Supplementary Material

Plasma Metabolomics

Plasma metabolomics analyses were performed by The Metabolomics Innovation Centre (TMIC, Calgary, Canada) using custom assays employing targeted methods. Each assay involved a combination of direct injection mass spectrometry with reverse phase liquid chromatography (LC-MS/MS) using an ABSciex 4000 Qtrap® tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA, USA) coupled with an Agilent 1260 series UHPLC system (Agilent Technologies, Palo Alto, CA, USA). These instruments were used to quantify a targeted selection of up to 172 metabolites and can provide identification and quantification of numerous metabolite species (specifically, amino acids, acylcarnitines, biogenic amines & derivatives, uremic toxins, glycerophospholipids, sphingolipids and sugars, TMIC prime assay) and free fatty acids. Assays involved the derivatization and extraction of analytes, and the selective mass-spectrometric detection using multiple reaction monitoring pairs and metabolites that are subsequently quantified using Isotope-labelled internal standards and other internal standards. Further details of this assay have been described elsewhere (Zheng et al., 2020). Upon identification and quantification, metabolites were subsequently classified into four distinct metabolite groups (Amino Acids, Peptides and Analogues; Fatty Acids and Fatty Acid Conjugates; Acylcarnitines; and Glycerophosphocholines & Phosphosphingolipids) using the “ChemOnt” taxonomic classification technique (Feunang et al., 2016). Thirteen of our detected metabolites belonged to taxonomy groups for which <3 metabolites were measured; these metabolites were gathered into a single group labelled “Others”. We also calculated four metabolite sums (branched chain amino acids, gluconeogenic amino acids, essential amino acids, total acylcarnitine) and three metabolite ratios (acylcarnitine/carnitine, C2/C0 and
kynurenine/tryptophan ratios respectively), as this can reduce data variation and provide insight into whether metabolic processes may be altered when certain clusters of metabolites with similar bioactivities have changed in a collected fashion (Petersen et al., 2012). Eleven metabolites in our targeted panel were below the limit of detection (histamine, cis-hydroxyproline, dopamine, L-DOPA, carnosine, nitro-tyrosine, diacetyl-spermine, tyramine, phosphocreatine, phenylethylamine, docosapentaenoic acid). Two additional metabolites (glucose and citric acid) were removed from analysis as our choice of vacuette anticoagulant (ACD-A) contains both of these metabolites, therefore the respective concentrations of these metabolites in samples were detected at physiologically abnormal concentrations. This collectively left the total number of variables generated for analysis (individual metabolites and metabolite sum/ratios) at 166 (172 potentially identifiable metabolites on the targeted assay panel, minus 13 undetected or inaccurately estimated metabolites, plus seven calculated metabolite sums/ratios for a total of 166 variables prepared for analysis). All identified metabolites, metabolite taxonomies and sum/ratios and raw data are reported in the supplementary document.

**References**


**Note:** The raw data are available on request from brendan.egan@dcu.ie