# Branched-chain and aromatic amino acids and T2D in the PREDIMED Study

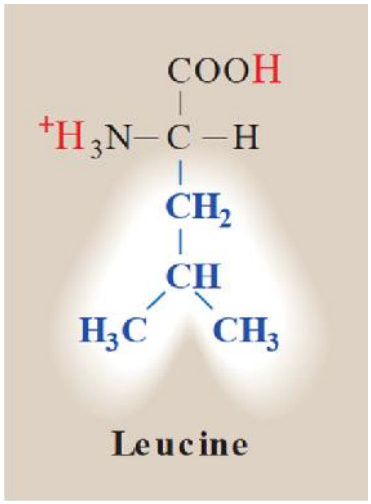
Miguel Ruiz-Canela, PharmD, MPH, PhD

www.unav.es/preventiva
www.predimed.es
www.predimedplus.com
www.ciberobn.es

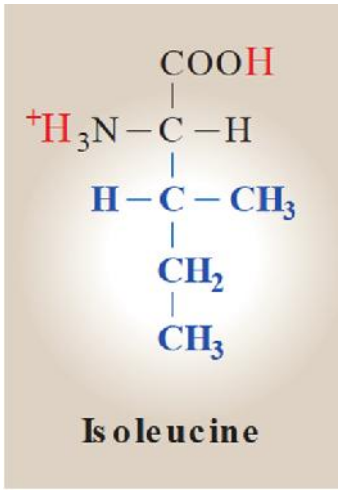
# Outline

1. Introduction
2. Methods
3. Results
4. Discussion/Conclusion

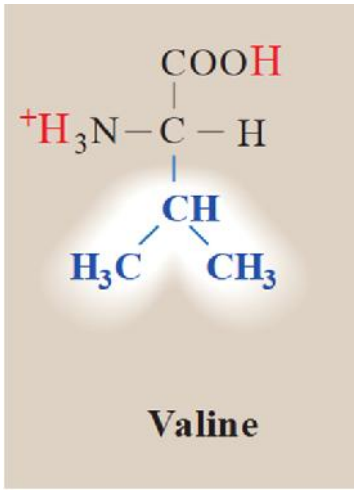
# Branched-chain amino acids (BCAA)



Leucine

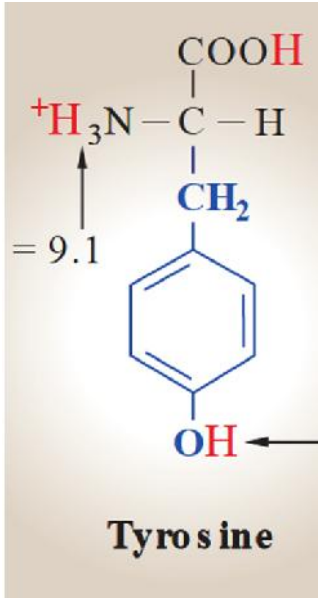


Isoleucine

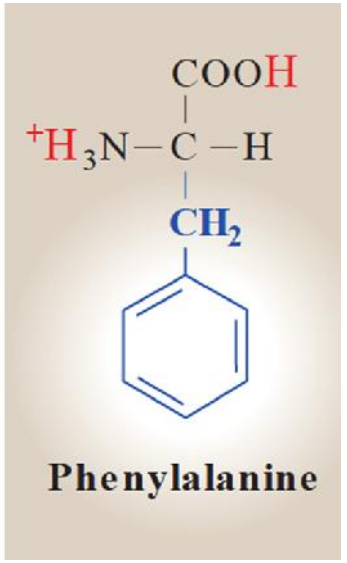


Valine

# Aromatic Amino Acids (AAAs)



Tyrosine

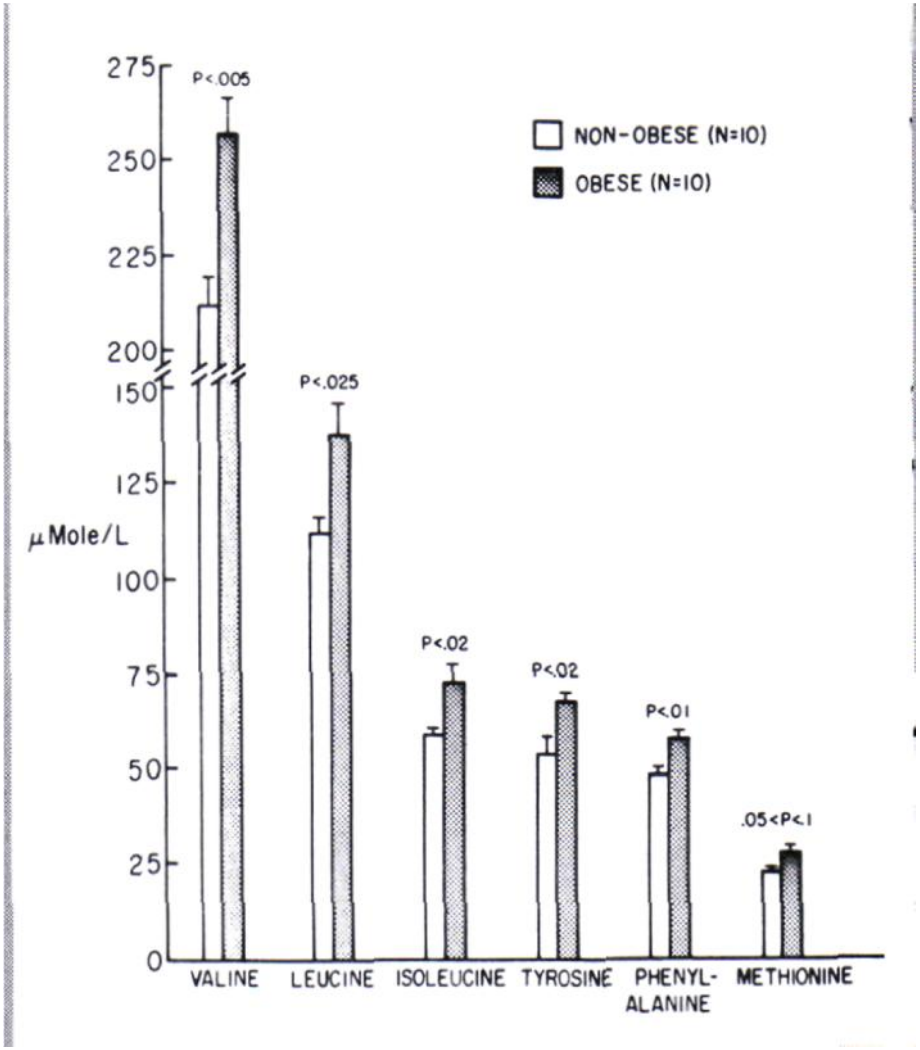


Phenylalanine

# Plasma Amino Acid Levels and Insulin Secretion in Obesity

Philip Felig, M.D., Errol Marliss, M.D., and George F. Cahill, Jr., M.D.

N Engl J Med 1969; 281:811-816 | October 9, 1969 | DOI: 10.1056/NEJM196910092811503

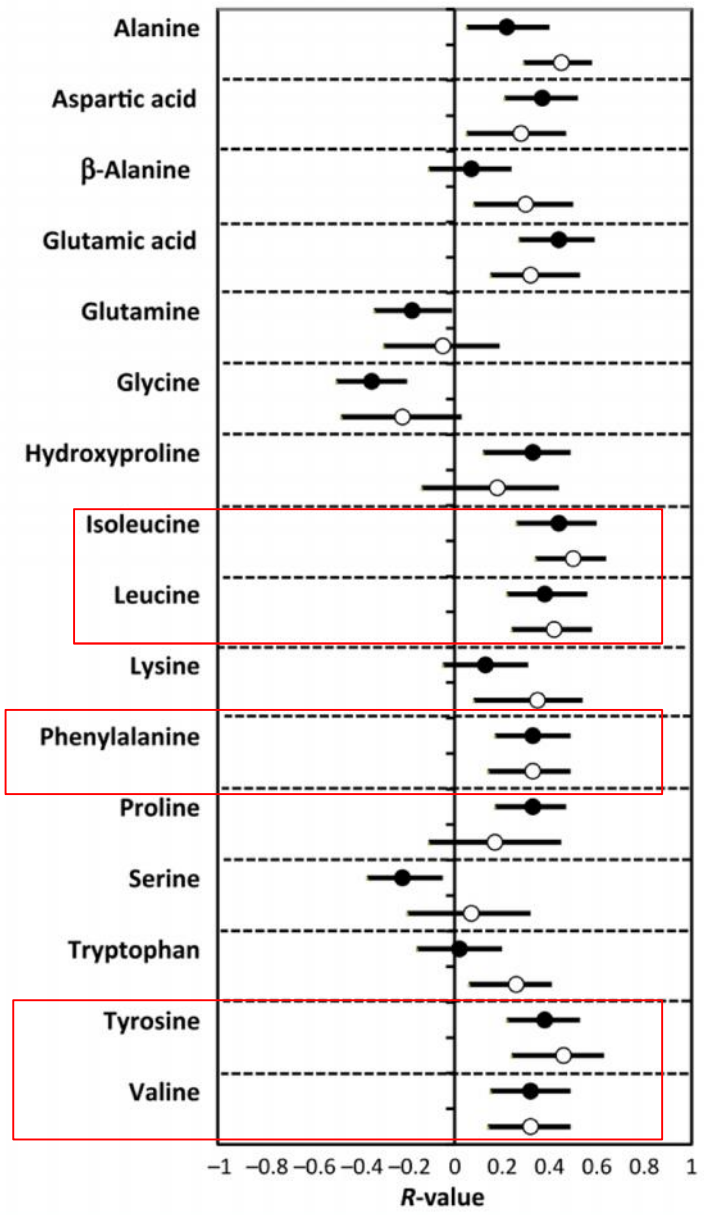



Background → Methods → Results → Discussion/Conclusion

# Relationship between insulin resistance and amino acids in women and men

Ryan Seibert[1], Fahim Abbasi[1], Feras M. Hantash[2], Michael P. Caulfield[2], Gerald Reaven[1] & Sun H. Kim[1]





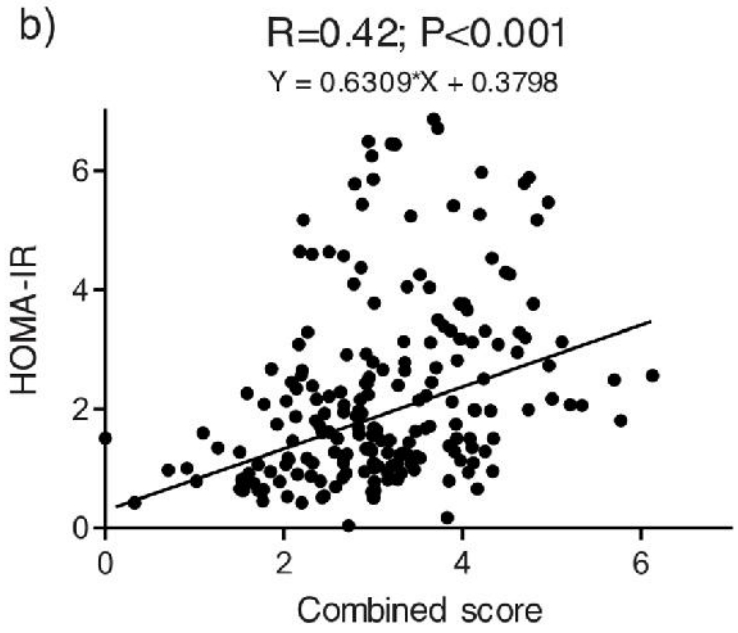

Background → Methods → Results → Discussion/Conclusion

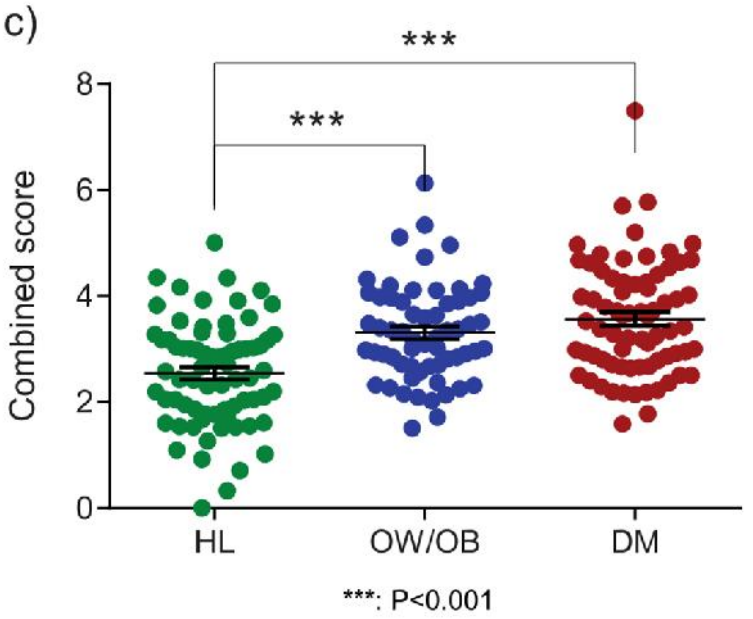
Tianlu Chen[1,*], Yan Ni[2,*], Xiaojing Ma[3], Yuqian Bao[1,3], Jiajian Liu[1], Fengjie Huang[1], Cheng Hu[1,3], Guoxiang Xie[2], Aihua Zhao[1], Weiping Jia[1,3] & Wei Jia[1,2]

