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Attenuation of Food Intake by Fragrant Odors: Comparison between *Osmanthus fragrans* and Grapefruit Odors

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Abstract

Odors affect various physiological and mental activities. Previous studies in rats have shown that the odors of grapefruit and *Osmanthus fragrans* (OSM, fragrant tea olive) attenuate food intake, leading to a reduction in body weight gain, but it is not yet clear whether the causative mechanisms underlying these effects are the same for both odors. The first part of the present study revealed that grapefruit odor had no effect on the expression of feeding-related neuropeptides, in contrast to the previous finding that OSM odor suppresses orexigenic and activates anorexi-genic neuropeptides in the hypothalamus of the rat. The second part revealed that OSM odor activated the parasympathetic nerve, in contrast to the previous finding demonstrating that grapefruit odor activates sympathetic nerve activity. The third part was performed to confirm the previous findings about the effects of OSM odor on appetitive reactions in humans. In human subjects, we found that continuous exposure to OSM odor attenuated appetite and consumption of snacks (cookies) and improved mood, when evaluated using the POMS (Profile of Mood States) data from university students. In conclusion, OSM odor attenuated appetite and decreased food intake in humans, and the underlying causative mechanisms differed from those mediating the effects of grapefruit odor, specifically in terms of the expression of hypothalamic feeding-related neuropeptides and autonomic nerve activity.

Keywords: odor, *Osmanthus fragrans*, grapefruit, feeding behavior, feeding-related neuropeptides, autonomic nerve, total mood disturbance

1. Introduction

Overeating leads to obesity, which heightens the risk of several chronic illnesses including hypertension, diabetes, high blood triglycerides, heart disease, stroke, kidney problems and cancer. One of the causes of overeating is palatability of foods, especially those containing sweet and fatty substances, which often promote ingestion over homeostatic repletion [1–3]. It is suggested that the palatability-induced ingestion is based on a sequential release of brain substances such as β -endorphin,

dopamine and orexigenic neuropeptides, corresponding to palatability (liking), motivation (wanting), and actual intake (eating), respectively [3–7]. Any attempts to suppress actions of one or more of these brain substances could be an effective approach to prevent from overeating.

Odors produce various physiological, psychoemotional, and behavioral reactions depending on their qualities and hedonic tones [8–15]. Concerning food intake behavior, it is our common experience that odors associated with pleasant foods enhance appetite, but repellent odors reduce appetite. Interestingly, some fragrant odors attenuate ingestive behavior and body weight gain. Studies using rats have demonstrated that grapefruit odor inhibits food intake, leading to a reduction in body weight gain. It is plausible that this effect is mainly caused by activation of sympathetic nerve activity, which enhances energy consumption and suppresses appetite [16, 17]. Another example is the odor of *Osmanthus fragrans* (OSM, fragrant tea olive), which also attenuates food intake in rats [18]. This effect, however, is suggested to be due to the reduced expression of feeding-related neuropeptides in the hypothalamus. More precisely, Yamamoto *et al.* [18] demonstrated that OSM odor decreased the messenger ribonucleic acid (mRNA) expression of orexigenic neuropeptides, such as agouti-related protein (AgRP), melanin-concentrating hormone (MCH), neuropeptide Y (NPY), and orexin, and increased the expression of anorexigenic neuropeptides, such as cocaine and amphetamine regulated transcript (CART) and proopiomelanocortin (POMC). It is also suggested that, in rats, OSM odor decreased the motivation to eat, food intake, and body weight, as well as caused sluggish masticatory movements [19].

OSM is an evergreen shrub that has been grown in Eastern Asia, especially in China, for more than 2500 years [20]. It produces small clusters of flowers in the late summer and autumn. The flowers are small, pale yellow, yellow, or orange-yellow and have a strong fragrant scent of ripe peaches or apricots. Because of its favorable fragrance, tea, wine, and jam with OSM flowers are traditionally very popular and are enjoyed on a daily basis in far-east Asia, especially in Taiwan and China. Since it has been traditionally believed to exert good effects on physical and mental health, the OSM plant has also been utilized as a Chinese herbal medicine. Among the volatile compounds of the scent of OSM, the essential ones are γ -decalactone, β -ionone, dihydro- β -ionone, linalool oxides [18, 21].

