seemed not to change food consumption over 20 weeks. A recent clinical study reported similar results using RKT, in which weight loss improved at 52 weeks after esophagectomy without a change in appetite score based on the FACT-E (Functional Assessment

of Cancer Therapy-Esophageal) scale.[18] However, in both cases, it was difficult to understand why the appetite did not change but the body weight increased with Kampo treatment. Although we could not prove the mechanism in this study, we identified a few research papers that might explain our findings. *A. Lancea*, an ingredient in YKSCH, was reported to stimulate gastric emptying or small intestinal motility by inhibiting dopamine D2 receptors and 5-HT3 receptors.[19] RKT, which contains similar ingredients to YKSCH, was reported to improve postprandial gastric motor dysfunction in a rat model of experimental stress.[20] From the above, we suggested that YKSCH might possess the potential to improve gastric and small intestinal functions and might enhance digestive and absorbing functions. However, the possibility that YKSCH decreased energy consumption was not excluded. For example, YKSCH might have lowered the activity in the cage or the basal metabolic rate. Either way, more precise measurements of food consumption per day per mouse, calories in food, and activity in the cage will be required in future studies.

We proposed the "long-term operant test method" for follow-up study of the long-term use of Kampo medicine. We switched from 6 times-a-week training sessions to twice-a-week test sessions without any dropout and conducted the test over 20 weeks to evaluate the long-term effects of YKSCH. We noted that once the mice were successfully transferred from training sessions to test sessions, all those transferred were maintained, even at a once-a-week schedule. Therefore, it is possible that an abrupt switch causes dropouts and a gradual switch prevents them. In this study, we chose a twice-a-week schedule for the long-term observation; however, the use of the gradual switching could be used to establish a once-a-week schedule, which is less laborious.

In the study of the long-term effects of YKSCH, we did not find any significant difference in the break point or cognitive function index between the two groups. The Kampo medicine approach aims to adjust the diseased body's condition back to a healthy balance.[2] Based on this approach, we could postulate that C57BL/6J mice were in a healthy state, and therefore, YKSCH did not have any effect. To evaluate this hypothesis, we should conduct the "long-term operant test method" using disease model mice that show a lack of motivation in future research. For example, with respect to the disease model mice, Valencia-Torres *et al.* reported that activation of ventral tegmental area 5-HT2C receptors reduced incentive motivation.[21] Tsutui-Kimura *et al.* recently reported that a dysfunction of the ventrolateral striatal dopamine receptor type 2-expressing medium spiny neurons impaired instrumental motivation.[22] These model mice may enable the proper evaluation of the effects of YKSCH.

YKSCH reproduced the effect of increasing body weight when food was restricted to 2.3 g twice a week. The effect of YKSCH on increasing body weight seemed to be reliable and we hope this result will be useful in the treatment of patients with dementia who exhibit a decreased body weight in daily clinic.

CONCLUSION

We showed that YKSCH had the potential to increase body weight, but not motivation, in wild-type mice. In this study, we evaluated only wild-type mice using the "long-term operant test method"; however, disease model mice that show lack of motivation should be used in the future to evaluate food-incentive motivation. Even so, because YKSCH caused an increase in body weight, YKSCH may be a good treatment option for preventing decreased body weight in patients with AD.

SUPPLEMENTARY INFORMATION

This study was presented as a poster at the 30th Collegium Internationale Neuro-Psychopharmacologicum (CINP), Seoul, Korea, July 3–5, 2016.

CONFLICT OF INTEREST

This work was supported by Tsumura & Co. (Tokyo, Japan), who also provided the YKSCH extract powder.

