Acylcarnitines, gut-microbiota related metabolites, T2D and CVD in the PREDIMED Study

Omics, advances, applications and translation into Nutrition and Epidemiology

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HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH

Powerful ideas for a healthier world

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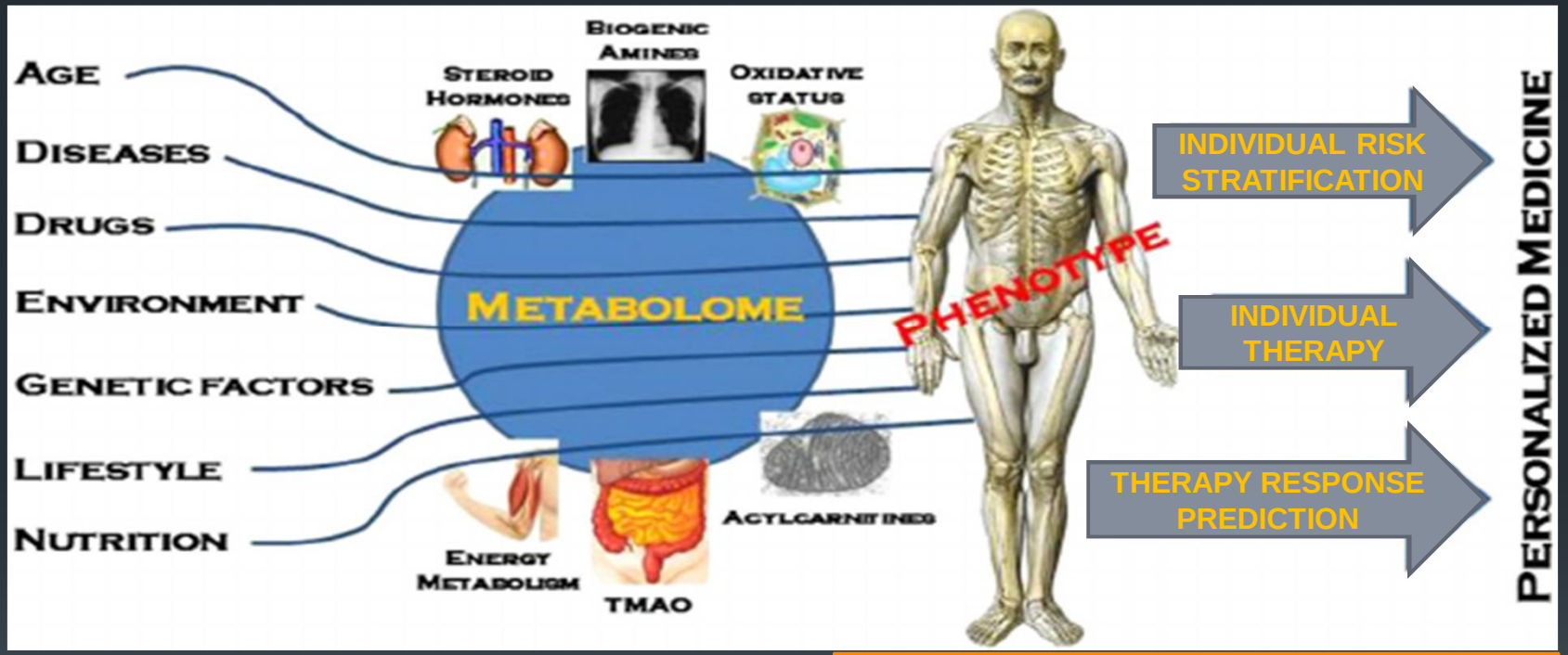
Metabolomics to improve outcomes prediction



Metabolomics is More Time Sensitive Than Other "Omics"

TYPE 2 DIABETES MELLITUS
CARDIOVASCULAR DISEASE

Acylcarnitines and type 2 diabetes

Previous evidence

Early Prediction of Developing Type 2 Diabetes by Plasma Acylcarnitines: A Population-Based Study

Diabetes Care 2016;39:1563–1570 | DOI: 10.2337/dc16-0232

Liang Sun,[1] Liming Liang,[2,3] Xianfu Gao,[4] Huiping Zhang,[4] Pang Yao,[1] Yao Hu,[1] Yiwei Ma,[1] Feijie Wang,[1] Qianlu Jin,[1] Huaixing Li,[1] Rongxia Li,[4] Yong Liu,[1] Frank B. Hu,[2,5,6] Rong Zeng,[4,7] Xu Lin,[1] and Jiarui Wu[4,7,8]

N= 2,103 individuals. **Aged**: 50-70 **Follow-up**: 6 years **Cases**=207

A panel of plasma acylcarnitines, especially long chain, was significantly associated with increased risk of T2D

Table 2—Selected models and risk of incident diabetes

	Variables in model	RR per SD increase of predictive model score	*P*-trend
Model 1	Age, sex, region, residence, smoking, drinking, physical activity, family history of diabetes, BMI, fasting glucose, HbA_{1c}, and systolic blood pressure	2.48 (2.20–2.78)	<0.001
Model 2 (full model)	Age, sex, region, residence, smoking, drinking, physical activity, family history of diabetes, BMI, fasting glucose, HbA_{1c}, systolic blood pressure, and 3-dehydroxycarnitine, 3-dehydrocarnitine, C0, C3, C3DC, C4, C5, C5OH, C6OH, C6DC, C7DC, C8:1, C10, C10DC, C12:1, C12DC, C14:1OH, C16, C16:1, C16:2, C18, C18OH, C18:1, C18:2, and C20:4	9.41 (7.62–11.62), among which 6.94 (5.73–8.41) was attributed to the 25 acylcarnitines	<0.001

The predictive model scores were computed as the weighted sum of all covariates with weights equal to the regression coefficients from the predictive models built by the elastic net regression model.

Previous evidence

A panel of acylcarnitines, mainly involving mitochondrial lipid dysregulation, significantly improved predictive ability for type 2 diabetes beyond conventional risk factors. These findings need to be replicated in other populations, and the underlying mechanisms should be elucidated.

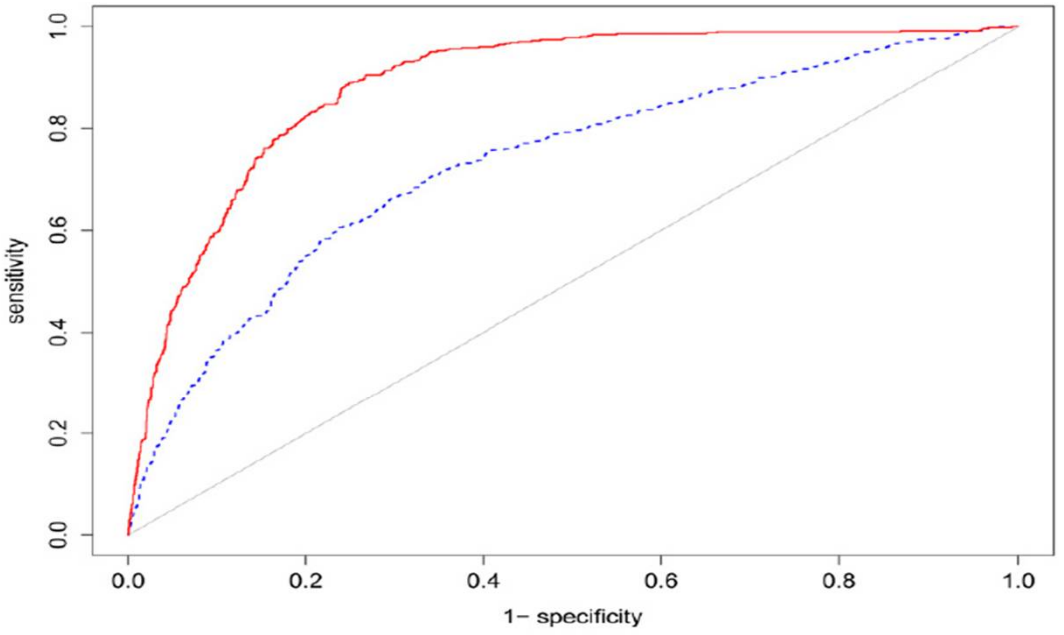


Figure 2—ROC curves for prediction of incident diabetes. Blue curve, conventional model including age, sex, region, residence, smoking, drinking, physical activity, family history of diabetes, BMI, fasting glucose, HbA_{1c}, and systolic blood pressure, AUC = 0.73 (95% CI 0.70–0.76); red curve, conventional model + acylcarnitines selected by elastic net model with s = lambda.min, AUC = 0.89 (95% CI 0.87–0.90).

