Diets Varying in Carbohydrate Content Differentially Alter Brain Activity in Homeostatic and Reward Regions in Adults

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ABSTRACT

Background: Obesity has one of the highest refractory rates of all chronic diseases, in part because weight loss induced by calorie restriction, the first-line treatment for obesity, elicits biological adaptations that promote weight regain. Although acute feeding trials suggest a role for macronutrient composition in modifying brain activity related to hunger and satiety, relevance of these findings to weight-loss maintenance has not been studied.

Objectives: We investigated effects of weight-loss maintenance diets varying in macronutrient content on regional cerebral blood flow (rCBF) in brain regions involved in hunger and reward.

Methods: In conjunction with a randomized controlled feeding trial, we investigated the effects of weight-loss maintenance diets varying in carbohydrate content [high, 60% of total energy: n = 20; 6 men/14 women; mean age: 32.5 y; mean BMI (in kg/m²): 27.4; moderate, 40% of total energy: n = 22; 10 men/12 women; mean age: 32.5 y; mean BMI: 29.0; low, 20% of total energy: n = 28; 12 men/16 women; mean age: 33.2 y; mean BMI: 27.7] on rCBF in brain regions involved in hunger and reward preprandial and 4 h postprandial after 14–20 wk on the diets. The primary outcome was rCBF in the nucleus accumbens (NAcc) at 4 h postprandial; the secondary outcome was preprandial rCBF in the hypothalamus.

Results: Consistent with a priori hypothesis, at 4 h postprandial, NAcc rCBF was 43% higher in adults assigned to the high- compared with low-carbohydrate diet (P [family-wise error (FWE)-corrected] < 0.05). Preprandial hypothalamus rCBF was 41% higher on high-carbohydrate diet (P [FWE-corrected] < 0.001). Exploratory analyses revealed that elevated rCBF on high-carbohydrate diet was not specific to prandial state: preprandial NAcc rCBF (P [FWE-corrected] < 0.001) and 4 h postprandial rCBF in hypothalamus (P [FWE-corrected] < 0.001). Insulin secretion predicted differential postprandial activation of the NAcc by diet.

Conclusions: We report significant differences in rCBF in adults assigned to diets varying in carbohydrate content for several months, which appear to be partially associated with insulin secretion. These findings suggest that chronic intake of a high-carbohydrate diet may affect brain reward and homeostatic activity in ways that could impede weight-loss maintenance. This trial was registered at clinicaltrials.gov as NCT02300857. J Nutr 2021;151:2465–2476.

Keywords: weight loss maintenance, dietary carbohydrate, brain activity, reward, obesity

Introduction

Obesity has one of the highest refractory rates of all chronic diseases in the United States and worldwide, with few indicators from epidemiological studies to suggest that recent research and policy efforts have led to an attenuation in prevalence (1). The first-line treatment for obesity, lifestyle modification (dietary restriction and physical activity), may achieve initial weight loss, but maintenance of reduced body weight remains challenging for most individuals (2, 3). A feature of many contemporary diets that might contribute to this challenge is macronutrient content. According to the carbohydrate–insulin model of obesity (4–6), the high insulin-to-glucagon ratio on high-carbohydrate/low-fat diets induces a cascade of metabolic events that lower energy expenditure and promote overeating among susceptible individuals.
Mechanisms that might drive weight regain on a high-carbohydrate/low-fat diet remain poorly understood. Neuroimaging studies indicate that brain activity in hedonic and homeostatic regions could play a critical role. Studies have demonstrated increased activity during periods of short-term hypoglycemia in regions involved in energy balance, particularly the hypothalamus, and those associated with reward processing 

[nucleus accumbens (NAcc)] (7–10).

Previous research from our group suggests that a high-glycemic-load meal lowers blood glucose and/or total circulating metabolic fuels in the late postprandial period (11–13) and increases regional cerebral blood flow (rCBF) in the NAcc (14), part of the mesoaccumbal reward circuitry implicated in craving and addiction (15–17). Thus, a high-carbohydrate/low-fat diet could elicit homeostatic and hedonic neurophysiological responses, leading to increased hunger and cravings with special preference for high-glycemic-index carbohydrates (9, 18, 19), and may also alter energy expenditure (20, 21), thereby propagating repeated cycles of overeating and weight gain. Most, but not all, single-meal studies support this possibility of a link between high-carbohydrate/low-fat diets elevating hunger and cravings (22, 23).

However, the relevance of these experimental and single-meal studies to mechanisms controlling chronic food intake remains uncertain. Here, we utilized the infrastructure of a large randomized control feeding trial (24) to investigate the effects of relatively long-term adherence to weight-loss maintenance diets varying in macronutrient content (high-, moderate-, or low-carbohydrate) on rCBF in key brain regions. Based on our prior findings at a late postprandial time point, the primary hypothesis was that rCBF in the NAcc at 4 h postprandial would vary by group, being higher on the high-carbohydrate diet than on the other two diets. In addition, we hypothesized that preprandial rCBF in the hypothalamus would vary similarly. In addition to these a priori hypotheses, we conducted exploratory analyses to evaluate whether group differences in rCBF in these regions and at the whole-brain level were specific to prandial state and to examine for unique individual susceptibility by testing whether diet groups differed in the relation between insulin secretion and postprandial rCBF in the NAcc.

Methods

Subjects

Subjects were recruited from the Framingham State Food Study [hereafter referred to as the parent study (24)], a randomized controlled trial examining long-term weight-loss maintenance on 3 diets varying in carbohydrate content (see Supplemental Figure 1 for study design). Subjects, recruited from the Framingham and Assabet Valley communities in Massachusetts, completed a 10- to 12-wk run-in phase during which they lost 12 ± 2% of their baseline body weight on a standard diet (45% of total energy carbohydrate/30% of total energy fat/25% of total energy protein) providing 60% of individually estimated energy needs. Subjects were then randomly assigned to 1 of 3 diets for 20 wk (the test diet phase), during which calorie prescription was individually adjusted to maintain weight within 2 kg of the post-weight-loss anchor. The macronutrient composition of the test diets (as a percentage of total energy) was as follows: high-carbohydrate (HIGH), 60% carbohydrate/20% fat/20% protein; moderate-carbohydrate (MOD), 40%/40%/20%; low-carbohydrate (LOW), 20%/60%/20% [examples of test meals available in Ref. (24)]. Energy content for the test diets was distributed throughout the day (22.5% for breakfast, 32.5% for lunch, 32.5% for dinner, 12.5% for evening snack), and the macronutrient composition of each meal and snack reflected the composition of respective test diets. All meals and snacks were prepared and provided by trained chefs, with weigh-backs at supervised meals and self-report at unsupervised meals to ascertain intake. Additional details of study design were previously published (24).

