Restriction of dietary fat, but not carbohydrate, alters brain reward circuitry in adults with obesity

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IN BRIEF

Darcey et al. found that selective reduction of dietary fat, but not carbohydrate, resulted in changes in brain dopamine receptor binding potential and neural activity in response to food cues, consistent with increased tonic dopamine and resulting in greater subsequent consumption of rewarding foods high in both fat and carbohydrate.

HIGHLIGHTS

- Compared to a eucaloric baseline diet, selective reduction of dietary fat, but not carbohydrate, significantly decreased both dopamine D2/3 receptor binding potential and neural activity to visual food cues in brain reward regions.
- Multimodal neuroimaging suggests that reduction of dietary fat, but not carbohydrate, increased tonic brain dopamine and influenced food choices in ways that may hamper diet adherence.

SUMMARY

Weight loss diets often restrict either fat or carbohydrate, macronutrients that are sensed via distinct gut-brain pathways and differentially affect peripheral hormones and metabolism. To investigate whether reductions in dietary fat versus carbohydrate alter brain reward circuitry, we measured dopamine D2/3 receptor binding potential (D2BP) using PET and neural activity in response to visual food cues using fMRI in 17 inpatient adults with obesity during a eucaloric baseline diet and on the fifth day of isocaloric diets selectively reduced in either dietary fat or carbohydrate, in random order. Reduction of dietary fat, but not carbohydrate, decreased D2BP and decreased neural activity to food cues in brain reward regions. After the reduced fat diet, *ad libitum* intake shifted towards foods high in both fat and carbohydrates. These results suggest that dietary fat restriction increases tonic dopamine in brain reward regions thereby affecting food choice in ways that may hamper diet adherence.

KEYWORDS: Obesity, diet, dopamine, macronutrients, reduced-fat, reduced-carbohydrate, PET, fMRI

INTRODUCTION

Among dietary approaches to treating obesity (Makris and Foster 2011), popularity has waxed and waned between strategies that target restriction of dietary fat versus carbohydrate – macronutrients that elicit divergent peripheral metabolic and endocrine states (Hall, Bemis et al. 2015). Furthermore, ingestion of dietary carbohydrate and fat engage distinct gut-brain pathways affecting brain dopamine (de Araujo, Oliveira-Maia et al. 2008, Tellez, Han et al. 2016, Alhadeff, Goldstein et al. 2019) and may thereby affect food choice and eating behavior because dopamine is a neuromodulator that influences motivation, reinforcement learning, habit formation, and compulsion (Wise 2004). Indeed, rodent models clearly demonstrate that brain dopamine is integral to eating behavior (Sotak, Hnasko et al. 2005) and body weight regulation (Johnson and Kenny 2010).

People with obesity have reduced dopamine synthetic capacity and tone (Wilcox, Braskie et al. 2010, Wallace, Aarts et al. 2014, Lee, Kroemer et al. 2018) and availability of striatal dopamine type 2/3 receptor binding potential (D2BP) is correlated with adiposity (Wang, Volkow et al. 2001, Guo, Simmons et al. 2014, Horstmann, Fenske et al. 2015). Brain dopamine has also been linked to facets of human eating behavior (Kaye, Frank et al. 1999, Bello and Hajnal 2010, Guo, Simmons et al. 2014, Eisenstein, Bischoff et al. 2015) and food reward processing (Frank, Veit et al. 2015) independent of body weight.

Whether diets restricting carbohydrates versus fat differentially affect brain dopamine and eating behavior in humans is unknown. Here, we investigated whether five days of selective restriction of dietary fat versus carbohydrate differentially affects the neurochemistry of food reward in the human brain as compared to a eucaloric baseline diet. Specifically, our prespecified primary multi-modal neuroimaging aims were to measure diet-induced changes in brain reward circuitry in adults with obesity, as assessed by D2BP measured using positron emission tomography (PET), and the activity of brain reward-processing regions in response to visual food cues using fMRI.

RESULTS

A total of 8 male and 9 female weight-stable adults with obesity (Table 1 and Supplemental Figure 1) had fMRI and PET neuroimaging at baseline and completed at least one of two 14-day visits to the Metabolic Research Unit at the NIH Clinical Center. As previously described (Hall, Bemis et al. 2015), for two days prior to each inpatient admission participants were asked to completely consume a provided standard eucaloric baseline diet (50% calories from carbohydrate, 35% calories from fat, 15% protein) that was continued for the first five days of admission. For the next six days, participants were randomized to consume a 30% calorie-restricted diet achieved either via selective reduction in fat (RF) or carbohydrate (RC), while keeping other two macronutrients unchanged from the eucaloric baseline (Figure 1). For the final three days of each inpatient period, participants were given *ad libitum* access to vending machines stocked with a variety of supermarket foods. After a washout period of 2-4 weeks, participants were readmitted and consumed the eucaloric baseline diet for five days followed by the alternate restricted diet for six days and *ad libitum* vending machine access for three days. Details of each diet phase are provided as Supplemental Information. The main text reports data from the 17 participants who completed fMRI at baseline and at least one of

the RC or RF diet periods, as well as the 15 participants completing PET at baseline and RF and 17 participants completing PET at baseline at RC. The Supplementary Materials presents the results for 13 participants with complete PET and fMRI data during baseline, RC, and RF diets, as well as the 15 participants with complete PET data and 15 participants with complete fMRI data.

