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# Appetite-Enhancing Effects: The Influence of Concentrations of Benzylacetone and *trans*-Cinnamaldehyde and Their Inhalation Time, as Well as the Effect of Aroma, on Body Weight in Mice

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Received November 24, 2015; accepted February 15, 2016

**Benzylacetone has appetite-enhancing and locomotor-reducing effects. The effective doses for these two outcomes overlap, and the weight gain of mice exposed to benzylacetone is caused by both appetite-enhancement and a reduction in locomotor activity. The appetite-enhancing effects of *trans*-cinnamaldehyde and benzylacetone have been reported previously. In this study, these appetite-enhancing effects were seen in mice after short-term, high-dose exposure.**

**Key words** benzylacetone; *trans*-cinnamaldehyde; appetite enhancement; inhalation; body weight; chronic administration

Obesity is a current major societal issue and studies into its prevention have been widely conducted.[1–3] However, many women and elderly people with low body weight have superior mesenteric artery syndrome (SMA), which can lead to ileus and subsequently lowered quality of life.[4]

Carcinostatic agents and some antidepressants carry the risk of side effects that reduce patient appetite.[5,6] In these cases, agents that improve appetite are required to improve the patient's response to treatment. In our previous study, *trans*-cinnamaldehyde, the main component of cinnamon essential oil, and benzylacetone, a compound derived from heated agarwood, showed appetite-enhancing effects *via* inhalation and their effects were stronger than that of ghrelin.[7] It is known that chronic administration of ghrelin leads to increased body weight in rats.[8] However, it is not yet known whether the appetite-enhancing effects are affected by concentration,[9] and/or inhalation time,[10] and the results differ among inhalation tests. Because appetite may increase after inhalation of the active compounds, weight gain after chronic administration should be investigated. In this study, the effects of concentration and inhalation time on appetite-enhancement, and the effects of chronic administration for 14 d on weight gain were investigated.

## MATERIALS AND METHODS

**Materials** The compounds used in this study (Fig. 1) were as follows: Benzylacetone (purity >95%) was purchased from Tokyo Chemical Industry (Tokyo, Japan), *trans*-cinnamaldehyde (purity 98%) was purchased from Nacalai Tesque (Kyoto, Japan). Triethyl citrate (purity 98%), the odourless solvent used to dissolve and dilute the compounds, was purchased from Merck KGaA (Darmstadt, Germany). All other chemicals and reagents used in this study were of the highest grade available.

**Animals** The animal studies were designed according to the guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006) and the recommendations of the Animal Research Committee of Kyoto University (Kyoto, Japan; authorisation numbers, 2014−17). Four-week-old male ddY mice (approximately 16 g at the time of purchase) were obtained from Japan SLC. The mice were housed in colony cages (six mice per cage) at an ambient temperature of 25±2°C under a 12-h light–dark cycle. The mice used to measure locomotor activity (25 g at the time of the experiments) and those used to evaluate weight gain (20 g at the time of the experiments) were fed standard pellet chow and water *ad libitum*. Mice used in the feeding tests (19 g at the time of the experiments) were fasted for up to but not more than 24 h before starting the tests; water was available *ad libitum*. Fasting time was determined according to the previous studies conducted by Tankam *et al.* on the time taken to empty the stomach of 4 week-old ddY mouse.[11] All studies were conducted from 08 : 00 to 17 : 00.

**Feeding Test** Feeding tests were performed according to the previous study.[7] The aroma compounds were dissolved in and diluted with triethyl citrate (400 $\mu$L) and dropped onto four filter paper discs attached to the four corners of the glass cage (30 cm ×60 cm ×34 cm; 61.2 L). The cage was filled with the vaporised solution by natural diffusion for 60 min. Fasted mice inhaled the aromas in the cage for 1 h before the end of the fast. At the end of the fast, mice were taken from the glass cage, placed in plastic cages, and given approximately 10 g of weighed standard pellet chow. After 4 h of feeding, they were removed from the plastic cages and the remaining pellets were weighed to calculate food intake. Food intake was tested at various applied dosages ($7.4\times10^{-8}$–$7.4\times10^{-2}$ mg/L) and periods of inhalation (5, 15, 30, 60 min) of benzylacetone. All feeding tests were conducted between 10 : 00 and 14 : 00. The

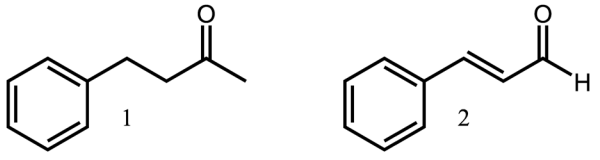


Fig. 1. Compounds Tested in This Study
1. Benzylacetone; 2. *trans*-Cinnamaldehyde

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control group inhaled air after triethyl citrate was introduced.