Although both grapefruit and OSM odors suppress appetite, food intake and body weight gain, the underlying causative mechanisms appear to differ to those described above. However, there are a lack of comparative data on the possible effects of grapefruit odor on feeding-related neuropeptides and effects of OSM odor on autonomic nerve activity. The present study, therefore, was designed to examine possible effects of grapefruit odor on the expression of orexigenic and anorexigenic neuropeptides in rats. We also examined effects of OSM odor, together with odors of lavender, jasmine, and milk on the autonomic nervous activity in humans. Finally, we examined how OSM odor affects appetitive reactions in humans.

2. Methods

2.1 Measurement of mRNAs for feeding-related neuropeptides

A total of 18 Wistar male rats were used. They were randomly divided into experimental and control groups ($n = 9$ each). Rats were individually housed in plastic cages, with freely available food and water, in a temperature- and humidity-controlled room (23°C, 60%). All animals were handled in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals (National

Institute of Health Guide), and this study was approved by the institutional committee on animal research (Animal Research Committee of Kio University).

The experimental rats received grapefruit essential oil as an olfactory stimulus with the same method as described in our previous paper [18]. Briefly, a drop (100 μ l) of the oil was put on a filter paper, which was inserted between two metal mesh plates, and placed on the floor of the cage. The control rats were exposed to a filter paper containing a drop of water instead of the olfactory stimulus. The brains were removed 60 min after the onset of stimulation and the hypothalamus was removed, and preserved at -80 degrees. To examine the changes in the expression of mRNAs for prepro-orexin, MCH, AgRP, NPY, CART, and POMC, we used the same quantitative real time (RT)-PCR technique as that in the previous study [18].

2.2 Effects of odors on autonomic nerve activity

We used the essential oils of lavender, jasmine, milk (these three are products of Takasago International Corp. Japan), and *Osmanthus fragrans* (product of Argeville, France) as olfactory stimuli, and distilled water as an odorless control stimulus. To examine the effects of odors on autonomic nerve activity, a total of 60 university students (20–21 years old, 54 females and six males) were used. They were all in good health, without symptoms of nasal congestion, and their olfactory sensitivity was within the normal range, as judged using a T & T olfactometer [22, 23]. They were randomly divided into four groups ($n = 15$ each). Subjects in each group sniffed either one of the four odors (diluted to 2.5% with triethyl citrate) soaked in filter papers fixed in front of each subject's nose; the control stimulus was prepared in the same way. The order of presentation of the odors and control stimuli was counterbalanced within the group. Heart rate variation (HRV) was measured with a pulse analyzer device (TAS9, YKC Co. Ltd., Tokyo, Japan). This device was designed to evaluate autonomic nervous activity using acceleration pulse waves obtained from the tip of the index finger of the left hand. HRV was recorded for five minutes for each stimulus under the relaxed condition in a seated position following rest for 15 minutes.

The technical procedures and physiological interpretation of the HRV analysis have been reported by a number of researchers [24–29] with a useful guideline for HRV measurement and physiological interpretation [30]. The heart rate data were transferred to a personal computer, and the frequency domain measurements of HRV were determined by spectral analysis using fast Fourier transformation. The power spectrum was decomposed into its frequency components and quantified in terms of the relative power of each component. We used three frequency domain variables as an index of HRV. These frequency domain variables included low-frequency (LF: 0.04–0.15 Hz), high-frequency (HF: 0.15–0.40 Hz) and the ratio of LF to HF (LF/HF). The LF component reflects both parasympathetic and sympathetic nervous activities, the HF component reflects parasympathetic nervous activity, and the LF/HF ratio is considered an index of sympathetic nervous activity.