Published: 05 February 2016

SCIENTIFIC REPORTS | 6:20594 | DOI: 10.1038/srep20594

# Branched-chain and aromatic amino acid profiles and diabetes risk in Chinese populations



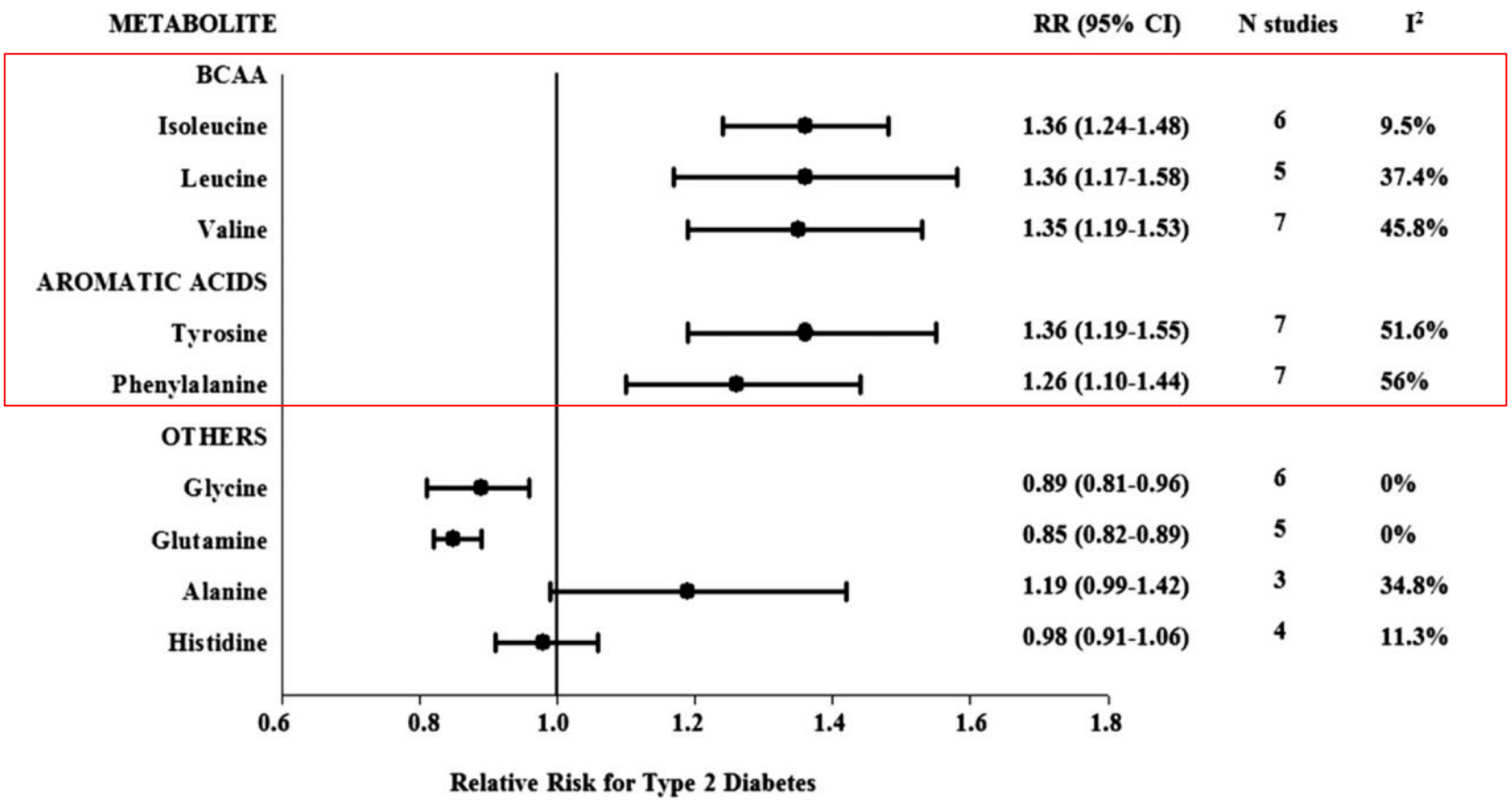



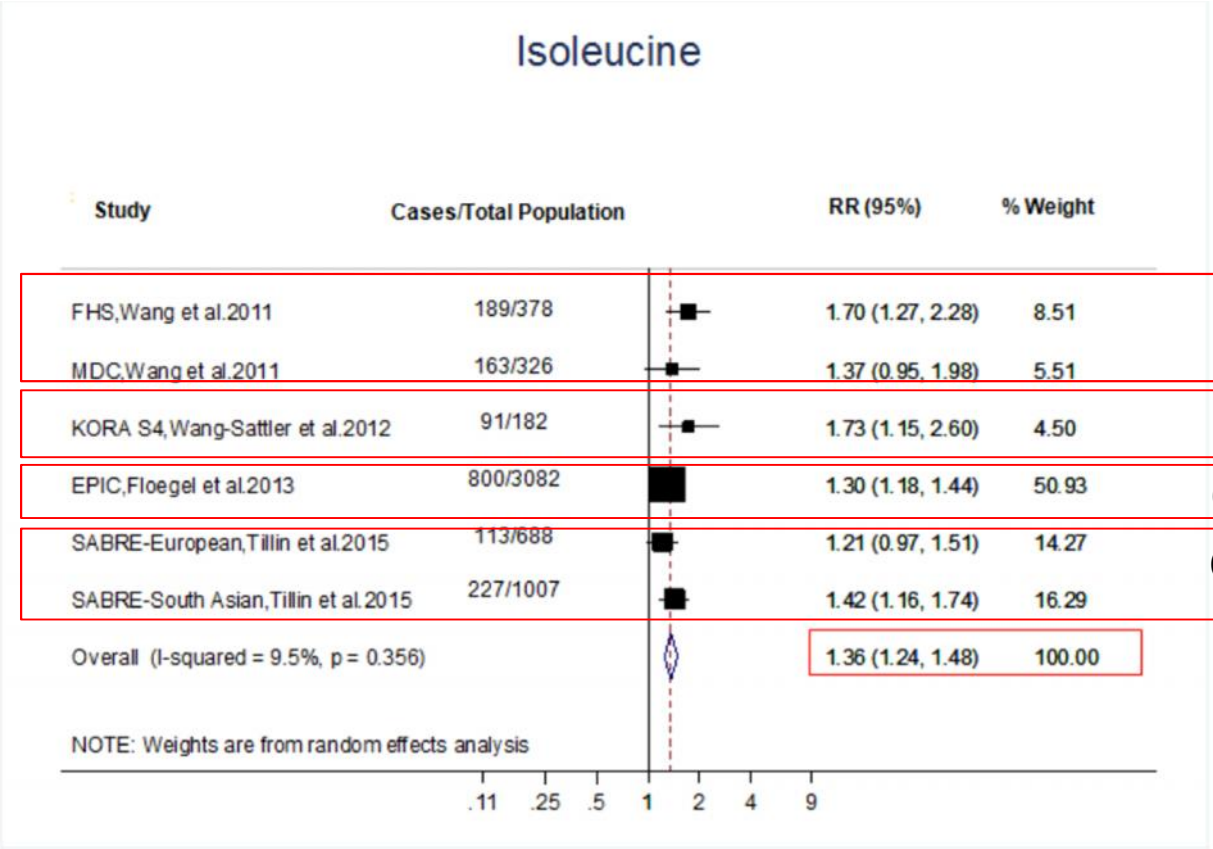

Background → Methods → Results → Discussion/Conclusion

# Metabolomics in Prediabetes and Diabetes: A Systematic Review and Meta-analysis

*Marta Guasch-Ferré,[1,2,3] Adela Hruby,[1] Estefanía Toledo,[3,4] Clary B. Clish,[5] Miguel A. Martínez-González,[3,4] Jordi Salas-Salvadó,[2,3] and Frank B. Hu[1,6,7]*

