nature portfolio

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Last updated by author(s): Apr 16, 2023

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	The online randomization tool available on http://www.graphpad.com/quickcalcs/index.cfm was used for the randomization and allocation procedures.
Data analysis	fMRI data were preprocessed using FMRIPREP v1.2.3. Subject level analysis was performed using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL 6.0, Oxford, UK). Whole-brain voxel-wise analysis was performed using Permutation Analysis of Linear Models (PALM) v.alpha116. Freesurfer (version 5.3.0) was used to obtain ROI masks from individual T1w MRI scans. Statistical analyses were performed using IBM SPSS Statistics v26 (Armonk, NY, USA) and R v3.6.1. Computer codes used for data analyses will be published in on the GitHub platform: https://github.com/MJMSerlie/SPIN-Study.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Individual data (subject characteristics) were entered in a Castor database; laboratory results were entered into a hospital based electronic record (EPIC).
Masks of ROIs were obtained from the Harvard-Oxford subcortical atlas (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases). Subject level analysis was performed using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL 6.0, Oxford, UK; http://www.fmrib.ox.ac.uk/fsl).
Free access to the individual data is restricted due to ethical/legal concerns. However, upon request the data may be made available for scientific collaborations after the execution of appropriate data sharing agreements, after review and approval of requests by the medical ethics committee, participants and investigators in line with existing local/national regulations and data sharing agreements. Requests can be send to the corresponding author (Mireille J. Serlie - mireille.serlie@yale.edu). A first response to requests will follow within 4 weeks.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	We included female and male subjects. The study has not enough statistical power to perform a valid analysis between sexes. To minimize sex differences with regard to the effect of sex hormones, we only included postmenopausal women. We did not collect any gender data.
Population characteristics	Shown in detail in Table 1.
Recruitment	Participants were recruited from the general population in the Amsterdam metropolitan area via local advertisements. There was no self-selection bias regarding inclusion of participants or study design. Subjects included in this trial might not be representative for the general population, subjects < 50 years (due to the restriction of exposing healthy and younger subjects to procedures involving radiation) and premenopausal women because of strict exclusion criteria.
Ethics oversight	The protocol was approved by the Academic Medical Center medical ethics committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	There has been substantial debate in the field regarding the power and statistical analysis of fMRI data. Between-group analyses are complicated by the large inter-subject variability in brain activity assessed by fMRI. The between-subject variability is caused by a mixture of random and deterministic or structured factors that are not easily studied and hard to predict. This unpredictable inter-subject variability complicates sample size calculation for fMRI studies. Therefore, the necessary sample size to reliably evaluate intervention effects on brain activity between groups has been an active topic of research, and conventional sample size calculations are no longer recommended for studies where fMRI outcomes are a primary objective. One analysis suggested that functional neuroimaging studies should include at least 27 subjects per treatment group to have sufficient power for inter-group analyses. Another study performed subsample analyses (consisting of a variable number of subjects selected from the total population of 130 subjects) to determine the effect of sample size on the precision of task outcomes. This showed that by increasing the sample size from 20 to 30 (i.e. selected from the total population), the precision of the outcome estimation increased substantially: outcomes in n = 30 subsamples correlated 95% with outcomes in the total population, and this was associated with a variance of 0.0002 in the outcome between different compositions of the 30/120 subsample. On the basis of these insights, in order to reliably assess inter-group differences in the effects of nutrients on brain activity, we intended to include 30 subjects per group: 30 lean and 30 subjects with obesity. However, since then new reports showed that this number of subjects is sufficient to reliably assess within-subject differences, but not between-subject differences. We therefore assessed the within-group effects of intragastric glucose and lipids in the lean subjects and in the subjects with obesity separately.

The main aim of the SPECT scan procedures in lean subjects was to compare the nutrient (glucose and Intralipid) effects on striatal dopamine release. We expected both glucose and intralipid to induce striatal dopamine release. No previous study assessed the effect of nutrient infusion on striatal IBZM BPnd (measure of dopamine release). Consumption of a meal resulted in a 12.4% decrease of [11C]raclopride BPnd in the putamen. Using this effect size, a sample size of n=27 with a power of 80% (paired t-test) was needed. Since the radiotracer [11C]raclopride is slightly more sensitive to fluctuations in endogenous dopamine (due to a lower affinity for the dopamine receptor), we included a total of 30 lean subjects.

