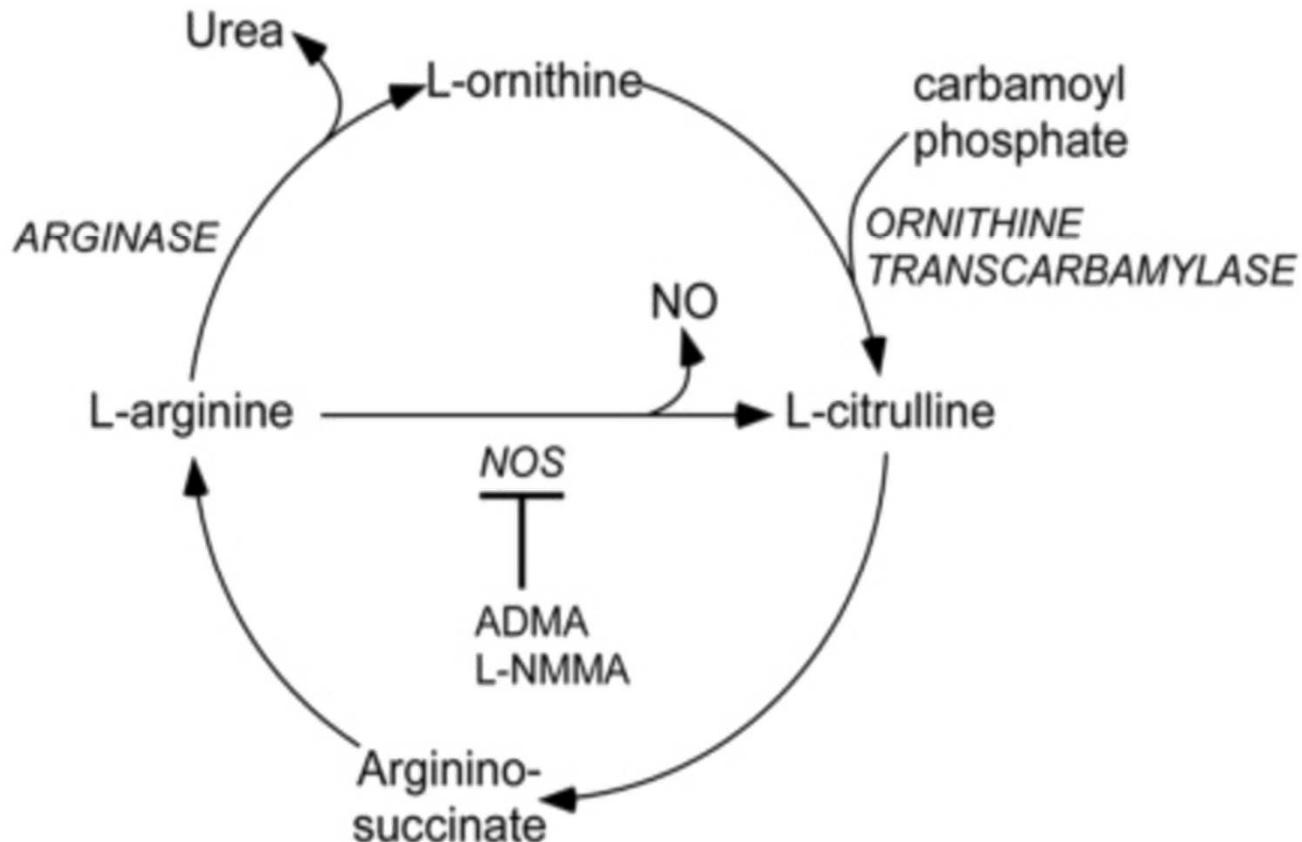


Urea Cycle, Tryptophan Metabolites, and Type 2 Diabetes



Edward Yu, MSc
Nutrition Omics Conference
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Background: Urea Cycle



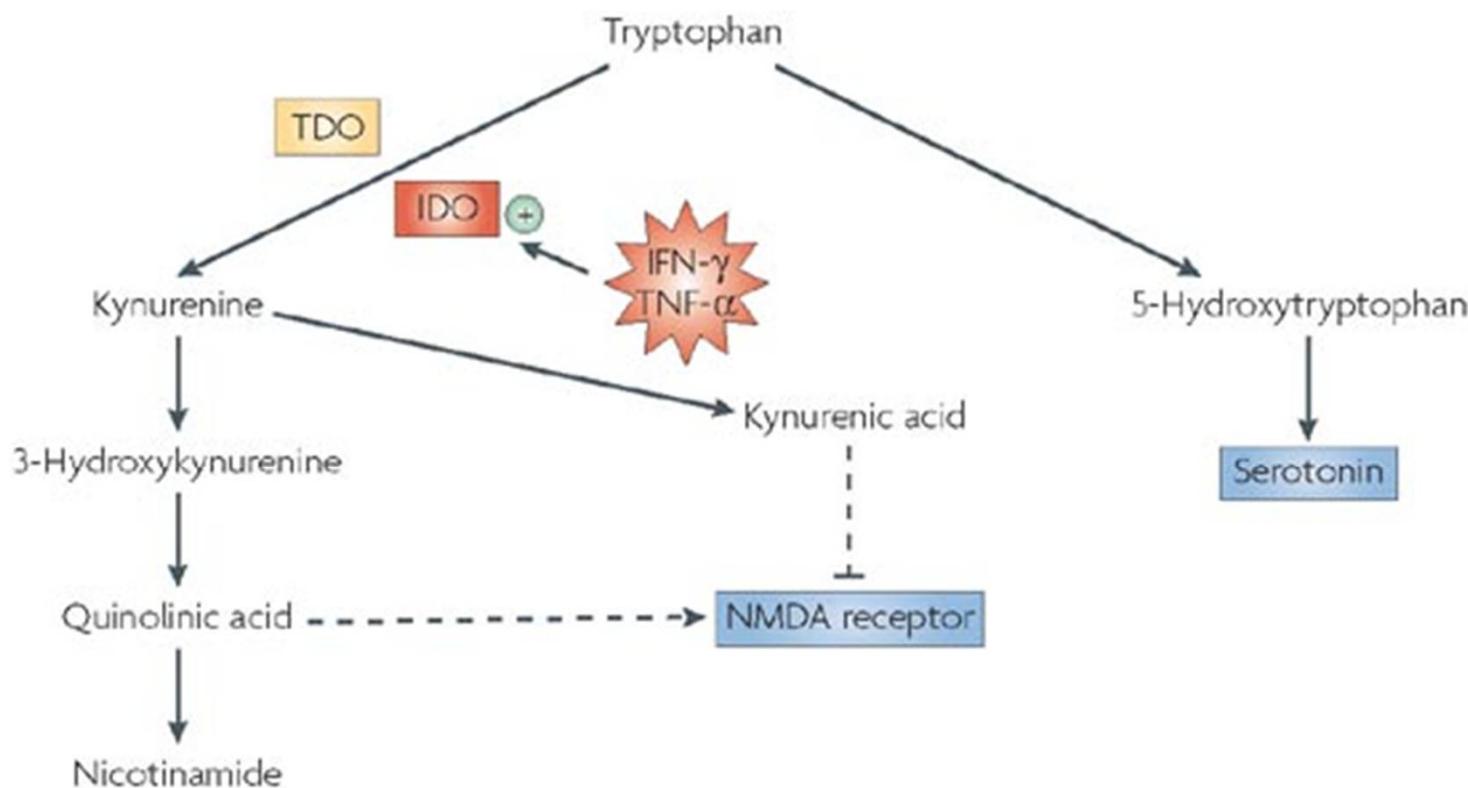
Plasma Arginine/Asymmetric Dimethylarginine Ratio and Incidence of Cardiovascular Events: A Case-Cohort Study