*Diabetes Care 2016;39:833–846 | DOI: 10.2337/dc15-2251*





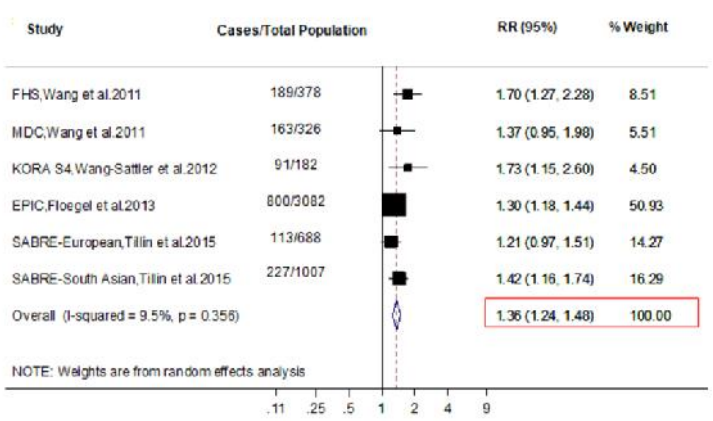

Nested case-control

Nested case-control

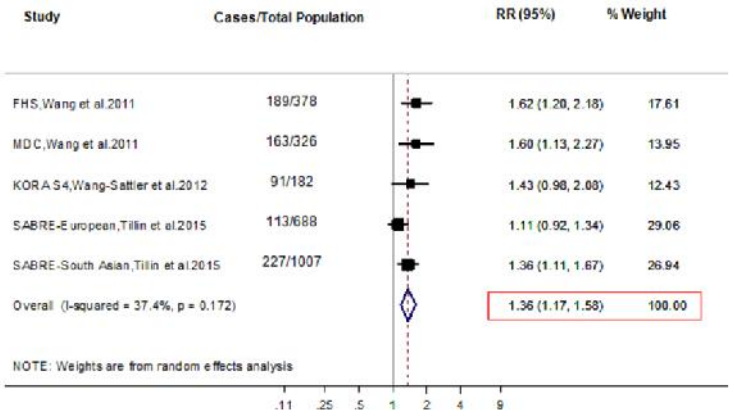
Case cohort, n=3,082

Cohort, n=688 / 1,007

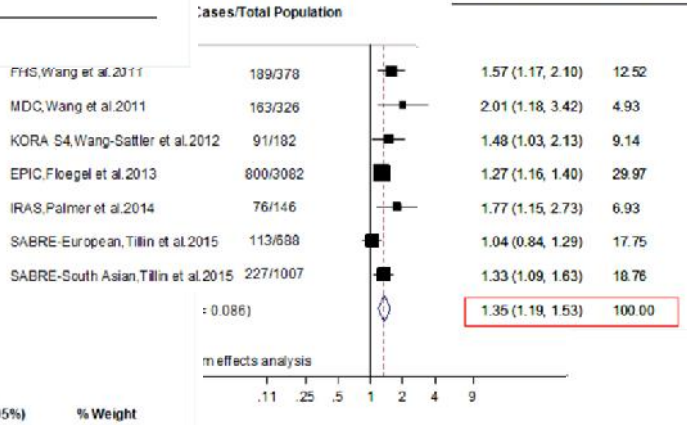
## Isoleucine



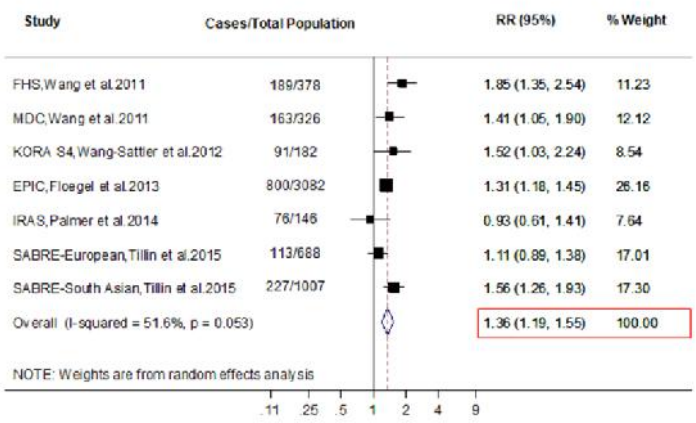

## Leucine



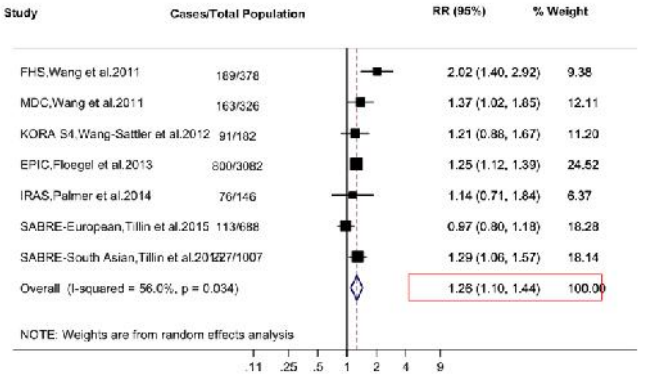

## Valine




## Tyrosine



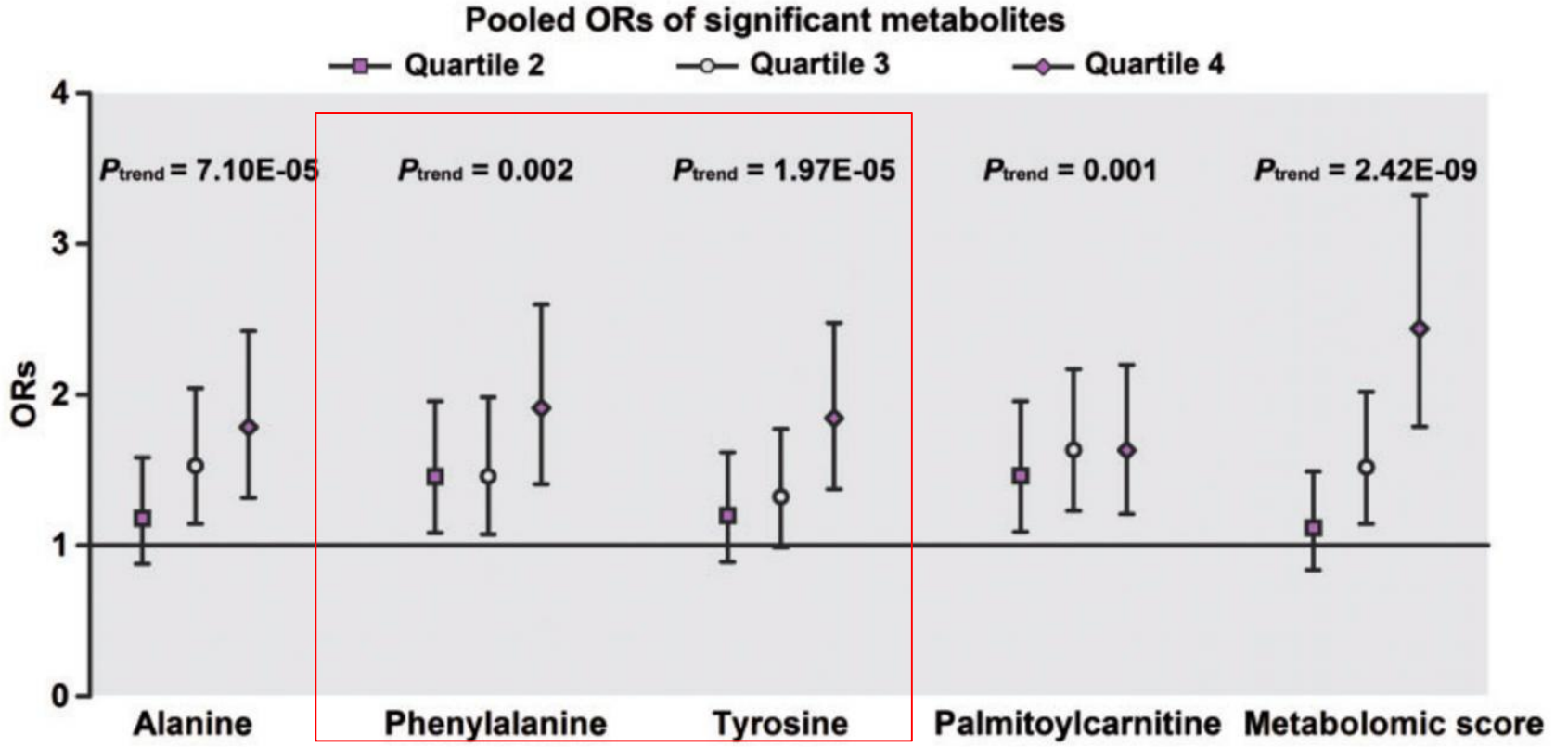

## Phenylalanine



Gaokun Qiu,[1] Yan Zheng,[2] Hao Wang,[1] Jie Sun,[3] Hongxia Ma,[3] Yang Xiao,[1] Yizhun Li,[1] Yu Yuan,[1] Handong Yang,[4] Xiulou Li,[4] Xinwen Min,[4] Ce Zhang,[4] Chengwei Xu,[4] Yue Jiang,[3] Xiaomin Zhang,[1] Meian He,[1] Ming Yang,[1] Zhibin Hu,[3] Huiru Tang,[5,6] Hongbing Shen,[3] Frank B. Hu,[2] An Pan[1]* and Tangchun Wu[1]*

*International Journal of Epidemiology*, 2016, 1–10

# Plasma metabolomics identified novel metabolites associated with risk of type 2 diabetes in two prospective cohorts of Chinese adults




Background → Methods → Results → Discussion/Conclusion

Yonghai Lu[1] · Yeli Wang[1] · Choon-Nam Ong[1,2] · Tavintharan Subramaniam[3] · Hyung Won Choi[1] · Jian-Min Yuan[4,5] · Woon-Puay Koh[1,6] · An Pan[7,8]

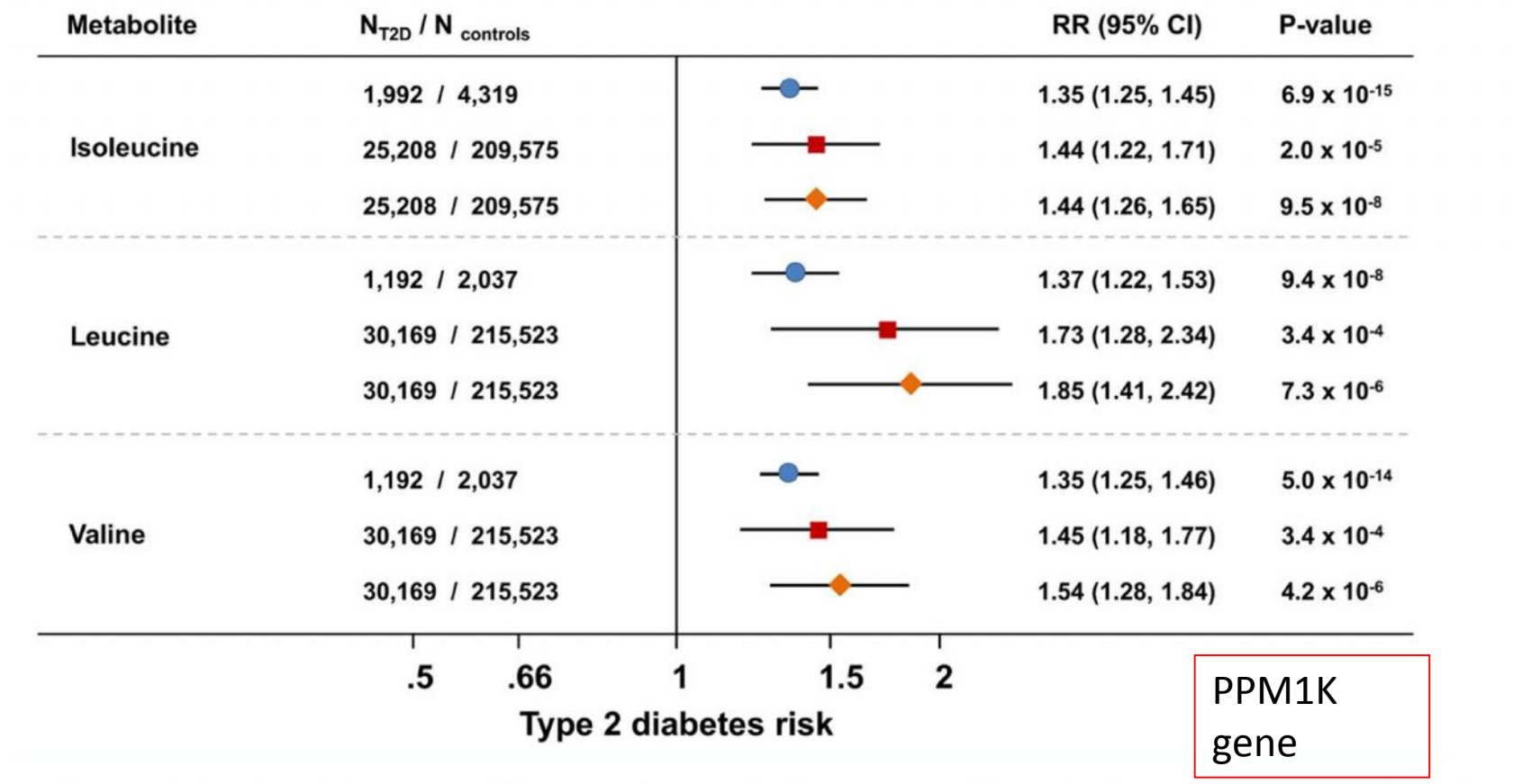
Diabetologia (2016) 59:2349–2359

# Metabolic signatures and risk of type 2 diabetes in a Chinese population: an untargeted metabolomics study using both LC-MS and GC-MS