A subsample of subjects from the parent study enrolled in the brain imaging ancillary study (the current study) prior to test diet assignment, preserving the strength of the randomized design. During the run-in phase, subjects were informed about the current study through email advertisements and flyers. Subjects expressing interest underwent phone screening to assess eligibility. Provisionally eligible subjects were invited to an informational visit, at which we explained the current study in detail and obtained written informed consent. The study was approved by Partners Healthcare Human Research Committee. The initial recruitment date was October 29, 2014. Subjects were recruited from each of 3 consecutive cohorts (1 per academic year) enrolled in the parent study. See Supplemental Methods for inclusion/exclusion criteria.

We screened 124 subjects from the parent study, of whom 76 were enrolled in the current study, randomly assigned to test diets, and scheduled for study visits. Of the subjects who did not participate, 19 were excluded for not meeting criteria for the ancillary study, 12 for inability to commit to ancillary study participation, 15 were dismissed or withdrew from the parent study, and 2 were excluded for other reasons (Figure 1). After 4 withdrawals due to scheduling conflicts, data were acquired for 72 subjects [28 men, 44 women; mean BMI (in kg/m²) at MRI visit: 28.0; mean age: 32.8 y]. Subject characteristics, overall and according to test diet group, are provided in Table 1.

Experimental design and procedure

Following 14–20 wk on the test diets, subjects completed a morning visit after a 12-h overnight fast (see Supplemental Figure 2 for study protocol schematic). During this visit, subjects underwent 2 MRI sessions (pre- and postprandial), which included a pulsed arterial spin labeling (pASL) scan for quantification of CBF.

Subjects arrived at Brigham and Women’s Hospital MRI Research Center at 07:00 and completed appetite-related visual analog scales (VASS; 8 in total). Women completed a urine sample collection for human chorionic gonadotropin to rule out pregnancy. Height and weight were ascertained from the parent study for measurements taken the day prior to or following the MRI visit via scales equipped with wireless transmission of data to the parent study data manager. Each subject then completed the preprandial scanning session to examine chronic effects of diet on brain function in the fasting state; exited the scanner; completed the VAS measurements; and consumed their HIGH, MOD, or LOW breakfast meal within a 15-min period to standardize rate of intake across subjects. We used representative meals for each diet to examine chronic diet effects on brain function in the postprandial period, although we recognize that choice of other (standardized) meals...
with similar macronutrients could have had somewhat different effects due to the influence of other uncontrolled dietary factors. Meal onset was set as Time 0 (T0). For the following 3.5 h, subjects rested quietly in a comfortable room. Every 30 min through the end of the visit, subjects completed the appetite VASs (see Supplemental Methods). At 4 h following the completion of the meal, they underwent a late postprandial scanning session. This time point was chosen based on prior work demonstrating that the nadir of circulating metabolic fuels occurs between 3 and 5 h after intake of a high-carbohydrate meal (11, 25). After exiting the scanner, subjects completed the Food Craving Inventory (FCI; see Supplemental Methods).

**MRI scanning protocol**

Each subject completed 2 MRI scanning sessions (preprandial and postprandial) on a 3 Tesla Skyra whole-body system (Siemens Healthineers) with a 12-channel phased-array receive radiofrequency head coil. Each session included a pASL scan (Siemens PICORE Q2TIPS) and a T1-weighted structural scan (see Supplemental Methods).

**ASL data processing**

pASL data were converted to a quantitative CBF image and processed using Advanced Normalization Tools (University of Pennsylvania), Functional MRI of the Brain Software Library (Oxford University), and SPM version 12 (SPM12; University College London). See Supplemental Methods for additional ASL data processing details.

**Statistical analysis**

**ASL data statistical analysis.**

To address main hypotheses, following processing at the individual level, CBF images were analyzed at the group level using ANCOVAs for voxelwise, whole-brain comparisons between diet groups at each prandial time point. The (adjusted) ANCOVA models included the following variables: sex, age, percentage BMI change (pre-weight-loss baseline to MRI visit), and time on the test diet (all variables mean centered around the overall group mean, with the exception of categorical variables). In addition to the adjusted ANCOVA models, CBF images were similarly analyzed at the group level using unadjusted ANOVA models that did not include covariates. Proportional scaling of the CBF images was applied to normalize each subject’s CBF image and set to a grand mean scaled value of 50 mL·dL⁻¹·min⁻¹ to minimize intersubject variability. In addition to the overall model (F test) to test for any differences between groups, independent t tests were conducted to compare individual groups (HIGH compared with MOD, HIGH compared with LOW). Statistical significance was assessed according to Gaussian random field theory in SPM12 (26, 27) and set at P < 0.05 family-wise error (FWE; to control for multiple comparisons across whole brain) corrected at whole-brain peak voxel level, with a minimum cluster size of k = 50 for these a priori hypotheses. Based on specified hypotheses, search volumes for rCBF for these analyses were restricted to bilateral anatomical masks for a priori regions of interest (ROIs; preprandial: hypothalamus; postprandial: NAnc). Prior to statistical testing, anatomical coregistration between individual T1-weighted, magnetization-prepared, rapid gradient-echo images, individual CBF maps, the normalized Montreal Neurological Institute (MNI) standard T1, and bilateral anatomical masks for each ROI was manually checked for each subject to verify precision of coregistration and adequate coverage of the CBF map for each ROI. Anatomical borders of hypothesized regions were defined using a manually segmented MNI brain [based on methods established by the Center for Morphometric Analysis at Massachusetts General Hospital and Harvard Medical School (28, 29)]. Using REX (http://www.nitrc.org/projects/rex; Massachusetts Institute of Technology), rCBF values were extracted from clusters with in each a priori ROI that met statistical thresholds specified previously and exported to SPSS version 19 (IBM) for data visualization.

