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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/m ^{2}): 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.

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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.

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Supplemental Methods, Supplemental Tables 1–5, and Supplemental Figures 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn.
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Abbreviations used: ASL, arterial spin labeling; DA, dopamine; FCI, Food Craving Inventory; FWE, family-wise error; HIGH, high-carbohydrate diet; LOW, low-carbohydrate diet; MOD, moderate-carbohydrate diet; NAcc, nucleus accumbens; pASL, pulsed arterial spin labeling; rCBF, regional cerebral blood flow; ROI, region of interest; SVC, small volume correction; VAS, visual analog scale.

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/m^2) 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 study 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

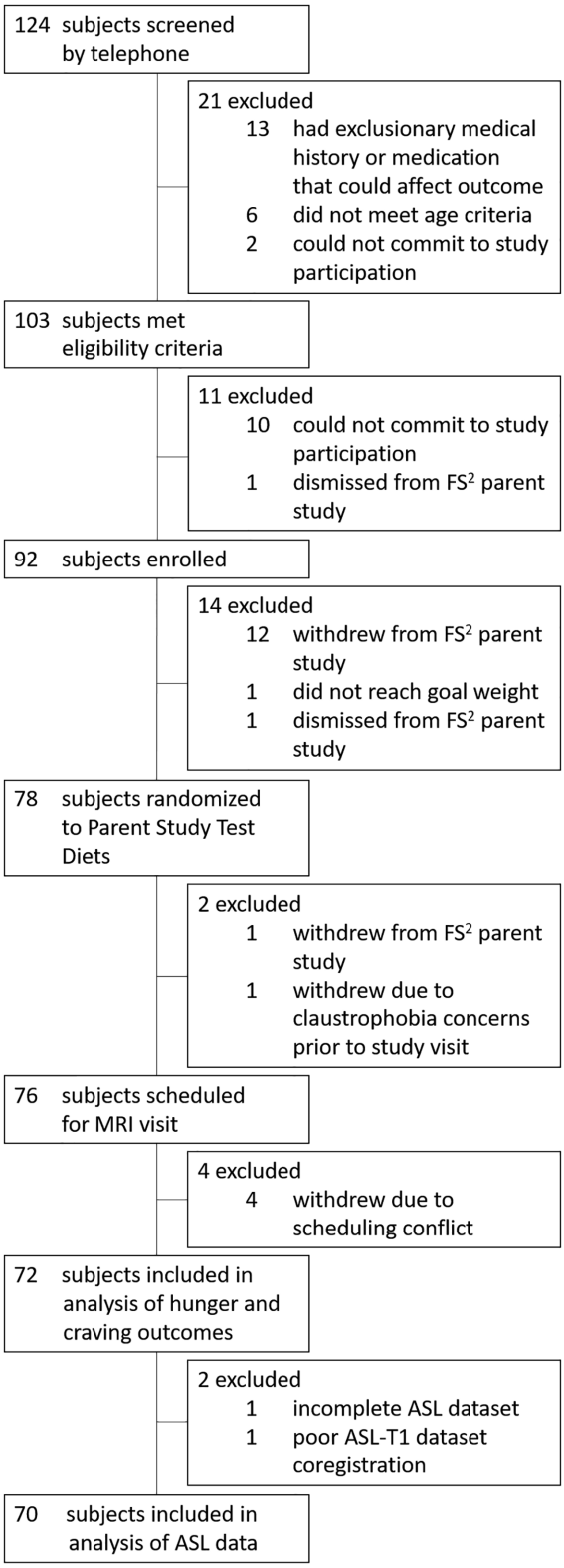

FIGURE 1 Subject flow schematic. ASL, arterial spin labeling.

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 $mL \cdot dL^{-1} \cdot min^{-1}$ 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: NAcc). 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 within 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

TABLE 1 Characteristics of adults assigned to LOW, MOD, and HIGH diets[1]

Characteristics	All participants ($n = 72$)	Test diet group		
		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/m^2				
Pre-weight-loss baseline	31.8 ± 4.7	31.7 ± 5.0	32.8 ± 4.5	31.0 ± 4.4
MRI visit	28.0 ± 4.3	27.7 ± 4.6	29.0 ± 4.2	27.4 ± 4.2
Percentage change[2]	12.0 ± 2.4	12.5 ± 2.7	11.6 ± 2.3	11.8 ± 2.3
Percentage change/diet week	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. (%)				
Sex				
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)

[1] HIGH, high-carbohydrate diet; LOW, low-carbohydrate diet; MOD, moderate-carbohydrate diet.
[2] Change from pre-weight-loss baseline to MRI visit.

