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

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Abstract

Aims—The use of walnuts is recommended for obesity and type 2 diabetes, although the mechanisms through which walnuts may improve appetite and/or glycemic 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 one month.

Results—Walnut consumption decreased feelings of hunger and appetite assessed using visual analog scales and increased the 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.

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 cardiovascular disease (CVD) risk and outcomes [2–4]. For instance, the Nurses' Health Study showed that consumption of one ounce or more of nuts at least five times a week reduces CVD risk 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

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Author Contributions

OMF, DT, JU, SO, CSM conducted research. OMF analyzed the data and wrote the manuscript. All authors reviewed and revised the final manuscript. OMF has primary responsibility for final content.

Conflicts of Interest

All authors have no conflicts of interest to disclose.

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high alpha-linoleic acid (ALA) content, which may contribute towards reduced obesity and diabetes risk [6, 7], it remains unknown whether walnuts may also act to activate central nervous system (CNS) mechanisms implicated in energy homeostasis or insulin resistance/glycemic control.

Our group has previously demonstrated that walnuts increase satiety and fullness [7]. One potential mechanism underlying the impacts of walnuts on satiety could be changes of CNS activations 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 have suggested that they may also have some neuroprotective effects and that 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 behaviors, 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 impacts 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 flavoring, to disguise which smoothie contained walnuts and to allow for equal caloric and fat content, as previously described [6, 7], during both five-day-long study admissions. The cross-over design allows participants to serve as their own controls and increase power.

Methods

Ten adult participants with obesity (defined as Body Mass Index, BMI 30kg/m^2) provided written informed consent to participate in this double-blind, randomized (1:1), cross-over inpatient study to test the effects of either 48g of walnuts per day vs. an isocaloric diet without walnuts on appetite and satiety, which was approved by the Beth Israel Deaconess Medical Center (BIDMC) Institutional Review Board. Participants stayed 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 one month long 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 fat content of walnuts) and walnut flavoring in the placebo smoothie but all other content was the same. Participants do not report a taste difference between the two smoothies, which allows for blinding [6, 7]. Isocaloric diets were designed by the study nutritionist on the basis of the patient's gender, weight and height utilizing established formulas. Each study subject was fed an identical diet during both 5-day-long admissions to minimize variability and maximize power.

For each of the inpatient series, subjects were admitted to CRC the night before the first day of the study. On day 1, subjects had baseline measures such as resting metabolic rate, body composition measurements, and blood draws. Prior to leaving the CRC on day 5, subjects had 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 will resume 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.

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. Next, five seven-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= 3.5s, echo time, TE= 25ms, in-plane resolution= 2.5×2.5mm, matrix size= 96×96, field of view= 24×24cm, voxel bandwidth= 83.33kHz, slice thickness= 3mm. E-Prime software controlled stimulus presentation. Images were presented in blocks, and each block was presented in a counterbalanced order and interspersed with periods of visual fixation.

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

BOLD data was 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 6mm. A general linear model (GLM) was constructed for each individual subject, using the 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 walnut and

placebo conditions, and means $\pm$ standard deviations are shown 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.

Neurocognitive Testing

Neurocognitive testing was done after the completion of the fasting fMRI scan on the CANTAB (Cambridge Cognition; Cambridge, UK) device as per previous [14].

Results

Of the ten participants who participated in this study, nine completed both fMRI scans and are included in subsequent analyses; one was unable to complete the fMRI due to an inability to stay awake in the scanner. Participants were 51 ± 3 years of age, BMI $37.0 \pm 2.6 \text{ kg/m}^2$, and consisted of 4 females (1 did not complete)/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 on walnut and the same 9 on placebo. Participants reported feeling less hungry while on walnuts as compared to placebo on a VAS (placebo: $7.65 \pm .99$; walnut: 6.12 ± 1.16 ; $t = 2.26$; $p < .05$) as well as feeling like they could eat less quantity of food while on walnuts (placebo: $7.55 \pm .99$; walnut: 6.44 ± 1.01 ; $t = 2.46$; $p < .04$).