[TABLE 1]

[FIGURE 1]

Only the RF diet decreased activity in brain reward regions in response to food cues

Participants rated the pleasantness of a variety of food images during fMRI 4.5 hours after lunch on the third inpatient day of the first eucaloric baseline diet period and on the fifth day of the carbohydrate and fat restricted diets. Voxel-wise blood-oxygen-level-dependent responses were compared to fixation within an *a priori* reward circuit mask - as previously reported in participants without obesity (Simmons, Rapuano et al. 2014). On average, the restricted diets did not significantly impact food pleasantness ratings in the scanner (Supplementary Figure 2). Compared to reward circuit response to food images at baseline, only the RF diet resulted in reduced activity in bilateral striatal clusters in caudate and putamen (Figure 2A; Table 2). In contrast, the RC diet did not change reward circuit response to visual food cues from baseline. Compared to reward circuit activity during the RC diet, the reduced fat diet decreased activity in a ventrolateral region of right putamen (Supplementary Figure 3B; Supplementary Table 1). Similar results were observed in the 15 participants with complete fMRI data during baseline, RF, and RC diets (Supplementary Figure 3A; Supplementary Table 1), as well as the subset of 13 participants who also had complete PET data (Supplementary Figures 3C & 3D; Supplementary Table 1).

Only the RF diet led to decreased dopamine D2/D3 receptor binding potential

Participants completed PET imaging with the radiolabeled D2/3 receptor subtype antagonist [18F]fallypride 2 hours after breakfast on the third inpatient day as well as on the fifth day of the RC and RF diets. [18F]fallypride time-activity curves using the cerebellum as a reference tissue were used with kinetic modeling to measure D2BP as previously described (Guo, Simmons et al. 2014). A small volume correction (D2BP > 1.5) applied to whole brain analyses was used to isolate voxel-wise D2BP analyses to the striatum.

Compared to D2BP at baseline, only the RF diet significantly decreased D2BP in bilateral striatal clusters spanning the left putamen and right caudate/putamen (Figure 2B; Table 2). There was no significant effect of the RC diet on D2BP as compared to baseline or the RF diet. Similar results were observed in the 15 participants with complete PET data during baseline, RF, and RC diets (Supplementary Table 1), as well as the subset of 13 participants with complete PET data who also had complete fMRI data (Supplementary Figure 3E; Supplementary Table 1).

[FIGURE 2]

[TABLE 2]

The RF diet resulted in greater *ad libitum* intake of foods high in both carbohydrate and fat

We explored *ad libitum* food intake for three days after the RF and RC diets. Participants selected foods from computerized vending machines stocked with calories in excess of maintenance energy requirements. Average energy intake was (mean ± SE) 25.9 ± 9.5% greater than the eucaloric baseline diet and was not significantly different following RF versus RC diets (Table 3). While overall macronutrient intake was similar after RC and RF diets, participants consumed a greater percentage of total calories from foods high in both carbohydrate and fat (HCHF) as well as high in both sugar and fat (HSHF) following the RF diet as compared to the RC diet (RF diet 28.8 ± 2.4% vs. RC diet 23.1 ± 2.4%; p=0.010) and consumed more calories from sugar sweetened beverages (SSB) (RF diet 9.8 ± 1.1% vs. RC diet 8.4 ± 1.1%; p=0.032) such that the combination of HCHF, HSHF, and SSB as a fraction of total calories consumed was greater following the RF diet (RF diet 38.6 ± 3.0% vs. RC diet 31.4 ± 3.0%; p<0.001).

[TABLE 3]

DISCUSSION

We previously showed that the RC diet led to widespread metabolic and endocrine changes compared to the eucaloric baseline diet, including increased fat oxidation as well as decreased energy expenditure and decreased daily insulin secretion, whereas the RF diet did not lead to substantial peripheral metabolic or endocrine changes (Hall, Bemis et al. 2015). Therefore, we expected that the RC diet would have a greater effect on brain reward circuitry than the RF diet, especially given insulin's effects on dopamine levels (Liu and Borgland 2019, Kullmann, Blum et al. 2021). Surprisingly, it was the RF diet that significantly decreased both D2BP and neural activity in response to visual food cues in brain reward regions. Furthermore, *ad libitum* food intake after the RF diet was shifted towards high fat, high carbohydrate foods as compared to the RC diet. These results suggest that a calorie is *not* a calorie when it comes to macronutrient effects on reward circuitry in the human brain.

The most likely interpretation of our data is that the RF diet increased tonic dopamine in the striatum. This would explain the observed decrease in D2BP because increased endogenous dopamine would be expected to displace the [18F]fallypride tracer (Laruelle, Abi-Dargham et al. 1995, Laruelle, Souza et al. 1997). Furthermore, an increase in tonic dopamine would be expected to activate high affinity D2/3 receptors thereby inhibiting neural activity (Stoof and Kebabian 1981) and explaining the observed decrease in brain activity to food cues with the RF diet. Indeed, pharmacological agonism of the D2/3 receptor has been demonstrated to decrease the fMRI signal in both rats (Chen, Choi et al. 2005) and non-human primates (Sander, Hooker et al. 2016).

It is unlikely that the observed reduction in D2BP during the RF diet was due to decreased D2/3 receptor density because neither dopamine depletion over 2-5 days (Ginovart, Farde et al. 1997, Laruelle, Souza et al. 1997) nor dopamine stimulation over five weeks (Blunt, Jenner et al. 1992) appreciably impacts receptor density. Moreover, a reduction in D2/3 receptor density would be expected to both minimize D2/3 receptor inhibitory signaling and produce a net increase in the stimulatory effect of dopamine at neurons expressing D1/5 receptors (Sander, Hooker et al. 2016) which is inconsistent with the observed decrease in fMRI BOLD response during the RF diet. This observed decrease in neural activity during the RF diet is consistent with increased tonic dopamine preferentially engaging inhibitory D2 receptor expressing neurons with a high affinity for dopamine. By contrast, stimulation of activity in neurons expressing the lower affinity D1/5 receptor requires phasic dopamine responses (Grace 2000), which are expected to reduce – not increase - dopamine binding at the D2/3 receptor (Dreyer, Herrik et al. 2010). Phasic dopamine responses are not expected under our experimental conditions given that the scans were conducted without providing rewards or reward-predicting stimuli. Indeed, visual food stimuli do not result in detectable changes in dopamine in humans (Volkow, Wang et al. 2002, Wang, Geliebter et al. 2011). Therefore, our multi-modal neuroimaging results are most likely explained by increases in tonic striatal dopamine resulting from the RF diet.

