STATISTICAL ANALYSES PLAN

A replicated crossover study to explore individual variability of appetite responses to a standardised meal and any moderating influence of the FTO gene

Version 1

Investigators
Professor David Stensel
Professor Greg Atkinson
Ms Fernanda Reistenbach Goltz

Address
School of Sport, Exercise and Health Sciences
Loughborough University
Leicestershire
LE11 3TU
United Kingdom

Correspondence
Professor David Stensel: +44(0)1509 226344, D.J.Stensel@lboro.ac.uk

Statistical analyses plan

Between-genotype differences in participant characteristics will be quantified using linear mixed models with group (AA versus TT) modelled as a fixed factor. The presence of interindividual differences in blood marker and perceived appetite responses to a standardised meal will be examined according to three analytical approaches (Atkinson and Batterham, 2015; Senn et al., 2011; Senn, 2016). The three approaches, detailed recently by Goltz et al. (2018), will be as follows:

(i) The association between the first and second replicate of control-adjusted treatment effect will be quantified for each outcome using Pearson's correlation coefficients (Senn, 2016). The first meal condition in any participant's sequence will be paired to the first control condition in the same individual's sequence. Thresholds of 0.1, 0.3 and 0.5 will be used to label correlation coefficients as small, moderate and large, respectively (Cohen, 1988). This correlation coefficient quantifies the consistency of meal effect across the replicated experimental conditions.

(ii) The following equation (Atkinson and Batterham, 2015) will be used to provide an overall estimate of the true (control condition adjusted) between-subject differences in treatment response:

$$SD_{IR} = \sqrt{SD_M^2 - SD_C^2}$$

SD_{IR} represents the true interindividual variation in treatment effect. SD_M and SD_C are the standard deviations of the pre-to-post change scores for the meal and fasted control conditions (averaged over the two replicates using the relevant equation for pooling SDs (Higgins and Green, 2011)).

(iii) While the equation in (ii) estimates response variance adjusted for control condition change variance, the associated standard errors and confidence intervals (CI) are not appropriate for our within-subjects crossover study design, hence our adjunct approach of within-subjects general linear modelling. Using the MIXED procedure in SAS OnDemand for Academics (https://www.sas.com/en_us/software/on-demand-for-academics.html), a within-participant linear mixed model will be formulated to quantify any participant-by-condition interaction for each outcome. Condition and period (sequence), and their interaction effects,

will be modelled as fixed effects, and participant and participant-by-condition terms will be modelled as random effects. Standard residual diagnostics will be undertaken to assess the "influence diagnostics" of a potential set of observations on the adequacy and the stability of the modelled covariance parameter estimates (Oman, 1995; Schabenberger, 2004; West and Galecki, 2011).

The grand mean differences between conditions, and associated confidence intervals will be quantified with a within-subjects linear mixed model run in version 23 of SPSS (IBM Corporation, New York, USA) without the participant-by-condition random effect, but with a covariate of baseline values. The FTO genotype will be included in this model as a fixed between-subjects effect, and the genotype-by-condition interaction will be quantified.

Absolute standardised effect sizes (ES) will be calculated, with a standardised ES of 0.2 denoting the minimum important mean difference for all outcomes, 0.5 moderate and 0.8 large (Cohen, 1988). To calculate the minimal clinically important difference (MCID) for individual responses, the threshold of 0.2 for interpreting standardised mean changes (Cohen, 1988) will be halved, i.e. 0.1, and multiplied by the baseline between-subject SD (Atkinson and Batterham, 2015; Williamson et al., 2018). Pearson's correlation coefficients will be quantified between the mean control-adjusted meal response for each of the appetite measures and body adiposity measurements. Pearson's correlation coefficients will also be quantified between the pooled mean pre-to-post change in concentrations of plasma constituents and the pooled mean pre-to-post change in appetite perceptions across the four conditions.

Data will be presented as mean (SD). Mean differences or changes and correlation coefficients will be presented along with respective 95% CI. Statistical significance will be accepted as $P < 0.050$ and P values will be expressed in exact terms apart for very low values, which will be expressed as $P < 0.001$.

References

Atkinson G, Batterham AM. True and false interindividual differences in the physiological response to an intervention. *Exp Physiol*. 2015;100(6): 577–88.

Cohen J. Statistical Power Analysis for the Behavioural Sciences. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates, 1988.

Goltz FR, Thackray AE, King JA, Dorling JL, Atkinson G, Stensel DJ. Interindividual responses of appetite to acute exercise: a replicated crossover study. *Med Sci Sports Exerc*. 2018;50(4): 758–68.

Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from: www.handbook.cochrane.org.

Oman SD. Checking the assumptions in mixed-model analysis of variance: a residual analysis approach. *Comput Stat Data An*. 1995;20(3): 309–30.

Schabenberger O. Mixed model influence diagnostics. Proceedings of the Twenty-Ninth Annual SAS Users Group International Conference. 2004. Paper 189-29, Cary, NC: SAS Institute.

Senn S, Rolfe K, Julious SA. Investigating variability in patient response to treatment – a case study from a replicate cross-over study. *Stat Methods Med Res*. 2011;20(6):657–66.

Senn S. Mastering variation: variance components and personalised medicine. *Stat Med*. 2016;35: 966–77.

West BT, Galecki AT. An overview of current software procedures for fitting linear mixed models. *Am Stat*. 2012;65(4): 274–82.

Williamson PJ, Atkinson G, Batterham AM. Inter-individual differences in weight change following exercise interventions: a systematic review and meta-analysis of randomized controlled trials. *Obes Rev*. 2018;19(7): 960–75.