Cell Metabolism
Clinical and Translational Report

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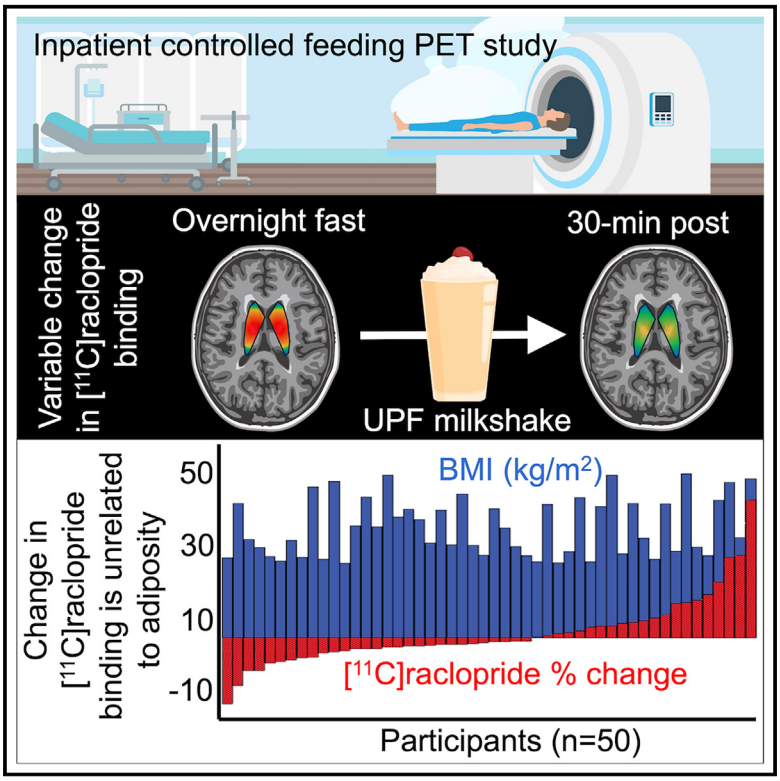
## Graphical abstract




## Highlights

- PET scans found no significant mean dopamine response to ultra-processed milkshakes
- Individual brain dopamine responses were not significantly related to adiposity
- Greater brain dopamine responses were correlated with fasting hunger levels
- *Ad libitum* cookie intake was correlated with brain dopamine responses

## Authors

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## In brief

Darcey et al. used standard PET methods in 50 adults to measure their brain's dopamine responses after consuming ultra-processed milkshakes high in fat and sugar. The milkshakes elicited no statistically significant mean brain dopamine response, and the variable individual post-ingestive dopamine responses were not significantly related to adiposity.

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# Brain dopamine responses to ultra-processed milkshakes are highly variable and not significantly related to adiposity in humans

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## SUMMARY

Ultra-processed foods high in fat and sugar have been theorized to be addictive due to their purported ability to induce an exaggerated post-ingestive brain dopamine response akin to drugs of abuse. Using [$^{11}$C]raclopride positron emission tomography (PET) displacement methods used to measure brain dopamine responses to addictive drugs, we measured striatal dopamine responses beginning 30 min after ingesting an ultra-processed milkshake high in fat and sugar in 50 young, healthy adults over a wide body mass index (BMI) range (20–45 kg/m$^2$). Surprisingly, milkshake consumption did not result in a significant post-ingestive dopamine response in the striatum ($p = 0.62$) nor in any striatal subregion ($p > 0.33$), and the highly variable interindividual responses were not significantly related to adiposity (BMI: $r = 0.076$, $p = 0.51$; % body fat: $r = 0.16$, $p = 0.28$). Thus, post-ingestive striatal dopamine responses to an ultra-processed milkshake were likely substantially smaller than for many addictive drugs and below the limits of detection using standard PET methods.

## INTRODUCTION

Ultra-processed foods often contain high levels of both sugar and fat[1]—a highly palatable combination that rarely occurs in natural foods.[2] Diets high in ultra-processed foods can cause excess calorie intake and weight gain[3] and have been linked with increased risk of obesity and several other chronic diseases.[4] Ultra-processed foods have been hypothesized to alter the normal gut-brain nutrient-sensing pathways in ways that may enhance their reinforcing effects.[5] In animal models, brain dopamine responds rapidly to the orosensory properties of food and is related to palatability.[6,7] Post-ingestive nutrient sensing of fat and sugar elicits prolonged dopamine responses primarily in the dorsal striatum via separate gut-brain pathways,[8–12] and their combination results in a synergistic effect.[13] Functional MRI studies suggest that similar synergistic effects may occur in humans[14,15] and may be impacted by adiposity such that blunted dopamine responses to consuming sugar alone are observed in people with obesity.[16]

Ultra-processed foods have been theorized to be addictive, in part, due to their consumption eliciting an outsized dopamine response in brain reward regions,[17] similar to drugs of abuse.[18] According to the dopamine theory of addiction, repeated consumption would eventually result in downregulation of dopamine type-2 receptors along with reduced post-ingestive dopamine responses in the striatum of the brain, thereby resulting in tolerance.[19] If people with obesity are addicted to ultra-processed foods via this mechanism, then adiposity should be associated with a reduction in striatal dopamine type-2 receptors and a blunted dopamine response to ultra-processed food consumption. A

seminal study suggested that obesity was indeed related to reduced striatal dopamine type-2 receptor availability.[20] However, we recently reported that previous observations are likely explained by adiposity being associated with increased dopamine tone rather than reduced receptor number.[21]

Here, we investigated whether humans exhibit an exaggerated post-ingestive brain dopamine response to ultra-processed milkshakes high in both fat and sugar and whether the dopamine response is related to adiposity. Brain dopamine response was measured beginning 30 min after consuming an ultra-processed milkshake using a standard positron emission tomography (PET) [$^{11}$C]raclopride displacement method used to investigate drugs of abuse.[22–26] In our preregistered aims, we hypothesized that striatal dopamine D2-like receptor binding potential (D2BP) would significantly decrease after milkshake consumption relative to the fasted state, indicating increased dopamine release displacing the radiotracer from dopamine D2 receptors. We further hypothesized that post-ingestive dopamine responses to milkshake consumption would be negatively correlated with adiposity. Instead, we found that post-ingestive striatal dopamine responses were highly variable, not statistically significant, and not significantly related to adiposity.

## RESULTS

A description for this preregistered clinical trial has been described elsewhere.[21] In brief, 61 weight-stable adults (65% female) completed 3–5 days of outpatient dietary stabilization through a eucaloric standardized diet (50% calories from carbohydrate, 35% from fat, 15% from protein; see STAR Methods) provided by the NIH Metabolic Kitchen, which was continued into the 5-day inpatient stay at the NIH Clinical Center that immediately followed (Table 1; Figure S1). Participants consumed the eucaloric stabilization diet for 4.5 ± 1.0 days outpatient prior to admission and completed [$^{11}$C]raclopride scanning after 2.4 ± 0.9 days inpatient (corresponding to 6.8 ± 1.1 days of total diet stabilization by the time of [$^{11}$C]raclopride PET scanning).

Data for both fasting and post-milkshake D2BP are available for $n$ = 50 participants (Figure S2).

### No significant post-ingestive striatal dopamine response to an ultra-processed milkshake

Participants completed the first of two [$^{11}$C]raclopride PET scans in a confirmed overnight fasted state. Upon completion of the fasted scan, participants rested quietly in an adjacent room for approximately 75 min, at the end of which they were allotted 5 min to consume a vanilla milkshake (226 mL) (see STAR Methods). Participants began their second and final [$^{11}$C]raclopride scan 30 min after initiating consumption of the milkshake. A paired-samples analysis across the entire sample revealed that the mean D2BP after the milkshake was not significantly different from mean D2BP at fasting (whole striatal D2BP post-milkshake 2.9 [0.06 SEM] vs. whole striatal D2BP fasting 2.9 [0.06 SEM]; $p$ = 0.616) (Figure 1A). D2BP was not significantly different between fasting and post-milkshake in any striatal subregion of interest ($p$'s > 0.33) (Figure S3). Further, no clusters emerged from corresponding voxelwise analyses (see Figure S4 for unthresholded voxelwise D2BP maps). Whole striatal dopamine response to milkshake did not significantly differ by sex ($p$ = 0.207).

Given that the only human study to assess temporal dynamics of dopamine responses to milkshake ingestion suggested that the peak response may occur roughly 20 min after initiating intake,[27] we sought to investigate whether we may have missed an early striatal dopamine response to the ultra-processed milkshake when using the complete time-activity curves collected over the full 70 min PET session. To address this possibility, we calculated striatal D2BP from time-activity curves excluding frames from late in the PET session. Compared with D2BP calculated using the full-time-activity curves after the milkshake, D2BP calculated using only the first 30 min of scanning decreased slightly by 0.06 ± 0.02 ($p$ = 0.006) but was similar to the D2BP decrease using the first 30 min of scanning in the fasted state (0.05 ± 0.03; $p$ = 0.13). These negligible differences in striatal D2BP suggest that our methods likely did not mask a post-ingestive dopamine signal earlier in the scan time course.

### Adiposity was not significantly correlated with post-ingestive striatal dopamine responses

We hypothesized that dopamine responses to the milkshake (percent decrease in D2BP between post-milkshake and fasting) would be dampened at higher adiposity. Body mass index (BMI) tended to be weakly related to dopamine response such that leaner individuals had a slightly greater decrease in D2BP from fasting (whole striatum D2BP, $r$ = 0.276, $\beta$ = 0.320, $p$ = 0.052; Figure 2A). However, this relationship was not robust to influential data points within the striatum as a whole (robust regression $\beta$ = 0.076, $p$ = 0.507; Figure 2A) or in individual striatal regions of interest (Figure S5). Adjusting dopamine response by age and sex did not reveal a relationship with BMI in any region of interest (Figure S6), and there were no permutations of BMI, age, and sex that significantly improved prediction of "responder" group membership as indicated by binary logistic regression. No clusters emerged from corresponding voxelwise analyses correlating BMI and milkshake response (ΔD2BP [milkshake − fasting]) (see Figure S4B for unthresholded voxelwise maps). Furthermore, neither kg of fat mass ($r$ = 0.219, $p$ = 0.126, $n$ = 50), body fat percentage ($r$ = 0.155, $p$ = 0.282, $n$ = 50), age ($r$ = 0.139, $p$ = 0.337, $n$ = 50), fasting glucose ($r$ = 0.159, $p$ = 0.280, $n$ = 48), fasting insulin ($r$ = 0.137, $p$ = 0.360, $n$ = 47), nor insulin sensitivity (HOMA-IR; $r$ = 0.112, $p$ = 0.459, $n$ = 46) were significantly correlated with whole striatal dopamine response to the post-ingestive milkshake state.

While the milkshake was provided as the same absolute amount to all participants (418 kcal), this amount varied as a proportion of each participant's resting energy expenditure (REE). Nevertheless, milkshake energy intake adjusted for REE was not significantly related to the striatal dopamine response (% of REE; $r$ = −0.175, $p$ = 0.228, $n$ = 49).

### Post-ingestive striatal dopamine responses may be related to perceived hunger and hedonic responses to the milkshake

To explore correlates of the highly variable interindividual dopamine responses to the ultra-processed milkshakes (Figure 1B), we investigated features that distinguished those who demonstrated a dopamine response in the expected direction

**Table 1. Participant characteristics and group differences between milkshake responders and non-responders at the whole striatum level**