An increase in tonic dopamine during the RF diet may have contributed to the tendency to increase selection of high-fat, high-carbohydrate foods observed during the subsequent *ad libitum* period. Elevations in tonic dopamine alter the balance with phasic dopamine responses (Grace 2000) and may increase incentive salience (Wise 2013, Samaha, Khoo et al. 2020), enhance the 'wanting' of foods high in both carbohydrate and fat that are particularly rewarding (DiFeliceantonio, Coppin et al. 2018, Perszyk, Hutelin et al. 2021), promote selection of these foods previously experienced to deliver reward (Beeler, Daw et al. 2010) and reduce the influence of any ensuing negative outcomes on changing behavior (Cox, Frank et al. 2015). Thus, our results suggest that diet approaches targeting the reduction of dietary fat may alter brain dopamine in a way that promotes consumption of highly rewarding foods high in both carbohydrate and fat. Such effects may thereby make it difficult for people to stick to low fat diets, at least in the short term.

How does reduction of dietary fat result in increased tonic dopamine in the brain? Dietary fat is detected and signaled to the brain throughout the alimentary canal from tastebud cells in the oral cavity to enteroendocrine and enterocyte cells in the gut (Berland, Small et al. 2021). One of several mechanisms by which dietary fats modulate feeding includes intestinal production of oleoylethanolamide (OEA), a lipid messenger produced from dietary oleic acid which can signal to the brain via the vagus nerve (Rodríguez de Fonseca, Navarro et al. 2001, Fu, Gaetani et al. 2003, Gaetani, Oveisi et al. 2003, Schwartz, Fu et al. 2008, Tellez, Medina et al. 2013). Despite OEA being produced from dietary fat, chronic consumption of high fat diets in rodents decreases intestinal production of OEA and decreases brain dopamine (Tellez, Medina et al. 2013). Perhaps because our study participants with established obesity reduced their fat intake by ~90 grams per day during the RF diet, their intestinal OEA production may have increased

thereby resulting in increased brain dopamine. In that case, the effect of increased intestinal OEA production might be expected to enhance satiety during the RF diet (Rodríguez de Fonseca, Navarro et al. 2001, Fu, Gaetani et al. 2003, Gaetani, Oveisi et al. 2003, Schwartz, Fu et al. 2008, Tellez, Medina et al. 2013) while at the same time the increased tonic dopamine might have steered food choices away from such a diet towards more rewarding foods. In other words, sticking to a low-fat diet might be difficult despite it potentially being more satiating and leading to decreased *ad libitum* energy intake in a setting where "off diet" foods are unavailable (Hall, Guo et al. 2021).

Another potential mechanism for increased brain dopamine during the RF diet involves decreased postprandial plasma triglycerides that peak several hours after a meal in proportion to the amount of fat consumed (Bozzetto, Della Pepa et al. 2020). Triglycerides have been shown to suppress dopamine synthesis and excitability of D2/3 receptor expressing neurons (Berland, Montalban et al. 2020) as well as influence the preference for palatable food and reward seeking in mice (Cansell, Castel et al. 2014). Compared to the baseline and RC diets, the RF diet would be expected to result in reduced postprandial triglycerides and therefore increased brain dopamine at the times of the neuroimaging scans conducted 2-3 hours postprandially.

Why did the RC diet have no effect on brain D2BP or neural activity in response to food cues? We found this result surprising particularly because the RC diet significantly decreased daily insulin secretion (Hall, Bemis et al. 2015) and would be expected to be reflected by decreased insulin in the brain (Banks 2004) which is thought to influence multiple aspects of the dopamine system. For example, dopaminergic neurons express insulin receptors (Figlewicz, Evans et al. 2003) and insulin decreases synaptic dopamine by increasing clearance from striatal synapses via enhanced dopamine transporter activity (Carvelli, Morón et al. 2002, Mebel, Wong et al. 2012). Consistent with a decrease in synaptic dopamine, intranasal insulin delivery was recently observed to increase D2BP in humans (Kullmann, Blum et al. 2021). Therefore, the lack of effect of the RC diet on brain dopamine remains a mystery. Whereas previous studies have demonstrated that calorie restriction potentiates dopaminergic signaling in both rodents and humans (Carr, Tsimberg et al. 2003, Dunn, Abumrad et al. 2017), our results using 30% calorie restricted RC and RF diets suggest that restriction of dietary fat has a more potent effect on brain dopamine than isocaloric restriction of carbohydrates.

How might changes in brain dopamine in response to different diets relate more generally to body weight regulation? Recent mouse data suggest that the effects of brain dopamine may not be isolated to canonical hedonic pathways of food reward. For example, striatal dopamine can also influence downstream hypothalamic circuits traditionally attributed to control homeostatic feeding and regulate body weight (Alhadeff, Goldstein et al. 2019, Mazzone, Liang-Guallpa et al. 2020) ultimately promoting intake of foods that cause obesity and devaluing foods that do not result in obesity (Mazzone, Liang-Guallpa et al. 2020). It is therefore intriguing to speculate that diet composition may contribute to altering the homeostatic body weight "set point" via changes in brain dopamine function.

