Official Title of the study: The Omnibus Satiety Metric (OmniSaM)
Date of the document: 1 March 2019

Full study protocol and statistical analysis plan

Contents

1. Participant Flow
Recruitment Details. Participants were recruited via convenience sample via the website www.forsøgsperson.dk **.** The recruitment started oct 2017 and lasted around approx. 16months.
Pre-assignment Details. Via a phone meeting we first assess if the volunteers fulfill the inclusion-exclusion criteria. Those who meet the initial criteria for inclusion will participate in an information meeting where information about procedures will be delivered and participants can ask questions. Those who agree to participate in the study will give written consent, deliver a blood sample to be analyze for: hemoglobin, liver and kidney function and inflammatory state, and weight, height and blood pressure will be measured, to ensure the recruited people meet all the inclusion and exclusion criteria. These criteria are listed in the main clinical trials document.
Enrollment (total, anticipatory). Total whom started the study 28; Anticipated completion of all four test days 25; Fully completion of all four test days 23; Excluded from analyses 5
Allocation (randomized). This is a single arm study in which all conditions of a 2x2 factorial design and allocated to the same group in a randomized order.
Follow-up (immediately after intervention). There is no follow up.
Arm/Group Information. There is 1 arm in the study.
Arm/Group Description. All participants in this single arm study undergo a dietary intervention, comprising the 2x2 factorial design in which they are tested under four conditions in which only the dietary preload changes.
1. high protein2carb high calorie
2. high protein2carb low calorie
3. low protein2carb high calorie
4. low protein2carb low calorie

Period(s). There is only one stage in the study, comprising the four test days, one for each of the experimental conditions
Period Title. Overall Study.
Started. 28 started the study.
Completed. 23 finished the study.
Milestone Title. There are no follow ups so therefore no milestones.
Reason Not Completed Type. The reasons for not completing the study included only the following, and were fully recorded: Metal thread behind front teeth (1); Excluded by project staff due to frivolous attitude towards the study (1); Physiological hindrance of drawing blood from subject (1); Blood sampling not possible (2); Personal issues without the possibility of rescheduling (1)

2. Baseline Characteristics
Arm/Group Information. Baseline characteristics will be computed in the form of standard summary statistics (means, standard deviations, Bayesian credibility intervals)
Baseline Measure Information. A group of demographic characteristics will be measured in the study, including: age; gender; weight; height; educational level; income;
Baseline Measure Title. Age: Continuous (years)**;** Gender: Categorical (female, male); Weight: Continuous (kg); Height Continuous (meter); Educational level: Categorical (lower secondary, higher secondary, higher secondary with trainee, short-length higher education, medium-length higher education, long-length higher education); Income: Categorical (< 100.000 Dkkr, 100.000-199.999 Dkkr, 200.000-299.999 Dkkr, 300.000-399.999 Dkkr, 400.000-599.999 Dkkr, 600.000-799.999 Dkkr, ≥ 800.000 Dkkr, do not wish to inform)
Measure of Dispersion. Standard Deviation**,** Interquartile ranges**,** Range

3. Outcome Measures
Outcome Measure Information. The primary outcome is the energy consumed in the ad libitum meal.
Outcome Measure Type. Primary
Outcome Measure Title. Energy consumed
Outcome Measure unit. kJ
Outcome Measure Time Frame. There will be four such measurements per participant, one for each condition. These are taken at the end of the experimental testing day.
Overall Number of Participants Analyzed. There will be data analysed from 23 participants.
Measure Type. Means
Measure of Dispersion/Precision. Standard Deviation and Bayesian central credibility intervals
Outcome Data. The measurement value(s) for each outcome measure cannot obtain right now since data collection has not finished yet.
Comparison Group Selection. There is only 1 group.

Statistical Testing.
Statistical modelling of multimodal metric
A Bayesian linear regression will be implemented in JASP software, with the primary outcome of energy consumption as the dependent variable. The regressor variables will be variables derived from all three data modalities (described below). This regression model computes all possible combinations of regressors, with each combination specified as a separate model and assigned an equal prior probability. The regressors to be tested are defined operationally as follows: we compute a Principal component analysis (PCA) to identify the components within the data necessary to explain 95% of the variance in the data, with the PCA performed independently for the three modalities of data, brain, blood and behavior. For all of these different models, Bayes Factors can be computed comparing each model against the best model, or alternatively against the null model. The null model is taken to include all covariates not included in the neuroimaging data, subjective report data, or endocrine and metabolite data. Inclusion probabilities will be computed for all regressors, which allows us to infer Bayes factors that indicate the evidence for models including each regressor against all models that do not. This indicates how much the data favors the inclusion of the regressor in the best model. Inclusion probabilities and their associated Bayes factors will be computed for all regressors and their groupings into the three data modalities. This affords inference into the how much the data favors the inclusion of a particular data modality. Bayes factors will also be computed between data modalities, to evaluate the relative importance of each modality in predicting energy consumption. The regressors can also be grouped according to timepoint, affording comparison of inclusion probabilities between distant and proximal times to the point of consumption.

