

Metabolic and neural responses to ultraprocessed foods: a randomized, controlled, crossover study

Corresponding Author: Dr Alexandra DiFeliceantonio

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The manuscript addresses a timely and increasingly relevant topic in nutritional science: the growing global consumption of ultraprocessed foods (UPFs) and their potential role in driving metabolic disease beyond what can be explained by caloric intake or macronutrient composition alone. The study's central hypothesis—that food processing alters post-ingestive metabolic and neural responses in ways that may contribute to long-term health outcomes—promises to advance our understanding regarding the link between diet quality and metabolic and behavioural health.

The authors present findings from an intervention study involving 57 healthy-weight adults that compare the metabolic and neural responses to nutritionally matched UPF and non-UPF meals, or using an auction task based on food cues, respectively. However, despite the study addressing a critical question with high translational relevance, the manuscript currently reads as preliminary and underdeveloped in several critical areas. The analytical approach is largely descriptive, with key findings presented as correlations that lack sufficient theoretical or empirical justification. The interpretation of neural correlates—particularly those linking peak carbohydrate oxidation to brain activation in the striatum and superior temporal gyrus—rests on associations that are not adequately contextualised within existing neuroimaging or metabolic literature or related to established models of incentive salience and decision-making.

To that end, the manuscript requires substantial revision to strengthen its analytical depth, clarify its conceptual contributions, and ensure that its conclusions are appropriately grounded in the data. Such an approach would also require a substantial reanalysis of data, likely a continued recruitment to acquire further data.

Here are a couple of immediate concerns and suggestions:

The auction task is used as a behavioural measure of subjective value (under the assumption that “willingness to pay” = a higher internal value signal). It is important to note that “value” here is a choice-based, subjective value, not an absolute or unitary notion of value. As this should be made obvious somewhere in the manuscript, the association of value to “reward” regions in the brain is wrong.

The authors report an association of carbohydrate oxidation after meals in response to food cues with neural responses (in the superior temporal gyrus). How can it be assured that this is not just correlative but has a mechanistic (causal) underpinning – what is the putative mechanism? This would possibly better suggest the somewhat implausible (or surprising) finding of a brain response in a brain area that is typically not involved in interoceptive, metabolic, salience or motivational processing: the superior temporal gyrus. Has this analysis been pre-registered? Otherwise, it would be very hard to argue for it, at least in a classical behavioural psychology standard.

In that respect, also (line 309): “a larger increase in peak carbohydrate oxidation for non-UPF relative to UPF was associated with the inverse response”, what does this mean?

Brain responses seem strongly lateralised. This is not at all discussed. Please expand.

Also related to brain areas, the brain response in the “striatum” (which is not a “reward-related region”) is analysed in relation to prior findings in the auction task. While this is correct on a phenomenological level, the reporting and discussion need significant nuance, especially when differentiating between dorsal striatal areas and the ventral striatum and in a context of predicted value; see in neuroscience literature, value inference (ventral striatum, updates beliefs about expected value), policy refinement (dorsolateral striatum, action policies rather than outcome values), sensory model (dorsal striatum receiving dense sensory cortical input support learning of sensory statistics independent of reward or action), etc. To avoid

further complications, I would suggest focusing on cue responses related to salience. This involves a broader discussion and analysis of the circuit, but it is logically clearer to argue.

The authors report that some food ratings, particularly healthiness and frequency of consumption (Figure 3), differ significantly between non-UPF and UPF cues. This is potentially very concerning, even if familiarity ratings do not differ between cue groupings. The difference in the ratings may introduce a potential bias in willingness to pay (which has not been tested or corrected for), but, even more concerning, it will bias the internal value signal and therefore compromise the study as a whole (or at least its interpretation).

Along the line, pre- and post-scan ratings of internal state also differ significantly, suggesting an effect of time and potentially altering cue saliency. Hence, this needs to be taken into account in the analysis and discussion. Consider accounting for metabolic rate and fat mass in the respective analyses.

The finding of a differential association of willingness to pay with UPF and non-UPF in areas of the visual association cortex (Figure 4), especially the ventral visual stream, lingual, as well as the fusiform gyrus, is interesting but potentially also very concerning:

(a) First of all, it needs to be ruled out (and shown) that there is no display effect between both conditions (cue sets), in terms of a main effect.

(b) Above and beyond this, the finding is concerning, relating to the above-discussed finding that healthiness ratings and frequency of consumption differ significantly between non-UPF and UPF pictures. Besides, these ratings likely affect willingness to pay; they will interact to drive visual responses, particularly visual associations. This needs to be tested for.

Furthermore, beyond showing differential associations of willingness to pay for non-UPF cues and UPF cues separately, there needs to be a test for statistical interaction.

(c) The finding of the brain response in the Fusiform gyrus is discussed according to the classical “what” association for the ventral visual stream, yet with a focus on food cues. The equally significant response in the Lingual gyrus is not discussed. But the issue here is not necessarily the attribution but the assumption of a predicted response. There has been work in mice showing that the visual association cortex can track not only stimulus identity but also predicted outcome. And also that outcome-tracking, but not identity-tracking, neurons can have a response bias to food-predicting cues and sensitivity to reward history (e.g. work by Mark Andemann’s group). However, given that the visual association cortex in mice has no clear homologue in Humans, this needs elaboration.

Related to the drop-out of participants in single sessions. If this was not consistent across sessions, consider a flexible-factorial design.

The MRI protocol parameters are missing in the Methods part.

Reviewer #2

(Remarks to the Author)

The epidemiological evidence linking ultra-processed food (UPF) intake to increased disease risk is now well established. What is needed are studies that elucidate the underlying mechanisms, and randomized cross-over trials are particularly valuable in this context because they control for both known and unknown confounders. A key debate in the literature is whether these associations are driven by nutrient composition rather than processing per se. The authors address this issue by comparing nutrient-matched UPF and non-UPF meals. In addition to examining postprandial metabolic responses, the study investigates the relationship between these metabolic changes and brain activity. This is a novel and important contribution, as the brain ultimately regulates eating behavior, including signals related to satiety and the decision to stop eating.

Please note that my review focuses specifically on the first two objectives of the study: the postprandial metabolic responses to UPF versus non-UPF meals and the associated neural reactivity. I have not evaluated the 'Willingness to Pay' (WTP) data or its neural correlates, as the behavioral economic modeling falls outside my primary area of expertise.

This study (data collection between 2023 and 2025) aimed to compare the effects of ultra-processed foods (UPF) on metabolism and neural responses compared to non-UPF in 57 enrolled healthy-weight adults aged 18–45. To do so, researchers compared the neural responses of participants to 5-second images of 14 non-UPF and 14 UPF items (matched on 26 characteristics) presented in a random order (n=52).

A subset of 32 participants took part in a cross-over RCT to compare changes in postprandial metabolism relative to baseline from 0 to 180 minutes post-meal between UPF and non-UPF meals. The meals consisted of items purchasable at a grocery store and were nutrient-matched (including glycemic index and glycemic load). These 300 kcal meals, to be consumed in less than 10 minutes, were a subset of the 28 foods used in the picture set and represented the most nutritionally matched set of UPF and non-UPF meals. The primary outcomes included mean, area under the curve (AUC), and trajectory of blood glucose, blood insulin, postprandial metabolic rate, respiratory exchange ratio (RER), and carbohydrate and fat oxidation.

The results showed that, relative to baseline, the 32 participants during the UPF meals had higher mean increases in blood insulin, metabolic rate, and fat oxidation, and lower mean increases in respiratory exchange ratios and carbohydrate oxidation than during the non-UPF meals. In 29 participants with both metabolic measures and fMRI data, greater positive differences between non-UPF versus UPF meals in exchange ratios and carbohydrate oxidation were associated with brain

activity (inverse correlation).

Some specific comments are provided below:

Introduction.

‘Concerningly, higher UPF intake is associated with poorer health outcomes, including cancer, overweight and obesity, and metabolic dysfunction (Barbaresco et al., 2025; Hasenböhler et al., 2026; Lane et al., 2024).’

I suggest citing a recently published paper that presents evidence on the association between UPF intake and health outcomes, based on meta-analyses including only prospective studies:

‘Monteiro CA, Louzada ML, Steele-Martinez E, Cannon G, Andrade GC, Baker P, Bes-Rastrollo M, Bonaccio M, Gearhardt AN, Khandpur N, Kolby M, Levy RB, Machado PP, Moubarac JC, Rezende LFM, Rivera JA, Scrinis G, Srouf B, Swinburn B, Touvier M. Ultra-processed foods and human health: the main thesis and the evidence. *Lancet*. 2025 Dec 6;406(10520):2667-2684. doi: 10.1016/S0140-6736(25)01565-X. Epub 2025 Nov 18. PMID: 41270766.’

Results.

‘To investigate the effect of processing level on post-ingestive metabolic response to foods with matched nutrient content, 32 participants consumed ~300kcal nutritionally matched meals composed entirely of either UPF or non-UPF’.

It would be helpful for the authors to clarify whether participants were required to consume the entire meal. Given the 10-minute time constraint and the subsequent metabolic analyses, confirming total consumption is essential for validating the nutrient-matched design.

‘The meals were consumed on separate days in a randomized crossover design, allowing within-participant comparison of indirect calorimetry and blood-derived metabolic outcomes.’

The authors should clarify the timing of the crossover sessions. Specifically, how many days apart were the two meals consumed, and was a standardized washout period implemented?

‘The UPF meal was consumed more slowly than the non-UPF meal by an average of 1.5 minutes ($t(30.43) = 5.13$, $p < 0.001$; Figure S3.a).’

It should be clarified whether participants were required to consume the entire meal. If total consumption was mandatory within the allotted 10-minute window, the findings suggest that the UPF meal may have been consumed more slowly than the non-UPF meal, given the specific time constraint. This would contrast with previous randomized trials—most notably Hall et al. (2019) and Hamano et al. (2024)—which consistently demonstrate significantly faster eating rates for ultra-processed foods. The authors should address this potential discrepancy and discuss how the fixed consumption time might have influenced the observed postprandial metabolic responses.

‘Associations between metabolic and brain responses. Next, we examined whether postprandial metabolic responses were associated with brain response to food pictures, a metric of food cue reactivity.’

Though described in the Methods, to better explain the experiment, it would be relevant to mention in this section as well that the non-UPF and UPF meals used in the metabolic sessions were derived from a 300 kcal subset of the 28 foods used in the picture set, representing the most nutritionally matched set of non-UPF and UPF meals.

‘The same participants on a separate day completed a Becker–DeGroot–Marschak (BDM) auction task during fMRI to quantify subjective food value.’

I'm interested in the rationale behind the study design. If the hypothesis is that the metabolism of a UPF meal affects neural responses differently than a non-UPF meal, why was the fMRI assessment conducted on a separate day using food images, rather than capturing the postprandial effect directly? Was this decision primarily due to logistical or other sort of constraints? It would be useful to describe when exactly the fMRI was performed in relation to the metabolic sessions (Meal 1 and Meal 2), as all were conducted on different days. Specifically, the authors should clarify the sequence of these sessions: Was the fMRI performed at baseline before any meal exposure, or was it conducted after participants had already completed the UPF and non-UPF meal interventions?

To better understand the neural component of the study, it would be extremely helpful if the authors described all the brain regions that were assessed for reactivity and the rationale for their selection (e.g., regions associated with reward processing...). Providing this anatomical context is essential for interpreting the functional significance of the findings; I think the authors should explicitly cite Table 2 here to guide the reader through these regional data.

‘Regressing the between-condition change in peak carbohydrate oxidation on participant-level non-UPF > UPF contrast estimates revealed a whole-brain FWE-corrected association in the left superior temporal gyrus [-44 , -24 , -8], $t = 7.33$, $p_{FWE} = 0.013$; Figure 2.b).’

Of all the brain regions evaluated for reactivity, it is unclear whether Figure 2 and Table 2 only plot those that were significantly associated with the changes in metabolic peaks, or if all studied brain regions showed such associations. This distinction should be made explicit within the captions and headers for Figure 2 and Table 2. To ensure full transparency and avoid selective reporting, I recommend that the authors report all associations—regardless of whether they reached statistical significance—perhaps by including the non-significant data in Table 2 (or a Supplemental Table).

‘Prior work in both humans and rodents has shown that peak metabolic responses to sugar-sweetened beverages are associated with striatal activation (de Araujo et al., 2013; Ren et al., 2010; Veldhuizen et al., 2017); therefore, we examined both whole brain corrected and small volume corrected results within an a priori striatal mask’.

Could the authors clarify the source of the a priori striatal mask?

‘Specifically, a larger increase in peak carbohydrate oxidation for non-UPF relative to UPF was associated with the inverse

neural response (Figure 2.b-d). As expected, given the close correspondence between these measures, peak RER exhibited a similar relationship in the left superior temporal gyrus ($[-44, -24, -8]$, $t = 7.63$, $p_{FWE} = 0.007$), and small volume corrected right caudate ($[4, 8, 2]$, $t = 5.77$, $SVC\ p_{FWE} = 0.014$; Figure 2.e). In addition, peak RER was also associated with a second locus of activation in the right caudate ($[22, -12, 24]$, $t = 5.48$, $SVC\ p_{FWE} = 0.026$; Figure 2.f)."

The inverse correlation suggests that as the metabolic response to non-UPF meals improves, the neural disparity between UPF and non-UPF cues diminishes. Is there a physiological rationale for this relationship?

"Between-condition changes in peak metabolic rate, fat oxidation, blood glucose, and blood insulin were not significantly associated with neural responses."

I recommend that these correlation results be included in Table 2 (or in Supplemental Table).

Discussion.

'Food processing can modify post-ingestive physiology, often by increasing carbohydrate availability and elevating glycemic index, thereby producing larger postprandial blood glucose and insulin responses (Huang et al., 2022; Tosh & Chu, 2015). Yet, this explanation is unlikely to account for our findings as the test meals were matched on glycemic index and yielded comparable blood glucose AUC, while the UPF condition elicited larger insulinemic and energetic responses with attenuated carbohydrate oxidation.'