LIMITATIONS

While our interpretation of increased tonic dopamine is supported by relative pharmacokinetic properties of D1/5 and D2/3 receptors, and literature on D2/3 receptor PET occupancy and fMRI activity, we did not directly measure brain dopamine. Consumption of dietary fat elicits rapid dopaminergic response in reward circuitry (Ferreira, Tellez et al. 2012, Tellez, Ferreira et al. 2013). While we observed an effect of reduced fat diet at the D2 receptor via tonic levels of dopamine, it is possible that the relatively high proportion of dietary fat in the reduced carbohydrate diet may have influenced DA system via mechanisms dependent on D1/5 receptors not examined here. Future studies are needed to delineate the effect of exposure duration (single meal versus multi-day), receptor subtype-specific effects (availability of D1/5 versus D2/3 receptors after exposure), and subsequent effect on *ad libitum* eating behavior.

Ad libitum eating behavior subsequent to the five-day period of dietary restriction supports our interpretation of increased incentive salience for rewarding foods after the RF diet. However, our study was not specifically powered to detect differences in this exploratory outcome and analyses were not corrected for multiple comparisons.

Finally, our interpretation on the effect of RC and RF diets on brain dopamine is limited to the early stages of initiating a reduced energy diets and does not address long term changes or adaptations in neurochemistry or reward circuit function. Future studies should investigate changes in neurochemistry and reward circuit activity in relation to diet composition over longer periods of weight loss.

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FIGURE TITLES AND LEGENDS

Figure 1.Study design. Seventeen men and women with obesity were admitted as inpatients to the Metabolic Clinical Research Unit at the NIH Clinical Center and completed fMRI and PET scans on the third day of a five-day eucaloric baseline diet after which they were randomized to either a 30% reduced calorie diet achieved by selective restriction of dietary fat (RF diet) or carbohydrate (RC diet). Neuroimaging was repeated on the fifth day of the reduced energy diet after which, on days 12-14 of the inpatient stay, participants consumed food *ad libitum* from vending machines. After a two to four-week washout period, participants were readmitted as inpatients to complete the five-day eucaloric baseline diet and neuroimaging was repeated on the fifth day of the alternate 30% reduced calorie diet. During the final three inpatient days, participants again consumed food *ad libitum* from vending machines.

Figure 2. **Selective reduction of dietary fat, but not carbohydrate, alters brain reward circuitry as compared to a eucaloric baseline diet**. A) Decreased striatal activity during the RF diet versus baseline in response to visual food cues using fMRI (n=17); B) Reduced dopamine D2/3 receptor binding potential during the RF diet versus baseline using [18F]fallypride PET (n=15).

TABLES

Table 1. Characteristics of participants completing baseline and portions of neuroimaging during reduced calorie interventions.

	Mean ± SD (Range)	N
Age (years)	34.8 ± 7.6 (23-46)	17
Body weight (kg)	106.2 ± 17.2 (80.9-134.2)	17
BMI (kg/m^2)	36.0 ± 4.9 (29.4-44.6)	17
% Body fat	39.8 ± 8.9 (22.4-51.5)	17
Resting metabolic rate (kcal/day)	1826 ± 351 (1279-2323)	17
Energy intake, baseline (kcal/day)	2569 ± 483 (1942-3399)	17
Carbohydrate intake (g/day)	324.0 ± 60.0 (245.3-425.2)	17
Fat intake (g/day)	100.2 ± 18.7 (74.7-130.0)	17
Protein intake (g/day)	110.7 ± 22.4 (72.7-132.9)	17
Sex	**Percent (%)**	**N**
Female	52.9	9
Male	47.1	8
Race/Ethnicity		
Black	70.6	12
White	11.8	2
Hispanic	11.8	2
Asian	5.9	1

Table 2. Locations of clusters with significant changes in D2BP or BOLD responses to reduced fat or carbohydrate diets after correction for multiple comparisons.

	Location of peak			*Voxels*	*Size (mm^3)*	*Z-score*	*alpha*
	X	***y***	***z***				
fMRI BOLD							
RF diet vs. Baseline (paired t-test, n=17)							
Right putamen	-23.0	-3.0	-2.0	113	904	-3.30	<0.01
Left putamen	19.0	-9.0	6.0	84	672	-3.59	<0.02
Left caudate	15.0	-11.0	6.0	47	376	-3.42	<0.04
RC diet vs. Baseline (paired t-test, n=17)							
No clusters	--	--	--	--	--	--	--
PET D2BP							
RF diet vs. Baseline (paired t-test, n=15)							
Left putamen	26.2	-9.5	6.5	165	7074	-2.26	<0.01
Right caudate	-8.8	-20.0	3.0	56	2401	-2.34	<0.01
Right putamen	-22.8	-2.5	17.0	44	1886	-2.09	<0.01
RC diet vs. Baseline (paired t-test, n=17)							
No clusters	--	--	--	--	--	--	--

Table 3. *Ad libitum* intake from vending machines after RF and RC diets (mean ± SE; n=17).