| Variable | Enrolled participants (n) | Enrolled participants | Milkshake completers (*n*) | Milkshake completers | Milkshake responders (*n*) | Milkshake responders | Milkshake non-responders (n) | Milkshake non-responders | p (responders vs. on-responders) |
|---|---|---|---|---|---|---|---|---|---|
| Total *N* | 61 | – | 50 | – | 29 | – | 21 | – | – |
| Females | 40 | 65% | 38 | 66.7% | 19 | 65.5% | 14 | 66.7% | 0.933 |
| Cycle day | 31 | 17.4 ± 9.9 | 26 | 17.9 ± 10.1 | 16 | 15.6 ± 10.5 | 10 | 21.6 ± 8.7 | 0.141 |
| Race | – | – | – | – | – | – | – | – | 0.741 |
| Black | 32 | 52.5% | 27 | 54.0% | 15 | 51.7% | 12 | 57.1% | – |
| White | 18 | 29.5% | 15 | 30.0% | 10 | 34.5% | 5 | 23.8% | – |
| Asian | 7 | 11.5% | 5 | 10.0% | 3 | 10.3% | 2 | 9.5% | – |
| Other/multiple | 4 | 6.6% | 3 | 6.0% | 1 | 3.4% | 2 | 9.5% | – |
| Age (years) | 61 | 32.2 ± 7.2 | 50 | 31.9 ± 7.2 | 29 | 30.8 ± 7.5 | 21 | 33.4 ± 6.8 | 0.218 |
| Body weight (kg) | | | | | | | | | |
| Mean | 61 | 85.9 ± 25.3 | 50 | 86.1 ± 25.0 | 29 | 84.6 ± 23.1 | 21 | 88.2 ± 27.7 | 0.622 |
| Range | 61 | 45.9–148.6 | 50 | 45.9–148.6 | 29 | 57.2–148.6 | 21 | 45.9–133.9 | – |
| Body fat (%) | | | | | | | | | |
| Mean | 61 | 35.0 ± 12.6 | 50 | 35.1 ± 12.3 | 29 | 35.9 ± 11.5 | 21 | 33.9 ± 13.6 | 0.571 |
| Range | 61 | 11.3–59.0 | 50 | 11.3–52.4 | 29 | 12.1–52.4 | 21 | 11.3–51.2 | – |
| BMI (kg/m$^2$) | | | | | | | | | |
| Mean | 61 | 30.1 ± 8.2 | 50 | 30.2 ± 7.9 | 29 | 29.5 ± 7.2 | 21 | 31.0 ± 8.9 | 0.540 |
| Range | 61 | 20.3–52.8 | 50 | 20.3–44.8 | 29 | 20.3–44.4 | 21 | 20.5–44.8 | – |
| Resting energy expenditure (kcal/day) | 60 | 1,624 ± 319 | 49 | 1,626 ± 321 | 29 | 1,607 ± 299 | 20 | 1,655 ± 357 | 0.608 |
| Glucose, fasting (mg/dL) | 55 | 91.8 ± 7.7 | 48 | 92.8 ± 7.3 | 27 | 93.0 ± 7.5 | 21 | 92.5 ± 7.2 | 0.826 |
| Insulin, Fasting (μU/mL) | 54 | 12.6 ± 7.4 | 47 | 12.6 ± 7.0 | 28 | 13.0 ± 7.8 | 19 | 12.1 ± 5.6 | 0.689 |
| HOMA-IR | 52 | 2.9 ± 1.9 | 46 | 2.9 ± 1.8 | 27 | 3.1 ± 2.1 | 19 | 2.8 ± 1.3 | 0.563 |
| Habitual diet (food frequency questionnaire) | | | | | | | | | |
| Usual energy intake (kcal/day) | 52 | 1,497 ± 662 | 45 | 1,481 ± 642 | 28 | 1,464 ± 664 | 17 | 1,510 ± 622 | 0.821 |
| Protein (% kcal) | 52 | 15.7 ± 4.2 | 45 | 15.7 ± 4.2 | 28 | 15.5 ± 3.3 | 17 | 16.0 ± 5.5 | 0.697 |
| Fat, total (% kcal) | 52 | 33.0 ± 8.2 | 45 | 33.3 ± 8.1 | 28 | 33.6 ± 6.6 | 17 | 32.7 ± 10.5 | 0.709 |
| Saturated fat (% kcal) | 52 | 10.5 ± 3.0 | 45 | 10.5 ± 3.1 | 28 | 10.9 ±2.8 | 17 | 9.8 ± 3.6 | 0.256 |
| Fatty acid ratio (unsat:sat) | 52 | 1.9 ± 0.4 | 45 | 2.0 ± 0.4 | 28 | 1.9 ± 0.3 | 17 | 2.1 ± 0.4 | 0.032 |
| Carbohydrate, total (% kcal) | 52 | 51.6 ± 11.7 | 45 | 51.2 ± 11.7 | 28 | 50.5 ± 9.5 | 17 | 52.4 ± 14.8 | 0.641 |
| Added sugars (g) | 52 | 47.0 ± 40.3 | 45 | 46.1 ± 39.7 | 28 | 43.4 ± 37.0 | 17 | 50.5 ± 44.7 | 0.562 |

(*Continued on next page*)

**Table 1.** ***Continued***

| Variable | Enrolled participants (n) | Enrolled participants | Milkshake completers (*n*) | Milkshake completers | Milkshake responders (*n*) | Milkshake responders | Milkshake non-responders (n) | Milkshake non-responders | p (responders vs. on-responders) |
|---|---|---|---|---|---|---|---|---|---|
| Taste preferences | | | | | | | | | |
| Fat taste preference (% milkfat; w/v) | 49 | 11.1 ± 6.0 | 41 | 11.6 ± 6.5 | 24 | 10.6 ± 5.4 | 17 | 12.9 ± 7.7 | 0.271 |
| Sweet taste preference (g sucrose/1,000 mL water) | 51 | 11.9 ± 9.1 | 42 | 12.6 ± 9.1 | 25 | 12.0 ± 8.5 | 17 | 13.6 ± 10.0 | 0.576 |
| Three factor eating questionnaire | | | | | | | | | |
| Cognitive restraint | 61 | 8.3 ± 4.7 | 59 | 8.5 ± 4.6 | 29 | 8.8 ± 4.0 | 21 | 8.1 ±5.3 | 0.617 |
| Disinhibition | 61 | 4.8 ± 2.7 | 50 | 5.0 ± 2.8 | 29 | 5.3 ± 2.7 | 21 | 4.4 ± 2.8 | 0.256 |
| Hunger | 61 | 3.2 ± 2.6 | 50 | 3.4 ± 2.7 | 29 | 3.3 ± 2.9 | 21 | 3.5 ± 2.5 | 0.799 |
| Yale food addiction scale | | | | | | | | | |
| Continuous symptom count | 60 | 1.1 ± 1.0 | 48 | 1.1 ± 0.9 | 29 | 1.2 ±1.1 | 19 | 1.0 ± 0.7 | 0.293 |
| Beck depression inventory | | | | | | | | | |
| Total score | 61 | 1.9 ± 2.6 | 50 | 2.0 ± 2.8 | 29 | 1.8 ± 2.8 | 21 | 2.3 ± 2.7 | 0.598 |

Participant characteristics and group differences between participants demonstrating a post-ingestive decrease in D2BP as a result of milkshake (responders) and those demonstrating an increase in D2BP (non-responders). Means and standard deviations indicated.

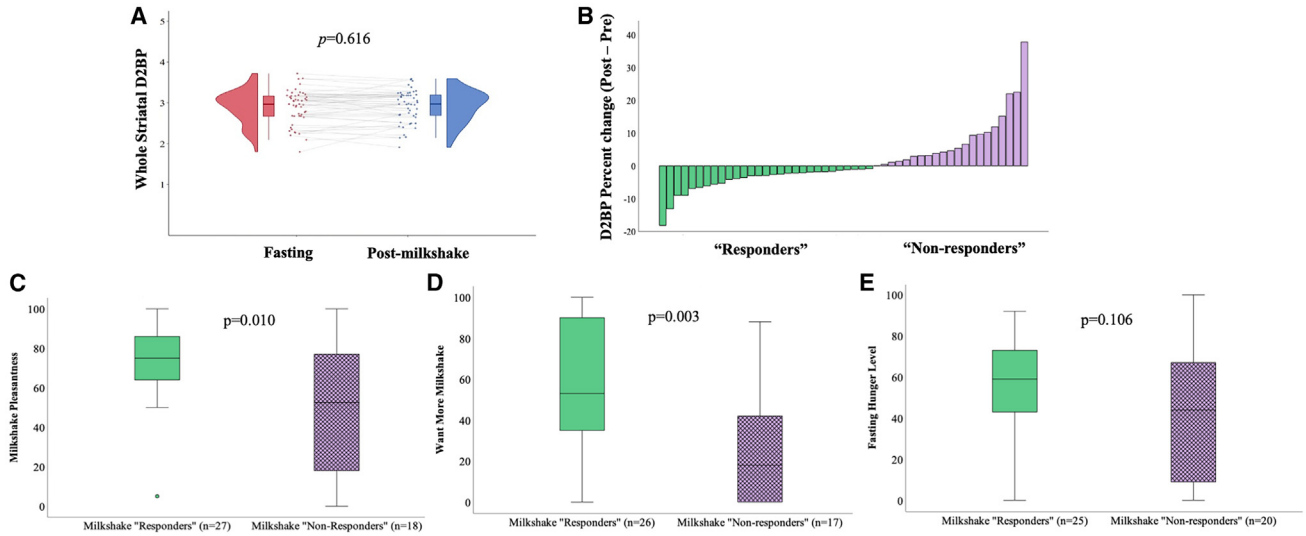


**Figure 1. Variability in dopamine response to post-ingestive effects of an ultra-processed milkshake**

(A) An ultra-processed milkshake did not significantly impact whole striatal D2-like receptor binding potential (D2BP) across the whole sample ($n$ = 50). See also Figures S3 and S4A.

(B) Distribution of percent change between fasting whole striatal D2BP and post-milkshake whole striatal D2BP, with individuals displaying dopamine release (green, left, "responders," $n$ = 29) and those who did not (purple, right "non-responders," $n$ = 21).

(C–E) (C) Those classified as milkshake responders rated the milkshake as more pleasant (0 = "neutral," 100 = "extremely pleasant") (D) and reported greater wanting (0 = "I don't want any more," 100 = "I want much more of the milkshake") (E) but similar levels of hunger after an overnight fast compared with non-responders. Significance values reflect paired t tests for visualization purposes. Box plots indicate the median as the horizontal center line, the box edges indicate the interquartile range, and the whiskers indicate 1.5 times the interquartile range from the box.

("responders") compared with those who demonstrated an increase in D2BP after milkshake, opposite to that expected ("non-responders") (Table 2).

Responders perceived the milkshake to be more pleasant (73.3 [4.1] vs. 48.2 [8.0], $p$ = 0.010), and they wanted more of the milkshake (56.4 [6.4] vs. 25.8 [6.8] $p$ = 0.003) and tended to be hungrier in the overnight fasted state (55.7 [5.1] vs. 41.3 [7.4], $p$ = 0.106) as compared with the non-responders (Figures 1C–1E; Table 2). Furthermore, non-responders tended to report an increase in perceived hunger after the milkshake compared with responders (Table 2). Both groups indicated similar preferences for fat ($p$ = 0.271) and sweet ($p$ = 0.576) tastes (Table 1) and similarly considered the milkshake to have "met expectations" ($p$ = 0.365; Table 2).

Across the group as a whole, there were no significant correlations between whole striatal dopamine response and degree to which the milkshake met expectations ($r$ = −0.064, $p$ = 0.681, $n$ = 43), perceived milkshake pleasantness ($r$ = −0.194, $p$ = 0.201, $n$ = 45), or wanting more milkshake ($r$ = −0.237, $p$ = 0.126, $n$ = 43). Further, these relationships were also not evident in striatal region of interest (ROI) subregions ($p$'s > 0.111, not shown). Finally, there were no significant correlations between adiposity and the degree to which the milkshake met expectations (BMI: $r$ = −0.049, $p$ = 0.754, $n$ = 43; percent body fat: $r$ = 0.011, $p$ = 0.947, $n$ = 43), perceived milkshake pleasantness (BMI: $r$ = −0.094, $p$ = 0.54, $n$ = 45; percent body fat: $r$ = 0.092, $p$ = 0.55, $n$ = 45), or wanting more milkshake (BMI: $r$ = 0.027, $p$ = 0.862, $n$ = 43; percent body fat: $r$ = 0.051, $p$ = 0.744, $n$ = 43).

While perceived hunger after an overnight fast was not significantly related to adiposity (BMI: $r$ = −0.185, $p$ = 0.223, $n$ = 45; percent body fat: $r$ = −0.030, $p$ = 0.844, $n$ = 45), hunger level was weakly related to whole striatal dopamine responses to the milkshakes ($r$ = 0.288, $p$ = 0.055, $n$ = 45) driven largely by responses in the right caudate ($r$ = 0.311, $p$ = 0.037), right pallidum ($r$ = −0.309, $p$ = 0.039), and left putamen ($r$ = −0.390, $p$ = 0.008) (Figure 3A). These regional associations were largely supported by voxelwise analyses (Figure 3B), revealing clusters in the left putamen and right caudate where the magnitude of milkshake response is correlated with perceived hunger after an overnight fast (Table S1 for cluster details). The change in hunger between the fasted and post-milkshake states correlated with whole striatal dopamine responses to the milkshakes ($r$ = 0.393, $p$ = 0.019, $n$ = 35) such that the more hunger was suppressed by the milkshake, the greater the degree of observed dopamine release. This effect was largely driven by dorsal rather than ventral striatal ROIs.

The milkshake increased blood glucose and insulin at both 30 and 90 min post-milkshake, but neither the overall increase in glucose nor insulin nor rates of increases were correlated with the milkshake dopamine responses at the whole striatal or sub-striatal ROI levels (not shown). Furthermore, we did not observe significant differences in either postprandial glucose or insulin changes between responders and non-responders (Figure S7).