In addition to hypotheses for a priori ROIs, group differences in whole-brain activation (i.e., not restricted to a priori ROI masks) were examined at a conservative threshold to guard against spurious findings: P < 0.001, FWE-corrected at whole-brain peak voxel level, with a more conservative minimum cluster size of k = 100. Following the overall model (F test) to test for any differences between groups at the whole-brain level, post hoc, independent t tests were conducted to compare individual groups (HIGH compared with MOD, HIGH compared with LOW).

Furthermore, post hoc analyses examined 2 questions of interest. First, recognizing that analysis of a priori hypotheses might mask group
differences in each ROI at the alternative time point (hypothalamus
at 4 h postprandial and NAcc at preprandial), post hoc ROI analyses
were examined within each region at this additional time point,
for both the adjusted ANCOVA and unadjusted ANCOVA models
as described previously and using identical methods and statistical
thresholds. Furthermore, overall effects of diet group, prandial state,
and the diet group × prandial state interaction were explored
using adjusted repeated-measures ANCOVA and unadjusted repeated-
measures ANOVA models incorporating data from both time points.
For these repeated-measures models, statistical significance was assessed
at 0.05 FWE-corrected, using small volume correction (SVC)
restricting search area to the anatomical mask for each ROI, with a
minimum cluster size of k = 10.

A second set of exploratory analyses assessed relations between
postprandial rCBF in the NAcc and insulin secretion. (The NAcc
was chosen as a focus for these post hoc analyses given that it is a
relatively discrete, homogeneous region whose primary functionality
in the domain of reward anticipation and processing has been firmly
characterized. This is in contrast to the hypothalamus, which includes
distinct nuclei involved in hunger and in satiety, the size and extent of
which cannot be discerned at the spatial resolution available using ASL.)
Pre-weight-loss early phase insulin secretion [insulin concentration
30 min after oral glucose (insulin-30) obtained at the pre-weight-loss
time point (prior to the run-in diet) as part of the parent study protocol]
was chosen based on our prior data suggesting an effect modification
by pre-weight-loss insulin secretion on group differences between the
HIGH and LOW groups for the primary outcome of the parent study
change in total energy expenditure).

A multiple regression model, implemented in SPM12, was used to
test the model of slope differences between the HIGH and LOW
groups. To examine whether relations were maintained at a time point
concurrent with measurement of rCBF, early phase insulin secretion
(insulin-30, obtained at the end of the test diet−weeks 18−20) was
additionally examined in a separate model. These analyses explored
whether diet groups differed in the relation between insulin secretion
and rCBF in the right NAcc. Additional relevant covariates [age, sex,
percentage BMI change (pre-weight-loss baseline to MRI visit), time on
the test diet] were also included in these adjusted regression models,
as in primary ANCOVA models discussed previously. Variables (with
the exception of sex) were mean centered for the overall mean of
the individual group. In addition to adjusted regression models, slope

differences between the HIGH and LOW groups in the relation between
NAcc rCBF and insulin secretion (baseline and end of test diet) were
similarly analyzed at the group level using unadjusted regression models
that did not include covariates. Statistical significance was assessed
at P < 0.05, FWE-corrected (minimum cluster size: k = 10), using
small volume correction (SVC), which restricted the search area to the
right NAcc cluster that was identified in the primary ANCOVA (or
ANOVA for the unadjusted models) model as showing a difference
between groups. This approach was used to ensure that the results of
this exploratory multiple regression analysis would translate to effect
modification of any ANCOVA (or ANOVA for the unadjusted models)
effects. rCBF values were extracted from the single cluster meeting this
threshold using REX and exported to SPSS version 19 for data visual-
ization and calculation of individual group correlation coefficients.

Behavioral data analysis.
Behavioral data (VAS ratings, FCI total and subscale scores) were
analyzed using SPSS version 19. Comparisons between the 3 diet groups
were completed using 1-way ANOVAs. Post hoc independent samples t
tests were conducted following 1-way ANOVAs for which the omnibus
F test was significant. Statistical significance was set at a threshold of
P < 0.05.

Results
Effect of diet on 4-h postprandial blood flow
Resting rCBF in the NAcc at 4 h postprandial, the primary
endpoint, differed significantly by group [adjusted model:
P(FWE-corrected) < 0.01; Table 2, Figure 2]. The peak voxel
of this cluster was localized in the right NAcc. In comparisons
between groups, postprandial NAcc rCBF in the HIGH group
was 43% higher than that in the LOW group [adjusted model:
P(FWE-corrected) < 0.05; Table 2]. HIGH and MOD
groups did not differ significantly in postprandial NAcc rCBF.
At the whole-brain level (outside of the a priori ROI), the
HIGH group exhibited higher postprandial rCBF compared
with the LOW group in the cerebellum (Supplemental Table
1, Figure 3). Unadjusted models yielded similar results in the

| Characteristics | All participants (n = 72) | LOW (n = 28) | MOD (n = 23) | HIGH (n = 21) |
|-----------------|--------------------------|-------------|-------------|--------------|
| Continuous variables, mean ± SD |
| Age, y          | 32.8 ± 11.3              | 33.2 ± 11.0 | 32.5 ± 12.4 | 32.5 ± 11.1 |
| BMI, kg/m²      | 28.0 ± 4.3               | 27.7 ± 4.6  | 29.0 ± 4.2  | 27.4 ± 4.2  |
| Pre-weight-loss baseline | 12.0 ± 2.4       | 12.5 ± 2.7  | 11.6 ± 2.3  | 11.8 ± 2.3  |
| Percentage change2 | 0.7 ± 0.2            | 0.7 ± 0.2   | 0.7 ± 0.1   | 0.7 ± 0.1   |
| Test diet week at MRI visit | 16.9 ± 1.5     | 17.2 ± 1.6  | 16.5 ± 1.4  | 16.9 ± 1.4  |
| Categorical variables, no. (%) |
| Male            | 28 (39)                  | 12 (43)     | 10 (43)     | 6 (29)       |
| Female          | 44 (61)                  | 16 (57)     | 13 (57)     | 15 (71)      |
| Race            |
| Caucasian       | 54 (75)                  | 19 (68)     | 18 (78)     | 17 (81)      |
| African American| 10 (14)                  | 5 (18)      | 4 (17)      | 1 (5)        |
| Asian           | 3 (4)                    | 1 (3)       | 1 (4)       | 1 (5)        |
| Other           | 5 (7)                    | 3 (11)      | 0 (0)       | 2 (9)        |
| Hispanic ethnicity | 10 (14)              | 4 (14)      | 3 (13)      | 3 (14)       |