Using a paired t-test, we observe an increased activation of the right insula with walnut consumption after five days to highly 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). The activation of the insula while on walnuts correlated with verbal memory free recall and recognition recall, suggesting that increased activation of insula to food cues corresponds with better verbal memory ($r = .82$; $p < .006$ and $r = .69$; $p < .04$, respectively). The change in activation of the insula between walnut and placebo conditions correlates inversely with the change in ratings of hunger and the quantity the participants felt they could eat, suggesting that the greater the increase in activation of insula after walnuts, the less hungry and amount of food participants could consume ($r = -.63$, $p < .045$ and $r = -.67$, $p < .035$, respectively).

Discussion

This study examines for the first time the impact of walnuts in the diet (as a smoothie consumed at a standardized time every day every five days for breakfast), on neuroimaging 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 five-day walnut consumption to highly desirable (e.g. high fat or high calorie) food cues amongst obese patients. As nuts are currently recommended for CVD and diabetes [5], these findings extend this knowledge to understand the mechanisms by which walnuts may promote

healthier eating/weight and may indicate the mechanisms by which nuts may decrease CVD and diabetes risk.

The increased activation of the insula to highly desirable foods observed herein could indicate a few clinically applicable possibilities. The primary function of the insula with relation to the behavior of eating is to correspond to taste concentration and pleasantness as well as to fullness/satiety [15–17]. However, since we are showing visual cues which would not have a physical taste component, the insula is likely involved in other processes. Typically, the activation of the insula to food cues is higher in individuals with obesity and/or type 2 diabetes [18, 19], and this is likely due to 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 saliency processing, or the detection of critical/relevant cues, and cognitive control [24, 25]. For instance, activation of the insula has been shown to inversely correlate with trait impulsivity [24], increase during response inhibition indicating the ability to inhibit a prepotent response (such as eating foods you know you 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 located 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 three main functional components [28]. In studying these areas more closely, our activation is located within the dorsoanterior insula, which was related to a network of the anterior cingulate cortex and dorsolateral prefrontal cortex, areas which relate strongly to cognitive/inhibitory control [28]. Considering we also observe correlations with reported measures of satiety, our findings support a role of the insula in improving cognitive control over food choices with dietary walnut supplementation. Thus, increased activation of the insula may indicate increased inhibitory control towards highly desirable (unhealthy, high fat) food cues, which in turn, may lead to less high fat or high calorie food consumption, and eventually, the previously observed improvement of 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.

The study conducted herein has some important strengths and limitations. This study 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, patients were kept inpatient during their participation in the study, which allowed for the control of environmental, dietary, and lifestyle factors and light-dark periods which would have standardized their exposures and/or enhanced compliance and thus limited variability in terms of outcomes. The sample size is relatively small, but this was an appropriately powered study on the basis of clinical outcomes and an inpatient study with a controlled diet (patients consumed an identical isocaloric diet during each visit) and cross-over design, which decreased potential differences/confounders and increased power. The patients were all obese and these results may not be generalizable to the lean/healthy population. Additionally, we examined changes after five days of walnut consumption, so we can only

remark on short-term neuroscientific changes. Our group has observed changes in satiety after four days in a previous study [7], which was why we chose this length. Future studies may need to examine how these changes may be altered with longer-term walnut consumption and amongst more subjects stratified by gender and possibly age. Furthermore, future studies should determine if these results are specific to walnuts or generalizable to other tree, or all, nuts. This study points to mechanisms by which walnut consumption may promote healthier weight and by which they may reduce the risk of diabetes, for which they are currently recommended by the ADA [5]. These results indicate that walnuts and/or other nuts may also be helpful to recommend to patients with obesity without diabetes, potentially as part of a Mediterranean diet.

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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

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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 desirable vs. 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).