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Investigating physiological variation in healthy Irish adults using a combination of traditional techniques and emerging metabolomic technologies

A. O'Sullivan[1], L. Brennan[1], B. Mion[1], A. O'Connor[1], S. Kaluskar[1], K. D. Cashman[2,3], A. Flynn[2], J. Marchesi[4], F. Shanahan[3] and M. J. Gibney[1]

[1]*Institute of Food and Health, School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Republic of Ireland and Departments of* [2]*Food and Nutritional Sciences,* [3]*Medicine and* [4]*Microbiology, University College Cork, Cork, Republic of Ireland*

Metabolomics is described as the comprehensive study of small molecules or metabolites present in biological samples[(1)]. Relatively new in human nutrition, metabolomics has the potential to provide an alternative to the traditional single-biomarker method currently used to assess health and disease[(2)]. To progress this field in nutrition research it is important to fully understand the effects of physiological variation on human profiles. The aim of the present study is to investigate metabolic variation in a group of healthy Irish adults using a combination of biochemical and metabolomic techniques.

Healthy volunteers (*n* 160) were recruited at two research centres, in Dublin and Cork. Subject characteristics and anthropometric measurements (e.g. height, weight and BMI) were recorded. Food diaries (3 d) were used to assess habitual diet and FFQ were completed to estimate Ca and vitamin D intake. Blood, urine and faecal samples were collected following an overnight fast. Serum 25-hydroxyvitamin D (25(OH)D) and factors related to the metabolic syndrome (e.g. glucose, insulin, C-peptide, IL-6, C-reactive protein, TAG, NEFA, cholesterol, fatty acid profiles, leptin, resistin, adiponectin and TNFα) were measured using standard biochemical assays and statistically analysed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). ^{1}H NMR spectra were acquired for biofluid samples and the data processed, reduced and imported into SIMCA P+ (Umetrics Inc., Kinnelon, NJ, USA) for multivariate data analysis.

In total seventy-five males and eighty-five females aged between 18 and 63 years and within a healthy BMI range (24.3 (SD 3.1) kg/m^2) were recruited. Mean plasma glucose was 5.11 (SD 0.67) mmol/l, 17% of participants presented with impaired fasting glucose according to the International Diabetes Federation definition[(3)]. Mean plasma cholesterol was 4.69 (SD 1.02) mmol/l and TAG 1.32 (SD 0.65) mmol/l. Of the total study group 38% had cholesterol levels above the desirable range (>5.0 mmol/l), while 23% presented with TAG levels >1.7 mmol/l. Average serum 25(OH)D was 59.2 (SD 27.1) nmol/l. Grouping subjects according to vitamin D status indicated that 3% of all participants were vitamin D deficient, 40% insufficient and 32% were classified as hypovitaminosis D, while only 25% had adequate vitamin D levels. Regression analysis indicated that 25(OH)D levels were inversely correlated with plasma TAG ($P=0.045$). Principal component analysis of metabolomic urine data showed that there was a separation between male and female subjects. To probe this further a partial least-squares discriminant analysis (PLS-DA) model was built (R^2 0.3, Q^2 0.5). Urine from male subjects had higher concentrations of creatinine, trimethylamine *N*-oxide (TMAO) and carnitine and lower concentrations of citrate and hippurate compared with female urine. A PLS-DA model was built to investigate the effects of age on the metabolic profile. Interrogation of the corresponding loadings plot revealed that the older subjects were characterised by higher concentrations of citrate, TMAO and phenylalanine and lower concentrations of 3-hydroxybutyrate compared with younger participants.

In conclusion, biochemical analysis showed an inverse relationship between serum 25(OH)D and levels of plasma TAG. Initial analysis of metabolomic data highlights gender and age as the primary influential factors on the metabolic profiles of urine samples. Future work will look at the integration of biochemical, dietary and metabolomic data.

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