Metabolic Signatures of Adherence to the Mediterranean Diet

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Dietary biomarkers

Measuring habitual dietary intake should be both accurate and applicable to large numbers of free-living individuals, hence measuring dietary exposure is one of the greatest challenges in nutritional research.

Population-based nutritional studies, in which food intake is not accurately defined and controlled, have traditionally evaluated the nutritional status using practical *self-reporting tools* such as FFQs, dietary records, and 24-h recall. However, all of these are subject to *measurement error* and *recall bias*.

O'Gorman A, Brennan L. The role of metabolomics in determination of new dietary biomarkers. Proc Nutr Soc. 2017 Aug;76(3):295-302.

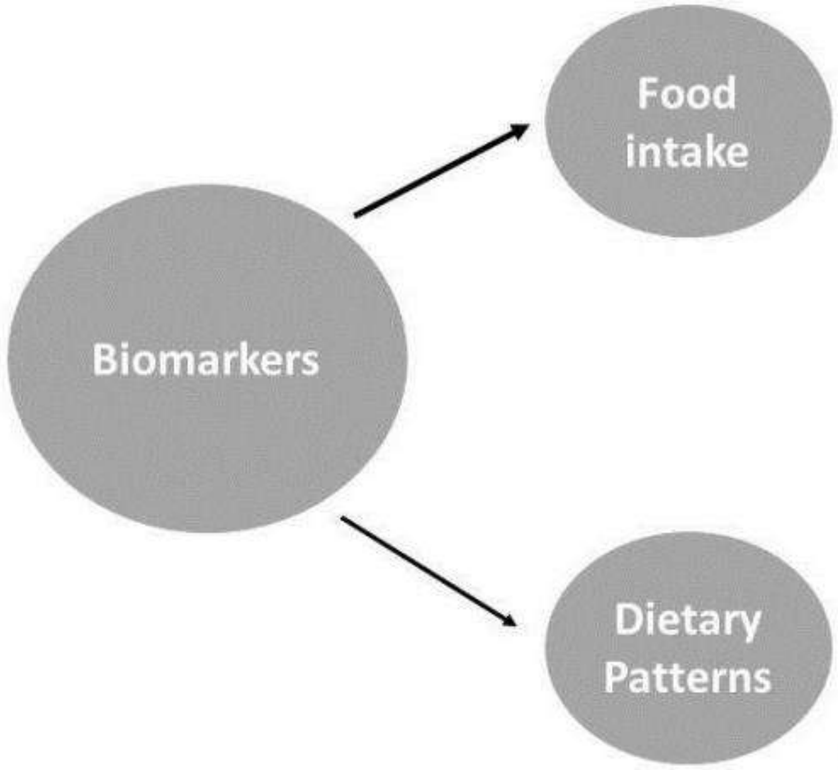
Dietary biomarkers

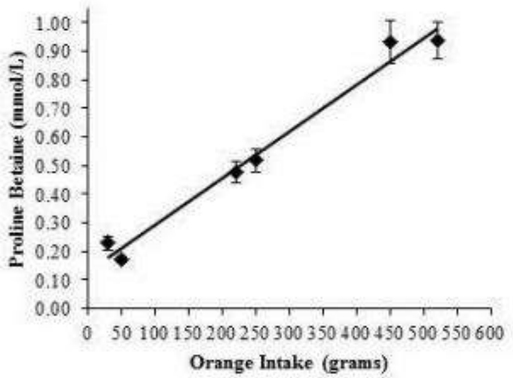
Another issue in interpreting the findings of dietary intervention is *compliance assessments*.

“Every 15 days, volunteers will be requested for an interview with the dietitian to monitor the compliance with the intervention and to *pick up the almond sachets* (for intervention group). We will also assess compliance with the almond consumption through measuring changes in *plasma concentrations of alpha-tocopherol* assessed by liquid chromatography.”

O'Gorman A, Brennan L. The role of metabolomics in determination of new dietary biomarkers. Proc Nutr Soc. 2017 Aug;76(3):295-302.

Dietary biomarkers





Metabolic profile

Dietary pattern classification

Brennan L, Hu FB. Metabolomics-Based Dietary Biomarkers in Nutritional Epidemiology-Current Status and Future Opportunities. Mol Nutr Food Res. 2019 Jan;63(1):e1701064.

Dietary biomarkers

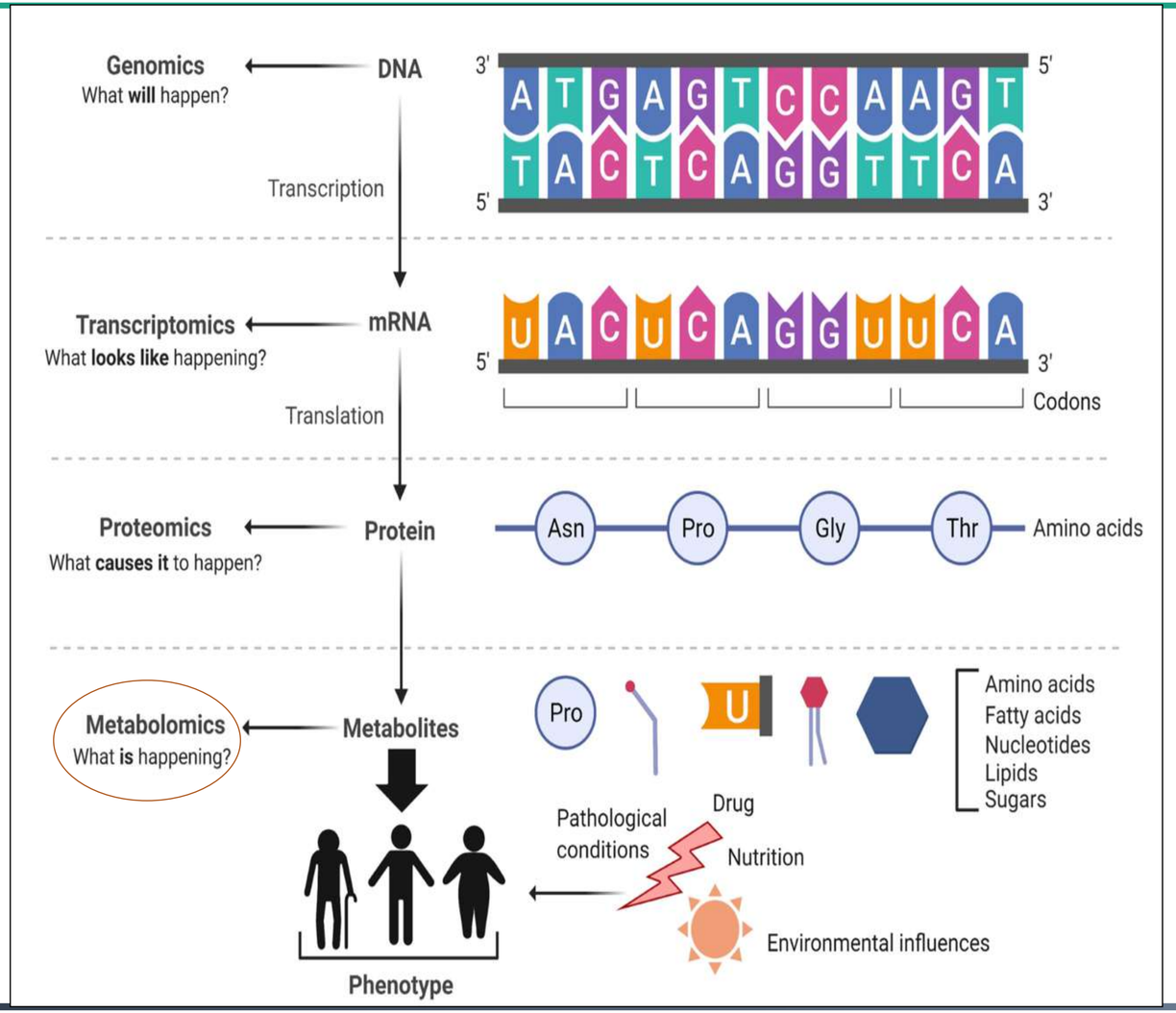
A single biomarker may be insufficient to fully represent the *complex* and *multi-dimensional nature* of dietary patterns.

Metabolomics can provide a comprehensive picture of overall dietary intake by measuring the full profile of small molecule metabolites in biological samples.

Nutritional metabolomics can be applied to discover new *biomarkers* of nutritional exposure and status and can help disentangle the *molecular mechanisms* by which diet affects health and disease.

Guasch-Ferré M, Bhupathiraju SN, Hu FB. Use of Metabolomics in Improving Assessment of Dietary Intake. Clin Chem. 2018 Jan;64(1):82-98.