## Post-ingestive dopamine responses correlated with *ad libitum* intake of ultra-processed cookies high in fat and sugar

On their last inpatient day, participants were offered an *ad libitum* buffet (Figure S8) in a metabolic state similar to that of milkshake

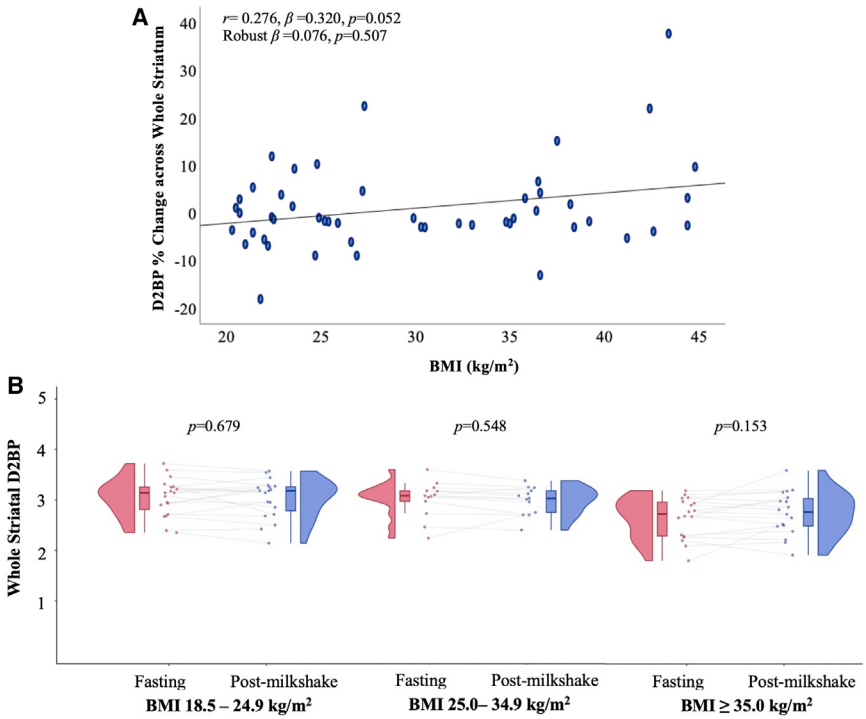


**Figure 2. Variability in dopamine response to milkshake was not related to adiposity**

(A) Relationship between BMI and whole striatal post-ingestive dopamine response to milkshake (% change from fasting) was not robust to influential data points.

(B) An ultra-processed milkshake did not significantly impact percent change in whole striatal [$^{11}$C]raclopride binding potential from fasting across three BMI strata (BMI 18.5–24.9 kg/m$^2$, $n$ = 19; BMI 25.0–34.9 kg/m$^2$, $n$ = 13; BMI ≥ 35.0 kg/m$^2$, $n$ = 18; 3-way ANOVA F = 1.41, $p$ = 0.254). Significance values reflect paired t tests for visualization purposes.

See also Figures S4B–S6. Box plots indicate the median as the horizontal center line, the box edges indicate the interquartile range, and the whiskers indicate 1.5 times the interquartile range from the box.

ingestion on a previous day and were instructed to eat as much or as little as they desired. Energy consumed (kcal) was calculated after remaining food was weighed back by Metabolic Kitchen staff. Exploratory analyses of energy intake are adjusted by REE measured during the inpatient stay.

REE-adjusted total energy intake was not correlated with dopamine response to milkshake across the striatum as a whole ($r$ = −0.205, $p$ = 0.176) but tended to be weakly correlated with post-ingestive dopamine response again in the left putamen ($r$ = −0.279, $p$ = 0.064).

We separated energy intake from the sole high-fat, high-sugar ultra-processed food item offered at the meal test, chocolate chip cookies (REE-adjusted cookie energy intake, "cookie EI"), from energy consumed from other foods (REE-adjusted non-cookie energy intake, "non-cookie EI"). While non-cookie EI was not related to dopamine response to milkshake in any striatal ROI ($p$'s > 0.131), cookie EI specifically tended to weakly correlate with whole striatal ($r$ = −0.283, $p$ = 0.06) and left caudate ($r$ = −0.276, $p$ = 0.067) response and was significantly correlated with dopamine response in the left pallidum ($r$ = −0.332, $p$ = 0.026) and again in the left putamen ($r$ = −0.323, $p$ = 0.031) (Figure 3C).

Voxelwise analyses support the ROI analyses, revealing bilateral clusters in the putamen where the magnitude of milkshake response is correlated with REE-adjusted *ad libitum* cookie energy intake (Figure 3D; cluster information in Table S1).

## DISCUSSION

Contrary to our hypotheses, we did not find evidence for a significant average increase in post-ingestive striatal dopamine release in response to consuming ultra-processed milkshakes high in fat and sugar. Furthermore, interindividual variation in the post-ingestive dopamine response was not significantly related to adiposity. Instead, our exploratory analyses suggest that variability in post-ingestive dopamine response may be related to perceived hunger, hedonic responses, and may predict future ultra-processed food eating behaviors.

Our study was designed to elicit a post-ingestive dopamine response as well as to minimize several sources of variability by delivering a single exposure to a novel milkshake formulation that participants experienced as a non-random, unconditioned stimulus at the time of PET scanning after a confirmed, standardized overnight fast following a period of controlled feeding in weight-stable adults. This design minimized psychological and behavioral influences (e.g., pre-exposure to milkshake,[28] cue-expectation,[29] and variability in physiological state).[30,31]

The [$^{11}$C]raclopride PET displacement method used in our study[32,33] has high reproducibility,[34] with test-retest absolute D2BP differences in the striatum of ∼6%.[35–37] This method has been regularly used to measure significant mean striatal dopamine responses following ingestion of substances with the greatest potential for abuse and addiction, such as psychostimulants that produce ∼10%–20% decreases in mean striatal D2BP.[24,25,38] However, relatively large increases in extracellular dopamine, as documented by simultaneous microdialysis measurements[39–42] are required to detect acute displacement of [$^{11}$C]raclopride in the striatum using PET. Thus, the ultra-processed milkshake post-ingestive signals may have resulted in striatal dopamine responses that were too small to detect using the standard [$^{11}$C]raclopride PET method.

In other words, despite expecting the high-fat and sugar ultra-processed milkshake to produce a synergistic effect on striatal dopaminergic activity,[13,14] our data suggest that any extracellular dopamine responses following milkshake consumption were much smaller than those following ingestion of *most* drugs of abuse. Thus, the narrative that the combination of high-fat and high-sugar content frequently found in

**Table 2. Group differences between participants demonstrating a decrease in whole striatal D2BP as a result of milkshake (responders) and those demonstrating an increase in D2BP (non-responders)**

| Variable | Milkshake completers (*n*) | Milkshake completers (mean SEM]) | Milkshake responders (*n*) | Milkshake responders (mean SEM]) | Milkshake non-responders (*n*) | Milkshake non-responders (mean [SEM]) | *p* (responders vs. non-responders) |
|---|---|---|---|---|---|---|---|
| D2BP % change, whole striatum (milkshake − fasting) | | | | | | | |
| Mean percent change | 50 | 1.1 (1.3) | 29 | −4.3 (0.73) | 21 | 8.5 (2.0) | <0.0001 |
| Range | 50 | −18.1 to 37.7 | 29 | −18.1 to −0.9 | 21 | 0.03–37.7 | – |
| Milkshake ratings | | | | | | | |
| Pleasantness | 45 | 63.3 (4.4) | 27 | 73.3 (4.1) | 18 | 48.2 (8.0) | 0.010 |
| Wanting more | 43 | 44.3 (5.2) | 26 | 56.4 (6.4) | 17 | 25.8 (6.8) | 0.003 |
| Met expectations | 43 | 57.0 (4.1) | 26 | 60.1 (5.4) | 17 | 52.4 (6.4) | 0.365 |
| Hunger ratings | | | | | | | |
| After overnight fast | 45 | 49.3 (4.4) | 25 | 55.7 (5.1) | 20 | 41.3 (7.4) | 0.106 |
| Effect of milkshake(% change from fasting) | 35 | 16.9 (13.9) | 20 | −8.4 (8.6) | 15 | 50.8 (28.7) | 0.065 |
| *Ad libitum* energy intake (REE-adjusted) | | | | | | | |
| Total (kcal) | 45 | 956.7 (70.3) | 28 | 1,007.0 (76.8) | 17 | 873.8 (137.4) | 0.364 |
| Cookie-only (kcal) | 45 | 109.9 (19.0) | 28 | 134.1 (23.6) | 17 | 69.9 (30.2) | 0.102 |
| Non-cookie (kcal) | 45 | 846.3 (58.9) | 28 | 872.9 (64.3) | 17 | 803.8 (116.5) | 0.575 |
| Glycemic response to milkshake | | | | | | | |
| Glucose | | | | | | | |
| 90-min weighted average (mg/dL) | 44 | 99.8 (1.3) | 25 | 99.0 (1.5) | 19 | 100.8 (2.3) | 0.506 |
| Change, 0–30 min (mg/dL) | 46 | 3.4 (1.4) | 26 | 4.0 (1.6) | 20 | 2.7 (2.3) | 0.640 |
| Change, 30–90 min (mg/dL) | 45 | 11.5 (2.6) | 26 | 7.0 (2.8) | 19 | 17.6 (4.4) | 0.041 |
| Peak, 0–90 min (mg/dL) | 44 | 110.7 (2.1) | 25 | 107.4 (2.2) | 19 | 115.4 (3.8) | 0.094 |
| Insulin | | | | | | | |
| 90-min weighted average (μU/mL) | 36 | 36.1 (4.4) | 23 | 38.2 (6.6) | 13 | 32.6 (3.8) | 0.468 |
| Change, 0–30 min (μU/mL) | 43 | 26.5 (5.4) | 25 | 31.1 (8.9) | 18 | 20.1 (4.0) | 0.267 |
| Change, 30–90 min (μU/mL) | 36 | −5.0 (5.8) | 23 | −10.2 (7.8) | 13 | 4.1 (7.9) | 0.239 |
| Peak, 0–90 min (μU/mL) | 36 | 52.0 (6.5) | 23 | 53.6 (9.9) | 13 | 49.1 (5.2) | 0.689 |

Means and standard errors reported.

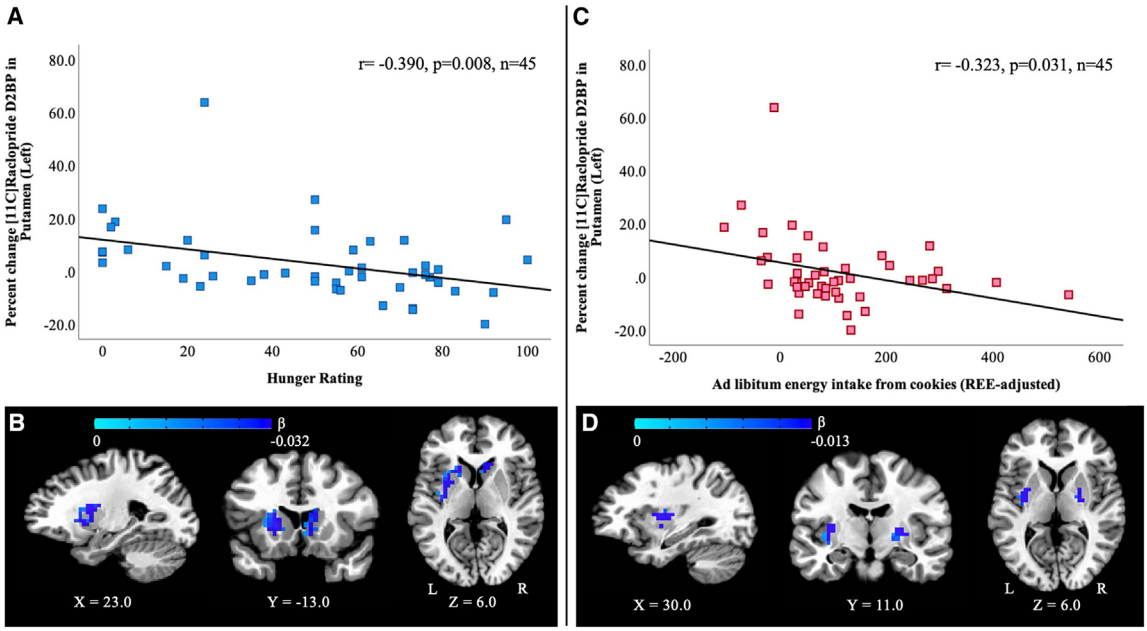


**Figure 3. Post-ingestive dopamine responses to milkshakes correlated with prior fasting hunger and subsequent *ad libitum* cookie energy intake**

(A) Region of interest (ROI) analyses indicate that self-reported hunger after an overnight fast correlated with dopamine response to milkshake consumption, particularly in the left putamen.

(B) The ROI relationship between hunger and dopamine response, was supported by voxelwise correlation analysis which identified two clusters surviving correction for multiple comparisons (left putamen: 106 voxels; x = 22.8, y = –6.0, z = 13.5; $p < 0.01$; and right caudate: 39 voxels; x = −15.8, y = −20.0, z = 6.5; $p < 0.05$).

(C) Additionally, ROI analyses indicate that the post-ingestive dopamine response to milkshake, particularly in the left putamen, was correlated with *ad libitum* intake of energy from cookies at a subsequent meal test in the overnight fasted state (see also Figure S8).

(D) Voxelwise analyses identified clusters in bilateral putamen surviving correction for multiple comparisons where dopamine response was correlated with subsequent *ad libitum* cookie consumption (left putamen: 41 voxels, x = 29.8, y = 11.5, z = 6.6; $p < 0.02$; right putamen: 34 voxels, x = −26.2, y = 11.5, z = 6.5; $p = 0.05$). All clusters are defined by first-nearest neighbor clustering (faces touching), $k_e = 20$, bi-sided $p_{uncorr} < 0.1$, and cluster corrected at $p < 0.05$.
See also Table S2.

ultra-processed foods can be as addictive as drugs of abuse based largely on their post-ingestive potential to elicit an outsized dopamine response in brain reward regions was not supported by our data.

