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Exploration of SCFA in human multi-compartments using 3-nitrophenylhydrazine-LC-MS

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Introduction

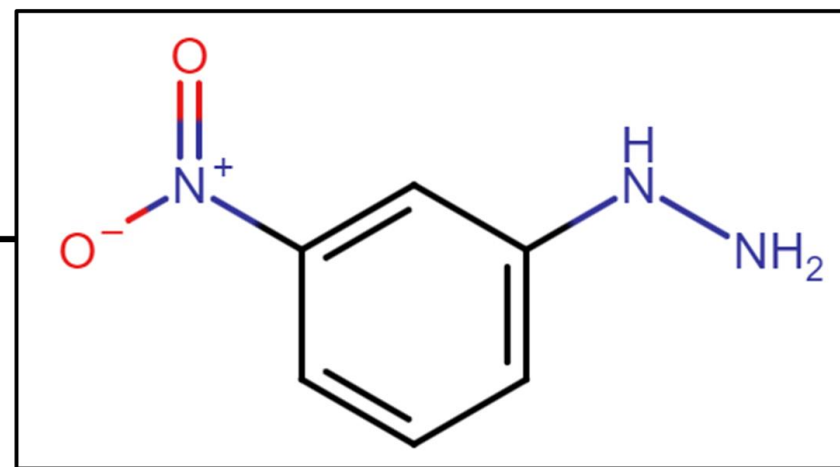
Short-chain fatty acids (SCFAs) are metabolites involved in many physiological processes. A disruption of their metabolism is suspected in diseases of the digestive system (inflammatory diseases) but also in neurodegenerative diseases such as Alzheimer's disease. Currently, one of the reference methodologies for the quantitative analysis of SCFA is based on the use of GC-MS due to its sensitivity and reproducibility. However, its technical implementation is delicate, limiting in particular the throughput of the analyzes carried out. Our objective is to propose a method for absolute quantification of SCFA that is easier to implement and for future use in a clinical environment for a large number of types of biological samples (stool, urine, serum, saliva, CSF, and dried blood spot).

With this objective we selected, 3-nitrophenylhydrazine (3NPH) to promote the detection and analytical separation of these metabolites. After methodological validation (robustness, repeatability, matrix effect, carryover, LLOQ), its use makes it possible to propose reference values of SCFA concentrations for each type of sample. This quantitative methodology also allows longitudinal monitoring of SCFA concentrations.