| Metabolite | Chemical class | Compound ID | | VIP | *p* value | Trend[a] | Association | | AUC[b] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | METLIN | HMDB | | | | OR (95% CI) | FDR | Low | High |
| 2-Aminooctanoic acid | Amino acid | 5923 | 00991 | 1.39 | 0.003 | Down | 0.69 (0.51, 0.93) | 0.016 | 0.733 | 0.779 |
| Aminomalonic acid | Amino acid | 58024 | 01147 | 3.50 | <0.001 | Up | 2.03 (1.48, 2.78) | <0.001 | 0.733 | 0.808 |
| Glycine | Amino acid | 20 | 00123 | 3.67 | <0.001 | Up | 2.66 (1.72, 4.12) | <0.001 | 0.731 | 0.798 |
| Isoleucine | Amino acid | 23 | 00172 | 1.50 | 0.002 | Up | 1.44 (1.07, 1.93) | 0.015 | 0.725 | 0.768 |
| Leucine | Amino acid | 24 | 00687 | 1.50 | 0.002 | Up | 1.44 (1.07, 1.93) | 0.015 | 0.725 | 0.768 |
| Ornithine | Amino acid | 27 | 00214 | 1.20 | 0.001 | Down | 0.63 (0.45, 0.88) | 0.007 | 0.738 | 0.783 |
| Phosphoserine | Amino acid | 297 | 00272 | 1.71 | 0.033 | Down | 0.74 (0.57, 0.96) | 0.023 | 0.748 | 0.771 |
| Proline | Amino acid | 29 | 00162 | 5.54 | <0.001 | Down | 0.44 (0.30, 0.66) | <0.001 | 0.728 | 0.850 |
| Serine | Amino acid | 30 | 00187 | 1.32 | 0.013 | Down | 0.68 (0.48, 0.94) | 0.022 | 0.743 | 0.765 |
| Threonine | Amino acid | 32 | 00167 | 1.80 | <0.001 | Up | 1.53 (1.16, 2.02) | 0.003 | 0.732 | 0.780 |
| Valine | Amino acid | 35 | 00883 | 1.78 | <0.001 | Up | 1.66 (1.21, 2.26) | 0.001 | 0.731 | 0.766 |

PLOS Medicine | DOI:10.1371/journal.pmed.1002179 November 29, 2016

# Genetic Predisposition to an Impaired Metabolism of the Branched-Chain Amino Acids and Risk of Type 2 Diabetes: A Mendelian Randomisation Analysis




PPM1K gene

Background → Methods → Results → Discussion/Conclusion

# Limitations in previous studies

- Did not used repeated measurements over time of these amino-acids
- No evaluation about how dietary interventions can influence changes in the levels of these plasma amino-acids and the risk of disease

# Hypotheses

1) **Baseline plasma** levels of BCAA and AA are directly associated with a higher subsequent risk of developing T2D;

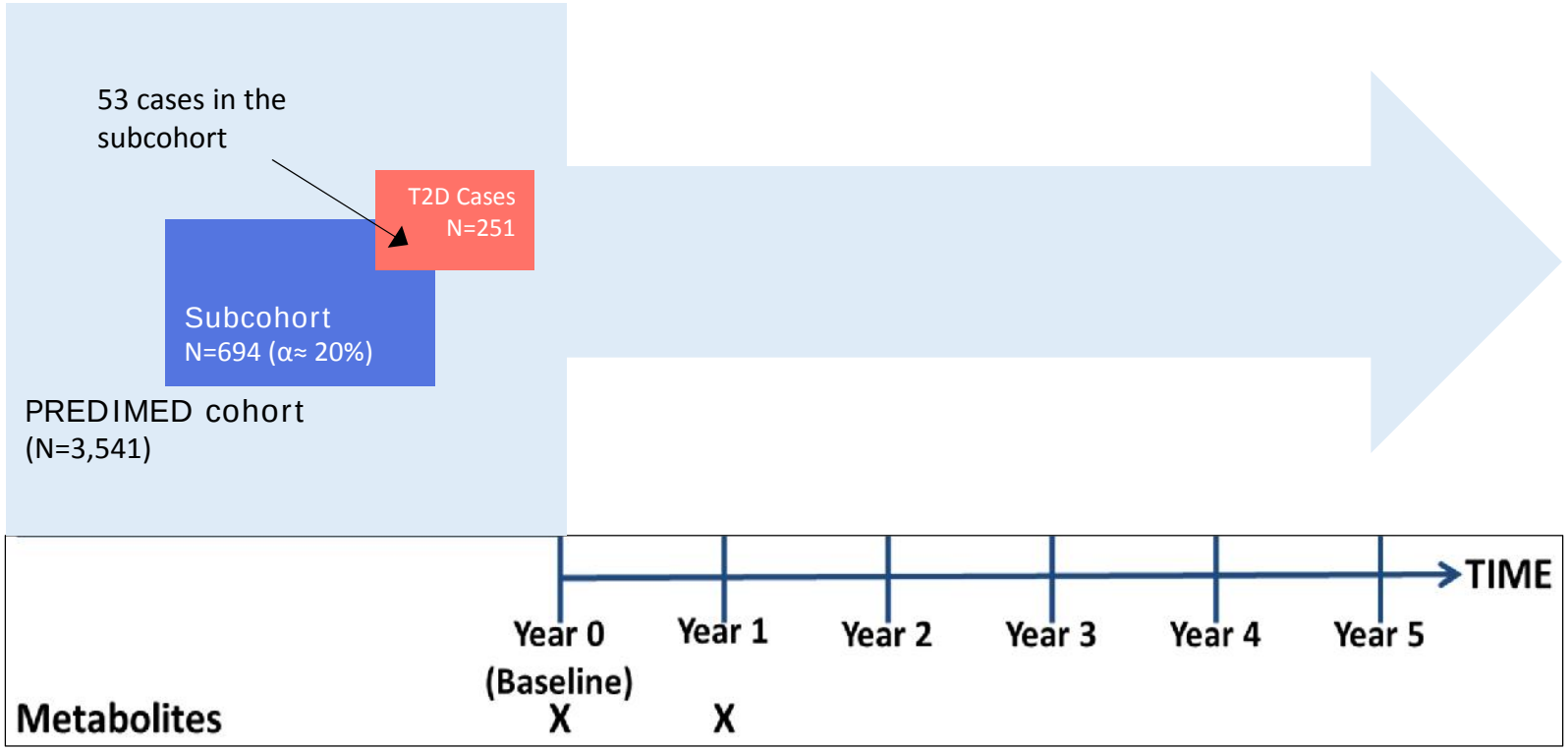
2) **1-year changes** in the plasma levels of these amino-acids are associated with a higher subsequent risk of developing T2D;

3) An intervention with a **Mediterranean diet** can attenuate the direct association between **baseline plasma levels** of these amino-acids and the risk of developing T2D;

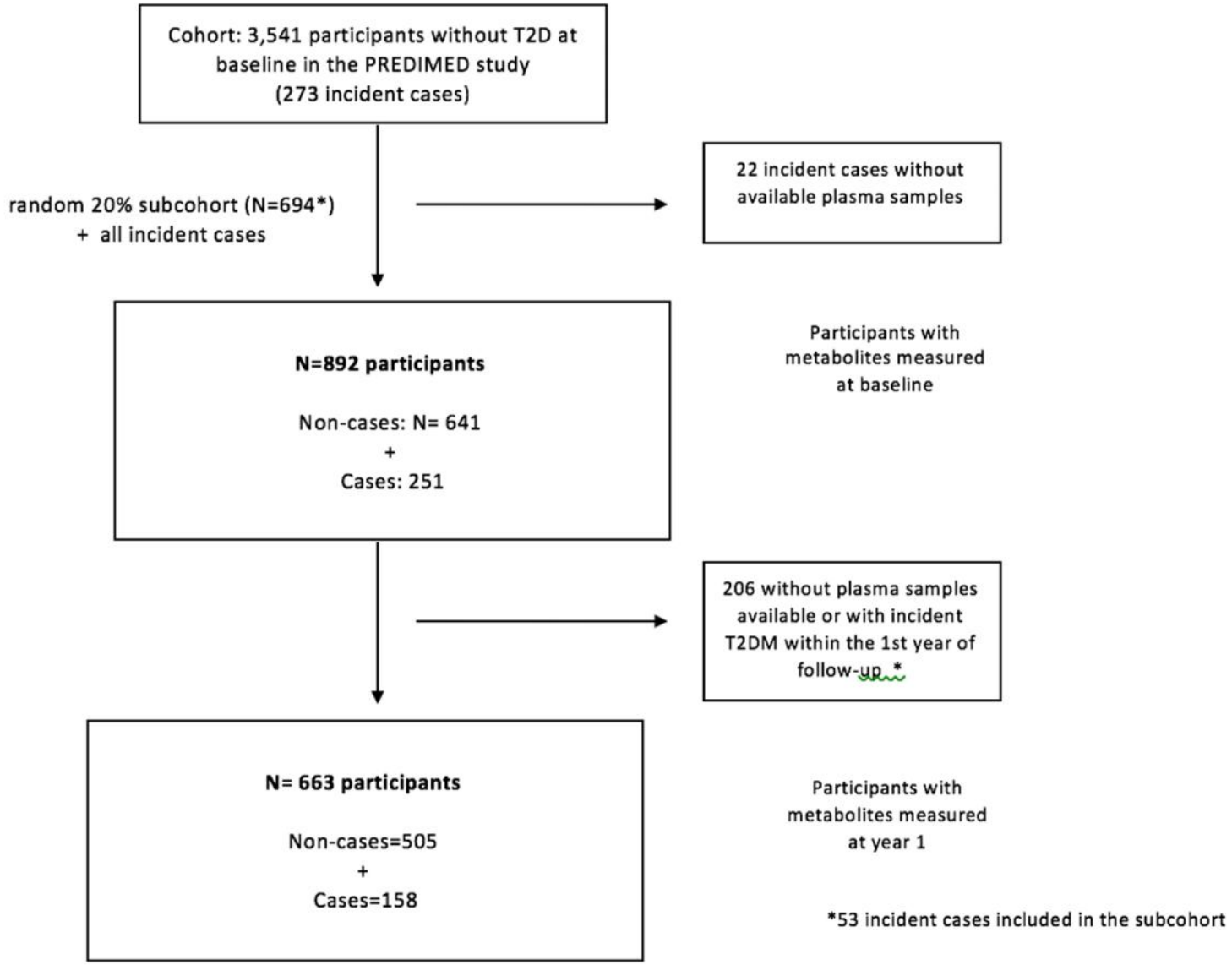
4) A **Mediterranean diet** intervention **during one year time** is able to reduce the plasma levels of these amino-acids.