**Evaluation of Weight Gain Caused by Aroma Compound Administration over 14 d** Daily administration was performed according to the study of Yamamoto *et al.* to investigate the weight gain caused by administration of the aroma compounds once a day over 14 d.[3] The triethyl citrate solution containing the aroma compounds (85 $\mu$L) was dropped onto a filter paper disc attached to the upper wall of a plastic cage (13 L). Mice ($n$=5) were placed in the cage for 60 min after they and the pellet chow were weighed, and then returned to the colony cages. The first day of exposure to the compounds was regarded as day 0, and the body weights of the mice were recorded daily for 14 d.

**Evaluation of Spontaneous Motor Activity** In a 1-h test, benzylacetone and *trans*-cinnamaldehyde were vaporised in a glass cage (61.2 L) using the same procedure followed for the feeding tests. Sixty minutes after charging the solution, mice were placed individually in the centre of the cage, and monitored with a video camera for 60 min. The total spontaneous locomotor activity is represented by the area under the curve (*AUC*) with time (min) on the *x*-axis, and counts per 5 min of the mouse crossing over lines drawn at 10 cm intervals on the bottom of the cage on the *y*-axis.[12] Most of the effective compounds showed a two-phasic effect and effects at lower doses were considered true activities. This is because excitable activities such as jumping and rearing are observed when mice inhale higher doses of the compounds.[9] In 1-d test, benzylacetone and *trans*-cinnamaldehyde were vaporised in a plastic cage (13 L) using the same procedure followed for evaluation of weight gain. Mice were placed in the cages for 60 min, and subsequently moved to colony cages containing Igloo Fast-Tracs running wheels with counters for mice (MK-713/CU01, Muromachi Kikai Co., Tokyo, Japan). A single mouse was placed in each cage, and spontaneous locomotor activity was measured by rotations of the running wheel.

**Statistical Analysis** Results are presented as the mean±standard error of the mean (S.E.M). Statistical analyses were performed by Dunnett's test, two-way ANOVA and subsequent *t*-tests with Bonferroni testing for multiple comparisons[3,9] using GraphPad InStat (GraphPad Software Inc.) and R (R Development Core Team, R Foundation for Statistical Computing). A probability level of $p<0.05$ was considered statistically significant.[9]

## RESULTS

Benzylacetone showed significant appetite-enhancing effects at doses of $7.4\times10^{-6}$ to $7.4\times10^{-4}$ mg/L. The strongest effect was observed at $7.4\times10^{-6}$ mg/L (Fig. 2).

Food intake was compared at different exposure times (Fig. 3). Mice were administered the most effective amount of benzylacetone ($7.4\times10^{-6}$ mg/L) and the inhalation times were set to 5, 15, 30, and 60 min. Food intake increased in line with exposure times, namely, 1.158-fold (15 min), 1.203-fold (30 min), and 1.241-fold (60 min) compared with the control group. These results might have indicated that food intake increased in proportion to the total amount of benzylacetone administered, and would increase with more concentrated benzylacetone solutions ($7.4\times10^{-3}$ mg/L) applied for a shorter time (5 min). However, the food intake of mice exposed to more concentrated solutions was the same as that of mice

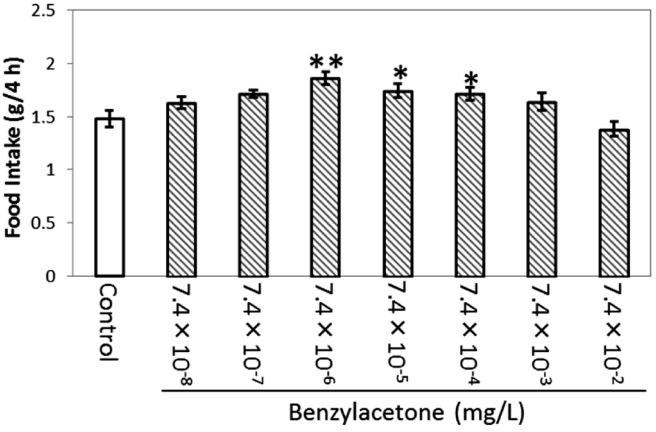


Fig. 2. Appetite Enhancement in Mice Treated with Several Applied Amounts of Benzylacetone

Control: triethyl citrate inhalation. Data are expressed as the mean±S.E.M. for 8 mice. *$p<0.05$, **$p<0.01$ *vs.* the control group. (One-way ANOVA followed by Dunnett's test.)