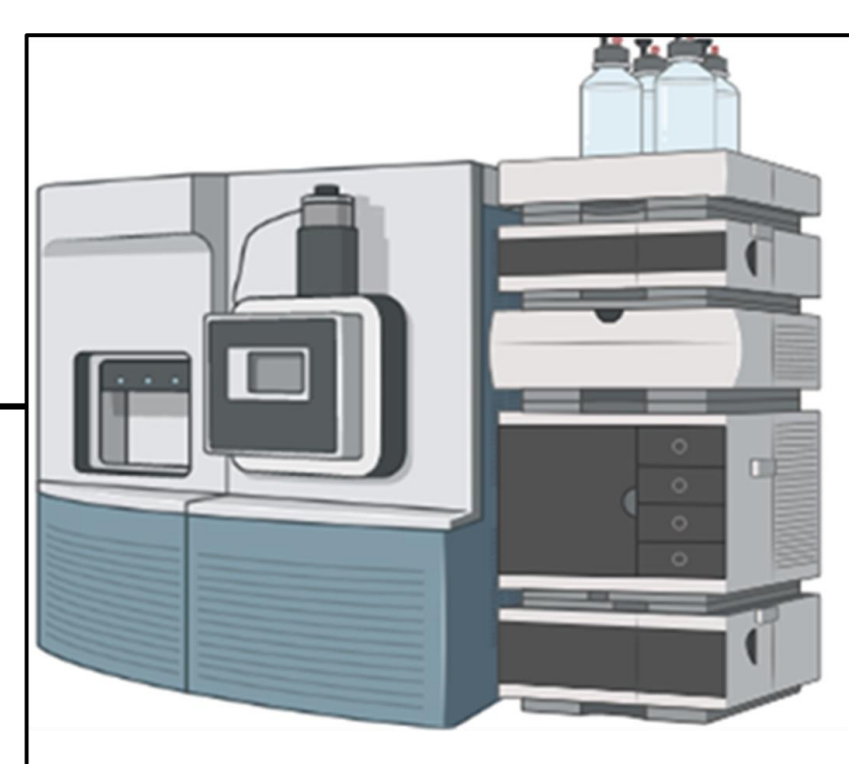
Material & Methods

Specific Pre-Analytical Preparation

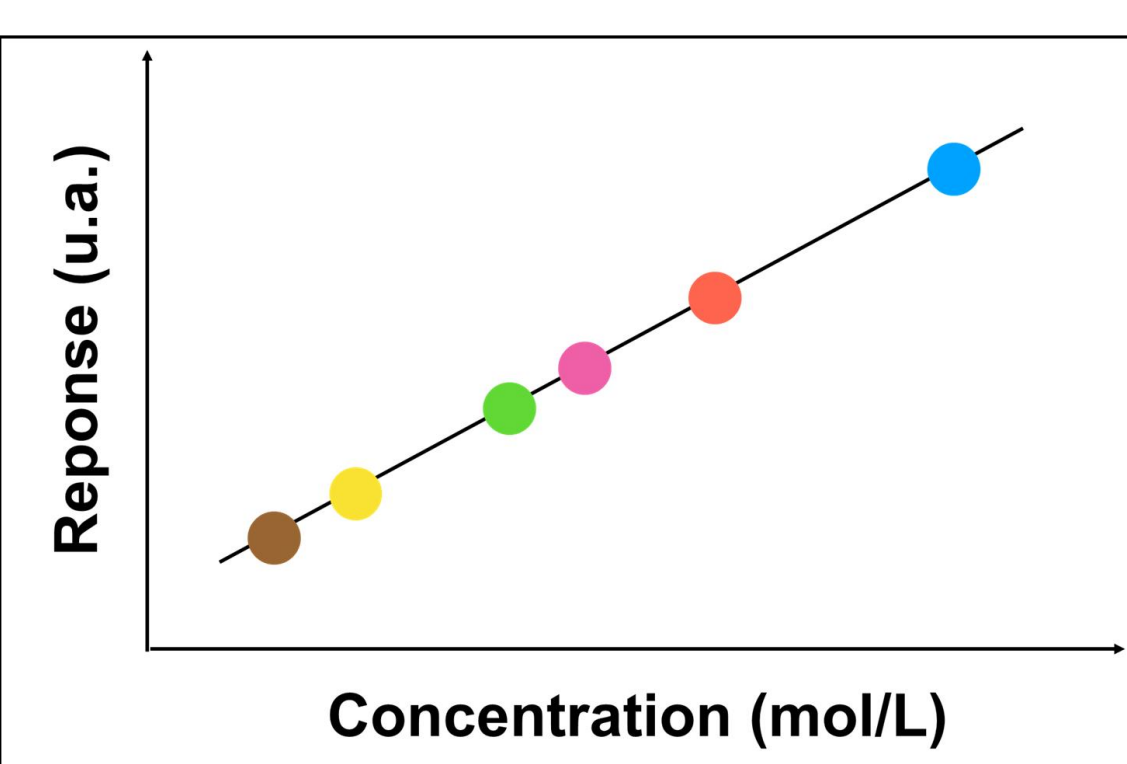
3-nitrophenylhydrazine derivatization: detection & sensitivity enhancement



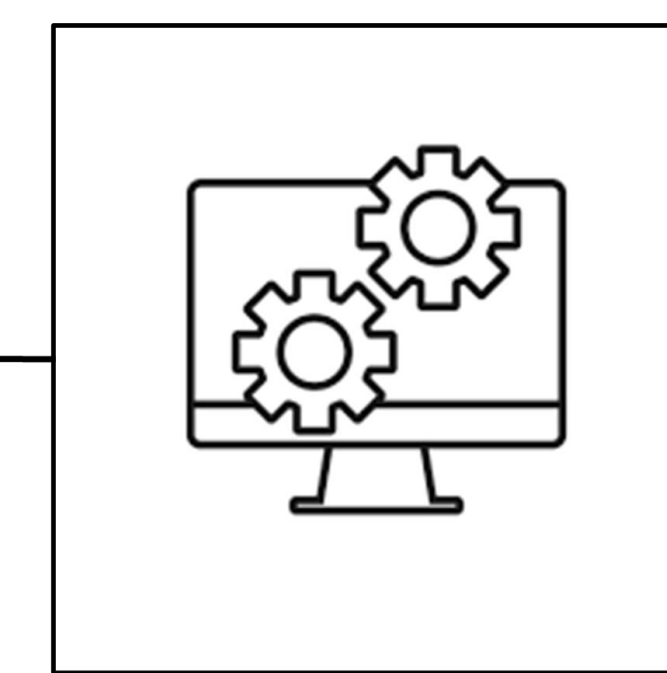
High Throughput Analysis: A unique calibration curve for all matrices / Compounds



Example of Acetic Acid: Distribution of the biological concentration within the curve



Clinical Context: Evaluation of intra- & inter-day repetibilities on n=10 pooled samples