	After RC	After RF	*p*
***Ad Libitum* Energy Intake (kcal/d)**	3225 ± 306	3297 ± 306	0.629
Protein (%kcal)	16.5 ± 0.9	16.0 ± 0.9	0.315
Fat (%kcal)	39.6 ± 1.2	39.3 ± 1.2	0.770
Carbohydrate (%kcal)	44.3 ± 1.1	45.4 ± 1.1	0.365
Sugar, total (%kcal)	20.0 ± 0.9	21.0 ± 0.9	0.231
Items selected per day (total)	19.3 ± 1.2	19.9 ± 1.2	0.427
Ultra-processed foods (%kcal)	80.7 ± 2.3	81.2 ± 2.3	0.748
Hyperpalatable foods (%kcal)	70.7 ± 2.8	71.2 ± 2.8	0.762
HCHF foods (%kcal)	9.8 ± 1.7	12.8 ± 1.7	0.072
HCLF foods (%kcal)	11.9 ± 1.5	10.2 ± 1.5	0.246
HPHF foods (%kcal)	25.5 ± 2.0	21.7 ± 2.0	0.049
HPLF foods (%kcal)	10.2 ± 1.6	11.4 ± 1.6	0.429
HSHF foods (%kcal)	13.2 ± 1.5	16.0 ± 1.5	0.099
HSLF foods (%kcal)	6.6 ± 1.3	5.5 ± 1.3	0.159
SSB (%kcal)	8.4 ± 1.1	9.8 ± 1.1	0.032
HCHF+HSHF (%kcal)	23.1 ± 2.4	28.8 ± 2.4	0.010
HCHF+HSHF+SSB (%kcal)	31.4 ± 3.0	38.6 ± 3.0	<0.001

HCHF=high carbohydrate, high fat; HCLF=high carbohydrate, low fat; HPHF=high protein, high fat; HPLF=high protein, low fat; HSHF=high sugar, high fat; HSLF=high sugar, low fat; SSB=sugar sweetened beverages

METHODS

Experimental Model and Subject Detail

Twenty-one adults provided informed consent to participate in a randomized crossover trial investigating the effects of selective isocaloric reduction of dietary fat versus carbohydrate on macronutrient metabolism, striatal dopamine type 2 receptor binding potential, and neural activity in response to food stimuli in brain reward regions (ClinicalTrials.gov NCT00846040). Study details regarding the primary metabolic outcomes were reported elsewhere (Hall et al., 2015). In brief, right-handed non-smokers between 18-45 years of age with a reported BMI greater than 30 kg/m^2 (body weight < 350 pounds) were recruited from the Washington DC metro area. All were free from diabetes, recent weight change (> ± 5 kg in the past 6 months), physical mobility impairments, past or present history of drug abuse, neurological, or psychiatric disorders (including eating disorders such as binge eating) as assessed by an abbreviated Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders. Furthermore, participants were free from evidence of diseases or medications interfering with study outcomes, allergies to food or local anesthetics, evidence of regular excessive use of caffeinated drinks and alcohol or had strict dietary concerns (vegetarian or kosher diet). Premenopausal women were studied in the follicular phase for each inpatient visit and were excluded if they were pregnant or breastfeeding. All study procedures were approved by the Institutional Review Board of the National Institute of Diabetes & Digestive & Kidney Diseases and participants were compensated for their participation.

Method Details

Volunteers were admitted to the NIH Clinical Center for a 14-day period to receive the eucaloric baseline diet for 5 days followed by either the RC or the RF diet for the next 6 days followed by 3 days of *ad libitum* feeding from computerized vending machine, as detailed below (Figure 1). Participants were readmitted after a 2- to 4-week washout period to repeat the 5-day eucaloric baseline diet followed by 6 days of the alternate reduced calorie diet and 3 days of *ad libitum* feeding. Every day, participants completed 60 min of treadmill walking at a fixed self-selected pace and incline determined during screening to mimic free-living levels of physical activity.

The CONSORT diagram reiterates enrollment details provided in (Hall, Bemis et al. 2015) (Supplementary Figure 1). Two participants withdrew during the first baseline diet, and did not complete any neuroimaging. Of the 19 participants who completed the initial baseline diet, 10 were randomized to next receive the RC diet and 9 were randomized to next receive the RF diet. Among 10 participants receiving the RC diet on their first admission, 1 participant completed PET but not fMRI procedures during the RC diet and 2 withdrew before receiving the RF diet on the second planned admission. Among the 9 participants receiving the RF diet on their first admission, 2 completed fMRI but not PET on their first admission (participant-declined PET scans), and 1 participant did not have available fMRI data during their second admission on the RC diet. Full neuroimaging data (PET and fMRI) across all 3 diet conditions are available for n=13 participants and the results are provided in Supplementary Materials. Complete PET data are available in

n=15 participants and complete fMRI data are available from n=15 participants and the results are provided in Supplementary Materials.

Anthropometrics.
Height was measured in centimeters using a wall stadiometer (Seca 242, Hanover, MD, USA) and weight was measured in kilograms using a digital scale (Scale-Tronix 5702, Carol Steam, IL, USA). All measurements were obtained after an overnight fast while participants were wearing only hospital scrubs.

Diets.
All subjects were confined to the metabolic ward throughout the study without access to outside food. Meals were consumed under observation and any uneaten food was weighed back to subsequent meals were adjusted to account for uneaten food as needed. Diets were designed using ProNutra software (version 3.4, Viocare, Inc.).

Baseline eucaloric diet. The daily caloric content during the initial out-patient segment and the weight-maintenance phase was based on the resting energy expenditure measured at screening with an activity factor of 1.5. Beginning 2 days before each admission, participants were provided with a weight-maintenance diet using a standard diet composition of 50% carbohydrate, 35% fat, and 15% protein, which continued for the next 5 days. All participants were provided with the standard diet during the first inpatient admission for at least one day prior to measuring baseline fMRI and D2BP. Energy and macronutrient intake during the baseline eucaloric diet are presented in Table 1.

