Consumption of a variety of vegetables to meet Dietary Guidelines for Americans recommendations does not induce sensitization of vegetable reinforcement among adults with overweight and obesity: a randomized controlled trial

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Abbreviations: VI- Vegetable Intervention; AC – Attention Control; RRV – Relative Reinforcing Value; DGA – Dietary Guidelines for Americans; Body Mass Index – BMI; Resonance Raman Spectroscopy – RRS; 6-n-propylthiouracil – PROP; Three-Factor Eating Questionnaire – TFEQ; Standard Deviation – SD; Analysis of Covariance – ANCOVA; Bayesian Information Criterion – BIC

ABSTRACT

Background: Food reinforcement, or the motivation to obtain food, can predict choice and consumption. Vegetable consumption is well below recommended amounts for adults, so understanding how to increase vegetable reinforcement could provide valuable insight on how to increase consumption.

Objective: We sought to determine whether daily consumption of the Dietary Guidelines for Americans (DGA) recommendations for vegetable intake induces sensitization of vegetable reinforcement in adults with overweight and obesity.

Methods: Healthy adults with a BMI > 25 kg/m^2 and consuming ≤1 cup-equivalent of vegetables/day were randomly assigned to a vegetable intervention (VI; *n*=55) or an attention control (AC; *n*=55) group. The vegetable intervention consisted of the daily provision of vegetables in the amounts and types recommended by the DGA (~270 g/day) for 8 weeks. Participants were followed for an additional 8 weeks to assess sustained consumption. Compliance was measured weekly by resonance Raman light-scattering spectroscopy (RRS). Vegetable reinforcement was tested at weeks 0, 8, 12 and 16 using a computer choice paradigm.

Results: In the VI, RRS intensity increased from week 0 to 8 (22,990 and 37,220, respectively) returning to baseline by week 16 (27,300). No change was observed in the AC. There was no main effect of treatment (*P*=0.974) or time (*P*=0.14) and no treatment x time interaction (*P*=0.44) on vegetable reinforcement. There was no moderating effect of sex (*P*=0.07), age (*P*=0.60), BMI (*P*=0.46), delay discounting (*P*=0.24), PROP (6-*n*-propylthiouracil) taster status (*P*=0.15), or dietary disinhibition (*P*=0.82) on the change in vegetable reinforcement.

Conclusions: These findings suggest no effects of the provision of a variety of vegetables to meet DGA recommendations for 8 weeks on vegetable reinforcement and highlight the difficulty in increasing vegetable consumption in adults.

Key words: Vegetables, Relative Reinforcing Value, Food Reinforcement, Overweight, Obese, Incentive Sensitization

INTRODUCTION

Even though eating vegetables promotes health, consumption remains low (1, 2). Behavioral Choice Theory provides a framework to understand food choices and how to shift the choice from less healthy to healthier food options (3-5). Behavioral Choice Theory predicts that food choice can be shifted toward healthier options by increasing the reinforcing value of healthy foods relative to less healthy foods. A food's reinforcing value is defined as the amount of work a person will perform to gain access to food(s) of interest using an established operant responding paradigm (6-13) that has been shown to predict choice and intake (14). Therefore, it would be advantageous to develop ways to increase the reinforcing value of healthier foods such as vegetables, especially relative to less healthy food options; thus, tipping the decision-making process towards choosing to eat the vegetable.

Originally theorized in the biopsychological literature of drugs of abuse, incentive sensitization is the process by which the reinforcing value of a substance is increased through repeated exposure independent of hedonic factors such as liking (15). However, incentive sensitization occurs because of specific circumstances with individual differences in responsiveness to incentive sensitization paradigms. For example, among individuals with obesity, the reinforcing value of energy-dense foods (e.g., candy, cookies, chips) increases with daily exposure in as little as two weeks, even as liking wanes, whereas a decrease occurs in individuals with a healthy body weight (10, 12). Furthermore, the increase in the reinforcing value of energy-dense foods in individuals with obesity is dependent on how much is consumed – a larger portion (300 kcal) increases the reinforcing value more readily than a smaller portion (100 kcal) (12). These findings demonstrate that individuals with obesity are more susceptible to incentive sensitation, especially when a greater amount is consumed. Still, there are some psychological constructs known to affect food reinforcement that may impact the incentive sensitization process (16-18). Dietary disinhibition interacts with food reinforcement and moderates the relationship between food reinforcement and intake (16). There is a strong relationship between dietary disinhibition and impulsivity (assessed by delay discounting) (18). Impulsivity also moderates food reinforcement and influences intake (17). Furthermore, genetic variants in the ability to taste bitter flavors impact liking and consumption of vegetables and may influence the ability to increase vegetable consumption (19, 20).

