NUTRIOME

NUTRIOME workshop1

Large-scale data handling and using tools to visualise multi-layered data from meal studies

Funded by the European Union

Marie Skłodowska-Curie Actions

1

NUTRIOME

Techniques to measure metabolomics

Overview

- Introduction
- Approaches
- Study Design
- Quality Control
- Applications

2

Approaches

Study of small molecules called metabolites: alterations in metabolic pathways under different conditions

NMR

LC-MS
GC-MS

3

Targeted and non-targeted approach

Targeted: prior knowledge of metabolites to be measured

Non-targeted: no prior selection of metabolites

4

NUTRIOME

Advantages of targeted data

- Quantitative
- Cross cohort comparisons
- Reproducibility
- Use as diagnostic biomarkers
- Re-use of data is easier

Advantages of un-targeted data

- Broader coverage of metabolome
- New discoveries possible
- Hypothesis generating

5

NUTRIOME

Data Format

Targeted

Concentration [µM]																	
Sample Identification	C0	C2	C3	C4	C14	C16	C18:1	Trigonellin	TMAO	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly
	Acylcarniti	Acylcarniti	Acylcarniti	Acylcarniti	Acylcarniti	Acylcarniti	Acylcarniti	Alkaloids	Amine Oxi	Aminoacid	Aminoacid	Aminoacid	Aminoacid	Aminoacid	Aminoacid	Aminoacid	Aminoacid
P_11	21.9	13.7	0.539	0.792	0.058	0.114	0.106	3.125	18.9	609	158	82	9.954	46.3	931	49	440
P_12	23.1	16.2	0.734	0.779	0.043	0.103	0.137	1.366	9.654	672	80.6	145	13.4	30.2	1075	84.3	458
P_13	24.9	8.466	0.206	0.171	0.03	0.095	0.06	1.387	9.097	483	116	49.7	7.034	35.1	724	42.5	269
P_14	29.1	14.2	0.428	0.362	0.063	0.114	0.128	0.932	9.652	423	136	49	11.3	39.3	721	46	313
P_2	25.9	9.573	0.427	0.399	0.037	0.076	0.092	0.916	4.274	718	85.6	107	13.5	29.5	842	81	389
P_3	26.4	8.969	0.417	0.328	0.036	0.126	0.11	2.846	25.2	414	178	51.2	9.11	29.9	656	36.8	313
P_30	26.1	10.9	0.407	0.587	0.024	0.133	0.105	5.068	88	624	181	66.9	10.7	30.8	722	38.2	317
P_31	17.4	9.942	0.46	0.445	0.019	0.117	0.079	4.452	147	418	144	40.2	10.9	18.2	560	28.2	217
P_34	16.1	6.566	0.469	0.44	0.023	0.11	0.06	4.485	175	633	235	73.7	11.8	28.2	770	35	297
P_35	13.3	6.075	0.436	0.303	0.023	0.109	0.145	4.391	225	403	134	51.7	9.041	30.6	562	25.7	262
P_36	18.2	6.508	0.385	0.382	0.013	0.068	0.06	3.645	53.5	379	159	43.9	6.527	34.8	503	26.4	201

