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ORIGINAL ARTICLE

Walnut consumption increases activation of the insula to highly desirable food cues: A randomized, double-blind, placebo-controlled, cross-over fMRI study

Olivia M. Farr Ph.D. | Dario Tuccinardi M.D. | Jagriti Upadhyay M.D. | Sabrina M. Oussaada | Christos S. Mantzoros M.D., D.Sc., Ph.D.

Division of Endocrinology, Beth Israel Deaconess Medical Center, Boston, Massachusetts

Correspondence
Olivia M. Farr, PhD, Division of Endocrinology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, ST820, Boston, MA 02215.
Email: ofarr@bidmc.harvard.edu

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Aims: The use of walnuts is recommended for obesity and type 2 diabetes, although the mechanisms through which walnuts may improve appetite control and/or glycaemic control remain largely unknown.

Materials and Methods: To determine whether short-term walnut consumption could alter the neural control of appetite using functional magnetic resonance imaging, we performed a randomized, placebo-controlled, double-blind, cross-over trial of 10 patients who received, while living in the controlled environment of a clinical research center, either walnuts or placebo (using a validated smoothie delivery system) for 5 days each, separated by a wash-out period of 1 month.

Results: Walnut consumption decreased feelings of hunger and appetite, assessed using visual analog scales, and increased activation of the right insula to highly desirable food cues.

Conclusions: These findings suggest that walnut consumption may increase salience and cognitive control processing of highly desirable food cues, leading to the beneficial metabolic effects observed.

KEYWORDS

appetite control, obesity therapy, randomised trial, type 2 diabetes

1 | INTRODUCTION

Obesity and type 2 diabetes are growing problems in industrialized countries.[1] A better understanding of dietary changes that may improve these disorders is required. Epidemiologic studies suggest that nut consumption reduces the risk of cardiovascular disease (CVD) and improves outcomes.[2–4] For instance, the Nurses' Health Study showed that consumption of 1 ounce or more of nuts at least 5 times weekly reduces the risk of CVD by 35% and improves lipid levels.[2] Additionally, nuts are included in the current standard of care dietary guidelines for diabetes released by the American Diabetes Association (ADA) because of their noted health benefits.[5] Although walnuts have specific properties, such as high alpha-linoleic acid (ALA) content, which may contribute towards reduced obesity and diabetes risk,[6,7] it remains unknown whether walnuts may also activate central nervous system (CNS) mechanisms implicated in energy homeostasis and/or insulin resistance/glycaemic control.

Our group has previously demonstrated that consumption of walnuts increases satiety and fullness.[7] One potential mechanism underlying the impact of walnuts on satiety could be changes in CNS activation to food cues with walnut consumption. Indeed, walnuts have previously been shown to improve memory and increase hippocampal N-methyl-D-aspartate (NMDA) receptor expression in rats, suggesting that they may have effects on the brain.[8] Some studies

The authors Olivia M. Farr and Dario Tuccinardi contributed equally to this work.

Abbreviations: ADA, American Diabetes Association; ALA, alpha-linoleic acid; BMI, body mass index; BOLD, blood oxygenation level dependent; CNS, central nervous system; CRC, clinical research center; CVD, cardiovascular disease; fMRI, functional magnetic resonance; FWE, family-wise error; GLM, general linear modeling; MPRAGE, Magnetization Prepared Rapid Gradient Echo; NMDA, N-methyl-D-aspartate imaging; TR, repetition time; TE, echo time; VAS, visual analog scales.

have suggested that they may also have neuroprotective effects and that they may mitigate some of the effects of aging.[9,10] These studies suggest that walnuts could impact many CNS areas, a hypothesis that remains to be confirmed in humans with well-controlled interventional studies. Herein, we focus specifically on how walnuts may impact eating behaviours and, more specifically, we examined how they may alter neural responses to visual food cues.

By understanding how walnut consumption may influence neurocognitive processes in obesity, we may be able to understand the mechanisms by which walnut consumption confers weight loss and other benefits, such as decreased risk of diabetes or improvement of glucose and lipid levels. Towards this end, we performed a randomized, placebo-controlled, double-blind, cross-over study to determine the impact of walnuts on the neural response to food cues using functional magnetic resonance imaging (fMRI). We employed a fruit smoothie delivery system with walnuts, or safflower oil and walnut flavouring, to disguise which smoothie contained walnuts and to allow for equal caloric and fat content, as previously described,[6,7] during 2 study admissions of 5 days each. The cross-over design allowed participants to serve as their own controls, which provided greater statistical power.