The “omics” cascade



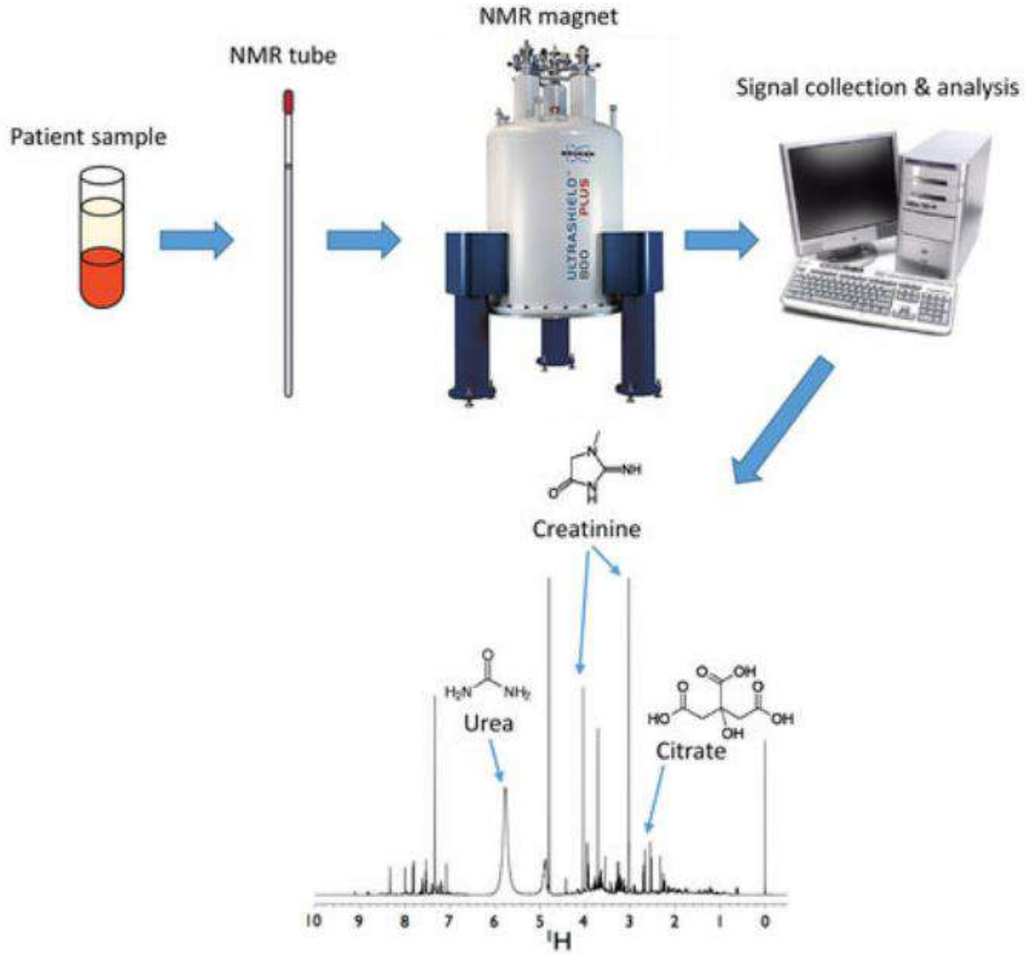
Untargeted vs Targeted Metabolomics

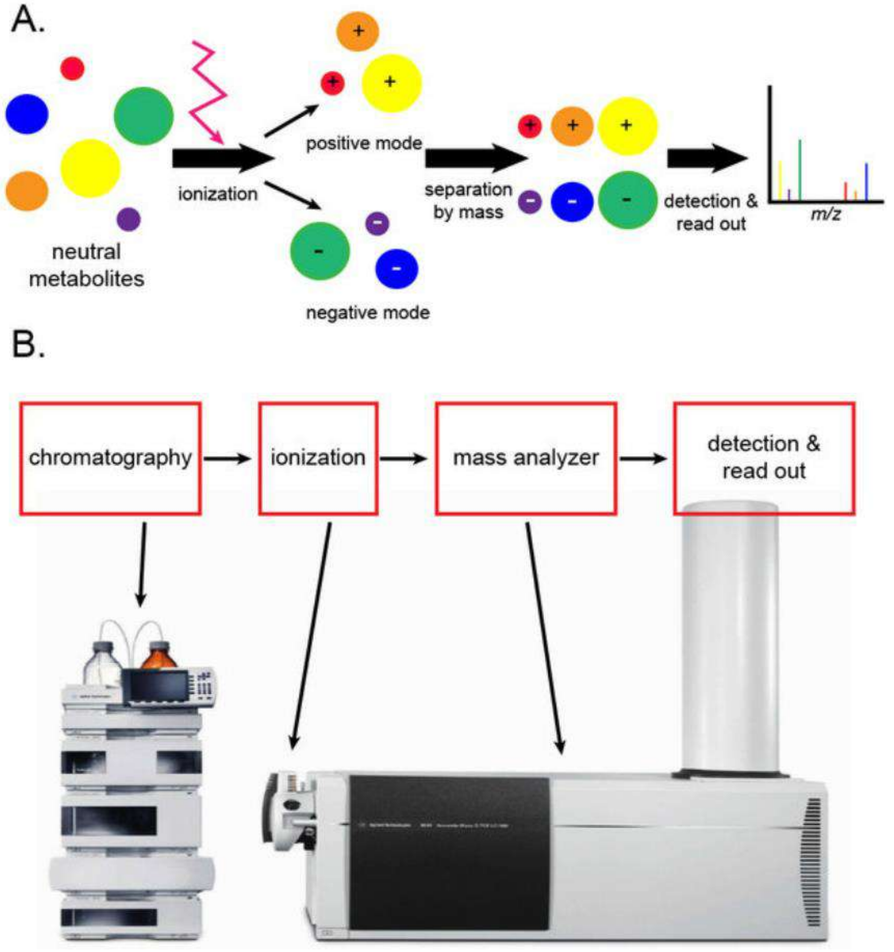
- *Untargeted metabolomics approaches involve global profiling of the metabolome*. This approach is typically employed in hypothesis-generating studies such as biomarker discovery.

- *Targeted metabolomics refers to the quantitative measurement of a select group of metabolites* (e.g. amino acids, lipids, sugars, and/or fatty acids) in order to investigate specific metabolic pathways or to validate biomarkers identified using untargeted metabolic profiling.

Roberts LD, et al. Targeted metabolomics. Curr Protoc Mol Biol. 2012; Chapter 30:Unit 30.2.1–24.;
Vinayavekhin N, Saghatelian A. Untargeted metabolomics. Curr Protoc Mol Biol. 2010; Chapter 30:Unit 30.1.1–24.

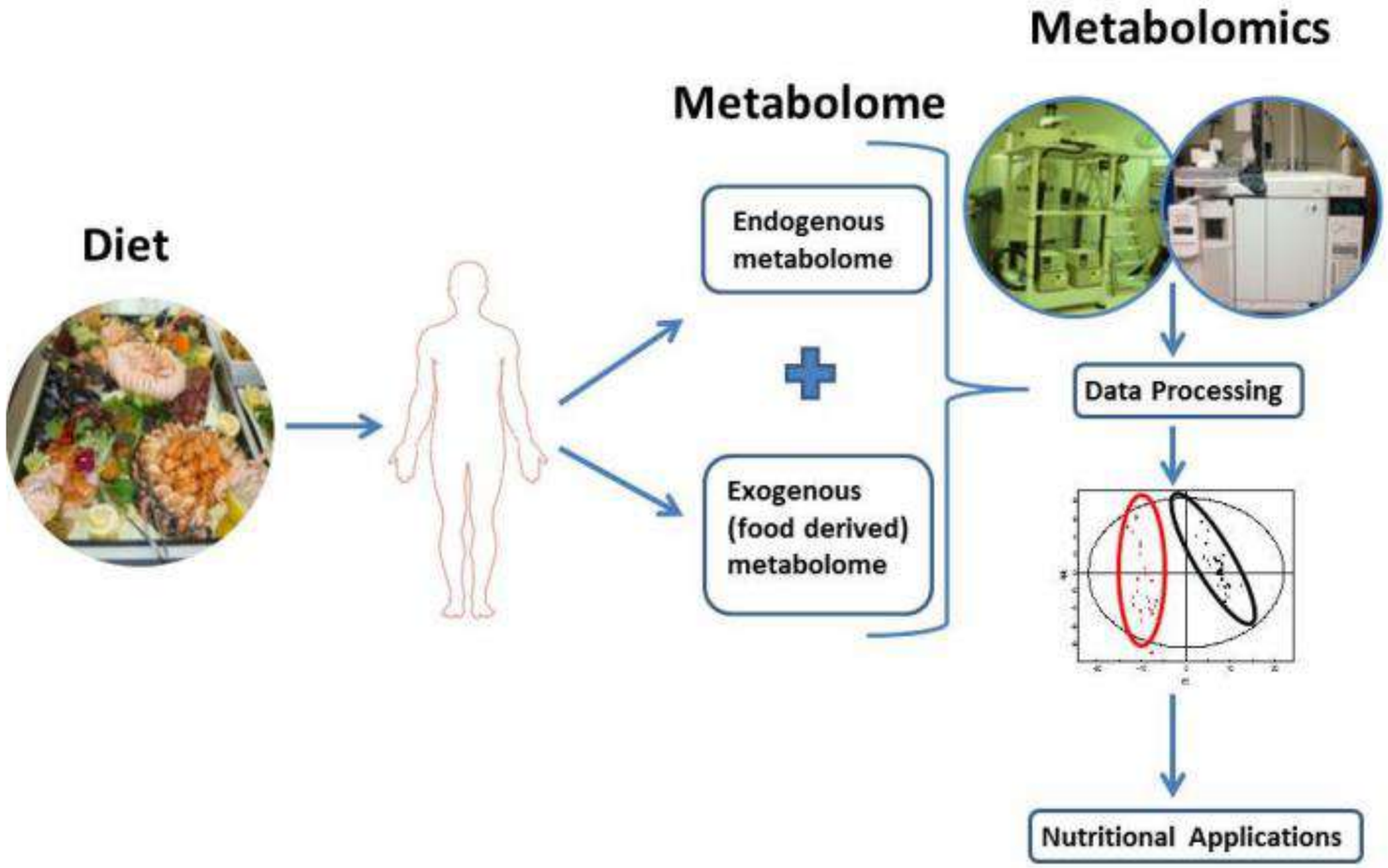
Analysis of metabolites by NMR and MS





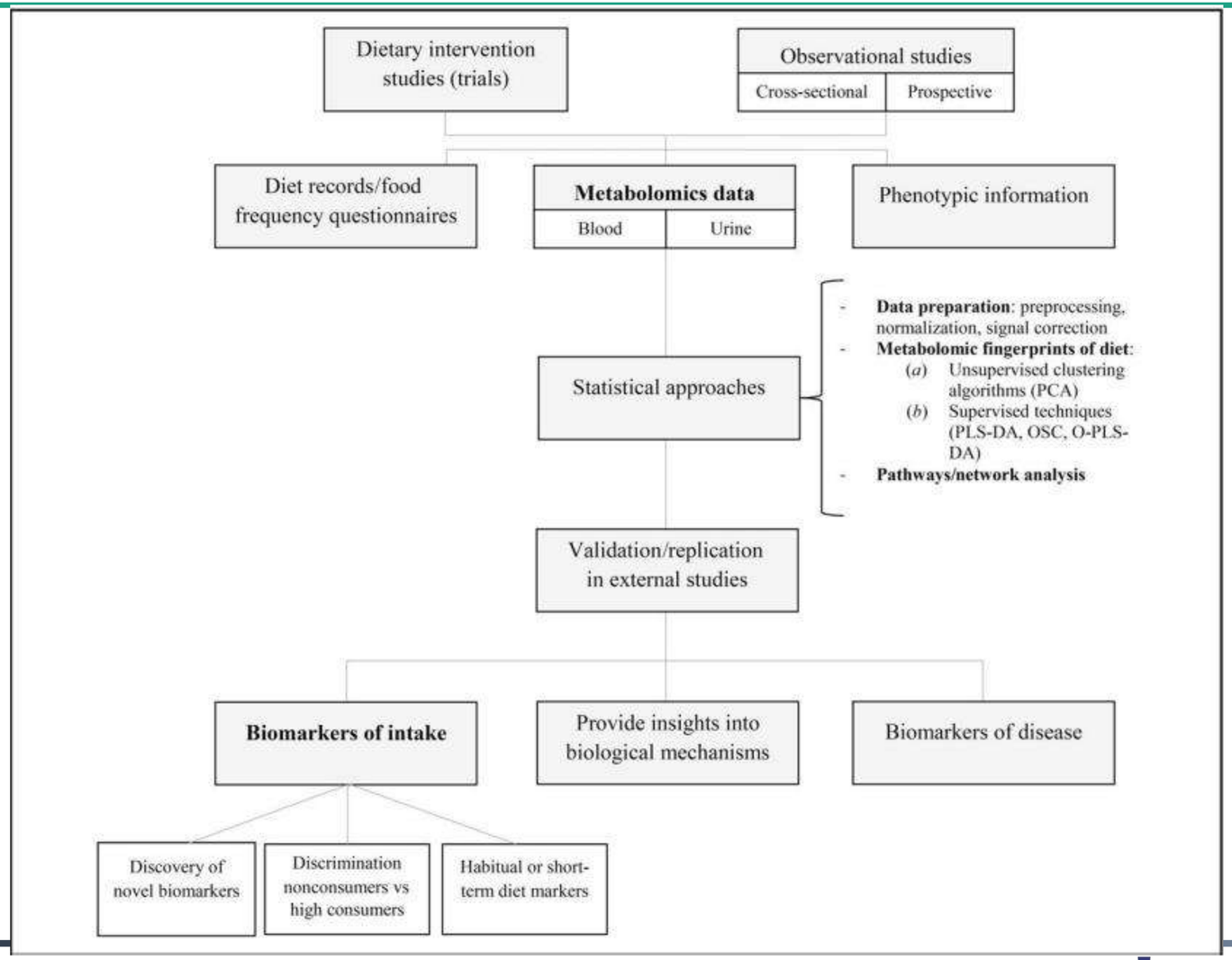
Stringer KA, et al. Metabolomics and Its Application to Acute Lung Diseases. Front Immunol. 2016 Feb 29;7:44.