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REFERENCES

1) Matsunaga S, Kishi T, Iwata N. Yokukansan in the treatment of behavioral and psychological symptoms of dementia: An updated meta-analysis of randomized controlled trials. *J Alzheimers Dis*, 2016; 54: 635–643.
2) The Japan Society for Oriental Medicine. Introduction to Kampo, Japanese traditional medicine. pp. 13, 49, 140, 2005, Elsevier Japan K. K. Tokyo.
3) Zhao QF, Tan L, Wang HF, Jiang T, Tan MS, Tan L, *et al.* The prevalence of neuropsychiatric symptoms in Alzheimer's disease: systematic review and meta-analysis. *J Affect Disord*, 2016; 190: 264–271.
4) Gao S, Nguyen JT, Hendrie HC, Unverzagt FW, Hake A, Smith-Gamble V, *et al.* Accelerated weight loss and incident dementia in an elderly African-American cohort. *J Am Geriatr Soc*, 2011; 59: 18–25.
5) Cova I, Clerici F, Rossi A, Cucumo V, Ghiretti R, Maggiore L, *et al.* Weight loss predicts progression of mild cognitive impairment to Alzheimer's disease. *PLoS One*, 2016; 11: e0151710.
6) Sergi G, De Rui M, Coin A, Inelmen EM, Manzato E. Weight loss and Alzheimer's disease: temporal and aetiologic connections. *Proc Nutr Soc*, 2013; 72: 160–165.
7) Terasawa K. Evidence-based reconstruction of Kampo medicine: part II-the concept of sho. *Evid Based Complement Alternat Med*, 2004; 1: 119–123.
8) Arai M, Arai K, Hioki C, Takashi M, Honda M. Evaluation of Kampo education with a focus on the selected core concepts. *Tokai J Exp Clin Med*, 2013; 38: 12–20.
9) World Health Organization. International Classification of Diseases 11 (ICD-11) Revision. Available at: <http://www.who.int/classifications/icd/en/> [Accessed Jan 27th 2017], <http://id.who.int/ictm/entity/1235397351> [Permalink].
10) Katayama K, Yamaguchi R, Imoto S, Watanabe K, Miyano S. Analysis of questionnaire for traditional medicine and development of decision support system. *Evid Based Complement Alternat Med*, 2014; 2014: 974139.
11) Tsumura & Co.. Package insert of Yokukansankachimpihange extract granules. Available at: <http://www.tsumura.co.jp/english/products/pi/JPR_T083.pdf> [Accessed Feb 8th 2017].
12) Richardson NR, Roberts DC. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *J Neurosci Methods*, 1996; 66: 1–11.
13) Hamaguchi T, Yoshino T, Horiba Y, Watanabe K. Goshajinkigan for low back pain: an observational study. *J Altern Complement Med*, 2016; Dec 19. [Epub ahead of print]
14) Mizoguchi K, Tanaka Y, Tabira T. Anxiolytic effect of a herbal medicine, yokukansan, in aged rats: involvement of serotonergic and dopaminergic transmissions in the prefrontal cortex. *J Ethnopharmacol*, 2010; 127: 70–76.
15) Tamano H, Yusuke E, Ide K, Takeda A. Influences of yokukansankachimpihange on aggressive behavior of zinc-deficient mice and actions of the ingredients on excessive neural exocytosis in the hippocampus of

zinc-deficient rats. *Exp Anim*, 2016; 65: 353–361.

16) Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 1999; 402: 656–660.

17) Matsumura T, Arai M, Yonemitsu Y, Maruoka D, Tanaka T, Suzuki T, *et al.* The traditional Japanese medicine Rikkunshito increases the plasma level of ghrelin in humans and mice. *J Gastroenterol*, 2010; 45: 300–307.

18) Nakamura M, Nakamori M, Ojima T, Katsuda M, Hayata K, Iwahashi M, *et al.* The effects of rikkunshito on body weight loss after esophagectomy. *J Surg Res*, 2016; 204: 130–138.

19) Kimura Y, Sumiyoshi M. Effects of an Atractylodes lancea rhizome extract and a volatile component β-eudesmol on gastrointestinal motility in mice. *J Ethnopharmacol*, 2012; 141: 530–536.

20) Harada Y, Ro S, Ochiai M, Hayashi K, Hosomi E, Fujitsuka N, *et al.* Ghrelin enhancer, rikkunshito, improves postprandial gastric motor dysfunction in an experimental stress model. *Neurogastroenterol Motil*, 2015; 27: 1089–1097.

21) Valencia-Torres L, Olarte-Sánchez CM, Lyons DJ, Georgescu T, Greenwald-Yarnell M, Myers MG, *et al.* Activation of ventral tegmental area 5-HT2C receptors reduces incentive motivation. *Neuropsychopharmacology*, 2016 Dec 21. doi: 10.1038/npp.2016.264. [Epub ahead of print]

22) Tsutsui-Kimura I, Takiue H, Yoshida K, Xu M, Yano R, Ohta H, *et al.* Dysfunction of ventrolateral striatal dopamine receptor type 2-expressing medium spiny neurons impairs instrumental motivation. *Nat Communications*, 2017; 8: 14304.