Edward Yu,¹ Miguel Ruiz-Canela,^{2,3} Frank B. Hu,^{1,4,5} Clary B. Clish,⁶ Dolores Corella,^{3,7} Jordi Salas-Salvadó,^{3,8} Adela Hruby,⁹ Montserrat Fitó,^{3,10} Liming Liang,¹¹ Estefania Toledo,^{2,3} Emilio Ros,^{3,12} Ramón Estruch,^{3,13} Enrique Gómez-Gracia,^{3,14} Jose Lapetra,^{3,15} Fernando Arós,^{3,16} Dora Romaguera,^{3,17,18} Lluís Serra-Majem,^{3,19} Marta Guasch-Ferré,¹ Dong D. Wang,¹ and Miguel A. Martínez-González^{2,3}

	Baseline (n=984, cases=231)	
	Arginine / ADMA Ratio	Global Arginine Availability Score
Model 1, trait as continuous variable, per SD		
HR (95% CI)	0.79 (0.66 – 0.94)	0.82 (0.66 – 0.98)
p	0.01	0.03
Trait in quartile categories, as compared to Q1 (reference)		
Q2	0.54 (0.35 – 0.83)	0.49 (0.32 – 0.77)
Q3	0.54 (0.35 – 0.84)	0.65 (0.43 – 0.98)
Q4	0.66 (0.44 – 1.01)	0.66 (0.43 – 1.01)
p-trend	0.08	0.16
Model 2, trait as continuous variable, per SD		
HR (95% CI)	0.80 (0.67 – 0.96)	0.83 (0.69 – 0.999)
p	0.02	0.048
Trait in quartile categories, as compared to Q1 (reference)		
Q2	0.55 (0.35 – 0.86)	0.48 (0.30 – 0.76)
Q3	0.56 (0.36 – 0.88)	0.69 (0.45 – 1.05)
Q4	0.69 (0.45 – 1.06)	0.71 (0.45 – 1.10)
p-trend	0.12	0.35

Global Arginine Availability Score = Arginine / (Citrulline + Ornithine)

Background: Tryptophan



Nature Reviews | Neuroscience

Increases in Plasma Tryptophan Are Inversely Associated with Incident Cardiovascular Disease in the Prevención con Dieta Mediterránea (PREDIMED) Study¹⁻³

Edward Yu,⁴ Miguel Ruiz-Canela,⁷⁻⁹ Marta Guasch-Ferré,^{4,8,9} Yan Zheng,⁴ Estefania Toledo,⁷⁻⁹ Clary B Clish,¹¹ Jordi Salas-Salvadó,^{9,10} Liming Liang,⁵ Dong D Wang,⁴ Dolores Corella,^{9,12} Montse Fitó,^{9,13} Enrique Gómez-Gracia,¹⁴ José Lapetra,^{9,15} Ramón Estruch,^{9,16} Emilio Ros,^{9,17} Montserrat Cofán,^{9,17} Fernando Arós,^{9,18} Dora Romaguera,^{9,19} Lluis Serra-Majem,^{9,20} Jose V Sorlí,^{9,13} Frank B Hu,^{4,6,21} and Miguel A Martinez-Gonzalez^{4,7-9*}

Departments of ⁴Nutrition, ⁵Biostatistics, and ⁶Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA; ⁷Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; ⁸Navarra Institute for Health Research, Pamplona, Spain; ⁹Biomedical Research Centre in Physiopathology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain; ¹⁰Human Nutrition Unit, Faculty of Medicine and Health Sciences, Institut d'Investigació Sanitària Pere Virgili, Rovira i Virgili University, Reus, Spain; ¹¹Broad Institute of MIT and Harvard University, Cambridge, MA; ¹²Department of Preventive Medicine, University of Valencia, Valencia, Spain; ¹³Cardiovascular and Nutrition Research Group, Institut de Recerca Hospital del Mar, Barcelona, Spain; ¹⁴Department of Preventive Medicine, University of Málaga, Málaga, Spain; ¹⁵Department of Family Medicine, Unit Research, Distrito Sanitario Atención Primaria Sevilla, Sevilla, Spain; ¹⁶Department of Internal Medicine Institut d'Investigacions Biomèdiques August Pi Sunyer, Hospital Clinic, and ¹⁷Lipid Clinic, Department of Endocrinology and Nutrition and University of Barcelona, Barcelona, Spain; ¹⁸Department of Cardiology, University Hospital of Álava, Vitoria, Spain; ¹⁹Health Research Institute of Palma, University Hospital Son Espases, Palma de Mallorca, Spain; ²⁰Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Las Palmas, Spain; and ²¹Channing Division for Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

TABLE 2 HRs (95% CIs) for composite CVD (stroke, nonstroke, death from vascular causes) by baseline and 1-y changes in plasma metabolites (tryptophan, kynurenine, kynurenic acid, 3-HAA, and quinolinic acid) as continuous and categorical variables¹