6

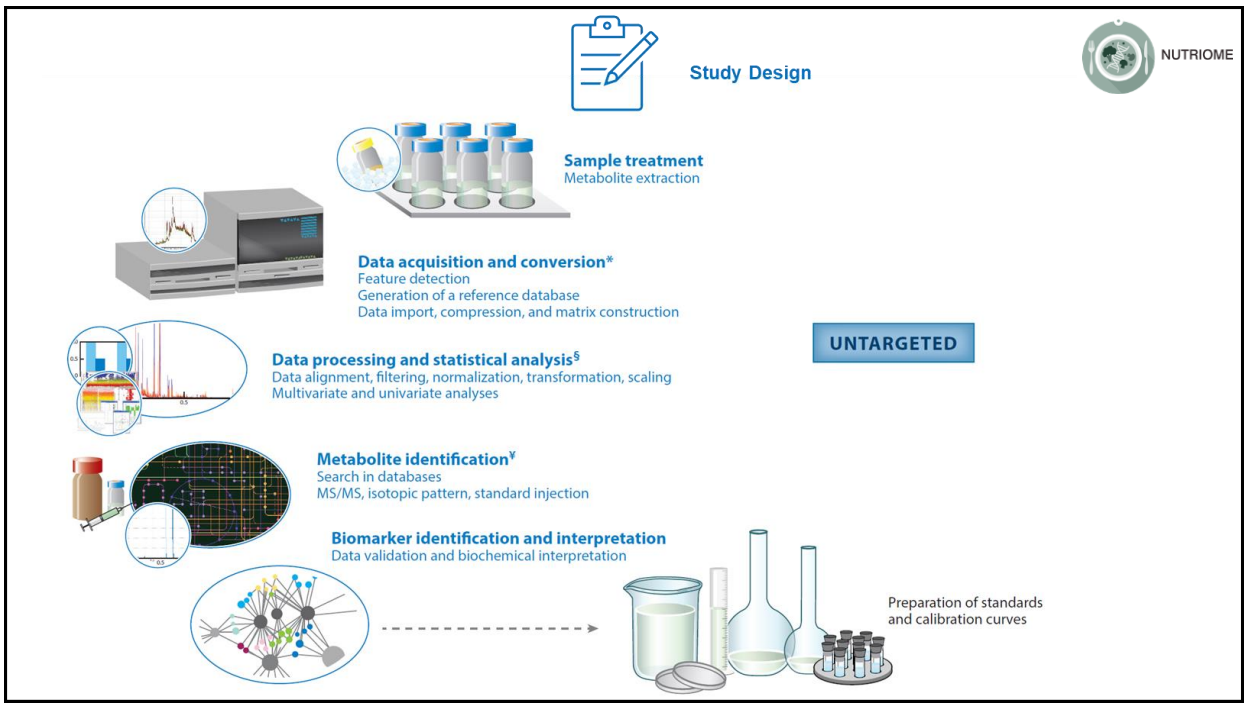
NUTRIOME

Data Format

Un-targeted

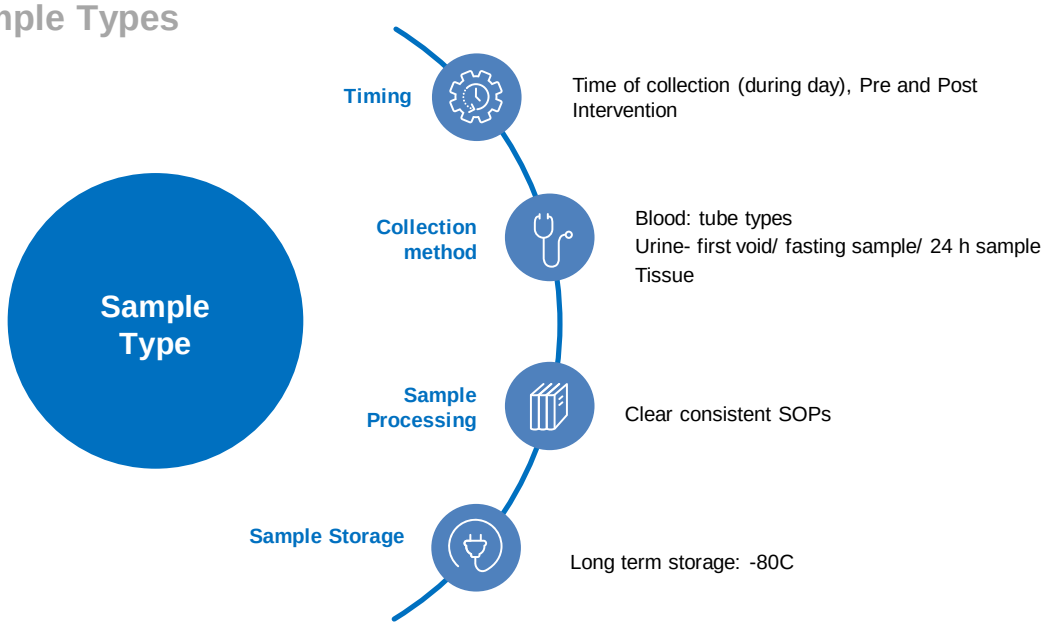
Compound Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
RT	0.543	0.551	0.586	0.586	0.59	0.591	0.592	0.595	0.597	0.604	0.605	0.611	0.616	0.623	0.624
Mass	155.0692	103.9641	196.0581	171.9075	166.0476	155.9339	218.0551	246.0502	216.0396	125.9988	186.0292	113.0589	180.0631	150.0527	301.0457
P100 V1 0h neg	3262	1541	119397	1063	3965	3396.5	4236	1856	6153.5	56552	5088	7109	9009	12818.5	1134
P100 V1 2h neg	3262	1541	90628	14178	3965	3396.5	4236	1856	6153.5	1764.5	5088	52467	9009	12818.5	1134
P100 V1 4h neg	3262	1541	178767	23173	3965	3396.5	4236	1856	6153.5	48314	5088	7109	9009	12818.5	1134
P100 V1 6h neg	3262	1541	10734	1063	3965	3396.5	4236	1856	6153.5	74181	5088	44811	9009	12818.5	1134
P100 V1 24h neg	3262	1541	10734	1063	3965	3396.5	4236	1856	6153.5	61569	5088	7109	9009	12818.5	15658
P102 V2 0h neg	34749	1541	77624	2126	104976	3396.5	4236	1856	69028	29558	5088	30851	120668	228586	12174
P102 V2 2h neg	22252	42764	24998	1063	20624	9740	18385	4484	32677	1764.5	23395	15908	18018	40522	2268
P102 V2 4h neg	12729	1541	32949	2328	28703	16898	21068	1856	40997	1764.5	5088	7109	9009	108444	1134
P102 V2 6h neg	10440	21988	72304	3322	57946	23795	30742	10753	58762	29674	5088	7109	67997	175683	4927
P102 V2 24h neg	17642	1541	111298	1063	76775	17374	4236	1856	6153.5	36856	5088	22362	191573	244794	12604
P105 V3 0h neg	34245	9329	10734	1063	3965	3396.5	4236	1856	48548	43415	10176	61865	62103	12818.5	1134

7



8

Sample Types



9

Biofluids

NUTRIOME

Blood: plasma, and serum

Plasma – need to decide on the anti coagulant and be consistent

Serum – clotting time and temperature needs to be consistent

Strict SOPs should be in place for collection of samples
some key items: time of collection
fasting state
consistent centrifugal speeds and temperature to be used

serum: clotting time
plasma: time from collection to processing

Storage: -80C and minimal freeze thaw cycles

10

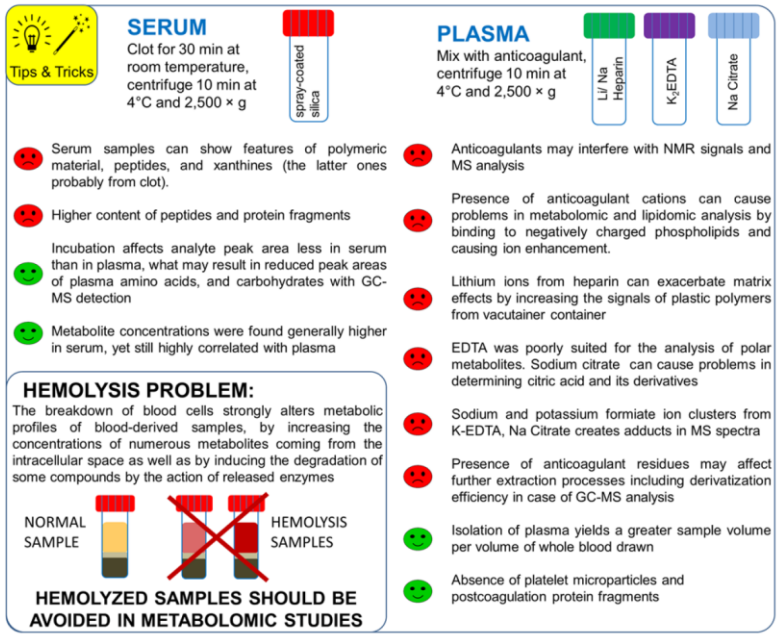
Blood: plasma, and serum

NUTRIOME

Key recommendations

- Design – decide on collection type (serum/ plasma)
- SOP for collection (clotting times, processing times, temperature, centrifugal speed)
- Storage: -80C
- Minimise Freeze Thaw cycles

11



NUTRIOME

Mol. Nutr. Food Res. **2018**, 1800384

12

Urine

- Urine is composed of endogenous and exogenous metabolites that have been filtered from the bloodstream by the kidneys
- Collected at home by participants (keep chilled)
- Issues to consider: first void/ mid stream/ time of day/ fasting
  24 h urine collection
- Processing: centrifugation before freezing
- Storage: -80C. Minimise freeze thaw cycles

13

Urine

Recommendations

- SOP – for collection – clear instructions to participants
- Processing prior to storage
- Storage: -80C
- Limit freeze thaw cycles