Contrary to our results, previous smaller studies using [$^{11}$C]raclopride displacement PET have shown significant decreases in post-ingestive striatal D2BP. A classic study of 7 people without obesity showed that consuming a favorite mixed meal decreased D2BP in the dorsal striatum.[43] In a study of 11 people using an 8 oz milkshake nearly identical in macronutrient composition to this study, decreased D2BP was observed in regions of the striatum, and this was driven predominantly by 5 participants without obesity.[44] Differences in post-ingestive striatal dopamine response between glucose vs. sucralose beverages in 19 adults were found to be negatively related to BMI, but no significant overall differences in D2BP between the beverages were reported.[16] In 10 individuals with obesity, no significant difference in D2BP was found between satiated and fasted conditions, and the authors suggested that obesity could blunt the post-ingestive dopamine response.[45] We believe our null results in 50 adults suggest that previous findings of post-ingestive striatal dopamine responses in studies with substantially smaller numbers of subjects may have been due to type 1 statistical error.

Recently, a rapid orosensory dopamine response followed by a later post-ingestive response was observed in a study using a novel [$^{11}$C]raclopride PET procedure in 10 men without obesity who sipped milkshakes at random intervals via a gustometer over a 10 min period during a 60 min scan.[27] Interestingly, consistent with the present results, these regionally and temporally specific results were not evident from traditional analysis of time-activity curves.[46] Perhaps our lack of ability to measure a dopamine response to the milkshake using a standard [$^{11}$C]raclopride PET procedure was because the post-milkshake PET scan started 30 min after the milkshake was consumed. However, we believe this is unlikely because brief intragastric nutrient infusions in rodents produce long-lasting (∼hours) striatal dopamine responses.[8,11,13] The milkshake used in our study would be expected to result in a relatively constant gastric emptying rate given that the milkshake contained appreciable amounts of cream and whole milk[47] with ongoing gut nutrient sensing and postprandial glucose and insulin increases over the duration of the subsequent 75-min PET scan, reflecting potential for a long-lasting dopamine release event. This same timing was also reported in a recent pilot study.[44] Nevertheless, if the peak post-prandial dopamine response was early and dissipated by the end of the scan, then calculating binding potential using time-activity curves over the entire duration of the scan

may have attenuated the effect of the milkshake on the calculated D2BP. However, our results indicate that truncating the PET time-activity curves to a minimum of 30 min had no appreciable effect on D2BP.

Our data suggest that the variable post-ingestive dopamine responses to the milkshakes were unrelated to adiposity. This was surprising because animal studies suggested that diet-induced obesity blunts dopamine response to nutrients in the gut,[48] and human functional MRI work suggested that obesity blunts striatal activity to food consumption.[49] A recent metabolic imaging study using the single photon emission computed tomography radiotracer [$^{123}$I]iodobenzamide observed that in both people with and without obesity, while nasogastric delivery of sugar caused dopamine release, the post-ingestive dopamine response to fat alone was only significant in those without obesity,[50] though the groups were not statistically compared. It is interesting to speculate that apparent dopamine responses to nasogastric nutrient infusion in this previous study may have occurred because these stimuli were not preceded by oral sensations as occurred with milkshake ingestion in our study.

Our data are limited to individuals free from a history of disordered eating or addiction to drugs and alcohol. Though we did not exclude participants reporting a history of "food addiction," our participants reported minimal endorsement of behaviors consistent with this construct. Food addiction is reported to have a 14% prevalence in non-clinical adult samples[51] and is comorbid with binge eating disorder[52] which has been associated with altered dopamine signaling, specifically anticipatory dorsal striatal dopamine release to food cues, independent of adiposity.[53] It is interesting to speculate that the post-ingestive striatal dopamine response to an ultra-processed food high in fat and sugar may be more pronounced in those endorsing behavioral features of food addiction or receiving a clinical diagnosis of binge eating disorder.

Even in the absence of a clinical eating disorder or food addiction, it is possible that some individuals may experience large post-ingestive dopamine responses to ultra-processed foods high in both fat and sugar under some conditions. Our exploratory analyses indicated individuals who displayed the expected post-ingestive dopamine response reported wanting more of the milkshake and rated it as more pleasant, consistent with prior work.[43] Individual variability in post-ingestive striatal dopamine responses may be related to the degree of hunger in the fasted state. Some of our study participants displayed dopamine responses to the post-ingestive signals from milkshake in the putamen, consistent with post-ingestive component in other studies[27] who displayed the expected response to milkshake consistently in left putamen, encompassing a region where interoceptive signals are registered.[54] Inducing hunger via restricted food access enhances development of addiction to drugs in animal studies,[55] possibly by enhancing post-ingestive dopamine responses.

Our results do not imply that ultra-processed foods high in fat and sugar are not addictive but rather call into question the mechanism by which this may occur. For some individuals, the post-ingestive dopamine response to an ultra-processed food high in fat and sugar may be sufficient but *not necessary* to promote problematic consumption. An outsized dopamine response may not be required for addiction. For example, nicotine is a drug widely acknowledged to promote addiction,[56] but nicotine ingestion produces only modest (~5%) reduction in striatal D2BP in human studies.[57] Some small studies have failed to show a significant dopamine response to nicotine,[58] possibly due to different modes of nicotine delivery. Others have questioned the dopamine theory of addiction despite its widespread acceptance.[19] It is intriguing to consider that there may be other neurochemical systems or other features of the stimulus separate from post-ingestive signals per se that may act in concert to promote problematic consumption.[3]

We believe the most likely interpretation of our data is that consuming ultra-processed milkshakes high in fat and sugar produces small but highly variable changes in post-ingestive striatal dopamine that were unrelated to adiposity but possibly related to perceived hunger and hedonic responses. Furthermore, individual post-ingestive striatal dopamine responses may predict food choices given that they correlated with *ad libitum* consumption of ultra-processed cookies high in both fat and sugar, which were the only such items available in a buffet lunch. Our results do not discount the experience of individuals who report difficulty in controlling their intake of ultra-processed foods high in fat and sugar but rather call into question the narrative that post-ingestive striatal dopamine responses similar in magnitude to illicit drugs perpetuate the consumption of ultra-processed foods and promote their excess intake.[3] The etiology of common obesity is more complex than dopamine-mediated ultra-processed food addiction, and the neurochemistry associated with excess adiposity, such as increased dopamine tone,[21] is not analogous to a state of drug tolerance.

## Limitations of the study

One limitation of this study is that we have assumed that measured changes in D2BP after milkshake consumption relative to the prior baseline period reflect striatal dopamine responses to the milkshake. However, the study did not include another comparator condition (e.g., a positive control known to stimulate dopamine release, a non-nutritive control of equal volume, a minimally processed milkshake matched for nutrients, etc.), which prohibits estimation of intrasubject variations or more accurate categorizations of whether one is truly a milkshake responder or not.[59] Future work would benefit from expanding on the exploratory classifications applied here by comparing any D2BP change induced by a control stimulus to any D2BP change induced by a milkshake. Further, we expected an ultra-processed formulation to be an especially potent stimulus given popular theories. However, the lack of a macronutrient-matched minimally processed comparator condition limits interpretations regarding specificity to ultra-processing per se.

The order of the fasting and post-ingestive scans was not random; the post-ingestive scan was consistently collected ~3 h after the morning fasting scan, which may pose potential concerns for both order effects and influence of diurnal variation in dopamine signaling. Preclinical studies suggest that striatal dopamine signaling varies diurnally.[60,61] However, limited human PET results suggest that D2BP measured by [$^{11}$C]raclopride is stable across the day and evening,[62,63] suggesting that the influence of scan time on D2BP is likely minimal. Another timing issue regards when the measurements occurred with respect

to menstrual phase in female participants that may have affected their dopamine response.

Finally, the change in binding potential itself is a snapshot of the *net* dopamine displacement resulting from the balance between dopamine release and clearance, predominantly achieved through dopamine transporters. It is possible that the magnitude of dopamine clearance overwhelmed the extent of dopamine release in response to the milkshake. Though available evidence suggests dopamine transporter availability is not related to adiposity,[64] future studies should investigate additional features of dopamine signaling (e.g., synthesis and clearance) as potential mechanisms by which ultra-processed foods high in fat and sugar may promote overconsumption.

## RESOURCE AVAILABILITY

### Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Kevin Hall (kevin.hall@nih.gov).

### Materials availability

This study did not generate materials.

### Data and code availability

Data from consenting individual subjects are available for download at the Open Science Framework (https://osf.io/z23xt/).

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## AUTHOR CONTRIBUTIONS

V.L.D., P.H., and K.D.H. designed the research study. A.B.C., P.V.J., S.T., S.Y., and S.T.C. contributed to research design, data collection, and analysis. M.C., I.G., R.H., M.L.N., L.M., A.S., M.S., N.U., N.Z., and M.S.Z. conducted experiments and collected data. V.L.D. and J.G. analyzed data and performed statistical analysis. V.L.D. and K.D.H. drafted the manuscript. All authors contributed intellectually and approved the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:



## SUPPLEMENTAL INFORMATION

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Chemicals, peptides, and recombinant proteins | | |
| [11C]Raclopride | PET Radiochemistry Department, National Institutes of Health | N/A |
| Software and algorithms | | |
| MATLAB version R2017a | Mathworks | https://www.mathworks.com/products/matlab.html |
| Analysis of Functional Neuroimages (AFNI_20.2.00 'Aulus Vitellius') | National Institutes of Health | https://afni.nimh.nih.gov/ |
| IBM SPSS Statistics (Version 28.0.1.1) | IBM SPSS | https://www.ibm.com/products/spss-statistics |

### EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Sixty-one adults provided informed consent to participate in a dual PET radiotracer study investigating the relationship between D2R availability and BMI under controlled dietary conditions (ClinicalTrials.gov NCT03648892). Participants were recruited from the community over a wide BMI range and approximately evenly sampled in each of three BMI categories (18.5 kg/m$^2$ $\leq$ BMI < 25 kg/m$^2$, 25 kg/m$^2$ $\leq$ BMI < 35 kg/m$^2$, BMI $\geq$ 35 kg/m$^2$) to ensure sufficient BMI range to test the hypothesis that BMI and dopamine D2 receptor availability are quadratically related.[21,65] Eligible volunteers were English-speaking, weight stable (less than ± 5% change in the past month), between 18-45 years of age, BMI $\geq$18.5 kg/m$^2$. They had no history of bariatric surgery, metabolic disorders, previous traumatic head injury or neurological disorders, severe food allergies (e.g., dairy, gluten) impaired activities of daily living, high blood pressure (>140/90 mm Hg), or current use of medication influencing metabolism or psychiatric medications. They did not have psychiatric conditions or disordered eating (EDE-Q, DSM Cross Cutting Symptom Measure Self Rated Level 1), nicotine dependence, drug use or in past 12 months (confirmed via urine toxicology at screening visit), binge drinking over previous 6 months, excessive caffeine consumption, or safety contraindications to MRI. Females were excluded if they were pregnant or lactating.

In the current sample (n=50), women reporting regular menses (not using hormonal contraceptives) (n=26) started inpatient admissions on day 17.9±10.1 of their cycle (luteal phase). Participants self-identified race and ethnicity at the time of admission to the NIH Clinical Center. Handedness was not exclusionary. Participants completed the 10-item Edinburgh Handedness questionnaire to determine laterality quotient[66] and 96.7% of participants (n=59) were determined to be right-handed (laterality quotient >0).

### METHOD DETAILS

This study was conducted between September 26, 2018 and February 17, 2023. On average, [$^{11}$C]raclopride scans were completed after 6.8±1.1 total days of dietary stabilization.

The enrollment and data distillation details can be found Figure S1. No participants withdrew from the inpatient portion after enrollment. The same day [$^{11}$C]raclopride scan order (fasted scan followed by milkshake scan) was standard across all participants. Of 61 enrolled participants, fasting [$^{11}$C]raclopride scan data are available for n=56 (n=1 participant declined, n=2 scans not performed due to tracer production issue, n=2 scans completed but did not pass quality control on time activity curves). Of n=56 participants with fasting [$^{11}$C]raclopride data, post-milkshake [$^{11}$C]raclopride scan data are available for n=50 (n=3 scans not performed due to a tracer production issue, n=3 scans completed but images did not pass quality control. Full PET data for fasting and milkshake [$^{11}$C]raclopride scans are available on n=50 participants (Table 1). All participants completed structural MRI. All study procedures were approved by the Institutional Review Board of the National Institute of Diabetes & Digestive & Kidney Diseases and the NIH Radiation Safety Committee; participants were compensated for their participation.

#### Metabolic diet

Participants were placed on a standard eucaloric diet (50% carbohydrate, 15% protein, 35% fat) with daily energy needs calculated using the Mifflin-St Jeor equation and standard activity factor of 1.5. All meals were prepared in the NIH Clinical Center Nutrition Department Metabolic Kitchen with all foods and beverages weighed on a gram scale (Mettler Toledo Model MS12001L/03).

For the run-in phase, participants were provided with 3-5 days of meals for retrieval from the NIH Clinical Center and consumed them at home prior to admission. Participants were instructed to consume all foods and beverages provided. Any food or beverage

not consumed was returned and weighed back. Participants were also instructed to continue their usual caffeine intake in calorie-free forms (e.g., black coffee, diet soda) and abstain from alcohol during this period. For any foods or beverages participants consumed that were not part of the standardized run-in diet, participants were asked to provide a description and amount of what was consumed so that total daily nutrient intake was captured. The eucaloric standardized outpatient diet was provided for an average of 4.5±1.0 days (range 0 – 5 days). Due to COVID-19 pandemic precautions, one participant was admitted without having completed a diet stabilization, and 3 participants completed some or all of their 3–5-day diet stabilization in the inpatient setting. The remainder of the full sample (n=57) consumed their stabilization diet as outpatients.

