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Untargeted metabolomics based on ultra-high-performance liquid chromatography-ion mobility-quadrupole time-of-flight mass spectrometry for biomarker discovery of orange intake in a cross-over trial

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Introduction

Diet is one of the most important lifestyle factors associated with health status. Currently, one of the main limitations of nutritional epidemiology and nutritional genomics is the difficulty in the measurements of dietary intake. In observational studies carried out in a large number of participants, the most commonly applied tools for estimating dietary intake are based on self-reporting, including food frequency questionnaires (FFQ) for the assessment of regular consumption (usually 1-year), or 24-h recalls for 1-day assessment. However, such instruments for data collection may contain several recall bias and other systematic or random errors that may have a great effect in the subsequent associations found. Although in recent years, it has been an improvement in increasing the validity and precision of food questionnaires due to the use of the new information technologies (Figure 1), these instruments are still biased and additional information based on objective biomarkers of food intake is needed.

Figure 1: Dietary assessment based on the use of food questionnaires

Biomarkers of food intake (BFIs) are promising tools to provide more objective food consumption measurements. Therefore, a major challenge nowadays for nutritional epidemiology and nutritional genomics is to identify novel biomarkers. Metabolomics has opened new opportunities for BFI discovery by metabolic profiling of biological samples (plasma, urine, etc.), following the intake of specific foods, meals, or diets (Figure 2).

Figure 2: Dietary assessment based on the determination of food intake biomarkers

The arrival of new and powerful analytical technologies like ion mobility separation coupled to high resolution mass spectrometry (IMS-HRMS) has provided new tools to facilitate the complicated task of biomarkers elucidation in metabolomics. IMS allows to maximize the detection of markers without increasing the analysis time, obtaining additional structural information (independent of the retention time) given by the collision cross-section (CCS) of the molecules. In addition, the fast time scale of the IMS separations (a few ms) facilitates its coupling after ultra-high pressure liquid chromatographic (UHPLC) separations, with 3-6s peak widths

AIMS:

To investigate the biomarkers associated with orange consumption in plasma samples taken in acute (4h after consumption) and in a medium-term intervention study (after 1 month of continued intake) using untargeted metabolomics based on ultra-high-performance liquid chromatography-ion mobility-quadrupole time-of-flight mass spectrometry (UHPLC-IMS-QTOF) MS in a cross-over randomized and controlled trial.

Methods

We carried out a cross-over randomized and controlled acute feeding trial including 30 healthy participants. After a minimum of 8 hours fasting participants were randomly allocated to eat 500 g of oranges or an isocaloric (same energy as the oranges) solution of sucrose in water. No other food is allowed for 4 hours. At the start and after 4 hours blood and urine samples were taken as well as blood pressure and body measurements and questionnaire data. BFIs were measured in plasma samples. After a 1-week break the two groups swap over and the study was repeated. In addition, In a longer study with a subgroup of the initial volunteers, participants were randomly allocated to be told to either eat oranges every day for a month, or to reduce their intake of oranges for a month. At the start and after 1 month, samples of blood and urine are taken. BFIs were measured in plasma samples. This trial (ORANGOMICS), was registered as ISRCTN17330010. We investigated

plasma BFIs associated with orange consumption using untargeted metabolomics based on UHPLC-IMS-QTOF MS (Figure 3).
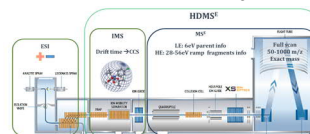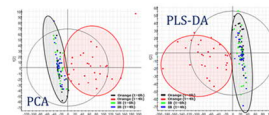
Figure 3: UHPLC-IMS-QTOF MS equipment

In the acute feeding trial, baseline and 4-h plasma samples were analyzed. Plasma samples were collected at baseline (after fasting), and at 4h postprandial. Samples were treated with acetonitrile for deproteinization followed by centrifugation . Then, 1μL of the supernatant was directly injected in both RP and HILIC chromatography. The UHPLC-IM-QTOF MS data were extracted and processed with

Results

In the acute trial we enrolled 30 healthy participants aged 25±2.8 years, and plasma samples were analyzed for each subject at baseline and after 4h, both for the orange intervention and for the control intervention (sucrose). Figure 4 shows the principal component analysis (PCA) (A) and the PLS-DA (B).

Figure 4: PSA (A) and PLS-DA (B) for all the samples (oranges and isocaloric control) at baseline (t=0) and at t=4h

When we focused specifically on the samples for the intervention with oranges, we obtained a clear separation between baseline and at 4 h. Figure 5 shows the corresponding OPLS-DA and Figure 6 shows the S-plot.
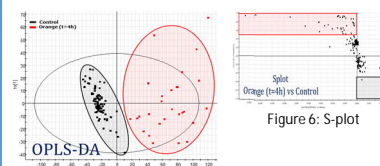
Figure 5: OPLS-DA

Figure 6: S-plot

We identified 2 markers (M1 and M2) with the higher trend-plots (Figure 7 and Figure 8), being excellent markers.

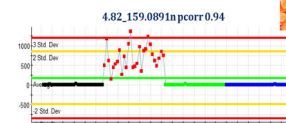4.82_159.0891n pcorr 0.94

Figure 7: Trend-plot for marker M1
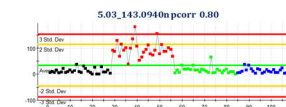
5.03_143.0940n pcorr 0.80

Figure 8: Trend-plot for marker M2

We further identified the markers as betonicine (4-hydroxybetaine proline) for M1 and Stachydrine (betaine proline) for M2 (Figures 9 and 10, respectively).
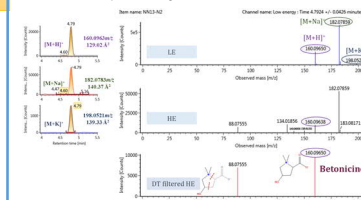
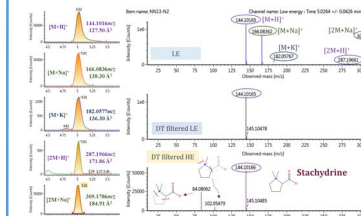Figure 9: Betonicine identification

Figure 10: Stachydrine identification

Our results in the short-term trial, clearly identified these biomarkers as excellent biomarkers of acute orange intake. Additionally, we also carried out a 1-month observational study (comparing a low versus a high orange intake in the diet), and differentially identified these BIFs