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 $P < 0.05$ 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) (20).

A multiple regression model, implemented in SPM12, was used to examine 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 visualization 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

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

	Peak *F* or *t* value[2]	Peak *Z* value[3]	R/L[4]	*k*(*E*)[5]	*P* (FWE-corrected)[6]	*P* (uncorrected)	*x*[7]	*y*	*z*
Preprandial: hypothalamus									
Any group difference	38.30	6.61	R	179	<0.001	<0.001	4	0	−12
HIGH > MOD	No significant clusters								
HIGH > LOW	8.68	6.96	R	179	<0.001	<0.001	4	0	−12
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	16	16	−6
HIGH > MOD	No significant clusters								
HIGH > LOW	5.14	4.66	R	63	<0.05	<0.001	12	16	−6
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; L, left; LOW, low-carbohydrate diet; MNI, Montreal Neurologic Institute; MOD, moderate-carbohydrate diet; NAcc, nucleus accumbens; R, right.
[2] Degrees of freedom for overall ANCOVA (*F*): 2, 60; degrees of freedom for post hoc between-group comparisons (*t* tests): 60.
[3] Peak *Z* value indicates the *z* score associated with the peak *F*/*t* value within the cluster identified as meeting statistical thresholds.
[4] R/L denotes hemisphere in which peak voxel within each cluster was localized.
[5] Cluster size (contiguous voxels).
[6] Statistical significance was assessed at $P < 0.05$ FWE (to control for multiple comparisons across whole brain) corrected at whole-brain peak voxel level, with a minimum cluster size of $k = 50$.
[7] Coordinates are presented in MNI space, with *x* corresponding to the sagittal plan, *y* to the coronal plane, and *z* to the axial plane.

NAcc (**Supplemental Table 2**) and at the whole-brain level (**Supplemental Table 3**).

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 hypothalamus rCBF in the HIGH group was 41% higher than the LOW group [adjusted model: *P*(FWE-corrected) < 0.001; **Table 2**]. Preprandial hypothalamus rCBF in the HIGH group did not differ significantly from 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 hypothalamus 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 hypothalamus 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 × 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 × 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 6**A, 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