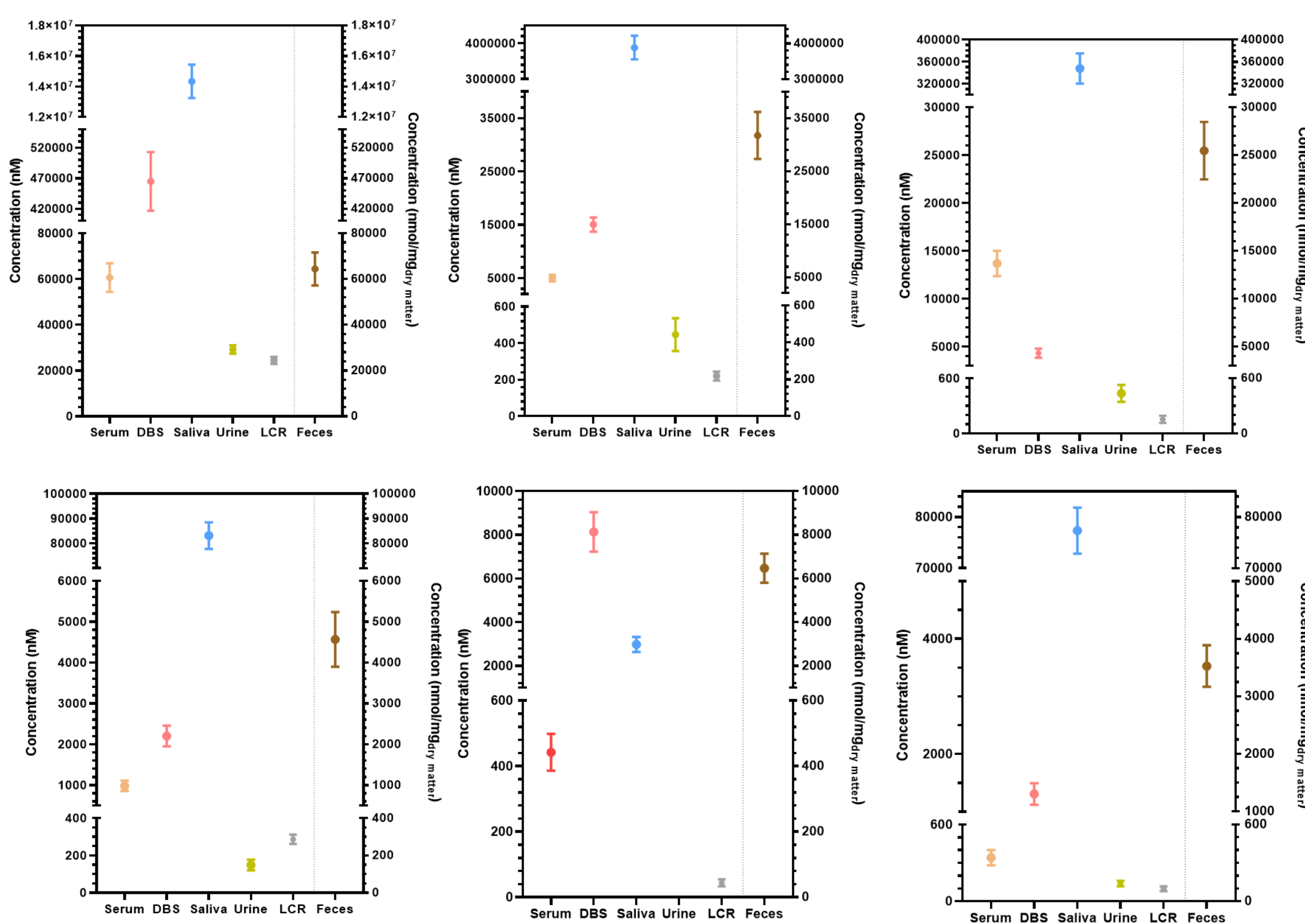
Quantitative Data

Matrix	Metabolite	Mean ± s.d. (n=10)	CV (%)	Mean ± s.d. (n=10)	CV (%)
FECES	Acetic Acid	58.25 ± 3.36	5.66	71.30 ± 5.50	7.71
	Propionic Acid	28.42 ± 2.03	7.09	35.24 ± 2.93	8.33
	Butyric Acid	-	-	-	-
	Isovaleric Acid	-	-	-	-
SALIVA	Acetic Acid	14.357 ± 676	4.71	14.323 ± 1469	10.26
	Propionic Acid	-	-	-	-
	Butyric Acid	-	-	-	-
	Isovaleric Acid	-	-	-	-
URINE	Acetic Acid	3499 ± 268	7.75	3418 ± 150	4.39
	Propionic Acid	58.6 ± 6.2	10.32	44.4 ± 8.0	17.98
	Butyric Acid	58.6 ± 3.2	5.35	42.3 ± 8.6	20.44
	Isovaleric Acid	18.7 ± 2.5	13.11	15.2 ± 4.0	26.52