Metabolomic applications in nutritional research



O'Gorman A, Brennan L. Metabolomic applications in nutritional research: a perspective. J Sci Food Agric. 2015 Oct;95(13):2567-70.

Work flow of nutritional metabolomics approaches

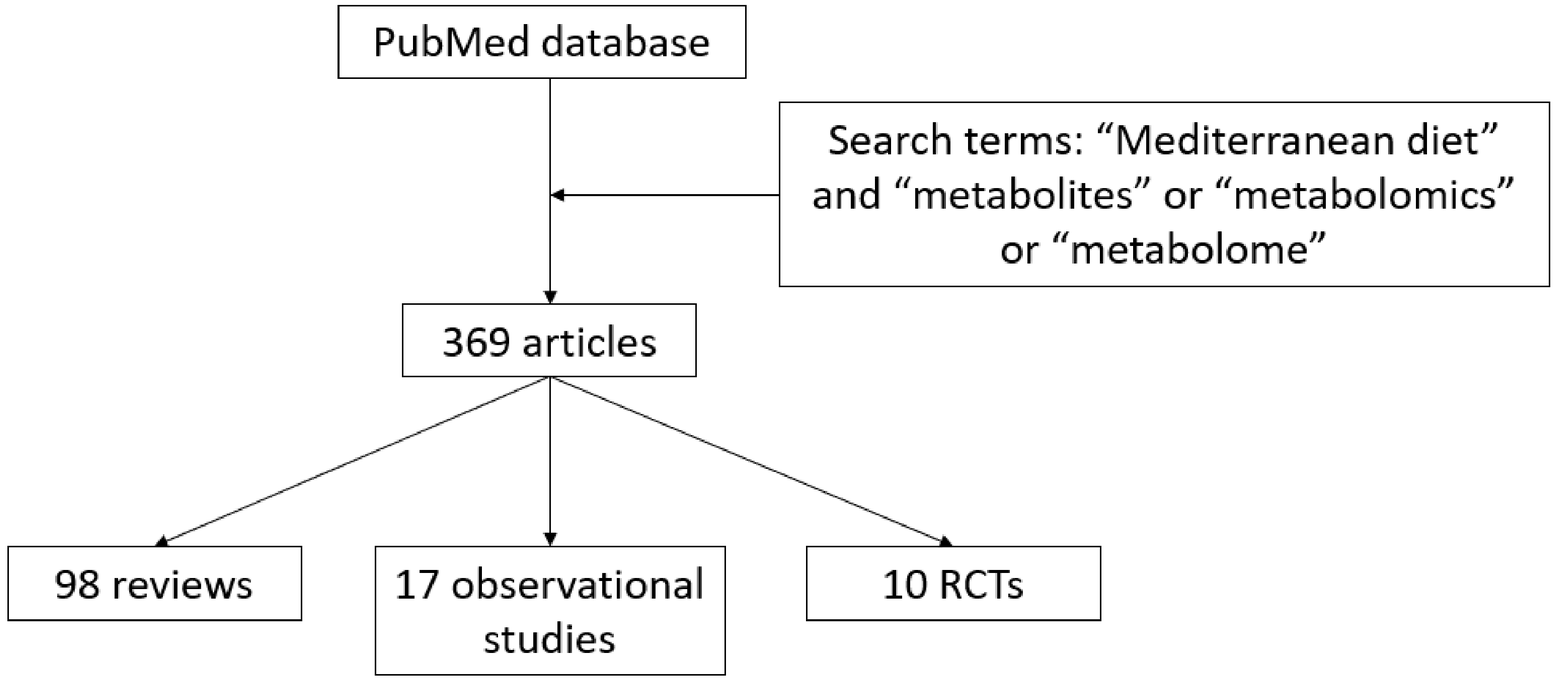


Guasch-Ferré M, et al. Clin Chem. 2018 Jan;64(1):82-98.

Even though the Mediterranean diet has been recognized as a powerful strategy to improve health, the accurate assessment of exposure to this diet has been a major challenge in epidemiological and clinical studies

Literature Search

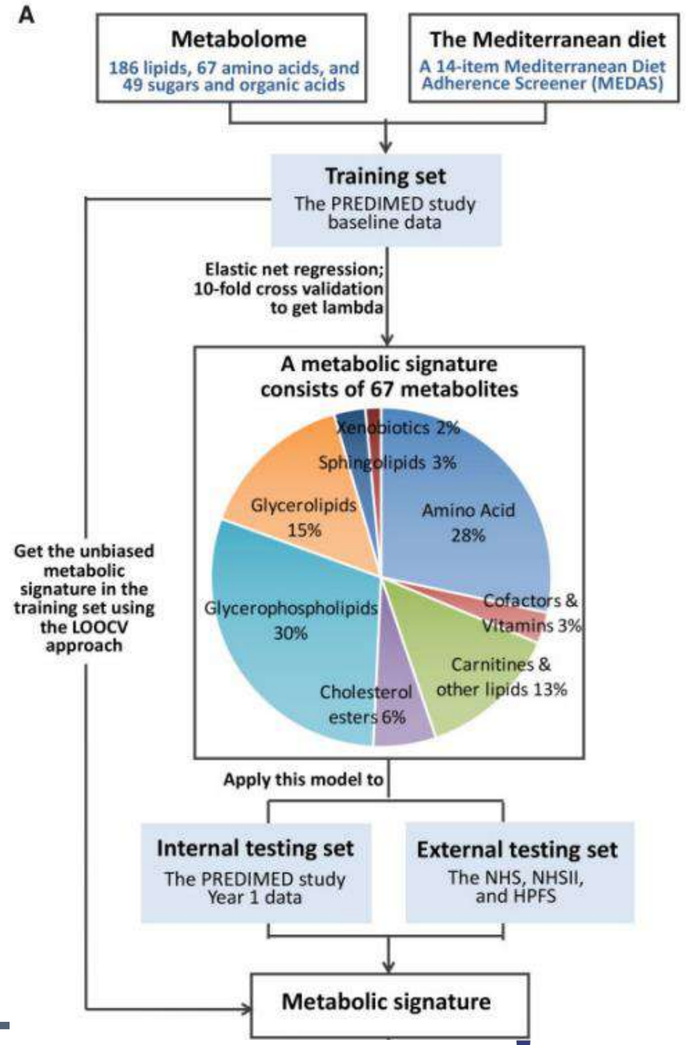
- I summarized the evidence from observational studies and randomized controlled trials (RCTs) investigating the impact of the Mediterranean diet on metabolome in adults.
- A comprehensive literature search was conducted in PubMed (last accessed 6th of September 2023).



The Mediterranean diet, plasma metabolome, and cardiovascular disease risk

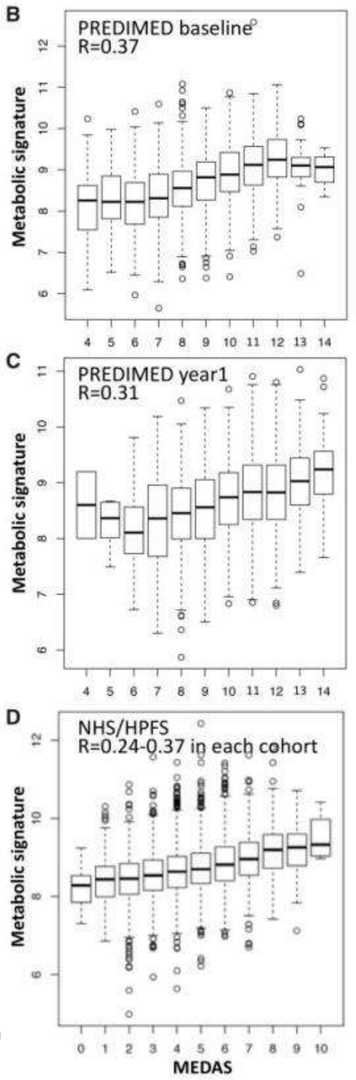
"In the present study, leveraging dietary and LC–MS/MS derived metabolomic data in the Spanish PREDIMED trial and three US prospective cohorts (the Nurses' Health Study [NHS], NHSII, and Health Professionals Follow-Up Study [HPFS]), we identified and validated a metabolic signature reflecting adherence to a Mediterranean diet."

Li J, et al. Eur Heart J. 2020 Jul 21;41(28):2645-2656.



The Mediterranean diet, plasma metabolome, and cardiovascular disease risk

- The metabolic signature was trained using PREDIMED baseline measurements and tested with PREDIMED year-1 (interval validation) and the NHS/HPFS baseline measurements (external validation).
- In the training set, the unbiased metabolic signature acquired using the leave-one-out cross-validation approach was significantly correlated with MEDAS with a similar magnitude (r = 0.37). At PREDIMED year-1 (internal testing set) and NHS/HPFS baseline (external testing set), we found that the metabolic signature was significantly correlated with MEDAS (PREDIMED year-1: r = 0.31; NHS/HPFS: r = 0.28).



Li J, et al. Eur Heart J. 2020 Jul 21;41(28):2645-2656.

The Mediterranean diet, plasma metabolome, and cardiovascular disease risk

- The blood metabolome reflects the overall metabolic homeostasis resulting from the interactive effects of all metabolism-influencing factors, including diet and genetics.
- Self-reported dietary instruments (MEDAS) is a combination of true dietary intakes and reporting errors.
- The metabolome can be changed by diet but is expected to be independent of reporting errors (A).
- By regressing MEDAS on metabolites (B formula #3), the resulting metabolic signature captured cumulative changes in the metabolome that are correlated with dietary adherence, incorporated individual metabolic variations from other factors that influence dietary metabolism, while minimized measurement errors from self-reported dietary assessments (A and B).

B

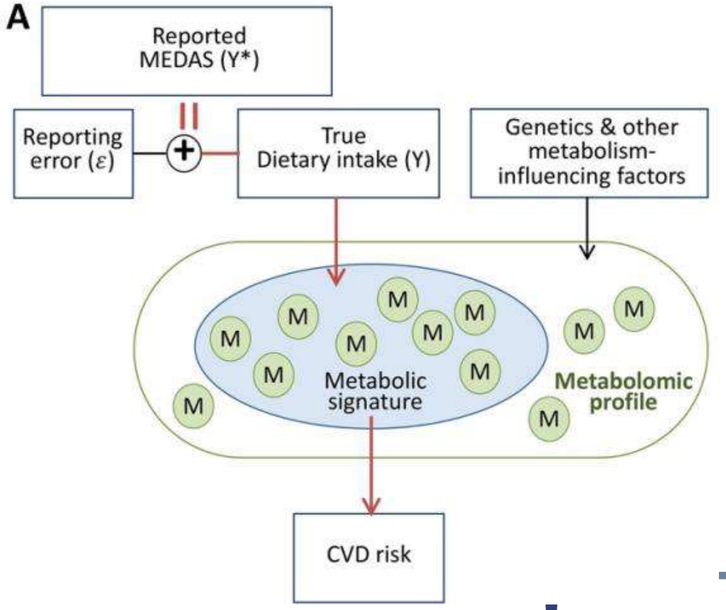
1) $Y = \sum_{i=1}^{k} \alpha_i M_i + \delta$

2) $Y^* = Y + \varepsilon$

3) $Y^* = \sum_{i=1}^{k} \alpha_i M_i + \varepsilon + \delta$

Y = true dietary intake; M = metabolites; α = coefficients for metabolites; δ = residual error, which is the variation in true dietary intake not explainable by metabolites; Y* = reported MEDAS; and ε = reporting error.