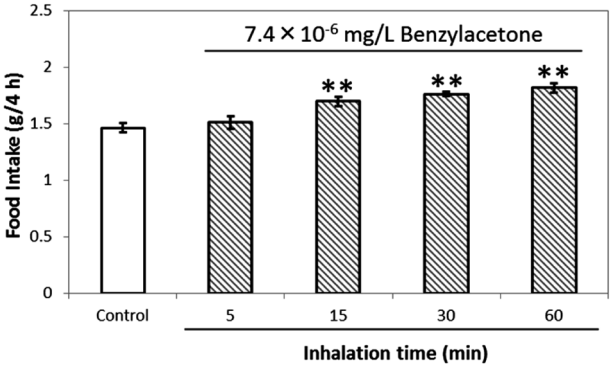


Fig. 3. Appetite Enhancement in Mice Exposed to Different Periods of Benzylacetone Inhalation

Control: triethyl citrate inhalation. Benzylacetone ($7.4\times10^{-6}$ mg/L) was inhaled by mice in the treated groups. Data are expressed as the mean±S.E.M. for 8 mice. **$p<0.01$ *vs.* the control group. (One-way ANOVA followed by Dunnett's test.)

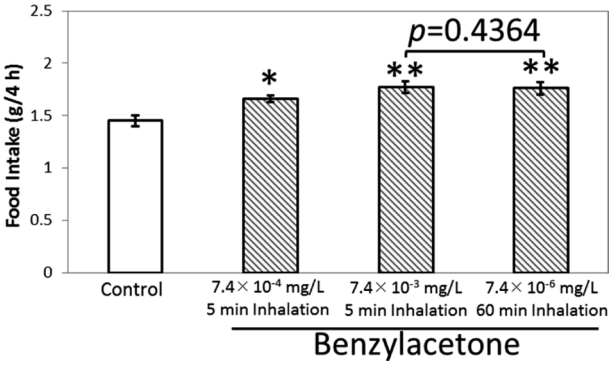


Fig. 4. Appetite Enhancement in Mice Inhaled $7.4\times10^{-3}$ mg/L of Benzylacetone for 5 min

Control: triethyl citrate inhalation. Data are expressed as the mean±S.E.M. for 8 mice. *$p<0.05$, **$p<0.01$ *vs.* the control group. (One-way ANOVA followed by Dunnett's test.)

treated with $7.4\times10^{-6}$ mg/L for 60 min (Fig. 4).

The body weights of mice exposed to benzylacetone and *trans*-cinnamaldehyde *via* inhalation increased during the 14 d of administration, and the groups gained weight of about 2.5 g and 1.5 g compared with the control groups, respectively (Fig. 5). A two-way (group×day) ANOVA using