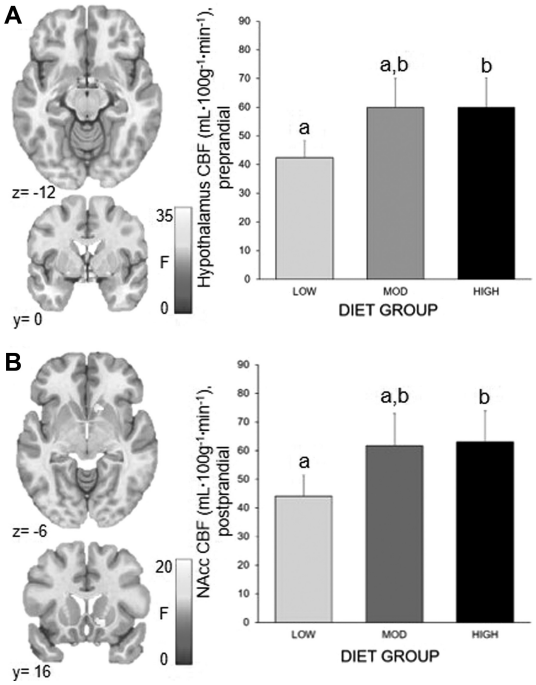

FIGURE 2 Adjusted model effect of diets varying in carbohydrate-to-fat ratio on preprandial blood flow in the hypothalamus and 4-h postprandial blood flow in the NAcc (primary endpoint) in adults assigned to LOW, MOD, and HIGH diets. (A, left) Preprandial rCBF in the hypothalamus differs between test diet groups (HIGH: $n = 20$; MOD: $n = 22$; LOW: $n = 28$), $P < 0.001$, FWE-corrected. The F scale and P value reflect the 3-way (HIGH, MOD, LOW) ANCOVA. Statistical maps for rCBF are overlaid on a normalized canonical image (MNI ICBM 152 nonlinear asymmetric T1 template), with SPM color map corresponding to relative F value. Coordinates (y, z) are presented in MNI space, with y corresponding to the coronal plane and z to the axial plane. (Right) Preprandial rCBF in the hypothalamus is 41% higher in individuals assigned to the high-carbohydrate diet compared with those assigned to the low-carbohydrate diet. Bar graph depicts mean rCBF within the cluster ± SEM. Means without a common letter differ, $P < 0.05$, FWE-corrected. The P value reflects the post hoc comparison (independent samples t test) between HIGH and LOW groups. (B, left) Late postprandial rCBF in the NAcc differs between test diet groups (HIGH: $n = 20$; MOD: $n = 22$; LOW: $n = 28$), $P < 0.01$, FWE-corrected. The F scale and P value reflect the 3-way (HIGH, MOD, LOW) ANCOVA. Statistical maps for the rCBF are overlaid on a normalized canonical image (MNI ICBM 152 nonlinear asymmetric T1 template), with SPM color map corresponding to relative F value. Coordinates (y, z) are presented in MNI space, with y corresponding to the coronal plane and z to the axial plane. (Right) Late postprandial rCBF in the NAcc is 43% higher in individuals assigned to the high-carbohydrate diet compared with those assigned to the low-carbohydrate diet. Bar graph depicts mean rCBF within the cluster ± SEM. Means without a common letter differ, $P < 0.001$, FWE-corrected. The P value reflects the post hoc comparison (independent samples t test) between HIGH and LOW groups. CBF, cerebral blood flow; FWE, family-wise error; HIGH, high-carbohydrate diet; LOW, low-carbohydrate diet; MNI, Montreal Neurologic Institute; MOD, moderate-carbohydrate diet; NAcc, nucleus accumbens; rCBF, regional cerebral blood flow; SPM, Statistical Parametric Mapping.

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: 12, 20, –8]. An unadjusted model yielded similar results for the effect modification by group [$t = 3.22$; $k = 42$; 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 6**B]. 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.