I recommend that the authors expand the Discussion to include a comparison with the metabolic findings of Hall et al. (2019) and Hamano et al. (2024). Placing these results in the context of existing UPF randomized controlled trials is important to validate the study's conclusions.

'Here, we provided novel evidence that UPFs influence metabolic and neural responses differently from nutrient-matched non-UPFs. First, we demonstrated that even when meals were matched across multiple nutritional factors, degree of food processing classified by Nova significantly altered post-ingestive metabolic responses. Next, we showed that these metabolic differences were associated with neural responses to non-UPF versus UPF food cues.'

A significant limitation is the methodological divergence between the metabolic and neuroimaging assessments. The metabolic outcomes reflect an acute postprandial response to meal ingestion, whereas the neural data reflects cue-reactivity to visual stimuli (food images). This discrepancy in the 'exposure' stimulus—coupled with the multi-day gap between experiments—introduces potential confounders that could account for the observed associations. The authors should explicitly address this as a constraint on the study's internal validity and its ability to establish a direct causal link between the metabolism of specific foods and subsequent neural reward processing.

Supplemental material.

p. 5 'This project aims to study the effects of food processing level on post-ingestive metabolic signals and reward through the consumption of snack meals between 200-400 kcal which have been paired for macronutrient content across processing level as defined by the NOVA (not an acronym) food classification system.'

It should be clarified whether all the meals provided were considered snacks. If so, it is important to state this explicitly in the main text, as the metabolic and neural responses to a 300 kcal snack may differ significantly from those of a full, ad libitum meal.

p. 11 'Participants will also undergo smell testing. They will be prompted to answer a short series of questions and then take the smell test. They will be provided cards. Each card contains three lift and sniff patches. Participants will be instructed to lift the plastic cover over each patch and smell each one. Only one patch will contain the odor, while the other two will be odorless. The smell test involves three tasks: (1) identifying which one of three patches contains an odor (detection task), (2) rating the intensity of the odor on a scale from 0- 100 (where 100 is the strongest odor) and (3) choosing from four picture and label alternatives, what the odor smells like (identification task).'

Were the smelling tests carried out afterall? If so, I think this should be described in the main text.

p. 15 'Meal consumption: There are no known risks known with consuming the proposed meals. Participants will be screened by research personnel prior to their first meal for known food allergies. Participants will be able to select the meals pairings they participate in.'

What do you mean by 'Participants will be able to select the meals pairings they participate in'?

Carlos Augusto Monteiro

Reviewer #3

(Remarks to the Author)

This is an interesting and timely manuscript examining whether ultraprocessed foods elicit distinct metabolic and neural responses compared with nutritionally matched non-ultraprocessed foods. The study is conceptually strong because it combines controlled postprandial metabolic phenotyping with fMRI-based food-cue reactivity and food valuation. The central neuroimaging claims are that differences in postprandial carbohydrate oxidation between UPF and non-UPF meals are associated with cue-related activation in superior temporal gyrus and striatal regions, and that neural encoding of food value differs between UPF and non-UPF cues despite similar willingness-to-pay behaviour. These findings are potentially important and suitable for Nature Metabolism if the authors more clearly address several methodological and interpretational issues.

The strongest neuroimaging aspect is the use of an independently validated, nutritionally and visually matched food-picture set, together with whole-brain FWE correction and a pre-specified striatal small-volume correction. The main weakness is

that the metabolic challenge and fMRI task were not performed in the same session or in response to the same acute meal state. Thus, the link between post-ingestive metabolism and neural cue reactivity is correlational across sessions rather than a direct measurement of acute meal-induced neural responses.

Major comments

1. The link between metabolic responses and fMRI responses should be framed more cautiously

The manuscript states that processing-related metabolic differences were associated with neural food-cue responses. This is supported statistically, but the study design does not show that the acute UPF or non-UPF meal altered brain responses in the same session. The metabolic sessions involved consumption of matched meals during whole-room indirect calorimetry, whereas the fMRI session was conducted separately using a BDM food-auction task after participants arrived “neither hungry nor full” and 3 hours fasted. The fMRI analysis then related between-condition metabolic differences from the chamber sessions to the non-UPF > UPF cue contrast in the scanner. The authors describe this design clearly in the methods, but the Results and Discussion sometimes imply a more direct post-ingestive brain effect than the design can establish.

Suggested revision: use language such as “individual differences in metabolic responses were associated with neural cue reactivity” rather than implying that the meals themselves acutely changed brain responses.

2. Clarification of power analyses

The power analysis requires clarification. The manuscript’s central mechanistic claim appears to rely on the association between postprandial metabolic responses and neural food-cue reactivity. However, the reported power analysis was based on detecting differences in willingness to pay and, separately, on detecting differences in metabolic responses, rather than on the brain–metabolism association that forms a key neuroimaging result. The relevant analysis appears to involve the subset of participants with both metabolic and fMRI data, which is smaller than the full fMRI sample. The authors should clarify whether the study was adequately powered for these brain–metabolism association analyses, report the exact sample size used for each association, and either provide a corresponding power/sensitivity analysis or explicitly frame these analyses as exploratory.

3. Clarify directionality and interpretation of the Figure 2 associations

The direction of the Figure 2 effects is somewhat difficult to follow. The text states that a larger increase in peak carbohydrate oxidation for non-UPF relative to UPF was associated with the “inverse neural response,” and the figure legend states that higher STG response to non-UPF pictures correlated with greater carbohydrate oxidation to UPF. This is confusing because the metabolic variable is defined as non-UPF – UPF, while the neural contrast is also non-UPF > UPF.

The authors should explicitly state whether individuals with relatively greater carbohydrate oxidation after UPF showed stronger non-UPF cue responses, or whether individuals with relatively greater carbohydrate oxidation after non-UPF showed stronger UPF cue responses. This matters for the interpretation.

Suggested addition: a schematic explaining the sign of the contrast would help.

4. The striatal small-volume correction is justified, but the ROI definition needs more detail

The striatum is a well-justified a priori region given prior work on nutrient reinforcement and food valuation. The manuscript reports significant SVC findings in right caudate and left ventral striatum for carbohydrate oxidation, and right caudate for RER. However, the methods only state that the striatal mask included caudate, putamen, and nucleus accumbens from the Harvard–Oxford atlas. The authors should provide more detail on thresholding and laterality of the ROI mask, whether the ROI was bilateral, whether probabilistic thresholds were applied, and whether the same mask was used for all SVC analyses.

Given that several SVC tests were performed across multiple metabolic predictors and two fMRI models, the authors should clarify the family of tests and whether correction across multiple SVC analyses was considered.

5. Multiple testing across metabolic predictors should be handled more transparently

The authors tested associations between the neural food-cue contrast and several metabolic variables: peak carbohydrate oxidation, peak RER, metabolic rate, fat oxidation, blood glucose, and blood insulin. They report that only carbohydrate oxidation and RER were significant. Because carbohydrate oxidation and RER are closely related, this is biologically coherent, but the analysis still involves multiple predictors and both whole-brain and ROI tests.

The authors should state whether these analyses were pre-specified and how they controlled or interpreted multiple testing across metabolic predictors. If carbohydrate oxidation was the primary hypothesis-driven predictor, this should be made explicit. Otherwise, the claim should be framed as exploratory.

6. The value-related fMRI findings are interesting but need more careful interpretation

The BDM analysis is a strength, and the positive control is convincing: WTP was associated with canonical valuation regions including OFC and pregenual ACC. The processing-level contrast showed opposing value associations for non-UPF versus UPF in fusiform and lingual gyri, with SVC effects in putamen and caudate.

However, because WTP itself did not differ between UPF and non-UPF, the biological meaning of a positive neural value association for non-UPF and negative association for UPF requires more cautious explanation. The authors interpret this as altered neural encoding of food value. That is plausible, but they should consider alternative explanations: residual visual differences, semantic categorisation, healthiness/frequency effects, or cognitive control/health beliefs influencing value coding.

7. Hunger and internal state should be considered as covariates.

Hunger increased, thirst increased, and fullness decreased during the fMRI session. Since internal state strongly modulates food-cue reactivity, the authors should clarify whether pre-scan hunger, change in hunger, or session-level hunger was included as a covariate in fMRI analyses. This is particularly important for the valuation analyses, as subjective value and striatal responses can vary with hunger.

8. The authors should avoid overinterpreting STG as food-specific multisensory integration

The STG finding is whole-brain corrected and therefore robust statistically. The interpretation as multisensory integration of post-ingestive signals with food cues is plausible but somewhat speculative. The STG is not a canonical metabolic or

reward region, and the fMRI task was visual only. The discussion does acknowledge the STG's broader role in multisensory integration. However, the manuscript should soften the conclusion and mention that the STG result may also reflect semantic, attentional, or perceptual processing of food category differences.

9. The authors should report whether UPF > non-UPF cue reactivity was tested independently

The paper states that the contrast UPF > non-UPF did not yield significant voxels for the value analysis. It would be useful to also state whether the simple food-cue reactivity contrast UPF > non-UPF, independent of metabolic covariates, showed any effects. This would help distinguish whether UPF cues generally engage reward/visual regions more strongly, or whether the main findings are specifically driven by individual differences in metabolic response and value coding.

On a similar note, the authors state that the UPF and non-UPF picture sets were matched on nine visual properties, including pixel colour, object size, and brightness. However, it is unclear whether visual complexity was matched or quantified. Given that key value-related effects emerged in occipitotemporal regions, including fusiform and lingual gyri, the authors should report whether the stimulus categories differed in visual complexity, for example edge density, entropy, spatial frequency, clutter, or number of objects. If these measures were not assessed, this should be acknowledged as a limitation, and the interpretation of occipitotemporal effects should be tempered.

10. Clarify whether the fMRI picture stimuli and metabolic meals were sufficiently aligned

The metabolic meals were derived from a subset of the 28 food images, while the fMRI task used all 28 images. This is reasonable, but the authors should clarify whether the fMRI trials corresponding to the exact foods consumed in the metabolic sessions show similar effects to the full stimulus set.

11. The authors appropriately acknowledge that total protein, but not protein type, was matched; that the UPF meal contained more additives; that eating rate differed modestly; and that additional meal pairings are needed to establish generalizability. However, these limitations should be given greater weight in the interpretation. The current design demonstrates that two meals matched for conventional nutrient composition but differing in processing-related formulation and structure elicit distinct metabolic responses. It does not isolate NOVA processing per se from differences in food matrix, ingredient source, protein type, additive profile, texture, oral processing, bolus formation, or digestion kinetics. The Discussion should therefore more explicitly temper causal statements attributing the effects to "processing level" and instead frame the findings as effects of a nutrient-matched UPF versus non-UPF meal comparison.

Minor comments

In the abstract, the phrase "mesolimbic and superior temporal gyrus activation" could be more precise. Consider "striatal and superior temporal gyrus responses" unless the authors want to define mesolimbic regions explicitly.

The use of peak metabolic response is interesting, but AUC may be more robust. The authors should justify why peak was selected for brain associations.

The authors should report the exact n for each brain-metabolism association in the Results and figure legends.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors did a commendable effort to address all of my points from the earlier review. Particularly, the major methodological concerns have been addressed; additional analyses have been performed, and interpretations have been softened. This relates especially to the interpretation of the imaging findings.

However, after reading the revised manuscript, I remain somewhat unconvinced about its mechanistic depth and biological implications, particularly given Nature Metabolism's scope. And I now have a statistical concern (see specific remarks below).

Collectively, the manuscript clearly demonstrates that nutrient-matched UPF and non-UPF meals elicit distinct acute metabolic responses. It furthermore identifies associations between these metabolic measures and neural responses to food cues. However, the central link between these observations remains largely correlational. In particular, it still remains unclear why carbohydrate oxidation emerges as the relevant metabolic correlate (whereas other altered physiological measures, such as insulin, glucose, or metabolic rate, do not). Although the manuscript now much better discusses prior work implicating post-ingestive signalling and striatal reinforcement, the present findings are not yet integrated into a coherent biological framework that explains the specificity of the observed associations.

Throughout the Discussion, the authors appropriately acknowledge that additional experiments are required to establish the functional significance of several neural findings. This caution is appreciated, but it also underscores that the manuscript primarily identifies novel associations rather than providing mechanistic insight into how altered food processing leads to altered neural responses. Given the Journal's scope, I would have liked to see a more substantial discussion of the potential biological mechanisms underlying these observations.

I do not think additional experiments are necessarily required at this stage, nor do I think the current interpretations are overstated (given that the authors can still demonstrate statistical rigour, see below). Rather, I believe the manuscript would

benefit from a more thoughtful discussion of what the present data can and cannot reveal mechanistically, and from a clearer articulation of why the observed metabolic-neural associations are biologically meaningful beyond their statistical significance.

Some specific remarks:

1. Regarding mechanistic depth or biological implications, the paper is observational despite its experimental elegance.

The key conclusions remain correlational: metabolism correlated with brain activity rather than metabolism causing changes in brain activity.

The paper essentially establishes three observations: nutrient-matched UPF and non-UPF meals elicit different metabolic responses; metabolic responses correlate with certain fMRI measures; value-related activity differs between UPF and non-UPF meals.

Each of these findings is interesting, but they are not integrated. Especially the neuroscience part, however, feels more like an add-on than the central mechanistic advance. It identifies intriguing, hypothesis-generating associations but doesn't yet explain how altered food processing translates into altered brain function. If the imaging component were removed, the metabolism paper would still tell a coherent story. The converse is less true.

In the bigger picture, why carbohydrate oxidation? And not insulin (changed), glucose (changed modestly), metabolic rate (changed). The Discussion never answers what is special about carbohydrate oxidation. Yet only carbohydrate oxidation predicts fMRI. Why?