1HIGH: high-carbohydrate diet; LOW: low-carbohydrate diet; MOD: moderate-carbohydrate diet.
2Change from pre-weight-loss baseline to MRI visit.
TABLE 2  Adjusted model\(^1\) of effect of diets varying in carbohydrate-to-fat ratio on preprandial blood flow in the hypothalamus and 4-h postprandial blood flow in the nucleus accumbens (primary endpoint) in adults assigned to LOW, MOD, and HIGH diets

| Table 2 | Peak For | Peak Z | R/L | k(E) | P (FWE-corrected) | P (uncorrected) |
|---------|---------|--------|-----|------|-----------------|----------------|
| Preprandial: hypothalamus | Any group difference | 38.30 | 6.61 | R | 179 | <0.001 | <0.001 |
| HIGH > MOD | No significant clusters | | | | | | |
| HIGH > LOW | 8.68 | 6.96 | R | 179 | <0.001 | <0.001 |
| MOD > HIGH | No significant clusters | | | | | | |
| LOW > HIGH | No significant clusters | | | | | | |
| 4 h postprandial: NAcc | Any group difference | 20.59 | 5.12 | R | 58 | <0.01 | <0.001 |
| HIGH > MOD | No significant clusters | | | | | | |
| HIGH > LOW | 5.14 | 4.66 | R | 63 | <0.05 | <0.001 |
| MOD > HIGH | No significant clusters | | | | | | |
| LOW > HIGH | No significant clusters | | | | | | |

\(^1\) Covariates included in adjusted (ANCOVA) model: sex, age, diet week, % BMI change. FWE, family-wise error; HIGH, high-carbohydrate diet; MOD, moderate-carbohydrate diet; NAcc, nucleus accumbens; R, right.

Effect of diet on preprandial blood flow

Preprandial resting rCBF in the hypothalamus differed significantly by group [adjusted model: \(P\) (FWE-corrected) < 0.001; Table 2, Figure 2]. The peak voxel of this cluster was localized in the right hypothalamus. In comparisons between groups, preprandial hypothalamic rCBF in the HIGH group was 41% higher than the LOW group [adjusted model: \(P\) (FWE-corrected) < 0.001; Table 2]. Preprandial hypothalamic rCBF in the HIGH group did not differ significantly from that in the MOD group. At the whole-brain level (outside of the a priori ROI), groups differed significantly in preprandial rCBF in the caudate, putamen, anterior cingulate gyrus, middle frontal gyrus, precentral gyrus, fusiform gyrus, and posterior cingulate gyrus. Post-hoc between group comparisons indicated higher preprandial rCBF in the HIGH vs. LOW group in the pulvinar nucleus, caudate, anterior cingulate gyrus, insula, angular gyrus, and occipital gyrus (Supplemental Table 1, Figure 4). Unadjusted models yielded similar results in the hypothalamus (Supplemental Table 2) and at the whole-brain level (Supplemental Table 3).

Exploratory analyses on preprandial blood flow in the nucleus accumbens and 4-h postprandial blood flow in the hypothalamus

To test whether diet group effects for each ROI (NAcc, hypothalamus) extended to the alternate prandial state, we conducted exploratory analyses on 1) preprandial resting rCBF in the NAcc and 2) 4-h postprandial rCBF in the hypothalamus. Preprandial resting rCBF in the NAcc differed significantly by group [adjusted model: \(P\) (FWE-corrected) < 0.001; Supplemental Table 4, Figure 5]. In comparisons between groups, preprandial NAcc rCBF in the HIGH group was 51% higher than that in the LOW group [adjusted model: \(P\) (FWE-corrected) < 0.001; Supplemental Table 4]. Preprandial NAcc rCBF in the HIGH group did not differ significantly from that in the MOD group. Resting rCBF in the hypothalamus at 4 h postprandial differed significantly by group [adjusted model: \(P\) (FWE-corrected) < 0.001; Supplemental Table 4, Figure 5]. Between-group comparisons revealed 4-h postprandial hypothalamic rCBF in the HIGH group was 36% higher than that in the LOW group [adjusted model: \(P\) (FWE-corrected) < 0.001; Supplemental Table 4]. Postprandial hypothalamic rCBF in the HIGH group did not differ significantly from that in the MOD group. Unadjusted models yielded similar results in the hypothalamus and NAcc (Supplemental Table 5).

These findings were further interrogated using a diet group \(\times\) prandial state repeated-measures ANCOVA, yielding a main effect of diet group in the hypothalamus [adjusted model: \(F = 6.73; k = 82; P\) (FWE-corrected) < 0.05; MNI coordinates: 4, 2, –14] and in the NAcc [adjusted model: \(F = 7.80; k = 47; P\) (FWE-corrected) < 0.05; MNI coordinates: 8, 20, –2]. At the whole-brain level (outside of the a priori ROI), there was no significant main effect of diet group. Unadjusted models yielded similar results in the hypothalamus [\(F = 6.55; k = 90; P\) (FWE-corrected) < 0.05; MNI coordinates: 4, 2, –14], but this model was not significant in the NAcc [\(F = 5.72; k = 36; P\) (FWE-corrected) = 0.11; MNI coordinates: 16, 16, –6]. There was no significant main effect of prandial state and no significant diet group \(\times\) prandial state interaction effect.

