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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	MassLynxV4.1 software (Waters Corporation)
Data analysis	MassLynxV4.1 software (Waters Corporation); Progenesis Bridge (Waters Corporation), Progenesis QI (Waters Corporation), and SIMCA (Sartorius)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Datasets relevant to our published supporting primary papers can be made available from the corresponding author upon reasonable request. The source data for figures is publicly available at Figshare repository:
Fig.3 https://doi.org/10.6084/m9.figshare.14258423.v1;
Fig. 5 https://doi.org/10.6084/m9.figshare.14258438.v1;
Fig. 6 https://doi.org/10.6084/m9.figshare.14258459.v2

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For performing LA-REIMS discriminative fingerprinting in a clinical context (i.e. type 2 diabetes and overweight/obesity), no sample size was calculated. Actually, samples were used from previous experiments, where samples were subjected to UHPLC-HRMS metabolomics analysis. For UHPLC-HRMS, sample size was calculated using MetaboAnalyst software (power of 0.8, FDR 0.2). Samples for which sufficient amounts of material were available after UHPLC-HRMS analyses (full-scan explorative and MS/MS fragmentation experiments) were also subjected to LA-REIMS analysis. Based on the present sample sizes, significant differences were defined, indicating sufficient power size for the used sample numbers.
Data exclusions	Outlier detection was performed using the established PCA-X models; using the Hotelling's T2 95% statistics. No data were excluded.
Replication	Reproducibility of the LA-REIMS methodology was evaluated for a biofluid representative (saliva) and semi-fluid representative (feces). Data have been reported in the manuscript. For demonstrating the application potential of LA-REIMS for discriminative fingerprinting in a clinical context, samples from adults (normal glycemic status (n=36) or type 2 diabetes (n=36)) or adolescents (healthy weight (n=35) or overweight/obesity (n=35)) were analyzed once. In this context, by considering two different clinical cases, valid proof-of-concept data for LA-REIMS being a valuable tool for rapid metabotyping according to pathophysiological status was repeatedly presented.
Randomization	Samples were initially classified according to pathophysiological origin/state based on well-established parameters (i.e. glycated hemoglobin in the case of type 2 diabetes and IOTF score for overweight/obesity in adolescents). During LA-REIMS analysis, samples were completely randomized.
Blinding	Investigators were not blinded during sample collection as it was the purpose to collect biological material from a sufficient number of participants from each class (i.e. health state) whereby knowledge regarding the health state was essential to achieve sufficient sample numbers for each health state. There awareness of health state had no influence on the biological material collected. During analysis (pre-treatments and LA-REIMS analysis) investigators were blinded (i.e. they had no information about the origin of the sample). Anonimity of samples was achieved by internal laboratory labeling. During data processing (i.e. supervised multivariate modelling), information about the health status was used.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	Population characteristics may be of importance when demonstrating the application potential of LA-REIMS for discriminative fingerprinting in a clinical context. 1) Type 2 diabetes clinical case: adults (> 18 years) (type 2 diabetes: average 61 years +- 8; euglycemia: average 47 years +- 9); BMI (type 2 diabetes: average 30.2 kg/m2 +- 3.8; euglycemia: average 23.6 kg/m2 +- 3.5); HbA1c (type 2 diabetes: average 7.06% +- 0.9; euglycemia: average 5.53% +- 0.3); gender (men and women (type 2 diabetes: 25% women; euglycemia: 65.2% women). Within the group of type 2 diabetes patients; there was a high prevalence of metformin treatment. 2) Overweight/obesity: adolescents (6 to 16 years) (healthy weight: average 14.2 years +- 1.7; overweight/obesity: average

	13.7 years +- 2.6), gender (boys and girls; 42.9% boys for both healthy weight and overweight/obesity).
Recruitment	With respect to the type 2 diabetes study; patients were recruited at University Hospital Ghent. Hereby, both the attending physician as Ghent University scientist were present to inform the patient about the experimental set-up and input needed from the patient. All patients that met the inclusion critera were contacted, so self-selection bias was not present. For the healthy group, participants (i.e. those with a healthy glycemic state) were recruited amongst lab personnel and friends/family of lab personnel. All lab personnel was contacted, for which no selection bias is expected. With respect to the obesity/overweight study: participants were recruited from the Obesity Prevention through Emotion Regulation in Adolescents (OPERA) study and from the paediatric obesity department of the Jan Palfijn hospital. Inclusion criteria included no severe underweight (IOTF ≥ -1), no endocrine diseases. No selection bias was present. All participants were invited to the Ghent University Hospital Campus. To create uniformity, all participants were assigned to a single researcher, who was trained to guide the participants. Appointments were only made on school days, outside school hours (Monday - Tuesday - Thursday between 4.30 pm and 5.50 pm, Wednesday between 2 pm and 2.30 pm and between 4.30 pm and 5.50 pm).
Ethics oversight	University Hospital Ghent Ethical Committee; Wales Regional Ethics Committee

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Not
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*