To that point, the authors simply state that rodent work implicates carbohydrate oxidation, which is true, but a much deeper discussion of why carbohydrate oxidation might uniquely index nutrient availability compared with insulin or glucose is needed.

2. Regarding the willingness to pay paradigm:

It is reported that there is no difference in behaviour, yet there is a difference in neural encoding. The author's report just says that there are neural representations despite similar behaviour. and that there are "follow-up experiments needed". This sounds like an escape argument.

If the behavioural endpoint is negative, interpreting interaction effects in BOLD is left without a behavioural anchor. That's not wrong, but it's inherently speculative unless there are further arguments.

This leads to a statistical concern: $n = 32$

The overall sample size was estimated based on behavioural data from an earlier study. While there is nothing wrong with this, the effective sample was now less than estimated ($n=52$), and thus the absence of effect in the behavioural data becomes an issue - it can certainly not be interpreted in a way that the effect is indeed absent. Unfortunately. The lack of behaviour despite neuronal representations is an interesting finding that has often been observed (and not really explained): brain responses to food cues (or other appetitive cues) are sustained longer than the behavioural effect lasts.

But anyway, for the following correlation analyses, this is a very small sample, especially given the whole-brain analyses with several metabolic variables and covariate analyses. What remains is the risk of being 'just' a collection of significant associations from a relatively small imaging cohort.

The authors need to discuss robustness and reproducibility in light of the actual effect sizes from their data.

3. Regarding the fMRI findings in visual association areas (an issue which was addressed in the prior review). The embedding is now much better, but given the vast extent of the activation areas, simply pointing towards a "fusiform food area" is not appropriate. In various fMRI tasks, we find neuronal responses to value modulation in the visual system. This is almost ubiquitous, likely reflecting familiarity or experience with certain cues, and is dynamically adjusted based on the motivational significance or behavioural relevance of a visual stimulus. These findings still need to be discussed more fully in light of system interactions (lightly).

4. More concern relates to the somewhat heavy use of "further experiments are needed". This seems to reflect that the manuscript repeatedly reaches the boundary of what the data can support. The authors are appropriately cautious, but caution also highlights that the central biological mechanism needs more plausibility than is demonstrated. That leaves the paper feeling like an important first step rather than a definitive mechanistic advance.

Reviewer #2

(Remarks to the Author)

The authors have addressed all my concerns. The manuscript is now in a condition to be published.

Reviewer #3

(Remarks to the Author)

The authors have addressed most of my concerns in a satisfactory manner.

Furthermore, the authors now clarify that the fMRI association analyses were corrected using whole-brain FWE correction or small-volume FWE correction within the a priori striatal mask. However, it remains unclear in the revised manuscript how multiplicity across the several metabolic predictors was handled. The authors tested carbohydrate oxidation, RER, metabolic rate, fat oxidation, blood glucose, and blood insulin, but no correction across these predictor-level tests appears to have been applied. If carbohydrate oxidation was the primary hypothesis-driven predictor, this should be stated explicitly.

Otherwise, the metabolism–brain association analyses should be framed as exploratory, or the authors should justify why no additional correction across metabolic predictors was applied.

Open Access This Peer Review File is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

Dear Dr. Nakhle and Reviewers,

Thank you for your time and attention to our manuscript. We greatly appreciate this fair and constructive review. We have run all additional requested analyses and made substantial changes to the text as requested. Addressing these comments has substantially improved the manuscript. In this letter, we reproduce the Editor and Reviewer comments in plain text and our response follows in *italics*:

Response to editors

1) We ask you to strengthen the analytical rigor and statistical transparency throughout the manuscript (all 3 Reviewers). Include appropriate sensitivity analyses for the reported metabolism-brain associations and ensure that key covariates including, but not limited to, hunger, food ratings, and fat mass are appropriately controlled for in the willingness-to-pay and valuation analyses. It will be very important for the reconsideration of the manuscript that the findings remain robust after accounting for these potential confounders.

Thank you for the opportunity to improve our statistical reporting. We agree these are important covariates to consider in our analysis. Two reviewers commented that the change in hunger, fullness, and thirst across the session was an important covariate, in addition to body composition. To address this, we re-ran all our models that produced the fMRI findings adding within session change in hunger, fullness, or thirst to the covariates of sex and age that were in our original model. The tables of these results are now shown in Supplemental Tables 1, 2, and 3. The main pattern of findings was unchanged, some values did change slightly, but adding these variables further reduced degrees of freedom for the analysis, so this is to be expected. For fat mass, we did not have a direct measure of fat mass (e.g., DXA), but we do have BMI information, which in our sample did not have a large amount of variance (mean = 22, SD = 1.87). We took the same approach as in the session variables and added BMI to age and sex as covariates in all of our fMRI models. The results table is depicted in Supplemental Table 4. Overall, we found our results to be robust to these additional covariates. We have added sentences that reference these results to the appropriate parts of the results section.

2) Please refine the interpretation of the neuroimaging findings. The conclusions regarding the superior temporal gyrus and food-specific sensory or motivational region should be substantially toned down, as this region is not classically linked to food-specific motivation. Please consider alternative interpretations related to salience rather than reward per se (Reviewer #1), and discuss more cautiously the meaning of the value-related fMRI findings (Reviewers #1 and #3).

We have substantially revised the interpretation of the STG findings and made sure to be specific that we are measuring and discussing subjective value. We have revised the manuscript throughout with alternative explanations and more generally

refined our interpretations, including striatal activations. Some examples of our re-wording are reproduced below, however the manuscript was changed throughout.

The section on the STG now reads: “Beyond striatal regions, processing-related individual differences in carbohydrate oxidation were also associated with responses in the superior temporal gyrus (STG). While primarily known for its role in auditory and language processing, the STG is increasingly recognized as a hub for multisensory integration (Scheliga et al., 2023) and is responsive to food cues (St-Onge et al., 2005; G.-J. Wang et al., 2004). More work is needed to determine if the STG is involved in food specific multisensory integration, including interoceptive metabolic signals.”

The section on the lingual and fusiform gyri now reads: “Interestingly, we observed condition differences in subjective value associations in the occipitotemporal gyrus, specifically the fusiform and lingual gyri. While the fusiform gyrus is best known for its role in face processing within the ventral visual stream, a distinct region identified for food-specific visual processing has also been found and termed the fusiform food area (Adamson & Troiani, 2018; Jain et al., 2023) Response in the fusiform food area is modulated by circulating glucose, insulin, and ghrelin (Kroemer, Krebs, Kobiella, Grimm, Pilhatsch, et al., 2013; Kroemer, Krebs, Kobiella, Grimm, Vollstädt-Klein, et al., 2013; Malik et al., 2008). The lingual gyrus has been reported in studies of food cues (Stice et al., 2013) and the visual association cortex in mice can be sensitive to reward associations (Nguyen et al., 2024). However, further experiments are needed to establish if visual regions are sensitive to food value.”

The rest of the discussion is also substantially reworked.

3) Because postprandial metabolic responses and neural food-cue responses were measured on separate days, we ask you to tone down causal language throughout the manuscript. Please clarify the rationale for this design, discuss its limitations explicitly, and interpret the relationship between carbohydrate oxidation and neural responses more cautiously (all 3 Reviewers).

Thank you for this comment. We agree causal language is not appropriate and have replaced it with language describing associations throughout the text. In regard to the design, here, we are working within and testing the framework that post-ingestive signals provide information from the periphery to the brain on the nutritional value of foods and those signals shape food value (de Araujo, et al 2020 and Weber et al 2025). A key component of this model is that these associations can be learned but should be relatively stable across the time frame in which we conducted our sessions, meaning that although we tested the metabolic response to UPF vs non-UPF foods on a different day, individual differences in responses to UPF vs non-UPF meals within each person should still be present on the fMRI session day. We are not testing the acute effects of these foods on brain response, but the neural encoding that is presumed to be a result of learning the post-ingestive consequences of these familiar foods. We can then observe differential encoding of those learned responses as in DiFeliceantonio, et al

2018 and Tang, et al 2014. We have revised the manuscript throughout to help clarify this point and removed causal language.

For example, we now introduce the correlation section of the results with: “Next, we examined whether postprandial metabolic responses **were associated** with brain response to food pictures, a metric of food cue reactivity, **testing the hypothesis that post-ingestive metabolic signals shape the learned value of food through gut-to-brain signaling** (de Araujo et al., 2020; Weber et al., 2025).”

The rest of the paper is substantially revised to reflect these changes.

de Araujo IE, Schatzker M, Small DM. Rethinking Food Reward. *Annu Rev Psychol.* 2020 Jan 4;71:139-164. doi: 10.1146/annurev-psych-122216-011643. Epub 2019 Sep 27. PMID: 31561741.

Weber LA, Yee DM, Small DM, Petzschnner FH. The interoceptive origin of reinforcement learning. *Trends Cogn Sci.* 2025 Sep;29(9):840-854. doi: 10.1016/j.tics.2025.05.008. Epub 2025 Jun 10. PMID: 40500611; PMCID: PMC12400946.

DiFeliceantonio AG, Coppin G, Rigoux L, Edwin Thanarajah S, Dagher A, Tittgemeyer M, Small DM. Supra-Additive Effects of Combining Fat and Carbohydrate on Food Reward. *Cell Metab.* 2018 Jul 3;28(1):33-44.e3. doi: 10.1016/j.cmet.2018.05.018. Epub 2018 Jun 14. PMID: 29909968.

Tang DW, Fellows LK, Dagher A. Behavioral and neural valuation of foods is driven by implicit knowledge of caloric content. *Psychol Sci.* 2014 Dec;25(12):2168-76. doi: 10.1177/0956797614552081. Epub 2014 Oct 10. PMID: 25304885.

4) Please address potential confounders and rule out alternative explanations for food-cue responses such as display effects, visual familiarity or non-specific perceptual bias that may influence food ratings and neural responses (Reviewers #1 and #3).

We agree testing for these factors is important. We previously reported the creation of the picture set used here (Hutelin, et al 2024). We report our methods in matching on three classes of characteristics: food (nutrient, cost, etc.), visual (complexity, colors, etc.), and ratings (familiarity, estimated calories, etc.). In that study, we surveyed 197 participants on the same ratings reported here and created the two sets of images (UPF and non-UPF) that were matched on all factors, except for perceived healthiness which was not possible to match on. Here, we observed that in our current sample, frequency of consumption also differed between conditions. Therefore, we included healthiness and frequency of consumption as a co-variate in all analyses of willingness to pay data, both neural and behavioral in the original manuscript. For completeness, we now provide a table for the willingness to pay results with the model with no covariates, separately, and together as we report in the text

(Supplemental Table 6). The neural data reported in the main figures and text reflect the model adjusting for the two covariates on a trial-by-trial basis as parametric modulators, in the revision we have clarified this is the reported model.

We agree matching on visual variables is important. In our previous publication, we provide details on the matching of visual stimuli (red, green, blue, object size, brightness, within object contrasts, complexity, normed complexity, and spatial frequencies; Hutelin, et al. 2024). For completeness, here, we have created bar charts of all 9 visual properties as Supplemental Figure 1. None of them differ by condition. We chose not to include them in the original models as previous work has matched stimuli sets on these variables and not adjusted for them in the first level fMRI models (Tang, et al 2014, DiFeliceantonio et al., 2018). However, here, to more rigorously test these factors beyond matching them, we entered object size and complexity as parametric modulators on a trial-by-trial basis in our analyses to adjust for differences in visual information across all images. Object size and visual complexity were chosen as examples of higher-level visual processing. This is a more stringent and direct way of accounting for potential influence of these variables on our outcomes compared to reporting the picture viewing (UPF vs non-UPF) contrast. We provide tables of these effects on the willingness to pay results in Supplemental Figure 5. Adding these variables reduces degrees of freedom, but did not substantially change the pattern of results. We now reference these tables in the results.

Hutelin Z, Ahrens M, Baugh ME, Oster ME, Hanlon AL, DiFeliceantonio AG. Creation and validation of a NOVA scored picture set to evaluate ultra-processed foods. *Appetite*. 2024 Jul 1;198:107358. doi: 10.1016/j.appet.2024.107358. Epub 2024 Apr 15. PMID: 38621591; PMCID: PMC11092385.

DiFeliceantonio AG, Coppin G, Rigoux L, Edwin Thanarajah S, Dagher A, Tittgemeyer M, Small DM. Supra-Additive Effects of Combining Fat and Carbohydrate on Food Reward. *Cell Metab*. 2018 Jul 3;28(1):33-44.e3. doi: 10.1016/j.cmet.2018.05.018. Epub 2018 Jun 14. PMID: 29909968.

Tang DW, Fellows LK, Dagher A. Behavioral and neural valuation of foods is driven by implicit knowledge of caloric content. *Psychol Sci*. 2014 Dec;25(12):2168-76. doi: 10.1177/0956797614552081. Epub 2014 Oct 10. PMID: 25304885.

5) Finally, we ask you to resubmit the revised manuscript as a LETTER. Letters contain up to 4 main figures, 10 extended data figures, and unlimited supplementary data figures. More information about our content types can be found here:

<https://www.nature.com/natmetab/content>

Thank you for the opportunity to revise. In this resubmission we have relabeled all our figures. We have 4 main display items, and all other items are marked as extended data figures. We now submit a separate document containing supplemental

tables and figures that contain the results from the additional requested analyses.

Response to Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The manuscript addresses a timely and increasingly relevant topic in nutritional science: the growing global consumption of ultraprocessed foods (UPFs) and their potential role in driving metabolic disease beyond what can be explained by caloric intake or macronutrient composition alone. The study's central hypothesis—that food processing alters post-ingestive metabolic and neural responses in ways that may contribute to long-term health outcomes—promises to advance our understanding regarding the link between diet quality and metabolic and behavioural health.