2 | METHODS

Ten adult participants with obesity (defined as body mass index, BMI ≥ 30 kg/m^2) provided written informed consent to participate in this double-blind, randomized (1:1), cross-over inpatient study to test the effects on appetite and satiety of either 48 g of walnuts per day or an isocaloric diet without walnuts. The study was approved by the Beth Israel Deaconess Medical Center (BIDMC) Institutional Review Board. Participants remained in the controlled environment of the BIDMC Clinical Research Center (CRC) for the entire duration of each arm of the study (5 days), which was separated from the other arm by a 1-month wash-out period. Walnuts/placebo were administered in the form of a smoothie with identical macronutrient content as previously described.[6,7] The walnuts were replaced by safflower oil (to replace the fat content of walnuts) and by walnut flavouring in the placebo smoothie, but all other content was the same. Participants did not report a taste difference between the 2 smoothies, which allows for blinding.[6,7] Isocaloric diets were designed by the study nutritionist on the basis of gender, weight and height, utilizing established formulas. Each study subject received an identical diet during both 5-day admissions to minimize variability and maximize power.

For each of the inpatient series, subjects were admitted to the CRC the night before the first day of the study. On day 1, subjects underwent baseline measurements, such as resting metabolic rates and body composition, and blood draws. Prior to leaving the CRC on day 5, subjects underwent resting metabolic rate and body composition measures. On this last day, they also underwent neurocognitive testing and an fMRI in the fasting state while viewing food cues. Before and after the scan, participants completed visual analog scales (VAS) to measure subjective feelings of hunger, appetite, and fullness. Subjects were discharged from the CRC after their final testing on day 5 and resumed their normal diet for a period of 5 weeks, after which time they returned to the CRC for a further 5 days for the second study visit. If they received placebo smoothie on the first visit, they had the walnut smoothie on the second visit and vice-versa.

2.1 | fMRI protocol and analysis

Participants viewed food and non-food items within a 3 Tesla GE MRI scanner at the MRI center at BIDMC in the fasting state with an InVivo Therapeutics 8-channel HD receiver head coil. Scanning was carried out using a protocol similar to that previously described.[11] First, in each of the scanning sessions, a T1-weighted MPRAGE (Magnetization Prepared Rapid Gradient Echo) structural MR image was acquired. Five 7-minute gradient-echo T2-weighted echo planar images depicting blood oxygenation level-dependent (BOLD) contrast were acquired from non-contiguous near-axial planes: repetition time, TR = 3.5 seconds; echo time, TE = 25 ms; in-plane resolution = 2.5 × 2.5 mm; matrix size = 96 × 96; field of view = 24 × 24 cm; voxel bandwidth = 83.33 kHz; slice thickness = 3 mm. E-Prime software controlled stimulus presentation. Images were presented in blocks, and each block was presented in a counter-balanced order, interspersed with periods of visual fixation.

The fMRI protocol consisted of 5 runs, during which subjects viewed blocks of highly desirable foods (high-calorie or high-fat images, eg, cakes, onion rings and similar foods), blocks of less desirable foods (low-calorie or low-fat images, eg, vegetables and fruits) and non-food images (eg, flowers, rocks, trees). The protocol allowed assessment of how subjects, using a response box held in their right hand, as previously described,[12,13] could imagine/visualize each image. Approximately 150 images were shown in random order. Blocks consisted of 5 images each, where each image was shown for 3 seconds (a total of 15 seconds for each block), with 10 seconds of fixation/rest between blocks, and 16 blocks were shown during each of the 5 runs.

BOLD data were preprocessed using the SPM8 (Statistical Parametric Mapping; The Wellcome Trust Centre of Neuroimaging; London, UK). Briefly, images of each individual subject were flipped, realigned (motion-corrected), normalized to an EPI template with affine registration followed by nonlinear transformation, and smoothed with a Gaussian kernel of 6 mm. A general linear model (GLM) was constructed for each individual subject, using onsets of the food or non-food image blocks with realignment parameters in 6 dimensions. The data were high-pass filtered to remove low-frequency signal drifts. The contrast images (highly desirable > less desirable food images; all food (highly and less desirable) > non-food images) of the first-level analysis were used for the second-level group statistics. A paired *t*-test was used to compare brain activations between consumption of walnuts and placebo; means ± standard deviations are given in the text. Given the multiple areas studied herein, activations which pass a corrected threshold of $P < .05$, family-wise error (FWE) corrected for multiple comparisons for cluster and/or peak activation are reported.