Other studies

Plasma Acylcarnitine Profiles Suggest Incomplete Long-Chain Fatty Acid β-Oxidation and Altered Tricarboxylic Acid Cycle Activity in Type 2 Diabetic African-American Women[1–3]

Adams et al. 2009

Increased Levels of Plasma Acylcarnitines in Obesity and Type 2 Diabetes and Identification of a Marker of Glucolipotoxicity

Mihalik et al. 2010

Plasma Metabolomic Profiles Reflective of Glucose Homeostasis in Non-Diabetic and Type 2 Diabetic Obese African-American Women

Fiehn et al. 2010

Novel biomarkers for pre-diabetes identified by metabolomics

Wang-Sattler et al. 2012

Hypothesis:

- Plasma acylcarnitine species are associated with the incidence of T2D; and these asssociations might be modified by a Mediterranean diet intervention.

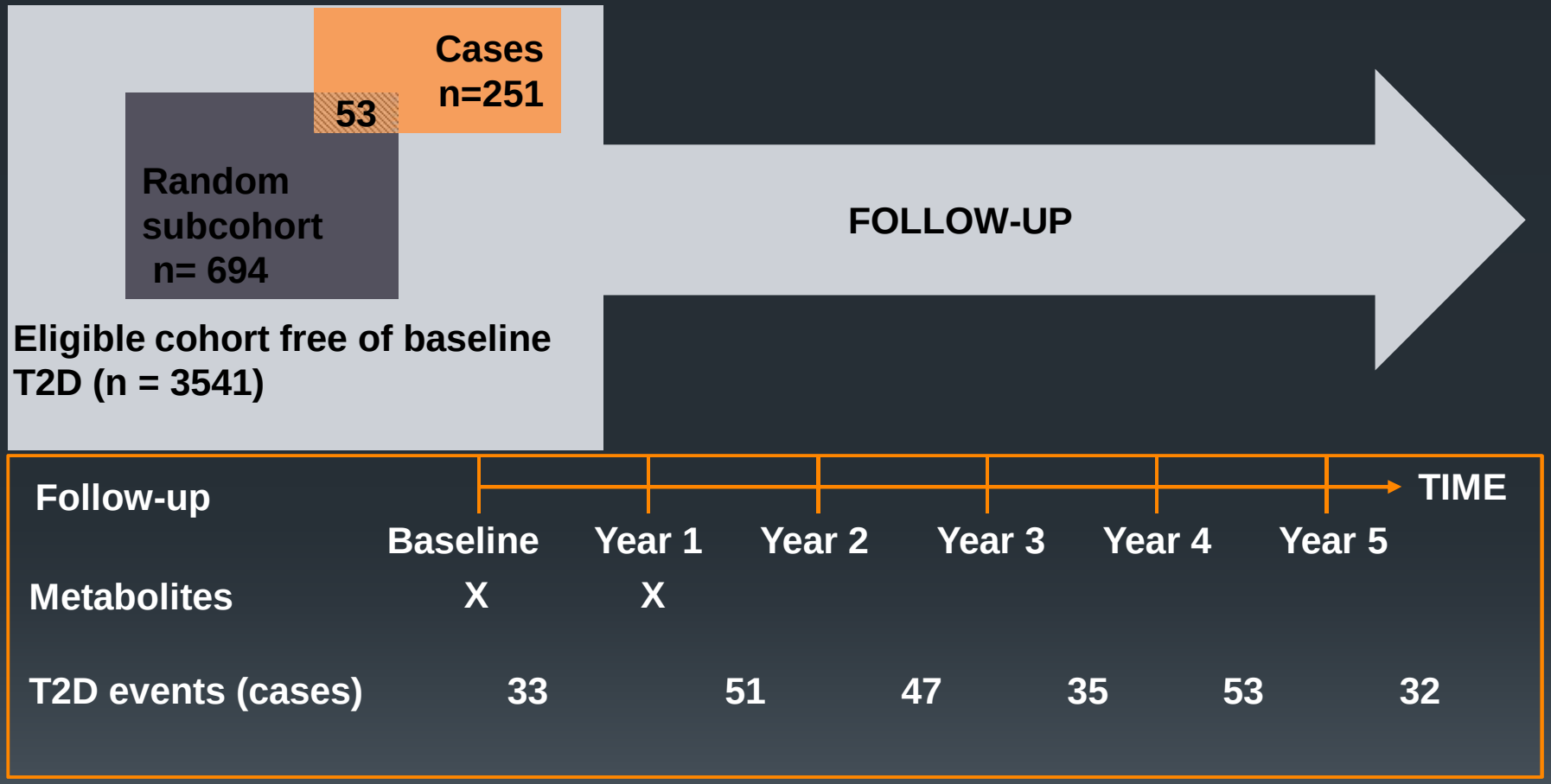
Objectives:

1) To evaluate the associations between plasma acylcarnitine profiles and the risk of T2D in individuals at high cardiovascular risk.

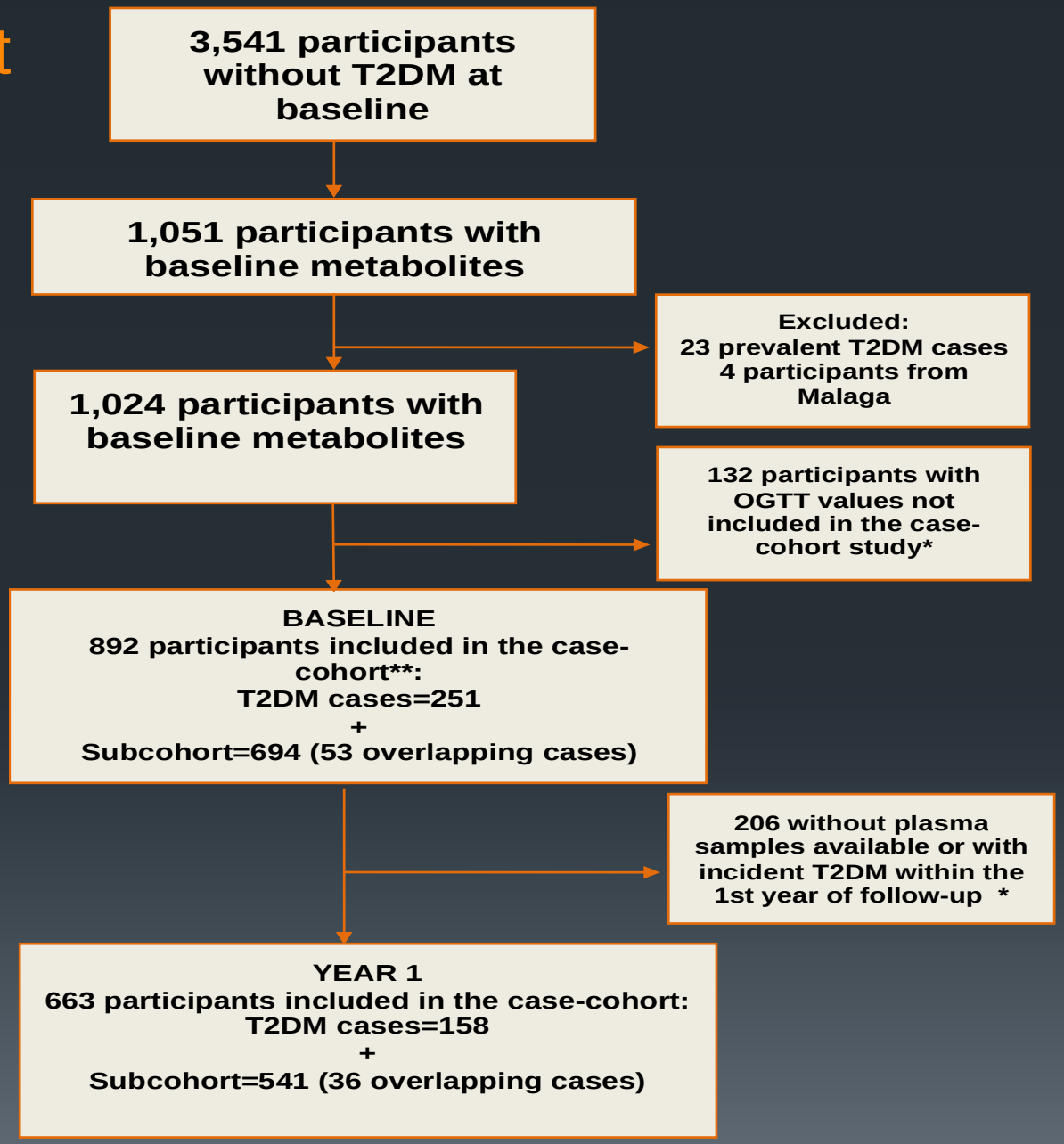
2) To examine whether these associations might be mitigated by Mediterranean Diet interventions.