	Tryptophan	Kynurenine	Kynurenic acid	3-HAA	Quinolinic acid
Baseline ²					
Model 1, metabolite as continuous variable, per SD					
HR (95% CI)	0.88 (0.74, 1.04)	1.00 (0.86, 1.16)	1.14 (0.99, 1.31)	0.82 (0.69, 0.98)	1.03 (0.86, 1.22)
P	0.13	0.99	0.08	0.03	0.77
Metabolite in Q categories as compared with Q1 (reference)					
Q2	0.91 (0.59, 1.40)	0.86 (0.55, 1.35)	1.49 (0.91, 2.42)	0.83 (0.53, 1.30)	0.93 (0.59, 1.47)
Q3	0.67 (0.42, 1.05)	0.93 (0.61, 1.44)	1.39 (0.86, 2.24)	0.62 (0.38, 1.03)	0.99 (0.64, 1.54)
Q4	0.75 (0.48, 1.19)	0.91 (0.59, 1.40)	1.49 (0.92, 2.40)	0.69 (0.43, 1.11)	1.17 (0.75, 1.83)
P-trend	0.12	0.76	0.14	0.07	0.46
Model 2, metabolite as continuous variable, per SD					
HR (95% CI)	0.87 (0.67, 1.11)	1.06 (0.89, 1.26)	1.23 (1.02, 1.48)	0.82 (0.67, 1.01)	1.15 (0.94, 1.41)
P	0.28	0.52	0.03	0.06	0.19
Metabolite in Q categories as compared with Q1 (reference)					
Q2	0.95 (0.58, 1.57)	1.27 (0.75, 2.13)	1.58 (0.91, 2.76)	0.72 (0.42, 1.23)	1.00 (0.60, 1.67)
Q3	0.58 (0.33, 1.01)	1.17 (0.72, 1.92)	1.62 (0.92, 2.85)	0.84 (0.46, 1.50)	1.08 (0.65, 1.82)
Q4	0.75 (0.40, 1.44)	1.21 (0.73, 1.99)	1.73 (0.97, 3.10)	0.64 (0.36, 1.16)	1.53 (0.91, 2.57)
P-trend	0.21	0.55	0.08	0.22	0.11
1-y changes ³					
Model 1, metabolite as continuous variable, per SD					
HR (95% CI)	0.77 (0.64, 0.94)	0.86 (0.69, 1.06)	0.87 (0.73, 1.04)	1.15 (0.91, 1.45)	0.96 (0.80, 1.15)
P	0.01	0.15	0.13	0.26	0.64
Metabolite in Q categories as compared with Q1 (reference)					
Q2	1.18 (0.72, 1.94)	0.79 (0.48, 1.30)	0.95 (0.56, 1.61)	1.59 (0.79, 3.19)	0.84 (0.48, 1.47)
Q3	0.75 (0.44, 1.27)	0.37 (0.20, 0.70)	0.88 (0.50, 1.53)	1.91 (0.91, 3.99)	0.95 (0.54, 1.66)
Q4	0.49 (0.27, 0.87)	0.68 (0.41, 1.13)	0.69 (0.40, 1.20)	1.46 (0.70, 3.05)	0.89 (0.52, 1.51)
P-trend	0.005	0.02	0.18	0.27	0.78
Model 2, metabolite as continuous variable, per SD					
HR (95% CI)	0.79 (0.63, 0.98)	0.85 (0.67, 1.06)	0.93 (0.75, 1.15)	1.15 (0.90, 1.49)	0.92 (0.75, 1.13)
P	0.03	0.15	0.52	0.27	0.42
Metabolite in Q categories as compared with Q1 (reference)					
Q2	1.19 (0.70, 2.02)	0.78 (0.45, 1.34)	1.00 (0.53, 1.87)	1.26 (0.56, 2.84)	0.82 (0.43, 1.57)
Q3	0.72 (0.40, 1.28)	0.39 (0.20, 0.77)	1.00 (0.52, 1.93)	1.37 (0.55, 3.43)	0.97 (0.51, 1.85)
Q4	0.49 (0.26, 0.95)	0.60 (0.34, 1.07)	0.84 (0.44, 1.58)	1.32 (0.57, 3.03)	0.78 (0.43, 1.42)
P-trend	0.01	0.02	0.60	0.50	0.55

RESEARCH ARTICLE

Tryptophan Predicts the Risk for Future Type 2 Diabetes

Tianlu Chen¹, Xiaojiao Zheng¹, Xiaojing Ma², Yuqian Bao², Yan Ni³, Cheng Hu², Cynthia Rajani³, Fengjie Huang¹, Aihua Zhao¹, Weiping Jia^{1,2*}, Wei Jia^{1,3*}

1 Shanghai Key Laboratory of Diabetes Mellitus and Center for Translational Medicine, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China, **2** Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai Diabetes Institute, Shanghai, China, **3** University of Hawaii Cancer Center, Honolulu, United States of America

* wjia@cc.hawaii.edu (WJ); wpjia@sjtu.edu.cn (WPJ)

Table 3. Predictive performance of baseline amino acids and combined scores in discriminating individuals who developed diabetes in 10 years (T2D, n = 51) from those who remained metabolically healthy (NGT, n = 162).

Amino acids and combined scores	p1	FC	Basic logistic model	
			Crude OR (95% CI)	p2
Valine	<0.001	2.52	1.83 (1.51, 2.23)	<0.001
Leucine	<0.001	2.06	1.94 (1.37, 2.76)	<0.001
Isoleucine	<0.001	2.60	1.50 (1.27, 1.83)	<0.001
Phenylalanine	<0.001	2.01	1.42 (1.18, 1.71)	<0.001
Tyrosine	<0.001	2.28	1.50 (1.24, 1.81)	<0.001
Tryptophan	<0.001	2.60	2.00 (1.65, 2.41)	<0.001
5AAs-Combined score	<0.001	2.72	1.47 (1.25, 1.75)	<0.001
6AAs-Combined score	<0.001	3.73	3.50(1.85, 6.60)	<0.001