14

Feces

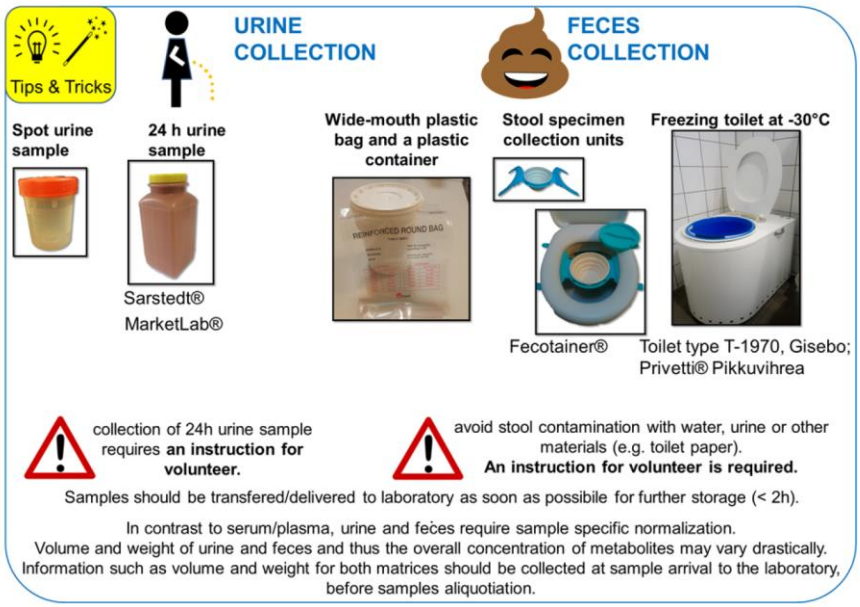
- Feces are considered a non-invasive proxy for the study of the intestinal microbiome and metabolome.
- The faecal metabolome- representation of host metabolism, microbial metabolism and host-microbial co metabolism

15

Feces

- Sample collection: (1) spot sample
  (2) bulk collection
- Specialised collection kit recommended
- Recent dietary intake recorded
- Clear instructions to volunteers
- Site of sampling needs to be consistent across the study
- Multiple sites if possible

16



NUTRIOME

Mol. Nutr. Food Res. **2018**, 1800384

17

NUTRIOME

Feces

Recommendations

- Bulk stool if possible (collection device consistent throughout study)
- Spot sampling may introduce variation
- Minimise freeze thaw
- Storage – at -80C recommended

18

NUTRIOME

Clinical Chemistry 64:8
1158–1182 (2018)

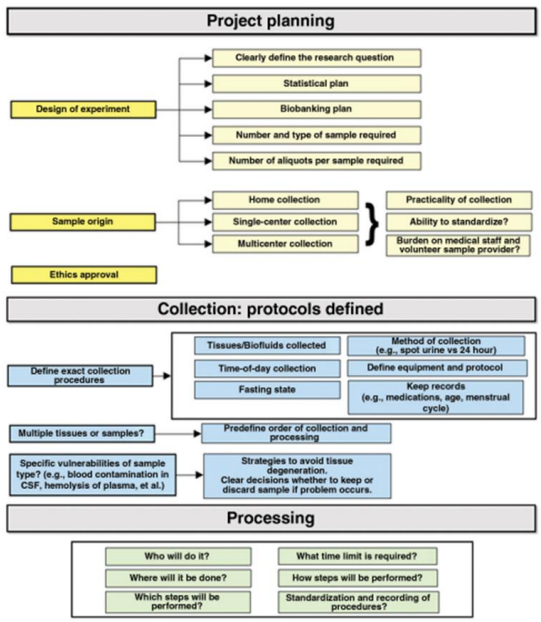
Review

Preanalytical Processing and Biobanking Procedures of Biological Samples for Metabolomics Research: A White Paper, Community Perspective (for "Precision Medicine and Pharmacometabolomics Task Group"—The Metabolomics Society Initiative)

Jennifer A. Kirwan,[1,2*] Lorraine Brennan,[3] David Broadhurst,[4] Oliver Fiehn,[5] Marta Cascante,[6] Warwick B. Dunn,[7] Michael A. Schmidt,[8,9] and Vidya Velagapudi[10*]

19

NUTRIOME



Kirwan et al. 2018

20

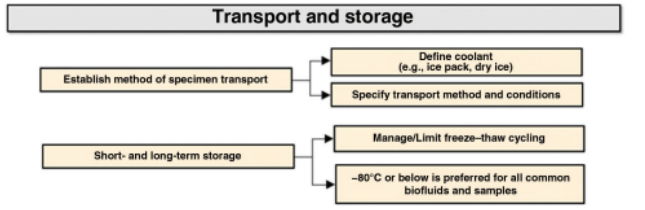


Fig. 1. Schematic of standard stages in planning tissue or body fluid collection for a biobank or tissue repository.

Kirwan et al. 2018

21

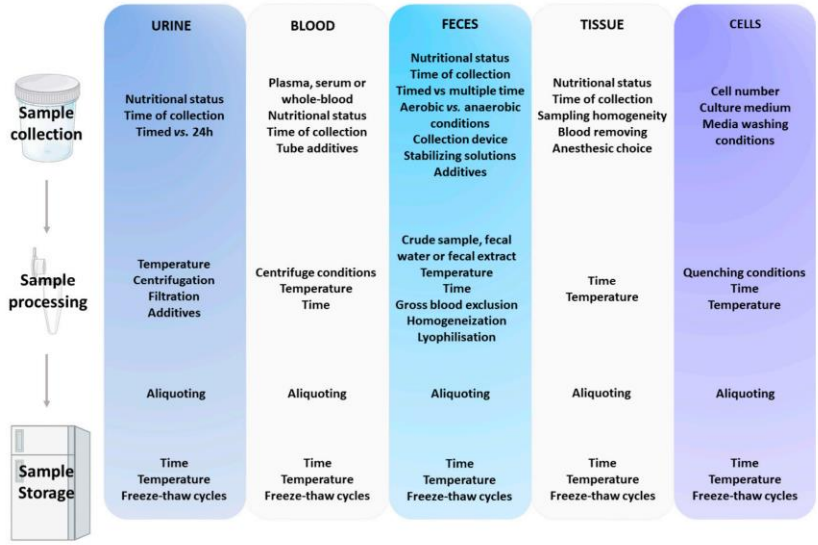


Figure 1. Summary of pre-analytical factors that can affect metabolite profiles in various matrices.