Prediméd
Prevención con Dieta Mediterránea

# DESIGN
# Case-cohort study



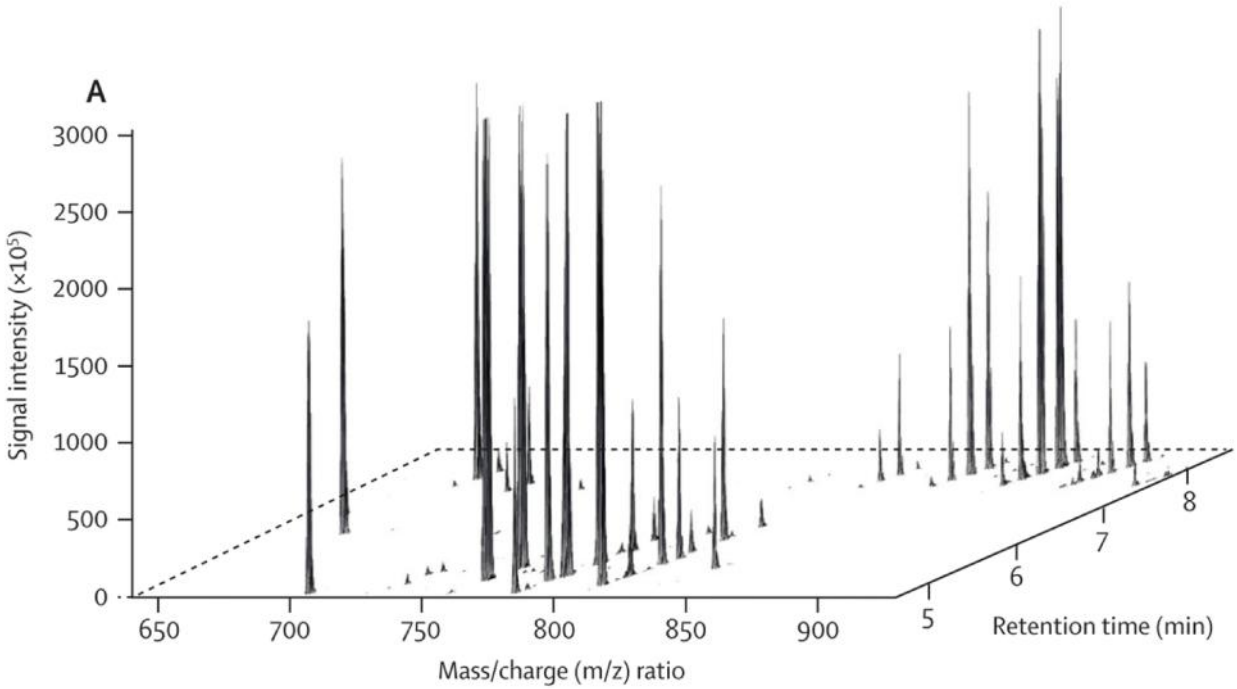
# Flow-chart of the case-cohort design



# Metabolomic analysis

- Blood samples in EDTA tubes: from Spain to Boston

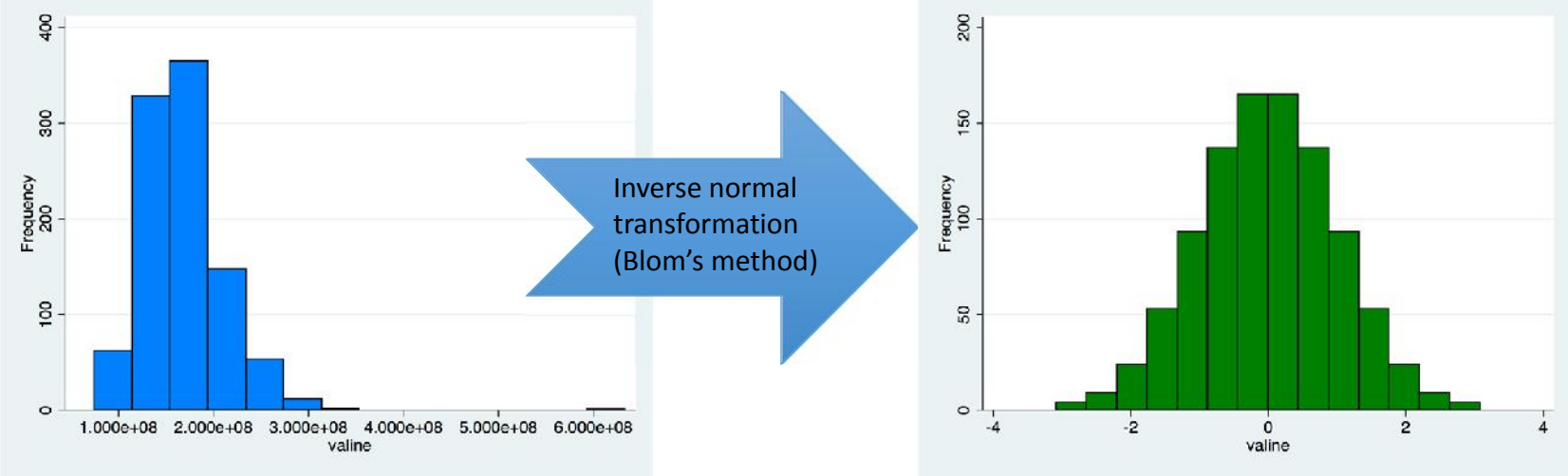
- Metabolite profiling of 137 metabolites:
- hydrophilic interaction liquid chromatography (HILIC)-positive with tandem Mass Spectrometry (Broad Institute)



# Individual AAs and scores

Leucine, isoleucine, valine
Phenylalanine, tyrosine
(signal intensity)

1




2

BCAA score: LEU + ISO+ VAL

AAA score: PHE + TYR

# Statistical analysis

- *Weighted* Cox regression models (Barlow weights)

**Model 1**: **age** (years), **sex** (male, female), **intervention group** (MedDiet+EVOO, MedDiet+nuts) (stratified by recruitment center)

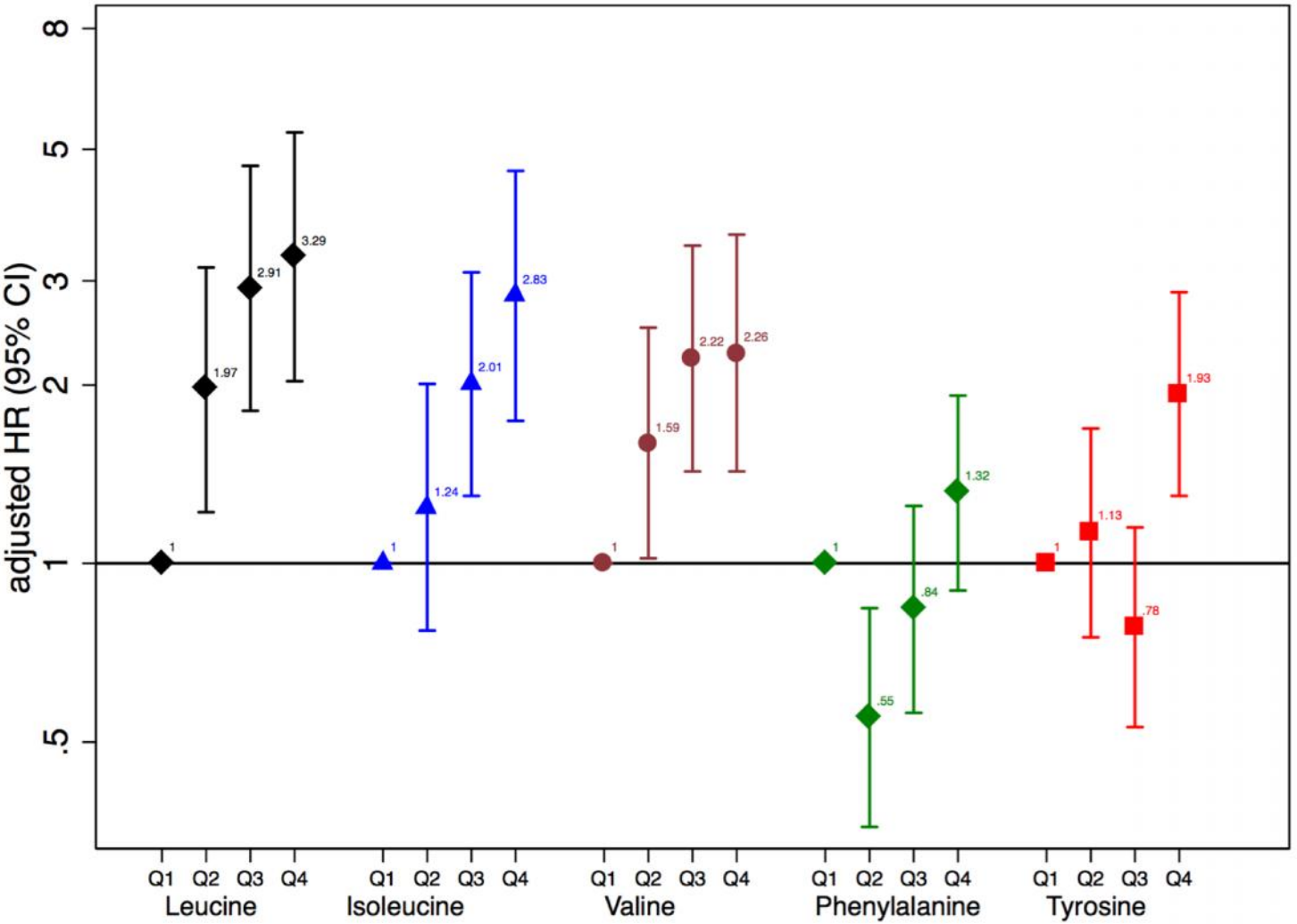
**Model 2**: *Model 1* + **body mass index** (kg/m$^2$), **smoking** (never, current, former), **leisure-time physical activity** (metabolic equivalent tasks in minutes/day), **dyslipidemia** and **hypertension**

**Model 3**: *Model 2* + **baseline fasting glucose** (mean + quadratic term of glucose centered mean)

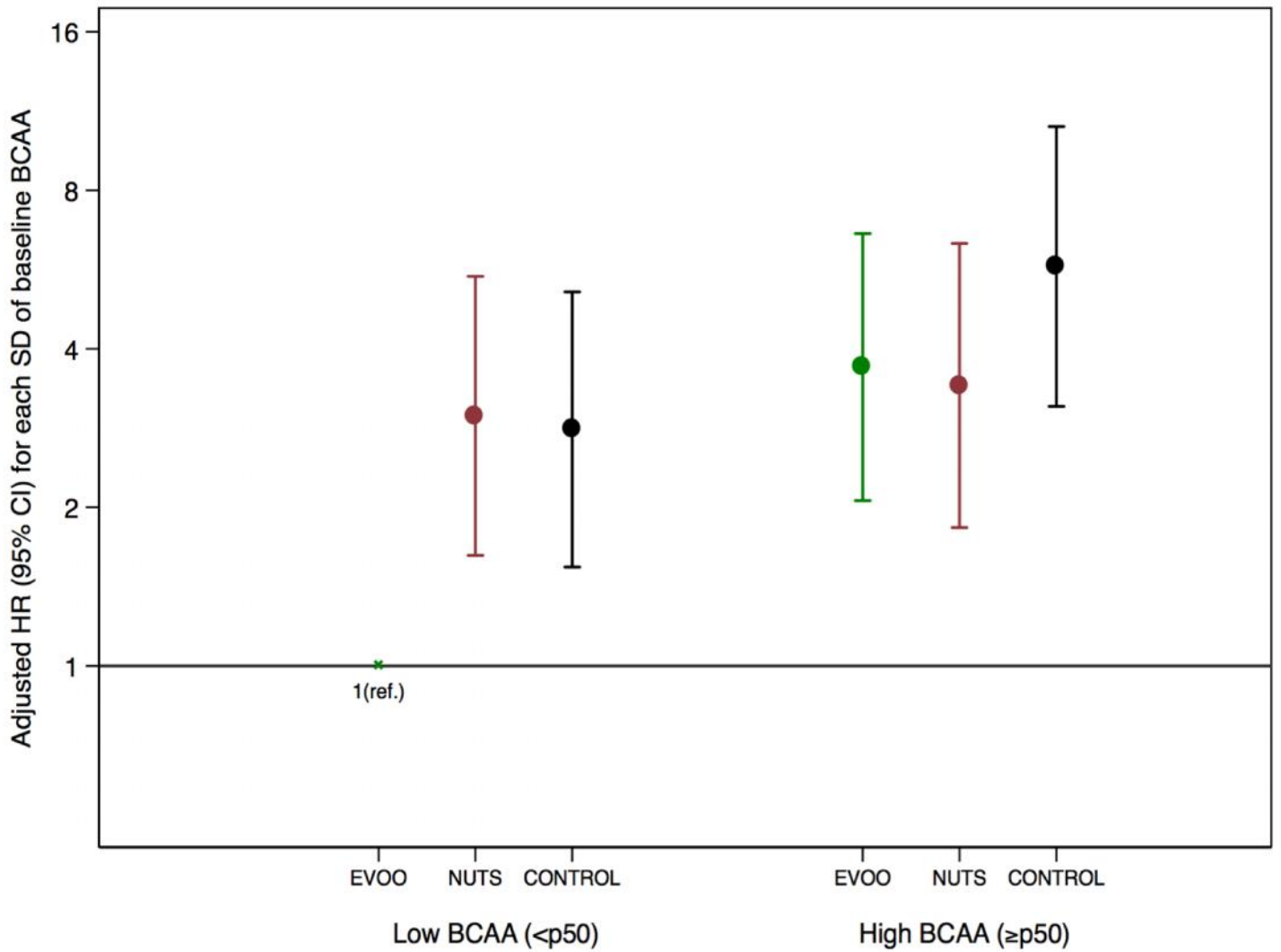
# Baseline participants characteristics

| | Subcohort[a] | 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 | 99.0 (10.7) | 103.4 (10.0) |
| Body mass index, kg/m$^2$ | 29.9 (3.6) | 30.8 (3.3) |
| Physical activity, METs/d | 238 (238) | 249 (232) |
| Education, % | | |
| Elementary or lower | 75.4 | 76.5 |
| Secondary or higher | 24.6 | 23.5 |
| Total energy intake, kcal/d | 2277 (566) | 2327 (622) |
| Mediterranean diet[b] | 8.6 (1.9) | 8.5 (1.8) |
| Glucose, mg/dl[c] | 99.7 (15.2) | 117.2 (17.6) |