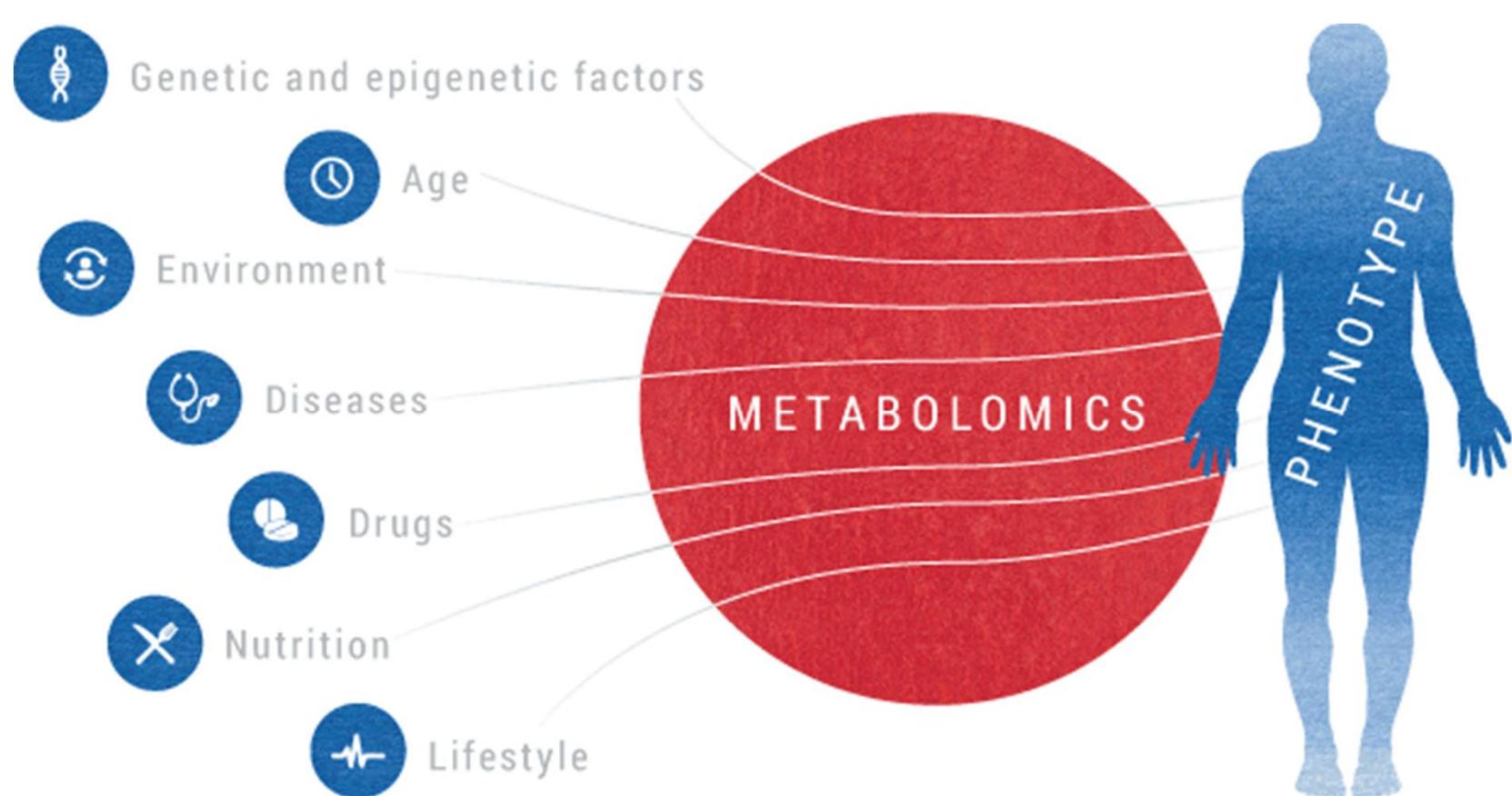
Abbreviations: 5AAs-Combined score: the combined score derived from the abundance of 5 AAs (valine, leucine, isoleucine, tyrosine, and phenylalanine); 6AAs-Combined score: the combined score derived from the abundance of 6 AAs (valine, leucine, isoleucine, tyrosine, phenylalanine, and tryptophan).

p1 was the p value from Mann Whitney U test compared T2D and NGT.

FC (fold change) represents the mean ratio of T2D to NGT.

Crude odds ratio (Crude OR) and confidence interval (CI) per s.d., and p2 were from basic logistic regression models and S.D. scaled data.

Background: Metabolomics



Predimed

Prevención con Dieta Mediterránea

Three arms

- Low fat control
- MedDiet + EVOO
- MedDiet + nuts

Case-cohort among diabetes-free patients at baseline (694 in subcohort)

All incident cases of T2D included (251 at baseline)

Samples collected at baseline and 1 year.

Approach: Cox Model

Weighted Cox Model:

$$h(t|A, \text{Covariates}) = h_0(t) * \exp(\beta_1 A + \beta_2 \text{Covariate}_1 + \dots + \beta_n \text{Covariate}_N)$$

Barlow weights (1/Probability of selection).

Continuous and categorical (quartiles).

Test for trend using median values.

Two-sided p-values with $\alpha = 0.05$.

Approach: Risk Score

Risk score construction:

$$\text{Individual Risk Score} = \sum_{i=1}^n \text{INT_Metabolite}_{ij} * \beta_i$$

$\text{INT_Metabolite}_{ij}$ = quantity of INT metabolite i in individual j .

β_i = regression coefficient for the metabolite (continuous) in the fully adjusted Cox model.