Smith et al 2020

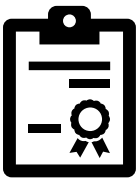
22

Statistical Plan

- Plan for analysis documented
- Data treatment
- Engage with statistician

23

Quality Control

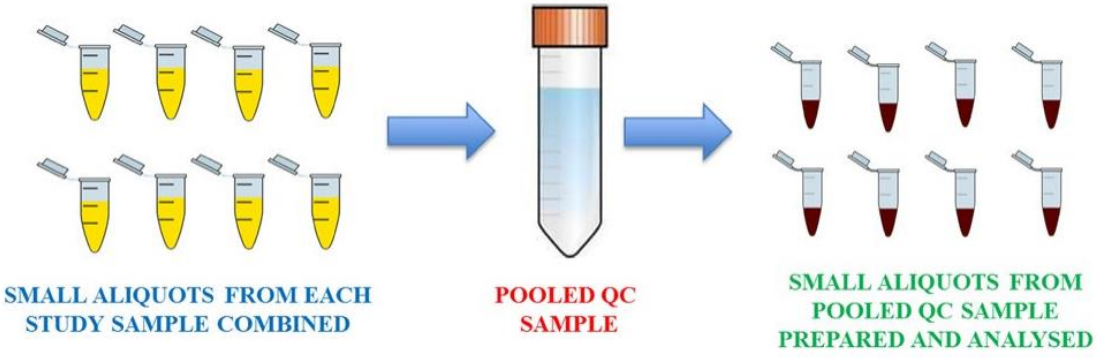


24

Quality Control

- Safeguards to be incorporated into a successful metabolomics study
- All samples that are to be directly compared to one another must be collected, processed, measured and statistically analysed in exactly the same way.
- Randomisation: samples should be extracted processed and measured in a random order.

25



Broadhurst et al. 2018 Metabolomics, 14, 72

26

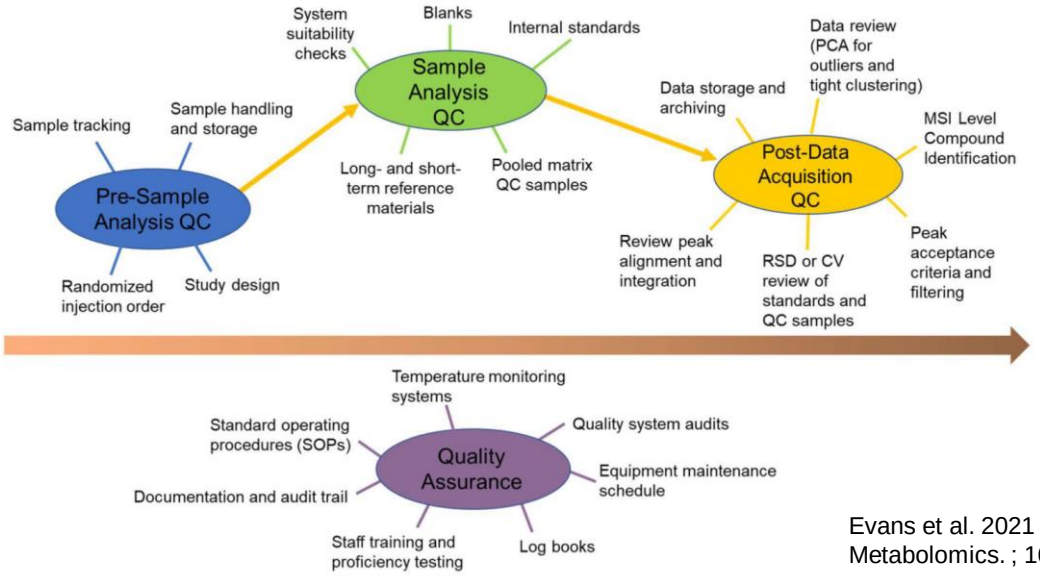
Standard Reference Material

a method to allow quality assessment across different laboratories

Injection Number	Sample Type
1	System Suitability Blank Sample
2	System Suitability Sample 1
3	System Conditioning QC sample 1
4	System Conditioning QC sample 2
5	System Conditioning QC sample 3
6	System Conditioning QC sample 4
7	Blank Extraction Sample 1
8	System Conditioning QC sample 5
9	System Conditioning QC sample 6
10	System Conditioning QC sample 7
11	System Conditioning QC sample 8
12	Pooled QC sample 9
13	Pooled QC sample 10
14	Standard Reference Material injection 1
15	Biological sample 1
16	Biological sample 2
17	Biological sample 3
18	Biological sample 4
19	Biological sample 5
20	Pooled QC Sample 11
21	Biological sample 6
22	Biological sample 7
23	Biological sample 8
24	Biological sample 9
25	Biological sample 10
26	Pooled QC Sample 12
27	Biological sample 11
28	Biological sample 12
29	Biological sample 13
30	Biological sample 14
31	Biological sample 15
32	Pooled QC Sample 13
33	Biological sample 16
34	Biological sample 17
35	Biological sample 18
36	Biological sample 19
37	Biological sample 20
38	Pooled QC Sample 16
39	Standard Reference Material injection 2

Broadhurst et al. 2018 Metabolomics, 14, 72

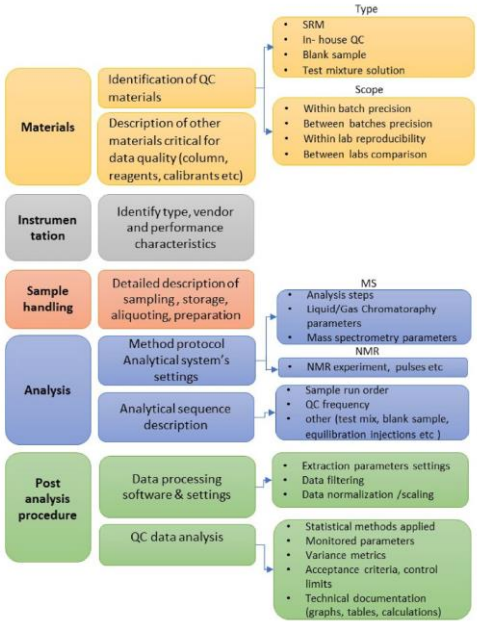
27



Evans et al. 2021 Metabolomics. ; 16(10)