Reduced energy diets. During the restricted diet phase (inpatient days 6 to 11), 30% of baseline calories were removed by selective reduction of either carbohydrate (RC diet) or fat (RF diet) while keeping other two macronutrients unchanged from eucaloric baseline diet.

Ad libitum vending machine diet. For the last 3 days of each inpatient stay, participants were given *ad libitum* access to a computerized vending machine (Starfoods, Necta, Valbrembo, Italy). The MSSP procedure was used to select items for stocking the vending machine (Geiselman, Anderson et al. 1998). This paradigm was selected for use in this study as it was developed to show preference between foods of differing fat and carbohydrate content. It is composed of six categories of food, including high complex carbohydrate/high fat (HCHF), high simple sugar /high fat (HSHF), low carbohydrate/high protein/high fat (HPHF), high complex carbohydrate/low fat (HCLF), high simple sugar/low fat (HSLF), and low carbohydrate/high protein/high fat/ (HPHF). A list of 76 foods that fit into these categories was provided to participants in a Food Questionnaire. This questionnaire contained Likert-type scales with questions in which the participant rated how much they liked each of the food items that could potentially be provided in the vending machine. The questionnaire also asked how often each of those food items were consumed normally by the participant (i.e., daily, weekly, or monthly). Of these foods, a total of 40 items that fit into the previously mentioned categories were chosen for inclusion in the vending machine if preference was rated from 4 to 9 on the 10-point Likert scale.

Vending machines were stocked with traditional breakfast, lunch, dinner and snack items. Beverages and condiments were also included in the vending machine and consumption of these items was also recorded. Sugar-sweetened beverages (SSB) included fruit juices, lemonade, chocolate milk and regular sodas. Each participant had access to one vending machine that only they could access. Once foods were selected, participants were instructed to eat in the dining area and no food was allowed in the participant's room. All uneaten food and wrappers were returned to the Metabolic Kitchen to be weighed. The vending machines were re-stocked daily at 8 am with items that had been removed in the previous 24 hours. All foods were weighed to the nearest tenth of a gram on a digital scale (Mettler Toledo MS Series, Columbus, OH, USA) prior to placing them in the computerized vending machine and the remainder of any uneaten foods were weighed after consumption. Energy and macronutrient composition of the foods consumed from the vending machine were calculated using a computerized nutrition database (ProNutra, Viocare Inc., Princeton, NJ, USA).

Vending machine foods were retrospectively categorized as either ultra-processed or non-ultra-processed based on NOVA categories (Monteiro, Cannon et al. 2018) and additionally categorized as hyperpalatable or non-hyperpalatable based on definitions presented by (Fazzino, Rohde et al. 2019).

Statistical analyses of caloric intake from Vending Machines were performed using IBM SPSS Statistics (28.0.1.1). Repeated-measure mixed model analyses were used to assess differences in intake of energy, macronutrients, percent of calories from MSSP and sugar sweetened beverages among 17 participants completing both 3-day *ad libitum* periods.

Magnetic Resonance Imaging

On the afternoon following the morning PET scanning, high resolution anatomical brain MRI was acquired with a HDx General Electric 3 Tesla scanner (TE = 2.7ms, TR 7.24 ms, flip angle 12°, voxel size 0.937*0.937*1.2mm) for each subject.

Under each diet condition, all subjects were scanned at 18:00, 4.5 h after a standardized, diet-appropriate meal. Functional and structural imaging was performed on a 3T General Electric scanner and a GE 8-channel receive-only head coil. High-resolution anatomical images were collected prior to functional scanning runs (TE = 2.7 ms, TR: 7.24 ms, flip angle: 12 degrees, voxel size: 0.937×0.937×1.2 mm). For the functional scans, 206 magnetic resonance (MR) volumes were acquired. Each echoplanar image (EPI) consisted of 44 2.8-mm slices (echo time [TE] = 27 ms, repetition time [TR] = 2500 ms, flip angle = 90 degrees, voxel size = 3.4375×3.4375×2.8 mm). All structural and functional images were collected with a Sensitivity Encoding (SENSE) factor of 2 used to reduce image collection time (for structural images) or minimize image distortions (in functional images) while reducing gradient coil heating over the course of the scan session.

The task is described in detail elsewhere (Simmons et al., 2014). In brief, 144 visual food cues ranging from highly processed, energy dense foods to raw fruits and vegetables were displayed to participants using E-prime software (www.pstnet.com). Images

projected to the scanner-room screen were viewed via head coil-mounted mirror. Each image was presented for 5 seconds during which time participants indicated their response to a question ("If given the opportunity right now, how pleasant would it be to eat this food?") using an MR compatible scroll wheel to select values along a number line positioned next to the image. A fixation cross was presented for varying durations between stimuli (mean ISI = 3.7 seconds; duration 2.5–7.5 seconds). The pleasantness rating scale ranged from 1 ("neutral") to 7, with 1 depicted as "neutral" and 7 as "extremely pleasant" and included an "unpleasant" option represented by the letter "X" located below the number line. For images that participants viewed as "unpleasant", they were instructed to select the "X" if they believed the depicted food would be at all unpleasant to eat. Food images rated as "unpleasant" were excluded from the MRI and behavioral analyses.

Analyses of functional neuroimaging were performed in AFNI (AFNI_20.2.00 'Aulus Vitellius'). Each individual's anatomical MRI was transformed into the Talairach space, and the transformation matrix was applied to the functional data during pre-processing. All functional volumes were aligned to a common base EPI represented by the third volume of the first functional run. The first three volumes of each EPI run were trimmed to allow the fMRI signal to reach steady state. A slice-time correction was applied to all functional volumes, which were also smoothed with a 6-mm full-width half-max Gaussian kernel. Additionally, the signal value for each EPI volume was normalized to the percent signal change from the voxel's mean signal across the time course.