Because vegetable consumption is well below the Dietary Guidelines for Americans (DGA)-recommended amounts for adults, understanding how to increase vegetable reinforcement provides valuable insight on how to increase consumption. Repeated exposure has been shown to increase the liking and intake of nutrient-dense foods (21-24); however, understanding whether incentive sensitization occurs for nutrient-dense foods is lacking. The primary aim of this randomized, controlled trial was to test whether sensitization of vegetable reinforcement occurs in overweight and obese individuals through daily exposure to amounts and types of vegetables recommended by the DGA. We hypothesized that daily consumption of vegetables to meet DGA recommendations would induce sensitization of vegetable reinforcement and that vegetable consumption would be increased above baseline after cessation of the intervention. In secondary *a priori* analyses, sex (25), BMI (10, 12), delay discounting (17, 18), PROP tasting status (19, 20), and dietary disinhibition (16) were also tested as potential *moderators* of the sensitization of vegetable reinforcement and sustained consumption.

SUBJECTS AND METHODS

Experimental protocol

The trial was conducted in accordance with CONSORT (**Supplemental Figure 1**) and registered at ClinicalTrials.gov as NCT02585102. All experimental procedures were conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983 and approved by the University of North Dakota Institutional Review Board. A comprehensive description of the study design and methods has been published (26). Briefly, men and women aged 18-65 years with a BMI ≥25 kg/m^2 and reporting consuming ≤1 serving of vegetables per day (excluding fried potatoes) were recruited for this 16-week randomized, parallel-group, non-blinded trial from the greater Grand Forks area through flyers and newspaper advertisements between October 2015 and January 2018. A serving of vegetables was defined as one cup-equivalent of raw leafy vegetables or 0.5 cup-equivalent cooked fresh, frozen, or canned vegetables, beans, and legumes [See **Supplemental Table 1** for gram amounts for each vegetable cup-equivalent]. Because DGA recommendations for vegetables are based on energy needs (27), individuals with overweight or obesity may find it more difficult to meet recommended intake amounts. Additionally, there is an inverse relationship between BMI and vegetable consumption (28). Taken together with findings that individuals with obesity are more susceptible to incentive sensitization, the current study focused on individuals with overweight and obesity in an effort to increase vegetable consumption in this population.

The study consisted of two arms – a vegetable intervention (VI) and an attention control (AC) – with a 1:1 allocation ratio. The VI group was provided a daily allotment of minimally processed (washed and portioned) DGA-recommended amounts and types of vegetables for the first 8 weeks to ensure recommendations from each vegetable group and subgroup (27) were achieved (**Supplemental Table 2**). Participants were then followed for an additional 8 weeks without provided vegetables to ascertain whether usual vegetable consumption changed from baseline amounts.

At the baseline study visit (week 0), energy needs were assessed in the morning after an overnight fast using indirect calorimetry (ParvoMedics, Salt Lake City, UT, USA) and adjusted for physical activity level (Stanford Brief Activity Survey (29)) to determine the amount of vegetables to provide as DGA recommendations are based on energy needs (e.g., 2.5 cup-equivalents for 2,000 kcal). Height was measured in triplicate to the nearest 0.1 cm using a stadiometer (Seca, Chino, CA, USA) and weight was measured in light clothing without shoes using a calibrated digital scale (Health-O-Meter Professional, McCook, IL, USA) to the nearest 0.1 kg.

To test potential moderators of incentive sensitization to vegetables and their sustained consumption participants completed validated questionnaires and the *n*-propylthiouracil (PROP) tasting test at baseline. The Three-Factor Eating Questionnaire (TFEQ) was used to measure cognitive control of eating behavior (dietary restraint), disinhibition of control (dietary disinhibition), and susceptibility to hunger (30). The Kirby Delay-Discounting Questionnaire (31) was used to measure temporal discounting of rewards. The Crowne-Marlowe Social Desirability Scale (32) was used to measure the potential influence of social desirability bias. PROP tasting status was measured by placing a piece of filter paper containing 6-*n*-propylthiouracil on the tongue for 30 seconds (20) and using the Labeled Magnitude Scale to rate the perceived intensity of the taste (33).