2.3 Effects of odors on feeding behavior

We used two odor stimuli (lavender and OSM), which were the same as those described in the previous section, and an odorless control stimulus (distilled water). To examine the effects of odors on feeding behavior, another cohort of 66 university students (20–21 years old, 60 females and six males) who belonged to one class of a nutritional course from Kio University were used. Experiments were conducted every Wednesday in three consecutive weeks.

The time schedule of an experimental day is shown in **Figure 1**. The experiment started at 12:00. After checking the physical condition, the subjects were randomly

Figure 1.
Time schedule of the conducted experiments on Wednesday. Subjects participated in the experiment with different odor stimuli on another two Wednesdays for three consecutive weeks.

divided into three groups of 22 subjects. Each subject was served with a box lunch and a bottle of water and ate the lunch between 12:15 and 12:50 (**Figure 2**). Then, each subject wore a mask with a small pocket inside in which a filter paper (one cm x five cm) was inserted (**Figure 3**). The filter papers were infiltrated with a few drops of 2.5% OSM, 2.5% lavender oil, or non-odor distilled water. Each group received either OSM odor, lavender odor, or no odor-containing filter papers during a lecture on the first experimental day. Similarly, on the second and third experimental days, each group received a different stimulus (of the three stimuli). Thus, every subject received all three stimuli throughout the three experimental days. Subjects attended two lectures with a 20-minute intermission from 13:00-to-15:40.

Figure 2.
A box of lunch and a bottle of water served on the first experimental day. A different box of lunch was served on the second and third experimental days.

Figure 3.
A mask with a filter paper in an inside pocket. The filter paper was soaked with 2.5% Osmanthus fragrans (OSM) for the OSM group, 2.5% lavender for the lavender group or odorless distilled water for the control group.

They took off their masks during the intermission and wore the masks again with new filter papers just before the second lecture. After stimulation, each subject was given a package of snacks, containing 16 pieces of small cookies (Bourbon Petit with French Butter flavor, Bourbon Co. Niigata, Japan), and they were allowed to eat them freely, as much as they desired (**Figure 4**). The numbers of leftover cookies were counted and compared among the three odor groups.

The following sensory tests were assessed in each subject. To evaluate intensity and pleasantness of odors (sensory test 1 in **Figure 1**), the subjects were asked to select the score from one of five values ranging from 1 (very weak), 2 (weak), 3 (neutral), 4 (strong) to 5 (very strong) soon after putting masks and soon after taking off masks. To evaluate the level of hunger (sensory test 2), the subjects selected the score from one of five values from 1 (not hungry), 2 (slightly hungry), 3 (medium), 4 (moderately hungry) to 5 (very hungry). To evaluate the sweetness and pleasantness of the cookies (sensory test 3), the subjects selected scores from one of five values, ranging from 1 (very weak) to 5 (very strong), soon after eating the cookies.

To examine mood changes before and after odor stimulation, we administered the Profile of Mood States (POMS), a short-form questionnaire translated into Japanese (Kaneko Shobo Co. Ltd. Tokyo, Japan), after finishing lunch (or before odor stimulation) and after taking off the mask (or after odor stimulation). The POMS test consisted of 35 questions about the current mood state. The 35 questions were classified into six subscales: T–A (tension and anxiety), D (depression and dejection), A–H (anger and hostility), V (vigor), F (fatigue), and C (confusion). The subjects selected

Figure 4.
A commercially available package of 16 small cookies served as snack after taking off mask.

the score from one of five values from 0 (not at all) to 4 (extremely). Total mood disturbance (TMD) was calculated by subtracting V from the sum of the other five subscale scores in each subject. Lower TMD scores were indicative of an improved mood.

For the experiments in humans (as described above), the study protocol was approved in advance by the Ethics Committee of Kio University and was performed in accordance with the Declaration of Helsinki of the World Medical Association. All subjects received an explanation of the nature of the research and agreed with the study protocol. We did not tell subjects about the names of the odors used in the experiments. All subjects signed written informed consent.