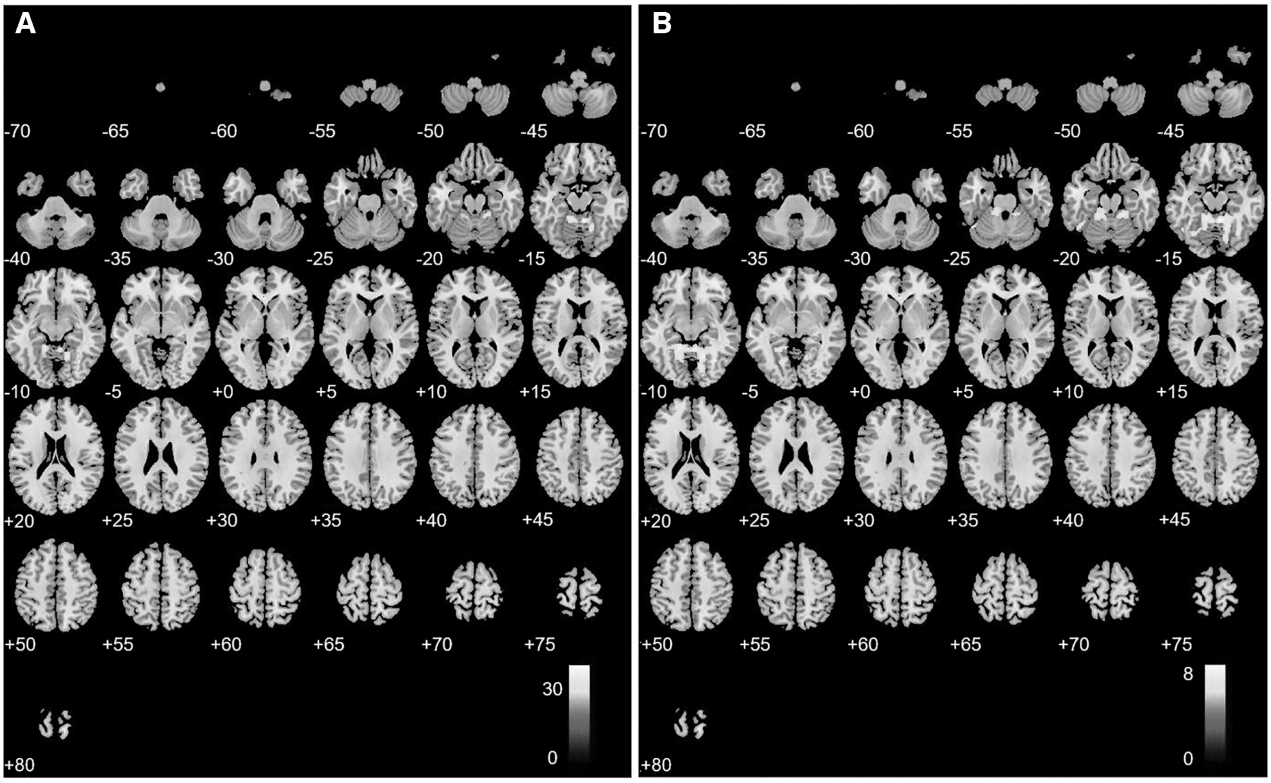

FIGURE 3 Adjusted model of effect of diets varying in carbohydrate-to-fat ratio on whole-brain 4-h postprandial blood flow in adults assigned to LOW, MOD, and HIGH diets. (A) Four-hour postprandial rCBF in the cerebellum differs between test diet groups (HIGH: $n = 20$, MOD: $n = 22$, LOW: $n = 28$), $P < 0.01$, FWE-corrected. The F scale and P value reflect the 3-way (HIGH, MOD, LOW) ANCOVA. Whole-brain statistical maps based on the ANCOVA show any group differences between LOW, MOD, and HIGH groups in rCBF, overlaid on axial slices of a normalized canonical image (ch2better template), with SPM color map corresponding to relative F value. Coordinates (z) are presented in MNI space, with z values corresponding to the axial plane. (B) Four-hour postprandial rCBF in the cerebellum is higher in individuals assigned to the high-carbohydrate diet compared with those assigned to the low-carbohydrate diet, $P < 0.05$, FWE-corrected. The t scale and P value reflect the post hoc comparison (independent samples t test) between HIGH and LOW groups. Whole-brain statistical maps based on post hoc group comparisons show HIGH > LOW group differences in rCBF, overlaid on axial slices of a normalized canonical image (ch2better template), with SPM color map corresponding to relative t value. Coordinates (z) are presented in MNI space, with z values corresponding to the axial plane. FWE, family-wise error; HIGH, high-carbohydrate diet; LOW, low-carbohydrate diet; MNI, Montreal Neurologic Institute; MOD, moderate-carbohydrate diet; rCBF, regional cerebral blood flow; SPM, Statistical Parametric Mapping.