During the inpatient phase, participants continued the same diet and were instructed to consume all foods and beverages provided. All subjects were confined to the NIH Clinical Center metabolic unit throughout their inpatient stay without access to outside food. Meals were consumed under observation. Any uneaten food was weighed back, and energy and macronutrients were replaced at the next available meal as needed. Diets were designed using ProNutra software (version 3., Viocare, Inc.). No adverse events, harms or unintended effects resulted from provision of standardized eucaloric diet.

### Milkshake

A 226 mL vanilla milkshake was prepared by mixing 40 g Vanilla Scandishake dry mix (Aptalis Pharma, US), 150 g whole milk, and 36 g heavy cream. The resulting milkshake contained a total of 418 kcals and 7.4 g protein (7.0% of kcal). Total fat was 28.1 g (60% of kcal) of which 14.9 g was saturated (32.1% of kcal). Total carbohydrate was 34.6 g (33% of kcal) of which 18 g comprised total sugar (17.2% of kcal), 9.4 g of which were added sugar (9% of kcal).

After completion of the 75 minute fasting [$^{11}$C]raclopride PET scan, participants were escorted to a quiet room adjacent to the scanner for a rest period (75 minutes). At the end of this rest period, the milkshake was served chilled in an opaque (Styrofoam) cup and consumed through a straw. Participants were allotted 5 minutes to consume the milkshake. Thus, the milkshake was consumed after an extended overnight fast (~17-18 hours) approximately 30 minutes prior to the start of the second [$^{11}$C]raclopride scan.

The energy and macronutrients provided to the participant in other meals on the shake day were adjusted to account for contents of the high fat shake, so that overall daily energy and macronutrient intake remained stable in comparison with intake over inpatient stay.

### *Ad libitum* lunch array

The night prior to their last day of inpatient admission, participants fasted between the end of their dinner (~6:30 pm) and the ad libitum lunch array the following day (~12:00 pm) to mimic time of day and metabolic conditions surrounding their completed milkshake [$^{11}$C]raclopride scan. Participants were presented with a standardized buffet lunch meal (>6000 kcals, 35% carbohydrate, 17% protein, 48% fat) that provided a variety of different foods. Participants were allowed to consume as much food as desired, with each food weighed before and after consumption to determine total nutrient intake.

The array (Figure S8) consisted of: eight slices of Ultimate Grains Whole Wheat Bread, 250g roast beef deli meat, 250g turkey deli meat, 220g Glenview Farms Swiss Cheese, 220g Glenview Farms American Cheese, 200g sliced tomatoes, 200g green leaf lettuce, 200g grapes, 18 Chips Ahoy! Chocolate Chip Cookies, 135g Hellmann's Real Mayonnaise, 135g Monarch Yellow Mustard, 375g El Pasado Mild Salsa, 200g baby carrots, 180g Tostito Tortilla Chips, and 850g sterile water. The eight slices of bread and 18 cookies were weighed before array administration, and the weight was recorded in grams.

A total of 5 participants data were unavailable or removed from analyses pertaining to ad libitum intake, leaving 45 participants for analysis (n=2 not collected due to truncated testing schedule due to pandemic, n=1 data was subject to weigh back error, n=1 scheduling error having erroneously completed the ad libitum test after consuming fat/sweet taste preloads, and n=1 failed to disclose a food aversion (wheat bread) prior to the test).

Energy intake was calculated in total and separately for cookie-only energy intake and non-cookie energy intake. Total energy intake and sub fractions were adjusted by resting energy expenditure using the means, residuals, intercept and slope of energy intake (total, cookie, non-cookie) versus resting energy expenditure for the subsample of participants with available array data (n=45).

### Taste testing

Sucrose and fat preference were assessed using a two-series paired comparison-tracking method developed at the Monell Center for Adults.[67–69] Subjects were presented with pairs of solutions differing in sucrose concentration (3, 6, 12, 24, and 36 g per 100 mL) and pairs of puddings differing in fat concentrations (0, 3.8, 8.4, 19, and 33 percent fat by weight, achieved via dilutions of skim 0% fat and heavy cream 33% fat in commercially available vanilla pudding powder). They were asked to taste the samples without swallowing and point to which of the pair they liked better. Subsequently, each pair presented was determined by the subject's preceding preference choice. The entire task was then repeated with the stimulus pairs presented in reverse order. After completion of the taste task, the geometric mean of the preferred concentrations was determined.[70,71] For the five sucrose solutions, the first pair presented was from the middle range (6 and 24% wt/vol), whereas for the pudding samples, the first pair was the two extremes (3.8 and 19% for fat). All stimuli were presented at room temperature. One drop of yellow food coloring (McCormick & Co., Inc. Hunt Valley, MD, USA) was added to the sample to mask color differences.

## Questionnaires

The following reflects questionnaire outcomes pertinent to the exploratory analyses presented in the current study. Other exploratory questionnaire outcomes not included will be reported elsewhere. All questionnaire data were collected and managed using Research Electronic Data Capture (REDCap)[72,73] electronic data capture tools hosted at NIDDK.

### *Post-milkshake ratings*

Immediately after consuming the milkshake and prior to their second and final [$^{11}$C]raclopride scan, participants responded to a series of questions pertaining to their orosensory and hedonic perception of the milkshake using a visual analog scale[74] with the following anchors: How pleasant was the milkshake? (0= "Neutral", 100= "Extremely pleasant"); How much do you want more of the milkshake? (0= "I don't want any more at all", 100= "I want much more of the milkshake"); How did the milkshake compare to your expectations? (0= "Worse than I expected", 50= "As I expected", 100= "Better than I expected").

### *Hunger and satiety visual analog scales*

Participants reported their perception of momentary hunger in the overnight fasted state prior to their first [$^{11}$C]raclopride and immediately following consumption of the milkshake: "How hungry do you feel? (0= "I am not hungry at all", 100= "I have never been more hungry").[75]

### *Three Factor Eating Questionnaire (TFEQ)*

Participants completed the TFEQ, a self-assessment questionnaire developed to measure eating behavior traits of dietary restraint, disinhibition and hunger.[76] at a standardized time during their inpatient stay.

### *Yale Food Addiction Scale (YFAS)*

Participants completed the YFAS, a self-report questionnaire designed to assess the presence and severity of addictive-like eating of high-fat, high-sugar foods in the preceding 12 months via items adopted from DSM-IV-R diagnostic criteria for substance use disorders.[77] Participants reported on the frequency of problematic behaviors (e.g. "I find that when I start eating certain foods, I end up eating much more than planned." 0= "Never" through 4= "4 or more times [a week] or daily") at a standardized time during their inpatient stay. We report the resulting Symptom Count Scores range from 0 – 7, computed by summing the scores for each of 7 criterion (0= "Criterion not met", 1= "Criterion met").

### *Food Frequency Questionnaire III (DHQIII; National Cancer Institute)*

Diet history questionnaire was completed at the initial visit. Participants were instructed to consider intake over the "past year" and report portion sizes consumed. Analyses included variable labeled "Added sugars by total sugar NDSR (grams)". Outliers were examined across completed questionnaires from all enrolled participants (n=56). We applied a conservative outlier rule to exclude implausible reported intakes (Q3 – (IQR*2.2) = max; Q1 – (IQR*2.2) = min)[78,79] and three participants were excluded for implausibly high intake. One participant was removed from the analysis for reporting an intake less than 500kcal/day. A total of 52 eligible dietary histories were eligible for analysis, 45 of which were from participants with available milkshake PET scanning (pre and post-milkshake).

### *Beck Depression Inventory (BDI-II)*

The BDI-II is a valid and reliable 21-item self-report surveying presence and severity of any depressive symptoms.[80] Participants completed this questionnaire at the start of their inpatient admission. Higher total scores indicate greater depression severity.

## 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 comfortable clothing.

## Body composition

During the inpatient stay, participants each completed one Dual Energy X-Ray Absorptiometry (DEXA) scan while wearing hospital gown/scrubs to determine body composition (General Electric Lunar iDXA; General Electric; Milwaukee, WI, USA).

## Resting energy expenditure

While inpatient, after a 12 hour overnight fast, participants underwent indirect calorimetry using the ventilated hood technique while supine. Data were collected for 30 minutes and the first 5 minutes were excluded from analysis. Resting energy expenditure was calculated using the principles of indirect calorimetry using the $VO_2$ and $VCO_2$ measurements.[81]

## Analytical measurements

Blood was collected at three timepoints: in the overnight fasted state, 30 minutes post-milkshake, 90 minutes post-milkshake. Blood samples were drawn into chilled EDTA-coated tubes containing preservative (glucose: GLT additive; insulin: SST additive) and kept on ice until centrifuged (1600 *g* for 15 min at 4°C) within 30 min of collection for isolation of plasma. Samples were processed immediately after collection and portions stored for future measurement of biomarkers. Glucose was analyzed using Hexokinase method assayed on Abbott Architect. Insulin was analyzed using electrochemiluminescence Immunoassay on Roche Cobas e601 analyzer.

Area under the glucose and insulin curves (AUC) were calculated using trapezoidal method. We report on exploratory Metrics of 90-minute weighted average (AUC / 90 minutes), absolute change in values between time points, and peak change from baseline over available data (at either 30 minutes or 90 minutes post-milkshake) and present a repeated measures ANOVA with 3 within

subjects factors (time) and group membership (whole striatal "Responder" vs "Non-responder") as between-subject factor (Figure S7). The HOMA-IR value was calculated as follows: [HOMA-IR = fasting glucose (mg/dL) × insulin (mcU/L)/405].

### Magnetic resonance imaging

During their inpatient stay, MRI was completed to collect high resolution T-1 weighted structural brain images on which to register individual subject PET data. Due to the duration of data collection, extended by the COVID-19 pandemic, T1 weighted structural MRIs were collected on 3T Siemens Verio (n=21; TE = 2.98 ms, TR = 2.3 ms, TI = 900 ms, flip angle 9°, slice thickness = 1.2 mm, voxel size 1*1*1.2mm), and on 3T GE MR-750 Discovery scanner (n=6, TE = 3.04 ms, TR = 7.648 ms, TI = 1060 ms, flip angle 8°, slice thickness = 1.0 mm, voxel size 1*1*1mm; n=32, TE= 3.46 ms, TR = 8.156 ms, TI = 900 ms, flip angle 7°, slice thickness = 1.0 mm, voxel size 1*1*1 mm) for each subject. Quality of individual subject data were checked by study team [VLD & JG].

The anatomical images were parcellated with FreeSurfer software to generate ROI binary mask volumes in each subject in the putamen, caudate, accumbens, pallidum, and the cerebellum (reference region) (http://surfer.nmr.mgh.harvard.edu). All individual ROI masks were visually checked.

### Positron emission tomography

Data for both fasting and post-milkshake dopamine D2 binding potential (D2BP) are available for n=50 participants (Figure S2).

All PET scanning was performed using a High-Resolution Research Tomograph (HRRT), (Siemens Healthcare, Malvern, PA), a dedicated brain PET scanner with resolution of 2.5 - 3.0 mm and a 25 cm axial field of view. Transmission scanning was performed with a $^{137}$Cs rotating point source scan to correct for attenuation. A bolus of approximately 20 mCi of [$^{11}$C]raclopride was infused intravenously using a Harvard® pump at both the fasting and post-milkshake scans.

The molar activity of [$^{11}$C]raclopride was approximately 4865 mCi/μmol and the radiochemical purity of the radiotracer was >90%. PET emission data for [$^{11}$C]raclopride were collected starting at radiotracer injection over one block lasting 75 minutes. Twenty-four frames were acquired in list mode at times 0, 0.5, 1, 1.5, 2.0, 2.5, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 min. During each scan, the room was illuminated and quiet, 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 using an optical head tracking sensor (Polaris Vicra, Northern Digital Inc., Shelburne, VT, USA).

Each scan consisted of 207 slices (slice separation = 1.2 mm). The fields of view were 31.2 cm and 25.2 cm for transverse and axial slices, respectively. 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.

For region of interest analyses, individual participants' anatomical MRI images were co-registered to the aligned PET images by minimizing a mutual information cost function for each individual participant. Time-activity curves for each tracer concentration in the Freesurfer-generated ROIs were extracted and kinetic parameters were fit to a two-compartment model (with the cerebellum used as the reference tissue given negligible D2/3R specific binding[82] to determine regional D2BP.[83]

For voxelwise analyses, each individual's anatomical MRI was nonlinearly transformed into the Talairach space using AFNI 3dQwarp, and the transformation matrix was applied to the PET images which were then smoothed with a 5-mm full-width, half-max Gaussian kernel. Final coregistration was visually checked. Data were exported from Talairach space to MATLAB where time-activity curves for tracer concentration in each voxel were fit to a kinetic model using the cerebellum as a reference tissue[83] to determine D2BP at each voxel and exported back to Talairach space for group level spatial analyses.