2.4 Data analysis

A two-way analysis of variance (ANOVA) was used to compare the expression of the feeding-related neuropeptides between grapefruit odor and non-odor conditions. To examine the effects of the four odors on the autonomic nerve activity in humans, a two-tailed paired *t*-test was used to compare each odor with the non-odor condition. With regards to the effects of odors on feeding behavior in humans, intensity and pleasantness scores of odors, and sweetness and palatability scores of cookies between OSM and lavender odors were analyzed using the Mann–Whitney *U* test, and comparisons of hunger scores and cookie intake among OSM, lavender, and control groups were performed using the Friedman test and *post hoc* Wilcoxon signed rank test. Values of $P < 0.05$ were considered statistically significant. Statistical analyses were performed using a software program (IBM SPSS Statistics, ver. 25).

3. Results

3.1 Effects of grapefruit odor on the feeding-related neuropeptides in rats

The expression of mRNAs for the hypothalamic orexigenic neuropeptides, such as AgRP, MCH, NPY and orexin, and anorexigenic neuropeptides, such as CART and POMC, was measured using a real-time polymerase chain reaction (RT-PCR) on the rat hypothalamic specimens taken 60 minutes after the onset of grapefruit odor stimulation. The results were compared to those of similar samples taken from non-odor control rats. **Figure 5** shows the expression of mRNAs for four orexigenic and two anorexigenic neuropeptides in the control and experimental groups. A two-way analysis of variance (ANOVA) with peptide (gene expression of six peptides) and odor (water and grapefruit) revealed a statistically significant main effect of peptide [$F(5;96) = 8.76, P < 0.001$]. However, there were no main effects of odor and no peptide-odor interaction.

3.2 Effects of OSM odor on the autonomic nerve activity in humans

The effects of four kinds of odors (lavender, jasmine, OSM, and milk) on autonomic nerve activity, in terms of frequency analysis, are graphically summarized in **Figure 6**. The mean high frequency (HF) component of R-R variation (variability of the time interval between R waves), an indicator of the parasympathetic activity, was statistically significantly ($P < 0.05$, paired *t*-test) higher for lavender and OSM, and highly significantly ($P < 0.01$) lower for milk compared with the comparative value for the non-odor control. The mean low frequency/high frequency (LF/HF) score, an indicator of sympathetic activity, was significantly ($P < 0.05$) lower for lavender and OSM than for controls.

Figure 5.
Effects of grapefruit odor on the expression of mRNAs for feeding-related neuropeptides in the hypothalamus of rats. Values are means $\pm$ SE. No difference was detected between the grapefruit odor group and the non-odor control group (two-way ANOVA, $P > 0.05$).

Figure 6.
Effects of odors on autonomic nerve activity. Odors are lavender (L), jasmine (J), OSM (O) and milk (M). C, non-odor control; HF, high-frequency component; LF, low-frequency component. Values are means $\pm$ SE. Asterisks denote that the autonomic activity in the presence of the odor is significantly different from that in non-odor condition (two-tailed paired *t*-test). * $P < 0.05$, ** $P < 0.01$. OSM, *Osmanthus fragrans*.

3.3 Effects of OSM odor on feeding behavior in humans

3.3.1 Intensity and pleasantness scores of odors

Five minutes after wearing masks with OSM odor, lavender odor or non-odor distilled water, the intensity score for the odors was 3.8 ± 0.9 (mean $\pm$ standard deviation [SD], $n = 66$) and 4.3 ± 0.7 ($n = 66$) for OSM and lavender groups, respectively. Soon after taking off the masks, the intensity score was significantly (Mann–Whitney U test, $P < 0.05$) lowered to 2.6 ± 1.0 and 2.8 ± 1.0 , respectively. Five minutes after wearing masks, the pleasantness score for the odors was 2.2 ± 0.8 and 2.6 ± 1.0 for OSM and lavender groups, respectively, and soon after taking off masks, the pleasantness score was elevated to 2.5 ± 0.8 ($P < 0.05$) and 2.7 ± 0.9 ($P > 0.05$), respectively.

