



School of Sport, Exercise and Health Sciences

Influence of High-fat Overfeeding on Circulating Hepatokine Concentrations: A Randomised Crossover Study

17th November 2017

OVEREAT Study Statistical Analysis Plan

The data collected will be analysed using the software package SPSS (SPSS Version 23, SPSS Inc., Chicago, IL) and an alpha value of $P < 0.05$ will be set to indicate statistical significance. Parametric assumptions will be checked using the Shapiro-Wilk test in order to confirm normal distribution of the data. Two-hour area under the curve (AUC) calculations for plasma glucose and insulin during the oral glucose tolerance tests will be performed using the trapezoidal method. Whole-body insulin sensitivity will be calculated using the Matsuda Insulin Sensitivity Index (ISI) equation:

$$\frac{10,000}{\sqrt{(Glucose_{0\ min} \times Insulin_{0\ min} \times mean\ Glucose_{OGTT} \times mean\ Insulin_{OGTT})}}$$

The homeostatic model assessment of insulin resistance (HOMA-IR) will also be calculated using the formula:

$$\frac{Glucose_{0\ min} \times Insulin_{0\ min}}{22.5}$$

Adipose tissue insulin resistance (ADIPO-IR) will be calculated using the formula:

$$Insulin_{0\ min} \times NEFA_{0\ min}$$

A one-way repeated measures analysis of variance (ANOVA) will be performed to assess differences in daily energy intake, daily fat intake and 7-day physical activity and sedentary behaviour (i.e sitting time, standing time, light physical activity, moderate-vigorous physical activity) between the high-fat condition, control condition and baseline measurement.

For the outcome measures which will be taken only pre- and post-dietary conditions, a 2 x 2 repeated measures ANOVA will be used for analysis. This includes the following outcome measures: Body fat percentage, resting metabolic rate, fat oxidation percentage, glucose AUC, insulin AUC and Matsuda ISI.

For the outcome measures which will be taken both pre-diet and post-diet as well as one and three days into the diet, a 2 x 4 repeated measures ANOVA will be used for analysis. This includes fasting plasma concentrations of: LECT2, FGF21, fetuin-A, acylated ghrelin, PYY, CTX, P1MP, glucose, insulin, NEFA and triglycerides. These outcome measures also include: HOMA-IR, ADIPO-IR, subjective ratings of appetite, food preference, body mass and both systolic and diastolic blood pressure.

Where a significant interaction effect is observed, *post hoc* paired samples *t*-tests with a Bonferroni correction will be used to locate any differences. If Mauchly's test of sphericity is violated by any variables, Greenhouse-Geisser corrected values will be used.