The main aim of the SPECT scan procedures in the subjects with obesity was to assess the effect of dietary weight loss on dopamine release induced by glucose or Intralipid. To our knowledge this has not been studied previously. Therefore we used study results from another study from our group with a similar population for this sample size calculation. Weight loss in that study induced a 15-16% increase in striatal BPnd. The standard deviation of the difference in BPnd before and after weight loss was 0.087 (arbitrary units). It follows that a sample size of n=15 has a power of 80% to find a difference in BPnd of 0.068 (arbitrary units). This translates to a difference of ±10% in basal BPnd. We considered this difference to be clinically relevant. We therefore included n=15 subjects with obesity per nutrient infusion group (i.e. 15 subjects for glucose infusion and 15 subjects for Intralipid infusion).

Data exclusions

Exclusion criteria were predefined, for details we refer to our method section.
Primarily due to the invasive nature of some of our study procedures, we have some missing data. Two lean subjects dropped out during the first fMRI session, before any of the SPECT study days. One lean subject and one subject with obesity completed only one fMRI session, but did complete all SPECT study days. In two subjects with obesity, the pre-diet, lipid fMRI session was interrupted and excluded from the analysis. One lean subject did not undergo the lipid SPECT session, whereas another lean subject did not undergo the glucose SPECT session. One subject with obesity did not continue with the diet intervention. Three subjects with obesity did not achieve >5% weight loss during the hypocaloric diet intervention and did not participate in post-diet study procedures. The post-diet, lipid fMRI session was interrupted in one subject and the glucose session in another. Finally, one subject's post-diet lipid SPECT session could not be completed due to technical failure of the scanner. For some subjects the sampling of blood during the fMRI session did not succeed due to failure of the iv cannula. We refer to the precise 'n' values in Figure 3.
Therefore, fMRI data could be analyzed for 27 lean subjects (both glucose and lipid) for 29 and 27 pre-diet subjects with obesity (glucose and lipid, resp.), and for 25 and 25 post-diet subjects (glucose and lipid, resp.). The SPECT data could be analyzed for 27 lean subjects (both glucose and lipid), for 30 pre-diet subjects with obesity (15 glucose and 15 lipid), and for 14 and 11 post-diet subjects (glucose and lipid, resp.). For an overview, we refer to Figure 2 (participant flow chart).

Replication

No replication was done. Due to the invasive nature of the study procedures (i.e. insertion of a nasogastric tube, radiation exposure) it was not considered ethical to increase the number of study procedures per participant any further.

Randomization

The order of the infusions for the fMRI study days was randomized and stratified by group. Subjects with obesity, were randomized to receive either intragastric glucose (n=15) or lipids (n=15) during SPECT study days. All randomization and allocation procedures were performed using the GraphPad QuickCalcs website.

Blinding

Participants were blinded for the type of intragastric infusion they received.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

Trial registration: Netherlands Trial Registry NTR7042

Study protocol

Included with submission.

Data collection

A pilot fMRI was performed in one subject who underwent nasogastric tube placement with infusion of lipids to assess feasibility and optimize the fMRI protocol. This subject was enrolled on December 13 2017. The data of this subject is not included in the analysis. Thirty subjects with a healthy body weight and 30 subjects with obesity were recruited from the general population in the

Amsterdam metropolitan area. These subjects were enrolled from March 15 2018 to November 22 2019. Data of these subjects was collected from March 30 2018 to March 4 2020. All data was collected at the Amsterdam UMC, location AMC, in Amsterdam.