3.3.2 Hunger score

Before and after eating cookies, we asked subjects how they evaluated their hunger status. The number of subjects was counted at each level, ranging from no-hunger (1), slightly hungry (2), medium hunger (3), moderately hungry (4), and very hungry (5). Since the numbers of subjects belonging to the no-hunger and very hungry groups were so small, we categorized the subjects into three groups: not hungry (1 + 2), medium hunger (3) and hungry (4 + 5), and the results for OSM, lavender, and control groups are shown in **Figure 7–A**. The proportion among the three levels was significantly ($P < 0.05$, Friedman test and *post hoc* Wilcoxon signed rank test) different between OSM and control groups before eating cookies, indicating that OSM odor reduces hunger in the subjects. After eating, no difference was detected among the three groups (**Figure 7–B**).

3.3.3 Cookie intake

After offering a snack package to each subject, which contained 16 pieces of small cookies that could be consumed at will, we counted the remaining cookies. The number of leftover cookies varied greatly among the subjects. To examine any difference of odor effects on cookie eating, the subjects were divided into three

Figure 7. Proportion of hunger status. The numbers of subjects in the OSM, lavender, and non-odor control groups are expressed in three categories of hunger status (1, no-hunger; 2, slightly hungry; 3, medium hunger; 4, moderately hungry; 5, very hungry) before and after eating snacks. The hunger status before snack eating was different between OSM and control groups (Friedman test, *post hoc* Wilcoxon signed rank test, $P < 0.01$). OSM, *Osmanthus fragrans*.

subgroups: a high-eating group (with leftovers ranging from zero-to-four cookies), a moderate-eating group (leftovers ranging from five-to-nine cookies), and a low-eating group (leftovers ranging from 10-to-16 cookies). The number of subjects belonging to each subgroup is shown for the three odor conditions in **Figure 8**. The graphical representation suggests that the subjects in OSM group ate less than those in control group, and this difference was statistically significant (Friedman test and *post hoc* Wilcoxon signed rank test, $P < 0.05$). No statistically significant difference was detected between either the OSM and lavender groups or between the lavender and control groups.

3.3.4 Sweetness and palatability scores

The palatability score for the cookies was 4.1 ± 0.7 ($n = 66$), 4.1 ± 0.6 ($n = 66$), and 4.2 ± 0.6 ($n = 66$) (mean $\pm$ SD) for OSM, lavender, and non-odor control groups, respectively. The sweetness score for the cookies was 3.5 ± 0.8 , 3.6 ± 0.7 and 3.6 ± 0.7 , respectively. No statistically significant difference in sweetness or palatability was observed among the three groups.

3.3.5 Total mood disturbance score

Total mood disturbance (TMD) scores of the Profile of Mood States (POMS) test are shown in **Figure 9**. The basal mood after lunch (or before putting on the odor mask) varied among the three groups: in the two odor (OSM and lavender) groups and the non-odor control group, the mean TMD scores were standardized to one. The rates of change in mood soon after taking off the mask (or before eating the cookie snack) were compared among the three groups. Statistically significantly (two-tailed paired *t*-test, $P < 0.01$) low TMD scores were detected after exposure to OSM odor, indicating a state of improved mood, while no significant difference was detected between pre- and post-mask-wearing in both the lavender and control groups.

Figure 8. The numbers of leftover cookies in the three groups. The numbers of subjects who left a small number (zero-to-four) of cookies, a moderate (five-to-nine) number of cookies, and many (10–16) cookies are shown for the OSM, lavender, and non-odor control groups. The OSM group ate fewer cookies compared with the control group (Friedman test, *post hoc* Wilcoxon signed rank test, $P = 0.05$). OSM, Osmanthus fragrans.

Figure 9. Total mood disturbance (TMD) scores before and after odor stimulation. The relative TMD score is shown after odor stimulation when the score before odor stimulation was set at unity. A statistically significant difference was apparent for OSM odor between the pre- and post-odor stimulation scores (two-tailed paired *t*-test). * $P < 0.05$. OSM, *Osmanthus fragrans*.