The authors present findings from an intervention study involving 57 healthy-weight adults that compare the metabolic and neural responses to nutritionally matched UPF and non-UPF meals, or using an auction task based on food cues, respectively. However, despite the study addressing a critical question with high translational relevance, the manuscript currently reads as preliminary and underdeveloped in several critical areas. The analytical approach is largely descriptive, with key findings presented as correlations that lack sufficient theoretical or empirical justification. The interpretation of neural correlates—particularly those linking peak carbohydrate oxidation to brain activation in the striatum and superior temporal gyrus—rests on associations that are not adequately contextualised within existing neuroimaging or metabolic literature or related to established models of incentive salience and decision-making.

To that end, the manuscript requires substantial revision to strengthen its analytical depth, clarify its conceptual contributions, and ensure that its conclusions are appropriately grounded in the data. Such an approach would also require a substantial reanalysis of data, likely a continued recruitment to acquire further data.

Thank you for this assessment of our work and manuscript. We greatly appreciate the time and constructive feedback. Incorporating these comments has substantially improved the manuscript. We provide point by point responses below.

Here are a couple of immediate concerns and suggestions:

The auction task is used as a behavioural measure of subjective value (under the assumption that “willingness to pay” = a higher internal value signal). It is important to note that “value” here is a choice-based, subjective value, not an absolute or unitary notion of value. As this should be made obvious somewhere in the manuscript, the association of value to “reward” regions in the brain is wrong.

We thank the reviewer for this important conceptual clarification. We agree that "value" as measured by the auction task reflects choice-based, subjective willingness to pay rather than an absolute or unitary construct, and that conflating this with "reward" in the neural interpretation is imprecise. We have revised all sections of the manuscript to use more precise language, referring to "subjective value".

The authors report an association of carbohydrate oxidation after meals in response to food cues with neural responses (in the superior temporal gyrus). How can it be assured that this is not just correlative but has a mechanistic (causal) underpinning – what is the putative mechanism? This would possibly better suggest the somewhat implausible (or surprising) finding of a brain response in a brain area that is typically not involved in interoceptive, metabolic, salience or motivational processing: the superior temporal gyrus. Has this analysis been pre-registered? Otherwise, it would be very hard to argue for it, at least in a classical behavioural psychology standard.

We were also surprised by the STG finding, but as it was whole brain corrected and our statistical analysis plan includes whole-brain corrected and ROI analyses, we reported it. We agree that the superior temporal gyrus (STG) is not a region traditionally implicated in interoceptive, metabolic, or motivational processing. In light of this point, we have revised our language in the discussion which now reads:

“Beyond striatal regions, processing-related individual differences in carbohydrate oxidation were also associated with responses in the superior temporal gyrus (STG). While primarily known for its role in auditory and language processing, the STG is increasingly recognized as a hub for multisensory integration (Scheliga et al., 2023) and is responsive to food cues (St-Onge et al., 2005; G.-J. Wang et al., 2004). More work is needed to determine if the STG is involved in food specific multisensory integration, including interoceptive metabolic signals.”

In that respect, also (line 309): “a larger increase in peak carbohydrate oxidation for non-UPF relative to UPF was associated with the inverse response”, what does this mean?

We thank the reviewer for pointing out this unclear phrasing. We agree that the sentence as written does not convey the intended relationship clearly. We have rewritten this passage to describe the direction of the association more directly. In response to this, and Reviewer 3’s comments, we have edited the axes of these figures to clarify the directions of the effects.

This sentence now reads: “Specifically, a larger increase in peak carbohydrate oxidation for non-UPF relative to UPF was negatively associated with the neural response (non-UPF>UPF) (Figure 2.b-d, Extended Data Table 2).”

Brain responses seem strongly lateralised. This is not at all discussed. Please expand.

We agree that our supra-threshold findings appeared lateralized. We therefore dropped our threshold and found sub-threshold findings bilaterally. We concluded the lateralization was likely due to the noisy nature of fMRI data and not a true lateralized finding, we therefore did not make claims on lateralization.

Also related to brain areas, the brain response in the “striatum” (which is not a “reward-related region”) is analysed in relation to prior findings in the auction task. While this is correct on a phenomenological level, the reporting and discussion need significant nuance, especially when differentiating between dorsal striatal areas and the ventral striatum and in a context of predicted value; see in neuroscience literature, value inference (ventral striatum, updates beliefs about expected value), policy refinement (dorsolateral striatum, action policies rather than outcome values), sensory model (dorsal striatum receiving dense sensory cortical input support learning of sensory statistics independent of reward or action), etc. To avoid further complications, I would suggest focusing on cue responses related to salience. This involves a broader discussion and analysis of the circuit, but it is logically clearer to argue.

We agree with the reviewer that the function of the dorsal and ventral striatum is complex, multi-faceted, and referring to it only as a reward region does not reflect this. We have corrected our language throughout. We sought to place our findings in the context of the feeding and eating behavior literature which implicates both the dorsal and ventral striatum. In rodent models, dopamine function in the dorsal, but not the ventral striatum, is essential for food intake (Palmiter, 2008). Also in rodents, post-ingestive responses to nutrients evoke dopamine efflux in the dorsal striatum (McDougle et al, 2024 and Tellez et al 2013) and changes in activity in dopamine neurons of the ventral tegmental area (presumably increasing dopamine in the nucleus accumbens; Fernandes et al 2020). In humans, the caudate has long been implicated in response to foods and that activity is associated with weight status and future weight gain (Stice et al 2008). Also in humans, ventral striatal (though the cluster extends to the ventral caudate) response to calorie-predictive flavors is associated with that flavor's previous metabolic response (Veldhuizen et al, 2017). These and other similar findings have led to a proposed framework for eating behavior and food reward by which subconscious interoceptive signals (including metabolic changes) engage striatal circuitry to guide eating behavior (de Araujo et al, 2020 and Weber, et al 2025). We have now updated the manuscript to better describe our findings in this framework.

Palmiter RD. Dopamine signaling in the dorsal striatum is essential for motivated behaviors: lessons from dopamine-deficient mice. Ann N Y Acad Sci. 2008;1129:35-46. doi: 10.1196/annals.1417.003. PMID: 18591467; PMCID: PMC2720267.

McDougle M, de Araujo A, Singh A, Yang M, Braga I, Paille V, Mendez-Hernandez R, Vergara M, Woodie LN, Gour A, Sharma A, Urs N, Warren B, de Lartigue G. Separate gut-brain circuits for fat and sugar reinforcement combine to promote overeating. Cell Metab. 2024 Feb 6;36(2):393-407.e7. doi: 10.1016/j.cmet.2023.12.014.

Epub 2024 Jan 18. Erratum in: Cell Metab. 2024 Jun 4;36(6):1431. doi: 10.1016/j.cmet.2024.05.004. PMID: 38242133; PMCID: PMC11898112.

Tellez LA, Medina S, Han W, Ferreira JG, Licon-Limón P, Ren X, Lam TT, Schwartz GJ, de Araujo IE. A gut lipid messenger links excess dietary fat to dopamine deficiency. Science. 2013 Aug 16;341(6147):800-2. doi: 10.1126/science.1239275. PMID: 23950538.

Fernandes AB, Alves da Silva J, Almeida J, Cui G, Gerfen CR, Costa RM, Oliveira-Maia AJ. Postingestive Modulation of Food Seeking Depends on Vagus-Mediated Dopamine Neuron Activity. Neuron. 2020 Jun 3;106(5):778-788.e6. doi: 10.1016/j.neuron.2020.03.009. Epub 2020 Apr 6. Erratum in: Neuron. 2023 Feb 15;111(4):595. doi: 10.1016/j.neuron.2023.01.025. PMID: 32259476; PMCID: PMC7710496.

Stice E, Spoor S, Bohon C, Small DM. Relation between obesity and blunted striatal response to food is moderated by Taq1A A1 allele. Science. 2008 Oct 17;322(5900):449-52. doi: 10.1126/science.1161550. PMID: 18927395; PMCID: PMC2681095.

Veldhuizen MG, Babbs RK, Patel B, Fobbs W, Kroemer NB, Garcia E, Yeomans MR, Small DM. Integration of Sweet Taste and Metabolism Determines Carbohydrate Reward. Curr Biol. 2017 Aug 21;27(16):2476-2485.e6. doi: 10.1016/j.cub.2017.07.018. Epub 2017 Aug 10. PMID: 28803868; PMCID: PMC5745144.

de Araujo IE, Schatzker M, Small DM. Rethinking Food Reward. Annu Rev Psychol. 2020 Jan 4;71:139-164. doi: 10.1146/annurev-psych-122216-011643. Epub 2019 Sep 27. PMID: 31561741.

Weber LA, Yee DM, Small DM, Petzschnner FH. The interoceptive origin of reinforcement learning. Trends Cogn Sci. 2025 Sep;29(9):840-854. doi: 10.1016/j.tics.2025.05.008. Epub 2025 Jun 10. PMID: 40500611; PMCID: PMC12400946.

The authors report that some food ratings, particularly healthiness and frequency of consumption (Figure 3), differ significantly between non-UPF and UPF cues. This is potentially very concerning, even if familiarity ratings do not differ between cue groupings. The difference in the ratings may introduce a potential bias in willingness to pay (which has not been tested or corrected for), but, even more concerning, it will bias the internal value signal and therefore compromise the study as a whole (or at least its interpretation).

We agree testing for these factors is important. We previously reported the creation of this picture set (Hutelin, et al 2024). In that study, we surveyed 197 participants on the same ratings reported here and created the two sets of images (UPF and non-UPF) that were matched on all factors, except for perceived healthiness which

was impossible to match on. Here, we observed that in this particular sample frequency of consumption also differed between groups. Therefore, we include healthiness and frequency of consumption as a co-variate in all analyses of willingness to pay data, both neural and behavioral, in the original manuscript. For completeness, we now provide a table for the willingness to pay results with the model with no covariates, separately, and together as we report in the text (Supplemental Table 6). The neural data reported in the text reflect the model adjusting for the two covariates on a trial by trial basis as parametric modulators, in the revision we clarify this was the reported model.

Hutelin Z, Ahrens M, Baugh ME, Oster ME, Hanlon AL, DiFeliceantonio AG. Creation and validation of a NOVA scored picture set to evaluate ultra-processed foods. Appetite. 2024 Jul 1;198:107358. doi: 10.1016/j.appet.2024.107358. Epub 2024 Apr 15. PMID: 38621591; PMCID: PMC11092385.

Along the line, pre- and post-scan ratings of internal state also differ significantly, suggesting an effect of time and potentially altering cue saliency. Hence, this needs to be taken into account in the analysis and discussion.

Thank you for bringing up this important point. To address this, we entered the change in hunger, fullness, and thirst across the session into the 2nd level model for all fMRI analyses. We found it did not substantially change the findings. We now report the findings from these models as Supplemental Tables 1-3. We have added information about those analyses to the analysis section of the methods.

Consider accounting for metabolic rate and fat mass in the respective analyses.

We agree this is an important consideration, we do not have a metric of fat mass, but calculated BMI in all participants (who were all healthy weight (mean = 22, SD = 1.87)). As above, we entered it as a covariate in our second level model which is included as Supplemental Table 4. It is not substantially different from our model with only age and sex as covariates. For metabolic rate, we take into account the baseline metabolic rate in the analysis and calculate change from baseline in our reported analyses.

The finding of a differential association of willingness to pay with UPF and non-UPF in areas of the visual association cortex (Figure 4), especially the ventral visual stream, lingual, as well as the fusiform gyrus, is interesting but potentially also very concerning:

(a) First of all, it needs to be ruled out (and shown) that there is no display effect between both conditions (cue sets), in terms of a main effect.

We agree this is an important point. In our previous publication, we provide details on the matching of visual stimuli (Hutelin, et al. 2024). We measured 9 visual properties red, green, blue, object size, brightness, within object contrasts, complexity, normed complexity, and spatial frequencies. Our stimuli did not differ by condition on

any of these properties. For completeness, here, we have created bar charts of all 9 measured visual properties as Supplemental Figure 1. Further, in response to this, the below comment, and comments from Reviewer 3, we adjusted for complexity and object size by using them as trial-by-trial parametric modulators. This is a more direct way of adjusting for potential influence of these variables on our outcomes compared to only viewing the picture viewing contrast. We chose object size and complexity as they are higher-level visual attributes. We provide a table of this model in Supplemental Table 5 and reference it in the results, our pattern of effects was largely unchanged, however some values changed due to reducing degrees of freedom by adding additional covariates.

(b) Above and beyond this, the finding is concerning, relating to the above-discussed finding that healthiness ratings and frequency of consumption differ significantly between non-UPF and UPF pictures. Besides, these ratings likely affect willingness to pay; they will interact to drive visual responses, particularly visual associations. This needs to be tested for.

Thank you for this important point. Adjusting our statistical models for these factors is important. Therefore, we included healthiness and frequency as a co-variate in all analyses of willingness to pay data, both neural and behavioral. In the revision we clarified these factors were in the original model reported. For completeness, we now provide a table for the willingness to pay results with the model with no covariates, separately, and together as we report in the text (Supplemental Table 6). The neural data reported in the text and main figures reflect the model adjusting for the two covariates on a trial-by-trial basis as parametric modulators.

Furthermore, beyond showing differential associations of willingness to pay for non-UPF cues and UPF cues separately, there needs to be a test for statistical interaction.

We agree. We report the contrast of non-UPF parametrically modulated with willingness to pay (and covariates) greater than UPF parametrically modulated with willingness to pay (and covariates). This directly tests the interaction (the difference between the two associations).

(c) The finding of the brain response in the Fusiform gyrus is discussed according to the classical “what” association for the ventral visual stream, yet with a focus on food cues. The equally significant response in the Lingual gyrus is not discussed. But the issue here is not necessarily the attribution but the assumption of a predicted response. There has been work in mice showing that the visual association cortex can track not only stimulus identity but also predicted outcome. And also that outcome-tracking, but not identity-tracking, neurons can have a response bias to food-predicting cues and sensitivity to reward history (e.g. work by Mark Andemann’s group). However, given that

the visual association cortex in mice has no clear homologue in Humans, this needs elaboration.