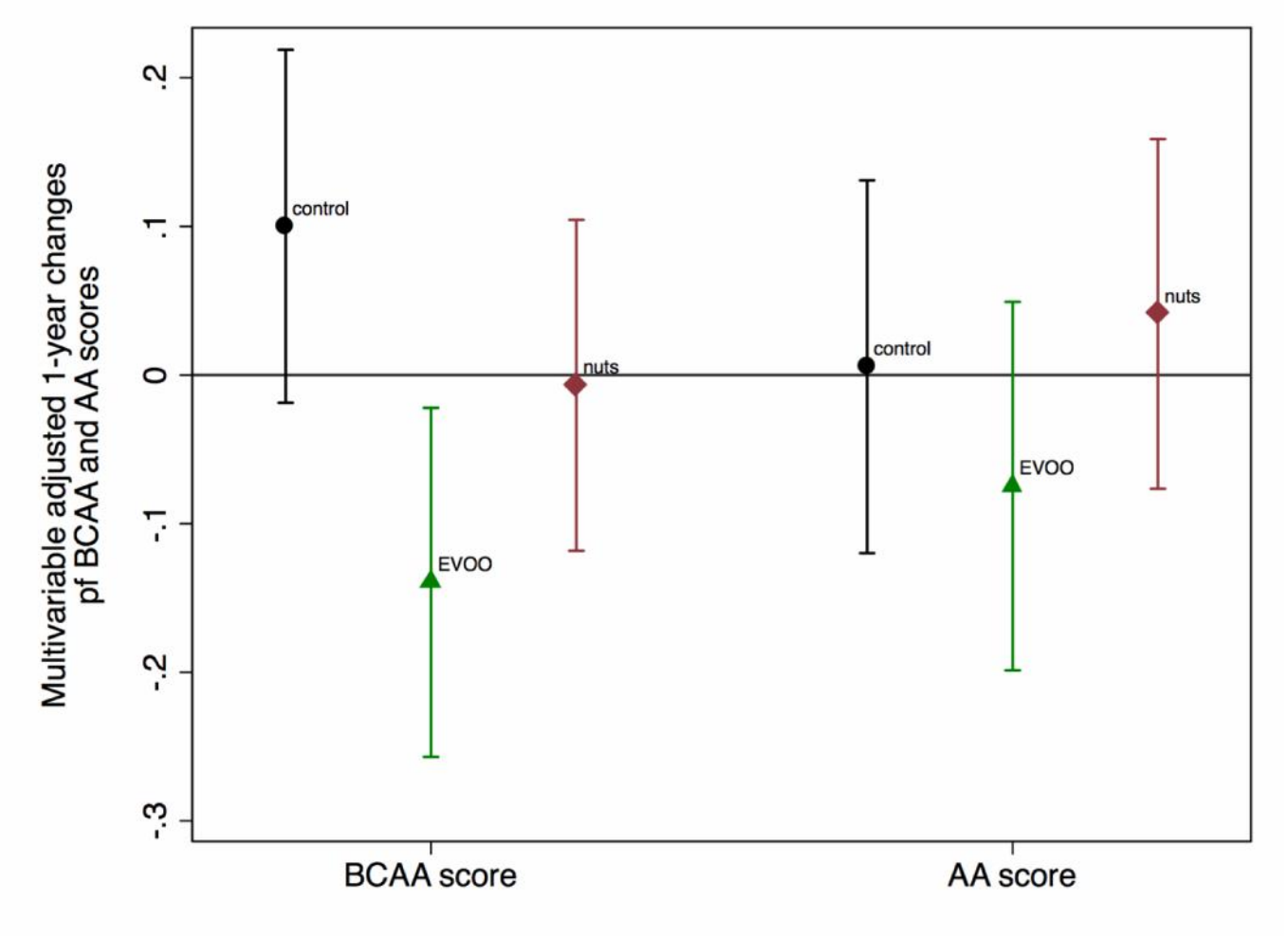
# Incident type 2 diabetes by Baseline Plasma Amino Acid Concentrations[a]



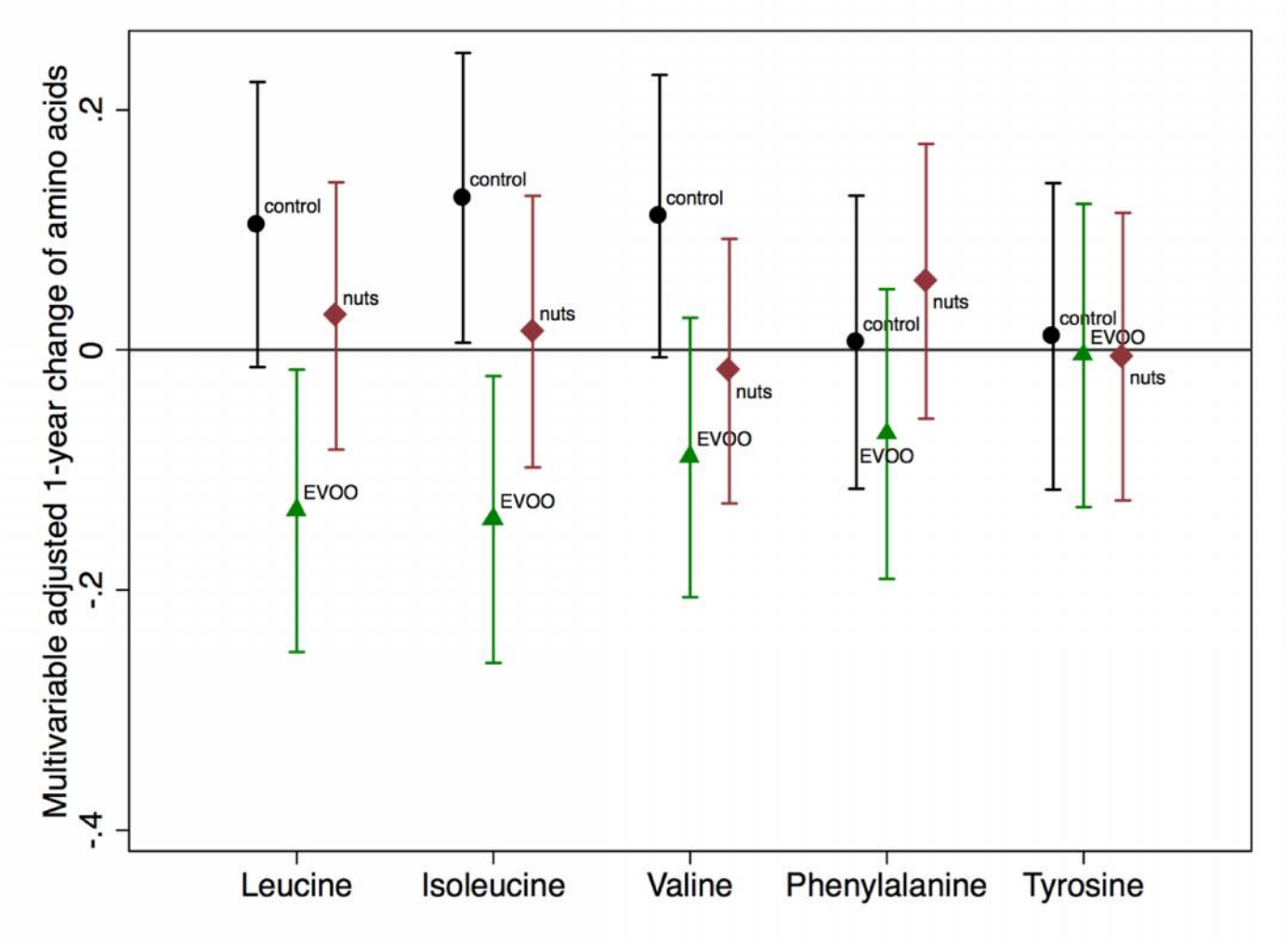
# Joint effect of MedDiet and baseline BCAA score



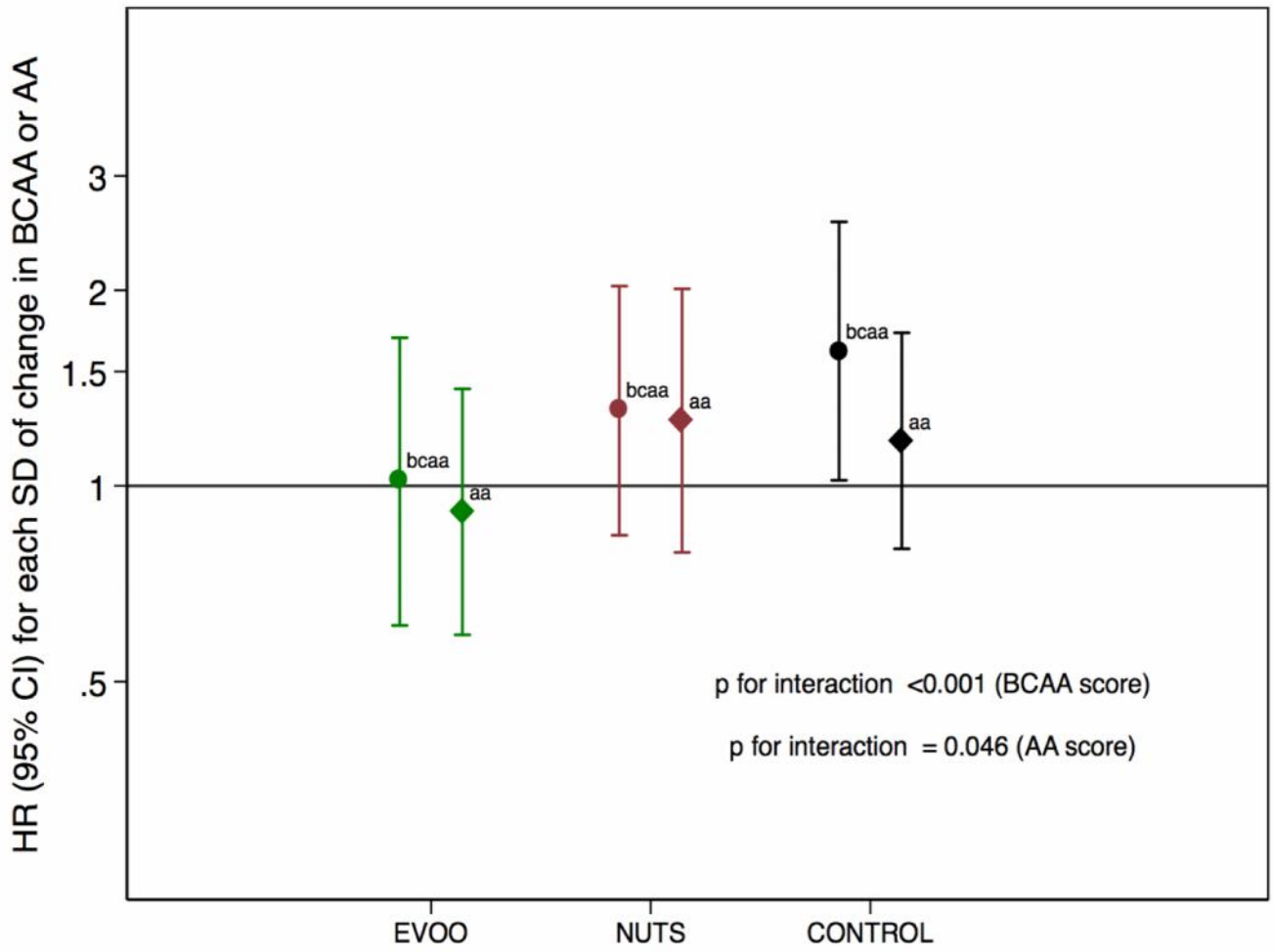
# Changes in BCAA and AA scores after 1 Year of Intervention, by Intervention Group.



