The profile of bile acids and their sulfate metabolites in post-prandial human serum suggest sex-related differences

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ABSTRACT

An isotopic dilution ultrahigh performance liquid chromatography tandem mass spectrometry method (UHPLC-MS/MS) was developed for the determination of 41 target and 8 additional bile acids isomers (BAs) in biological fluids. BAs were analysed by solid-phase extraction on 50 µL biofluid-aliquots, followed by a properly optimised 27 min-chromatographic run.

Application to a dietary intervention kinetic study with *Vaccinium corymbosum* (VC) and *Vaccinium myrtillus* (VM). confirmed the existence of possible metabotypes amongst the study population (n = 20). A trend differentiating males from females was observed suggesting that serum samples from women contained smaller amounts of certain bile acids.

STUDY DESING and EXPERIMENTS

Twenty healthy volunteers received 25 g of *V. myrtillus* or *V. corymbosum* supplement mixed with 500 mL of water. Ethical approval n. SPE 14.178 AOUC, 30th May 2016. Serm samples were collected at followinf times points: 0min, 0.5h, 1h, 2h, 3h, 4h, 5h, and 6h.

Serum samples were extracted with 96-well plate Ostro (Waters) and diluted 1:2. 14 internal standards of bile acids and external standard (h*ippuric acid-*d_5) were added for quantification purposes.

A combination of quantitative (LC-Triple Quad) and qualitative (LC-HR-Orbitrap) analysis for discovery of BAs isomers. Their presence was confirmed thanks to fragmentation pattern similarities with analytical standards and mass accuracy measurements in full scan and MS/MS modes.

Fig. 1 Discovery strategy: unscheduled LC-TripleQuad + neutral loss + screening LC-HR MS/MS Orbitrap. Panel A: unscheduled screening with LC-Triple Quad mass spectrometer; Panel B: untargeted screening and MS/MS analysis with LC-HR Orbitrap mass spectrometer