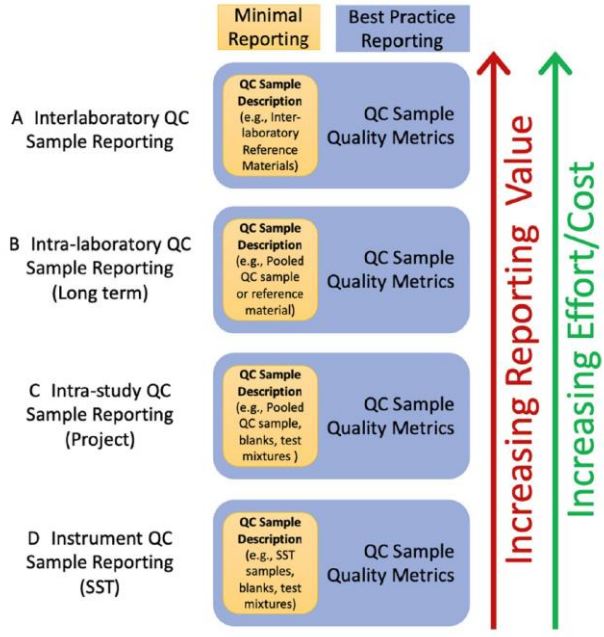
28

Information that should be documented in manuscripts



Kirwan et al 2022

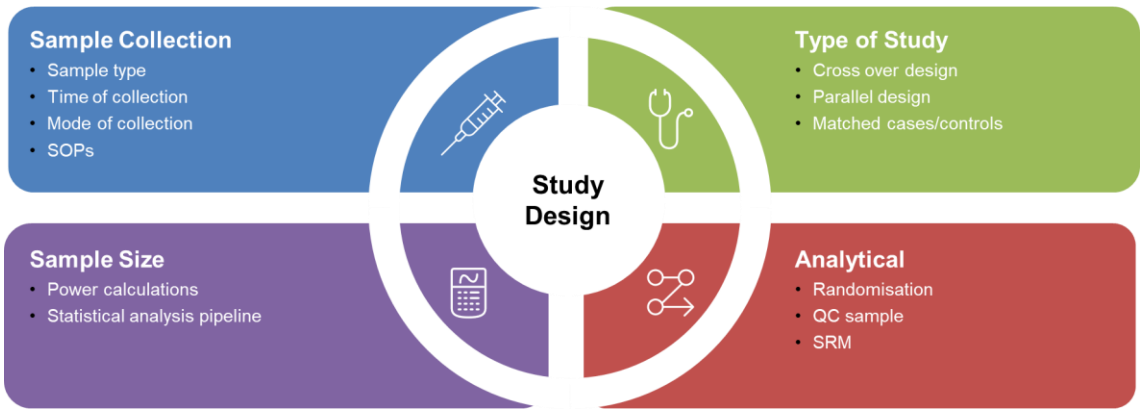
29



Kirwan et al 2022

30

Summary



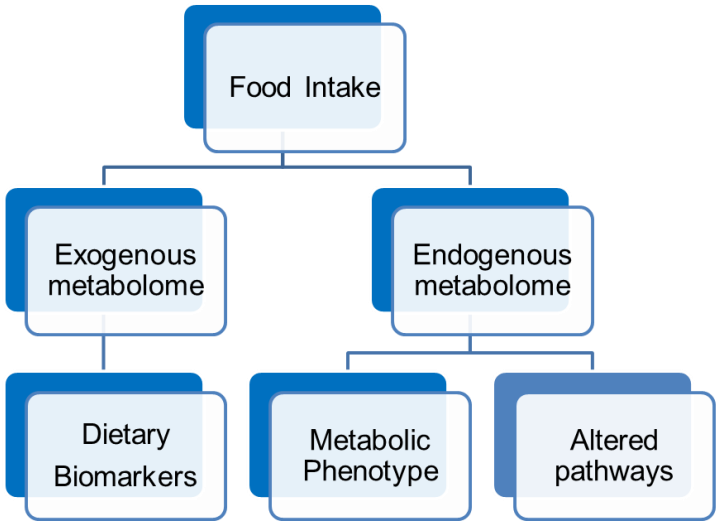
31

Metabolomics in nutrition

1. Biomarkers of food intake
2. Metabolic phenotyping / Precision Nutrition
3. Molecular understanding of impact of diets
4. Study of diet related diseases

32

Metabolomics in nutrition



33

Food Intake Biomarkers

34

NUTRIOME

Food Intake Assessment

Food diagries, FFQ, 24 hr recalls

Limitation-1
Measurements over short periods of time may be unrepresentative

Limitation-2
Recall of eating behaviour can be difficult

Limitation-3
Errors in reporting intakes

Limitation-4
Recording process alters dietary habits

35

NUTRIOME

Approach

Controlled Interventions
- Exact intakes

Dose Response
- Calibration curves

Determination of intake
- Independent studies

36

Food Intake Biomarkers

NUTRIOME

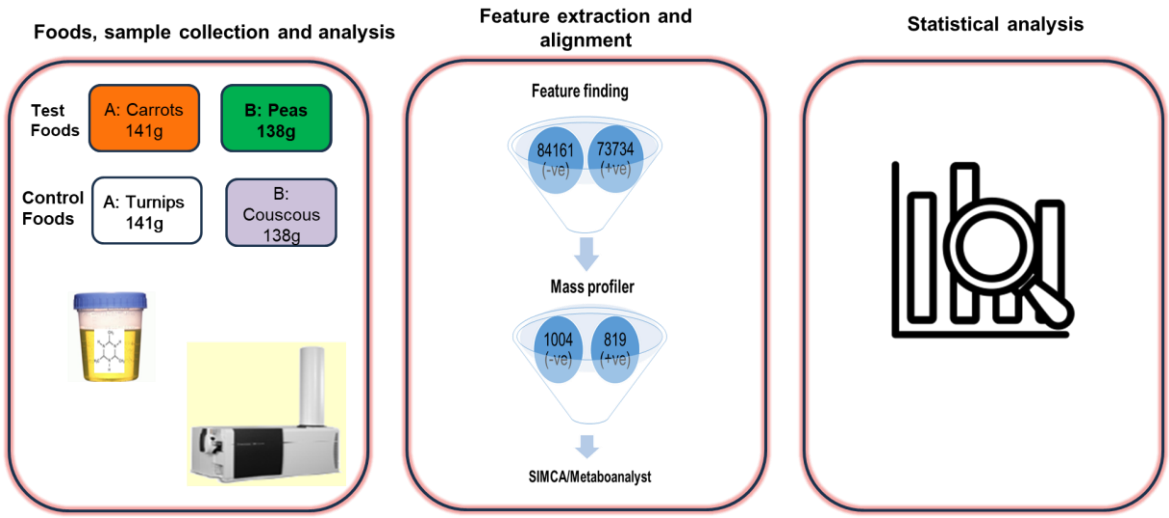
Identify biomarkers of legume intake

Confirmation of biomarkers

37

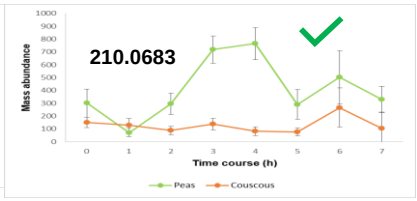
Legume biomarker discovery

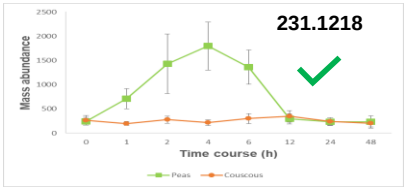
NUTRIOME

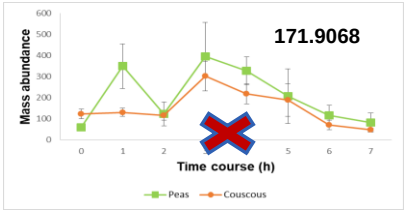


38

NUTRIOME







Accurate mass (M1 scan)