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

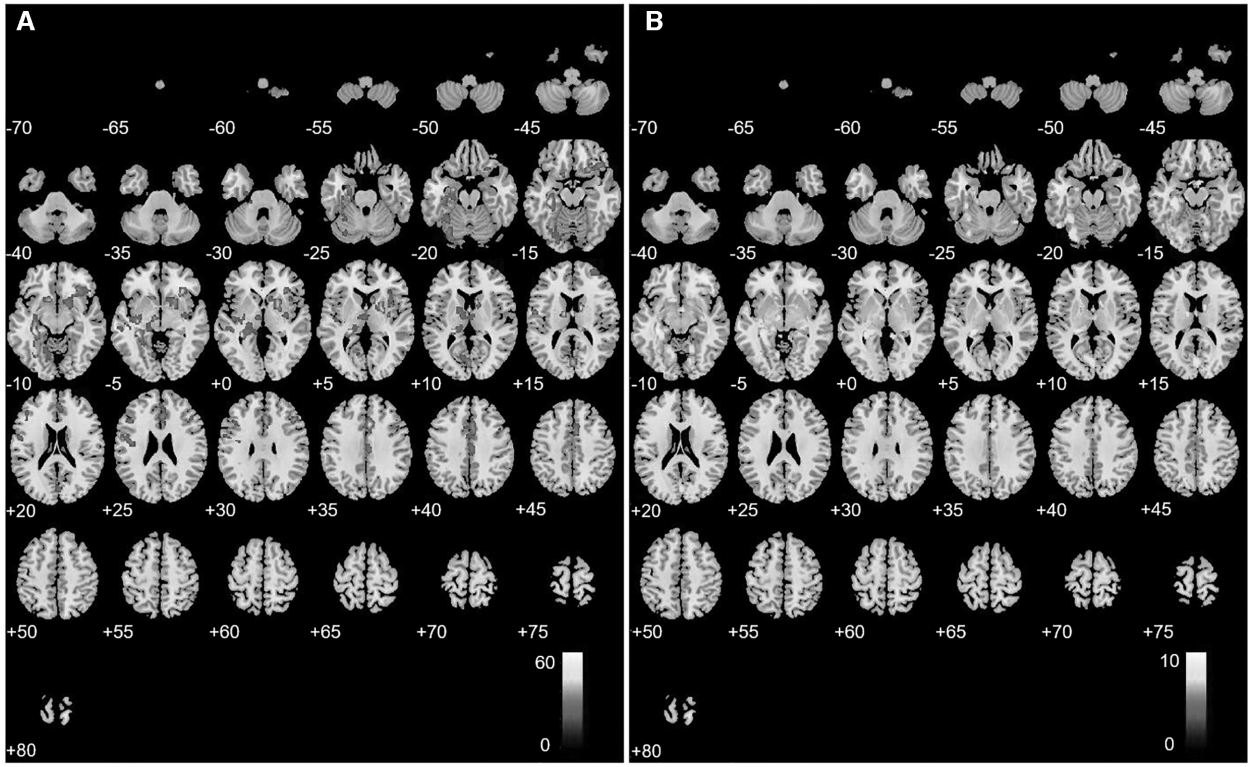

FIGURE 4 Adjusted model of effect of diets varying in carbohydrate-to-fat ratio on whole-brain preprandial blood flow in adults assigned to LOW, MOD, and HIGH diets. (A) Preprandial rCBF in the caudate, putamen, anterior cingulate gyrus, middle frontal gyrus, precentral gyrus, fusiform gyrus, and posterior cingulate gyrus differs between test diet groups (HIGH: $n = 20$; MOD: $n = 22$; LOW: $n = 28$), $P < 0.01$, FWE-corrected. The F scale and P value reflect the 3-way (HIGH, MOD, LOW) ANCOVA. Whole-brain statistical maps based on the ANCOVA show any group differences in rCBF, overlaid on axial slices of a normalized canonical image (ch2better template), with SPM color map corresponding to relative F value. Coordinates (z) are presented in MNI space, with z values corresponding to the axial plane. (B) Preprandial rCBF in the pulvinar nucleus, caudate, anterior cingulate gyrus, insula, angular gyrus, and occipital gyrus is higher in individuals assigned to the high-carbohydrate diet compared with those assigned to the low-carbohydrate diet, $P < 0.05$, FWE-corrected. The t scale and P value reflect the post hoc comparison (independent samples t test) between HIGH and LOW groups. Whole-brain statistical maps based on post hoc group comparisons show HIGH > LOW group differences in rCBF, overlaid on axial slices of a normalized canonical image (ch2better template), with SPM color map corresponding to relative t value. Coordinates (z) are presented in MNI space, with z values corresponding to the axial plane. FWE, family-wise error; HIGH, high-carbohydrate diet; LOW, low-carbohydrate diet; MNI, Montreal Neurologic Institute; MOD, moderate-carbohydrate diet; rCBF, regional cerebral blood flow; SPM, Statistical Parametric Mapping.