Formula (3) is the Elastic Net Regression to define metabolic signature



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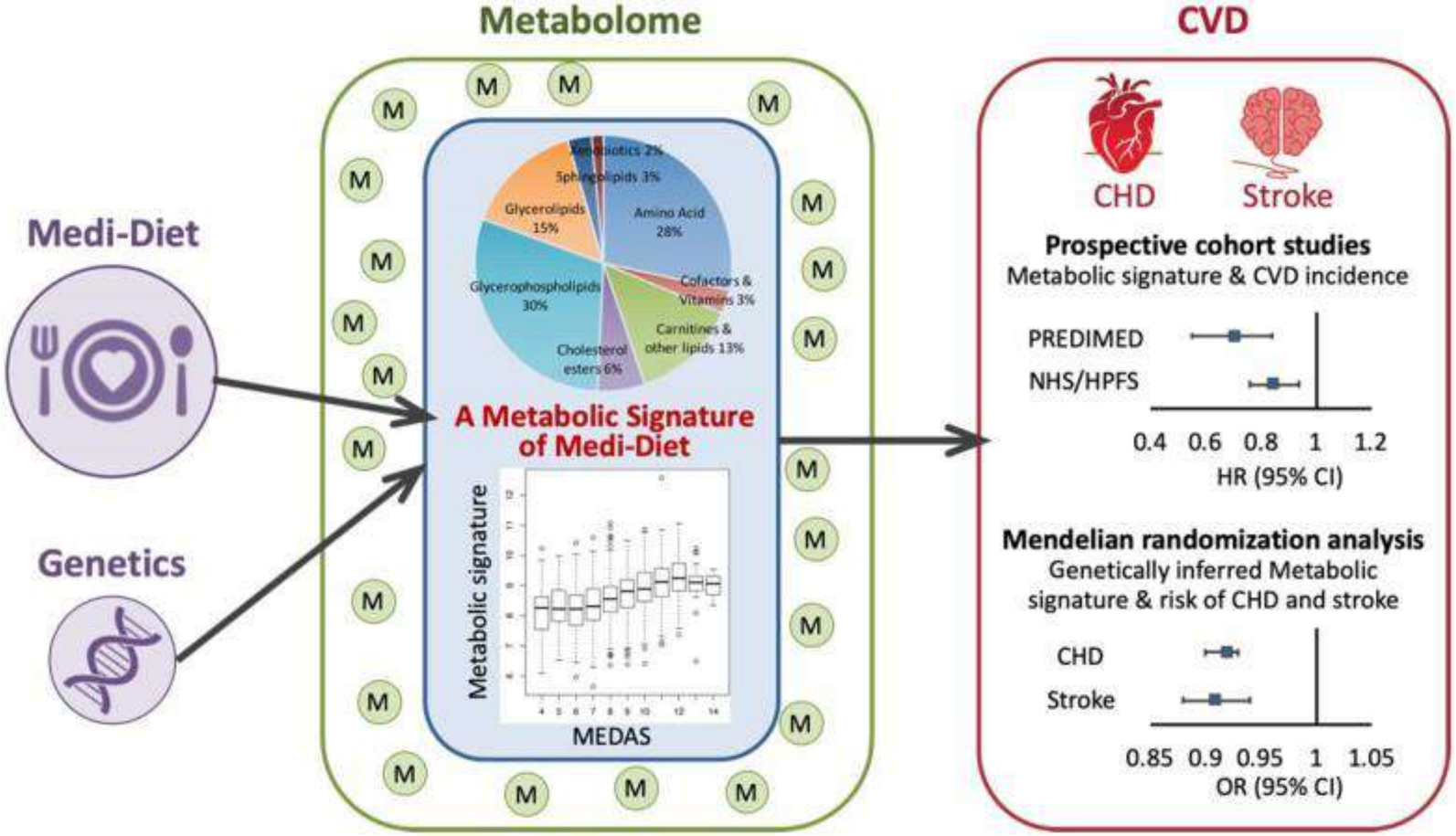
The Mediterranean diet, plasma metabolome, and cardiovascular disease risk



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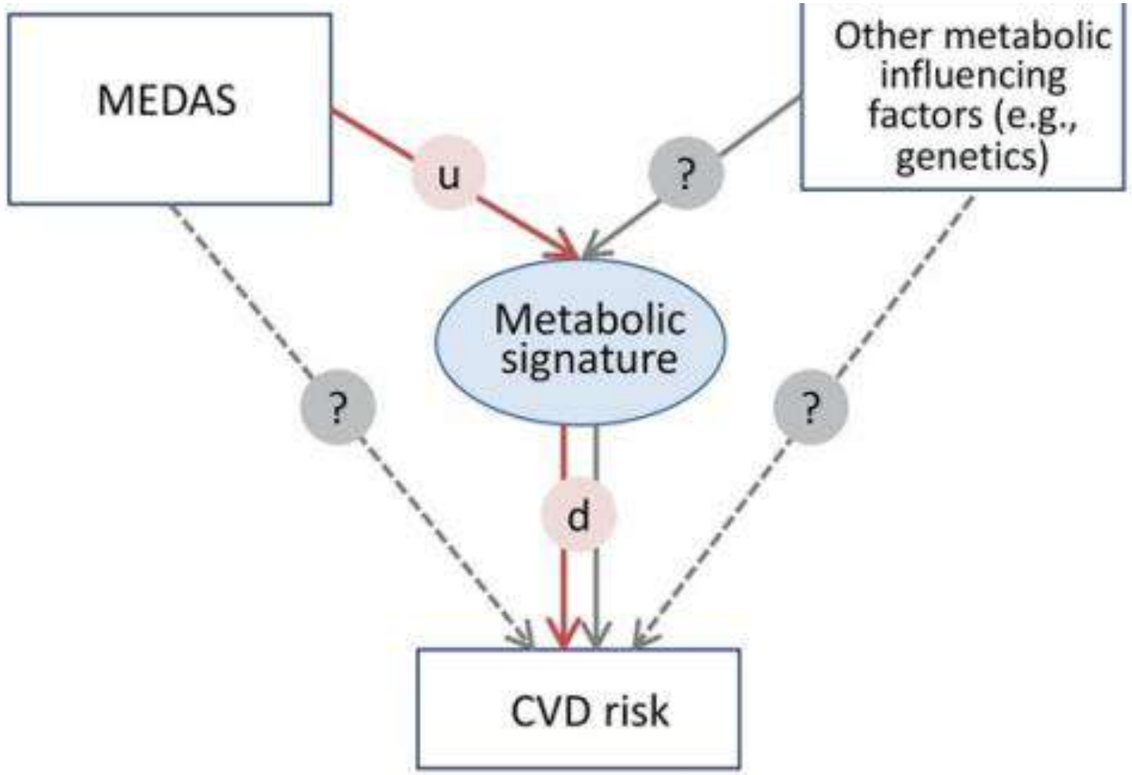
The Mediterranean diet, plasma metabolome, and cardiovascular disease risk

Association of MEDAS with CVD mediated by pathways represented by the signature:
In PREDIMED, PTE=37.2%, P<0.001
In NHS/HPFS, PTE=64.5%, P=0.004

u
d
Mechanisms up- or down-stream of the metabolic signature from adherence to Mediterranean diet to CVD

Association between other metabolic influencing factors (e.g., genetics) and CVD risk mediated by the signature

?
Other pathways through which diet and metabolic influencing factors impact CVD



Li J, et al. Eur Heart J. 2020 Jul 21;41(28):2645-2656.

Mediterranean diet related metabolite profiles and cognitive performance in a Greek population

Aims:

1) We aimed to analyze plasma metabolites using a high-throughput NMR platform and identify a metabolite profile of adherence to the MedDiet among 1250 Greek Middle-aged adults from the Epirus Health Study.

2) We also investigated cross-sectional associations between the identified metabolite profile and domain-specific cognitive tests.

The *Epirus Health Study* is an innovative prospective cohort study designed to investigate the aetiology of complex multifactorial chronic diseases in Greece. A total of 10,000 residents of Epirus, aged 25-70 years, will be recruited.

Papandreou C, et al. Clin Nutr. 2023 Feb;42(2):173-181.

Mediterranean diet related metabolite profiles and cognitive performance in a Greek population

14-item Questionnaire of Mediterranean diet adherence

Questions	Criteria for 1 point
1. Do you use olive oil as main culinary fat?	Yes
2. How much olive oil do you consume in a given day (including oil used for frying, salads, out-of-house meals, etc.)?	≥4 tbsp
3. How many vegetable servings do you consume per day? (1 serving : 200 g [consider side dishes as half a serving])	≥2 (≥1 portion raw or as a salad)
4. How many fruit units (including natural fruit juices) do you consume per day?	≥3
5. How many servings of red meat, hamburger, or meat products (ham, sausage, etc.) do you consume per day? (1 serving: 100–150 g)	<1
6. How many servings of butter, margarine, or cream do you consume per day? (1 serving: 12 g)	<1
7. How many sweet or carbonated beverages do you drink per day?	<1
8. How much wine do you drink per week?	≥7 glasses
9. How many servings of legumes do you consume per week? (1 serving : 150 g)	≥3
10. How many servings of fish or shellfish do you consume per week? (1 serving 100–150 g of fish or 4–5 units or 200 g of shellfish)	≥3
11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits, or custard?	<3
12. How many servings of nuts (including peanuts) do you consume per week? (1 serving 30 g)	≥3
13. Do you preferentially consume chicken, turkey, or rabbit meat instead of veal, pork, hamburger, or sausage?	Yes
14. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek, or garlic and simmered with olive oil)?	≥2