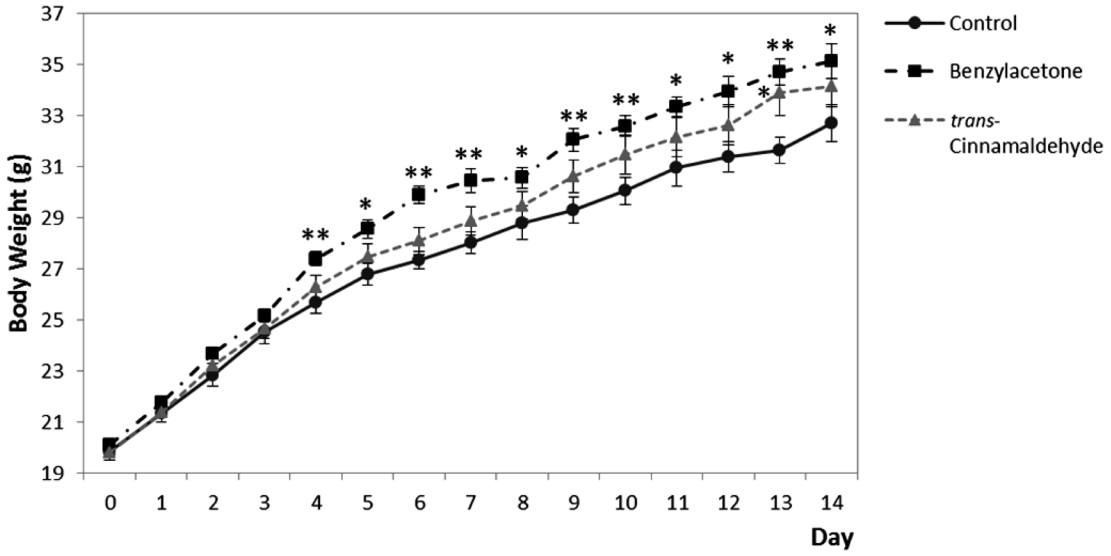


Fig. 5. Body Weights of Mice Treated Daily with Benzylacetone for 14 d

Data are expressed as the mean±S.E.M. for 5 mice. *$p<0.05$, **$p<0.01$ *vs.* the control group. (One-way ANOVA followed by Dunnett's test, and two-way ANOVA.)

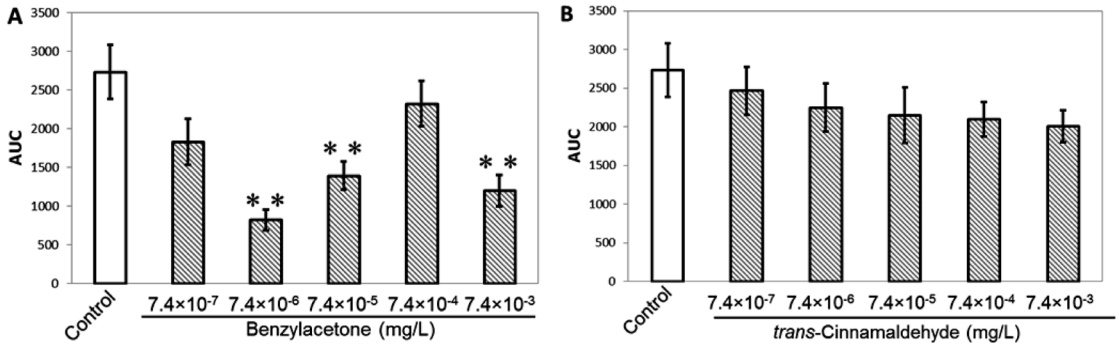


Fig. 6. Spontaneous Motor Activity after 1 h of Mice Treated with Benzylacetone (A) and *trans*-Cinnamaldehyde (B)

Control: triethyl citrate inhalation. Data are expressed as the mean±S.E.M. for 5 mice. **$p<0.01$ *vs.* the control group. (One-way ANOVA followed by Dunnett's test.)

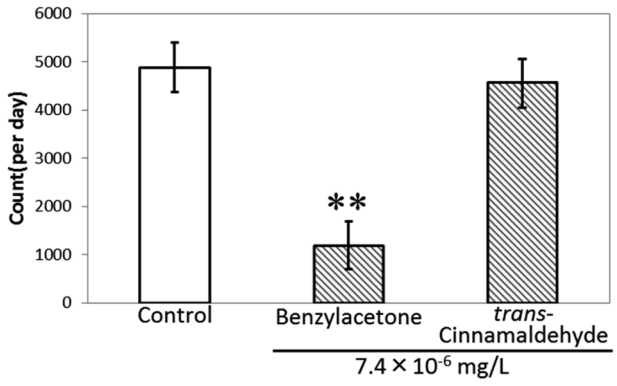


Fig. 7. Spontaneous Locomotor Activity throughout the Day after 1-h Inhalation of Aroma Compounds

Control: triethyl citrate inhalation. Data are expressed as the mean±S.E.M. for 5 mice. **$p<0.01$ *vs.* the control group. (One-way ANOVA followed by Dunnett's test.)

the results of the group exposed to benzylacetone revealed significant effects by both group [$F(1, 148)=7.0554$, $p<0.01$] and day [$F(14, 135)=96.818$, $p<0.001$]. The group×day interaction was shown to significantly affect body weight gain [$F(14, 135)=2.5238$, $p<0.01$]. *Post hoc* analysis revealed significant differences between benzylacetone and the control groups (days 5, 8, 11–12, and 14, $p<0.05$; days 4, 6–7, 9–10, and 13, $p<0.01$). A two-way ANOVA of the results after exposure to *trans*-cinnamaldehyde, revealed that the main effect was day [$F(14, 135)=125.25$, $p<0.001$]; *post hoc* analysis revealed a significant difference between *trans*-cinnamaldehyde and the control groups only on day 13 ($p<0.05$).