Results

UREA CYCLE



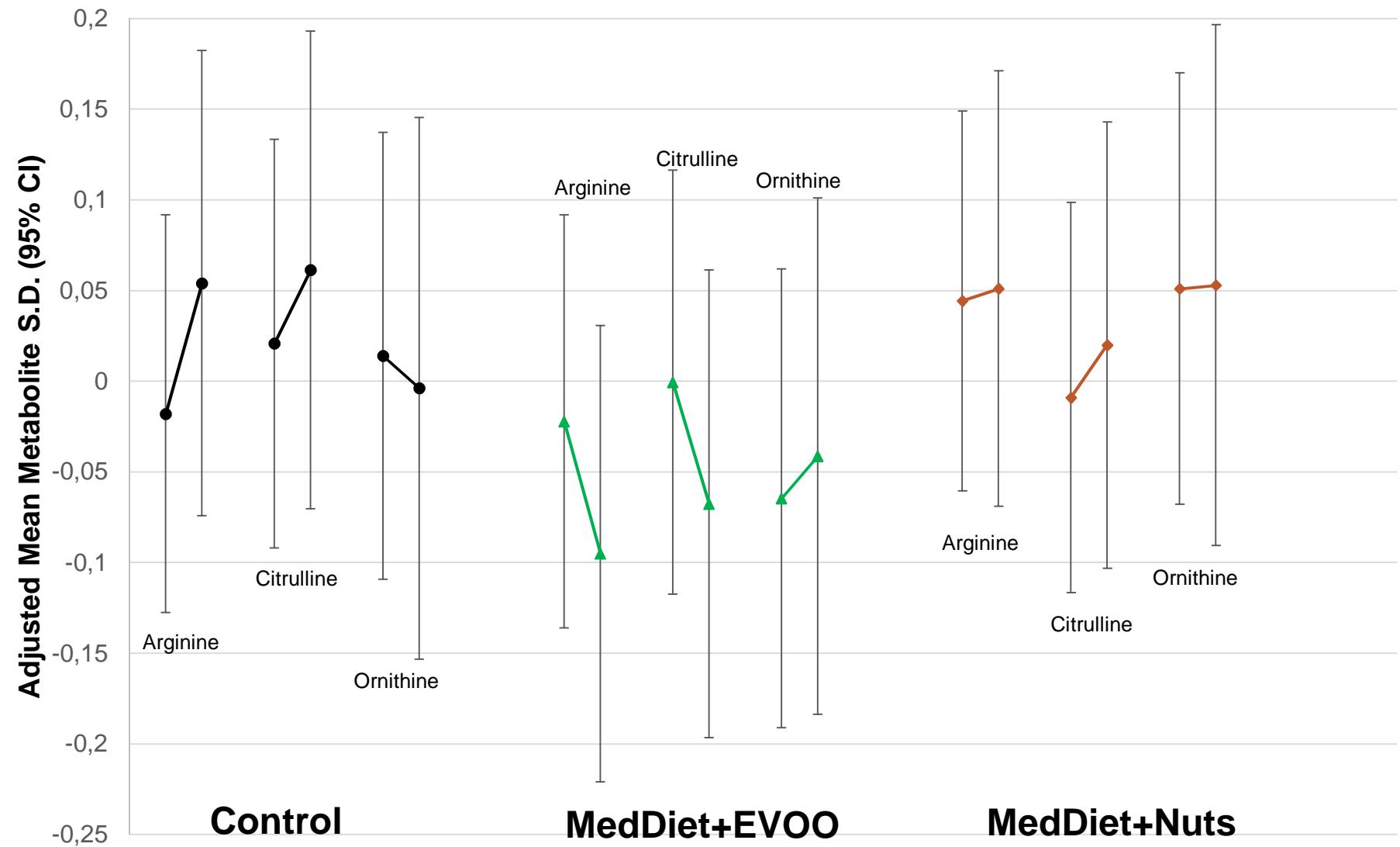
Results: Descriptives

	Subcohort	Cases
n	694	251
Age (years)	66.5 (5.7)	66.4 (5.7)
Sex (% women),	62.8	55.0
Intervention group, %		
MedDiet+EVOO	30.7	29.9
MedDiet+nuts	37.2	33.9
Control	32.1	36.3
Hypertension, %	90.8	96.0
Dyslipidemia, %	85.0	79.7
Smoking, %		
Never	61.0	52.6
Former	22.6	22.3
Current	16.4	25.1
Waist circumference, cm	99.5 (10.7)	103.4 (10.0)
Body mass index, kg/m²	29.9 (3.6)	30.8 (3.3)
Physical activity, METs/d	238 (238)	249 (232)
Total energy intake, kcal/d	2277 (566)	2327 (622)

Results: Correlation Matrix

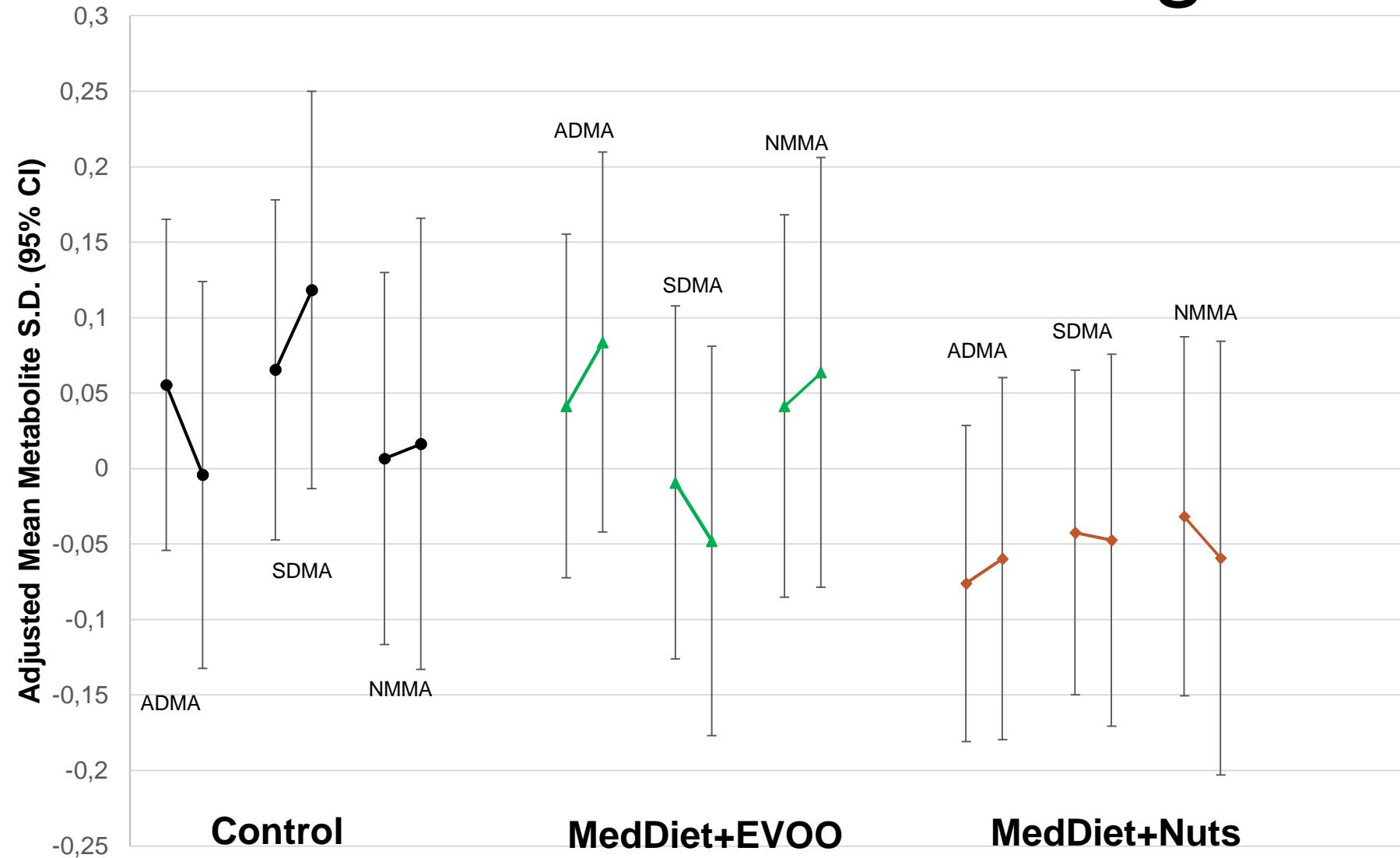
	Arginine	Citrulline	Ornithine	ADMA	SDMA	NMMA
Arginine	1	0.36	0.18	0.33	0.26	0.4
Citrulline	0.36	1	0.46	0.39	0.49	0.44
Ornithine	0.18	0.46	1	0.42	0.28	0.48
ADMA	0.33	0.39	0.42	1	0.59	0.62
SDMA	0.26	0.49	0.28	0.59	1	0.53
NMMA	0.4	0.44	0.48	0.62	0.53	1

Results: Intervention Changes



Changes are adjusted for age (years), sex (male, female), intervention group, body mass index (kg/m²), smoking (never, current, former), dyslipidemia, hypertension, leisure time physical activity and baseline fasting glucose.