doi:10.1371/journal.pone.0043134.t001

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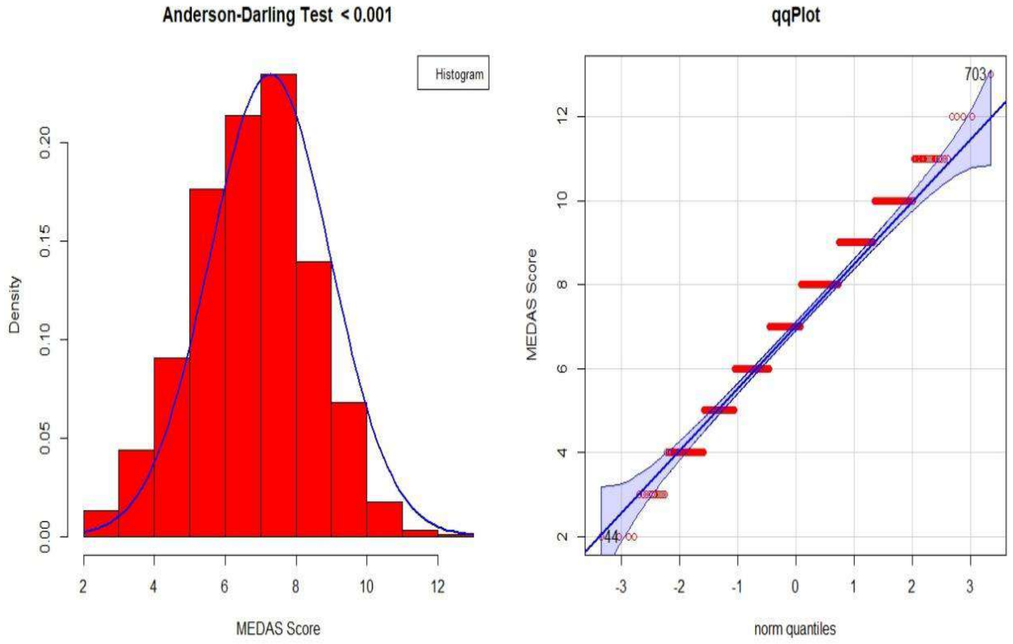
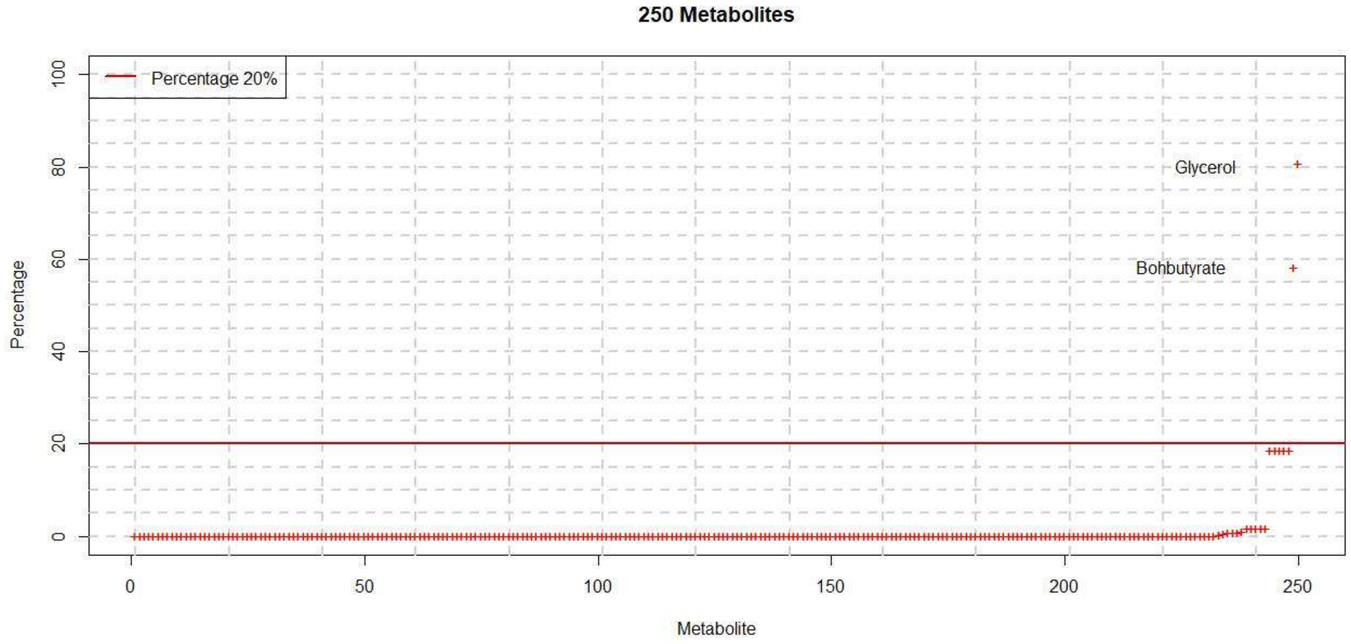
Distribution of the MEDAS score



Plot of the 250 metabolites according to the % of missingness



Missing values of these metabolites were imputed using the random forest imputation approach.
The inverse normal transformation, which generates a rank-based standard normal distribution (mean = 0, SD = 1) was applied to the 248 metabolites.

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Mediterranean diet related metabolite profiles and cognitive performance in a Greek population

To identify a metabolite profile for adherence to the MedDiet, we regressed MEDAS on the 248 NMR metabolites. Due to the high dimensionality and collinear nature of the data, we performed Gaussian linear regression with elastic net penalty.

1) We split 90% of the data selected randomly into training set and the remaining 10% sample into validation set.

2) We applied a 10-fold CV on the training set to find the best combination of alpha and lambda parameters keeping those with the lowest root mean-squared error (RMSE).

We evaluated the alpha parameter from 0.1 (we did not use Ridge regression [alpha = 0] as not being able to perform variable selection) to 1 (Lasso regression) in ~0.05 increments, together with the tuning parameter (lambda), using a 10-fold cross-validation (CV) approach, to identify the optimal combination regarding model accuracy and complexity.

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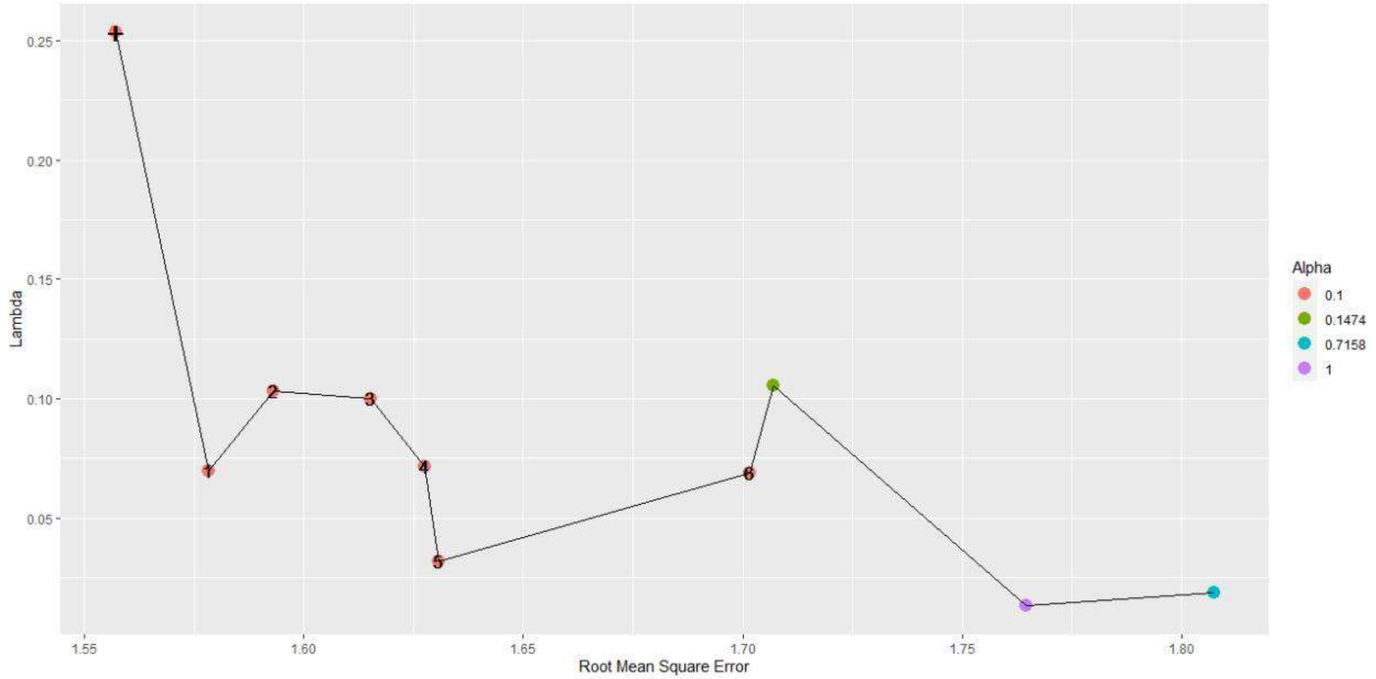
3) In the initial validation set we computed the RMSE and the Pearson correlation coefficient for these tuning parameters.

Running this loop 10 times, in 7 out of 10 iterations, the alpha parameter had a value of 0.1 giving the lowest RMSE.

Plot of the lambda and RMSE for different alpha values after the 10 iterations with the elastic net penalty



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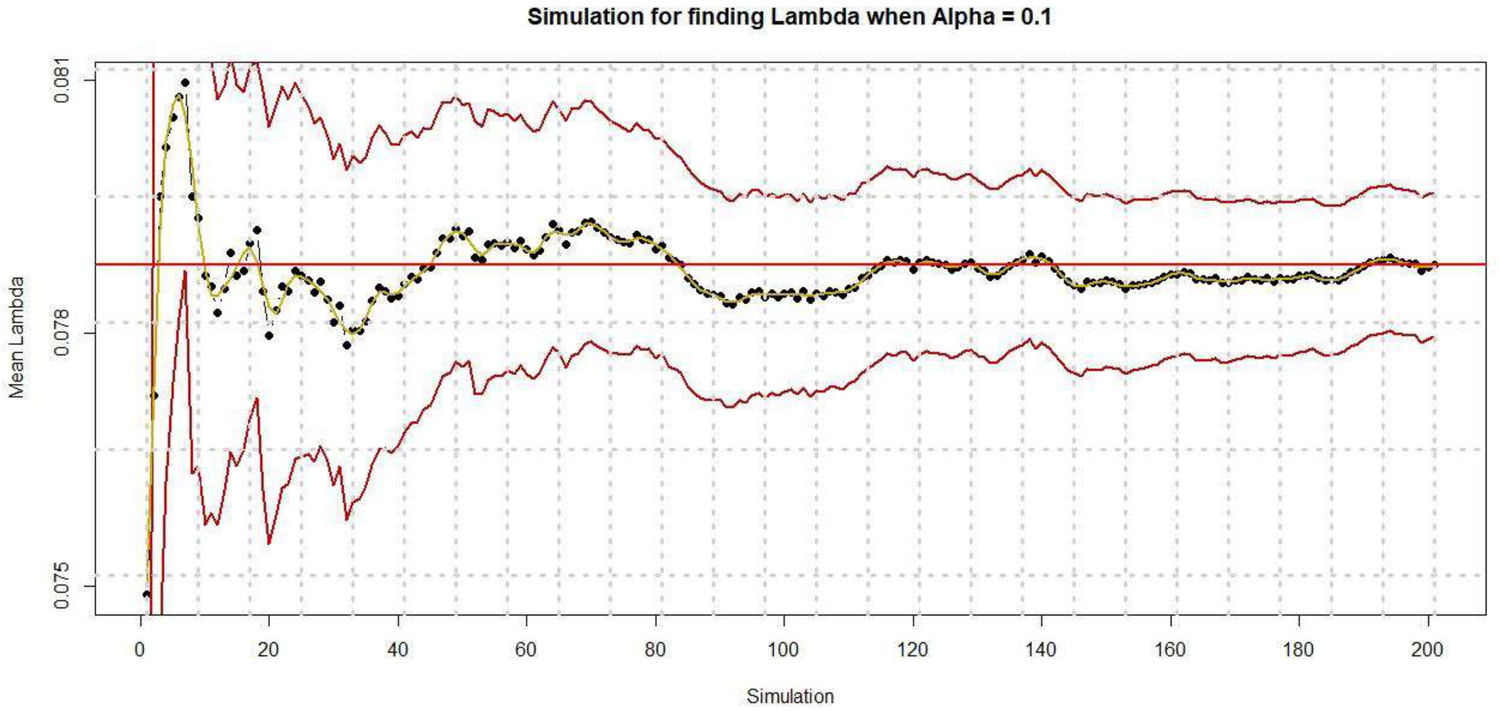
Mediterranean diet related metabolite profiles and cognitive performance in a Greek population

4) For this alpha value (alpha=0.1), we performed 200 times a 10-fold CV to our main data set to find 200 different values of lambda with the lowest RMSE, keeping the mean value.