The spontaneous locomotor activity of mice measured for 1 h after administration of benzylacetone was 30% that of the control group at a dose of $7.4\times10^{-6}$ mg/L (Fig. 6A); however, dose-dependent decreases in locomotor activity following *trans*-cinnamaldehyde administration were not significant (Fig. 6B). Total spontaneous locomotor activity for a day after 1-h administration of benzylacetone was also 30% of the control group at a dose of $7.4\times10^{-6}$ mg/L, yet *trans*-cinnamaldehyde exposure did not result in a similar decrease at this dose (Fig. 7).

## DISCUSSION

Our study revealed time-dependent appetite-enhancing effects, while Satou *et al.* reported time-dependency on anxiolytic-like effects in mice.[10] Furthermore, Satou *et al.* reported cases of weaker effects at longer inhalation times. In this study, food intake in mice exposed to higher doses of

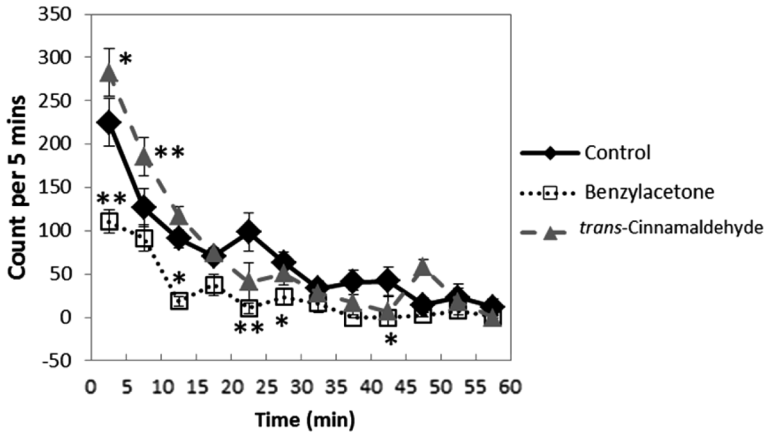


Fig. 8. Changes in Locomotor Activities in 1 h

Control: triethyl citrate inhalation. Data are expressed as the mean±S.E.M. for 5 mice. *$p<0.05$, **$p<0.01$ *vs.* the control group. (One-way ANOVA followed by Dunnett's test.)

the aroma compounds ($>7.4\times10^{-6}$ mg/L) was less than that in mice exposed to $7.4\times10^{-6}$ mg/L of benzylacetone (Fig. 2). Furthermore, food intake in mice exposed to either $7.4\times10^{-3}$ mg/L for 60 min or $7.4\times10^{-6}$ mg/L for 5 min did not increase. Food intake in mice exposed to $7.4\times10^{-3}$ mg/L for 5 min—the concentration and inhalation time that did not show appetite-enhancing effects—increased 1.2-fold compared with the control group (Fig. 4).

Hence, the results (Figs. 2, 3) would indicate that food intake increased in proportion to the total amount of benzylacetone administered to mice. Food intake in mice administered with higher doses ($7.4\times10^{-2}$ mg/L) of benzylacetone was lower than that of the control group (Fig. 2). This suggests that other mechanisms might have played a role after high dose administration.

Hur *et al.* reported that both food intake and body weight in rats are reduced after inhalation of high doses of fennel oil.[1] Choi *et al.* and Yamamoto *et al.*[2,3] also reported that high doses of geranium and *Osmanthus fragrans* essential oils reduce appetite. In these studies, the expression of pro-opiomelanocortin (POMC) mRNA increased and neuropeptide Y (NPY) mRNA expression decreased in the hypothalamus following inhalation of high doses of essential oils. Moreover, it is known that hypothalamic POMC reduces while hypothalamic NPY increases appetite. In our previous study, lower concentrations of geranium oil showed neither appetite-enhancing, nor appetite-reducing effects, and hypothalamic gene expression of NPY and POMC was similar to the control group.[7] These results suggest that higher doses of the aroma compounds might affect gene expression in the hypothalamus. Therefore, decreases in food intake at doses $>7.4\times10^{-6}$ mg/L might correlate with changes in the expression levels of NPY and POMC mRNA in the hypothalamus.

The results of two-way ANOVA showed that inhalation of the aroma compounds caused significant increases in body weight in the benzylacetone group; however, this was not the case in the *trans*-cinnamaldehyde group over 14 d, although both groups showed a tendency to gain body weight (Fig. 5). However, compared to total food intake in the control group over 14 d (418.9 g), food intake was 16.5 g higher in the benzylacetone group (total intake: 435.4 g) and 21.2 g higher in the *trans*-cinnamaldehyde group (total intake: 440.1 g). This would suggest that an appetite-enhancing effect is involved in the gain in body weight, but another cause may still exist to explain the weight gain observed in the benzylacetone group.