Individual subject data were checked for quality assurance, and outlying time points resulting from head motion were censored from the analyses. At the individual level, multiple regression was used to analyze the data, with regressors of non-interest included in the model to account for each run's signal mean, linear, quadratic, and cubic signal trends, as well as six motion parameters (three translations and three rotations) saved from the image registration step during pre-processing. The food pleasantness task regressor was constructed by convolving a box-car function with a width of 5 s beginning at the onset of the food image with a gamma-variate function to adjust the predictor variable for the delay and shape of the BOLD response.

Positron Emission Tomography

PET scanning was completed using Siemens HRRT (Siemens Healthcare, Malvern, PA) 2 hours after a standard breakfast and immediately after a bolus of 5 mCi of [18F]fallypride infused intravenously using a Harvard® pump. The specific activity was approximately 2000 mCi/μmol at time of injection and the radiochemical purity of the radiotracer was > 99%. PET emission data were collected over 3.5 hours in three blocks separated by two 10-minute breaks. Thirty-three volumes were acquired at times 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 12.5, 15, 20, 25, 30, 40, 50, 60, 90, 110, 130, 170, 200 min. During each scan block, the room was quiet and dimly lit and each subject was instructed to keep their head as still as possible, relax, and try to avoid falling asleep. The image reconstruction process corrected for head motion which was tracked throughout each scan.

Each scan consisted of 207 slices (slice separation = 1.22 mm). The fields of view were 31.2 cm and 25.2 cm for transverse and axial slices, respectively. A transmission scan was obtained with a 137Cs rotating pin source before radiotracer injection and before the emission scan to correct for attenuation. The PET images were aligned within each scan block with 6-parameter rigid registration using 7th order polynomial interpolation and each block was aligned to the volume taken at 20 min of the first block. The final alignments were visually checked, with translations varying by <5 mm and the rotations by <5 degrees.

Quantification and Statistical Analysis

fMRI images were included in AFNI's 3dttest++ to identify clusters of significant effects of the diet condition (RF>Baseline for n=17; RC>Baseline for n=17; RF>RC for n=15). Analyses using participants with complete neuroimaging data across 3 diet conditions (n=13) were analyzed via AFNI 3dANOVA (Supplementary Materials). Since diet condition did not have a significant impact on food pleasantness ratings, analysis of brain activity to food pictures was not modulated by pleasantness ratings to maximize study power. Small volume corrections were implemented within the ROI defined by the orbitofrontal cortex, striatal-pallidal reward neurocircuit previously described (Simmons, Rapuano et al. 2014) (Supplementary Figure 4) with a voxel-wise $p<0.005$ and cluster size threshold to achieve bi-sided correction for multiple comparisons at $p<0.05$ via AFNI 3dclustsim.

Individual participants' anatomical MRI images (see above) were co-registered to the aligned PET images by minimizing a mutual information cost function for each individual participant. Each individual's anatomical MRI was transformed into the Talairach space, and the transformation matrix was applied to the PET images which were then smoothed with a 5-mm full-width, half-max Gaussian kernel. Time-activity curves for [18F] fallypride concentration in each voxel were fit to a four-compartment model (with the cerebellum used as the reference tissue) to determine D2BP (Lammertsma and Hume 1996). Participants' D2BP maps were included in AFNI's 3dttest++ identify clusters of significant dietary effects (RF>Baseline for n=15; RC>Baseline for n=17; RF>RC for n=15). Analyses using participants with complete neuroimaging data across 3 diet conditions (n=13) were analyzed via AFNI 3dANOVA (Supplementary Materials). Since high D2BP occurs mainly in striatum, small volume corrections were implemented within each hemisphere where D2BP >1.5. A bi-sided voxel-wise threshold of $p<0.1$ was used, and cluster size threshold to achieve correction for multiple comparisons at $p<0.05$. Using a full mixed effects model (AFNI 3dANOVA3), clusters survive correction for multiple comparisons using 3dClustSim at alpha of 0.05 a threshold of 33 voxels.