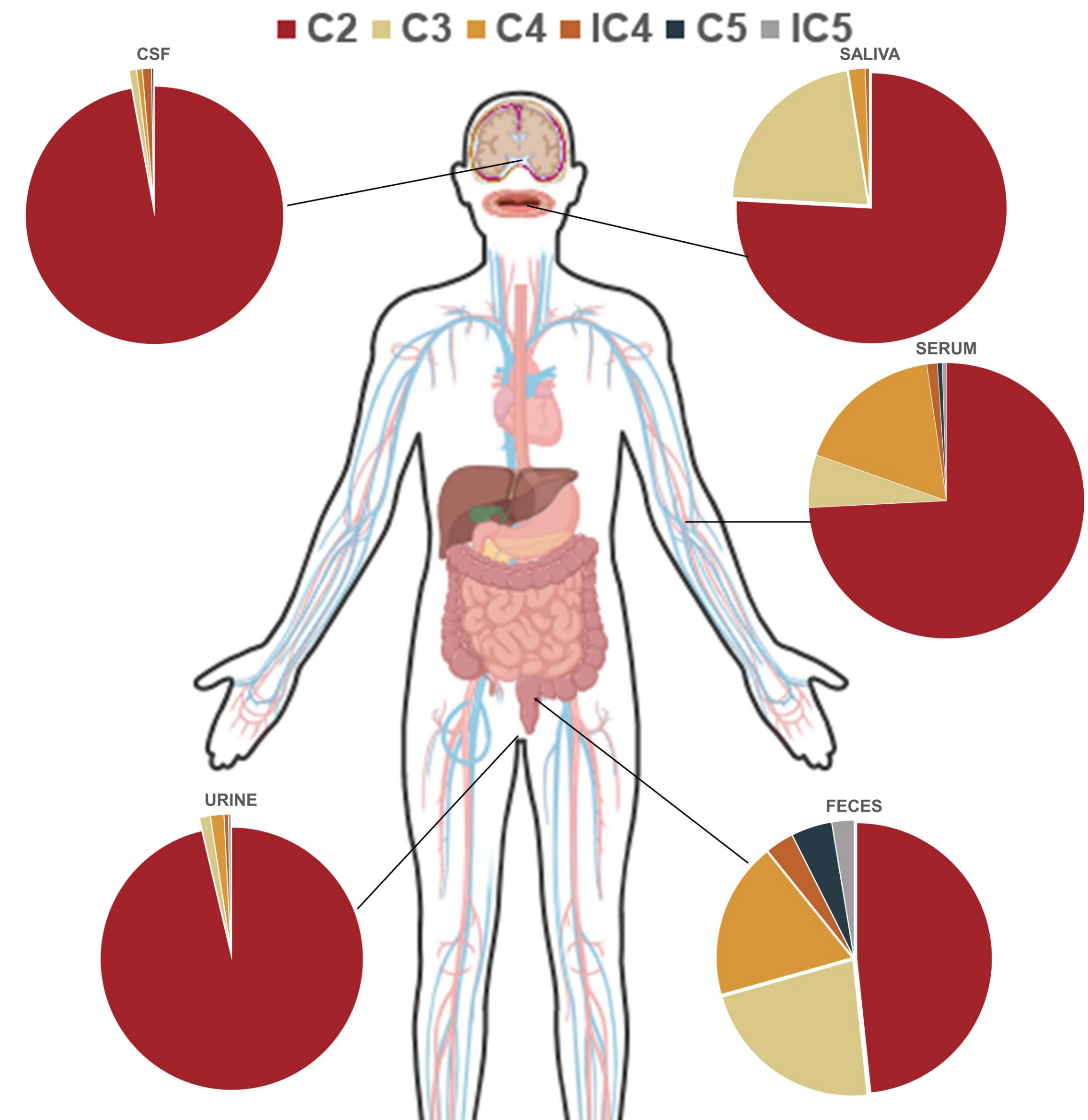
Results

(1) Comparison of biological concentrations of SCFAs

- concentration in feces are normalized to dried matter
- concentration in urine are normalized to [creatinine]



(2) Biological repartition of SCFAs in human (One pie chart = one matrix; the proportion is based on the concentration of compounds within the matrix studied)



Acetic acid is always the most concentrated SCFA, to the point of obscuring the others in the CSF and urine. In the faeces, all the SCFAs are concentrated: these are the main products of digestion. Note the high concentrations of propionic acid in saliva, produced by the enzymes it contains.

Comparison of different SCFA concentrations between biological matrices, from healthy patients. The results shown gather intra-day and inter-day repeatabilities. The concentrations found in saliva are in μM (left axis) whereas they are expressed in nM for the other matrices.

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. These validated approaches are now used to characterize metabolism and better investigate the inflammatory component of several diseases.