LC-MS/MS

Markers identified

2-Isopropylmalic acid (Level 1)

Asparaginyl valine (Level 2)

N-Carbamoyl-2-amino-2-(4-hydroxyphenyl)acetic acid (Level 2)

39

NUTRIOME

Biomarker Validation

Week 1
Low intake

1-week break

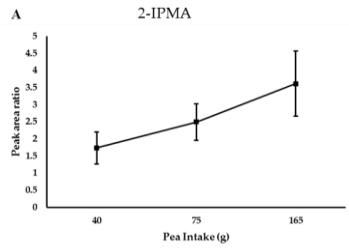
Week 2
Medium intake

1-week break

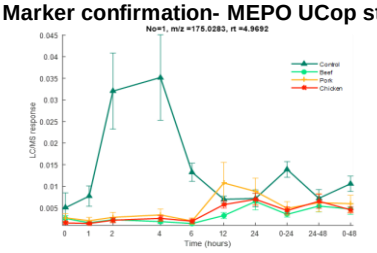
Week 3
High intake

- *Samples collected

Dose response study



Marker confirmation- MEPO UCop study



40

Biomarker Examples

NUTRIOME

Discovery and Validation of Banana Intake Biomarkers Using Untargeted Metabolomics in Human Intervention and Cross-sectional Studies

Natalia Vázquez-Manjarrez,[1,2] Christoph H Weinert,[3] Maria M Ulaszewska,[4] Carina I Mack,[3] Pierre Micheau,[1] Mélanie Pétéra,[5] Stephanie Durand,[5] Estelle Pujos-Guillot,[5] Björn Egert,[3] Fulvio Mattivi,[4,6] Achim Bub,[7] Lars Ove Dragsted,[2] Sabine E Kulling,[3] and Claudine Manach[1]

A metabolomic study of biomarkers of meat and fish intake[1,2]

*William Cheung,[3] Pekka Keski-Rahkonen,[3] Nada Assi,[3] Pietro Ferrari,[3] Heinz Freisling,[3] Sabina Rinaldi,[3] Nadia Slimani,[3] Raul Zamora-Ros,[3] Milena Rundle,[4] Gary Frost,[4] Helena Gibbons,[6] Eibhlin Carr,[6] Lorraine Brennan,[6] Amanda J Cross,[5] Valeria Pala,[7] Salvatore Panico,[8] Carlotta Sacerdote,[9] Domenico Palli,[10] Rosario Tumino,[11] Tilman Kühn,[12] Rudolf Kaaks,[12] Heiner Boeing,[13] Anna Floegel,[13] Francesca Mancini,[14,15] Marie-Christine Boutron-Ruault,[14–16] Laura Baglietto,[17,18] Antonia Trichopoulou,[19,20] Androniki Naska,[19,20] Philippos Orfanos,[19,20] and Augustin Scalbert[1]**

REVIEW — Open Access

Biomarkers of cereal food intake

Rikard Landberg[1*], Kati Hanhineva[2], Kieran Tuohy[3], Mar Garcia-Aloy[4,5], Izabela Biskup[1], Rafael Llorach[4,5], Xiaofei Yin[6], Lorraine Brennan[6] and Marjukka Kolehmainen[2]

Ulaszewska *et al. Genes & Nutrition* (2018) 13:29
https://doi.org/10.1186/s12263-018-0620-8

Genes & Nutrition

REVIEW — Open Access

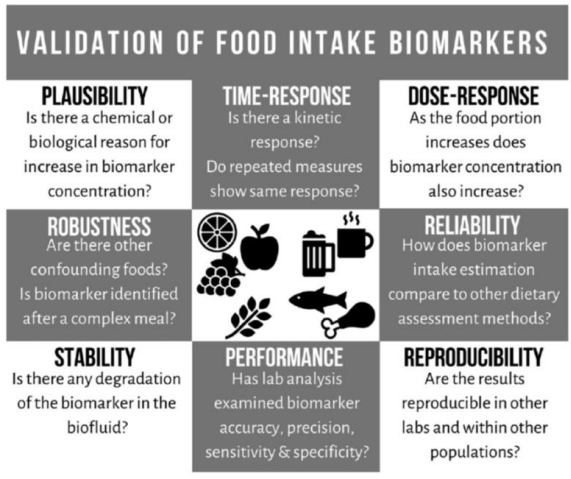
Food intake biomarkers for apple, pear, and stone fruit

Marynka Ulaszewska[1†], Natalia Vázquez-Manjarrez[2,3†], Mar Garcia-Aloy[4,5], Rafael Llorach[4,5], Fulvio Mattivi[1,6], Lars O. Dragsted[2], Giulia Praticò[2] and Claudine Manach[2*]

41

Biomarker Validation

NUTRIOME



JPI a healthy diet for a healthy life

FOODBALL The Food Biomarker Alliance

Dragsted et al 2018

42

NUTRIOME

How do we use the biomarkers?

43

NUTRIOME

- Measure of adherence to the dietary intervention
- Objective measures of dietary intake
- Correct self-reported data
- Relationships with health/disease parameters

44

NUTRIOME

Mol. Nutr. Food Res. 0, 0, 2017, 1700037 DOI 10.1002/mnfr.201700037 (1 of 8) 1700037

RESEARCH ARTICLE

Demonstration of the utility of biomarkers for dietary intake assessment; proline betaine as an example

Helena Gibbons[1], Charlotte J. R. Michielsen[1], Milena Rundle[2], Gary Frost[2], Breige A. McNulty[1], Anne P. Nugent[1], Janette Walton[3], Albert Flynn[3], Michael J. Gibney[1] and Lorraine Brennan[1]

[1] School of Agriculture and Food Science, Institute of Food and Health, University College Dublin, Dublin, Ireland

[2] Nutrition and Dietetic Research Group, Division of Endocrinology and Metabolism, Imperial College London, London, U.K.