2.2 | Neurocognitive testing

Neurocognitive testing was undertaken after completion of the fasting MRI scan on the CANTAB (Cambridge Cognition; Cambridge, UK) device as described previously.[14]

3 | RESULTS

Of the 10 participants who participated in this study, 9 completed both fMRI scans and are included in subsequent analyses; 1 was unable to complete the fMRI because of an inability to remain awake during the scanning procedure. Participants were 51 ± 3 years of age, with a BMI of 37.0 ± 2.6 kg/m^2, and included 4 females (1 who did not complete the study) and 6 males. BMI and body weight did not change during the 5-day inpatient stay. As this was a cross-over design with a wash-out period, demographics were identical for the placebo and walnut phases; thus, we studied 9 participants receiving walnuts and the same 9 receiving placebo. When participants received walnuts, they reported feeling less hungry as compared to when they received placebo on a VAS (placebo, 7.65 ± 0.99; walnut, 6.12 ± 1.16; t = 2.26; $P < .05$) as well as feeling that they could eat a smaller quantity of food while consuming walnuts (placebo, 7.55 ± 0.99; walnuts, 6.44 ± 1.01; t = 2.46; $P < .04$).

Using a paired *t*-test, we observed an increased activation of the right insula with walnut consumption after 5 days in response to highly desireable as compared to less desirable food cues (X, Y, Z, 32,10,10; z = 133; size = 2494 mm^3; $P < .001$, uncorrected and $P < .003$; FWE corrected for cluster) (Figure 1). There were no significant changes in neurocognitive measures tested by the CANTAB device in verbal or working memory, intra-extra dimensional set shift, spatial span or cognitive control (data not shown). Activation of the insula while receiving walnuts correlated with verbal memory free recall and recognition recall, suggesting that increased activation of insula to food cues corresponds with better verbal memory (r = .82; <.006 and r = .69; $P < .04$, respectively). Change in activation of the insula as a result of consuming walnuts vs placebo correlated inversely with change in ratings of hunger and the quantity of food participants felt they could eat, suggesting that the greater the increase in activation of insula after walnut consumption, the less hungry participants would feel and the less food they could consume (r = −.63, $P < .045$ and r = −.67, $P < .035$, respectively).

4 | DISCUSSION

This study examines, for the first time, the impact of walnuts in the diet (as a smoothie consumed for breakfast at a standardized time for each of 5 days), on neuroimaging findings and neurocognitive mechanisms of food intake, using a placebo-controlled, double-blind, cross-over design. Our key finding is increased activation of the right insula following walnut consumption for 5 days in response to highly desirable (eg, high-fat or high-calorie) food cues among obese patients. As nuts are currently recommended for CVD and diabetes,[5] these findings extend the understanding of the mechanisms by which walnuts may promote healthier eating and weight control, and may indicate the mechanisms by which walnuts could decrease the risk of CVD and diabetes.

The increased activation of the insula in response to highly desirable foods observed herein could indicate several clinically applicable possibilities. The primary function of the insula, with relation to the behaviour of eating, is to provide representations of taste concentration and pleasantness, as well as of satiety.[15–17] However, since we used visual cues which would not have a physical taste component, the insula is probably involved in other processes. Typically, activation of the insula in response to food cues is higher in individuals with obesity and/or type 2 diabetes,[18,19] and this is probably explained by the role that the insula plays in reward responses and/or emotion regulation.[20–23] However, it is important to note that other areas of the insula are involved in salience processing, or in detection of critical/relevant cues, and in cognitive control.[24,25] For instance, activation of the insula has been shown to inversely correlate with trait impulsivity,[24] to increase during response inhibition, indicating the ability to inhibit a prepotent response (eg, to eating foods one knows one should not),[26] and the insula activates to saliency over valuation during decision-making tasks.[27] Thus, it is critical to understand where, within the insula, the activation shown in our study is taking place, and how this particular area may relate to the way the brain is processing these highly desirable food cues.

Notably, a resting-state study recently examined network connectivity that differentiated sections of the right insula into 3 main functional components.[28] In studying these areas more closely, we determined that the activation we observed is taking place within the dorsoanterior insula, which was related to a network of the anterior cingulate cortex and dorsolateral prefrontal cortex, areas that relate strongly to cognitive/inhibitory control.[28] Considering that we also observed correlations with reported measures of satiety, our findings support a role of the insula in improving cognitive control over food choices with dietary supplementation of walnuts. Thus, increased activation of the insula may indicate increased inhibitory control in the face of highly desirable (unhealthy, high-fat) food cues which, in