# Changes in individual AAs after 1 Year of Intervention, by Intervention Group.

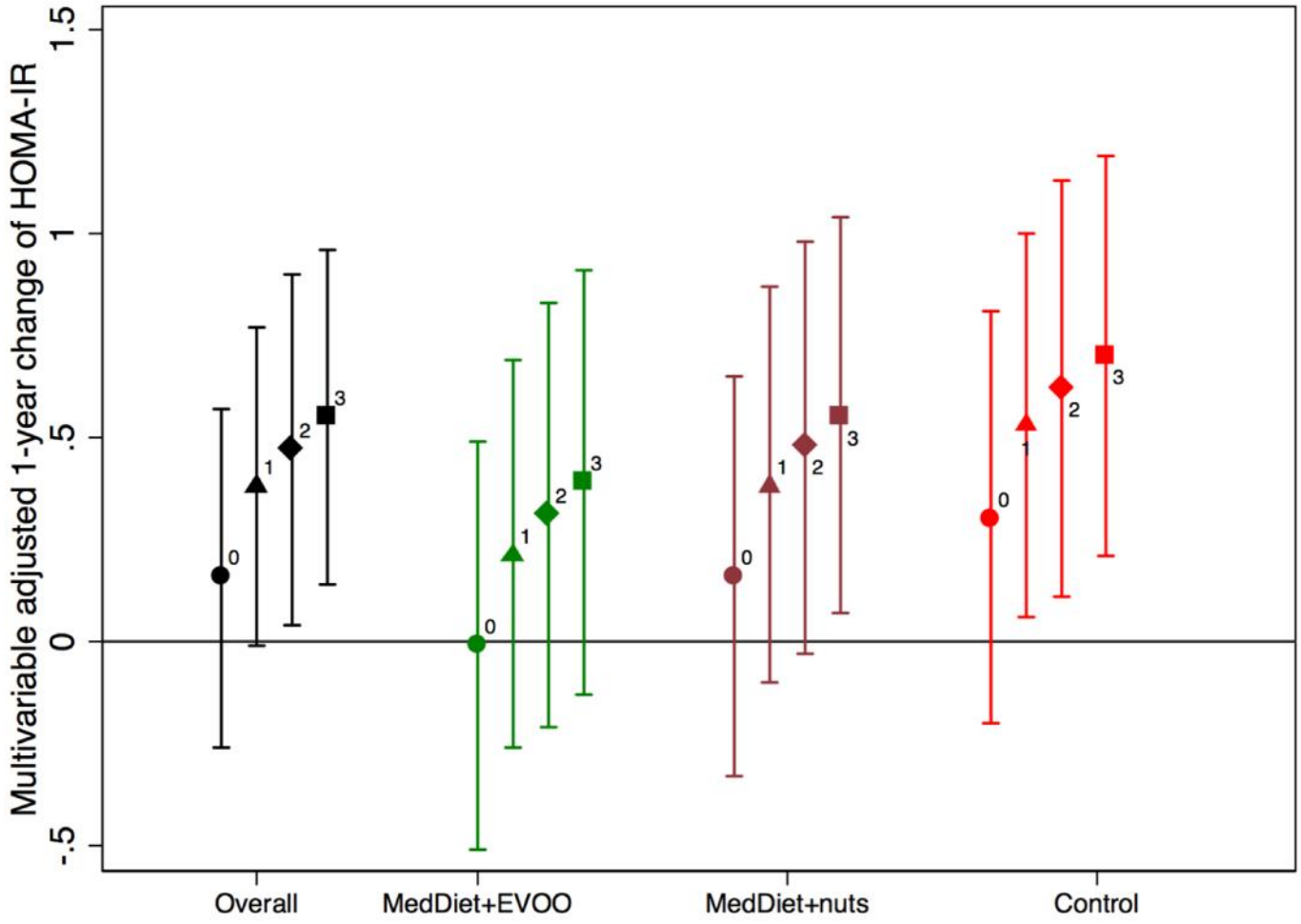


# Associations of 1-yr changes (per SD) in BCAA and AA score with the risk of incident type 2 diabetes stratified by intervention group

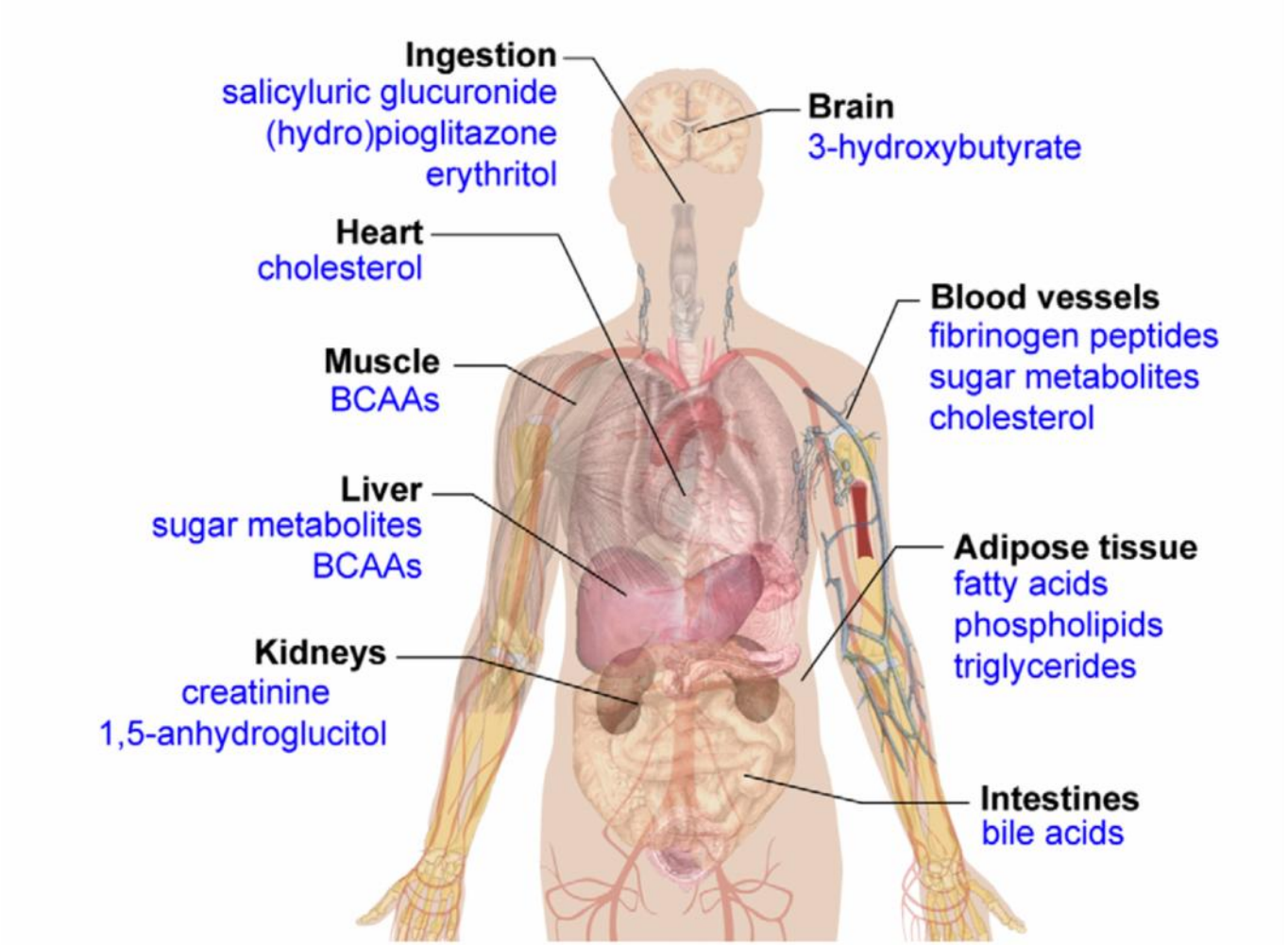



Background → Methods → Results → Discussion/Conclusion

# One-year Change of HOMA-IR Index 95% confidence intervals by Quartiles of Baseline Plasma Branched-Chain



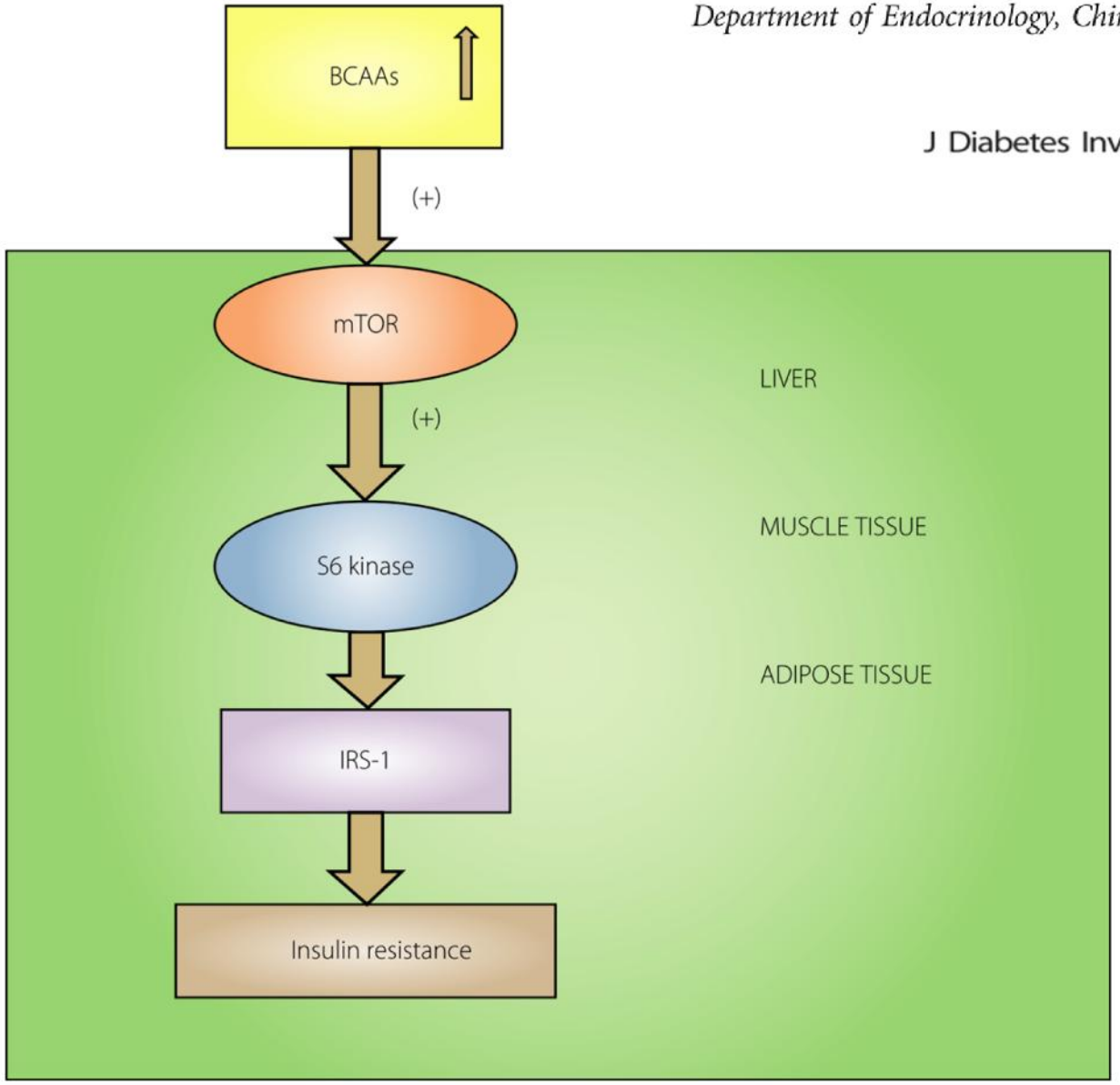
# Metabolic Footprint of Diabetes: A Multiplatform Metabolomics Study in an Epidemiological Setting