Relationship between insulin secretion and 4-h postprandial blood flow in the nucleus accumbens

We explored effect modification by insulin secretion, as previously hypothesized (4), as a basis for understanding individual differences in response to carbohydrate. Analysis of the effect modification was focused specifically within the (right) NAcc cluster in which HIGH and LOW groups showed significantly different rCBF. As depicted in Figure 6A, diet groups differed in their relation between postprandial right NAcc rCBF and pre-weight-loss insulin secretion (insulin-30 at pre-weight loss). Insulin-30 at pre-weight-loss baseline was
positively associated with right NAcc rCBF in the LOW group ($r = 0.35$) but negatively associated in the HIGH group ($r = -0.47$), with significant effect modification by group [$t = 2.87$; $k = 37$; $P$(FWE-corrected) < 0.05; $P$(uncorrected) < 0.005; MNI coordinates: 10, 18, -10]. There was no significant relation between pre-weight-loss insulin secretion and postprandial right NAcc rCBF in the MOD group.

Similarly, insulin-30 at end of test phase (18–20 wk after start of diet) was negatively associated in the HIGH group ($r = -0.48$), with effect modification by group significant at an uncorrected $P$ level [$t = 2.17$; $k = 16$; $P$(FWE-corrected) = 0.14; $P$(uncorrected) < 0.05; MNI coordinates: 14, 20, -8; Figure 6B]. This effect modification by group for postprandial right NAcc rCBF and insulin-30 at the end of test phase held at a trend level in an unadjusted model [$t = 2.59$; $k = 29$; $P$(FWE-corrected) = 0.07; $P$(uncorrected) < 0.01; MNI coordinates: 10, 18, -10]. There was no significant relation between end of test phase insulin secretion and postprandial right NAcc rCBF in the LOW or MOD groups.

**Effect of diet on pre- and postprandial appetite and recent food craving ratings**

Ratings of hunger and desire to eat one’s favorite food [4.5-h AUC and time-240 minutes (T240) measurements] ascertained via VASs did not differ significantly by group (Table 3, Supplemental Figure 3). Similarly, ratings of cravings for specific food categories (high-fat, fast food, carbohydrates/starches, sweets), as assessed via total and subscale scores on the FCI, did not differ significantly by group (Supplemental Figure 4).

**Discussion**

Despite advances in the understanding of effects of weight loss on central nervous system–mediated function (30–32) and evidence for the role of extrahypothalamic regions in modulating the response to variation in macronutrient content (14, 33, 34), translation of these results to weight-loss maintenance remains unclear. Here, we found evidence that intake of diets differing in carbohydrate content over 14–20 wk induces potentially clinically relevant effects on activity in brain regions involved in energy balance, reward, and addiction. In support of our hypotheses, individuals randomly assigned to the low- compared with high-carbohydrate diet demonstrated lower blood flow to the hypothalamus in the fasting state and also to the NAcc at 4 h following intake of diet-representative meals. In addition to these primary outcomes, exploratory follow-up analyses revealed that reductions in blood flow in the low-carbohydrate group were not specific to prandial time point but were evident both pre- and postprandially, suggesting the chronic impact of these diets on blood flow in NAcc and hypothalamus dominate acute responses to a meal. Furthermore, early phase insulin secretion emerged as an effect modifier in the relation between diet group and postprandial NAcc blood flow. Finally, in contrast to our earlier study on acute brain effects of high carbohydrate (14), we did not observe group differences in subjective hunger or food cravings, possibly due to habituation from recurrent intake of diet-specific foods and the lack of sensitivity of these rating scales to chronic effects. Taken together with findings from the parent study of lower total energy expenditure on the high-carbohydrate diet (20), these data provide insights into physiological and neural mechanisms underlying challenges to maintenance of diet-induced weight loss, potentially informing the design of more effective therapies.
Recent neuroimaging studies have illustrated the impact of obesity interventions on brain activity, including behavioral (30, 35, 36) and surgical (32, 37) approaches to weight loss. Although these findings have been informative as to mechanisms of initial weight loss, they do not address the prevalent occurrence of weight regain nor identify more effective therapeutic options. Drummen and colleagues examined whether diets varying in protein content induced differential effects on the BOLD response (38). Although brain activity was related to daily protein intake across groups, there were no differences between diet groups in brain activity. However, comparison of these findings with ours warrants consideration of differences in design, sample size, imaging modality, and duration of intervention.

The NAcc plays a pivotal role in reward processing. Preclinical studies indicate robust dopamine (DA) release following glucose ingestion (39) and modulation of DA signaling in NAcc following infusion of supraphysiologic concentrations of insulin (40). Furthermore, prolonged consumption of refined carbohydrate induced compulsive behavior via NAcc signaling, whereas impaired NAcc DA receptor function was associated with sucrose intake (34). These are consistent with human studies demonstrating short-term effects of glucose ingestion on BOLD reactivity in striatal regions (8, 41). Our data align with these studies and extend our previous findings (14), suggesting that alterations in NAcc activation occur both preprandially and in the late postprandial period following meal ingestion in the context of a long-term high-carbohydrate diet, indicative of chronic effects of carbohydrate-to-fat ratio diets on brain reward functioning. It is notable that postprandial differences were lateralized to the right NAcc, consistent with prior findings (14) and data demonstrating an effect of insulin sensitivity on glucose metabolism in the right ventral striatum (42). Although the significance of this lateralization remains unclear, preclinical data suggest differential hemispheric DA release in the right NAcc evoked via projections from the dentate nucleus of the cerebellum (43), a region in which we also found greater rCBF among those assigned to the high- vs. low-carbohydrate diet in the late postprandial phase. Future studies examining cerebellar–striatal circuits in the context of diets varying in macronutrient content would aid in establishing a more precise understanding of this lateralization.

In the context of the prandial time points at which we observed this effect, we propose that intake of high-carbohydrate foods elevates signaling in reward circuitry preprandially and...
during the late postprandial period, when metabolic fuels reach a nadir (25). In nonexperimental settings, increased reward signaling could result in hedonic food intake, propagating cycles of overeating that could impede long-term weight-loss maintenance. These findings raise the possibility that chronic intake of carbohydrates stimulates reward pathways analogous to some degree to drugs of abuse, eliciting behaviors and neurobiological responses that may manifest as “food addiction” (44, 45).