3) To evaluate the association between 1-year changes in acylcarnitines profiles and risk of T2D.

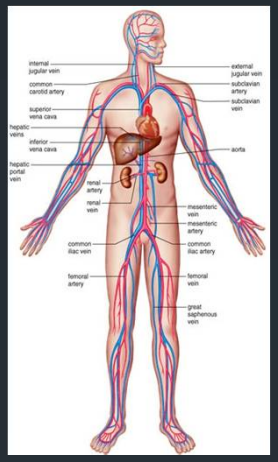
Case-cohort study in the PREDIMED trial



Flow-chart


3,541 participants without T2DM at baseline
1,051 participants with baseline metabolites
Excluded:
23 prevalent T2DM cases
4 participants from Malaga
1,024 participants with baseline metabolites
132 participants with OGTT values not included in the case-cohort study*
BASELINE
892 participants included in the case-cohort**:
T2DM cases=251
+
Subcohort=694 (53 overlapping cases)
206 without plasma samples available or with incident T2DM within the 1st year of follow-up *
YEAR 1
663 participants included in the case-cohort:
T2DM cases=158
+
Subcohort=541 (36 overlapping cases)

Statistical approach



Metabolomics

Free acylcarnitine and 27 acylcarnitine subtypes

- Natural logarithmic transformation to raw metabolite values
- 3 weighted acylcarnitine scores (β*AC1 + β*AC2 + β*AC3 …)
  - Short-chain acylcarnitine score: C2 – C7
  - Medium-chain acylcarnitine score: C8 – C14:2
  - Long-chain acylcarnitine score: C16 – C26
- Multivariable Cox regression models
  - Adjusted for potential confounders
  - Case-cohort design

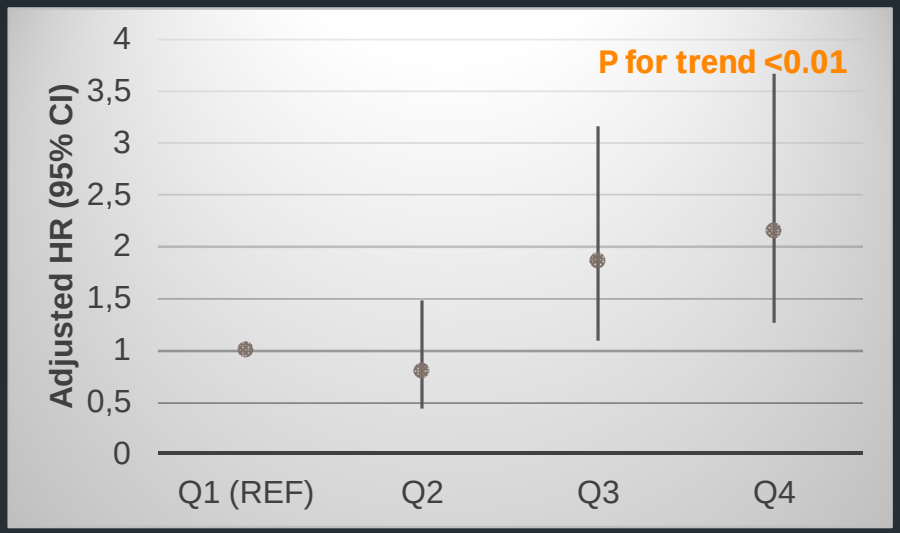
Baseline characteristics

Variable	Total (n=892)	Cases (n=251)	Non-cases (n=641)
Age (years)	66.5 (6)	66.4 (5.7)	66.5 (5.7)
Sex, n (% Women)	546 (61.2)	138 (54.9)	408 (63.6)
Body mass index, kg/m^2	30.0 (3.6)	30.8 (3.4)	29.7 (3.5)
Smokers, %	166 (18.6)	63 (25.1)	103 (16.0)
MedDiet+EVOO	273 (30.1)	75 (29.8)	198 (30.9)
MedDiet+Nuts	324 (36.3)	91 (36.2)	204 (31.8)
Control group	295 (33.1)	85 (33.8)	239 (37.2)
Hypertension, %	818 (91.7)	241 (96.0)	577 (90.0)
Dyslipidaemia, %	752 (84.3)	200 (79.6)	552 (86.1)
Fasting glucose, mg/dL	102.56 (19.23)	116.45 (19.09)	96.63 (16.22)
Total cholesterol, mg/dL	220.47 (38.81)	220.09 (41.44)	220.63 (37.75)
Physical activity, METS/d	240.7 (234.6)	249.2 (253.5)	237.38 (235.1)

Data are expressed as means ± SD or percentage (n). CHD, coronary heart disease; EVOO, extra-virgin olive oil; MedDiet, Mediterranean Diet.*significant

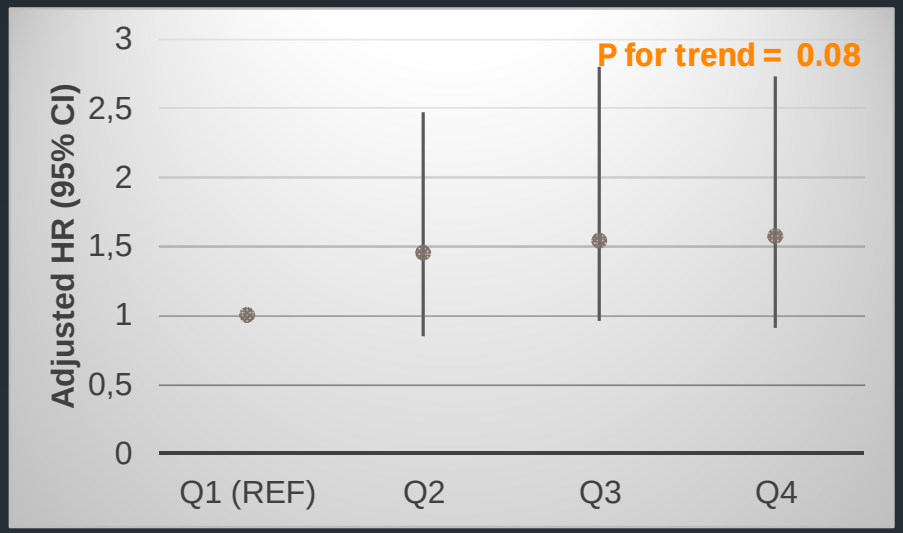
Baseline acylcarnitines and T2D risk

1) Short-chain acylcarnitine score

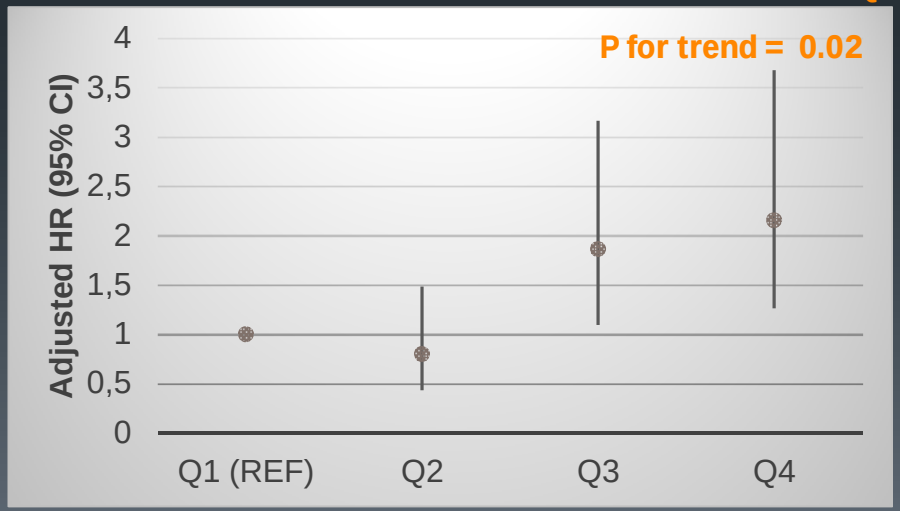


HR Q4 vs Q1: 2.14 (95% CI: 1.25-3.65)

2) Medium-chain acylcarnitine score



HR Q4 vs Q1: 1.57 (95% CI: 0.91-2.73)



3) Long-chain acylcarnitine score

HR Q4 vs Q1: 1.73 (95% CI: 1.04-1.90)

Models were adjusted for age, sex, BMI, family history of premature heart disease, smoking, physical activity (Mets/d), hypertension, dyslipidemia, fasting glucose and stratified by intervention group.