Thank you for this point, in response to this comment, that of the editor, and reviewer 3 we have substantially revised this section. We now discuss the lingual gyrus but highlight the need for more studies on food cues in visual cortex. This section now reads: "Interestingly, we observed condition differences in subjective value associations in the occipitotemporal gyrus, specifically the fusiform and lingual gyri. While the fusiform gyrus is best known for its role in face processing within the ventral visual stream, a distinct region identified for food-specific visual processing has also been found and termed the fusiform food area (Adamson & Troiani, 2018; Jain et al., 2023) Response in the fusiform food area is modulated by circulating glucose, insulin, and ghrelin (Kroemer, Krebs, Kobiella, Grimm, Pilhatsch, et al., 2013; Kroemer, Krebs, Kobiella, Grimm, Vollstädt-Klein, et al., 2013; Malik et al., 2008). The lingual gyrus has been reported in studies of food cues (Stice et al., 2013) and the visual association cortex in mice can be sensitive to reward associations (Nguyen et al., 2024). However, further experiments are needed to establish if visual regions are sensitive to food value"

Related to the drop-out of participants in single sessions. If this was not consistent across sessions, consider a flexible-factorial design.

We thank the reviewer for raising this point. We would like to clarify that all participant data used from the fMRI portion of the study had complete data across conditions. For analyses of measures collected outside the scanner, we used linear mixed-effects models throughout, to appropriately handle unbalanced data and missing observations.

The MRI protocol parameters are missing in the Methods part.

We thank the reviewer for this comment. The MRI acquisition parameters were included in the original submission; however, we agree they were not easy to locate. To improve visibility, we have now grouped them under a dedicated subsection titled "MRI acquisition parameters"

Reviewer #2 (Remarks to the Author):

The epidemiological evidence linking ultra-processed food (UPF) intake to increased disease risk is now well established. What is needed are studies that elucidate the underlying mechanisms, and randomized cross-over trials are particularly valuable in this context because they control for both known and unknown confounders. A key debate in the literature is whether these associations are driven by nutrient composition rather than processing per se. The authors address this issue by comparing nutrient-matched UPF and non-UPF meals. In addition to examining postprandial metabolic

responses, the study investigates the relationship between these metabolic changes and brain activity. This is a novel and important contribution, as the brain ultimately regulates eating behavior, including signals related to satiety and the decision to stop eating.

Please note that my review focuses specifically on the first two objectives of the study: the postprandial metabolic responses to UPF versus non-UPF meals and the associated neural reactivity. I have not evaluated the 'Willingness to Pay' (WTP) data or its neural correlates, as the behavioral economic modeling falls outside my primary area of expertise.

This study (data collection between 2023 and 2025) aimed to compare the effects of ultra-processed foods (UPF) on metabolism and neural responses compared to non-UPF in 57 enrolled healthy-weight adults aged 18–45. To do so, researchers compared the neural responses of participants to 5-second images of 14 non-UPF and 14 UPF items (matched on 26 characteristics) presented in a random order (n=52).

A subset of 32 participants took part in a cross-over RCT to compare changes in postprandial metabolism relative to baseline from 0 to 180 minutes post-meal between UPF and non-UPF meals. The meals consisted of items purchasable at a grocery store and were nutrient-matched (including glycemic index and glycemic load). These 300 kcal meals, to be consumed in less than 10 minutes, were a subset of the 28 foods used in the picture set and represented the most nutritionally matched set of UPF and non-UPF meals. The primary outcomes included mean, area under the curve (AUC), and trajectory of blood glucose, blood insulin, postprandial metabolic rate, respiratory exchange ratio (RER), and carbohydrate and fat oxidation.

The results showed that, relative to baseline, the 32 participants during the UPF meals had higher mean increases in blood insulin, metabolic rate, and fat oxidation, and lower mean increases in respiratory exchange ratios and carbohydrate oxidation than during the non-UPF meals. In 29 participants with both metabolic measures and fMRI data, greater positive differences between non-UPF versus UPF meals in exchange ratios and carbohydrate oxidation were associated with brain activity (inverse correlation).

Thank you, Dr. Monteiro, for your time and favorable assessment of our manuscript. We have incorporated your points below into our resubmission and we believe they have improved the manuscript. We appreciate this fair and constructive review.

Some specific comments are provided below:

Introduction.

‘Concerningly, higher UPF intake is associated with poorer health outcomes, including cancer, overweight and obesity, and metabolic dysfunction(Barbaresko et al., 2025; Hasenböhler et al., 2026; Lane et al., 2024).’

I suggest citing a recently published paper that presents evidence on the association between UPF intake and health outcomes, based on meta-analyses including only prospective studies:

‘Monteiro CA, Louzada ML, Steele-Martinez E, Cannon G, Andrade GC, Baker P, Bes-Rastrollo M, Bonaccio M, Gearhardt AN, Khandpur N, Kolby M, Levy RB, Machado PP, Moubarac JC, Rezende LFM, Rivera JA, Scrinis G, Srouf B, Swinburn B, Touvier M. Ultra-processed foods and human health: the main thesis and the evidence. Lancet. 2025 Dec 6;406(10520):2667-2684. doi: 10.1016/S0140-6736(25)01565-X. Epub 2025 Nov 18. PMID: 41270766.’

We thank the reviewer for this helpful suggestion. We agree that Monteiro et al. (2025) provides a valuable synthesis of prospective evidence linking UPF intake to adverse health outcomes. We have added this citation to the relevant sentence in the Introduction.

Results.

‘To investigate the effect of processing level on post-ingestive metabolic response to foods with matched nutrient content, 32 participants consumed ~300kcal nutritionally matched meals composed entirely of either UPF or non-UPF’.

It would be helpful for the authors to clarify whether participants were required to consume the entire meal. Given the 10-minute time constraint and the subsequent metabolic analyses, confirming total consumption is essential for validating the nutrient-matched design.

We thank the reviewer for raising this important point. Participants were indeed required to consume the entire meal within the 10-minute window, and all participants did so. This ensured that the nutrient-matched design was preserved across conditions and that subsequent metabolic measures reflected complete ingestion of the prescribed meal. We have updated the Methods section to clarify this requirement.

‘The meals were consumed on separate days in a randomized crossover design, allowing within-participant comparison of indirect calorimetry and blood-derived metabolic outcomes.’

The authors should clarify the timing of the crossover sessions. Specifically, how many days apart were the two meals consumed, and was a standardized washout period implemented?

We agree this is an important methodological consideration. Given the meals were only 300 kcals, we did not impose a washout period longer than 24 hours. We have added to the methods that seven participants had sessions within 3 days, 14 participants between 4 days and 2 weeks, and 7 participants between 15 days and four weeks, and finally 4 participants had sessions with a greater than 4 week gap. We have added this information to the Methods (bottom of page 28). We explored the effect of session spacing by entering it as a covariate in our analysis of blood and chamber

outcomes and found no effect.

‘The UPF meal was consumed more slowly than the non-UPF meal by an average of 1.5 minutes ($t(30.43) = 5.13$, $p < 0.001$; Figure S3.a).’

It should be clarified whether participants were required to consume the entire meal. If total consumption was mandatory within the allotted 10-minute window, the findings suggest that the UPF meal may have been consumed more slowly than the non-UPF meal, given the specific time constraint. This would contrast with previous randomized trials—most notably Hall et al. (2019) and Hamano et al. (2024)—which consistently demonstrate significantly faster eating rates for ultra-processed foods. The authors should address this potential discrepancy and discuss how the fixed consumption time might have influenced the observed postprandial metabolic responses.

We thank the reviewer for this thoughtful observation. As clarified in the revised Methods section, participants were expected to consume the entire meal within the 10-minute window. We have also expanded the Limitations section to further address this discrepancy in eating rate with the findings in prior work.

This section in the limitations now reads: “Prior work indicates that UPFs are consumed more rapidly under ad libitum conditions (Forde et al., 2020; Hall et al., 2019; Hamano et al., 2024). In contrast, in the present study, the meals were isocaloric (~300 kcal) and fully consumed, and the non-UPF meal was eaten slightly faster than the UPF meal. However, the modest 1.5-minute difference in eating time is unlikely to produce a physiologically meaningful effect (Alsalim & Ahrén, 2019; Angelopoulos et al., 2014).”

“Associations between metabolic and brain responses. Next, we examined whether postprandial metabolic responses were associated with brain response to food pictures, a metric of food cue reactivity.”

Though described in the Methods, to better explain the experiment, it would be relevant to mention in this section as well that the non-UPF and UPF meals used in the metabolic sessions were derived from a 300 kcal subset of the 28 foods used in the picture set, representing the most nutritionally matched set of non-UPF and UPF meals.

We agree that restating this design detail in the Results section will make the link between the metabolic and neuroimaging components of the study clearer to the reader. We have added clarifying text noting that the UPF and non-UPF meals used in the metabolic sessions were drawn from a 300 kcal subset of the 28 foods in the picture set, selected to represent the most nutritionally matched UPF and non-UPF meals. This section now reads: “The same participants on a separate day completed a Becker–DeGroot–Marschak (BDM) auction task during fMRI to quantify subjective food value; notably, the non-UPF and UPF meals used in the metabolic sessions were derived from a ~300 kcal subset of the foods used in the BDM task.”

I'm interested in the rationale behind the study design. If the hypothesis is that the metabolism of a UPF meal affects neural responses differently than a non-UPF meal, why was the fMRI assessment conducted on a separate day using food images, rather than capturing the postprandial effect directly? Was this decision primarily due to logistical or other sort of constraints?

Thank you for this opportunity to clarify. Here, we are working within and testing the framework that post-ingestive signals provide information from the periphery to the brain on the nutritional value of foods and those signals shape food value (de Araujo, et al 2020 and Weber et al 2025). A key component of this model that we also wished to test is that these associations can be learned but should be relatively stable across the time frame in which we conducted our sessions. Meaning that although we tested the metabolic response to UPF vs non-UPF foods on a different day, individual differences in responses to UPF vs non-UPF meals within each person should still be present on the fMRI session day. We can then observe differential encoding of those learned responses as in DiFeliceantonio, et al 2018 and Tang, et al 2014. We have revised the manuscript throughout to help clarify this point.

de Araujo IE, Schatzker M, Small DM. Rethinking Food Reward. Annu Rev Psychol. 2020 Jan 4;71:139-164. doi: 10.1146/annurev-psych-122216-011643. Epub 2019 Sep 27. PMID: 31561741.

Weber LA, Yee DM, Small DM, Petzschnner FH. The interoceptive origin of reinforcement learning. Trends Cogn Sci. 2025 Sep;29(9):840-854. doi: 10.1016/j.tics.2025.05.008. Epub 2025 Jun 10. PMID: 40500611; PMCID: PMC12400946.

DiFeliceantonio AG, Coppin G, Rigoux L, Edwin Thanarajah S, Dagher A, Tittgemeyer M, Small DM. Supra-Additive Effects of Combining Fat and Carbohydrate on Food Reward. Cell Metab. 2018 Jul 3;28(1):33-44.e3. doi: 10.1016/j.cmet.2018.05.018. Epub 2018 Jun 14. PMID: 29909968.

Tang DW, Fellows LK, Dagher A. Behavioral and neural valuation of foods is driven by implicit knowledge of caloric content. Psychol Sci. 2014 Dec;25(12):2168-76. doi: 10.1177/0956797614552081. Epub 2014 Oct 10. PMID: 25304885.

It would be useful to describe when exactly the fMRI was performed in relation to the metabolic sessions (Meal 1 and Meal 2), as all were conducted on different days. Specifically, the authors should clarify the sequence of these sessions: Was the fMRI performed at baseline before any meal exposure, or was it conducted after participants had already completed the UPF and non-UPF meal interventions?

We agree this is an important detail. We have now added to the methods that 6 participants had their fMRI after all sessions, 7 between sessions, and 16 before the metabolic sessions. As in our response to the above point, we expect these metabolic

responses have influenced the learned value of the food, and therefore would be stable within our testing time. We confirmed all of the foods tested were familiar and frequently consumed both in sample (Figure 3) and out of sample (Hutelin, et al 2024). We now include information on the timing of all sessions in the methods section.

To better understand the neural component of the study, it would be extremely helpful if the authors described all the brain regions that were assessed for reactivity and the rationale for their selection (e.g., regions associated with reward processing...). Providing this anatomical context is essential for interpreting the functional significance of the findings; I think the authors should explicitly cite Table 2 here to guide the reader through these regional data.

Thank you for pointing out that we do not refer to our fMRI results table in this section. This has been corrected. We followed our statistical analysis plan which outlined both a whole brain and ROI approach. Our ROI included the caudate, putamen, and nucleus accumbens (as a single large mask to increase robustness), as prior work has demonstrated these regions are associated with metabolic responses to food cues in humans and differences in willingness to pay. We have added additional information on our ROI selection and creation to the methods section.

‘Regressing the between-condition change in peak carbohydrate oxidation on participant-level non-UPF > UPF contrast estimates revealed a whole-brain FWE-corrected association in the left superior temporal gyrus ($[-44, -24, -8]$, $t = 7.33$, $p_{FWE} = 0.013$; Figure 2.b).’

Of all the brain regions evaluated for reactivity, it is unclear whether Figure 2 and Table 2 only plot those that were significantly associated with the changes in metabolic peaks, or if all studied brain regions showed such associations. This distinction should be made explicit within the captions and headers for Figure 2 and Table 2. To ensure full transparency and avoid selective reporting, I recommend that the authors report all associations—regardless of whether they reached statistical significance—perhaps by including the non-significant data in Table 2 (or a Supplemental Table).