In additional exploratory analyses that require cautious interpretation and replication, we found differences between diet groups in the association between insulin secretion (both pre-weight loss and at the end of the test diet at a time point concurrent with rCBF measurement) and postprandial NAcc rCBF. Insulin-30 is a marker for early phase insulin secretion and predicts weight loss or metabolic response in relation to macronutrient composition (20, 46–48). The observed correlations of insulin-30 and NAcc rCBF are consistent with the reward deficit theory of obesity and drug addiction (45, 49, 50). According to this model, NAcc DA neurons are activated by novel food rewards; with repeated exposure, consummatory activation decreases and is replaced by increased response to predictive cues. The resulting cue-based signaling with decreased reward response has been proposed to drive craving and habitual food intake because increased intake is needed to produce reward.

Exposure to a high-carbohydrate meal induces rapid shifts in insulin and blood glucose concentrations that would result in NAcc activation, as seen in response to high compared with low glycemic index test meals (14). High insulin secretion may be associated with greater blood glucose [or total metabolic fuel (13)] and insulin excursions following a meal (from the early postprandial peak to the late postprandial nadir) on the high-carbohydrate diet, and thus magnify brain exposure to these signals. Therefore, individuals with high insulin-30 would have a more pronounced NAcc response to a meal but be more prone to attenuation over time. The positive insulin-30–NAcc rCBF association in the low-carbohydrate group could represent more pronounced naïve meal response in the setting of high insulin secretion. The negative insulin-30–NAcc rCBF association in the high-carbohydrate group could represent stronger signal attenuation in the setting of chronic overstimulation.

Other studies have linked insulin to NAcc DA signaling and obesity. Heni et al. (51) reported that intranasal insulin...
administration affected peripheral insulin sensitivity, possibly mediated by the insula. Anthony et al. (42) found that peripheral insulin infusion increased metabolism in the striatum, an effect attenuated among those with insulin resistance. Stouffer et al. (52) showed that a chronic obesogenic diet reduced responsiveness of NAcc DA to insulin, whereas impaired NAcc insulin signaling can lead to mismatch between metabolic need and food intake (53). Our data suggest that individuals with
high insulin secretion are susceptible to the effects of chronically high-carbohydrate intake on mesoaccumbal reward circuitry. This aligns with discrepancies in weight-loss maintenance on low-carbohydrate compared with low-fat diets depending on insulin secretion status (20, 46–48).

We additionally observed relatively greater perfusion to the hypothalamus following a 12-h fast and at 4 h postprandial in individuals assigned to the high-carbohydrate diet. Together with evidence of elevated activity in the hypothalamus in mice fed a high-carbohydrate diet (54) and mesoaccumbal–hypothalamic activity coupling during fasting and post-glucose infusion in humans (55), these data suggest that chronic consumption of a high-carbohydrate diet may alter neuroendocrine pathways of hypothalamic nuclei responsible for regulating energy balance. Although it is acknowledged that the hypothalamus consists of several nuclei representing diverse cell types and that spatial resolution of the ASL sequence prevents localization to specific nuclei, the role of the hypothalamus in energy balance is well-established. Prior neuroimaging studies citing alterations in hypothalamus (7–10) have similarly reported elevated activation in the hypothalamus in general, interpreted as potentiation of appetitive signaling. Furthermore, we found evidence for hyperperfusion in the high-carbohydrate diet group, primarily in the fasting preprandial state, across regions associated with reward, appetite, and gustation that have previously been reported as overactive in individuals with obesity (56). Thus, hyperactivation of the hypothalamus, NAcc, and appetitive and reward regions primarily could lead to homeostatic and hedonic overconsumption.

Strengths of this study include partnership with a randomized controlled trial using rigorous feeding methodology, enrollment into our ancillary study prior to randomization (to avoid confounding of diet assignment on enrollment), use of ASL to examine perfusion absent of processing of food-related stimuli, large sample conferring relatively high power, prespecification of ROIs, and use of rigorous statistical treatments to minimize risk of type 1 error. Our study also has several notable limitations. First, we did not collect pre-diet brain perfusion data to examine longitudinal effects. However, randomization would protect against systematic bias from baseline variation on the interpretation of results at 14–20 wk. In addition, we controlled for change in BMI throughout the analyses. Second, key metabolic fuels and hormones were not measured on the scan day. Third, we used a diet-representative meal to examine postprandial effects of chronic intake; results might have differed to some degree with other meals. However, our findings are consistent with hypotheses and physiological mechanisms (14, 20), and we know of no other dietary factors varying among meals, independent of macronutrients, that would more plausibly account for findings. Finally, the study was underpowered to examine potential sex differences and dose-related effects, and it did not measure other potential outcomes, such as diet-dependent effects on cognitive function. Future studies should address these limitations.

In conclusion, we report significant differences in rCBF in individuals assigned to diets varying in carbohydrate content for several months—effects that appear to be modified by baseline insulin secretion. These findings suggest that long-term intake of a high-carbohydrate diet may affect brain reward and homeostatic activity in ways that could impede weight-loss maintenance. These data lay the foundation for future mechanistic studies, with potential to inform clinical treatments for weight control, such as by incorporating neuromodulation of reward and homeostatic circuitry in concert with diet interventions.

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References
1. Hales CM, Fryar CD, Carroll MD, Freedman DS, Ogden CL. Trends in obesity and severe obesity prevalence in US youth and adults by sex and age, 2007–2008 to 2015–2016. JAMA 2018;319:1723–5.
2. Kraschnewski JI, Boan J, Esposito J, Sherwood NE, Lehman EB, Kephart DK, Sciamanna CN. Long-term weight loss maintenance in the United States. Int J Obes 2010;34:1644–54.

3. Wing RR, Hill JO. Successful weight loss maintenance. Annu Rev Nutr 2001;21:323–41.

4. Ludwig DS, Ebbeling CB. The carbohydrate–insulin model of obesity: beyond “calories in, calories out.” JAMA Intern Med 2018;178:1098–103.