Takemoto *et al.* and Miyoshi *et al.* reported that the administration of benzylacetone leads to reduced locomotor activity in mice.[9,12] Likewise, locomotor-reducing effects were seen in the mice administered $7.4\times10^{-6}$ mg/L benzylacetone in our study, but not in mice exposed to the same dose of *trans*-cinnamaldehyde (Figs. 6A, B). Changes in locomotor activity measured for 1 h were compared between the benzylacetone and *trans*-cinnamaldehyde groups (Fig. 8). The locomotor activity of the former decreased immediately after inhalation commenced. In contrast, that of the *trans*-cinnamaldehyde group increased after 10 min, and thereafter appeared similar to the control group. This suggests that differences in spontaneous locomotor activities might affect body weight in mice; however, the locomotor-reducing effects observed for an hour in our study do not explain the results of the statistical analysis. Spontaneous locomotor activities during the day after exposure in the benzylacetone group were significantly decreased to 30% that of control group, but this effect was not seen in the *trans*-cinnamaldehyde group (Fig. 7). These results suggested that the gain in body weight in mice was affected not only by appetite-enhancing effects, but also by locomotor-reducing effects.

An imbalance between food intake and consumption of energy seemed to result in weight gain. Benzylacetone is thus an interesting compound because of its bifunctional locomotor-reducing and appetite-enhancing characteristics.

The appetite-enhancing effect of benzylacetone is thought to occur mainly *via* olfactory stimulation, because this effect is not observed when the compound is administered intraperitoneally.[7] Different from locomotor-reducing effects of benzylacetone,[13] its appetite-enhancing effects appear to result from olfactory stimulation by the aroma compound. To investigate the site of action for *trans*-cinnamaldehyde's appetite-enhancing effects, *trans*-cinnamaldehyde was dissolved and diluted in corn oil, and administered intraperitoneally (at doses of 0.01, 0.1 $\mu$g/kg).[7] The results indicated that food intake in the *trans*-cinnamaldehyde group was not significantly different from that in the control group (Fig. 9). This suggests that, like benzylacetone, *trans*-cinnamaldehyde has appetite-enhancing effects *via* olfactory stimulation, but whether this is the main mechanism of action remains un-

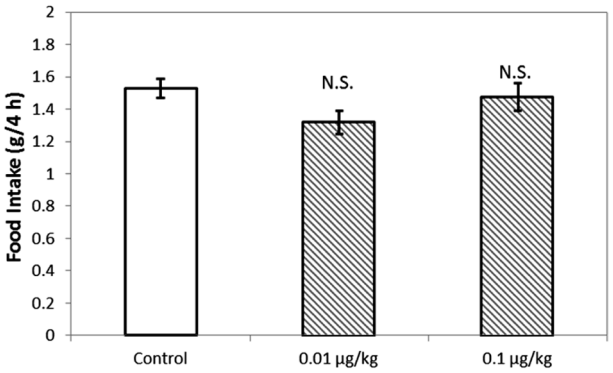


Fig. 9. Food Intake of Mice Treated with Intraperitoneal *trans*-Cinnamaldehyde

Control: triethyl citrate inhalation. Data are expressed as the mean±S.E.M. for 5 mice. N.S.: not significant ($p>0.05$). (One-way ANOVA followed by Dunnett's test.)

known. Therefore, increases in blood levels of benzylacetone and *trans*-cinnamaldehyde may not necessarily influence their appetite-enhancing effects.

The amounts of the compounds administered to the mice in this study were measured as milligrammes per cubic centimetre. This method is accurate and quantitative for measuring very small amounts of aroma compounds.

However, the amount of evaporating compound inhaled by mice is too small to measure accurately, and the results of these measurements are not reproducible. Thus, to examine any dose-dependency of the test compounds, the concentrations of the compound solutions applied to the disc papers in the cage were carefully adjusted during this study. It was assumed that the evaporation rates of the sample solutions were constant, and the results of experiments with benzylacetone showed a good association between dose and enhancement of appetite (Fig. 2).

Precise quantification of the volatile compounds will be required for the development of new techniques to enable further investigation into the biological effects of exposure to inhaled benzylacetone and *trans*-cinnamaldehyde.

**Conflict of Interest** The authors declare no conflict of interest.

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