Acylcarnitines and T2D risk by intervention group

	Both MedDiet groups	Control group
Cases, n	153	85
Non-cases, n	437	199
Short-chain acylcarnitine score		
Q1	1 (ref.)	1 (ref.)
Q2	0.73 (0.33, 1.58)	0.69 (0.25,1.94)
Q3	2.10 (1.06, 4.12)	0.62 (0.19, 1.99)
Q4	2.15 (0.87, 5.46)	1.25 (0.52, 3.00)
P for trend	0.08	0.45
Medium acylcarnitine score		
Q1	1 (ref.)	1 (ref.)
Q2	1.46 (0.73, 2.94)	1.01 (0.42, 2.43)
Q3	1.77 (0.87, 3.58)	1.61 (0.68, 3.82)
Q4	1.81 (0.86, 3.77)	0.81 (0.30, 2.17)
P for trend	0.08	0.95

Models were adjusted for age, sex, BMI, family history of premature heart disease, smoking, physical activity (Mets/d), hypertension, dyslipidemia, fasting glucose and stratified by intervention group.

Acylcarnitines and T2D risk by intervention group

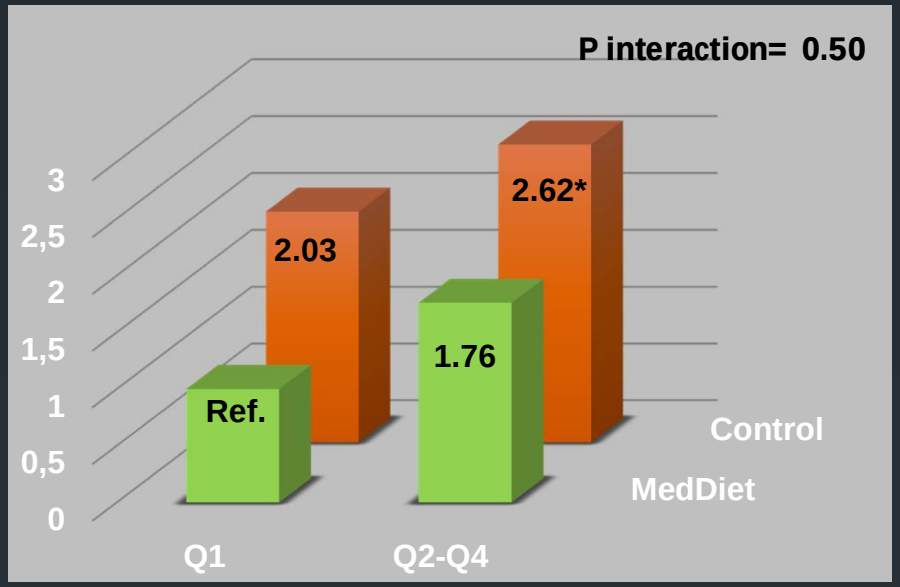
	Both MedDiet groups	Control group
Cases, n	148	81
Non-cases, n	423	194
Long-chain acylcarnitine score		
Q1	1 (ref.)	1 (ref.)
Q2	1.22 (0.58, 2.59)	1.89 (0.68, 5.24)
Q3	1.16 (0.57, 2.33)	2.37 (0.80, 6.97)
Q4	2.33 (1.20, 4.55)	1.32 (0.47, 3.75)
P for trend	<0.01	0.67

Models were adjusted for age, sex, BMI, family history of premature heart disease, smoking, physical activity (Mets/d), hypertension, dyslipidemia, fasting glucose and stratified by intervention group.

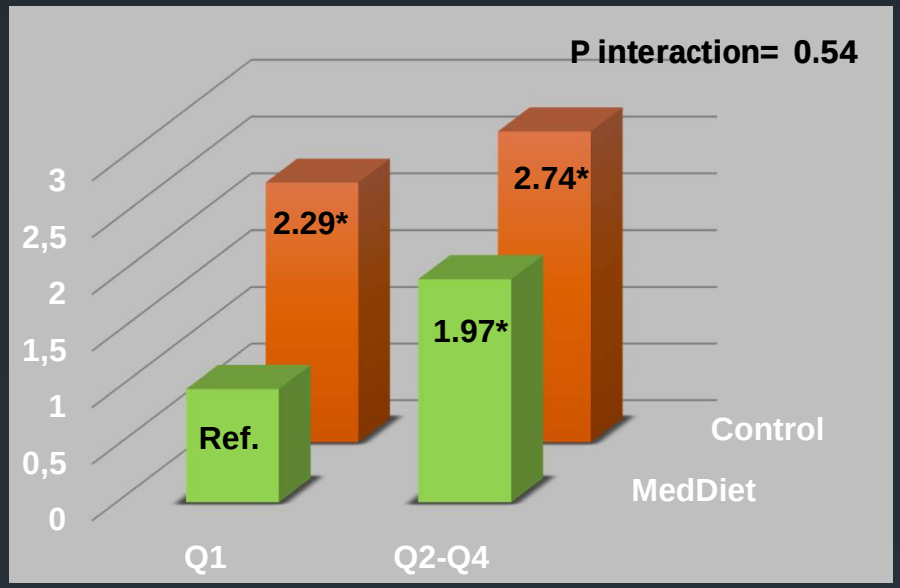
- Associations were not significant when fasting glucose was not included in the model or when stratifying the analysis with 3 intervention groups.