## QUANTIFICATION AND STATISTICAL ANALYSIS

Power calculations based on 80% of power and 5% of type I error indicated a sample size of 39 participants to detect a quadratic relationship between fasting striatal D2BP and BMI which was the first primary aim of this study.[21] To follow up on an exploratory preliminary finding using n=13 of BMI-dependent dopamine release in the ventral pallidum (r=0.586; p=0.045), we increased the sample size to 50 distributed evenly across 3 BMI strata to detect $r>0.6$ at $p<0.05$ and > 80% power. Our recruitment exceeded the minimum sample size requirement. We report here results for the full sample (n=19 in 18.5 kg/m$^2$ $\leq$ BMI < 25 kg/m$^2$; n=13 in 25 kg/m$^2$ $\leq$ BMI < 35 kg/m$^2$; n=18 in BMI $\geq$ 35 kg/m$^2$). The much smaller previous studies showing a dopamine effect suggested that this was more than ample to detect an effect of the milkshake.

Statistical analyses were performed using IBM SPSS Statistics (Version 28.0.1.1, Chicago, IL, USA). Tests were 2-sided and alpha was set to 0.05. In the ROI analyses, associations between either BMI or percent body fat and percent change in D2BP between fasting and milkshake scans were evaluated with regression analyses. Person correlation coefficients were also reported. Influence of wide variation in datapoint residuals on associations was tested using SPSS extension for Robust Regression, which uses maximum likelihood estimator loss function to down-weight points with comparatively large residuals.

In the voxel-wise analyses, regional clusters where D2BP's are highly correlated with BMI were identified with regression analysis in AFNI's 3dttest++ (https://afni.nimh.nih.gov/). Since high D2BP occurs mainly in striatum, small volume corrections were implemented within each hemisphere where D2BP >1.5. A bi-sided uncorrected voxel-wise threshold of $p<0.1$ was used with a cluster extent minimum of 20 voxels (faces touching). Resultant clusters were deemed to survive correction for multiple comparisons using 3dClustSim at alpha of <0.05 and a threshold of 34 voxels.

### Study approval

All study procedures were approved by the Institutional Review Board of the National Institute of Diabetes & Digestive & Kidney Diseases and the NIH Radiation Safety Committee. Written informed consent was received prior to participation and compensation was provided.

## ADDITIONAL RESOURCES

ClinicalTrials.gov Identifier: NCT03648892

**Cell Metabolism, Volume *37***

## Supplemental information

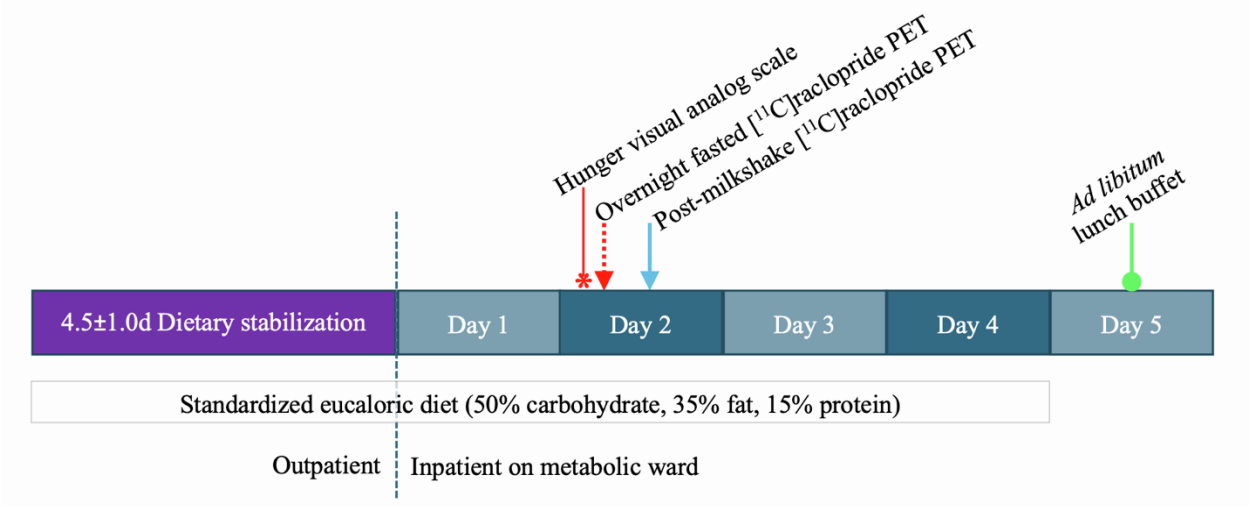
# Brain dopamine responses to ultra-processed milkshakes are highly variable and not significantly related to adiposity in humans

**Valerie L. Darcey, Juen Guo, Meible Chi, Stephanie T. Chung, Amber B. Courville, Isabelle Gallagher, Peter Herscovitch, Paule V. Joseph, Rebecca Howard, Melissa La Noire, Lauren Milley, Alex Schick, Michael Stagliano, Sara Turner, Nicholas Urbanski, Shanna Yang, Nan Zhai, Megan S. Zhou, and Kevin D. Hall**

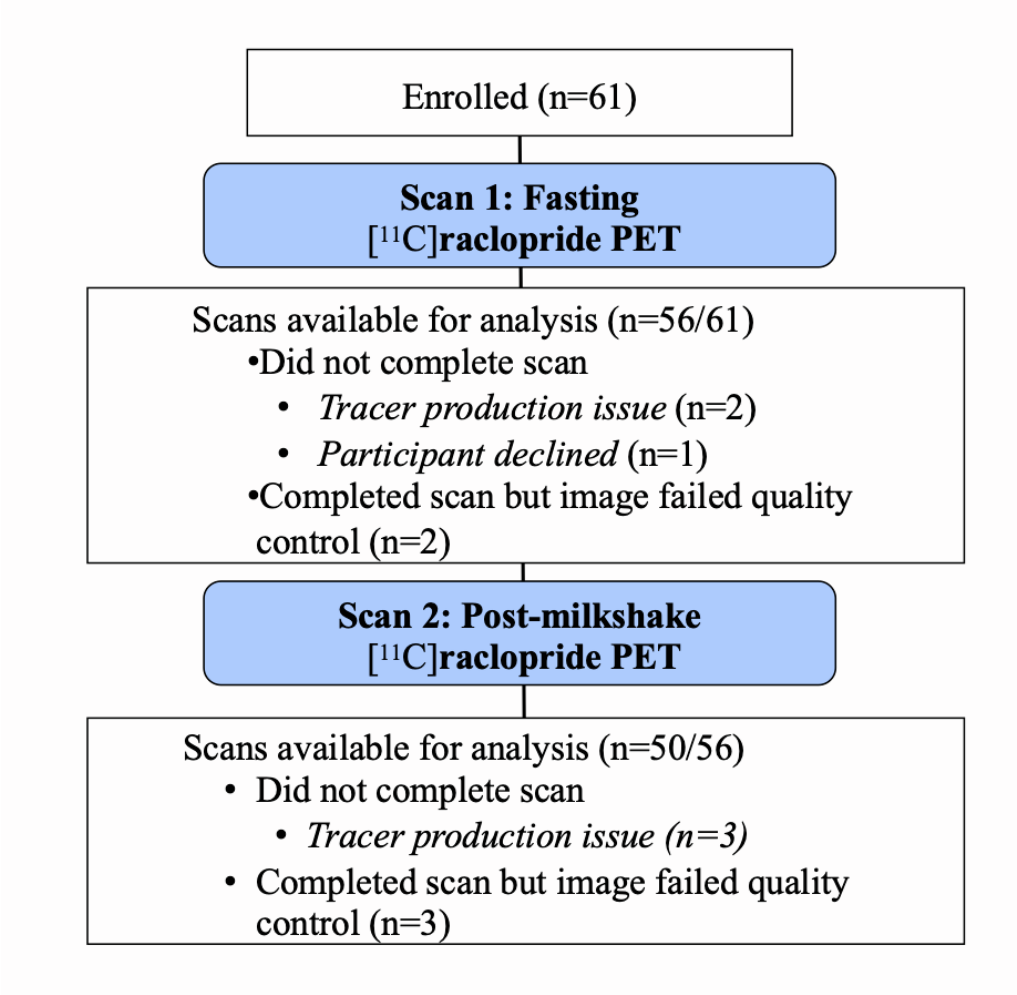
**SUPPLEMENTARY DATA**

**Brain dopamine responses to ultra-processed milkshakes are highly variable and not significantly related to adiposity in humans.**

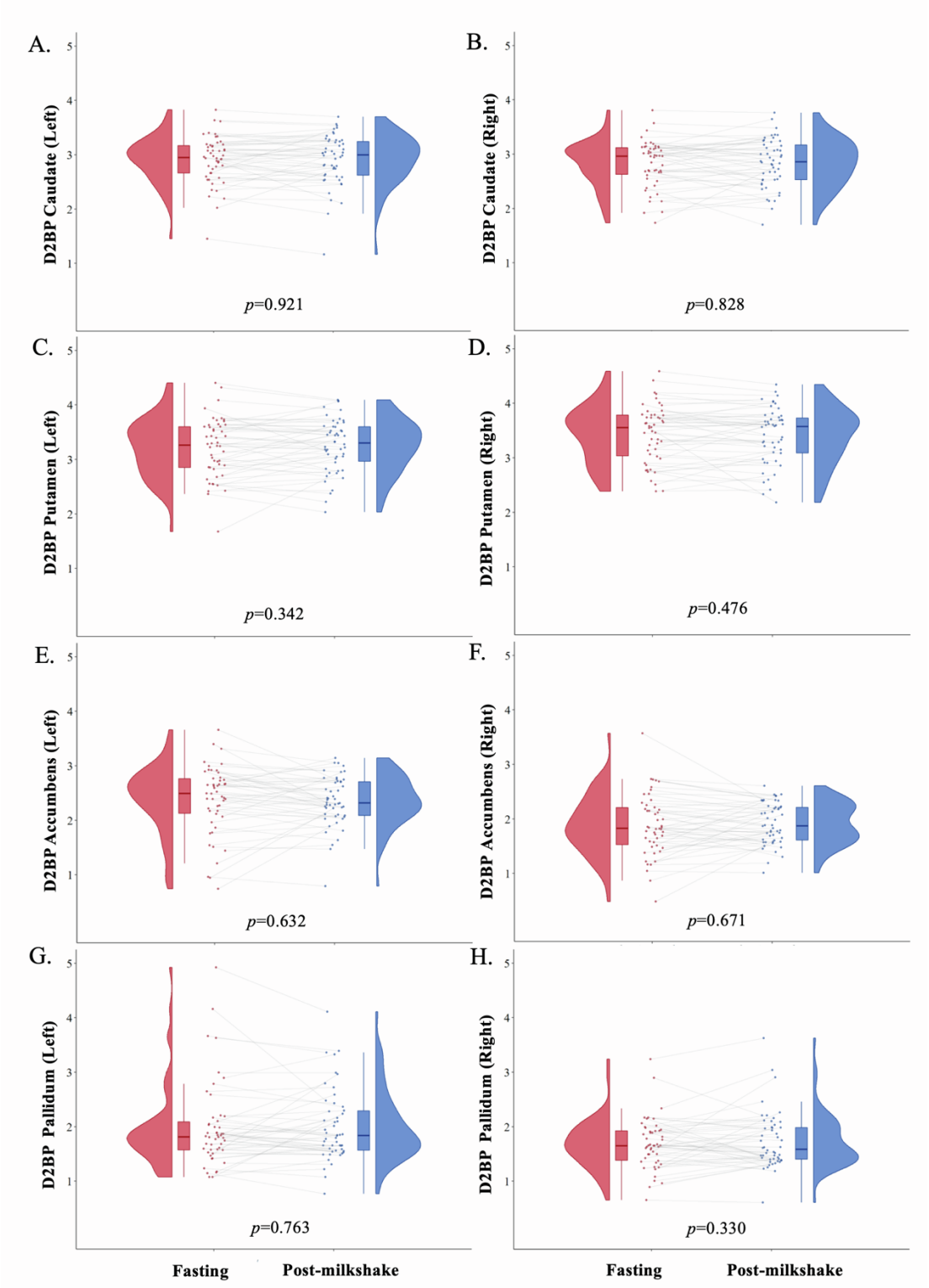
Valerie L. Darcey[1,2], Juen Guo[1], Meible Chi[1], Stephanie T. Chung[3], Amber B. Courville[4], Isabelle Gallagher[1], Peter Herscovitch[5], Paule V. Joseph[6,7,8], Rebecca Howard[1], Melissa La Noire[1], Lauren Milley[1], Alex Schick[1], Michael Stagliano[1], Sara Turner[9], Nicholas Urbanski[1], Shanna Yang[9], Nan Zhai[1], Megan S. Zhou[1], Kevin D. Hall[1]