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

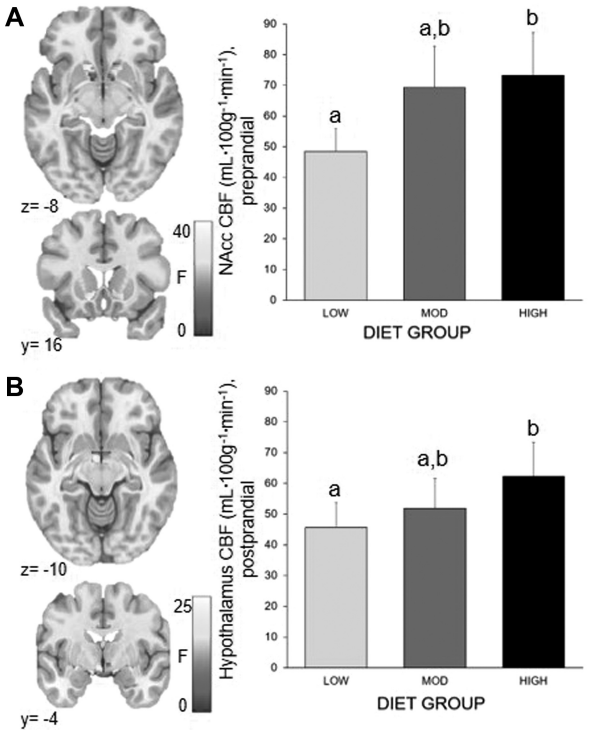

FIGURE 5 Adjusted model of effect of diets varying in carbohydrate-to-fat ratio on preprandial blood flow in the nucleus accumbens and 4-h postprandial blood flow in the hypothalamus in adults assigned to LOW, MOD, and HIGH diets. (A, left) Preprandial rCBF in the NAcc differs between test diet groups (HIGH: $n = 20$; MOD: $n = 22$; LOW: $n = 28$), $P < 0.01$, FWE-corrected. The F scale and P value reflect the 3-way (HIGH, MOD, LOW) ANCOVA. Statistical maps for the rCBF are overlaid on a normalized canonical image (MNI ICBM 152 nonlinear asymmetric T1 template), with SPM color map corresponding to relative F value. Coordinates (y, z) are presented in MNI space, with y corresponding to the coronal plane and z to the axial plane. (Right) Preprandial rCBF in the NAcc is 51% higher in individuals assigned to the high-carbohydrate diet compared with those assigned to the low-carbohydrate diet. Bar graph depicts mean rCBF within the cluster ± SEM. Means without a common letter differ, $P < 0.001$, FWE-corrected. The P value reflects the post hoc comparison (independent samples t test) between HIGH and LOW groups. (B, left) Late postprandial rCBF in the hypothalamus differs between test diet groups (HIGH: $n = 20$; MOD: $n = 22$; LOW: $n = 28$), $P < 0.01$, FWE-corrected. The F scale and P value reflect the 3-way (HIGH, MOD, LOW) ANCOVA. Statistical maps for the rCBF are overlaid on a normalized canonical image (MNI ICBM 152 nonlinear asymmetric T1 template), with SPM color map corresponding to relative F value. Coordinates (y, z) are presented in MNI space, with y corresponding to the coronal plane and z to the axial plane. (Right) Late postprandial rCBF in the hypothalamus is 36% higher in individuals assigned to the high-carbohydrate diet compared with those in the low-carbohydrate diet. Bar graph depicts mean rCBF within the cluster ± SEM. Means without a common letter differ, $P < 0.001$, FWE-corrected. The P value reflects the post hoc comparison (independent samples t test) between HIGH and LOW groups. CBF, cerebral blood flow; FWE, family-wise error; HIGH, high-carbohydrate diet; LOW, low-carbohydrate diet; MNI, Montreal Neurologic Institute; MOD, moderate-carbohydrate diet; NAcc, nucleus accumbens; rCBF, regional cerebral blood flow; SPM, Statistical Parametric Mapping.