4. Discussion

Previous studies in our laboratory have demonstrated that the odor of OSM attenuates food intake in rodents [18]. The present study was designed to confirm this effect in humans and also to compare the underlying causative mechanisms, in terms of autonomic nerve activity and expression of mRNA for feeding-related neuropeptides, between the OSM odor and grapefruit odor, which also attenuates food intake and body weight gain [16, 17, 31].

4.1 Feeding-related neuropeptides

It is well established that feeding-related neuropeptides in the hypothalamus play important roles in the elicitation, maintenance, and cessation of appetite and food intake [3, 32, 33]. Previously, our research group revealed that the neural information of OSM odor decreased mRNA expression of orexigenic neuropeptides (AgRP, NPY, MCH, and orexin) and increased expression of anorexigenic neuropeptides (CART and POMC) [18]. These findings are suggested to be, at least in part, the causative mechanisms underlying the effects of OSM odor on the decreased motivation to eat, sluggish masticatory movements, and the resulting reduction in body weight [18, 19]. Since comparative data are not available for the grapefruit odor, the present study examined the expression of feeding-related neuropeptides following exactly the same method we have previously used for the OSM odor. Consequently, we could not detect any difference in the expression of feeding-related neuropeptides between the grapefruit odor group and non-odor control group, indicating that grapefruit odor essentially had no effect on the expression of hypothalamic feeding-related neuropeptides.

4.2 Autonomic nerve activity

Fragrant odors are known to affect the autonomic nerve activity. For example, the odors of rose flowers [13, 15], lavender [34–36], and yuzu [37] activate parasympathetic neurons, whereas those of lemon [38], jasmine [39] and grapefruit [13, 17, 38, 40] activate sympathetic nerve activity. To our knowledge, there is only one previously published study that suggests that the OSM odor stimulates parasympathetic activity in humans [41]; therefore, more research is required to confirm these findings.

To examine how the OSM odor affects autonomic nerve activity in humans, we used the fingertip photoplethysmogram (PPG) to monitor autonomic nervous activation. Analysis of fingertip PPG signals is an important tool for assessing pulse wave components and their relation to vascular health. Several studies have demonstrated that the PPG waveform can provide clinical information on the dynamics of the autonomic nervous system, as well as the activity of the left ventricle, vascular aging, and arterial stiffness [42–44]. Although PPG is easy to set up, convenient, simple, and inexpensive, with only a single fingertip sensor, it has been proven that electrocardiogram and PPG signal recordings can be interchanged for heart rate variation (HRV) analysis including the time and frequency domains [45]. The PPG technique is also utilized for the assessment of arterial wall stiffening during aging [46] and for the assessment of the index of the periodontal condition [47].

The present HRV analysis on the basis of PPG has revealed that lavender odor significantly stimulates parasympathetic nerve activity, which is in agreement with previous results [34–36]. Jasmine odor tended to be a sympathetic activator, but the effect was not significant, which may have reflected an inter-individual difference in the preference for this odor, as suggested by Inoue *et al.* [48] and Kuroda *et al.* [49]. The important finding is that OSM odor significantly stimulated the parasympathetic nerve activity, which is opposite in action to grapefruit odor, which is a well-established sympathetic activator in animals [38] and humans [13, 40]. The milk odor, which was used as a control (or counter-part) odor for the OSM odor [18], tended to stimulate the sympathetic nerve activity.

The differences in the physiological actions between the OSM and grapefruit odors (as mentioned above) should be derived from the difference in volatile compounds in these odors. There are more than 10 active compounds detected in OSM odor, including major volatiles (such as ocimene, ionone, linalool, capraldehyde, and decalactone) [21, 50]. The major active volatile compound in grapefruit odor is limonene; additional compounds include myrcene, pinene, and linalool [51, 52]. It is noted that not only the major volatiles but some volatiles with low content also contribute to aroma [50]. Further study is required to elucidate the specific role of each compound.