Results: Intervention Changes



Changes are adjusted for age (years), sex (male, female), intervention group, body mass index (kg/m²), smoking (never, current, former), dyslipidemia, hypertension, leisure time physical activity and baseline fasting glucose.

Results: Baseline Metabolites

251 cases, 694 subcohort						
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend	HR per S.D.
Multivariable models						
Arginine	1.00 (ref)	0.97 (0.60 – 1.57)	0.74 (0.46 – 1.19)	1.05 (0.61 – 1.80)	0.88	0.94 (0.78 – 1.14)
Ornithine	1.00 (ref)	1.47 (0.91 – 2.39)	1.08 (0.67 – 1.75)	1.37 (0.79 – 2.37)	0.47	1.04 (0.86 – 1.25)
Citrulline	1.00 (ref)	0.97 (0.61 – 1.54)	0.80 (0.49 – 1.30)	0.61 (0.38 – 1.00)	0.04	0.82 (0.68 – 0.97)
ADMA	1.00 (ref)	1.02 (0.64 – 1.62)	0.69 (0.43 – 1.11)	0.96 (0.60 – 1.56)	0.52	0.90 (0.76 – 1.08)
SDMA	1.00 (ref)	0.66 (0.42 – 1.03)	0.66 (0.43 – 1.02)	0.41 (0.25 – 0.69)	<0.01	0.71 (0.59 – 0.85)
NMMA	1.00 (ref)	0.97 (0.62 – 1.54)	1.10 (0.71 – 1.71)	0.75 (0.46 – 1.24)	0.36	0.93 (0.79 – 1.11)
Arginine/ADMA	1.00 (ref)	0.92 (0.58 – 1.46)	0.68 (0.42 – 1.10)	1.24 (0.76 – 2.03)	0.66	1.03 (0.84 – 1.25)
GABR	1.00 (ref)	1.08 (0.67 – 1.73)	0.91 (0.56 – 1.46)	1.44 (0.86 – 2.41)	0.27	1.02 (0.84 – 1.23)
Multivariable models + baseline fasting glucose						
Arginine	1.00 (ref)	1.14 (0.61 – 2.13)	1.15 (0.64 – 2.07)	1.41 (0.73 – 2.76)	0.31	1.09 (0.87 – 1.38)
Ornithine	1.00 (ref)	1.61 (0.91 – 2.84)	1.40 (0.78 – 2.50)	1.74 (0.87 – 3.49)	0.17	1.21 (0.97 – 1.53)
Citrulline	1.00 (ref)	1.04 (0.60 – 1.80)	0.74 (0.39 – 1.39)	0.88 (0.49 – 1.56)	0.44	0.89 (0.72 – 1.09)
ADMA	1.00 (ref)	0.82 (0.46 – 1.44)	0.85 (0.51 – 1.42)	0.86 (0.48 – 1.53)	0.65	0.94 (0.76 – 1.16)
SDMA	1.00 (ref)	0.95 (0.56 – 1.62)	0.70 (0.42 – 1.17)	0.57 (0.29 – 1.12)	0.06	0.80 (0.65 – 0.99)
NMMA	1.00 (ref)	0.65 (0.37 – 1.14)	0.87 (0.52 – 1.48)	0.69 (0.40 – 1.21)	0.37	0.97 (0.79 – 1.19)
Arginine/ADMA	1.00 (ref)	1.23 (0.70 – 2.18)	1.09 (0.59 – 2.00)	1.72 (0.92 – 3.24)	0.13	1.16 (0.92 – 1.47)
GABR	1.00 (ref)	1.32 (0.73 – 2.38)	0.95 (0.51 – 1.75)	1.39 (0.76 – 2.55)	0.50	1.00 (0.79 – 1.27)

Multivariate models stratified by recruitment center and adjusted for age (years), sex (male, female), and intervention group (MedDiet+EVOO, MedDiet+nuts), body mass index (kg/m²), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia and hypertension.

Results: 1-Year Changes

	157 cases, 531 subcohort					
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend	HR per S.D.
Multivariable models						
Arginine	1.00 (ref)	1.12 (0.62 – 2.02)	0.62 (0.34 – 1.13)	0.63 (0.32 – 1.21)	0.06	0.77 (0.59 – 1.00)
Ornithine	1.00 (ref)	0.55 (0.28 – 1.07)	0.76 (0.40 – 1.45)	0.64 (0.33 – 1.25)	0.35	0.88 (0.68 – 1.12)
Citrulline	1.00 (ref)	0.82 (0.45 – 1.51)	1.02 (0.57 – 1.83)	0.64 (0.35 – 1.15)	0.23	0.85 (0.68 – 1.06)
ADMA	1.00 (ref)	1.30 (0.75 – 2.25)	0.86 (0.46 – 1.60)	0.97 (0.51 – 1.85)	0.63	0.93 (0.73 – 1.19)
SDMA	1.00 (ref)	1.19 (0.63 – 2.22)	0.64 (0.27 – 1.07)	0.84 (0.40 – 1.76)	0.28	0.84 (0.62 – 1.14)
NMMA	1.00 (ref)	0.85 (0.47 – 1.54)	1.18 (0.63 – 2.22)	1.07 (0.53 – 2.16)	0.64	1.06 (0.82 – 1.37)
Arginine/ADMA	1.00 (ref)	0.87 (0.47 – 1.59)	0.57 (0.30 – 1.08)	0.57 (0.27 – 1.23)	0.09	0.84 (0.64 – 1.11)
GABR	1.00 (ref)	0.56 (0.31 – 1.02)	0.72 (0.38 – 1.36)	0.55 (0.27 – 1.12)	0.18	0.87 (0.66 – 1.14)
Multivariable models + baseline fasting glucose						
Arginine	1.00 (ref)	1.30 (0.64 – 2.66)	0.64 (0.30 – 1.34)	0.49 (0.22 – 1.08)	0.03	0.71 (0.51 – 0.98)
Ornithine	1.00 (ref)	0.94 (0.45 – 2.01)	0.68 (0.32 – 1.44)	0.85 (0.37 – 1.94)	0.53	0.95 (0.70 – 1.31)
Citrulline	1.00 (ref)	1.23 (0.59 – 2.56)	1.45 (0.70 – 3.00)	0.80 (0.39 – 1.68)	0.68	0.87 (0.68 – 1.13)
ADMA	1.00 (ref)	1.15 (0.57 – 2.29)	0.84 (0.40 – 1.76)	0.87 (0.42 – 1.83)	0.54	0.94 (0.71 – 1.24)
SDMA	1.00 (ref)	0.81 (0.40 – 1.67)	0.44 (0.20 – 0.93)	0.71 (0.32 – 1.57)	0.20	0.82 (0.59 – 1.13)
NMMA	1.00 (ref)	1.00 (0.49 – 2.02)	1.09 (0.51 – 2.31)	0.91 (0.38 – 2.19)	0.89	1.07 (0.77 – 1.47)
Arginine/ADMA	1.00 (ref)	0.93 (0.46 – 1.88)	0.35 (0.15 – 0.81)	0.53 (0.23 – 1.24)	0.04	0.73 (0.51 – 1.05)
GABR	1.00 (ref)	0.38 (0.19 – 0.77)	0.52 (0.25 – 1.07)	0.56 (0.26 – 1.21)	0.27	0.83 (0.60 – 1.15)