For the operant responding paradigm to establish vegetable reinforcement, participants tasted and rated their highest-liked vegetable (choice of red bell pepper slice, baby carrot, cucumber slice, and cherry tomato) and flavored cracker (choice of cheddar, sour cream, white cheddar, and pizza flavored Goldfish® Cracker) on a 10-point scale anchored by "Don't like at all" (1) and "like very much" (10). Participant's highest liked vegetable and cracker were used for the determination of vegetable reinforcement (6-8).

Participants also indicated their liking of 26 vegetables representing the 5 subgroups recommended by the Healthy US-Style Eating Pattern found in the 2010-2015 DGA – Dark Green, Red and Orange, Starchy, Beans and Peas, and Other, using a scale ranging from 1 (Do not like at all) to 7 (Like very much) at baseline and week 16. Participants could also indicate that they had never tried the vegetable and whether they were willing to try it.

The VI group was given a list of 31 vegetables grouped according to the 5 subgroups of vegetables in the DGA. Participants could freely choose from the list but were required to pick the recommended number of servings from each subgroup (**Supplemental Table 3**). Each serving equaled a one-half cup equivalent portion (44-80 g). Participants could also choose 100% vegetable juice but were limited to 4 servings a week. Beans, potatoes, and sweet potatoes were packaged cooked with no seasonings and all other vegetables were raw or frozen. Participants were provided with a recipe booklet and instructed to consume the vegetables whenever they wished and prepared how they desired. The only caveat was that they had to consume all provided vegetables.

For the AC group, participants completed the same study procedures as the VI group. However, no vegetables were provided, and participants were told to continue their usual diet.

To assist with adherence, participants kept a daily log of their vegetable consumption and skin carotenoid concentration was measured using resonance Raman light-scattering spectroscopy (RRS) (34). RRS-measured skin carotenoid concentration is a valid and reliable objective measure of vegetable intake that correlates strongly with changes in blood carotenoid concentrations and responds quickly to changes in vegetable intake (35). Furthermore, the slope of the increase in RRS intensities in response to increasing vegetable consumption is similar in individuals with a relatively high or low baseline RRS-measured skin carotenoid concentration (35). It is important to note that RRS monitors changes over time and is not used as a reference biomarker of absolute intake. Participant's vegetable intake logs and RRS-measured skin carotenoid concentration were assessed weekly, to instill accountability in participants.

Primary Outcome Measures

Vegetable reinforcement was measured in the morning after an overnight fast at weeks 0, 8, 12, and 16 using a computer choice paradigm as previously described (6-8). Briefly, participants played a computer game that mimics a slot machine, with points earned by clicking on the left mouse button, on two separated computer stations to assess the reinforcing value of their most-liked vegetable and cracker. For every 5 points earned participants received a 5-10 g portion of their most-liked vegetable or cracker depending on which computer they were working on. Points were earned on concurrent progressive, variable-ratio (± 5%) schedules starting at 4 clicks per point and doubling after each time the participant earned 5 points. Participants could freely choose which food option (vegetable or cracker) they wished to work for. The last schedule of reinforcement completed (P_{max}) for both the vegetable and cracker option was recorded and vegetable reinforcement was calculated as P_{max} for the vegetable / (P_{max} for the vegetable + P_{max} for the cracker). As calculated, a reinforcement value greater than 0.5 indicates a greater reinforcing value of the vegetable relative to the cracker.

STATISTICAL ANALYSIS

Power analysis and randomization

Assuming α = 0.05 and a between-subject SD = 240 operant responses (13), 50 subjects per group was determined to have 90% power to detect a mean difference of 160 operant responses during the RRV task between the vegetable intervention and attention control group at the end of the 8-week treatment period.

After the initial visit, participants were randomized to the VI or AC group using Taves Minimization to balance groups with respect to age (≤30, 31-50, 51-65 years), sex, and weight status (with overweight, with obesity) (36). Participants were enrolled by the Primary Investigator or a designee and assigned treatments by a statistician. Participant identifications were blinded to the statistician but not the researchers.