We thank the reviewer for this thoughtful suggestion and for the opportunity to clarify our reporting. Figure 2 and Table 2 display only the peaks that survived family wise error (FWE) correction either whole-brain or small volume (ROI) corrected. We did not extract values and separately correlate them, rather we regressed those values onto every voxel in the brain (whole brain corrected) or every voxel in our single large ROI mask (small volume corrected), which is much more statistically robust. It would not be feasible to report every voxel in a table but, if published, we will provide raw data on OpenNeuro.

“Prior work in both humans and rodents has shown that peak metabolic responses to sugar-sweetened beverages are associated with striatal activation (de Araujo et al., 2013; Ren et al., 2010; Veldhuizen et al., 2017); therefore, we examined both whole

brain corrected and small volume corrected results within an a priori striatal mask". Could the authors clarify the source of the a priori striatal mask?

We thank the reviewer for this clarification request. The mask came from the Harvard-Oxford Subcortical Atlas. To clarify the source of the mask, we have added a parenthetical reference "(see Methods for details)" to the relevant section in the Results section. In response to this and Reviewer 3's comment we have provided additional detail in the methods about the mask generation and a figure of the mask in Supplemental Figure 2.

"Specifically, a larger increase in peak carbohydrate oxidation for non-UPF relative to UPF was associated with the inverse neural response (Figure 2.b-d). As expected, given the close correspondence between these measures, peak RER exhibited a similar relationship in the left superior temporal gyrus ($[-44, -24, -8]$, $t = 7.63$, $p_{FWE} = 0.007$), and small volume corrected right caudate ($[4, 8, 2]$, $t = 5.77$, $SVC\ p_{FWE} = 0.014$; Figure 2.e). In addition, peak RER was also associated with a second locus of activation in the right caudate ($[22, -12, 24]$, $t = 5.48$, $SVC\ p_{FWE} = 0.026$; Figure 2.f)." The inverse correlation suggests that as the metabolic response to non-UPF meals improves, the neural disparity between UPF and non-UPF cues diminishes. Is there a physiological rationale for this relationship?

We thank the reviewer for raising this important interpretive question. We agree that the direction of the association warrants explicit consideration, and we have expanded the Discussion to address the potential physiological rationale now reads: "Notably, prior human studies reported a positive association between post-ingestive metabolic responses and striatal activation (de Araujo et al., 2013; Veldhuizen et al., 2017), whereas the present findings indicate a negative association. This discrepancy may reflect a key methodological difference: prior studies measured neural responses during oral flavor receipt, whereas the present study assessed responses to visual food cues, which have been shown to evoke distinct and sometimes opposite neural responses (O'Doherty et al., 2002)."

"Between-condition changes in peak metabolic rate, fat oxidation, blood glucose, and blood insulin were not significantly associated with neural responses." I recommend that these correlation results be included in Table 2 (or in Supplemental Table).

We thank the reviewer for this suggestion. As noted in our response to the preceding comment, voxel-wise whole-brain fMRI analyses test hundreds of voxels, and it is not feasible to present sub-threshold results in a table. The analyses for peak metabolic rate, fat oxidation, blood glucose, and blood insulin were conducted using the same whole-brain and small volume corrected FWE-corrected approach as the carbohydrate oxidation analysis, and no clusters survived correction. If published, we will provide all raw data on OpenNeuro.

Discussion.

'Food processing can modify post-ingestive physiology, often by increasing carbohydrate availability and elevating glycemic index, thereby producing larger postprandial blood glucose and insulin responses (Huang et al., 2022; Tosh & Chu, 2015). Yet, this explanation is unlikely to account for our findings as the test meals were matched on glycemic index and yielded comparable blood glucose AUC, while the UPF condition elicited larger insulinemic and energetic responses with attenuated carbohydrate oxidation.'

I recommend that the authors expand the Discussion to include a comparison with the metabolic findings of Hall et al. (2019) and Hamano et al. (2024). Placing these results in the context of existing UPF randomized controlled trials is important to validate the study's conclusions.

We thank the reviewer for this valuable suggestion. We agree that situating our metabolic findings within the context of prior UPF randomized controlled trials strengthens the interpretation of our results. We have expanded the Discussion to compare our postprandial metabolic findings with those of prior work: "Food processing can modify post-ingestive physiology, often by increasing carbohydrate availability and elevating glycemic index, thereby producing larger postprandial blood glucose and insulin responses (Huang et al., 2022; Tosh & Chu, 2015). Prior work has reported differences in metabolic rate and respiratory quotient following meals differing in degree of processing; however, these effects are difficult to attribute directly to processing as the test meals also differed in total energy and macronutrient composition (Barr & Wright, 2010; Hall et al., 2019). The present findings address this limitation directly — the test meals were matched on energy, macronutrients, and glycemic index and yielded comparable blood glucose AUC, while the UPF condition elicited larger insulinemic and energetic responses with attenuated carbohydrate oxidation."

'Here, we provided novel evidence that UPFs influence metabolic and neural responses differently from nutrient-matched non-UPFs. First, we demonstrated that even when meals were matched across multiple nutritional factors, degree of food processing classified by Nova significantly altered post-ingestive metabolic responses. Next, we showed that these metabolic differences were associated with neural responses to non-UPF versus UPF food cues.'

A significant limitation is the methodological divergence between the metabolic and neuroimaging assessments. The metabolic outcomes reflect an acute postprandial response to meal ingestion, whereas the neural data reflects cue-reactivity to visual stimuli (food images). This discrepancy in the 'exposure' stimulus—coupled with the multi-day gap between experiments—introduces potential confounders that could account for the observed associations. The authors should explicitly address this as a constraint on the study's internal validity and its ability to establish a direct causal link between the metabolism of specific foods and subsequent neural reward processing.

In response to the above comment, and those of Reviewer's 1 and 3, we have clarified our design choice throughout the manuscript. We more explicitly state that we are working within and testing a framework in which post-ingestive signals provide information from the periphery to the brain on the nutritional value of foods and those signals shape food value (de Araujo et al 2020 and Weber et al 2025). In this model, these associations can be learned but should be relatively stable across the time frame in which we conducted our sessions. Meaning that although we tested the metabolic response to UPF vs non-UPF foods on a different day, individual differences in responses to UPF vs non-UPF meals within each person should still be present on the fMRI session day. We did not seek to test the acute effects of metabolism on neural representations but rather test if 1. the post-ingestive effects of UPF vs non-UPF foods differed and 2. if those learned differences influenced neural responses as has been demonstrated for other types of foods previously (ie. DiFeliceantonio et al., 2018, Tang et al. 2014, Veldhuizen, et al 2017).

de Araujo IE, Schatzker M, Small DM. Rethinking Food Reward. Annu Rev Psychol. 2020 Jan 4;71:139-164. doi: 10.1146/annurev-psych-122216-011643. Epub 2019 Sep 27. PMID: 31561741.

Weber LA, Yee DM, Small DM, Petzschnner FH. The interoceptive origin of reinforcement learning. Trends Cogn Sci. 2025 Sep;29(9):840-854. doi: 10.1016/j.tics.2025.05.008. Epub 2025 Jun 10. PMID: 40500611; PMCID: PMC12400946.

DiFeliceantonio AG, Coppin G, Rigoux L, Edwin Thanarajah S, Dagher A, Tittgemeyer M, Small DM. Supra-Additive Effects of Combining Fat and Carbohydrate on Food Reward. Cell Metab. 2018 Jul 3;28(1):33-44.e3. doi: 10.1016/j.cmet.2018.05.018. Epub 2018 Jun 14. PMID: 29909968.

Tang DW, Fellows LK, Dagher A. Behavioral and neural valuation of foods is driven by implicit knowledge of caloric content. Psychol Sci. 2014 Dec;25(12):2168-76. doi: 10.1177/0956797614552081. Epub 2014 Oct 10. PMID: 25304885.

Veldhuizen MG, Babbs RK, Patel B, Fobbs W, Kroemer NB, Garcia E, Yeomans MR, Small DM. Integration of Sweet Taste and Metabolism Determines Carbohydrate Reward. Curr Biol. 2017 Aug 21;27(16):2476-2485.e6. doi: 10.1016/j.cub.2017.07.018. Epub 2017 Aug 10. PMID: 28803868; PMCID: PMC5745144.

Supplemental material.

p. 5 'This project aims to study the effects of food processing level on post-ingestive metabolic signals and reward through the consumption of snack meals between 200-400 kcal which have been paired for macronutrient content across processing level as defined by the NOVA (not an acronym) food classification system.'

It should be clarified whether all the meals provided were considered snacks. If so, it is

important to state this explicitly in the main text, as the metabolic and neural responses to a 300 kcal snack may differ significantly from those of a full, ad libitum meal.

We thank the reviewer for raising this important point. We agree that the metabolic and neural responses elicited by a ~300 kcal snack-sized meal may differ from those following a larger meal, and that this distinction is important for interpreting our findings. We chose this meal size to capture the rising and falling curves of the post-ingestive responses. Large meals would necessitate more time in our smaller “flex” chamber (with no toilet) than is feasible to capture the falling end. We have added a statement to the Limitations section acknowledging that the test meals in this study were ~300 kcal, and that the generalizability of our findings to larger meals or ad libitum eating contexts remains to be established in future work.

p. 11 ‘Participants will also undergo smell testing. They will be prompted to answer a short series of questions and then take the smell test. They will be provided cards. Each card contains three lift and sniff patches. Participants will be instructed to lift the plastic cover over each patch and smell each one. Only one patch will contain the odor, while the other two will be odorless. The smell test involves three tasks: (1) identifying which one of three patches contains an odor (detection task), (2) rating the intensity of the odor on a scale from 0- 100 (where 100 is the strongest odor) and (3) choosing from four picture and label alternatives, what the odor smells like (identification task).’? Were the smelling tests carried out after all? If so, I think this should be described in the main text.

We thank the reviewer for raising this point. The smell testing protocol described on p. 11 was conducted in a subset of participants as a small pilot effort to collect preliminary data for future work and was not part of the primary outcomes or hypotheses of the present study. For this reason, the smell testing data are not analyzed or reported in this manuscript.

p. 15 ‘Meal consumption: There are no known risks known with consuming the proposed meals. Participants will be screened by research personnel prior to their first meal for known food allergies. Participants will be able to select the meals pairings they participate in.’

What do you mean by ‘Participants will be able to select the meals pairings they participate in’?

We thank the reviewer for flagging this ambiguous wording. The original sentence was intended to convey that participants could opt out of the metabolic (meal-consumption) sessions while still participating in the behavioral and fMRI portions of the study. It was also included to be clear to participants they could withdraw at any time, which is required by our Institution Review Board.

Carlos Augusto Monteiro

Reviewer #3 (Remarks to the Author):

This is an interesting and timely manuscript examining whether ultraprocessed foods elicit distinct metabolic and neural responses compared with nutritionally matched non-ultraprocessed foods. The study is conceptually strong because it combines controlled postprandial metabolic phenotyping with fMRI-based food-cue reactivity and food valuation. The central neuroimaging claims are that differences in postprandial carbohydrate oxidation between UPF and non-UPF meals are associated with cue-related activation in superior temporal gyrus and striatal regions, and that neural encoding of food value differs between UPF and non-UPF cues despite similar willingness-to-pay behaviour. These findings are potentially important and suitable for Nature Metabolism if the authors more clearly address several methodological and interpretational issues.

The strongest neuroimaging aspect is the use of an independently validated, nutritionally and visually matched food-picture set, together with whole-brain FWE correction and a pre-specified striatal small-volume correction. The main weakness is that the metabolic challenge and fMRI task were not performed in the same session or in response to the same acute meal state. Thus, the link between post-ingestive metabolism and neural cue reactivity is correlational across sessions rather than a direct measurement of acute meal-induced neural responses.

Thank you for this constructive and favorable assessment of our manuscript. We appreciate the time and effort needed for a thorough review. We have incorporated all comments into the manuscript and believe the resulting version is considerably improved.

Major comments

1. The link between metabolic responses and fMRI responses should be framed more cautiously

The manuscript states that processing-related metabolic differences were associated with neural food-cue responses. This is supported statistically, but the study design does not show that the acute UPF or non-UPF meal altered brain responses in the same session. The metabolic sessions involved consumption of matched meals during whole-room indirect calorimetry, whereas the fMRI session was conducted separately using a BDM food-auction task after participants arrived “neither hungry nor full” and 3 hours fasted. The fMRI analysis then related between-condition metabolic differences from the chamber sessions to the non-UPF > UPF cue contrast in the scanner. The authors describe this design clearly in the methods, but the Results and Discussion sometimes imply a more direct post-ingestive brain effect than the design can establish. Suggested revision: use language such as “individual differences in metabolic

responses were associated with neural cue reactivity” rather than implying that the meals themselves acutely changed brain responses.

We thank the reviewer for this important clarification and agree. We have changed our language accordingly to frame these findings. Specifically, we have revised the relevant passages on page 14 and page 22 to reflect this more cautious framing. An example from page 14: “Together, these findings are consistent with the possibility that a food processing-related shift in postprandial carbohydrate oxidation may be associated with food cue reactivity, learned through exposure to these foods, extending previous findings from beverages to whole foods and providing additional evidence consistent with pre-clinical findings that carbohydrate metabolism is important for neural response (de Araujo et al., 2013; Tellez et al., 2013; Veldhuizen et al., 2017).”

2. Clarification of power analyses

The power analysis requires clarification. The manuscript’s central mechanistic claim appears to rely on the association between postprandial metabolic responses and neural food-cue reactivity. However, the reported power analysis was based on detecting differences in willingness to pay and, separately, on detecting differences in metabolic responses, rather than on the brain–metabolism association that forms a key neuroimaging result. The relevant analysis appears to involve the subset of participants with both metabolic and fMRI data, which is smaller than the full fMRI sample. The authors should clarify whether the study was adequately powered for these brain–metabolism association analyses, report the exact sample size used for each association, and either provide a corresponding power/sensitivity analysis or explicitly frame these analyses as exploratory.