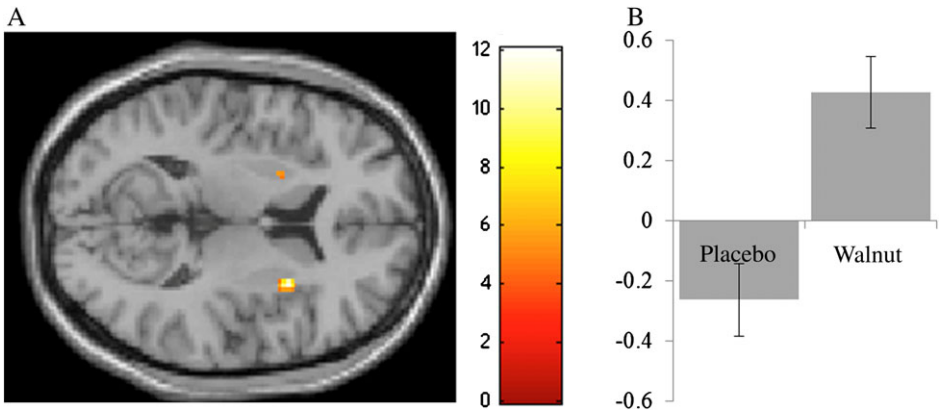

FIGURE 1 In a paired *t*-test, participants showed an increased activation of the right insula (A) after 5 days of walnut consumption vs placebo in response to highly as compared to less desirable food cues. Bold contrasts are superimposed on a T1 structural image in an axial section (z = 10, in neurological orientation). The colour bar represents the voxel *t*-score. Effect sizes (z-scores) are shown as mean with standard error bars (B)

turn, may lead to less high-fat or high-calorie food consumption and, eventually, to the previously observed improvement in metabolic parameters. These results suggest that eating walnuts may act at the level of the insula to alter food intake in obese participants. This will need to be confirmed in longer-term and larger studies.

This study has important strengths and limitations. It is strengthened by the use of a previously validated and tested placebo/walnut smoothie delivery system, which allowed for blinding of subjects and researchers.[6,7] Additionally, participants remained inpatients during the study, which allowed for control of environmental, dietary and lifestyle factors and for light-dark periods, which should have standardized their exposures to these factors and/or enhanced compliance, and thus limited variability in outcome. The sample size is relatively small, but this was an appropriately powered study, on the basis of clinical outcome, and was an inpatient study with a controlled diet (identical isocaloric diet during each visit) and with a cross-over design, which decreased potential differences/confounders and increased power. Patients were all obese, and these results may not be generalizable to the lean/healthy population. Additionally, we examined changes after 5 days of walnut consumption; thus, we can only comment on short-term neuroscientific changes. Our group has observed changes in satiety after 4 days in a previous study,[7] which is why we chose this duration. Future studies should examine how these changes may be altered with longer-term walnut consumption and among more subjects, stratified by gender and, possibly, by age. Furthermore, future studies should determine if these results are specific to walnuts or generalizable to other tree nuts, or to all nuts. This study points to mechanisms by which walnut consumption may promote healthier weight control and by which it may reduce the risk of diabetes, for which it is currently recommended by the ADA.[5] These results indicate that walnuts and/or other nuts may potentially be recommended to obese patients without diabetes, as part of a Mediterranean diet.

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Conflicts of interest

All authors have no conflicts of interest to disclose.

Author contributions

O. M. F., D. T., J. U., S. O., C. S. M. conducted research. O. M. F. analysed the data and wrote the manuscript. All authors reviewed and revised the final manuscript. O. M. F. has primary responsibility for final content.