Plot of the 200 simulated identified lambda for Alpha = 0.1. As the simulations increase the mean lambda converges to its value 0.0788



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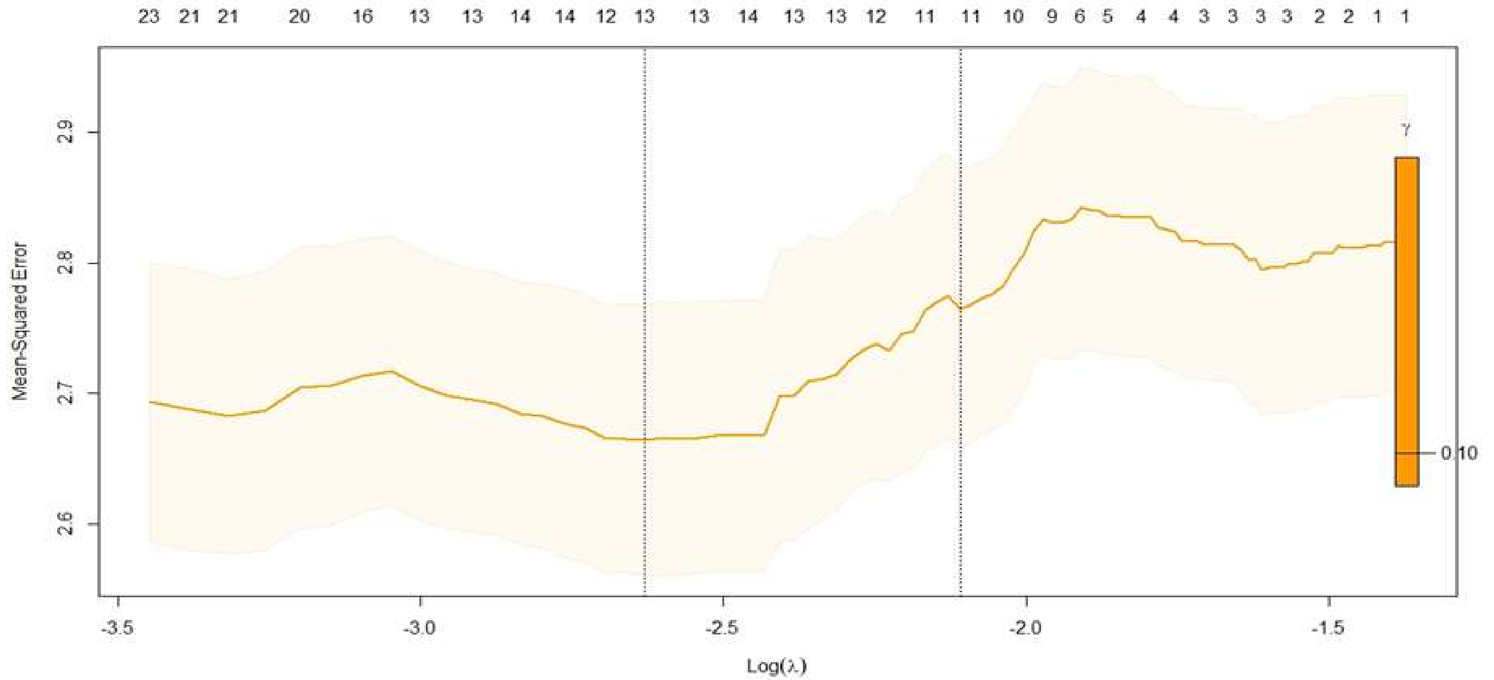
Finally, for lambda = 0.0788 (95% confidence interval [CI]: 0.0777-0.0797) and with permutation test to find the 95% CI, we had the expected lambda with the lowest RMSE.

Plot of the Mean-Squared Error as functions of log(λ) for the 200 times 10-fold cross-validation regression analyses with the elastic net penalty



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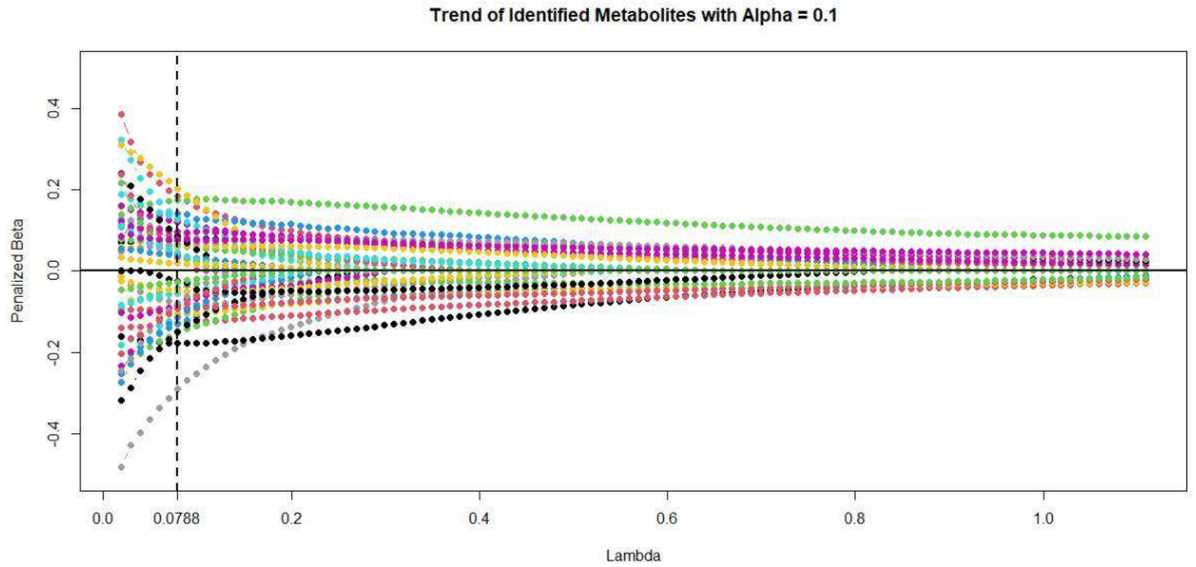
To obtain the metabolites coefficients, we randomly split the data 10 times to training and validation sets as before. From each of the 10 iterations, we applied these alpha and lambda values in each elastic net regression for every training set. Then, we built the metabolite model by keeping the mean value of those metabolites that were consistently selected on each iteration (i.e., metabolites selected 10 times). Finally, we calculated the metabolite score as the weighted sum of the averaged coefficients from the 10 iterations for each selected metabolite.

Plot of the beta coefficients of metabolites in the model as functions of Lambda for the 10-fold cross-validation analyses with the elastic net penalty



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Pearson correlation was calculated to evaluate the performance of the metabolite profile in assessing adherence to the MedDiet. To avoid overfitting, a 10-fold CV procedure was performed diving the complete dataset into training and validation sets (90% and 10%, respectively).

Associations of individual metabolites that with each food component and MEDAS were estimated using Spearman's rank correlations. After merging the two olive oil and the two fruit components, a P-value = 0.05/(12 x 15) was considered as statistically significant for the metabolites-food components associations (Bonferroni correction for 12 MEDAS components x 15 independent clusters of metabolites) and P-value = 0.05/15 for the associations with MEDAS.

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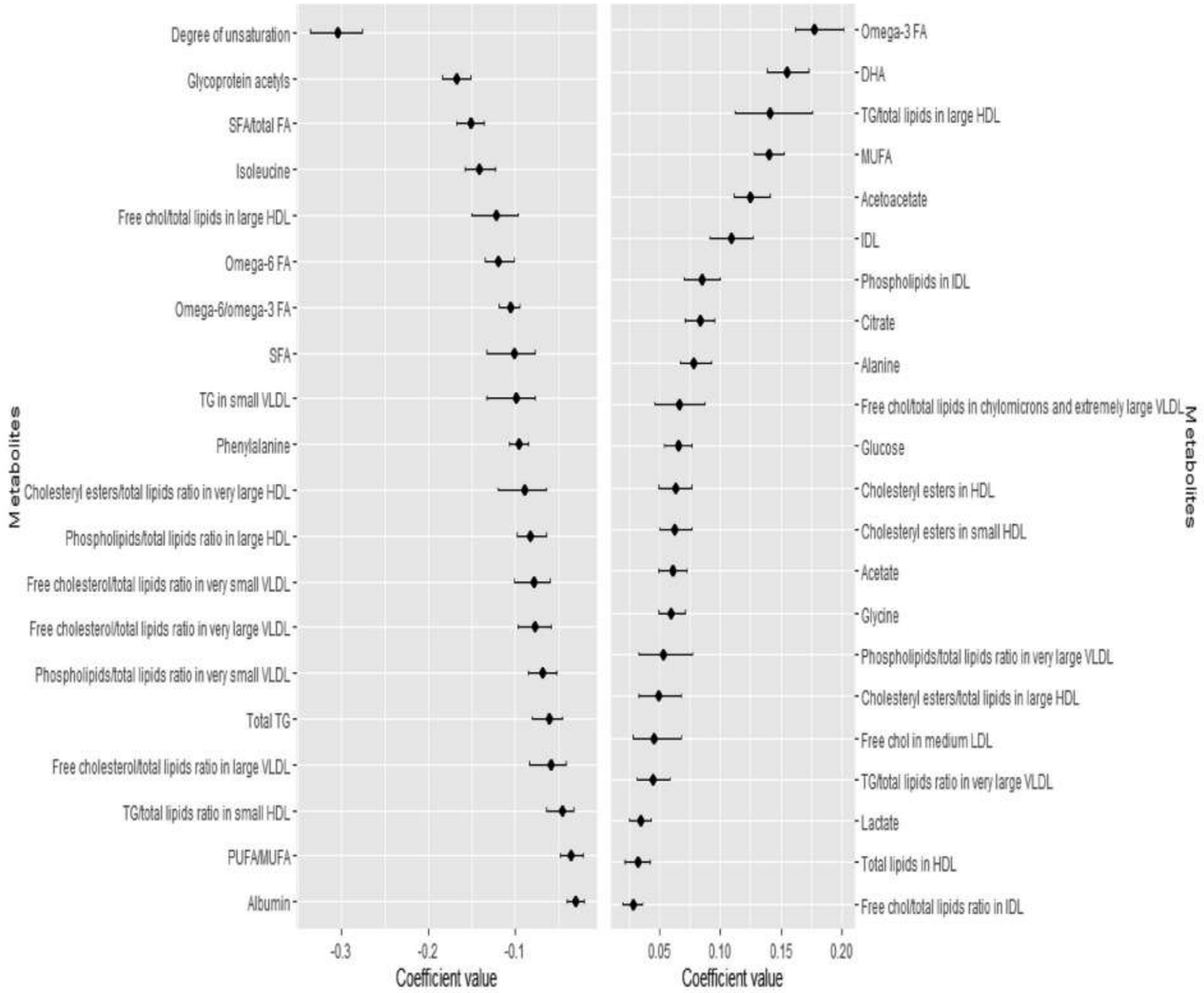