4.3 Effects of odors on cookie intake

To confirm our previous findings in rodents that the OSM odor attenuates appetite and food intake, we elaborated on an experimental design in which the effect of OSM odor on snack eating behavior was examined in university students. Since OSM odor activates parasympathetic nerve activity (as described above), we selected lavender odor which also stimulates parasympathetic nerve activity for a comparable stimulus. Although sweetness and palatability of cookies were not different after exposure to OSM or lavender odors and in non-odor control group, we found that the hunger level, TMD score, and the numbers of cookies eaten significantly changed in the OSM group, compared with lavender and control groups. After exposure to the odors, subjects in the OSM group felt less hungry than those exposed to lavender or subjects in the control group, suggesting that appetite is reduced after exposure to OSM odor. Consequently, the consumption of cookies after OSM odor was less than that after lavender or non-odor conditions. Such effects in feeding behavior are not due to disagreeable feelings to OSM odor because pleasantness of OSM odor after exposure was not statistically significantly different from that of lavender odor. Moreover, mood states were significantly improved after exposure to OSM odor compared with lavender odor or non-odor conditions, as shown by the POMS data. Thus, the previous finding that the odor of OSM decreases food intake in rodents was modestly confirmed in humans through the present experimental paradigm.

4.4 Application

How could the findings of this present study be utilized in our daily life? As it is expected that appetite and meal size could be reduced under the presence of OSM odor, you will be more satisfied (satiated) with a smaller meal size that otherwise would not fulfill your appetite (**Figure 10**). Repeating this procedure at every meal, you could adjust yourself to eating smaller meals, which could possibly lead to a reduction in body weight. To examine this possibility, we performed a pilot study [53] where five females were exposed to OSM odor daily from the hour of rising to bedtime for 12 days. For delivery of the odor, each subject hung a small case containing a filter paper soaked in OSM essential oils around their neck. At the end of the experiment, the subjects showed a reduction in total body fat and body weight, compared with five females in the non-odor control group. For a practical use, it is necessary to elucidate the most effective and convenient method of odor exposure, or exposure duration. A proper use of the OSM odor as well as grapefruit odor could be an attractive and promising tool to promote ecological eating and to improve and promote good health.

A limitation of our study pertains to the selection of subjects and the duration of odor stimulation. The number of subjects was not enough to analyze the results in terms of sex differences because the number of male subjects was too small to be compared with female subjects. Subjects wore masks with odor continuously for 70 minutes and another 70 minutes, separated by a 20-minute-intermission without masks. Adaptation to odors is a well-known phenomenon: repeated or prolonged exposure to an odorant leads to decreases in olfactory sensitivity to that odorant [54–56]. According to Inoue *et al.* [48], five-minutes continuous exposure to the odor of jasmine tea affected autonomic nerve responses for more than 60 minutes, suggesting that our prolonged odor presentation may have not been necessary. Proper duration of exposure and concentration of the odor should be determined more precisely in future studies.

Figure 10. A model showing that a less amount attains the same satiety level after exposure to the *Osmanthus fragrans* (OSM) odor. Suppose the degree of satiety increases linearly with the amount of food consumed, the amount of food intake “a” attains satiety level “a”. after exposure to the OSM odor, the line (relationship between the amount of food consumed and the degree of satiety) shifts upward, and the same satiety level “a” can be attained by taking less amount of food “b”, indicating that the OSM odor is effective in satisfying appetite with a smaller volume, otherwise you will be unhappy because you are not full and want to eat more. A proverb says that “moderation in eating is the best medicine”. Inlet picture denotes OSM flowers.

5. Conclusion

The present human experiments have shown that OSM odor is agreeable and elicits sedative effects, improves mood, attenuates hunger, and reduces food intake. Grapefruit odor, which has also been shown to attenuate food intake, activates sympathetic nerve activity and had no effects on expression of feeding-related neuropeptides in rats, which is contrary to the results obtained for OSM odor, indicating the difference of causative neural mechanisms between the two odors. Exposure to OSM odor before eating and that to grapefruit odor after eating may be recommended as the effective practical use for preventing from overeating and obesity.

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Author contributions

TY and KU designed the study, KU and TI performed the experiments on humans and animals, respectively, HM performed the data analyses, TY wrote the manuscript, and all authors reviewed and approved the paper.

Competing interests

The authors declare no competing interests.

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