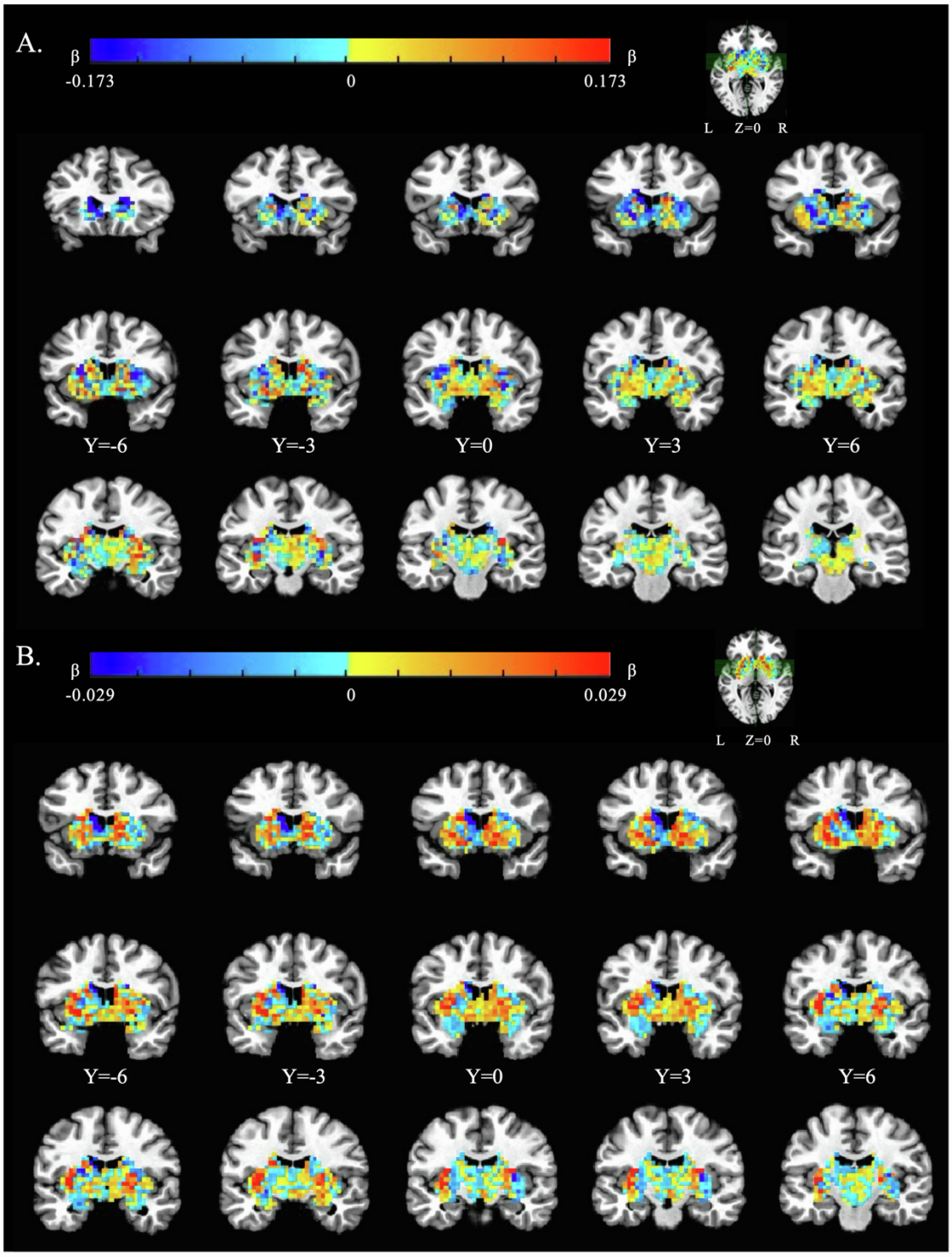

**Supplementary Figure 1. Inpatient controlled feeding study design, related to STAR Methods (Methods Detail).** Participants (n=50) consumed the provided weight-stabilizing standardized diet for an average of 4.5±1.0 days (mode 5 full days) prior to admission to the NIH Clinical Center for testing. During their inpatient stay, participants continued their dietary stabilization. [$^{11}$C]Raclopride displacement scan protocol was conducted on pseudo randomly assigned day during inpatient stay (2.4±0.9 days; mode 2 days), after approximately 6.8±1.1 total days (mode 7 full days) of dietary stabilization. Participants completed a confirmed overnight fast (~15 h) at which time hunger was assessed via digital visual analog scale prior to their first [$^{11}$C]raclopride scan. Upon completion, participants rested quietly in an adjacent room for roughly 75 minutes, at which time they consumed 226mL vanilla milkshake within 5 minutes and began their second and final [$^{11}$C]raclopride scan approximately 30 minutes after consuming the milkshake. On the final day of their inpatient stay, participants were presented with an ad libitum lunch buffet after a confirmed overnight fast.



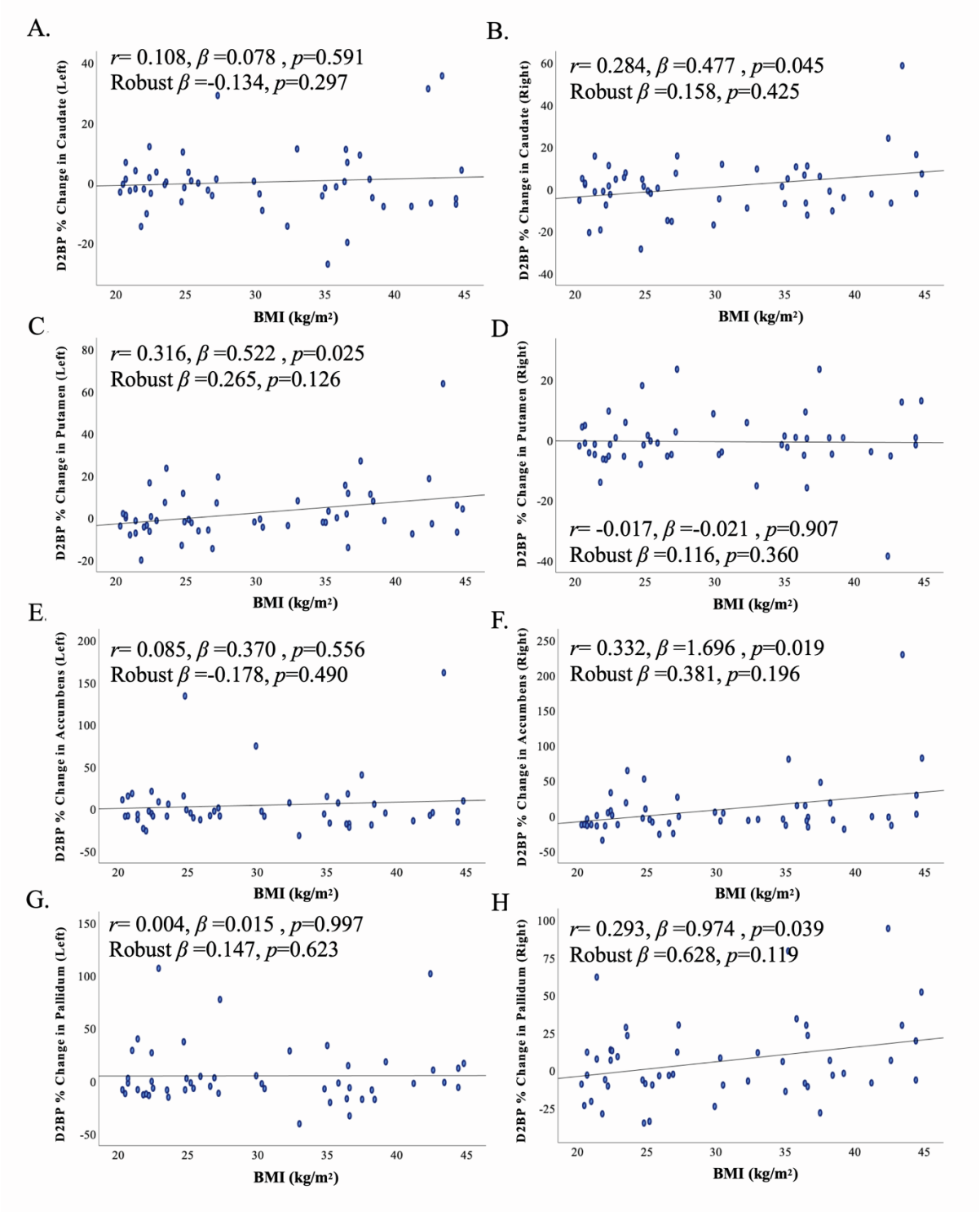

**Supplementary Figure 2. Enrollment and neuroimaging data distillation details related to STAR Methods (Positron Emission Tomography).** Sixty-one participants provided informed consent for enrollment in this preregistered clinical trial. Only the sample numbers pertinent to the current analysis for primary outcomes are presented here.



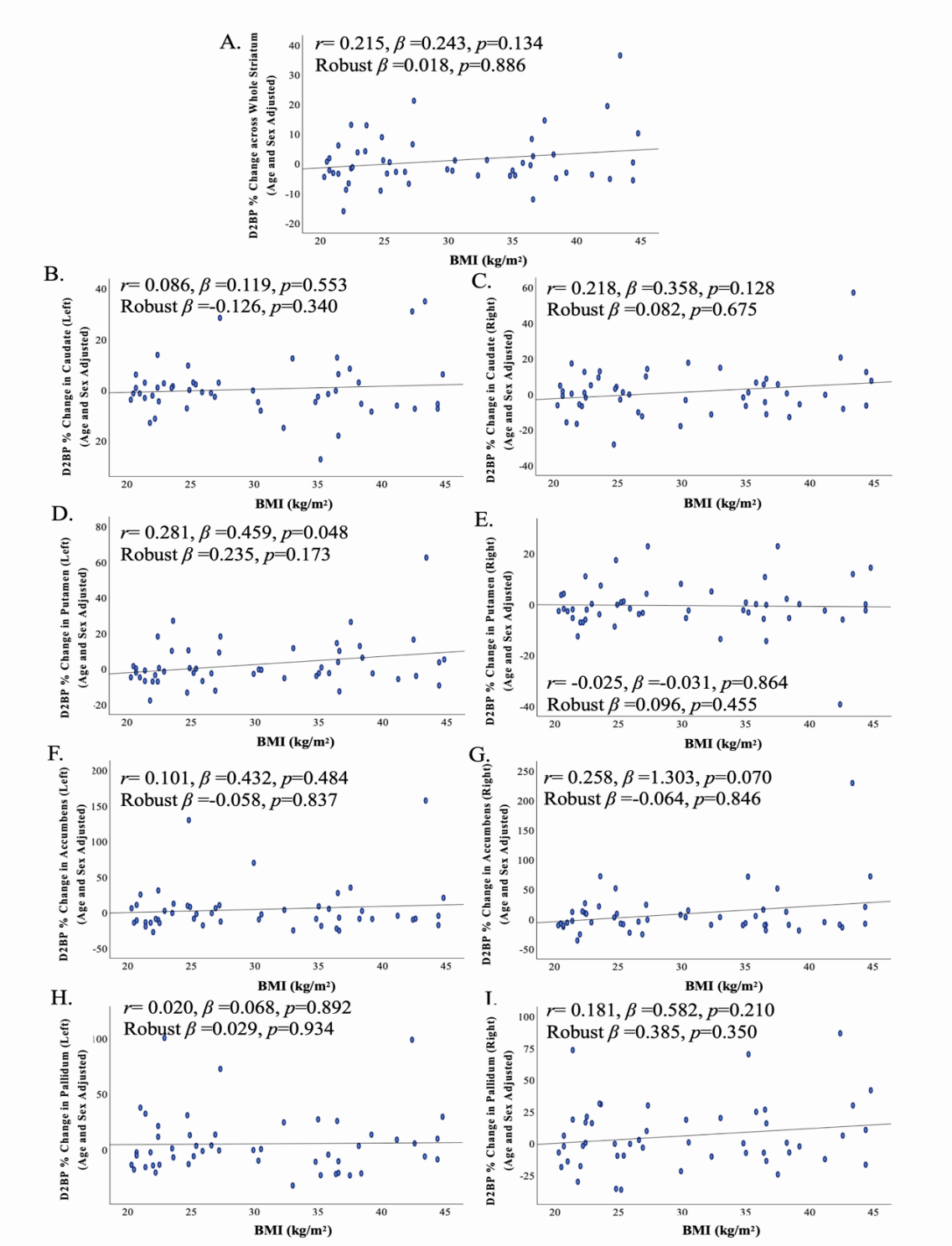

**Supplementary Figure 3. An ultra-processed milkshake did not significantly impact [$^{11}$C]raclopride binding potential across the whole sample (n=50) in striatal sub regions of interest, related to Figure 1A.** (A) left caudate, (B) right caudate, (C) left putamen, (D) right putamen, (E) left accumbens, (F) right accumbens, (G) left pallidum, and (H) right pallidum.