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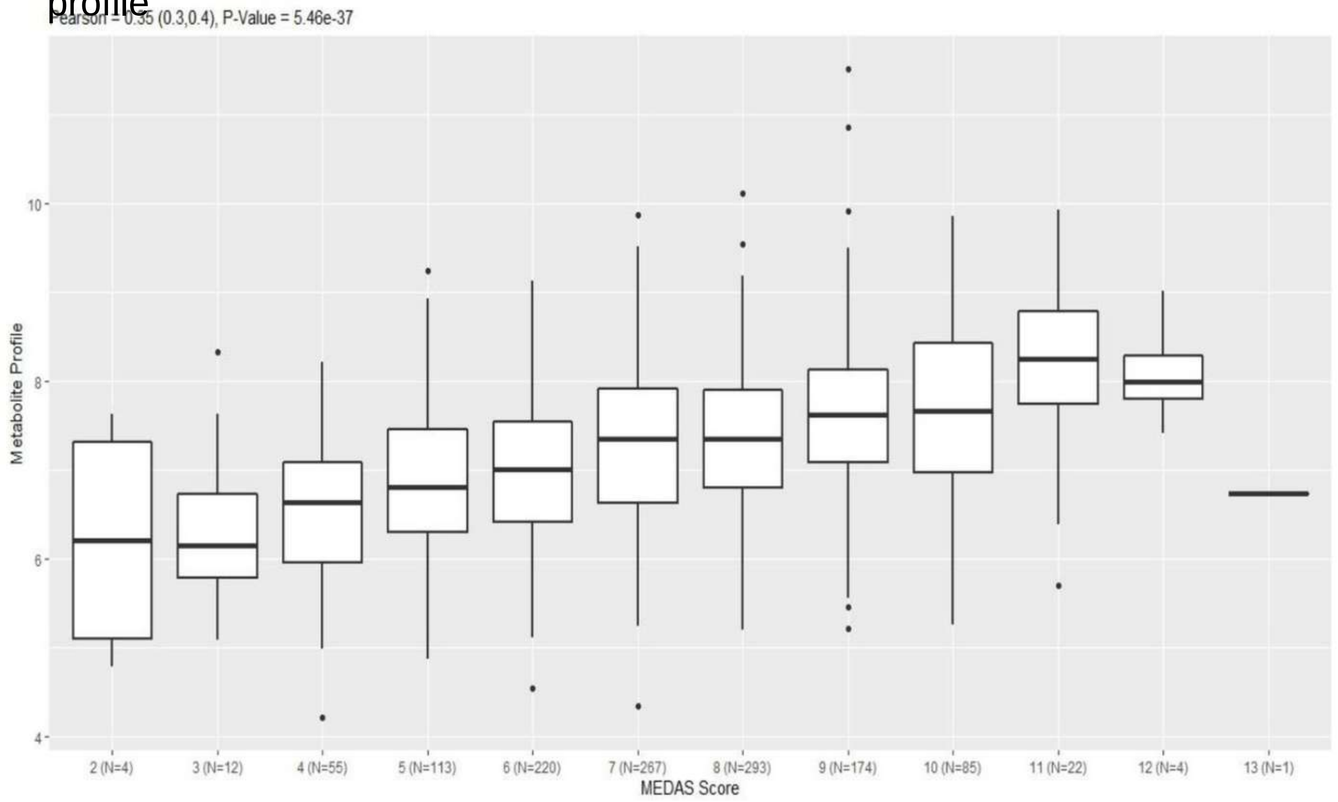
Mediterranean diet related metabolite profiles and cognitive performance in a Greek population

Pearson correlation between the MEDAS score and the metabolite profile



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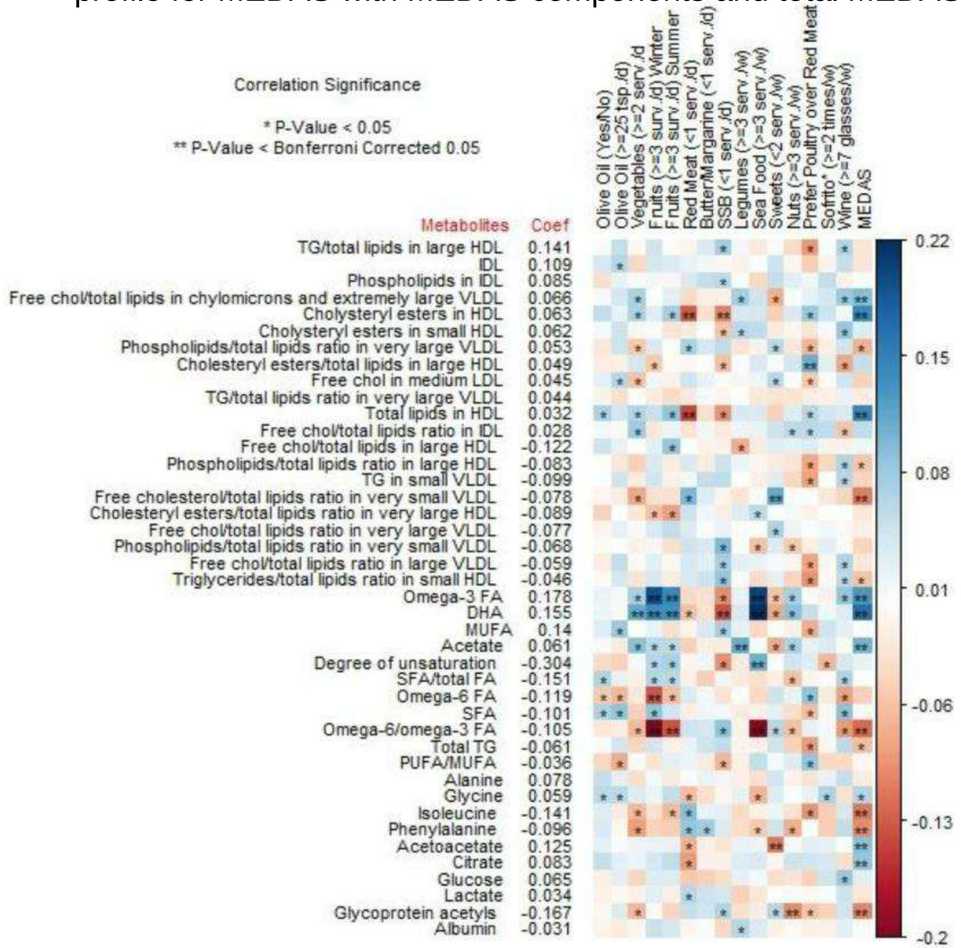
Spearman's correlations of the 42 metabolites selected in the metabolite profile for MEDAS with MEDAS components and total MEDAS score



Mediterranean diet related metabolite profiles and cognitive performance in a Greek population

The identified metabolite profile showed no associations with cognitive performance.

Associations of the metabolite profile with cognitive performance scores. Exposure contrast is per SD/z-score increase of the metabolite score.

Cognitive performance scores	Metabolic signature					
	Model 1[a]			Model 2[b]		
	β	(95% CI)	P value[c]	β	(95% CI)	P value[c]
Trail Making Test (Part A) (seconds)	−0.014	(-0.692,0.664)	0.968	0.054	(-0.645,0.753)	0.88
Trail Making Test (Part B) (seconds)	1.096	(0.116,2.075)	0.140	1.049	(0.038,2.06)	0.147
Verbal Fluency (Sematic) (counts)	0.193	(-0.194,0.579)	0.574	0.140	(-0.261,0.541)	0.692
Verbal Fluency (Phonetic) (counts)	0.087	(-0.151,0.326)	0.6622	0.093	(-0.155,0.341)	0.692
Logical Memory (Immediate Recall) (counts)	−0.160	(-0.454,0.135)	0.574	−0.166	(-0.47,0.138)	0.663
Logical Memory (Delayed Recall) (counts)	−0.160	(-0.313,-0.008)	0.140	−0.168	(-0.326,-0.011)	0.147
Cognitive Battery	−0.003	(-0.038,0.032)	0.968	−0.004	(-0.04,0.032)	0.880

Abbreviations: CI, Confidence Interval.

[a] Regressions were adjusted for age (continuous), sex, education (primary and secondary school, high school, higher education), body mass index (BMI; continuous), smoking status (current, former or never smokers), alcohol consumption (never, less than once/month, 1–3 times/month, 1–2 times/week, almost every day), recreational physical activity (measured in Metabolic Equivalents of Energy Expenditure (METs) per hour/week) (continuous), menopausal status (male, no, yes).

[b] Regressions were adjusted for age (continuous), sex, education (primary and secondary school, high school, higher education), body mass index (BMI; continuous), smoking status (current, former or never smokers), alcohol consumption (never, less than once/month, 1–3 times/month, 1–2 times/week, almost every day), recreational physical activity (measured in Metabolic Equivalents of Energy Expenditure (METs) per hour/week) (continuous), menopausal status (male, no, yes) and Mediterranean Diet Adherence Screener (MEDAS) score.

[c] Adjusted with the Benjamin-Hochberg False Discovery Rate method.

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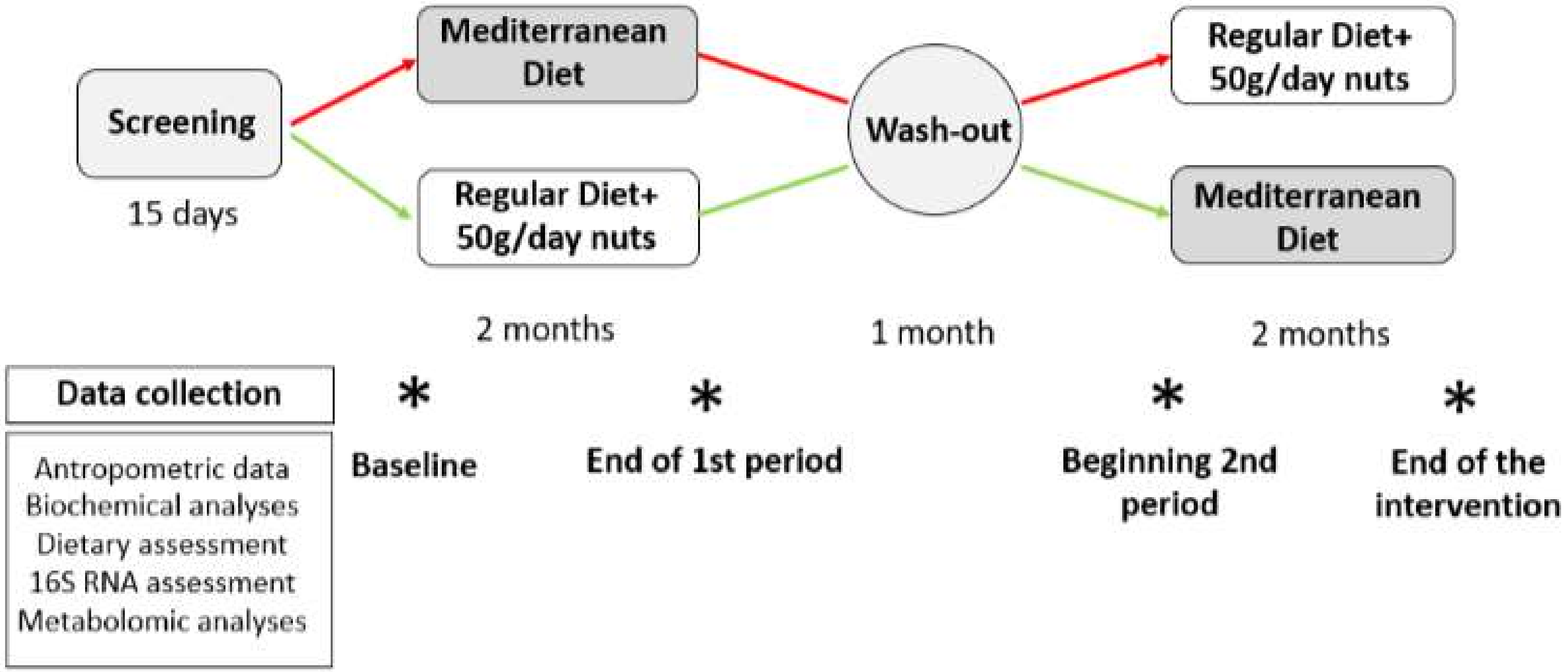
Effects of Mediterranean diet on Metabolome