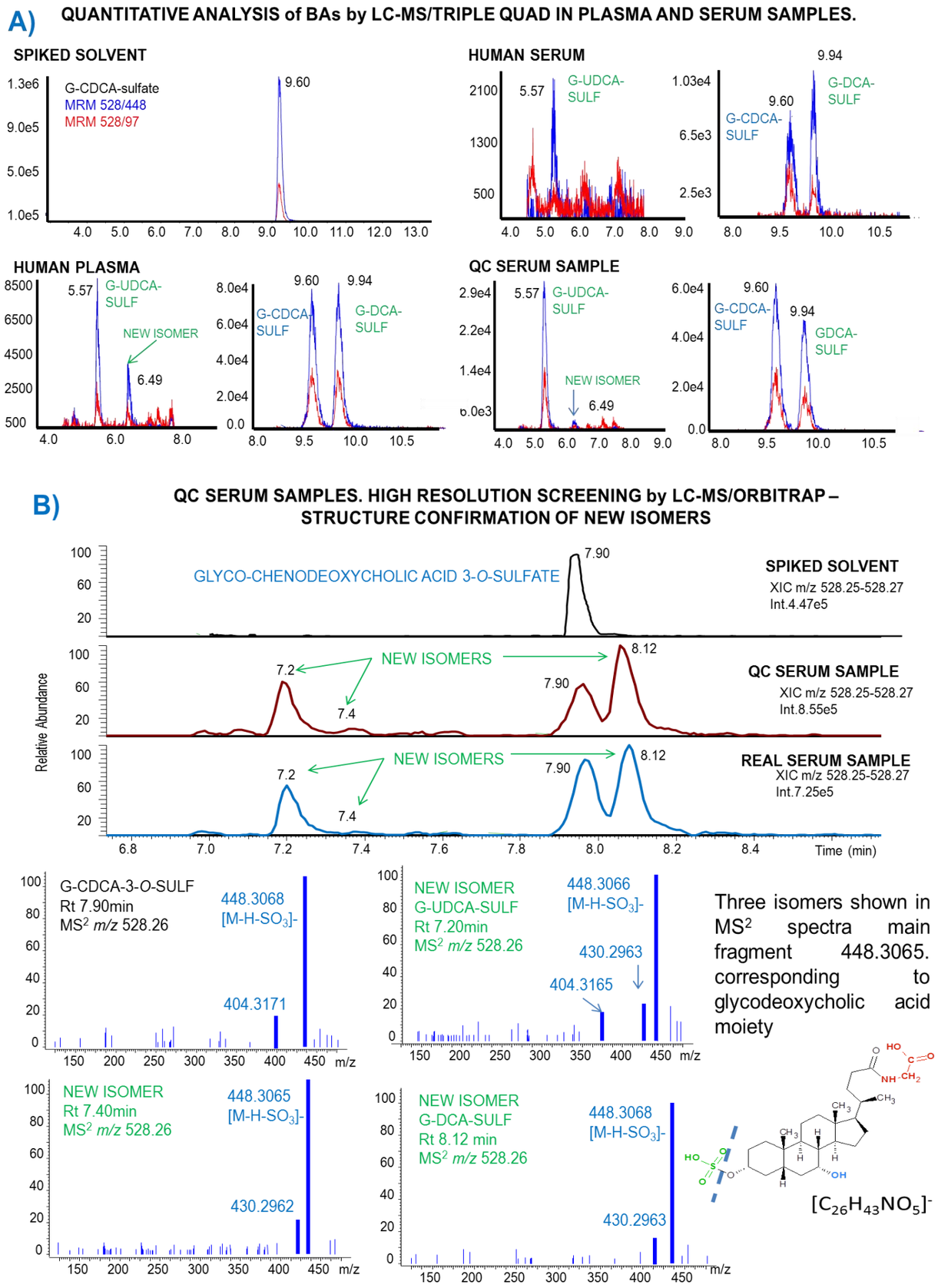


Fig. 2. Heatmap shows clustering of volunteers according to postprandial plasma bile acid kinetics.

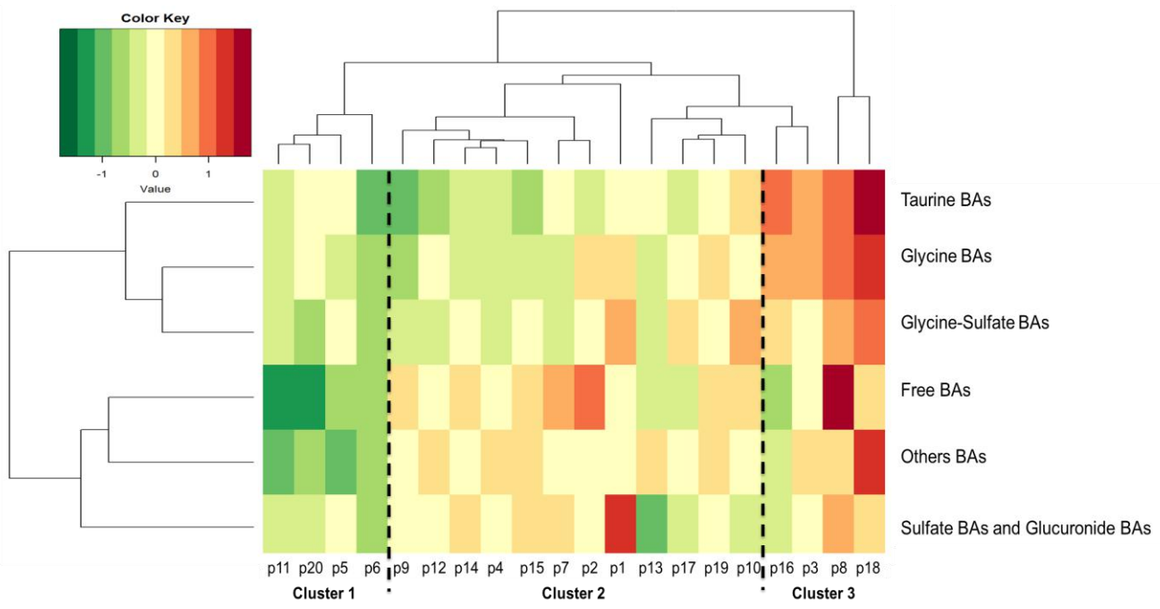
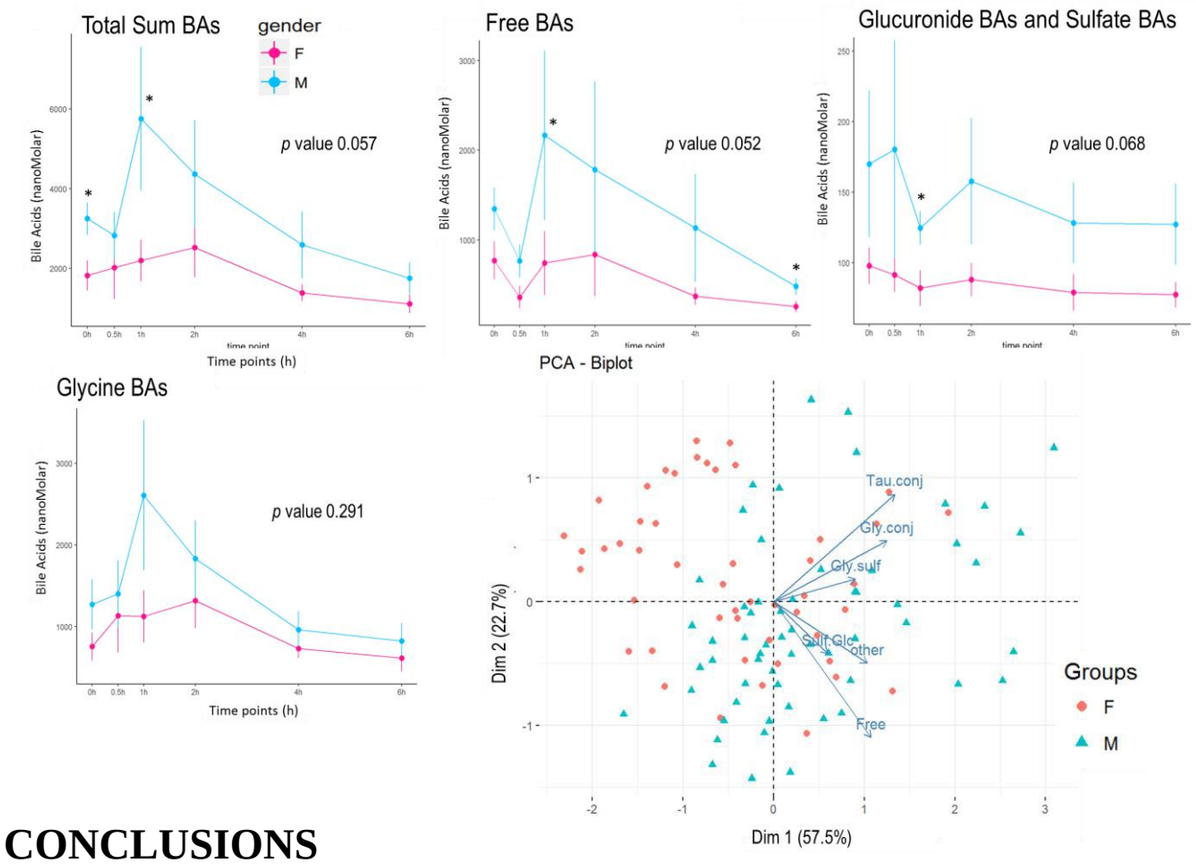


Fig 3. Kinetic curves for bile acids classes plotted for males and females with corresponding *p* values resulting from comparison of AUC curves



CONCLUSIONS

For the first time sulfates conjugated forms of bile acids were measured and quantified using deuterated bile acids internal standards. A strategy of scheduled/ unscheduled injections of real samples allowed us to find additional bile acid isomers not *a priori* included in the method, while high resolution full scan and MS/MS fragmentation analysis confirmed their structural adherence to the bile acid family.

The application to a nutrikinetic challenge study with two different types of berries, showed high inter-individual differences both in the concentrations and composition of plasmatic BAs in fasting and postprandial state.

We found differences in bile acids quantity and time reaching maximal BAs concentrations between males and females, suggesting higher BA concentrations in males. Additionally we found three possible subgroups through hierarchical clustering. Values regarding the variability among all study volunteers and proportional distribution of BAs were in agreement with previous data measured in independent populations.

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