REFERENCES

1. Hotamisligil G. Inflammation and metabolic disorders. *Nature*. 2006;542:177–185.
2. FB H, Stampfer MJ, Manson JE, et al. Frequent nut consumption and risk of coronary heart disease in women: prospective cohort study. *BMJ*. 1998;317:1341–1345.
3. Jiang R, Jacobs DR Jr, Mayer-Davis E, et al. Nut and seed consumption and inflammatory markers in the multi-ethnic study of atherosclerosis. *Am J Epidemiol*. 2006;163:222–231.
4. Albert CM, Gaziano JM, Willett WC, Manson JE. Nut consumption and decreased risk of sudden cardiac death in the Physicians' Health Study. *Arch Intern Med*. 2002;162:1382–1387.
5. Association AD. Standards of medical Care in Diabetes- 2017. *Diabetes Care*. 2017;40(suppl):S1–S135.
6. Aronis KN, Vamvini MT, Chamberland JP, et al. Short-term walnut consumption increases circulating total adiponectin and apolipoprotein A concentrations, but does not affect markers of inflammation or vascular injury in obese humans with the metabolic syndrome: data from a double-blinded, randomized, placebo-controlled study. *Metabolism*. 2012;61:577–582.
7. Brennan AM, Sweeney LL, Liu X, Mantzoros CS. Walnut consumption increases satiation but has no effect on insulin resistance or the metabolic profile over a 4-day period. *Obesity*. 2010;18:1176–1182.
8. Hicyilmaz H, Vural H, Delibas N, Sutcu R, Gultekin F, Yilmaz N. The effects of walnut supplementation on hippocampal NMDA receptor subunits NR2A and NR2B of rats. *Nutr Neurosci*. 2014;20:203–208.
9. Fisher DR, Poulose SM, Bielinski DF, Shukitt-Hale B. Serum metabolites from walnut-fed aged rats attenuate stress-induced neurotoxicity in BV-2 microglial cells. *Nutr Neurosci*. 2014;20:103–109.
10. Poulose SM, Miller MG, Shukitt-Hale B. Role of walnuts in maintaining brain health with age. *J Ntr*. 2014;144:561S–566S.
11. Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC. Leptin regulates striatal regions and human eating behavior. *Science*. 2007;317:1355.
12. Farr OM, Fiorenza C, Papageorgiou P, et al. Leptin therapy alters appetite and neural responses to food stimuli in brain areas of leptin-sensitive subjects without altering brain structure. *J Clin Endocrinol Metab*. 2014;99:E2529–2538.
13. Alonso-Alonso M, Ziemke F, Magkos F, et al. Brain responses to food images during the early and late follicular phase of the menstrual cycle in healthy young women: relation to fasting and feeding. *Am J Clin Nutr*. 2011;94:377–384.
14. Farr OM, Sofopoulos M, Tsoukas MA, et al. GLP-1 receptors exist in the parietal cortex, hypothalamus and medulla of human brains and the GLP-1 analogue liraglutide alters brain activity related to highly desirable food cues in individuals with diabetes: a crossover, randomised, placebo-controlled trial. *Diabetologia*. 2016;65:2943–2953.
15. Rolls ET. Taste, olfactory, and food reward value processing in the brain. *Prog Neurobiol*. 2015;127-128:64–90.
16. Dalenberg JR, Hoogeveen HR, Renken RJ, Langers DR, Horst GJ. Functional specialization of the male insula during taste perception. *Neuroimage*. 2015;119:210–220.
17. van Rijn I, de Graaf C, Smeets PA. Tasting calories differentially affects brain activation during hunger and satiety. *Behav Brain Res*. 2015;279:139–147.
18. Pursey KM, Stanwell P, Callister RJ, Brain K, Collins CE, Burrows TL. Neural responses to visual food cues according to weight status: a systematic review of functional magnetic resonance imaging studies. *Front Nutr*. 2014;1:7.
19. Ten Kulve JS, Veltman DJ, van Bloemendaal L, et al. Endogenous GLP-1 mediates postprandial reductions in activation in central reward and satiety areas in patients with type 2 diabetes. *Diabetologia*. 2015;58:2688–2698.
20. Berns GS, Capra CM, Moore S, Noussair C. Three studies on the neuroeconomics of decision-making when payoffs are real and negative. *Adv Health Econ Health Serv Res*. 2008;20:1–29.
21. Garrido MI, Barnes GR, Sahani M, Dolan RJ. Functional evidence for a dual route to amygdala. *Curr Biol*. 2012;22:129–134.

22. Vuilleumier P, Armony JL, Driver J, Dolan RJ. Distinct spatial frequency sensitivities for processing faces and emotional expressions. *Nat Neurosci*. 2003;6:624–631.
23. Seara-Cardoso A, Sebastian CL, Viding E, Roiser JP. Affective resonance in response to others' emotional faces varies with affective ratings and psychopathic traits in amygdala and anterior insula. *Soc Neurosci*. 2016;11:140–152.
24. Farr OM, Hu S, Zhang S, Li CS. Decreased saliency processing as a neural measure of Barratt impulsivity in healthy adults. *Neuroimage*. 2012;63:1070–1077.
25. Hendrick OM, Luo X, Zhang S, Li CS. Saliency processing and obesity: a preliminary imaging study of the stop signal task. *Obesity*. 2012;20:1796–1802.
26. Chikazoe J, Jimura K, Hirose S, Yamashita K, Miyashita Y, Konishi S. Preparation to inhibit a response complements response inhibition during performance of a stop-signal task. *J Neurosci*. 2009;29:15870–15877.
27. Litt A, Plassmann H, Shiv B, Rangel A. Dissociating valuation and saliency signals during decision-making. *Cereb Cortex*. 2011;21:95–102.
28. Chang LJ, Yarkoni T, Khaw MW, Sanfey AG. Decoding the role of the insula in human cognition: functional parcellation and large-scale reverse inference. *Cereb Cortex*. 2013;23:739–749.

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