Thank you for the opportunity to clarify, as stated in our power section, we used previously published data to estimate effect sizes of Cohens’s $f=0.40$ for the behavioral responses and Cohen’s d of 0.57 for the metabolic differences. For the metabolic correlations with brain response, the most relevant data are from Veldhuizen et al 2017. That effect size was reported as $r=.83$, which is much larger than the effect sizes for our other outcomes, which is why we chose to calculate power using the smaller effect sizes. If we perform a power analysis with $r=.83$ from that study, we need 16 participants to achieve 95% power a significance level of .0001. This subset of our study had 29 participants; therefore, we were sufficiently powered for these comparisons. We have added this information to our power analysis section.

We have carefully reviewed our CONSORT diagram and results sections to ensure the n for each analysis is in each section and included in each figure caption.

Veldhuizen MG, Babbs RK, Patel B, Fobbs W, Kroemer NB, Garcia E, Yeomans MR, Small DM. Integration of Sweet Taste and Metabolism Determines Carbohydrate Reward. Curr Biol. 2017 Aug 21;27(16):2476-2485.e6. doi: 10.1016/j.cub.2017.07.018. Epub 2017 Aug 10. PMID: 28803868; PMCID: PMC5745144.

3. Clarify directionality and interpretation of the Figure 2 associations

The direction of the Figure 2 effects is somewhat difficult to follow. The text states that a larger increase in peak carbohydrate oxidation for non-UPF relative to UPF was associated with the “inverse neural response,” and the figure legend states that higher STG response to non-UPF pictures correlated with greater carbohydrate oxidation to UPF. This is confusing because the metabolic variable is defined as non-UPF – UPF, while the neural contrast is also non-UPF > UPF.

The authors should explicitly state whether individuals with relatively greater carbohydrate oxidation after UPF showed stronger non-UPF cue responses, or whether individuals with relatively greater carbohydrate oxidation after non-UPF showed stronger UPF cue responses. This matters for the interpretation.

Suggested addition: a schematic explaining the sign of the contrast would help.

We agree that the directionality of the Figure 2 associations was not described as clearly as it could have been, and that the apparent inconsistency between the text and the figure legend created confusion about the direction of the relationship. We have revised the manuscript to describe the relationship more explicitly and updated the axes of Figure 2 (with a schematic as suggested) to more clearly describe the relationship.

4. The striatal small-volume correction is justified, but the ROI definition needs more detail

The striatum is a well-justified a priori region given prior work on nutrient reinforcement and food valuation. The manuscript reports significant SVC findings in right caudate and left ventral striatum for carbohydrate oxidation, and right caudate for RER. However, the methods only state that the striatal mask included caudate, putamen, and nucleus accumbens from the Harvard–Oxford atlas. The authors should provide more detail on thresholding and laterality of the ROI mask, whether the ROI was bilateral, whether probabilistic thresholds were applied, and whether the same mask was used for all SVC analyses.

Given that several SVC tests were performed across multiple metabolic predictors and two fMRI models, the authors should clarify the family of tests and whether correction across multiple SVC analyses was considered.

We agree that we should provide more information on the creation of the striatal mask. We have updated the methods to include that the mask was bilateral, we used the most lenient probability threshold (resulting in the largest mask), and created a single large mask of all regions. We provide an image of this mask in axial array in Supplemental Figure 2. We used a single large mask for SVC instead of multiple smaller masks to reduce potential alpha inflation from multiple small volume corrections, as indicated by the reviewer, and to correct across a larger number of voxels for robustness.

5. Multiple testing across metabolic predictors should be handled more transparently. The authors tested associations between the neural food-cue contrast and several metabolic variables: peak carbohydrate oxidation, peak RER, metabolic rate, fat oxidation, blood glucose, and blood insulin. They report that only carbohydrate oxidation and RER were significant. Because carbohydrate oxidation and RER are closely related, this is biologically coherent, but the analysis still involves multiple predictors and both whole-brain and ROI tests.

The authors should state whether these analyses were pre-specified and how they controlled or interpreted multiple testing across metabolic predictors. If carbohydrate oxidation was the primary hypothesis-driven predictor, this should be made explicit. Otherwise, the claim should be framed as exploratory.

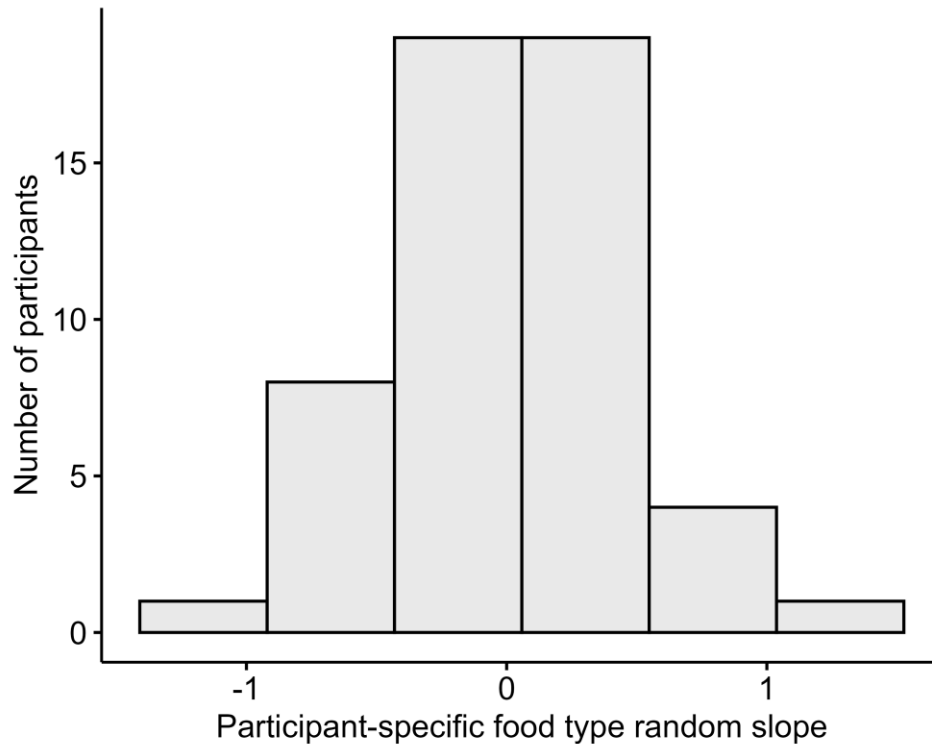
Thank you for this important point. There are multiple independent metabolic predictors that are tested against brain response. For each predictor, this was done in two phases. First, whole brain corrected results were examined and recorded, then a single small volume correction of the large a priori mask described previously was applied and recorded. This was according to our data analysis plan. As each test of metabolic response would be considered independently (even though some metabolic measures are by nature correlated), we did not further reduce the alpha level needed for significance beyond that necessary for each test type (whole brain vs SVC). We use the conservative peak level correction throughout the manuscript to increase robustness (Eklund et al 2016).

6. The value-related fMRI findings are interesting but need more careful interpretation. The BDM analysis is a strength, and the positive control is convincing: WTP was associated with canonical valuation regions including OFC and pregenual ACC. The processing-level contrast showed opposing value associations for non-UPF versus UPF in fusiform and lingual gyri, with SVC effects in putamen and caudate.

However, because WTP itself did not differ between UPF and non-UPF, the biological meaning of a positive neural value association for non-UPF and negative association for UPF requires more cautious explanation. The authors interpret this as altered neural encoding of food value. That is plausible, but they should consider alternative explanations: residual visual differences, semantic categorisation, healthiness/frequency effects, or cognitive control/health beliefs influencing value coding.

This is an important point; we did not see overall condition differences in willingness to pay. Food is inherently complex and hard to match on every possible variable. We matched on many: 9 visual properties, 11 food and nutrient characteristics, and 5 behavioral. The two additional behavioral characteristics we measured but were unable to match on were included as covariates. However, we agree that factors we did not measure could still be influencing our results. We have revised the discussion and limitations to reflect this.

The neural responses are individual, in that for each person brain response to the different foods had a different association with willingness to pay which was on average more positive for non-UPF and negative for UPF on the group level. To examine this type of individual level variability in our behavioral data we fit the willingness-to-pay model using linear mixed-effects modeling, including food type (UPF versus non-UPF) as the primary predictor and adjusted for perceived healthiness and frequency of consumption as fixed effects. Random intercepts for participants and food items, as well as a random slope for food type within participants, were included to account for repeated observations and food specific clustering. From this model, we extracted the predicted random slopes for each participant and examined them. The distribution of participant-specific food-type effects was symmetric and centered near zero (histogram below) indicating little overall difference in willingness to pay between UPF and non-UPF at group level, as we report in the paper. However, the spread of the distribution demonstrates heterogeneity in participants' relative valuation of UPF versus non-UPF, suggesting that while we didn't see an overall effect of willingness to pay across food type, this heterogeneity in participant slopes could be reflective of the differences seen in the brain. In performing this analysis, we realized there were two additional covariance structures to examine—one including random intercepts for participants and food only, and another that additionally included a participant-specific random slope for food type. It was found that the random intercepts plus participant-specific random slope for food type structure improved AIC by more than 2 points, we therefore now include this covariance structure for our behavioral willingness to pay findings, but it does not change the results.



7. Hunger and internal state should be considered as covariates.

Hunger increased, thirst increased, and fullness decreased during the fMRI session. Since internal state strongly modulates food-cue reactivity, the authors should clarify whether pre-scan hunger, change in hunger, or session-level hunger was included as a covariate in fMRI analyses. This is particularly important for the valuation analyses, as subjective value and striatal responses can vary with hunger.

Thank you for this point, it was also brought up by Reviewer 1. To address both comments. We entered the change in hunger, fullness, and thirst across the session into the 2nd level model for all fMRI analyses. We found it did not substantially change the findings. We now report the findings from these models as Supplemental Tables 1-3.

8. The authors should avoid overinterpreting STG as food-specific multisensory integration

The STG finding is whole-brain corrected and therefore robust statistically. The interpretation as multisensory integration of post-ingestive signals with food cues is plausible but somewhat speculative. The STG is not a canonical metabolic or reward region, and the fMRI task was visual only. The discussion does acknowledge the STG's broader role in multisensory integration. However, the manuscript should soften the conclusion and mention that the STG result may also reflect semantic, attentional, or perceptual processing of food category differences.

We were also surprised by the STG finding, but as it was whole brain corrected and our statistical analysis plan includes whole-brain corrected and ROI analyses, we reported it. We agree that the superior temporal gyrus (STG) is not a region traditionally implicated in interoceptive, metabolic, or motivational processing. In light of this point, brought up by 2 reviewers, we have substantially revised our language in the discussion. It now reads: “Beyond striatal regions, processing-related individual differences in carbohydrate oxidation were also associated with responses in the superior temporal gyrus (STG). While primarily known for its role in auditory and language processing, the STG is increasingly recognized as a hub for multisensory integration (Scheliga et al., 2023) and is responsive to food cues (St-Onge et al., 2005; G.-J. Wang et al., 2004). More work is needed to determine if the STG is involved in food specific multisensory integration, including interoceptive metabolic signals.”

9. The authors should report whether UPF > non-UPF cue reactivity was tested independently

The paper states that the contrast UPF > non-UPF did not yield significant voxels for the value analysis. It would be useful to also state whether the simple food-cue reactivity contrast UPF > non-UPF, independent of metabolic covariates, showed any effects. This would help distinguish whether UPF cues generally engage reward/visual regions more strongly, or whether the main findings are specifically driven by individual differences in metabolic response and value coding.

On a similar note, the authors state that the UPF and non-UPF picture sets were matched on nine visual properties, including pixel colour, object size, and brightness. However, it is unclear whether visual complexity was matched or quantified. Given that key value-related effects emerged in occipitotemporal regions, including fusiform and lingual gyri, the authors should report whether the stimulus categories differed in visual complexity, for example edge density, entropy, spatial frequency, clutter, or number of objects. If these measures were not assessed, this should be acknowledged as a limitation, and the interpretation of occipitotemporal effects should be tempered.

We agree these factors are important, especially in interpreting our visual cortex findings. In our pictures, we measured red, green, blue, object size, brightness, within object contrasts, complexity, normed complexity, and spatial frequencies of the two image sets. They did not statistically differ on any of these factors; we now provide the bar plots for these measures as Supplemental Figure 1.

In response to this comment and that of Reviewer 1, we sought to more stringently adjust for potential confounds from the picture set. We therefore took object size and complexity (two higher-order visual variables) and entered them as a parametric modulator in our analyses to more robustly adjust our analysis. It did not substantially change the results and is now included in Supplemental Table 5.

10. Clarify whether the fMRI picture stimuli and metabolic meals were sufficiently aligned

The metabolic meals were derived from a subset of the 28 food images, while the fMRI task used all 28 images. This is reasonable, but the authors should clarify whether the fMRI trials corresponding to the exact foods consumed in the metabolic sessions show similar effects to the full stimulus set.

Thank you for the opportunity to clarify. The UPF meal contained 5 items from the picture set and the non-UPF meal contained 3 items from the picture set. We did not seek to test if the metabolic response to each specific food item was associated with different neural activity but rather that responses to the class of foods would correlate with non-UPF vs UPF foods differently. We also sought to balance feasibility, consuming many different foods on different days to completely match what is needed for an fMRI design would greatly increase participant burden. Design efficiency and power for the fMRI design was calculated based on all 28 items, we therefore did not test if inclusion of only 8 items produced similar results.