Data analysis

Data are presented as means ± SD or *n* (%) unless otherwise indicated. Data were analyzed using SAS for Windows, version 9.4 (SAS Institute, Inc., Cary, NC, USA). Significance was established at $P < 0.05$. Data from all randomized participants were included in a primary intent-to-treat analysis; a secondary per-protocol analysis with only participants who adhered to and complied with all study requirements was used as a sensitivity analysis. The per-protocol analysis revealed the same results as the intent-to-treat analysis.

Mixed linear models were used to test the effect of the intervention across time on RRS intensities, the last schedule completed (P_{max}) for both the vegetable and the cracker options, and the RRV of vegetables with time (weeks 0, 8 ,12,16) as the within-subject factor using a repeated measures covariant structure AR(1), treatment (VI or AC) as the between-subjects factor and subject as a random effect. The interaction between treatment and time was included in the model. Tukey's contrasts were used for post-hoc pairwise comparison of means.

All models were fitted with the Glimmix procedure. The Gaussian distribution was used to model the P_{max} for the vegetable and the cracker using the Identity link function. For the RRV of vegetables a “zero-inflation” factor of 0.01 and a “one-inflation” factor of 0.99 was applied. Because RRV is a ratio, the beta distribution was used to model the RRV of vegetables with the Logit link function. The ratio of the generalized chi-square statistic and its degrees of freedom was “1” indicating that the variability in these data was properly modeled, and that there is no residual overdispersion.

In separate models, each of the *a priori* potential moderators - age, sex, BMI (continuous), PROP taster status (participants were categorized into three groups; supertasters, medium tasters, and non-tasters based upon the mm marked), delay discounting, TFEQ subscale scores and social desirability bias - were included as a covariate and tested separately by including interaction terms with time and treatment group. Models incorporating random slopes, random intercepts or random slopes and intercepts were initially investigated. The random slopes models fit better using the BIC criterion for all cases and was thus used for the final models. For each moderator investigated, the model included the baseline value of the dependent variable, treatment, time and the interaction between the moderator, treatment, and time.

In post-hoc analyses, participants were subdivided based upon whether the vegetable reinforcement increased (sensitizers) or whether there was no change or a decrease (satiators) from baseline to week 8. Two-sided Fisher’s exact test was used to examine the association between sensitization/satiation and sex. Two-way analysis of variance was used to test whether BMI or PROP taster status (based upon the mm marked) differed between sensitizers and satiators. A mixed model ANCOVA was used to test whether RRS intensity differed between sensitizers and satiators at weeks 8, 12 and 16.

RESULTS

Participant Characteristics

The flow of applicants through the study with intent-to-treat (n = 110) through completers (n = 102) is show in **Supplemental Figure 1**. Following randomization, reasons for participants dropping out included time conflict (n = 4), inability to eat all the provided vegetables (n = 1), health reasons (n = 1), personal reasons (n = 1), and unable to complete study requirements (n = 1). For the intervention group, one participant dropped out prior to the initial study visit (week 0) and three more dropped prior to completing week 8 testing leaving 51 participants completing all study requirements. For the control group, four participants dropped out prior to completing the initial study visit (week 0) with the remaining 51 participants completing all study requirements. Baseline participant characteristics for the randomized intervention (n = 55) and control (n = 55) groups are presented in **Table 1**. Participants were a mean of 40 years of age and had a BMI of 34 kg/m^2. Participants were primarily non-Hispanic white (97%), female (75%), with obesity (68%).

Participant Compliance

The mean estimated energy need was 3140 kcal/day, thus participants were provided with approximately 4 cup-equivalents (~270 g) of vegetables daily. As shown in **Figure 1**, compliance with the vegetable exposure intervention, as measured by RRS, was excellent with a significant treatment x time interaction (P < 0.0001). For the VI, RRS intensity increased from 22,990 at week 0 to 37,220 at week 8; however, by week 16 had returned to week 0 levels (27,300). There was no change over time in the AC.

Vegetable Reinforcement

Based on a 10-point Likert scale (anchored by 1 = Don't like at all and 10 = Like very much), liking for the vegetable and cracker used for the vegetable reinforcement testing was 7 ± 2 and 7 ± 1, respectively. **Figure 2** displays the P_{max} (reinforcing value) of the cracker (**A**) and vegetable (**B**). There was no main effect of treatment on the P_{max} of the vegetable ($P = 0.28$) or cracker ($P = 0.53$). There was a main effect of time on the P_{max} of the vegetable ($P = 0.03$) and cracker ($P = 0.003$). Post-hoc analysis revealed no significant difference between weeks 0, 8, 12, or 16 for the P_{max} of the vegetable. However, post-hoc analysis revealed a decrease in the P_{max} of the cracker from week 0 to week 12 ($P = 0.01$) and week 16 ($P = 0.002$). There was no interactive effect of treatment and time on the P_{max} of the vegetable ($P = 0.53$) or cracker ($P = 0.64$).