Statistical modelling of subjective measures
In modelling the subjective reports of appetitive sensations we will perform the following procedures: To address the question of how well subjective measures predict energy intake we will perform a Partial Least Squares analyses, to find the multidimensional direction in appetite sensation space, that explains the maximum variance in energy intake space. To isolate the subset of subjective factors that are best predictive of energy intake we will perform a Bayesian Linear regression with subjective reports and time as regressors, and with energy expenditure, weight and BMI as covariates. To address the question of between preload effects on subjective reports of hedonic- and appetite ratings and energy intake, and the discriminability of the different appetite ratings, we will perform a repeated measures 2-way ANOVA, with both preload dimensions and time as independent/explanatory variables and hedonic, appetite and intake data as dependent/response variables. The repeated-measure factor will be the within-subject factor. Post hoc pairwise multiple-comparisons will be used as follow-up tests to compare every group mean.

Statistical modelling of neural measures
The aim of neuroimaging analysis is to delineate the interoceptive neural systems underpinning satiety, and its interface with behavioral and endocrine systems. Broadly we hypothesise that tonic & food cue-evoked activity in viscerosensory & visceromotor networks, will be predictive of next-meal energy intake, specifically in Hypothalamus & Insula and more broadly in regions of interest: ACC, OFC, SGC, PBN, NTS, amygdala, striatum. We will engage in exploratory data analysis by mapping the neural modulations caused by post-prandial endocrine dynamics. Finally we will extract key features of data from regions of interest to assess how neural activity can predict energy intake. For the functional neuroimaging data, a multiple linear regression model will be constructed via the software Statistical Parametric Mapping. The onset of food and object images will be modelled as stimulus onsets using standard methods, convolving stick functions representing stimulus onsets with hemodynamic response functions. Regressors of interest will include food images classified according to high or low energy density, and objects. Resting state data will be modelled according to standardized pipelines. We will compute the main effect of caloric density of food cues. We will compute the Parametric modulations of blood and subjective covariates on these caloric density responses. We will compute the main effect of endocrine and subjective covariates as slow varying modulators of resting activity. Multivariate Bayesian decoding will be performed on regions of interest to ask which brain regions decode onto endocrine responses, subjective responses, and ultimately energy intake.

Statistical modelling of endocrine responses
In modelling the endocrine responses we will focus on 2 physiological modalities; appetite sensation (outcome=energy intake) and nutrient sensing (outcome=hormone responses).
1. Appetite sensation: To address the question of how well endocrine responses predict energy intake we will perform a linear regression models with energy intake as dependent variable and AUC of endocrine metabolic marker and energy expenditure (calculated from height and weight) as covariates (and subject id as random effect). A regression model will be performed for each of the different endocrine markers to find the marker that explains the maximum variance in energy intake.
In addition, we will perform a Bayesian linear regression (as described in preceding section), with energy intake as dependent variable. The regressors of interest will be based on area under the curve metrics computed from all of the endocrine and metabolic markers obtained from the blood sampling including GLP1, Ghrelin, PYY, CCK, glucose, insulin.
2. Nutrient sensing: To address the question of between preload effects on endocrine responses we will perform a repeated measures 2-way ANOVA with AUC of endocrine marker as dependent variable and both preload dimensions as independent fixed effects and individual subject id as an independent random effect. Post hoc pairwise multiple-comparisons will be used as follow-up tests to compare every group mean.

4. Adverse Event Information
Time Frame. Possible adverse events can include fainting at the point of drawing blood. This is monitored by two researchers at all times, where the participant is vulnerable to fainting.
Adverse Event Reporting Description. We will add relevant information about adverse event after finishing the study.

5. Limitations and Caveats
There may be several limitations or caveats. First, it is possible that the dietary intervention does not impact on energy consumption hours later. The dietary intervention is primarily used as a manipulation to drive variation in consumption. As long as consumption has variance across and within participants, then the main goal of using the data modalities to predict satiety is still achievable. Second, it is possible participants do not comply with instructions prior to their experimental day. This will be evident in the blood measures, indicating for instance if they have not fasted. Non-compliance could be evident in their tracker activity, for instance if they have engaged in extreme activity prior to testing. However not all such cases can be inferred from these measures.

6. Certain Agreements
Are all PIs Employees of Sponsor? No. There is one sponsor-investigator and none of the other principal investigators are employed by the sponsor.

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