5. Ludwig DS, Friedman MI. Increasing adiposity: consequence or cause of overeating? JAMA 2014;311:2167–8.

6. Taubes G. The science of obesity: what do we really know about what makes us fat? An essay by Gary Taubes. BMJ 2013;346:f1050.

7. Li J, An R, Zhang Y, Li X, Wang S. Correlations of macronutrient-induced magnetic resonance imaging signal changes in human brain and gut hormone responses. Am J Clin Nutr 2012;96:273–82.

8. Page KA, Chan O, Arora J, Belfort-Deaguiar R, Dzuira J, Roehmholdt B, Cline GW, Naik S, Sinha R, Constable RT, et al. Effects of fructose vs. glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways. JAMA 2013;309:63–70.

9. Page KA, Seo D, Belfort-DeAguiar R, Lacadie C, Dzuira J, Naik S, Amarnath S, Constable RT, Sherwin RS, Sinha R. Circulating glucose levels modulate neural control of desire for high-calorie foods in humans. J Clin Invest 2011;121:4161–9.

10. Purnell JQ, Klopfenstein BA, Adams SH, Dunn TN, Krisky C, Rooney WD. Brain functional magnetic resonance imaging response to glucose and fructose infusions in humans. Diabetes Obes Metab 2011;13:2239–34.

11. Walsh CO, Ebbeling CB, Swart SK, Markowitz RL, Feldman HA, Ludwig DS. Effects of diet composition on postprandial energy availability during weight loss maintenance. PLoS One 2013;8:e58172.

12. Ludwig DS, Majzoub JA, Al-Zahrani A, Dallal GE, Blanco I, Roberts SB. High glycemic index foods, overeating, and obesity. Pediatrics 1999;103:E26.

13. Shimy KJ, Feldman HA, Klein GL, Bielak L, Ebbeling CB, Ludwig DS. Effects of dietary carbohydrate content on circulating metabolic fuel availability in the postprandial state. J Endoc Soc 2020;4:bvaa062.

14. Lemmer B, Aslop DC, Holsen LM, Stern E, Rojas R, Ebbeling CB, Goldstein JM, Ludwig DS. Effects of dietary glycemic index on brain regions related to reward and craving in men. Am J Clin Nutr 2013;98(3):641–7.

15. Kooch GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology 2010;35:217–38.

16. Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma Y, Wong C. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. J Neurosci 2006;26:6538–43.

17. Henz A, Siessmeier T, Wrase J, Herrmann D, Klein S, Grusser SM, Flor H, Braus DF, Buchholz HG, Grunder G, et al. Correlation between dopamine D2 receptors in the ventral striatum and central processing of alcohol cues and craving. Am J Psychiatry 2004;161:1783–9.

18. Mayer-Gross W, Walker JW. Taste and selection of food in children. Arch Dis Child 1946;21:297–305.

19. Strachan MW, Ewing FM, Frier BM, Harper A, Deary IJ. Food cravings during acute hypoglycaemia in adults with type 1 diabetes. Physiol Behav 2004;80:675–82.

20. Ebbeling CB, Feldman HA, Klein GL, Wong JMW, Bielak L, Steltz SK, Luoto PK, Wolfe RR, Wong WW, Ludwig DS. Effects of a low carbohydrate diet on energy expenditure during weight loss maintenance: randomized trial. BMJ 2018;363:k4583.

21. Ludwig DS, Dickinson SL, Henschel B, Ebbeling CB, Allison DB. Do lower-carbohydrate diets increase total energy expenditure? An updated and reanalyzed meta-analysis of 29 controlled-feeding studies. J Nutr 2021;151:482–90.

22. Ludwig DS. Dietary glycemic index and obesity. J Nutr 2000;130:2805–35.

23. Roberts SB. Glycemic index and satiety. Nutr Clin Care 2003;6:20–6.

24. Ebbeling CB, Klein GL, Luoto PK, Wong JMW, Bielak L, Eddy RG, Steltz SK, Devlin C, Sandman M, Hron B, et al. A randomized study of dietary composition during weight-loss maintenance: rationale, study design, intervention, and assessment. Contemp Clin Trials 2018;65:76–86.

25. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. JAMA 2002;287:2414–23.

26. Worsley KJ, Evans AC, Marrett S, Neelin P. A three-dimensional statistical analysis for CBF activation studies in human brain. J Cereb Blood Flow Metab 1992;12:900–18.

27. Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, Evans AC. A unified statistical approach for determining significant signals in images of cerebral activation. Hum Brain Mapp 1996;4:58–73.

28. Makris N, Kaiser J, Hesselglove C, Seidman LJ, Biederman J, Dorling D, Valera EM, Papadimitriou GM, Fischl B, Caviness VS, Jr, et al. Human cerebral cortex: a system for the integration of volume- and surface-based representations. Neuroimage 2006;33:139–53.

29. Makris N, Swaab DF, van der Kouwe A, Abbs B, Biedrol H, Ratanthan J, Tobet S, Goldstein JM. Volumetric parcellation methodology of the human hypothalamus in neuroimaging: normative data and sex differences. Neuroimage 2013;69:1–10.

30. Espeeland MA, Luchsinger JA, Neiberg RH, Carmichael O, Laurienti PJ, Pi-Sunyer X, Wing RR, Cook D, Horton E, Casanova R, et al. Long-term effect of intensive lifestyle intervention on cerebral blood flow. J Am Geriatr Soc 2018;66:120–6.

31. Farr OM, Upadhyay J, Gavrielis A, Camp M, Sproun N, Kaye H, Mathew H, Yammin V, Koniaris A, Kilim H, et al. Lorcaparin administration decreases activation of brain centers in response to food cues and these emotion- and salience-related changes correlate with weight loss effects: a 4-week-long randomized, placebo-controlled, double-blind clinical trial. Diabetes 2016;65:2943–53.

32. Holsen LM, Davidson P, Cerit H, Hye T, Moonpura R, Haimovic F, Sogg S, Shikora S, Goldstein JM, Evans AE, et al. Neural predictors of 12-month weight loss outcomes following bariatric surgery. Int J Obes 2018;42:785–93.