Because vegetable reinforcement is calculated as a ratio, 0.5 is used as a cut-off to determine which alternative the participant finds more reinforcing. For the current study, a ratio > 0.5 means that the participant preferred the vegetable option over the cracker option. Our findings show that all but 12 participants preferred their most-liked vegetable over their most-liked cracker at all measured time points (**Figure 3**). There was no main effect of treatment ($P = 0.97$) or time ($P = 0.14$) and no treatment x time interaction ($P = 0.44$) for vegetable reinforcement.

Because the analysis revealed a preference for vegetables from the beginning of the study, the P_{max} of the vegetable and cracker and vegetable reinforcement were reanalyzed using baseline values as a covariate. There was no main effect of treatment on the P_{max} of the vegetable ($P = 0.28$) or cracker ($P = 0.40$). There was a main effect of time on the P_{max} of the vegetable ($P = 0.026$) and cracker ($P < 0.001$). Post-hoc analysis revealed a decrease in the P_{max} of the vegetable from week 0 to week 8 ($P = 0.049$) and a decrease in the P_{max} of the cracker from week

0 to week 12 ($P = 0.002$) and week 16 ($P < 0.001$) and from week 8 to week 16 ($P = 0.027$). There was no interactive effect of treatment and time on the P_{max} of the vegetable ($P = 0.52$) or cracker ($P = 0.58$). There was no main effect of treatment ($P = 0.20$) or time ($P = 0.095$) and no treatment x time interaction ($P = 0.40$) for vegetable reinforcement.

There was no main effect of treatment ($P = 0.94$), time ($P = 0.97$) and no treatment x time interaction ($P = 0.95$) on the liking of 26 vegetables representing the 5 subgroups.

Incentive Sensitization

Incentive sensitization (increased vegetable reinforcement after 8 weeks of repeated intake) occurred in 15 of the 51 VI participants. There was no sex ($P = 0.30$, two-sided Fisher's exact test), BMI ($P = 0.23$) or PROP taster status ($P = 0.61$) differences between the participants in which incentive sensitization occurred and the participants for which vegetable reinforcement stayed the same or decreased. Vegetable reinforcement also increased from week 0 to week 8 in 14 of the 51 AC participants. There was no sex ($P = 0.25$, two-sided Fisher's exact test), BMI ($P = 0.42$) or PROP taster status ($P = 0.26$) differences in the participants in which vegetable reinforcement increased between the two treatment groups. There was no interactive effect of treatment over time on RRS intensity in participants in which vegetable reinforcement increased from week 0 to week 8 ($P = 0.61$). Among the AC participants who demonstrated an increase in vegetable reinforcement RRS intensity was 25,067 ± 7,576 at week 0 and 25,009 ± 8,538 at week 8. Among the VI participants in which incentive sensitization occurred RRS intensity was 23,588 ± 10,255 at week 0 and 41,421 ± 14,898 at week 8. Among the VI participants in which incentive sensitization did not occur RRS intensity was 22,760 ± 8,447 and 35,469 ± 13,986 at weeks 0 and 8, respectively.

Putative Moderators of Incentive Sensitization

There was no moderating effect of sex ($P = 0.07$), age ($P = 0.60$), BMI ($P = 0.46$), delay discounting ($P = 0.24$), PROP taster status ($P = 0.15$), or dietary disinhibition ($P = 0.82$) on the change in vegetable reinforcement. Additionally, there was no moderating effect of dietary restraint ($P = 0.57$), susceptibility to hunger ($P = 0.40$), or social desirability ($P = 0.65$) on the change in vegetable reinforcement.