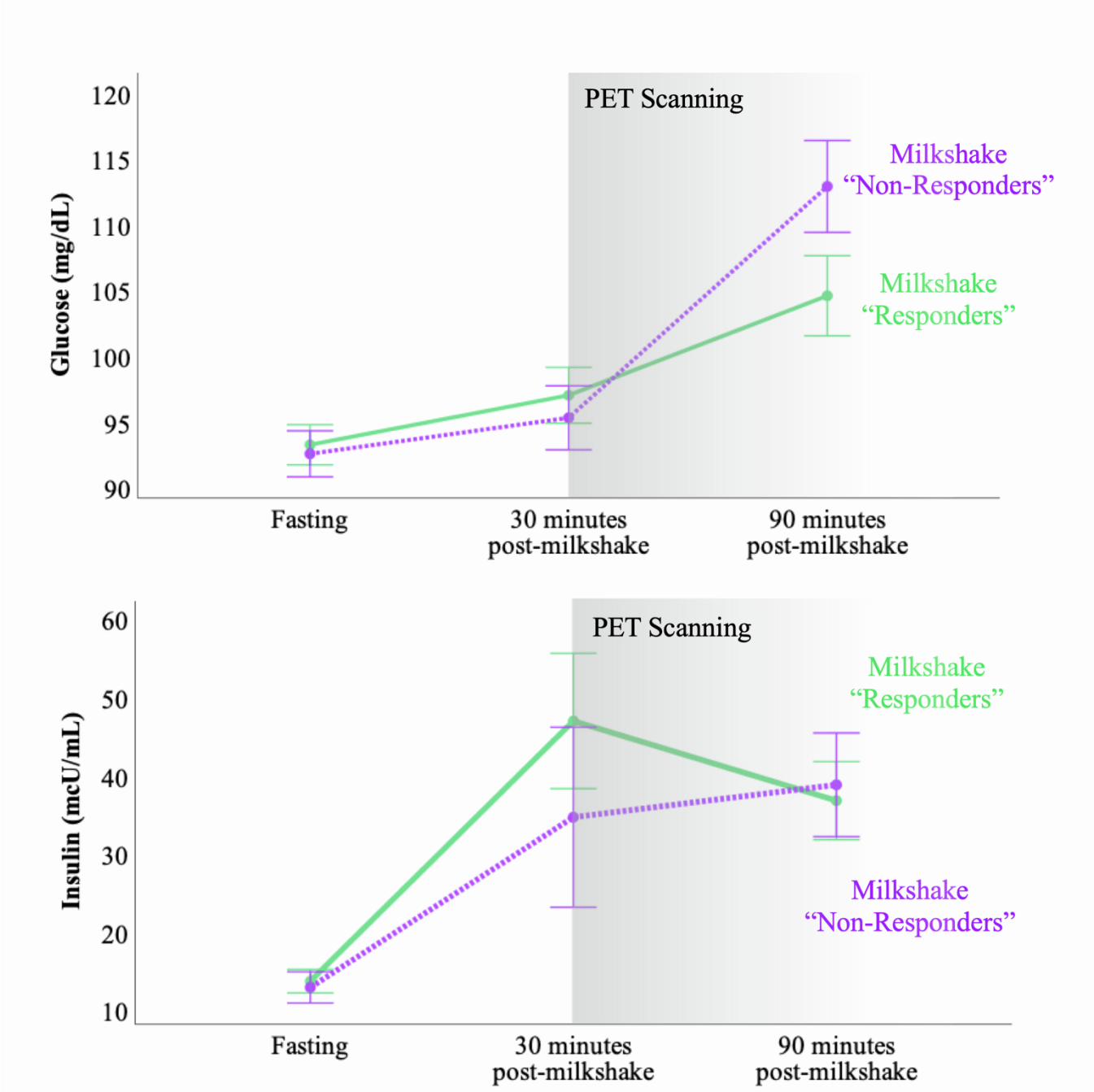

**Supplementary Figure 4. Unthresholded PET beta maps of dopamine response within striatal mask, related to Figure 1A and Figure 2. (A)** Response to milkshake across 50 adults contrasting D2BP post-milkshake vs D2BP fasting, AFNI 3dANOVA2. No clusters survive *a priori* correction for multiple comparisons (NN=1, $k_e$=20, $p_{uncorr}$=0.1) **(B)** Correlation between BMI and milkshake response (**Δ** D2BP fasting – post-milkshake) across 50 adults. AFNI 3dttest++. No clusters survive *a priori* correction for multiple comparisons (NN=1, $k_e$=20, $p_{uncon}$=0.1).




**Supplementary Figure 5. Relationships between BMI and response to milkshake (% change D2BP from fasting) across striatal subregions (A – H) are not robust to influential data points, related to Figure 2A.** (A) left caudate, (B) right caudate, (C) left putamen, (D) right putamen, (E) left accumbens, (F) right accumbens, (G) left pallidum, and (H) right pallidum.




**Supplementary Figure 6. Relationships between BMI and response to milkshake (% change D2BP from fasting) *adjusted for age and sex across* (A) whole striatum and striatal subregions (B – I) are not robust to influential data points, related to Figure 2A. (**B) Left caudate, (C) right caudate, (D) left putamen, (E) right putamen, (F) left accumbens, (G) right accumbens, (H) left pallidum, and (I) right pallidum.




**Supplementary Figure 7. Glycemic and insulinemic response to milkshake, related to STAR Methods (Analytical Measurements).** Overall, milkshake caused a significant increase from fasting levels of both glucose ($F$=27.0, $p$<0.001, n=44) and insulin ($F$=25.4, $p$<0.001, n=36) over the duration of PET scanning (gray shaded region). However, the interaction between time and dopamine response (group) was not significant for either glucose ($F$=2.2, $p$=0.125, n=44) or insulin responses ($F$=0.75, $p$=0.480, n=36). Error bars represent standard error.

**Supplementary Figure 8. Ad libitum buffet array offered for lunch (~12:00pm) after an overnight fast on the day of their discharge, related to Figure 3C and 3D.** Participants were presented with the above meal (>6000 kcal, 35% carbohydrate, 17% protein, 48% fat) and instructed to consume as much or as little as they wanted. Each food was weighed before and after consumption to determine total nutrient intake. Participants were presented with: 8 slices of Ultimate Grains Whole Wheat Bread, 250g roast beef deli meat, 250g turkey deli meat, 220g Glenview Farms Swiss Cheese, 220g Glenview Farms American cheese, 200g sliced tomatoes, 200g green leaf lettuce, 200g grapes, 18 Chips Ahoy! chocolate chip cookies, 135g Hellmann's Real mayonnaise, 135g Monarch yellow mustard, 375g Pasado mild salsa, 200g baby carrots, 180g Tostito tortilla chips, and 850g water. (Bread and cookies were weighed before array administration and the weight was recorded in grams.)

**Supplementary Table 1. Locations of striatal clusters with significant correlations, related to Figure 3 and Supplementary Figure 4.** PET resolution 3.5mm$^3$. Imaging analyses conducted in Analysis of Functional Neuroimaging (AFNI) within striatal region binding potential mask. Clusters defined by voxels with faces touching, cluster extent of 20, bi-sided $p_{uncorr}$<0.1.

| | Location of peak | | | Voxels | Size (mm$^3$) | t-stat | alpha |
|---|---|---|---|---|---|---|---|
| | x | y | z | | | | |
| **Δ D2BP (Post milkshake – Fasting)** [a] | | | | | | | |
| *No clusters* | -- | -- | -- | -- | -- | -- | -- |
| **Δ D2BP x BMI** [b] | | | | | | | |
| *No clusters* | -- | -- | -- | -- | -- | -- | -- |
| **Δ D2BP x Fasting Hunger** [c] | | | | | | | |
| Left putamen | 22.8 | -6.0 | 13.5 | 106 | 4545 | -2.44 | <0.01 |
| Right caudate | -15.8 | -20.0 | 6.5 | 39 | 1672 | -2.46 | <0.05 |
| Right putamen | -33.2 | 11.5 | -0.5 | 25 | 1072 | 2.53 | >0.10 |
| Right pallidum | -15.8 | -2.5 | -0.5 | 20 | 858 | -2.69 | >0.10 |
| **Δ D2BP x Ad Libitum Total Energy Intake**[d] | | | | | | | |
| Left putamen | 26.2 | -2.5 | 3.0 | 33 | 1415 | -3.74 | >0.05 |
| **Δ D2BP x Ad Libitum Non-cookie Energy Intake**[d] | | | | | | | |
| *No clusters* | -- | -- | -- | -- | -- | -- | -- |
| **Δ D2BP x Ad Libitum Cookie Energy Intake**[d] | | | | | | | |
| Left putamen | 29.8 | 11.5 | 6.6 | 41 | 1757 | -2.85 | <0.02 |
| Right putamen | -26.2 | 11.5 | 6.5 | 34 | 1458 | -2.52 | 0.05 |

*a.* Paired samples t-test, n=50
*b.* 1 sample t-test, n=50
*c.* 1 sample t-test, n=45
*d.* 1 sample t-test, n=45

Supplementary Table 2. **Group differences between participants across 3 BMI strata, related to Figure 2B.** Means and standard errors reported. BMI groups compared using one-way ANOVA except where indicated.

| | **BMI 1 (18.5 – 24.9 kg/m$^2$)** *(n)* | **BMI 1 (18.5 – 24.9 kg/m$^2$)** [Mean (SEM)] | **BMI 2 (25 – 34.9 kg/m$^2$)** *(n)* | **BMI 2 (25 – 34.9 kg/m$^2$)** [Mean (SEM)] | **BMI 3 (≥35 kg/m$^2$)** *(n)* | **BMI 3 (≥35 kg/m$^2$)** [Mean (SEM)] | ***p*** |
|---|---|---|---|---|---|---|---|
| **Total N** | 19 | | 13 | | 18 | | |
| *Females*[1] | 13 | 68.4% | 7 | 53.8% | 13 | 72.2% | 0.544 |
| **Age**[2] | 19 | 28.9 (1.6) | 13 | 29.5 (1.8) | 18 | 36.7 (1.3) | <0.001 |
| **BMI (kg/m$^2$)**[3] | 19 | 22.3 (0.3) | 13 | 28.9 (0.9) | 18 | 39.4 (0.8) | <0.001 |
| **D2BP % Change, Whole Striatum (Milkshake – Fasting)** | | | | | | | |
| *Mean percent change* | 19 | -0.6 (1.7) | 13 | -0.5 (2.1) | 18 | 4.0 (2.7) | 0.254 |
| *Range* | 19 | -18.1 – 11.9 | 13 | -9.0 – 22.5 | 18 | -13.1 – 37.7 | |
| **D2BP % Change, Whole Striatum (Milkshake – Fasting), adjusted for age and sex** | | | | | | | |
| *Mean percent change* | 19 | -0.2 (1.7) | 13 | 0.2 (2.0) | 18 | 3.0 (2.7) | 0.521 |
| *Range* | 19 | -16.0 – 13.1 | 13 | -6.8 – 21.2 | 18 | -12.0 – 36.5 | |
| **Milkshake ratings** | | | | | | | |
| *Pleasantness* | 18 | 60.1 (6.8) | 11 | 80.1 (5.8) | 16 | 55.3 (8.2) | 0.081 |
| *Wanting more*[4] | 18 | 31.1 (7.4) | 10 | 73.5 (8.5) | 15 | 40.5 (8.0) | 0.004 |
| *Met expectations* | 18 | 55.4 (6.3) | 10 | 67.7 (8.4) | 15 | 51.8 (7.1) | 0.343 |
| **Hunger ratings** | | | | | | | |
| *After overnight fast* | 15 | 55.7 (8.2) | 13 | 48.2 (8.1) | 17 | 44.5 (7.0) | 0.569 |
| *Effect of milkshake (% change from fasting)* | 10 | 17.2 (33.4) | 10 | 6.1 (11.7) | 15 | 24.0 (23.5) | 0.874 |
| **Ad libitum energy intake** (REE-adjusted) | | | | | | | |
| *Total (kcal)* | 17 | 924.3 (88.0) | 12 | 1026.9 (129.1) | 16 | 938.4 (150.0) | 0.038 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Cookie-only (kcal)* | 17 | 104.9 (23.8) | 12 | 107.4 (28.2) | 16 | 116.9 (43.4) | 0.963 |
| *Non-cookie (kcal)* | 17 | 819.4 (71.7) | 12 | 919.5 (108.2) | 16 | 821.5 (126.4) | 0.766 |
| **Glycemic response to milkshake** | | | | | | | |
| *Glucose* | | | | | | | |
| 90-minute weighted average (mg/dL) | 18 | 100.2 (2.2) | 11 | 98.8 (2.1) | 15 | 99.8 (2.5) | 0.920 |
| Change, 0 min – 30 min (mg/dL)[5] | 18 | 5.7 (2.0) | 12 | 7.3 (2.3) | 16 | -2.1 (2.2) | 0.010 |
| Change, 30 min – 90 min (mg/dL) | 18 | 15.2 (4.4) | 11 | 2.5 (5.4) | 16 | 13.4 (3.5) | 0.136 |
| Peak, 0 min – 90 min (mg/dL) | 18 | 112.9 (3.7) | 11 | 108.4 (3.3) | 15 | 109.9 (3.8) | 0.683 |
| *Insulin* | | | | | | | |
| 90-minute weighted average (μU/mL) | 11 | 20.0 (2.8) | 12 | 41.8 (10.5) | 13 | 44.6 (5.7) | 0.046 |
| Change, 0 min – 30 min (μU/mL) | 16 | 12.6 (2.8) | 12 | 46.0 (16.5) | 15 | 25.8 (6.3) | 0.046 |
| Change, 30 min – 90 min (μU/mL) | 11 | 0.8 (5.5) | 12 | -24.4 (13.1) | 13 | 8.0 (7.6) | 0.048 |
| Peak, 0 min – 90 min (μU/mL) | 11 | 29.1 (4.1) | 12 | 61.8 (16.5) | 13 | 62.3 (7.0) | 0.061 |

1. Sex distribution compared using chi-square statistic.
2. BMI 1 vs BMI 3, Bonferroni p=0.001. BMI 2 vs BMI 3, Bonferroni p=0.009.
3. BMI 1 vs BMI 2, Bonferroni p<0.001, BMI 1 vs BMI 3, Bonferroni p<0.001, BMI 2 vs BMI 3, Bonferroni p<0.001.
4. BMI 1 vs BMI 2, Bonferroni p=0.003. BMI 2 vs BMI 3, Bonferroni p=0.033.
5. BMI 1 vs BMI 3, Bonferroni p=0.034. BMI 2 vs BMI 3, Bonferroni p=0.019.