[3] School of Food and Nutritional Sciences, University College Cork, Cork, Ireland

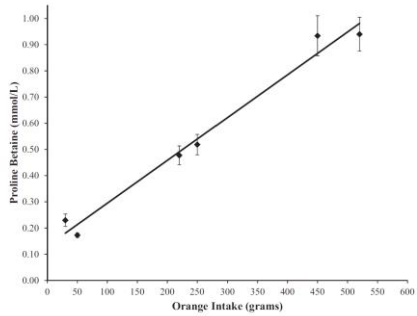
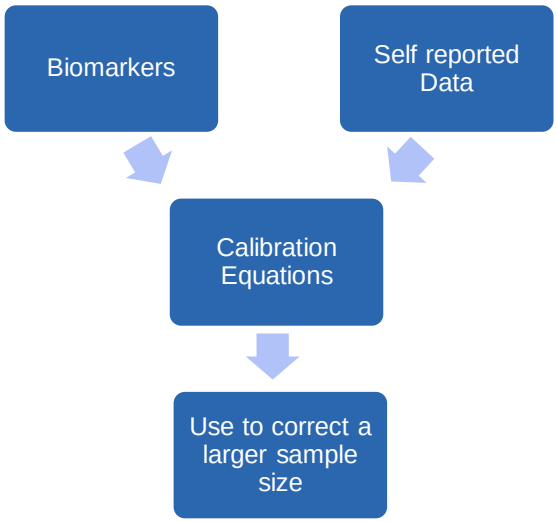


Figure 1. Calibration curve using the 24 h urine samples (mean and error bars (SEM) are presented). The x-axis; actual orange juice intake (grams) during the NutriTech food intake study, the y-axis; proline betaine concentrations measured in urine (mmol/L). Each point represents average proline betaine concentration for a particular orange juice intake. ($Y = 1.63 \times 10^{-03} * X + 1.31 \times 10^{-01}$).

45

NUTRIOME

Calibration method



46

Calibration method

NUTRIOME

Citrus intake data

- $i = 1, \ldots, n$: subject in the study (with $n = 565$);
- W_i : mean daily self-reported citrus intake (g/day);
- M_i : biomarker-derived estimates of citrus intake (g/day).

The aim is to **estimate** :

- $\widehat{X}_i$: calibrated mean daily citrus intake (g/day).

CALIBRATION EQUATIONS:

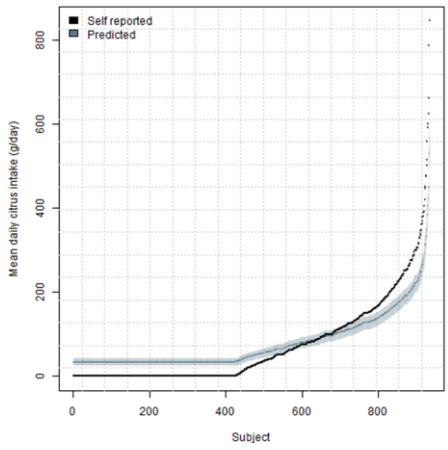
$$M_i = \beta_0 + \beta_1 W_i + \epsilon_i,$$

$$\widehat{X}_i = \widehat{\beta_0} + \widehat{\beta_1} W_i$$

With $\epsilon_i \sim N(0, \sigma^2)$.

47

NUTRIOME

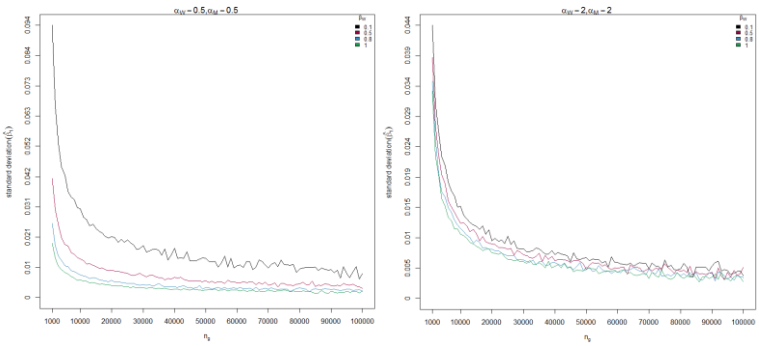


- Developed an on-line App
- https://adiet.shinyapps.io/Bio-Intake/

D'Angelo et al. 2019 AJCN

48

How much biomarker data do you need ?



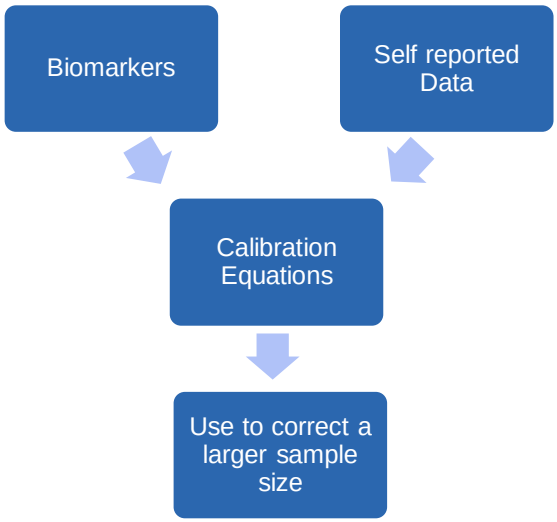
The figures above present the variability of the estimated slope coefficient $\widehat{\beta_1}$ in the "best" and "worst" case scenarios.

Biomarker data needed for approx. 20-30% of the population

D'Angelo et al. 2019 AJCN

49

Calibration method



D'Angelo et al. 2019 AJCN

50

Calibration method

NUTRIOME

The Journal of Nutrition
Nutritional Epidemiology

Biomarker-Calibrated Red and Combined Red and Processed Meat Intakes with Chronic Disease Risk in a Cohort of Postmenopausal Women

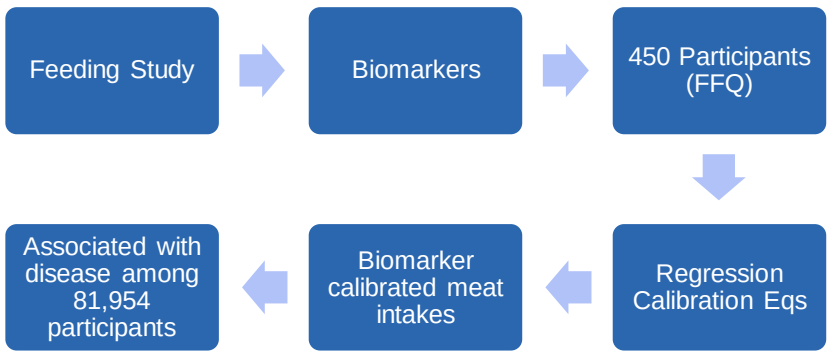
Cheng Zheng,[1] Mary Pettinger,[2] GA Nagana Gowda,[3] Johanna W Lampe,[2,4] Daniel Raftery,[3] Lesley F Tinker,[2] Ying Huang,[2,4] Sandi L Navarro,[2] Diane M O'Brien,[5] Linda Snetselaar,[6] Simin Liu,[7] Robert B Wallace,[6] Marian L Neuhouser,[2,4] and Ross L Prentice[2,4]