33. Di Feliceantonio AG, Coppin G, Rigouxs L, Edwin Tharnarajah S, Dagher A, Timgemeyer M, Small DM. Supra-additive effects of combining fat and carbohydrate on food reward. Cell Metab 2018;28:33–44.e3.

34. Michaelides M, Miller ML, DiNieri AJ, Jayne MJ, Schwartz E, Egervari W, Gwj G, Mobbs CS, Volkow ND, Hurd YL. Dopamine D2 receptor signaling in the nucleus accumbens comprises a metabolic-cognitive brain interface regulating metabolic components of glucose reinforcement. Neuropsychopharmacology 2017;42:2365–76.

35. Neseliler S, Hu W, Larcher K, Zacchia M, Dadar M, Scala SG, Lamarche M, Zeghinyam T, Stotland SC, Larocque M, et al. Neurocognitive and hormonal correlates of voluntary weight loss in humans. Cell Metab 2019;29:39–49.e4.

36. Garcia-Casares N, Bernal-Lopez MR, Roe-Vellve N, Gutierrez-Bedmar M, Fernandez-Garcia JC, Garcia-Arnes JA, Ramos-Rodriguez JR, Alfaro F, Santamaria-Fernandez S, Steward T, et al. Brain functional connectivity is modified by a hypocaloric Mediterranean diet and physical activity in obese women. Nutrients 2017;9:685.

37. Faulconbridge LF, Raparel K, Loughead J, Allison KC, Hesson LA, Fabricatore AN, Rochette A, Ritter S, Hopson RD, Sarwer DB, et al. Changes in neural responsivity to highly palatable foods following roux-en-y gastric bypass, sleeve gastrectomy, or weight stability: an fMRI study. Obesity 2016;24:1054–60.

38. Drummens M, Dorenbos E, Vreugdenhil ACE, Fabracolster A, Tittgemeyer M, Fernandez-Garcia JC, Caviness VS, Jr, et al. Association of brain response to food cues, weight loss, protein intake and dietary restraint during the PREVIEW intervention. Nutrients 2018;10:1771.

39. Oliveira-Mata AJ, Roberts CD, Walker QD, Luo B, Kuhn C, Simon SA, Nolices M, Zeghinyam T, Stotland SC, Larocque M, et al. Neurocognitive and hormonal correlates of voluntary weight loss in humans. Cell Metab 2019;29:39–49.e4.

40. Bello NT, Hajnal A. Alterations in blood glucose levels under dietary carbohydrate intake in healthy individuals. J Physiol Metab 2019;39:1–10.

41. de Araujo IE, Lin T, Veldhuizen MG, Small DM. Metabolic regulation of brain response to food cues. Curr Biol 2013;23:6583–8.

42. Anthony K, Reed LJ, Dunn JT, Bingham E, Hopkins D, Marsden PK, Amiel SA. Attenuation of insulin-evoked responses in brain networks controlling appetite and reward in insulin resistance: the cerebral basis for impaired control of food intake in metabolic syndrome? Diabetes 2006;55:2986–92.

43. Holloway ZR, Paige NB, Comstock JF, Nolen HG, Sable HJ, Lester DB. Cerebellar modulation of mesolimbic dopamine transmission is functionally asymmetrical. Cerebellum 2019;18:922–31.
44. Gearhardt AN, Davis C, Kuschner R, Brownell KD. The addiction potential of hyperpalatable foods. Curr Drug Abuse Rev 2011;4:140–5.

45. Lennerz B, Lennerz JK. Food addiction, high-glycemic-index carbohydrates, and obesity. Clin Chem 2018;64:64–71.

46. Chaput JP, Tremblay A, Rimm EB, Bouchard C, Ludwig DS. A novel interaction between dietary composition and insulin secretion: effects on weight gain in the Quebec Family Study. Am J Clin Nutr 2008;87:303–9.

47. Ebbeling CB, Leidig MM, Feldman HA, Lovesky MM, Ludwig DS. Effects of a low-glycemic load vs low-fat diet in obese young adults: a randomized trial. JAMA 2007;297:2092–102.

48. Hron BM, Ebbeling CB, Feldman HA, Ludwig DS. Relationship of insulin dynamics to body composition and resting energy expenditure following weight loss. Obesity 2015;23:2216–22.

49. Smith DG, Robbins TW. The neurobiological underpinnings of obesity and binge eating: a rationale for adopting the food addiction model. Biol Psychiatry 2013;73:804–10.

50. Volkow ND, Wang GJ, Tomasi D, Baler RD. Obesity and addiction: neurobiological overlaps. Obes Rev 2013;14:2–18.

51. Heni M, Kullmann S, Ketterer C, Guthoff M, Linder K, Wagner R, Stengl KT, Veit R, Staiger H, Haring HU, et al. Nasal insulin changes peripheral insulin sensitivity simultaneously with altered activity in homeostatic and reward-related human brain regions. Diabetologia 2012;55:1773–82.

52. Stouffer MA, Woods CA, Patel JC, Lee CR, Wirtzovsky P, Bao L, Machold RP, Jones KT, de Vaca SC, Reith ME, et al. Insulin enhances striatal dopamine release by activating cholinergic interneurons and thereby signals reward. Nat Commun 2015;6:8543.

53. Woods CA, Gutman ZR, Huang D, Kolaric RA, Rabinowitz AL, Jones KT, Cabeza de Vaca S, Sclafani A, Carr KD. Insulin receptor activation in the nucleus accumbens reflects nutritive value of a recently ingested meal. Physiol Behav 2016;159:52–63.

54. Zeeni N, Nadkarni N, Bell JD, Even PC, Fromentin G, Tome D, Darcel N. Peripherally injected cholecystokinin-induced neuronal activation is modified by dietary composition in mice. Neuroimage 2010;50:1560–5.

55. Ulrich M, Endres F, Kolle M, Adolph O, Widenhorn-Muller K, Gron G. Glucose modulates food-related salience coding of midbrain neurons in humans. Hum Brain Mapp 2016;37:4376–84.

56. Stoeckel LE, Weller RE, Cook EW, Twieg DB, Knowlton RC, Cox JE. Widespread reward-system activation in obese women in response to pictures of high-calorie foods. Neuroimage 2008;41:636–47.