Effect modification on MedDiet intervention

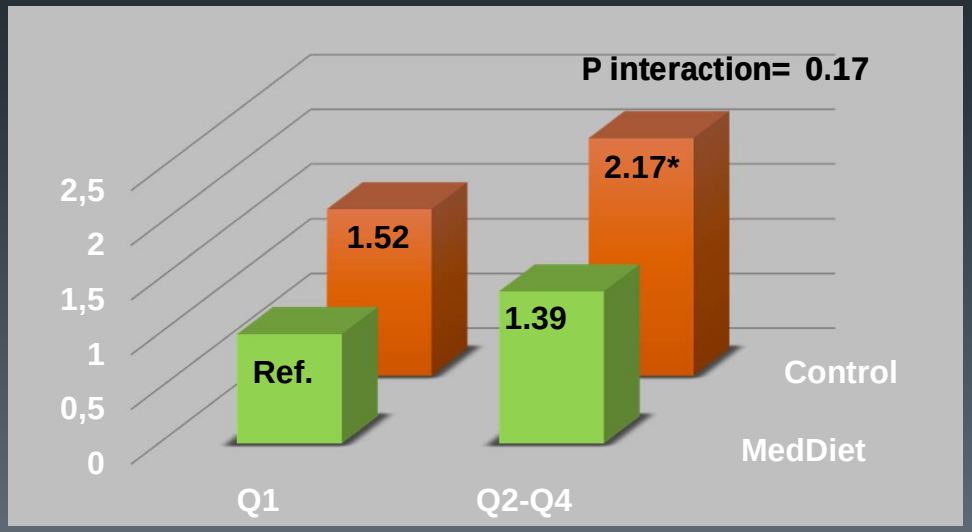
1) Short-chain acylcarnitine score



2) Medium-chain acylcarnitine score

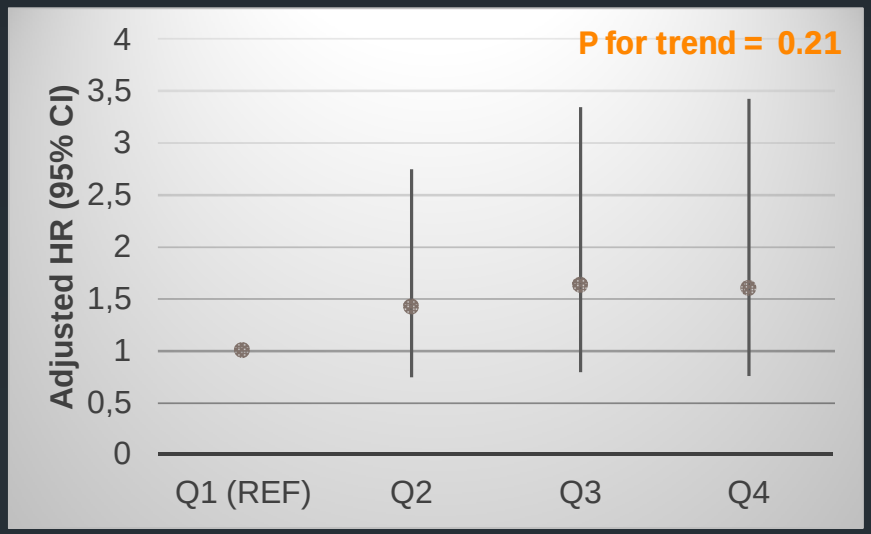


3) Long-chain acylcarnitine score



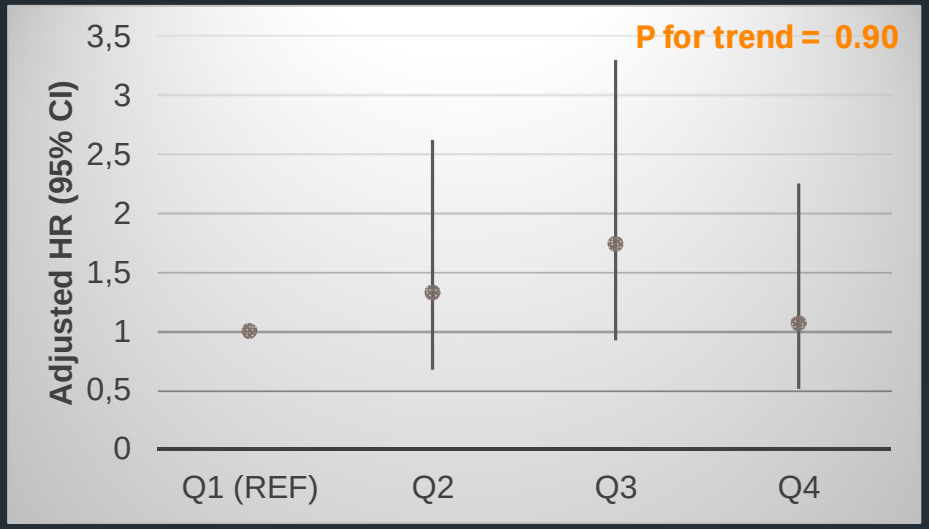
1-year changes in AC and risk of T2D

1) Short-chain acylcarnitine score

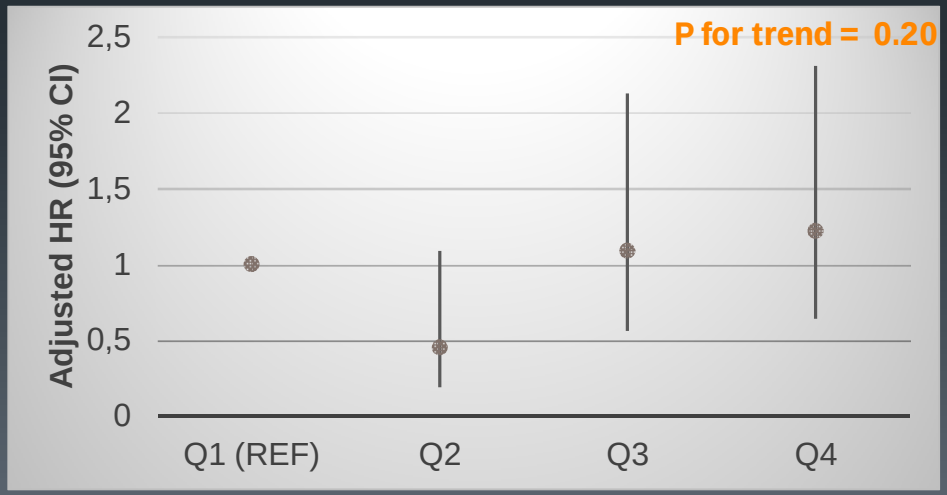


HR Q4 vs Q1: 1.60 (95% CI: 0.75-3.42)

2) Medium-chain acylcarnitine score



HR Q4 vs Q1: 1.07 (95% CI: 0.51-2.25)



Models were adjusted for age, sex, BMI, family history of premature heart disease, smoking, physical activity (Mets/d), hypertension, dyslipidemia, fasting glucose, baseline acylcarnitines and stratified by intervention group.

3) Long-chain acylcarnitine score **HR Q4 vs Q1: 1.22 (95% CI: 0.64-2.31)**

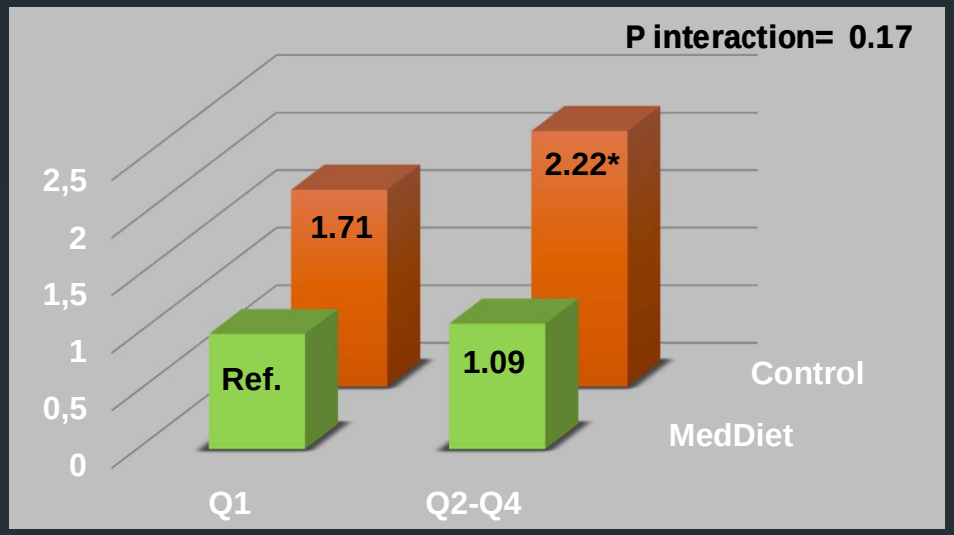
1-year changes in AC and risk of T2D per SD

	Multivariable HR and 95% CI per SD	P value
Short acylcarnitine score	1.93 (1.22, 3.04)	<0.01
Medium acylcarnitine score	1.13 (0.66, 1.92)	0.65
Long acylcarnitine score	1.73 (0.54, 5.57)	0.35

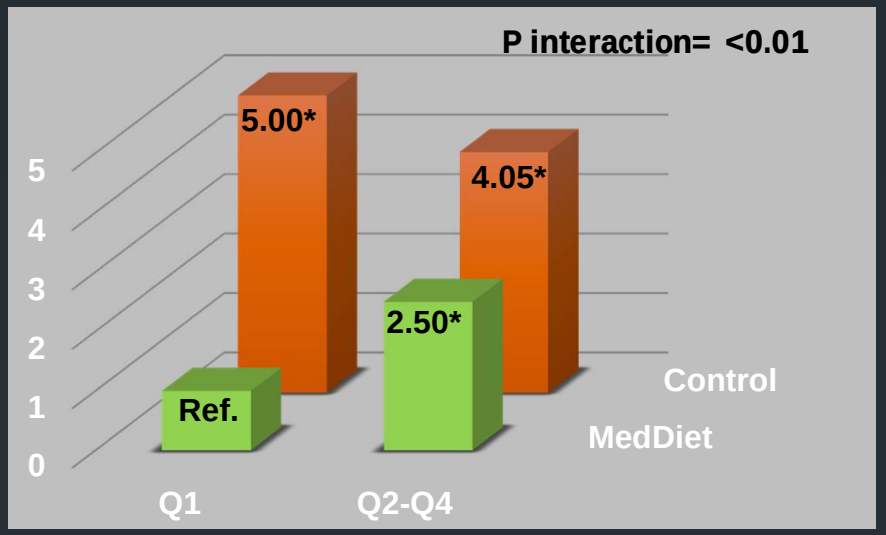
Models were adjusted for age, sex, BMI, family history of premature heart disease, smoking, physical activity (Mets/d), hypertension, dyslipidemia, fasting glucose, baseline acylcarnitines and stratified by intervention group.