[1]Department of Biostatistics, University of Nebraska Medical Center, Omaha, NE, USA; [2]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [3]Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA; [4]School of Public Health, University of Washington, Seattle, WA, USA; [5]Institute for Arctic Biology, University of Alaska, Fairbanks, AK, USA; [6]College of Public Health, University of Iowa, Iowa City, IA, USA; and [7]Department of Epidemiology, School of Public Health, Brown University, Providence, RI, USA

51

Calibration method

NUTRIOME



52

Calibration method

NUTRIOME

TABLE 5 T2D incidence HRs, and 95% CIs for a 40% increment above the median intake for red meat and for red plus processed meat in a Women's Health Initiative cohorts of 81,954 postmenopausal US women enrolled during 1993–1998 at 40 US clinical centers and followed through February 2020[1]

	No adjustment for BMI or dietary correlates				With adjustment for BMI and dietary correlates			
Outcome (n = participants with outcome)	Red meat intake, g/d	*P* value	Red + processed meat intake, g/d	*P* value	Red meat intake, g/d	*P* value	Red + processed meat intake, g/d	*P* value
T2D (12,145)	1.37 (1.34, 1.39)	<0.001	1.35 (1.33, 1.37)	<0.001	1.08 (1.04, 1.12)	<0.001	1.09 (1.05, 1.13)	<0.001

[1]Values are HRs (95% CIs). Red meat median intakes were 24.7 g/d with biomarker calibration and 31.8 g/d without biomarker calibration, and red plus processed meat median intakes were 34.8 g/d with biomarker calibration and 42.2 g/d without biomarker calibration. DM-C, dietary modification comparison group; OS, observational study; T2D, type 2 diabetes.
[2]HR estimates and 95% CIs are based on Cox models with baseline hazard rates stratified on study component (DM-C or OS), hormone therapy trial status (estrogen plus progestin, estrogen plus progestin placebo, estrogen-alone, estrogen-alone placebo, not randomized), age at enrollment (50–54, 55–59, 60–64, 65–69, 70–74, or ≥75 y), and with adjustment for a disease-specific set of potential confounding factors.

53

Calibration method

NUTRIOME

The Journal of Nutrition
Nutritional Epidemiology

Associations of Biomarker-Calibrated Healthy Eating Index-2010 Scores with Chronic Disease Risk and Their Dependency on Energy Intake and Body Mass Index in Postmenopausal Women

Marian L Neuhouser,[1] Mary Pettinger,[1] Lesley F Tinker,[1] Cynthia Thomson,[2] Linda Van Horn,[3] Bernhard Haring,[4] James M Shikany,[5] Marcia L Stefanick,[6] Ross L Prentice,[1] JoAnn E Manson,[7] Yasmin Mossavar-Rahmani,[8] and Johanna W Lampe[1]

54

Calibration method

NUTRIOME

TABLE 4 Associations between a 20% increment in HEI-2010 score with and without biomarker calibrations with additional for calibrated energy and adjustment BMI and type 2 diabetes in the WHI[1]

		HR (95% CI)[2]				
	No. of cases (rate per 1000 person-years)	Uncalibrated	Calibrated[3]	Calibrated, adjusted for calibrated total energy[4]	Calibrated, adjusted BMI	Calibrated, adjusted for calibrated total energy and BMI
Type 2 diabetes[5]	14,201 (10.14)	0.91 (0.90, 0.93)	0.45 (0.36, 0.55)	0.84 (0.70, 1.02)	0.85 (0.74 0.97)	0.87 (0.75, 0.99)

[1]CT, Clinical Trial; CVD, cardiovascular disease; FFQ, food-frequency questionnaire; E, estrogen; HEI, Healthy Eating Index; OS, Observational Study; P, progesterone; WHI, Women's Health Initiative.
[2]Cox proportional regression models stratified by 5-y age categories, study component (CT/OS), and HT(hormone therapy) trial arm, and adjusted for linear age, race/ethnicity (White, Black, Hispanic, other race/ethnicity), season of FFQ completion, education (≤high school, some post-high school, college degree or higher), income (<$20K, $20K to <$35K, $35K to <$50K, $50K to <$75K, ≥$75 K), current smoking, alcohol intake (nondrinker, <1 drink/wk, 1 to <7 drinks/wk, ≥7 drinks/wk), total recreational physical activity, dietary supplement use, postmenopausal hormone therapy (never, past, current E-alone, current E+P), history of CVD, and family history of diabetes.
[3]HEI-2010 scores are biomarker calibrated.
[4]Adjustment for calibrated total energy using doubly labeled water.
[5]Incidence.

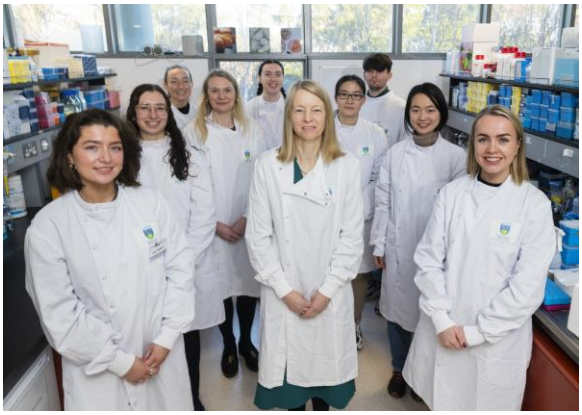
55

Conclusions

NUTRIOME

- Metabolomics – wide range of applications in nutrition research
- Precision Nutrition – metabotypes
- Food Intake Biomarkers

56



Dr Aoife O'Gorman
Claire Connolly
Fionnuala McDermott

Wenxuan Li
Rebecca Finlay
Max Ammann
Tilde Martinsen

Shubbham Gupta
Dr Xiaofei Yin
Dr Ya Gao
Aoife Courtney

NUTRIOME

Collaborators

HELMHOLTZ MUNICH

Prof. Jakob Linseisen
Dr. Anna Riedl

PROMED-COG: C MvEvoy, F Prinelli, C Trevisan
NANS: A Flynn, M Gibney, A Nugent, B McNulty, J Walton
FoodBall Partners
Food4me Partners
US-Ireland: Jayne Woodside (QUB), Frank Hu (Harvard) Qi Sun (Harvard)

Funding Agencies

CAPES HRB erc

Department of Agriculture, Fisheries and Food
An Roinn Talmhaíochta, Iascaigh agus Bia

European Commission

57