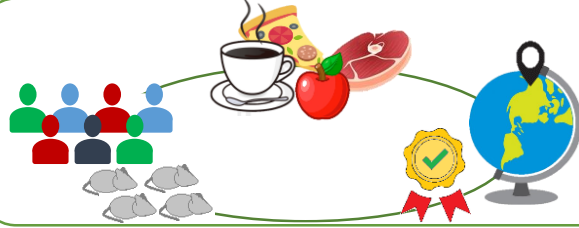


Biological question



Study Design *



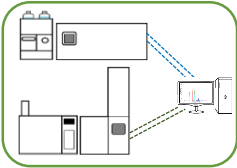
- Untargeted Metabolomics Approach*
- Intervention/observational study
- Groups definition: TEST vs CONTROL
- Control of variables: metadata collection

Sampling and Sample Preparation *



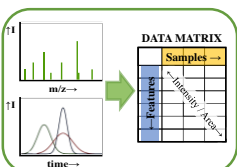
- Sampling plan
- Matrix and chemistry of the metabolites consideration
- Sample treatment selection

Data Acquisition *



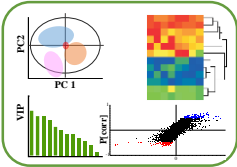
- LC-HRMS
- GC-HRMS
- MS acquisition method: FS, DDA, DIA, etc

Data Processing *



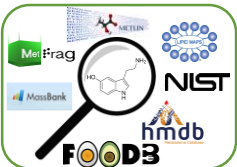
- Peak picking and spectra deconvolution
- Retention time alignment
- Gap filling
- Data matrix obtention
- Normalization, scaling, data reduction, possible annotation

Statistical Analysis *



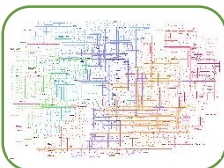
- Univariate: t-test, ANOVA, etc
- Multivariate: PCA, PLS-DA, OPLS-D, HCA, etc
- Discrimination: VIP, p-[corr], etc

(Bio)marker Structure Identification *

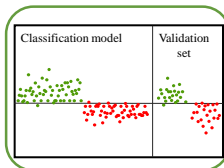


- Fragmentation spectra evaluation
- Putative annotation with spectral libraries
- Identity confirmation: comparison with reference standard

Biological Interpretation



Model Validation



*Aspects included in this review