Multivariate models stratified by recruitment center and adjusted for age (years), sex (male, female), and intervention group (MedDiet+EVOO, MedDiet+nuts), body mass index (kg/m²), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia, hypertension, and baseline metabolite values.

Results: Effect Modification by Baseline Arginine/ADMA Ratio

Characteristic		Continuous HR per 1 SD (95% CI)	P for interaction
Sex	Men (n=346)	0.88 (0.58 – 1.32)	<0.01
	Women (n=546)	1.60 (1.15 – 2.22)	
Age	< 65 years (n=357)	1.64 (1.00 – 2.69)	0.14
	≥ 65 years (n=535)	1.04 (0.78 – 1.39)	
Intervention group	Control group (n=295)	1.18 (0.77 – 1.81)	0.09
	Mediterranean diet + EVOO (n=273)	1.64 (0.99 – 2.71)	
	Mediterranean diet + nuts (n=324)	1.08 (0.62 – 1.90)	
Obesity	< 30 kg/m ² (n=473)	1.02 (0.73 – 1.42)	<0.01
	≥ 30 kg/m ² (n=419)	1.54 (1.08 – 2.19)	
Smoking status	Current/former smoking (n=366)	1.09 (0.76 – 1.56)	0.99
	Never smoking (n=526)	1.21 (0.83 – 1.75)	
Baseline Hypertension	Yes (n=818)	1.13 (0.89 – 1.45)	0.28
	No (n=74)	8.45 (1.86 – 38.36)	
Baseline Dyslipidemia	Yes (n=752)	1.30 (0.99 – 1.70)	<0.01
	No (n=140)	0.49 (0.24 – 1.01)	

Stratified by recruitment center and adjusted for age (years), sex (male, female), and intervention group (MedDiet+EVOO, MedDiet+nuts), body mass index (kg/m²), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia, hypertension, and baseline glucose.

Results

TRYPTOPHAN PATHWAY

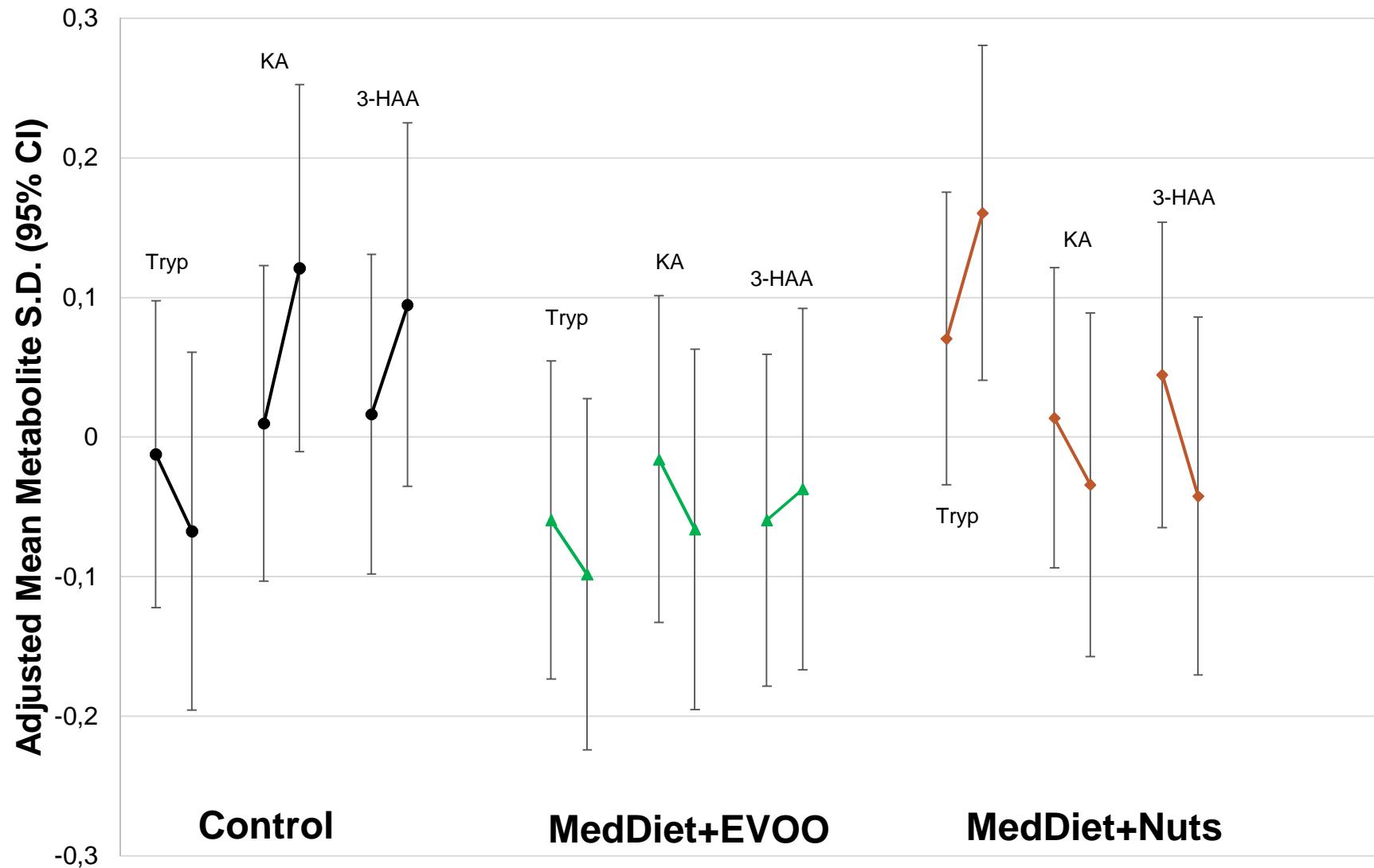


Results: Correlation Matrix

	Tryptophan	3-HAA	KA
Tryptophan	1	0.18	-0.01
3-HAA	0.18	1	-0.01
KA	-0.01	-0.01	1



Results: Intervention Changes



Changes are adjusted for age (years), sex (male, female), intervention group, body mass index (kg/m²), smoking (never, current, former), dyslipidemia, hypertension, leisure time physical activity and baseline fasting glucose.