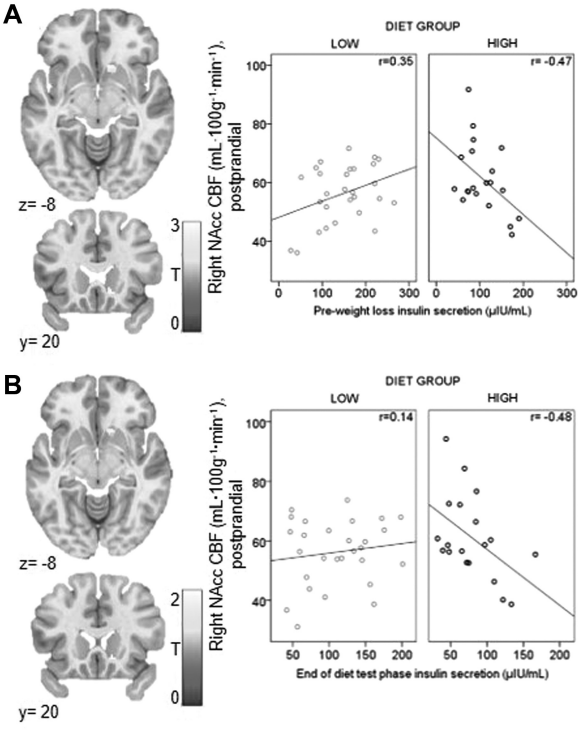

FIGURE 6 Relation between insulin secretion and 4-h postprandial blood flow in the nucleus accumbens in adults assigned to LOW, MOD, and HIGH diets. (A, left) Pre-weight-loss insulin secretion (insulin concentration 30 min after oral glucose, obtained at the pre-weight-loss time point as part of the parent study protocol) differentially predicted late postprandial right NAcc rCBF in those assigned to the low- compared with high-carbohydrate weight-loss maintenance diets (HIGH: $n = 20$; LOW: $n = 28$), $P < 0.05$, FWE-corrected using small volume correction ($P < 0.005$, uncorrected). Statistical maps are overlaid on the MNI ICBM 152 nonlinear asymmetric T1 template, with SPM color map corresponding to relative t value. Coordinates (y, z) are presented in MNI space, with y corresponding to the coronal plane and z to the axial plane. (Right) In the low-carbohydrate group, the relation between pre-weight-loss insulin secretion and late postprandial right NAcc rCBF was positive, whereas in the high-carbohydrate group there was an inverse relation. Scatterplots depict average rCBF within the cluster plotted against insulin secretion, with correlation coefficients specified for each group. (B, left) End of diet test insulin secretion (insulin concentration 30 min after oral glucose, obtained at the 18- to 20-wk time point as part of the parent study protocol) differentially predicted late postprandial right NAcc rCBF in those assigned to the low- compared with high-carbohydrate weight-loss maintenance diets (HIGH: $n = 19$; LOW: $n = 28$), $P = 0.14$, FWE-corrected using small volume correction ($P < 0.05$, uncorrected). Statistical maps are overlaid on the MNI ICBM 152 nonlinear asymmetric T1 template, with SPM color map corresponding to relative t value. Coordinates (y, z) are presented in MNI space, with y corresponding to the coronal plane and z to the axial plane. (Right) In the low-carbohydrate group, there was a positive relation between end of diet test insulin secretion and late postprandial right NAcc rCBF. The high-carbohydrate group showed an inverse relation. Scatterplots depict average rCBF within the cluster plotted against insulin secretion, with correlation coefficients specified for each group. CBF, cerebral blood flow; FWE, family-wise error; HIGH, high-carbohydrate diet; LOW, low-carbohydrate diet; MNI, Montreal Neurologic Institute; NAcc, nucleus accumbens; rCBF, regional cerebral blood flow; SPM, Statistical Parametric Mapping.

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

TABLE 3 Appetite and craving ratings in adults assigned to LOW, MOD, and HIGH diets[1]

	Test diet group				
	LOW	MOD	HIGH	*F*	*P*[2]
VAS hunger rating at T240	55.2 ± 27.2	53.5 ± 21.7	56.3 ± 29.0	0.07	0.94
VAS hunger rating AUC[3] T0–T270	7762 ± 3216	7881 ± 3340	7605 ± 3614	0.04	0.97
VAS desire to eat favorite food rating at T240	50.5 ± 28.9	49.2 ± 30.3	41.5 ± 31.1	0.59	0.56
VAS desire to eat favorite food rating AUC T0–T270	8324 ± 4858	8689 ± 5611	6393 ± 4513	1.30	0.28
Food Craving Inventory scores					
Total	62.4 ± 15.2	60.0 ± 15.7	56.0 ± 15.9	1.01	0.37
High-fat foods	14.3 ± 5.4	12.6 ± 4.9	13.1 ± 5.1	0.71	0.49
Fast-food fats	8.7 ± 3.0	8.0 ± 2.8	7.1 ± 2.6	1.90	0.16
Carbohydrates/starches	18.1 ± 5.1	16.5 ± 4.5	16.4 ± 5.4	0.93	0.40
Sweets	21.3 ± 5.4	22.9 ± 6.8	19.2 ± 5.4	2.06	0.14

[1]Values are means ± SDs. HIGH, high-carbohydrate diet; LOW, low-carbohydrate diet; MOD, moderate-carbohydrate diet; VAS, visual analog scale.
[2]*P* value specified for overall ANOVA model; because all results for the overall ANOVA models were nonsignificant, post hoc tests comparing individual diet groups were not completed.
[3]AUC: based on individual time point VAS ratings (on a scale from 0 to 100) across 11 time points (T0–T270).

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.

Acknowledgments

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