Outcomes

Primary study outcomes were the effects of the intragastric nutrient infusions on cerebral neuronal activity and striatal dopamine release. Secondary study outcomes were the effects of the intragastric nutrient infusions on: glucoregulatory and gut hormone release and objective and subjective hunger scores.
Lean subjects underwent three fMRI sessions on three separate study days to assess the effects of glucose, lipids, and tap water on cerebral neuronal activity. Subjects with obesity underwent three fMRI study days before the start of a 12-week hypocaloric diet intervention and three fMRI study days after completion of this diet intervention to assess the effect of diet-induced body weight loss.
In addition, all subjects underwent two [123I]IBZM SPECT study days to assess the post-ingestive nutrient effects of glucose and lipids on the striatal dopamine system. In lean subjects, in random order, the effect of glucose was assessed during one SPECT study day and the effect of lipids during the other SPECT day. Due to radiation exposure, the number of SPECT study days a subject is allowed to undergo is limited to two. Therefore, in subjects with obesity, the effect of either glucose (n=15) or lipids (n=15) on the striatal dopamine system was assessed both before and after the diet.
During the fMRI session, blood was sampled at baseline and at 5, 10, 15, 20, and 30 minutes after the start of the intra-gastric infusion. At all timepoints, plasma glucose was determined. At baseline, t=15, and t=30 plasma insulin and GLP-1 concentrations were determined. At baseline and t=30, concentrations of plasma acylated ghrelin were determined.
Subjects were asked to rate their feeling of hunger on a visual analogue scale (VAS) with a range from 0 to 10 before the start of the fMRI and shortly after the fMRI was finished. Twenty minutes after the removal of the nasogastric tube and i.v. cannula, subjects received a meal, consisting of a bowl of yoghurt (isocaloric vanilla or natural) mixed with muesli, and were asked to eat until satiated. The food was weighed before and after consuming the meal and the caloric intake was calculated as caloric content after versus before the meal.

Magnetic resonance imaging

Experimental design

Design type

Event-related (response to intra-gastric infusions).

Design specifications

During the functional brain scans, baseline activity was measured for eight minutes, after which the intragastric infusion of either glucose, lipids, or tap water was administered in five minutes. Imaging continued for another 27 minutes. Subjects had one fMRI session per day.

Behavioral performance measures

No behavioral performance measures were recorded during the functional brain scans.

Acquisition

Imaging type(s)

Functional.

Field strength

3T

Sequence & imaging parameters

TR/TE = 7.0/3.2ms; FOV = 256x240x180mm; voxel size = 1x1x1mm. fMRI was acquired using a gradient echo planar imaging (EPI) sequence with the following scan parameters: TR/TE = 1700/35ms; FOV = 216x216x124mm; voxel size = 2.7x2.7x2.7mm; 1410 dynamics; MB factor = 2; SENSE= 1.7; scan duration = 40 minutes.

Area of acquisition

Whole-brain.

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software

fMRI data were preprocessed using FMRIPREP v1.2.3 [RRID: SCR_016216]. Preprocessing of the functional scans included motion correction (FLIRT), distortion correction (3dQwarp), and co-registration to the anatomical T1-weighted scans. The functional scans were then non-aggressively denoised using independent component analysis (ICA) based Automatic Removal Of Motion Artifacts (AROMA) and spatially smoothed (6mm FWHM). For details see supplementary materials.

Normalization

Scans were spatially normalized to MNI space through nonlinear registration.

Normalization template

MNI space.

Noise and artifact removal

fMRI data were preprocessed using FMRIPREP v1.2.3[RRID: SCR_016216]. Anatomical T1-weighted scans were corrected for intensity non-uniformity, skull-stripped and spatially normalized to MNI space through nonlinear registration. In the brain-extracted T1-weighted image, brain tissue was segmented in CSF, white-matter (WM) and gray-matter (GM). Functional scans were corrected for motion (FLIRT) and distortion (TOPUP technique using 3dQwarp), and were co-registered to individual anatomical T1-weighted scans (boundary-based registration cost-function, 9 degrees of freedom). The motion correcting transformations, field distortion correcting warps, BOLD-to-T1-weighted transformations, and T1-weighted-to-MNI warp were performed with a single interpolation step using antsApplyTransforms (ANTs v2.1.0) with Lanczos interpolation. Independent component analysis (ICA) based on Automatic Removal Of Motion Artifacts (AROMA) was applied for non-aggressive denoising of the functional data. Finally, the functional data were spatially smoothened (6 mm full-width half-maximum).

Volume censoring | The first three volumes from each functional scan were removed.