Effect modification on MedDiet intervention for 1year changes

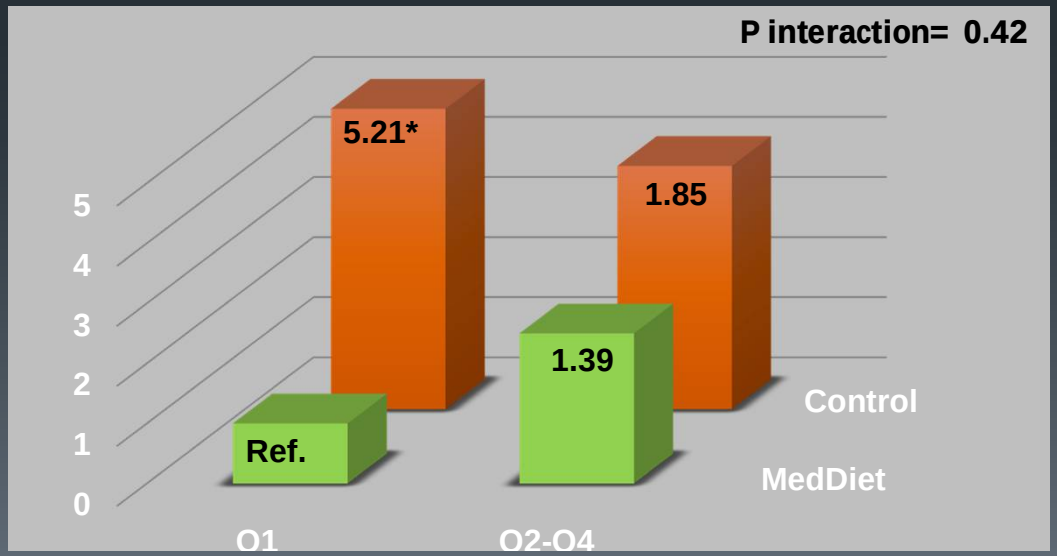
1) Short-chain acylcarnitine score



2) Medium-chain acylcarnitine score

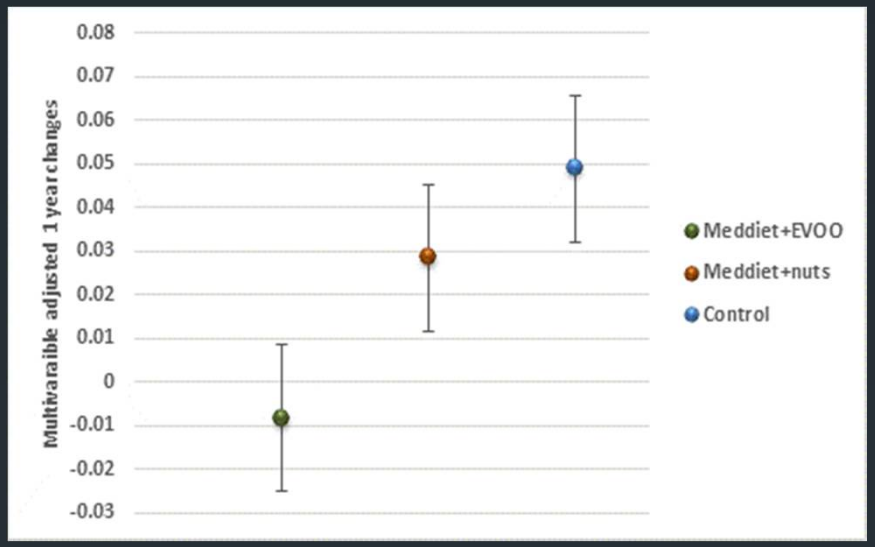


3) Long-chain acylcarnitine score

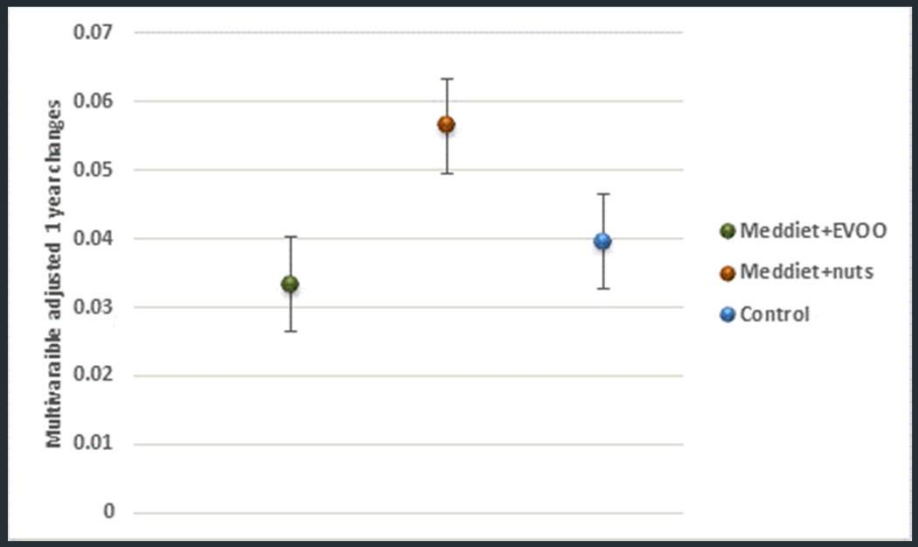


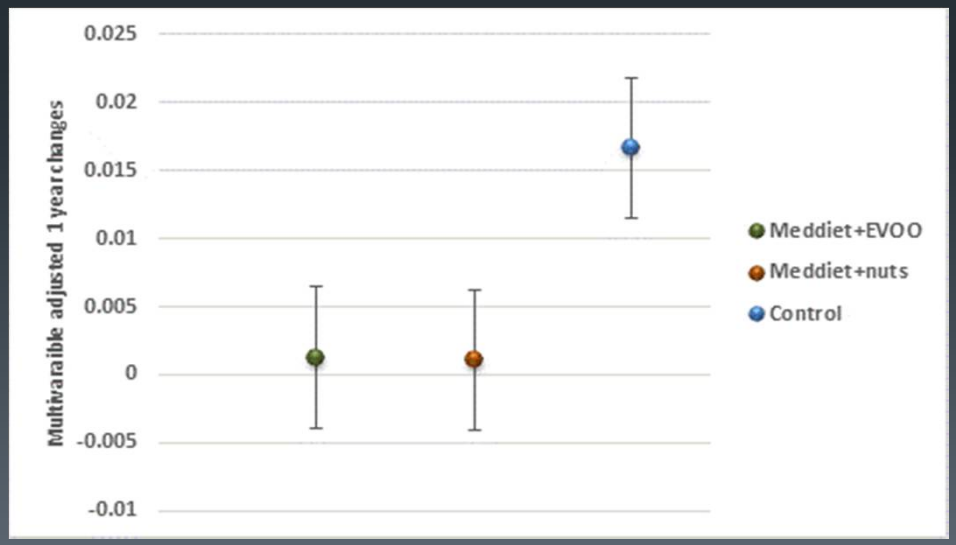
Changes in acylcarnitines scores by Intervention Group

1) Short-chain acylcarnitine score



2) Medium-chain acylcarnitine score





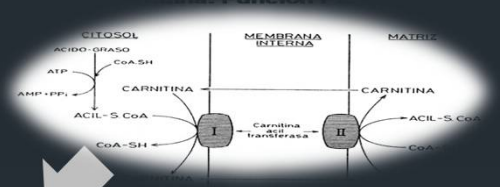
3) Long-chain acylcarnitine score

Models were adjusted for age, sex, BMI, family history of premature heart disease, smoking, physical activity (Mets/d), hypertension, dyslipidemia, fasting glucose, baseline acylcarnitines and stratified by intervention group.

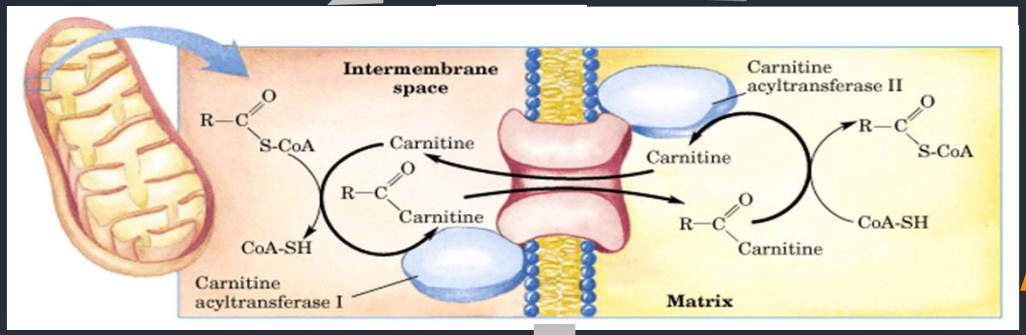
Potential mechanisms

Diet

Biosynthesis



carnitine



Acylcarnitines

Age

Inefficient B oxidation and mitochondrial dysfunction

Accumulation of acylcarnitines

Insulin resistance Type 2 diabetes

Conclusions

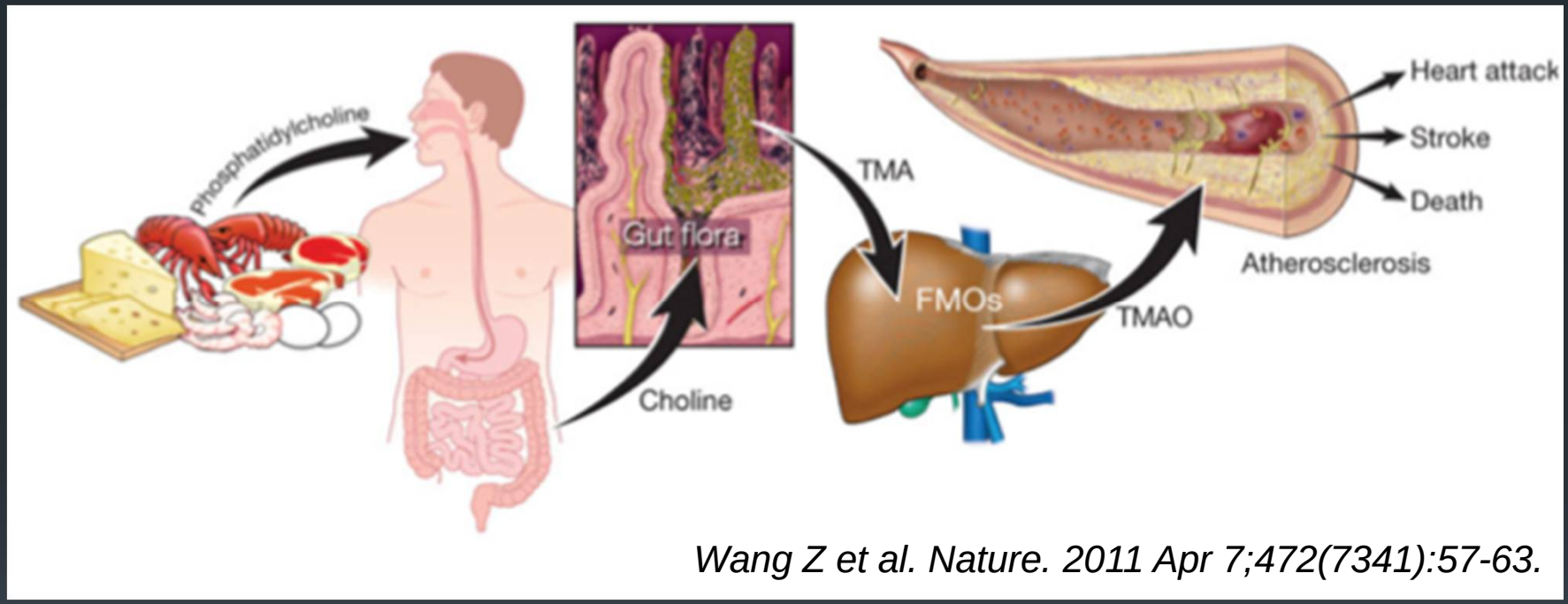
- Metabolite profiles composed of **elevated acylcarnitines** were associated with **higher risk of T2D** after 4.1 years of follow-up in individuals at high risk from the PREDIMED Study.

- Although no significant interactions were observed between MedDiet and acylcarnitine scores, those participants with higher concentrations of acylcarnitines and randomized to the control group had higher risk of T2D compared to those with lower concentrations and randomized to the MedDiet group.

- No significant associations were observed for MedDiet and 1 year changes in acylcarnitines.

Choline pathway metabolites and risk of CVD

Choline metabolites and risk of CVD



Wang Z et al. Nature. 2011 Apr 7;472(7341):57-63.

Betaine and choline metabolites and the gut-microbiota-dependent **TMAO** have been linked with **CVD**.

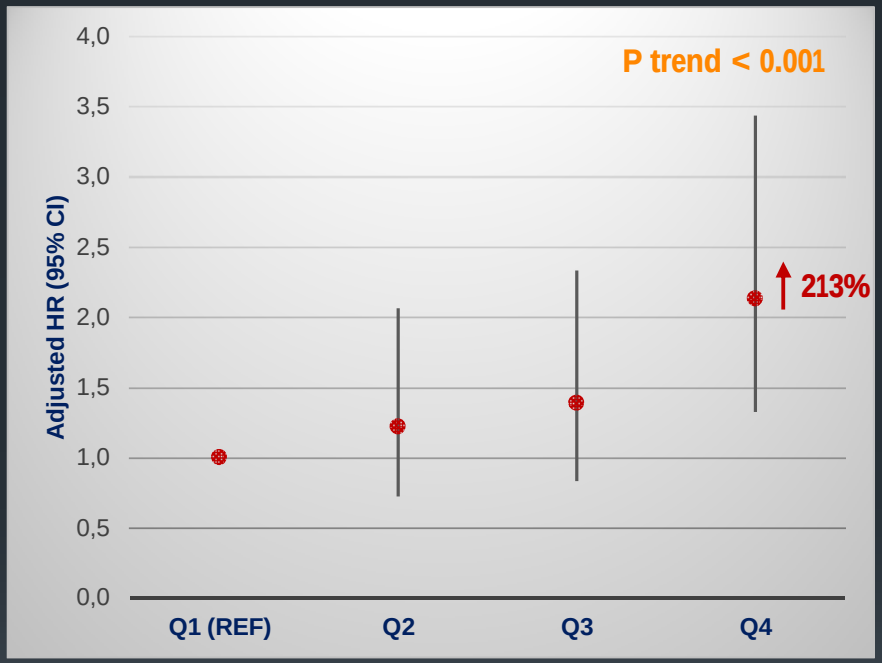
Choline pathway metabolites are associated with CVD

Baseline plasma concentrations of choline pathway metabolites and CVD in the PREDIMED Study						
	Q1	Q2	Q3	Q4	P_{trend}	1SD
TMAO						
Age-adjusted	Ref.	1.39 (0.89, 2.16)	1.16 (0.74, 1.84)	1.22 (0.78, 1.92)	0.59	1.04 (0.89, 1.20)
Multivariable Model	Ref.	1.37 (0.85, 2.22)	1.08 (0.66, 1.76)	1.09 (0.67, 1.77)	0.94	1.01 (0.85, 1.18)
Betaine						
Age-adjusted	Ref.	0.94 (0.59, 1.48)	1.03 (0.66, 1.61)	0.77 (0.49, 1.21)	0.30	0.89 (0.77, 1.03)
Multivariable Model	Ref.	1.00 (0.63, 1.60)	1.23 (0.77, 1.97)	0.91 (0.77, 1.49)	0.87	0.95 (0.82, 1.12)
Choline						
Age-adjusted	Ref.	1.17 (0.71, 1.91)	1.69 (1.06, 2.68)	1.93 (1.22, 3.07)	<0.01	1.30 (1.11, 1.52)
Multivariable Model	Ref.	1.08 (0.64, 1.81)	1.48 (0.89, 2.46)	1.72 (1.05, 2.81)	0.01	1.24 (1.05, 1.46)
Phosphocholine						
Age-adjusted	Ref.	1.07 (0.69, 1.67)	1.20 (0.76, 1.88)	1.37 (0.88, 2.13)	0.12	1.09 (0.93, 1.28)
Multivariable Model	Ref.	1.18 (0.73, 1.93)	1.27 (0.77, 2.08)	1.41 (0.87, 2.28)	0.15	1.09 (0.92, 1.30)
Alphaglycerophosphocholine						
Age-adjusted	Ref.	1.02 (0.66, 1.57)	0.86 (0.55, 1.36)	1.36 (0.89, 2.09)	0.19	1.21 (1.01, 1.44)
Multivariable Model	Ref.	0.99 (0.62, 1.57)	0.92 (0.57, 1.51)	1.42 (0.89, 2.28)	0.14	1.24 (1.03, 1.50)

Adjusted for age, sex, body mass index, family history of premature heart disease, smoking, physical activity, hypertension, dyslipidemia, and diabetes, and stratified by intervention group

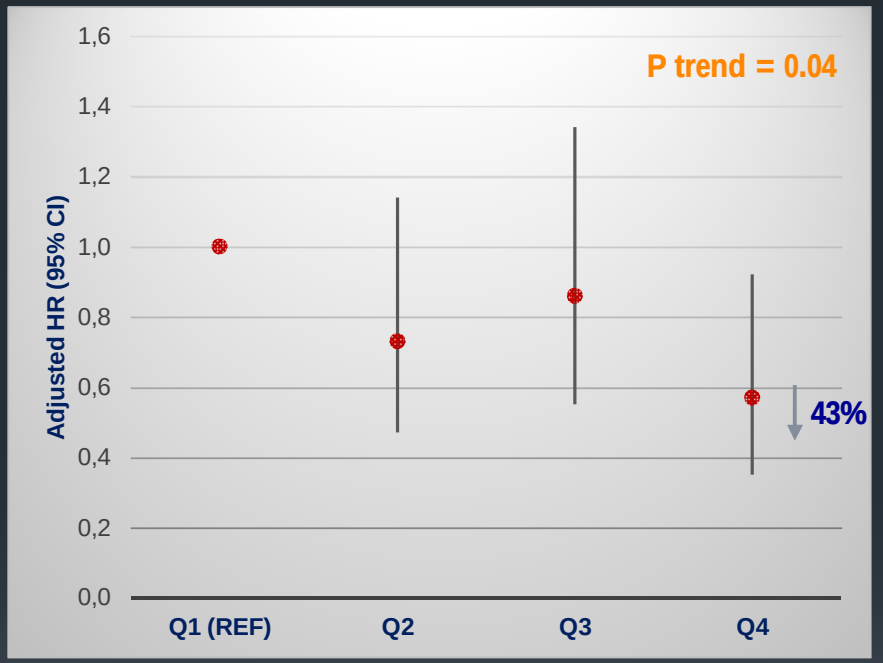
Overall metabolite score and risk of CVD

a) Metabolite score



Cases:	34	46	52	97

b) Betaine/choline ratio

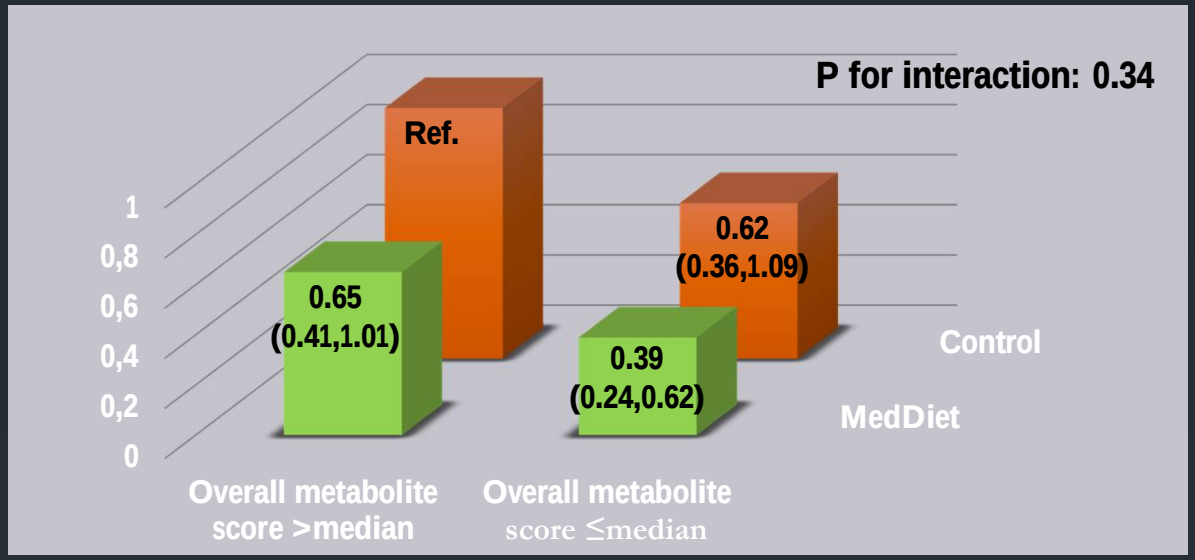


Cases:	74	52	63	40

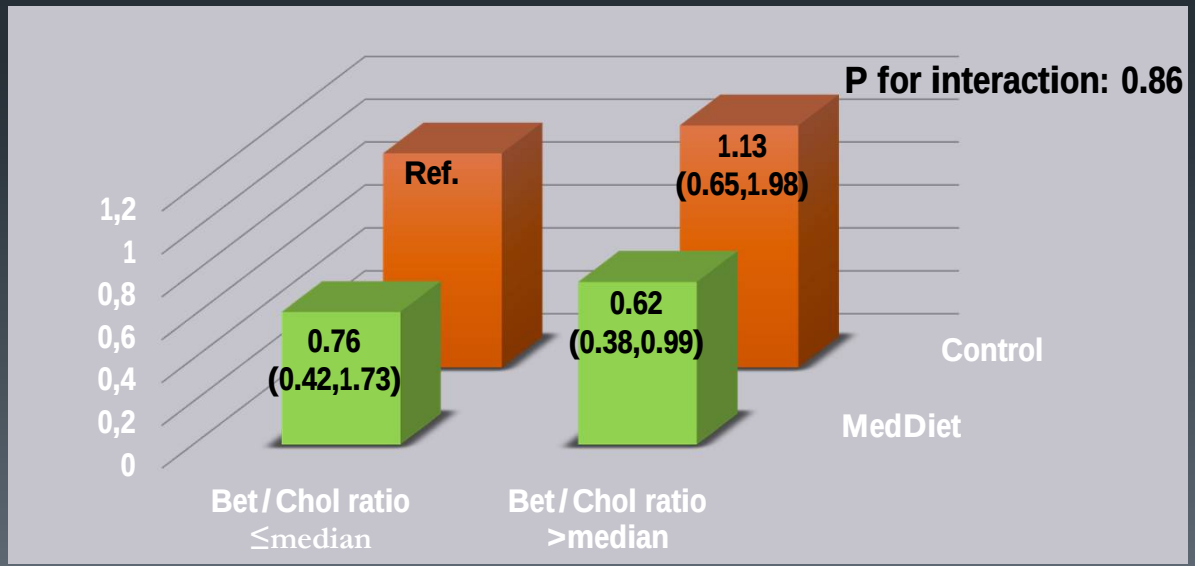
Adjusted for age, sex, body mass index, family history of premature heart disease, smoking, physical activity, hypertension, dyslipidemia, and diabetes, and stratified by intervention group

Effect modification by intervention group

a) Metabolite score



b) Betaine / choline ratio



LIMITATIONS

- Extrapolation to other populations
- Residual confounding
- Hypothesis driven specific metabolite pathways which could include other metabolites

STRENGTHS

- Prospective design
- Well-characterised cohort
- Blinded assessment of T2D and CVD cases
- Case-cohort design preserved randomization

Conclusions

- Our findings demonstrated that **choline pathway metabolites** were associated with an **increased risk of CVD**, independent of traditional risk factors, in a Mediterranean population at high cardiovascular risk.

- Identifying **novel metabolites** can improve our **understanding of diet and disease**.

Acknowledgements

PREDIMED Metabolomics team

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Dr. Jordi Salas-Salvadó

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Dr. Yan Zheng

Dr. Adela Hruby

Dr. Liming Liang

Dr. Daniel Wang

Dr. Mònica Bulló

Edward Yu

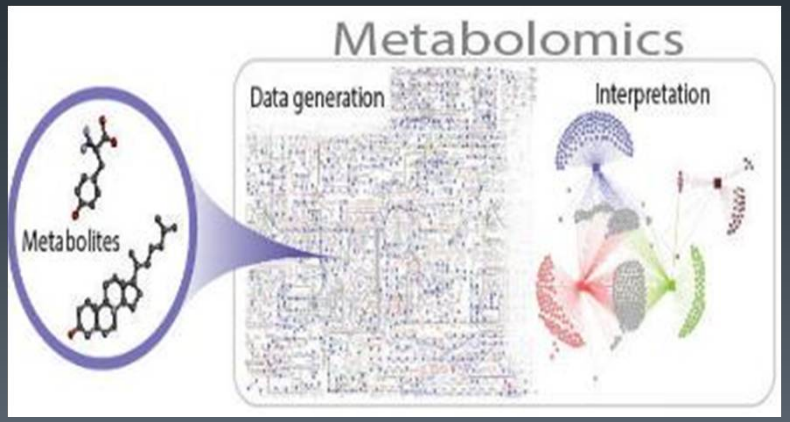
Dr. Jun Li

Dr. Christopher Papandreou

PREDIMED STEERING COMMITE

PREDIMED personnel and participants

Thank you for your attention



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