Additional Resources

ClinicalTrials.gov Identifier NCT00846040

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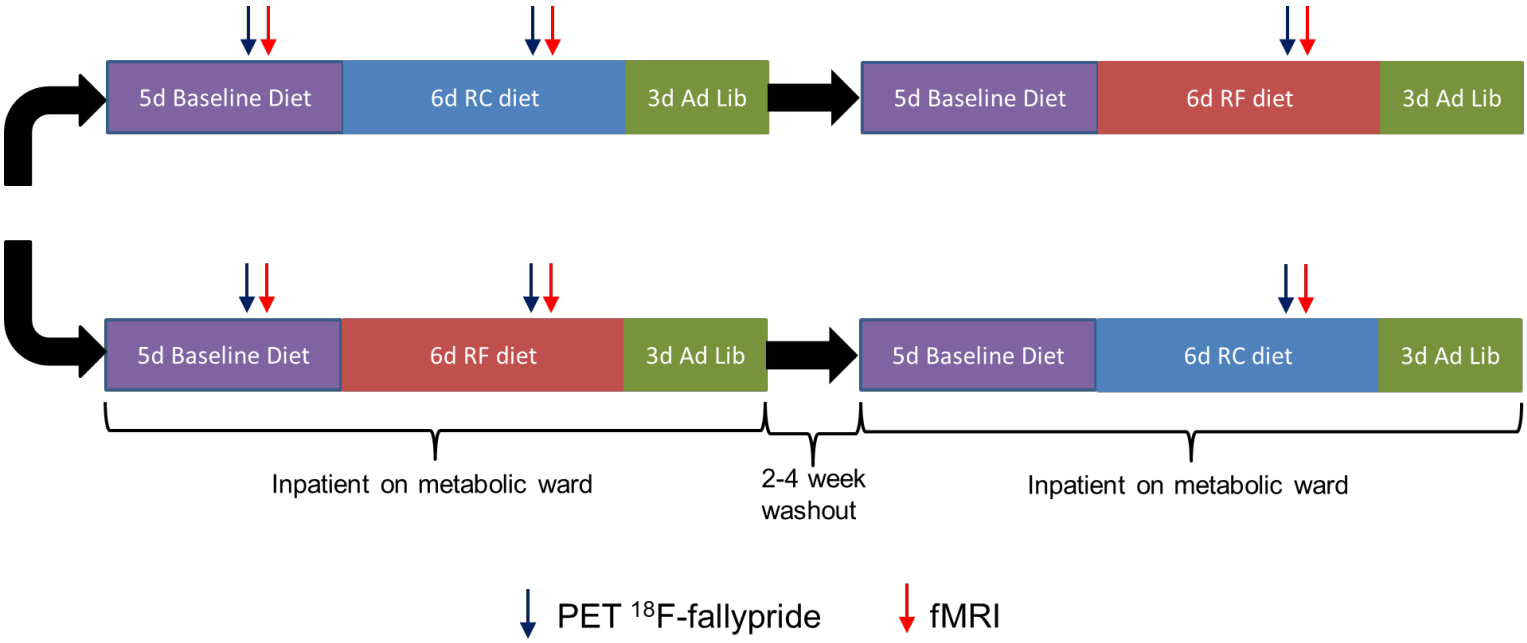

Figure 1

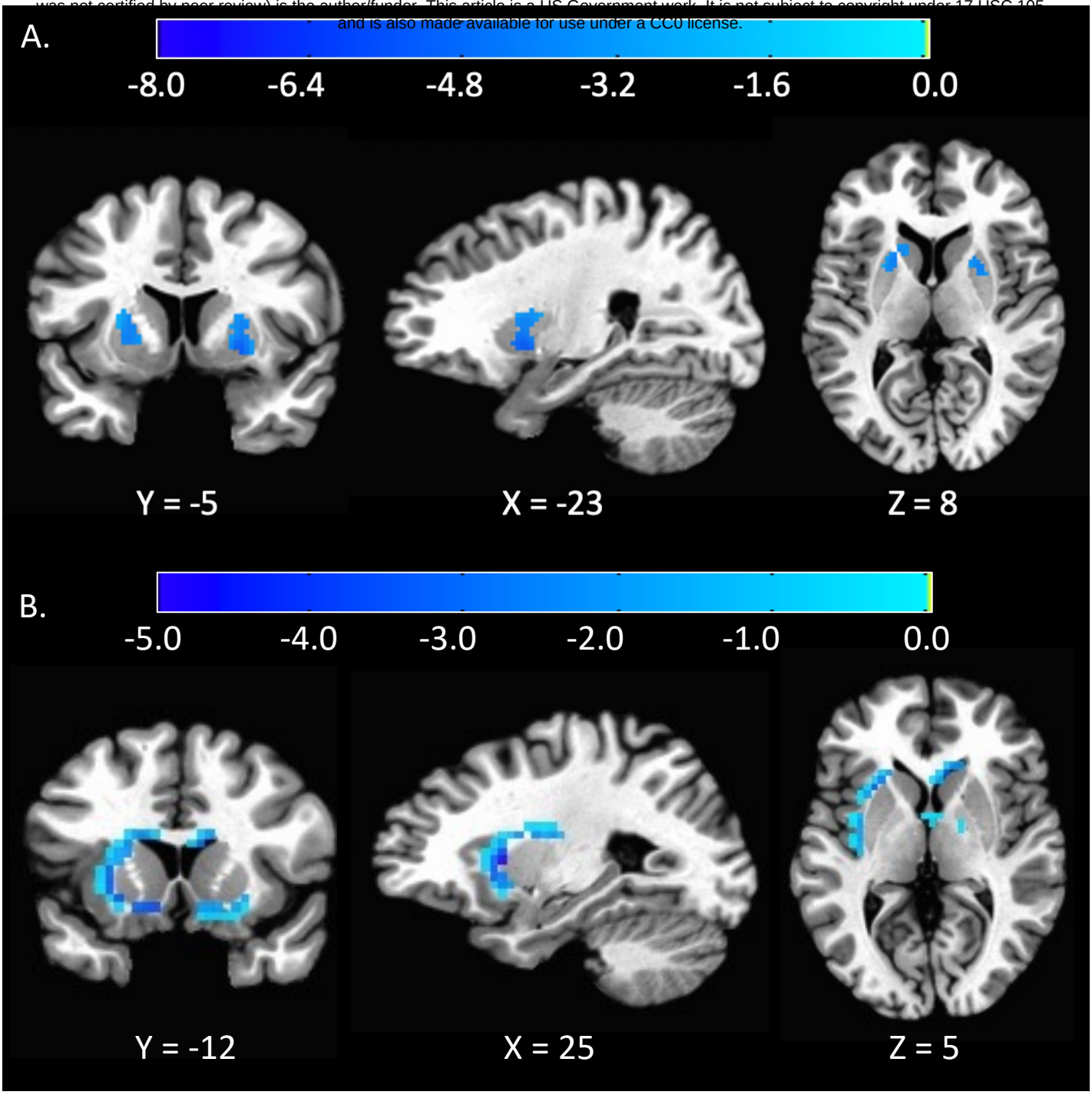

Figure 2