This randomized, controlled, crossover 2-months dietary-intervention trial, with a 1-month wash-out period, examined changes in plasma metabolites after following a MedDiet compared to the consumption of a single healthy food such as nuts in the context of a non-MedDiet among 44 adults with Metabolic Syndrome.



Galié S, García-Gavilán J, Papandreou C, et al. Clin Nutr. 2021 Jun;40(6):3798-3806.

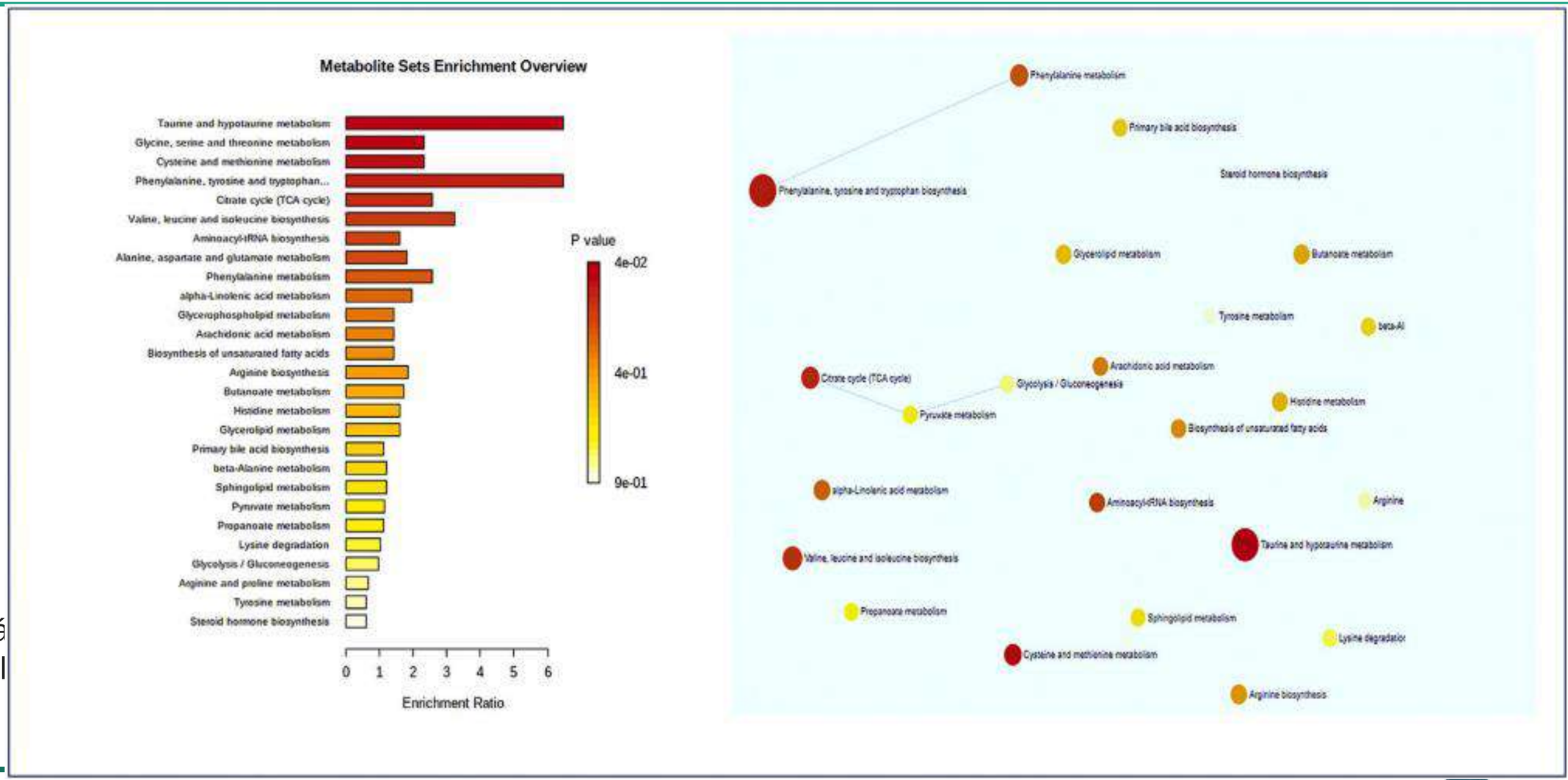
Effects of Mediterranean diet on Metabolome

Plasma metabolites ranked from the highest to the lowest elastic net positive or negative regression coefficients for the MedDiet intervention.

Metabolites	Coefficient	Metabolites	Coefficient
HpEPE	0.958	LPE18:3-sn1	−0.649
Testosterone	0.579	SM36:0	−0.563
PC40:6	0.537	9-OxoODE	−0.517
TMA	0.518	C12:0	−0.334
Succinic acid	0.407	C5-M-DC	−0.320
ChoE(17:0)	0.388	Taurine	−0.284
Taurolithocholic acid	0.380	Linolenic acid-iso2	−0.283
Threonine	0.356	SM41:1	−0.282
LPC19:0-sn1	0.306	Dehydroepiandrosterone sulfate	−0.281
C5−OH	0.304	Hydroxyproline trans	−0.236
3-Phosphoglyceric acid	0.291	PC33:2	−0.235
LPC 22:6	0.278	Linolenic acid-iso1	−0.229
Cystathione	0.273	androsterone sulfate-iso4	−0.222
Histidine	0.231	PC34:1 e	−0.218
C5:1	0.219	Taurocholic acid	−0.215
C18:2	0.215	PC32:0	−0.213
Glycoursodeoxycholic acid	0.205	TG47:0	−0.198
Phenylalanine	0.199	ChoE(22:5)	−0.191
C2:0	0.197	PC34:2	−0.188
Glycerol-1-phosphate	0.172	dihomo-y-linolenic acid-iso2	−0.188
TG56:6	0.166	androsterone sulfate-iso2	−0.178
PC38:2	0.161	C12_0-OH-a	−0.172
alpha-tocopherol	0.145	HODE-iso1	−0.140
TG56:7	0.139	HpODE	−0.134
15-HETE	0.131	Cystine	−0.112
ChoE(20:5)	0.128	9.12.13-TriHOME	−0.109
LPE20:5-sn1	0.095	SM32:2	−0.106
Fumaric acid	0.089	Phosphoethanolamine	−0.096
3-Hydroxybutyric acid	0.088	LPC18:3-sn1	−0.078
PC35:1	0.088	PC32:2	−0.059
Nervonic acid	0.075		
C16:0-OH	0.058		
cis-10 heptadecenoic acid	0.041		
LPC16:1	0.026		
TG56:5	0.025		

Galié S, García-Gavilán J, Papandreou C, et al. Clin Nutr. 2021 Jun;40(6):3798-3806.

Effects of Mediterranean diet on Metabolome



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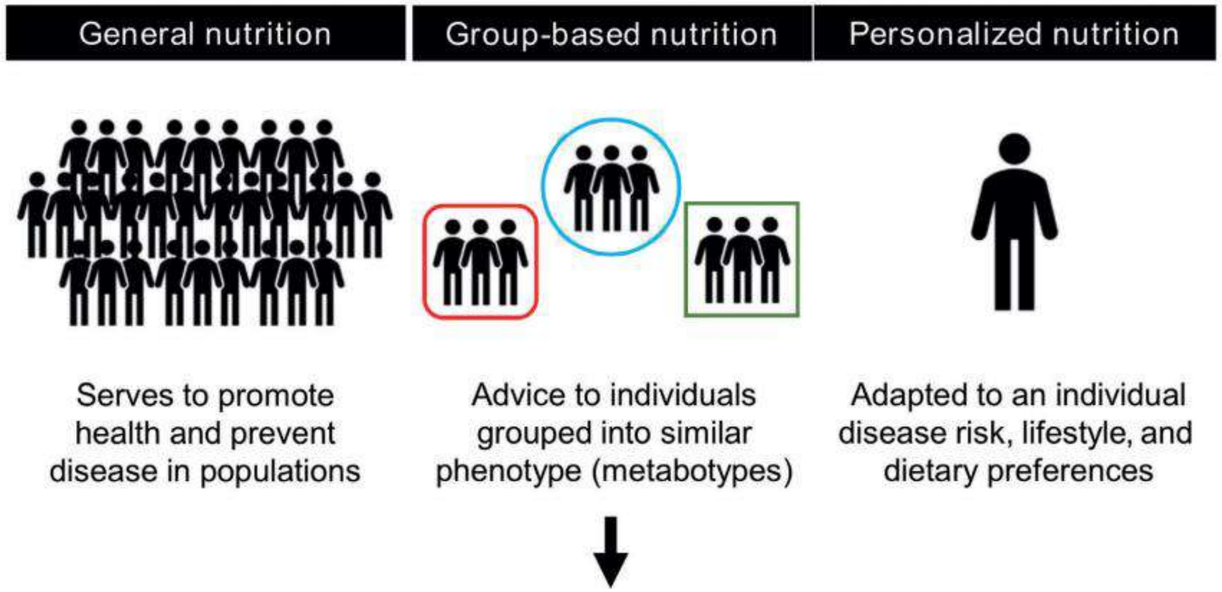
Metabotyping—A Potential Personalized Nutrition Strategy for Precision Prevention of Diseases

Grouping individuals based on similarities in their metabolic phenotype—that is, metabotypes—is a novel concept, and different definitions have been used.

The underlying idea behind metabotyping is to identify metabolic phenotypes based on factors such as diet, anthropometric measures, clinical parameters, metabolomics data, and the gut microbiota.

An optimal diet can then be tailored to fit each metabotype specifically.



Palmnäs M, et al. Adv Nutr. 2020 May 1;11(3):524-532.