DISCUSSION

This is the first study to investigate whether repeated exposure to vegetables elicits incentive sensitization of vegetables in adults. Contrary to our hypothesis, daily consumption of DGA-recommended amounts and types of vegetables did not increase vegetable reinforcement. In individuals in which sensitization of vegetable reinforcement occurred there did not appear to be any benefit of the repeated exposure to increase vegetable consumption beyond the 8-week intervention when the vegetables were provided, at least for the high carotenoid vegetables as measured by RRS intensities. Sex, BMI, delay discounting, PROP tasting status, and dietary disinhibition did not moderate the sensitization of vegetable reinforcement. Furthermore, an equal number of participants in the VI and AC groups had an increase in vegetable reinforcement from baseline to week 8. Taken together, these results suggest that vegetable consumption can be increased by providing people with vegetables and asking them to consume them for a study/payment, but that repeated exposure to vegetables, as presented in the current study, does not promote incentive sensitization of vegetables.

A number of factors can influence the incentive sensitization process including dose, frequency, interval or pattern of exposures, number of exposures, and duration of exposure (37). Temple *et al*. (12) found that a 300-kcal portion of an energy-dense food, which is highly reinforcing, was needed for incentive sensitzation to occur in women with obesity. In the present study, the DGA-recommened amount of vegetable intake supplied the same amount of calories as was used in the aforementioned study. While disappointing to find that the same dose of vegetables did not elicit the same increase in reinforcment as energy-dense foods, vegetables do not naturally contain substances that can stimulate dopaminergic neural pathways to the same level as high sugar/high fat foods (38-40). It may be that the reinforcing nature of a food – its ablitiy to stimulate dopaminergic neural pathways – is the most important factor in eliciting incentive sensitization to result in changes in reinforcement. In the only other study examining the impact of repeated exposure to nutrient-dense foods on food reinforcement the investigators found that consuming a small serving (60-g portion) of the same nutrient-dense food for two weeks did not change the reinforcing value for that food (10). In the current study, daily exposure to vegetables for 8 weeks in a much larger quantity produced similar results on vegetable reinforcement.

While 8 weeks of daily exposure to vegetables in the current study did not promote incentive sensitization of vegetables and did not increase consumption after the 8-week exposure intervention, other studies do suggest that exposure can increase consumption, presumably though other mechanisms. In a recent meta-analysis, Appleton *et al.* (41) demonstrate increased liking for and consumption of vegetables following repeated exposure compared to no exposure, and while this meta-analysis focused on studies that only targeted vegetables, similar effects are also found in studies targeting fruits and vegetables. For example, in a recent study of older adults who reported consuming ≤2 servings (defined as an 80g portion) of fruits and vegetables per day, the investigators found that consuming 5 portions of fruits and vegetables for 16 weeks led to increased intake 18 months post-intervention (42). When looking at vegetable intake specifically, consumption went from less than one portion per day to 1.5 portions per day (42).

Given the 16-week duration of exposure in the aforementioned study (42), it is possible that the dose and frequency of our intervention was appropriate, but the duration of exposure was not optimal to elicit incentive sensitization of vegetables.

Interestingly, participants favored vegetables from the beginning of the study through the intervention and follow up, indicating that participants either always found the vegetables more reinforcing than the crackers used as the alternative choice or possibly that social desirability played a role in the choice of which food to play for. Although there was no moderating effect of measured social desirability, an implicit bias toward choosing to work for the perceived healthier option cannot be ruled out. Eating behavior is influenced by social context and the need to fit in with perceived norms or desire to affiliate (43). As such, volunteering to participate in a research study investigating ways to increase vegetable consumption at a Human Nutrition Research Center may have influenced the incentive motivational effect of the vegetable compared to the cracker option. Another explanation for these results is the potential influence of an external motivator, such as a reward. In their meta-analysis, Appleton, *et al*. (41) found that pairing repeated vegetable exposure with a reward resulted in greater and longer-term vegetable consumption. Although incentive sensitization occurred in 29% of the intervention participants, 28% of participants in the attention control group also had an increase in vegetable reinforcement during the 8-week intervention time frame. Based on these findings it cannot be ruled out that some participants may have viewed the compensation for study participation, and, for the VI, the provision of vegetables (free food), as a reward and this may have prejudiced the results. Further studies are needed to better understand the impact of repeated exposure on the incentive sensitization of vegetables.