Statistical modeling & inference

Model type and settings | Subject level analysis was performed using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL 6.0, Oxford, UK; http://www.fmrib.ox.ac.uk/fsl). The first three volumes from each functional scan were removed. The remaining 1407 volumes were divided over 14 consecutive time bins: T0 (baseline, i.e. the 5 minutes before the start of the intragastric infusion) and T1-T13 (each including consecutive 2-2.5-minute intervals, with T1 beginning directly at the start of the intragastric infusion). First level analysis was applied with use of FMRI Expert Analysis Tool (FEAT) to compare T1-T13 with baseline (T0) within every functional brain scan. The time course of the BOLD signal of the cerebrospinal fluid (CSF) of each scan was extracted and included as covariate to adjust for general, non-infusion related changes in BOLD signal. Next, for each subject, the maps of T1-T13 of the water infusion session were subtracted from the maps of T1-T13 of the glucose and lipids infusion session. For each subject, this resulted in 13 maps for the glucose infusion and the lipid infusion that reflected the percentage change in BOLD signal from baseline (T0) for each time bin (T1-T13) for glucose and lipids, corrected for the effects of the infusion of tap water. These values were used as input for the group level analyses: the explorative whole-brain voxel-wise analysis and the ROI analysis.

Effect(s) tested | Whole-brain voxel-wise analysis - Clusters of grey-matter voxels that showed a significant increase or decrease from baseline (T0) for each time bin (T1-T13) were identified using Threshold Free Cluster Enhancement (TFCE) and permutation testing using Permutation Analysis of Linear Models (PALM) v.alpha116. Faster permutation inference was applied by fitting a generalized Pareto distribution to the tail of the permutation distribution, with 5000 permutations. The PALM options –corrcon and –corrmod were applied to perform FWER correction for multiple testing over the multiple contrasts and time bins, respectively. A FWER-corrected p-value (pFWER) <0.05 was considered significant. For clusters with a significant change in BOLD signal the locations of the peak and up to five local maxima (minimum distance 20mm) were interpreted using the Harvard-Oxford (sub)cortical atlas (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases). As within-subject designs are more powerful than between-subject design, this analysis was performed to assess the within-group effects of intragastric glucose and lipids in the lean subjects and in the subjects with obesity, pre-diet, separately. To evaluate the effect of the diet-intervention, the voxel-wise analysis was performed on the post-diet data after subtraction of the pre-diet data for each subject.

ROI analysis - Effects of the nutrient infusions on the striatal subregions the NAc, caudate nucleus, and putamen were assessed with a ROI analysis. Masks of these regions were obtained from the Harvard-Oxford subcortical atlas (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) with a threshold of 30%. The mean change (%) in BOLD signal from baseline (T0) was extracted for each time bin (T1-T13) for each ROI. To limit the number of comparisons, only at T3 (pre-absorption), T8 (early-absorption) and T12 (late-absorption) the significance of the change from baseline was evaluated by one-sample t-tests in lean subjects and subjects with obesity pre-diet. In addition, the effect of the diet-intervention was evaluated by comparing the change from baseline for these time bins (T3, T8 and T12) between the pre and post-diet condition by paired t-tests.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☒ Both

Anatomical location(s) | For clusters with a significant change in BOLD signal the locations of the peak and up to five local maxima (minimum distance 20mm) were interpreted using the Harvard-Oxford (sub)cortical atlas (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases).

ROI masks were obtained from the Harvard-Oxford subcortical atlas (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) with a threshold of 30%.

Statistic type for inference (See Eklund et al. 2016) | Clusters of grey-matter voxels that showed a significant increase or decrease from baseline (T0) for each time bin (T1-T13) were identified using Threshold Free Cluster Enhancement (TFCE) and permutation testing using Permutation Analysis of Linear Models (PALM) v.alpha116. Faster permutation inference was applied by fitting a generalized Pareto distribution to the tail of the permutation distribution, with 5000 permutations.

Correction | The PALM options –corrcon and –corrmod were applied to perform FWER correction for multiple testing over the multiple contrasts and time bins, respectively [61]. A FWER-corrected p-value (pFWER) <0.05 was considered significant.

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis