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3-NPH derivatization of SCFAs in human biofluids for longitudinal and multi-matrix exploration

Jérémy Monteiro¹, Antoine Lefèvre¹, Adeline Oury¹, Diane Dufour-Rainfray^{1,2}, Lydie Nadal-Desbarats¹, Patrick Emond^{1,2}

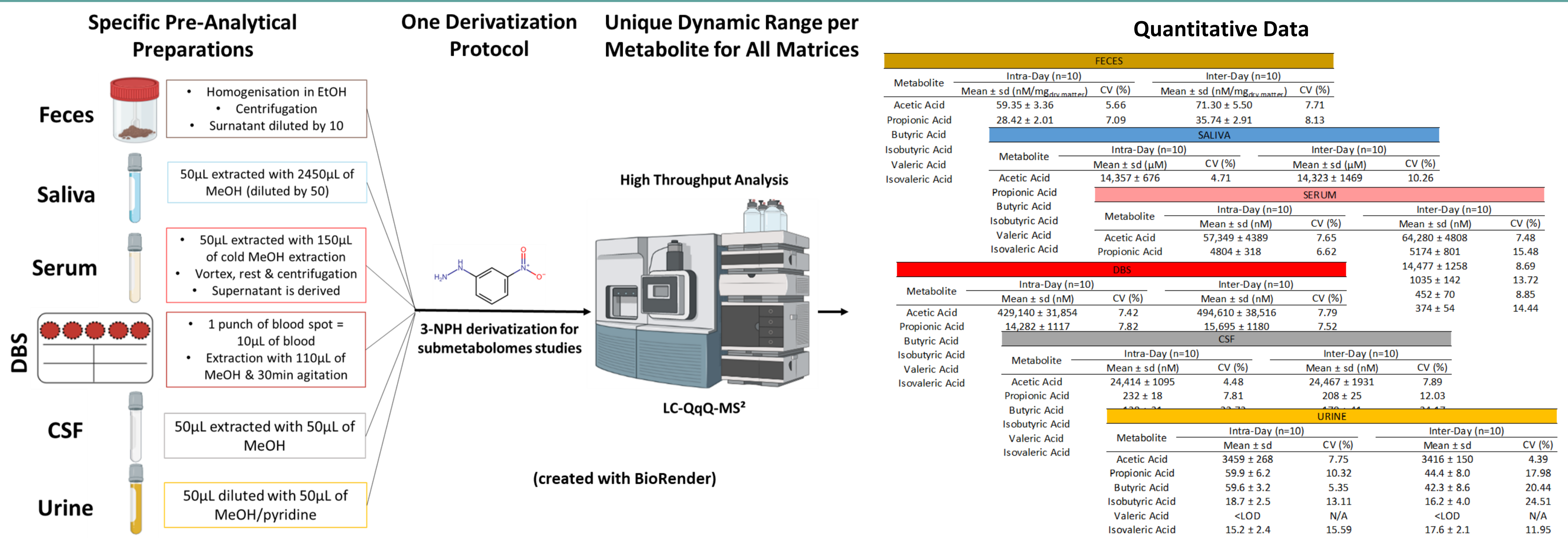
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Introduction

Short-chain fatty acids (SCFAs) play an important role in human biochemistry. They originate primarily from the digestive system through the microbial fermentation of carbohydrates and bind to the GPR41/43 receptor on the epithelial barrier or with cellular receptors to regulate glucose storage, fatty acid oxidation or fuel the TCA cycle. SCFAs are also involved in regulating the levels of several neurotransmitters such as GABA, glutamate/glutamine, dopamine, norepinephrine. Their role is also highlighted in many inflammatory and neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease.

In this work, we propose an absolute quantification method of SCFAs suitable for human biological samples such as feces, saliva, serum, DBS, CSF and urine using 3-NPH derivation and LC-MS/MS. We have adapted the pre-analytical parts for each sample with the aim of using a common calibration curve, facilitating multi-array quantification studies. A chromatographic gradient has been developed in order to separate, after derivatization, isomeric and isobaric SCFAs and to be free from interferences, whatever the matrix studied.

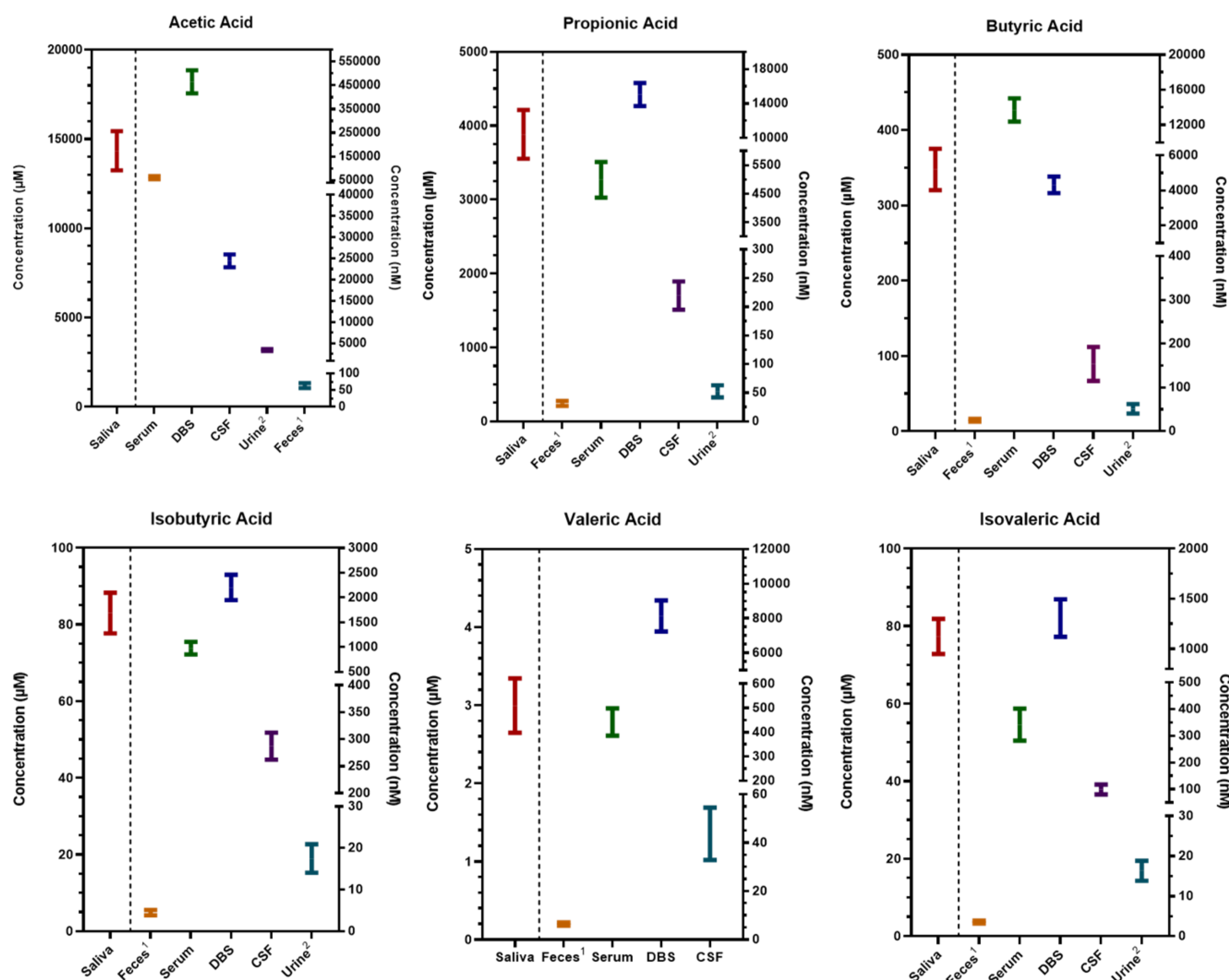
Material & Methods



Results

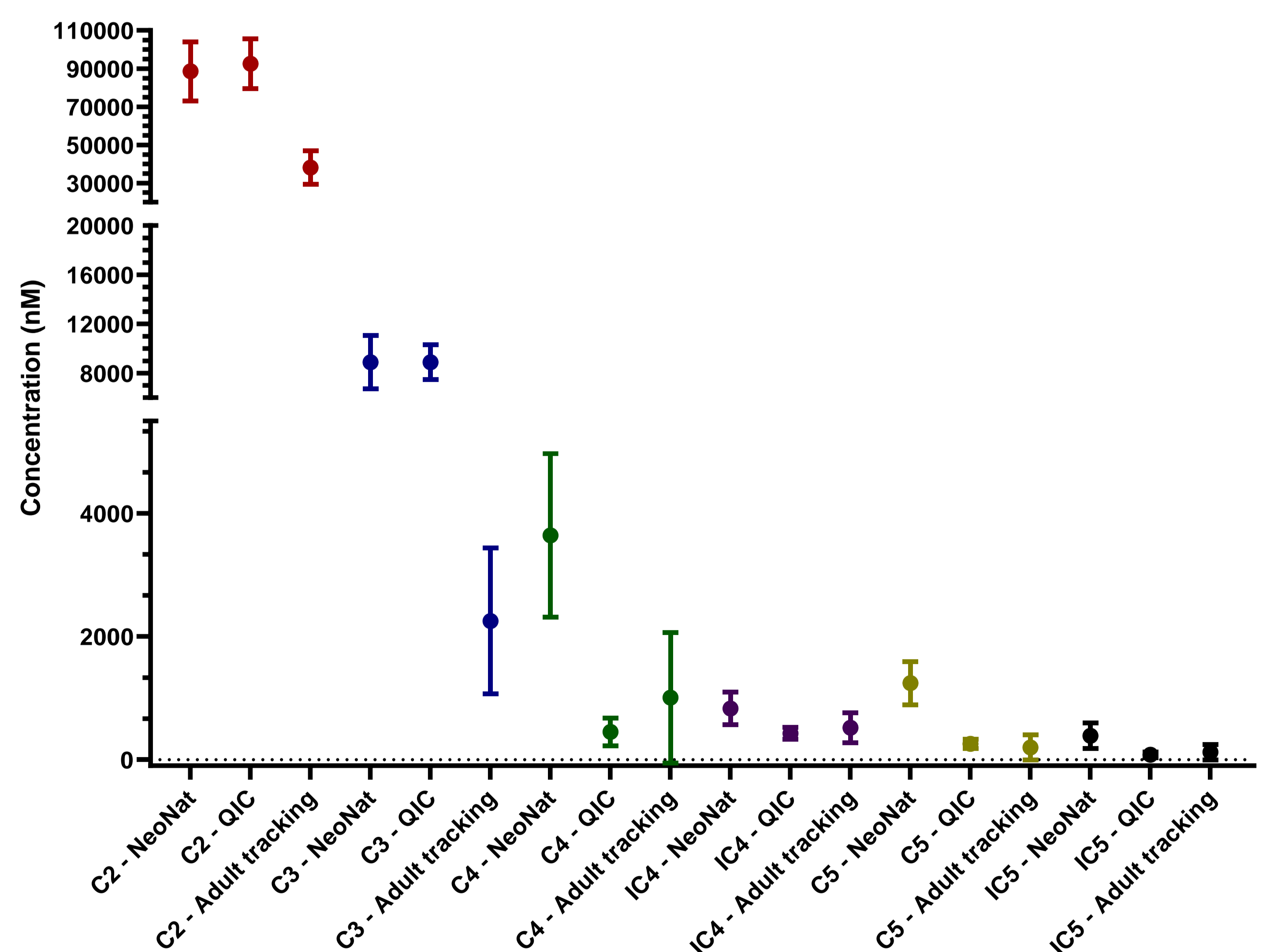
(1) Comparison of biological concentrations of SCFAs

1: concentration in feces are normalized to dried matter
2: concentration in urine are normalized to [creatinine]



(2) Concentration of SCFAs in 3 types of blank blotter papers

C2: Acetic Acid / C3: Propionic Acid / (I)C4: (Iso)Butyric Acid / (I)C5: (Iso)Valeric Acid



Determination of residual SCFA concentrations from blotting papers. Three types of blotter are used in clinical routine: neonatal screening (NeoNat), patient monitoring (Adult Tracking) and internal quality control (IQC) samples. The data was produced with 30 aliquots from a blotter, with spots made randomly over the entire surface. The results show that it is necessary to take into account the residual SCFA concentrations contained in blotters for any assays carried out on DBS.

Conclusion

SCFAs have been extensively and intensively studied by 3-nitrophenylhydrazine-LC-MS² derivatization due to their involvement in metabolisms and diseases. We propose a unique methodology to quantify SCFAs in 6 different biological matrices to generate FAIR data. Reference values are proposed for CSF and DBS. We also propose a new methodology for sampling patient faeces that could be widely applicable. These validated approaches are now used to characterize metabolism and better investigate the inflammatory component of several diseases.