The present study had some strengths that bear mentioning. First, the experimental design, DGA-recommended amounts and types of vegetables were provided for 8 weeks and our sample size was relatively large. Second, few studies have investigated whether incentive sensitization occurs in adults for nutrient-dense foods, and to date, none have focused on vegetables. Third, both women and men were included, in contrast to previous research on the RRV of nutrient-dense foods which was conducted only in women (10). Perhaps the greatest potential limitation of this study design was the possibility for habituation to occur (11, 44). Variety has been shown to decrease the rate of habituation (13, 45); however, individual differences in the rate of habituation are unknown. Although participants were free to choose from a variety of vegetables, habituation may have occurred within the 8-week time frame of the intervention in the participants who experienced a decrease in vegetable reinforcement. Further studies are needed to determine the optimal duration and variety needed for incentive sensitization to occur in adults.

In conclusion, repeated intake of vegetables in the amounts and types recommended by the DGA for 8 weeks did not cause incentive sensitization of vegetables. Decades of research and interventions have had limited success in increasing vegetable intake among adults, and our findings extend this body of literature to understand the impact of daily exposure to DGA-recommended amounts of vegetables on incentive sensitization. These results show that incentive sensitization does not readily occur with repeated vegetable consumption in adults suggesting that perhaps efforts need to be guided towards reducing the reinforcing value of energy-dense foods rather than increasing the reinforcing value of nutrient-dense foods in order to shift behavioral choice toward healthier food options.

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Table 1: Baseline characteristics of participants in the intent-to-treat analysis in the vegetable intervention (VI) and attention control (AC) groups[1]

Characteristics	**VI**	**AC**
Female	41 (75)	42 (76)
Weight Status		
Obese (BMI ≥ 30.0 kg/m^2)	37 (67)	38 (69)
PROP Tasting Status		
Medium	20 (36)	21 (38)
Super	9 (16)	9 (16)
Race/ethnicity		
White	54 (98)	52 (96)
Not Hispanic or Latino	51 (96)	48 (87)
Income		
<$50,000	23 (46)	28 (56)
$50,000-$99,999	14 (28)	15 (30)
≥$100,000	10 (20)	6 (12)
Age, *y*	40.4 ± 14.1	39.8 ± 15.8
BMI, *kg/m^2*	34.6 ± 7.3	34.2 ± 6.5
Estimated energy needs, *kcal/d*	3158 ± 822	3122 ± 849
RRS, *intensity*	22,990 ± 8,893	23,754 ± 8,835
TEFQ		
Dietary restraint score	6.8 ± 4.3	7.6 ± 4.1
Dietary disinhibition score	6.8 ± 3.0	7.8 ± 3.4
Susceptibility to hunger score	5.1 ± 3.0	5.8 ± 3.3
Delay discounting score	0.017 ± 0.023	0.016 ± 0.025
Social desirability score	20.0 ± 5.3	18.8 ± 5.8

[1]Values are mean ± SD, n=55 or frequency (percent). BMI - Body Mass Index; PROP - 6-n-propylthiouracil; RRS - Resonance Raman Spectroscopy; TEFQ - Three-Factor Eating Questionnaire

FIGURE LEGEND

Figure 1: Skin carotenoid status in adults with overweight or obesity who did or did not receive a vegetable intervention for 16 wk as measured by Raman light-scattering spectroscopy (RSS). Data are mean ± SD, n=55. *Different from AC at that time, P < 0.05. AC, attention control; VI, vegetable intervention.

Figure 2: Reinforcing value (P_{max}) of the cracker (A) and vegetable (B) in adults with overweight or obesity who did or did not receive a vegetable intervention for 16 wk. P_{max} represents the number of responses needed to earn a point for the last schedule of reinforcement completed. Data are expressed as back-transformed means (−1SE, +1SE), n = 55 per group. # Different from week 0 $P < 0.05$. AC, attention control; VI, vegetable intervention.

Figure 3: Relative reinforcing value of vegetables (RRV of vegetables) in adults with overweight or obesity who did or did not receive a vegetable intervention for 16 wk. Data are presented as box and whisker plots for both the intervention (participants who received a daily allotment of vegetables the amounts and types recommended by the Dietary Guidelines for American for 8 weeks, n = 55) and attention control groups (n = 55) at weeks 0, 8, 12 and 16. The dash line represents the breakpoint (RRV = 0.5) between preferring the vegetable option (RRV > 0.5) or the cracker option (RRV < 0.5). The box represents the 1st to the 3rd quartile, the vertical line in the box represents the median, the whiskers represent Tukey minimum and maximum values, and the dots represent outliers. AC, attention control; VI, vegetable intervention.