11. The authors appropriately acknowledge that total protein, but not protein type, was matched; that the UPF meal contained more additives; that eating rate differed modestly; and that additional meal pairings are needed to establish generalizability. However, these limitations should be given greater weight in the interpretation. The current design demonstrates that two meals matched for conventional nutrient composition but differing in processing-related formulation and structure elicit distinct metabolic responses. It does not isolate NOVA processing per se from differences in food matrix, ingredient source, protein type, additive profile, texture, oral processing, bolus formation, or digestion kinetics. The Discussion should therefore more explicitly temper causal statements attributing the effects to “processing level” and instead frame the findings as effects of a nutrient-matched UPF versus non-UPF meal comparison.

Thank you for this point, we have revised the discussion and limitations to reflect this. Though, we do point out that some factors such as additives and changes in the food matrix are inherent in Nova 4 items. We include a discussion of digestion kinetics in the interpretation of our results.

Minor comments

In the abstract, the phrase “mesolimbic and superior temporal gyrus activation” could be more precise. Consider “striatal and superior temporal gyrus responses” unless the authors want to define mesolimbic regions explicitly.

We agree and this has been fixed.

The use of peak metabolic response is interesting, but AUC may be more robust. The authors should justify why peak was selected for brain associations.

While AUC is a common summary measure of metabolic response, we chose to examine first local peak as it better addresses the question that magnitude of metabolic response is associated with brain response. A response can differ in magnitude (height)

and shape substantially but still produce the same AUC. For this reason, we chose peak rather than AUC.

The authors should report the exact n for each brain–metabolism association in the Results and figure legends.

We agree and this has been included.

We wish to thank the editor and all three reviewers for their comments. In general, reviewers recognized the importance of these findings within the context of better understanding the effects of ultra-processed foods. We greatly appreciate the thorough and fair review of our manuscript. We believe the additional analyses and changes to the text have greatly improved the manuscript.

Dear Dr. Nakhle and Reviewers,

Thank you for your time and attention to our manuscript. We have addressed the additional comments from the reviewers; there were no additional editorial comments. We have edited our interpretation in response to Rev 1 and provided clarifying information in response to Rev 1 and 3. In this letter, we reproduce the Reviewer comments in plain text, and our response follows in *italics*:

Reviewer #1 (Remarks to the Author):

The authors did a commendable effort to address all of my points from the earlier review. Particularly, the major methodological concerns have been addressed; additional analyses have been performed, and interpretations have been softened. This relates especially to the interpretation of the imaging findings.

Thank you for your comments and recognition of the substantial work for the revision; it has improved the manuscript.

However, after reading the revised manuscript, I remain somewhat unconvinced about its mechanistic depth and biological implications, particularly given Nature Metabolism's scope. And I now have a statistical concern (see specific remarks below).

Collectively, the manuscript clearly demonstrates that nutrient-matched UPF and non-UPF meals elicit distinct acute metabolic responses. It furthermore identifies associations between these metabolic measures and neural responses to food cues. However, the central link between these observations remains largely correlational. In particular, it still remains unclear why carbohydrate oxidation emerges as the relevant metabolic correlate (whereas other altered physiological measures, such as insulin, glucose, or metabolic rate, do not). Although the manuscript now much better discusses prior work implicating post-ingestive signalling and striatal reinforcement, the present findings are not yet integrated into a coherent biological framework that explains the specificity of the observed associations.

Throughout the Discussion, the authors appropriately acknowledge that additional experiments are required to establish the functional significance of several neural findings. This caution is appreciated, but it also underscores that the manuscript primarily identifies novel associations rather than providing mechanistic insight into how altered food processing leads to altered neural responses. Given the Journal's scope, I would have liked to see a more substantial discussion of the potential biological mechanisms underlying these observations.

We appreciate this point and have added additional discussion of mechanism of gut-brain signaling on page 9: "These metabolic differences may also shape neural responses to food cues. Post-ingestive metabolic signals are a key reinforcing stimulus linking orosensory food cues to their nutritional value (De Araujo et al., 2008; Myers,

2018; Sclafani & Ackroff, 2012), and these signals are thought to be integrated in the striatum (both dorsal and ventral) and guide eating behavior (de Araujo et al., 2020; Weber et al., 2025). This gut to brain signaling is thought to be macronutrient specific, with fat signaling through PPAR-alpha (Peroxisome Proliferator-Activated Receptor Alpha) receptors on the vagus nerve (Han et al., 2016). While, carbohydrate sensing has multiple proposed mechanisms including activation of the sodium glucose transporter (Sclafani & Ackroff, 2012), vagal and spinal afferent dependent pathways (Fernandes et al., 2020; Goldstein et al., 2021), and an unknown carbohydrate oxidation sensor (Tellez et al., 2013). Insulin secretion is not macronutrient specific and can be evoked by carbohydrates and proteins. In animal models there is some evidence it influences food preference (Stouffer et al., 2015), and in humans intranasal inulin increases response to sweet tastes (Wingrove et al., 2022) and that response is correlated with circulating insulin (Dalenberg et al., 2020). While we observed differences in multiple metabolic measures, our data provide additional evidence for the importance of carbohydrate oxidation in conveying nutrient information to the brain.”

I do not think additional experiments are necessarily required at this stage, nor do I think the current interpretations are overstated (given that the authors can still demonstrate statistical rigour, see below). Rather, I believe the manuscript would benefit from a more thoughtful discussion of what the present data can and cannot reveal mechanistically, and from a clearer articulation of why the observed metabolic-neural associations are biologically meaningful beyond their statistical significance.

Some specific remarks:

1. Regarding mechanistic depth or biological implications, the paper is observational despite its experimental elegance.

The key conclusions remain correlational: metabolism correlated with brain activity rather than metabolism causing changes in brain activity.

The paper essentially establishes three observations: nutrient-matched UPF and non-UPF meals elicit different metabolic responses; metabolic responses correlate with certain fMRI measures; value-related activity differs between UPF and non-UPF meals.

Each of these findings is interesting, but they are not integrated. Especially the neuroscience part, however, feels more like an add-on than the central mechanistic advance. It identifies intriguing, hypothesis-generating associations but doesn't yet explain how altered food processing translates into altered brain function. If the imaging component were removed, the metabolism paper would still tell a coherent story. The converse is less true.

In the bigger picture, why carbohydrate oxidation? And not insulin (changed), glucose (changed modestly), metabolic rate (changed). The Discussion never answers what is special about carbohydrate oxidation. Yet only carbohydrate oxidation predicts fMRI. Why?

To that point, the authors simply state that rodent work implicates carbohydrate oxidation, which is true, but a much deeper discussion of why carbohydrate oxidation might uniquely index nutrient availability compared with insulin or glucose is needed.

There are very few tools to manipulate the brain in humans and as such most fMRI studies are by nature correlational. Thank you for the assessment of our experimental design. We believe our experimental design allows us to make inferences about a potential biological mechanism, specifically altered post-ingestive signals driven by altered nutrient availability that are relayed to the brain. We now include a discussion of these signals and additional support for carbohydrate oxidation on page 9 in response to this and the above comments.

2. Regarding the willingness to pay paradigm:

It is reported that there is no difference in behaviour, yet there is a difference in neural encoding. The author's report just says that there are neural representations despite similar behaviour. and that there are "follow-up experiments needed". This sounds like an escape argument.

If the behavioural endpoint is negative, interpreting interaction effects in BOLD is left without a behavioural anchor. That's not wrong, but it's inherently speculative unless there are further arguments.

This leads to a statistical concern: $n = 32$

The overall sample size was estimated based on behavioural data from an earlier study. While there is nothing wrong with this, the effective sample was now less than estimated ($n=52$), and thus the absence of effect in the behavioural data becomes an issue - it can certainly not be interpreted in a way that the effect is indeed absent. Unfortunately. The lack of behaviour despite neuronal representations is an interesting finding that has often been observed (and not really explained): brain responses to food cues (or other appetitive cues) are sustained longer than the behavioural effect lasts.

But anyway, for the following correlation analyses, this is a very small sample, especially given the whole-brain analyses with several metabolic variables and covariate analyses. What remains is the risk of being 'just' a collection of significant associations from a relatively small imaging cohort.

The authors need to discuss robustness and reproducibility in light of the actual effect sizes from their data.

Thank you for these two points.

First, it is true, fMRI effects are often found without strong behavioral effects. We now discuss possible interpretations of these effects on lines in the discussion on page 10. We have removed the phrase about follow up experiments from the results section.

Second, we would like to clarify our power analyses and sample sizes. We did base our power analyses on previously published behavioral findings with a Cohen's f of 0.40. For our analysis, as stated, we needed 52 participants to be appropriately powered. We report findings from 52 participants for both the behavioral willingness to pay analysis and fMRI willingness to pay analysis (see CONSORT diagram).

For the metabolic outcomes, using previously published data, we estimated a Cohen's d of 0.57 and a needed sample size of 27. We report data from 32 participants (CONSORT).

For the correlation between the metabolic data and brain response, using previously published results of $r=0.83$, we needed 16 participants for an alpha of 0.0001. We report data from 29 participants for that analysis (CONSORT).

More details on the power analyses performed are in the power analysis section of the manuscript and reproduced for convenience below. For all of our analyses we report participants numbers either equal to or greater than what was estimated by our power analyses based on previously published effect sizes.

Reproduction of power section:

“Power analysis. Power analysis (Behavior). The overall sample size was estimated based on behavioral data from (DiFeliceantonio et al., 2018). Sample size was calculated in R (Bunn et al., 2007) based on a repeated measures ANOVA model, and detecting differences in willingness to pay (bid amount in USD) among the degree of food processing condition groups (Zhang et al., 2023). With a total sample size of 52 participants, pairwise comparisons among the two groups yielded 80% power to detect an effect size of Cohen's $f=0.40$ with a type I error rate of 5%. Based on this total sample size of 52, power was estimated for a generalized linear mixed-effects model, regressing the primary outcome (willingness to pay) on food processing, sex, their two-way interaction, and controlling for potential confounders (hunger, energy expenditure, blood glucose, blood insulin). This was accomplished using 5,000 iterations of a Monte Carlo simulation, expected assumptions were based on the bidding data published in DiFeliceantonio et al. (DiFeliceantonio et al., 2018). In these simulations, the model parameters were estimated via restricted maximum likelihood, such that the specified model was compared to the null model using the likelihood ratio test, yielding 99% power to detect a statistically significant test at the 5% level of significance. In summary, a sample size of 52 participants provides sufficient power to detect a main effect difference in willingness to pay by food processing group. **Power analysis (Associations between metabolism and fMRI).** An ad hoc power analysis was conducted to ensure power was achieved for the subset of participants in the correlations between metabolic with brain responses. In the literature, the most relevant data are from Veldhuizen et al 2017, who reported a correlation of $r=.83$, which is much larger than the effect sizes used to power the study (Veldhuizen et al., 2017). We performed a power analysis

based on a test of Pearson's correlation with that value. To achieve 95% power, 16 participants were needed at a significance level of .0001. Power analysis (Metabolism). A separate power analysis was conducted to evaluate the sample size needed to detect a difference in metabolic response to the meals consumed in the whole room indirect calorimeter. There was over 80% power to detect a difference, based on a paired t-test, in the AUC for energy expenditure, assuming a Cohen's d of .57 (medium size) and sample size of 27."

3. Regarding the fMRI findings in visual association areas (an issue which was addressed in the prior review). The embedding is now much better, but given the vast extent of the activation areas, simply pointing towards a "fusiform food area" is not appropriate. In various fMRI tasks, we find neuronal responses to value modulation in the visual system. This is almost ubiquitous, likely reflecting familiarity or experience with certain cues, and is dynamically adjusted based on the motivational significance or behavioural relevance of a visual stimulus. These findings still need to be discussed more fully in light of system interactions (lightly).

Thank you for this point. We report a peak level correction, but for increased transparency, we display all voxels with an uncorrected $p < 0.001$. This means the displayed cluster is larger than the significant peak. The significant peak is within the fusiform gyrus, which is what we report and interpret.

4. More concern relates to the somewhat heavy use of "further experiments are needed". This seems to reflect that the manuscript repeatedly reaches the boundary of what the data can support. The authors are appropriately cautious, but caution also highlights that the central biological mechanism needs more plausibility than is demonstrated. That leaves the paper feeling like an important first step rather than a definitive mechanistic advance.

We have removed all but one instance in favor of more appropriate interpretations. Given the complexity of the topic, we feel reaching the bounds of what is known and what can be interpreted is an important contribution to the literature. In response to this and the above comments, we have expanded the discussion of the potential underlying mechanisms.

Reviewer #2 (Remarks to the Author):

The authors have addressed all my concerns. The manuscript is now in a condition to be published.

Thank you for the careful evaluation of our manuscript and for the positive assessment of the revised version.

Reviewer #3 (Remarks to the Author):

The authors have addressed most of my concerns in a satisfactory manner. Furthermore, the authors now clarify that the fMRI association analyses were corrected using whole-brain FWE correction or small-volume FWE correction within the a priori striatal mask. However, it remains unclear in the revised manuscript how multiplicity across the several metabolic predictors was handled. The authors tested carbohydrate oxidation, RER, metabolic rate, fat oxidation, blood glucose, and blood insulin, but no correction across these predictor-level tests appears to have been applied. If carbohydrate oxidation was the primary hypothesis-driven predictor, this should be stated explicitly. Otherwise, the metabolism–brain association analyses should be framed as exploratory, or the authors should justify why no additional correction across metabolic predictors was applied.

Thank you for this important point. Our intent was to evaluate several a priori physiological outcomes rather than conduct an exploratory search across metabolic measures. Accordingly, we used whole-brain and small-volume FWE correction for each imaging analysis but did not apply an additional correction across metabolic measures, consistent with prior metabolism–brain association studies (de Araujo et al., 2013; Veldhuizen et al., 2017). We have now clarified this in the Methods (page 19).

We wish to thank the editor and all three reviewers for their comments. Overall, reviewers found us responsive to review and incorporation of these additional comments has improved the manuscript.