Results: Baseline Metabolites

251 cases, 694 subcohort						
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend	H.R. per S.D.
Multivariable models						
Tryptophan	1.00 (ref)	1.28 (0.80 – 2.05)	1.68 (1.02 – 2.75)	1.18 (0.67 – 2.08)	0.40	1.04 (0.86 – 1.26)
Kynurenic Acid	1.00 (ref)	0.79 (0.48 – 1.32)	1.17 (0.75 – 1.84)	1.21 (0.76 – 1.92)	0.21	1.10 (0.92 – 1.33)
3-HAA	1.00 (ref)	1.01 (0.64 – 1.58)	0.72 (0.44 – 1.18)	0.79 (0.47 – 1.32)	0.21	0.91 (0.75 – 1.10)
Tryptophan Score	1.00 (ref)	1.13 (0.68 – 1.89)	1.51 (0.89 – 2.55)	1.54 (0.89 – 2.66)	0.08	1.14 (0.93 – 1.39)
Multivariable models + baseline fasting glucose						
Tryptophan	1.00 (ref)	1.97 (1.10 – 3.52)	3.26 (1.75 – 6.09)	2.13 (1.12 – 4.05)	0.01	1.29 (1.04 – 1.61)
Kynurenic Acid	1.00 (ref)	0.88 (0.49 – 1.57)	0.99 (0.56 – 1.75)	1.13 (0.65 – 1.94)	0.59	1.07 (0.87 – 1.31)
3-HAA	1.00 (ref)	1.01 (0.56 – 1.80)	0.85 (0.48 – 1.53)	1.05 (0.58 – 1.91)	0.98	1.00 (0.80 – 1.24)
Tryptophan Score	1.00 (ref)	1.79 (0.89 – 3.61)	2.95 (1.47 – 5.93)	3.25 (1.63 – 6.47)	<0.01	1.38 (1.11 – 1.71)

Multivariate models stratified by recruitment center and adjusted for age (years), sex (male, female), and intervention group (MedDiet+EVOO, MedDiet+nuts), body mass index (kg/m²), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia and hypertension.

Results: 1-Year Changes

157 cases, 531 subcohort						
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend	H.R. per S.D.
Multivariable models						
Tryptophan	1.00 (ref)	1.22 (0.67 – 2.24)	0.82 (0.43 – 1.58)	0.93 (0.47 – 1.81)	0.57	0.97 (0.74 – 1.26)
Kynurenic Acid	1.00 (ref)	0.81 (0.44 – 1.46)	0.48 (0.25 – 0.93)	0.57 (0.32 – 1.03)	0.02	0.81 (0.64 – 1.03)
3-HAA	1.00 (ref)	0.85 (0.48 – 1.51)	0.88 (0.51 – 1.52)	1.05 (0.60 – 1.85)	0.85	1.03 (0.84 – 1.26)
Tryptophan Score	1.00 (ref)	1.45 (0.63 – 3.37)	1.31 (0.62 – 2.76)	0.97 (0.40 – 2.33)	0.88	0.99 (0.74 – 1.31)
Multivariable models + baseline fasting glucose						
Tryptophan	1.00 (ref)	0.97 (0.48 – 1.95)	0.91 (0.43 – 1.96)	0.61 (0.27 – 1.39)	0.25	0.92 (0.66 – 1.26)
Kynurenic Acid	1.00 (ref)	1.15 (0.59 – 2.24)	0.57 (0.25 – 1.31)	0.67 (0.35 – 1.29)	0.09	0.85 (0.66 – 1.10)
3-HAA	1.00 (ref)	0.87 (0.45 – 1.65)	0.91 (0.48 – 1.72)	0.97 (0.47 – 2.01)	0.97	1.02 (0.79 – 1.33)
Tryptophan Score	1.00 (ref)	1.16 (0.46 – 2.90)	1.33 (0.54 – 3.29)	0.83 (0.31 – 2.28)	0.80	0.93 (0.65 – 1.32)

Multivariate models stratified by recruitment center and adjusted for age (years), sex (male, female), and intervention group (MedDiet+EVOO, MedDiet+nuts), body mass index (kg/m²), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia, hypertension, and baseline metabolite values.

Results: Effect Modification

Characteristic		Continuous HR per 1 SD (95% CI)	P for interaction
Sex	Men (n=346)	1.64 (1.08 – 2.49)	0.16
	Women (n=546)	1.45 (1.08 – 1.94)	
Age	< 65 years (n=357)	1.44 (0.96 – 2.16)	0.01
	≥ 65 years (n=535)	1.65 (1.25 – 2.21)	
Intervention group	Control group (n=295)	1.71 (1.10 – 2.66)	<0.01
	Mediterranean diet + EVOO (n=273)	1.62 (1.05 – 2.47)	
	Mediterranean diet + nuts (n=324)	1.01 (0.69 – 1.47)	
Obesity	< 30 kg/m ² (n=473)	1.46 (1.08 – 1.98)	0.18
	≥ 30 kg/m ² (n=419)	1.36 (0.95 – 1.95)	
Smoking status	Current/former smoking (n=366)	1.44 (1.00 – 2.09)	0.30
	Never smoking (n=526)	1.48 (1.09 – 1.99)	
Baseline Hypertension	Yes (n=818)	1.39 (1.11 – 1.74)	0.32
	No (n=74)	*	
Baseline Dyslipidemia	Yes (n=752)	1.39 (1.10 – 1.74)	0.40
	No (n=140)	1.67 (0.53 – 5.20)	

Stratified by recruitment center and adjusted for age (years), sex (male, female), and intervention group (MedDiet+EVOO, MedDiet+nuts), body mass index (kg/m²), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia, hypertension, and baseline glucose.

Limitations

- Causal inference
 - Unmeasured confounding
 - Correlated metabolites
- Generalizability issues
 - Race
 - High-risk population
 - Eating patterns between U.S. and populations in the Mediterranean regions
- Differential loss to follow-up
 - If both baseline metabolite values and case status affect dropout

Future Directions

- Additional tryptophan metabolites (kynurenine and quinolinic acid)
- Alternate scores for urea cycle
- Mediation analysis
 - Diet → Metabolites → T2D
 - Metabolites → BMI → T2D
- Mutual adjustment for risk scores

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- Christopher Papandreou
- PREDIMED Steering Committee