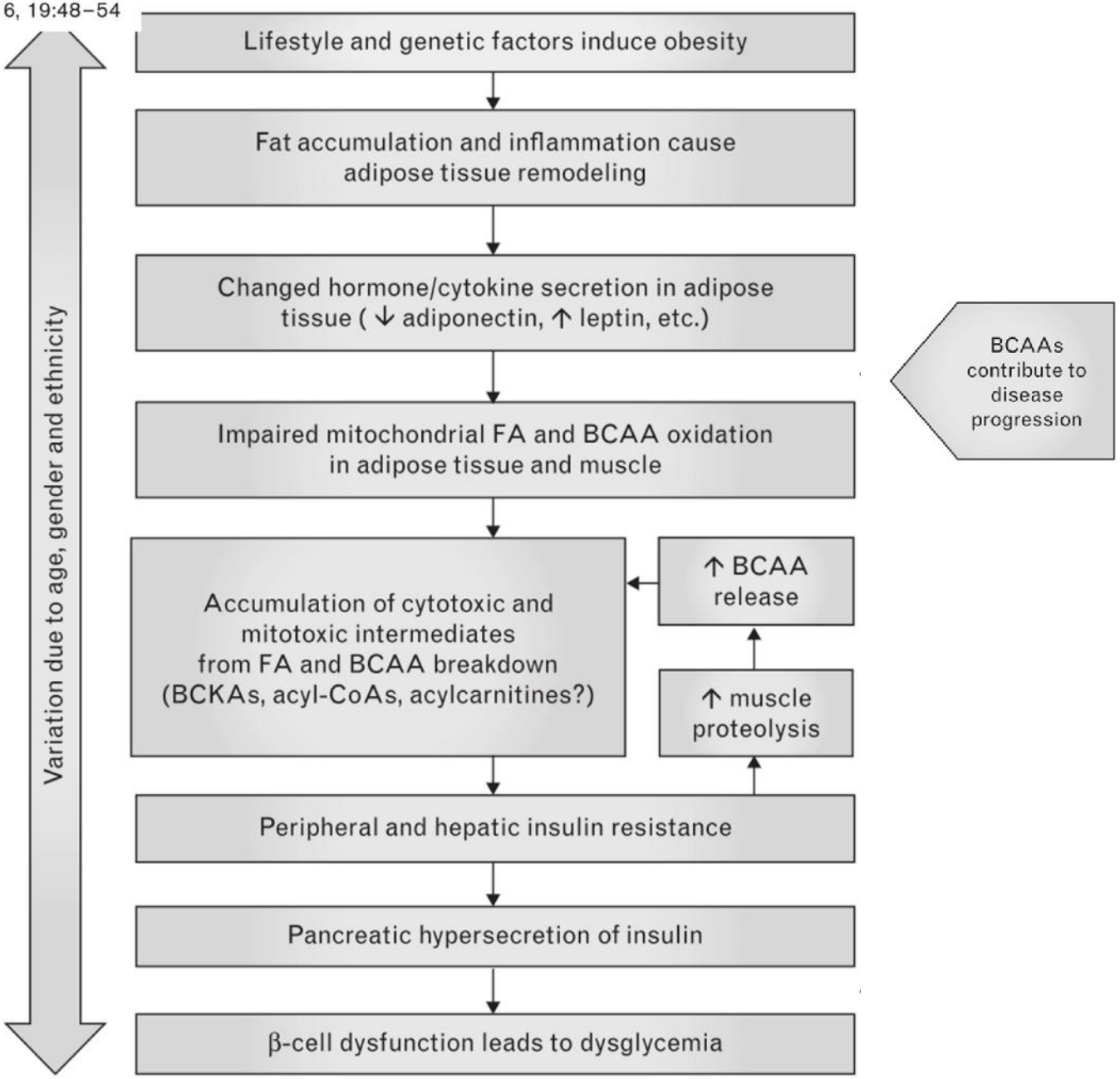

Background → Methods → Results → Discussion/Conclusion

Xiaoping Chen, Wenying Yang*
*Department of Endocrinology, China-Japan Friendship Hospital, Beijing, China*

J Diabetes Invest Vol. 6 No. 4 July 2015



**Curr Opin Clin Nutr Metab Care** 2016, 19:48–54

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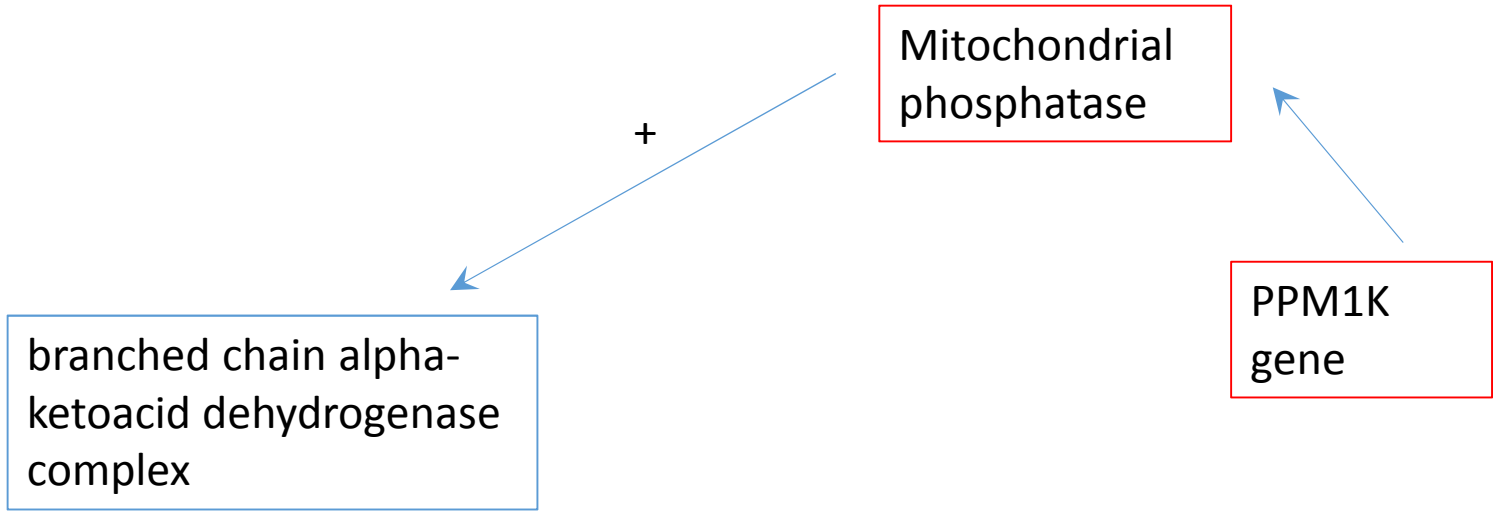
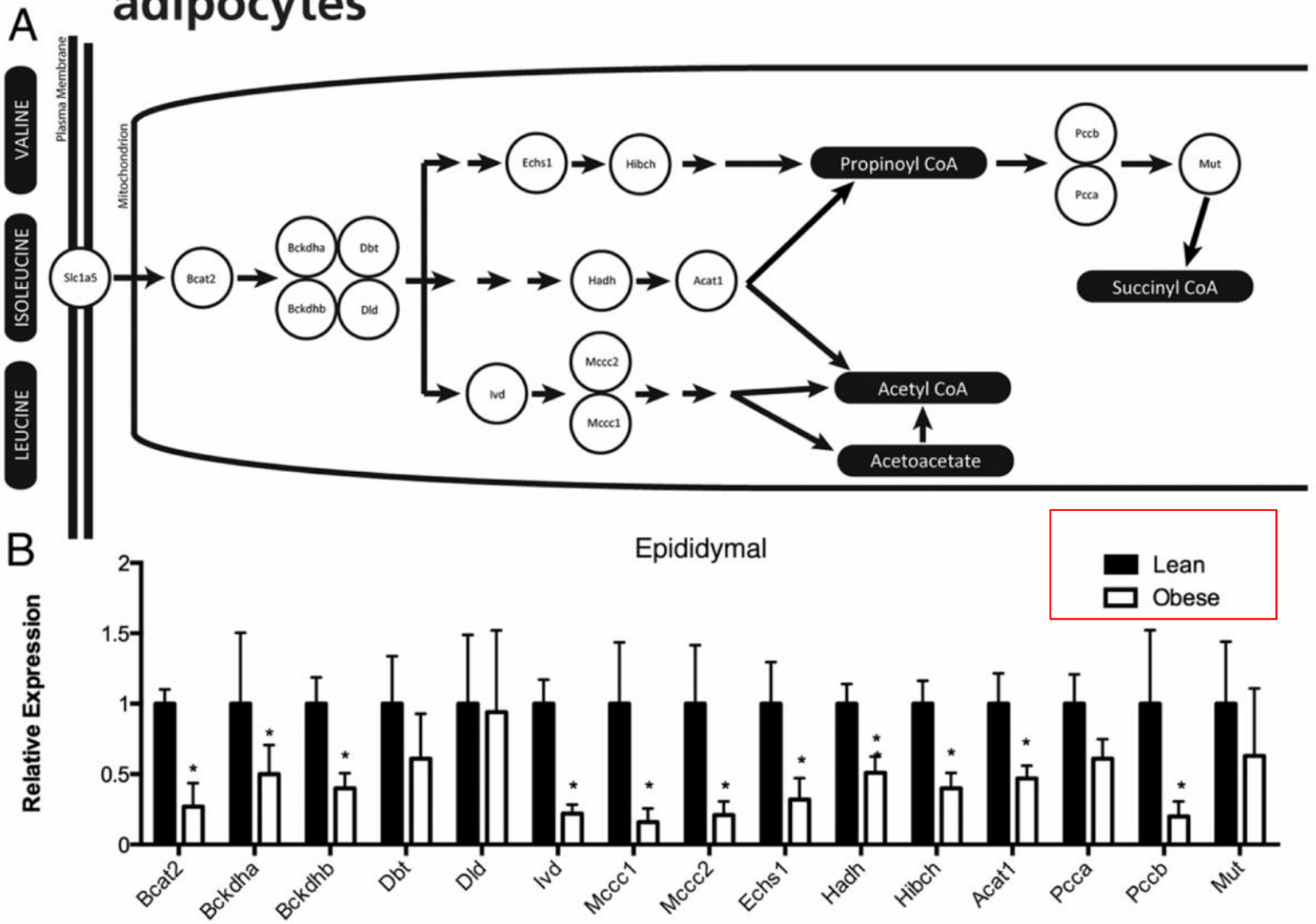



PLOS Medicine | DOI:10.1371/journal.pmed.1002179 November 29, 2016

# Genetic Predisposition to an Impaired Metabolism of the Branched-Chain Amino Acids and Risk of Type 2 Diabetes: A Mendelian Randomisation Analysis

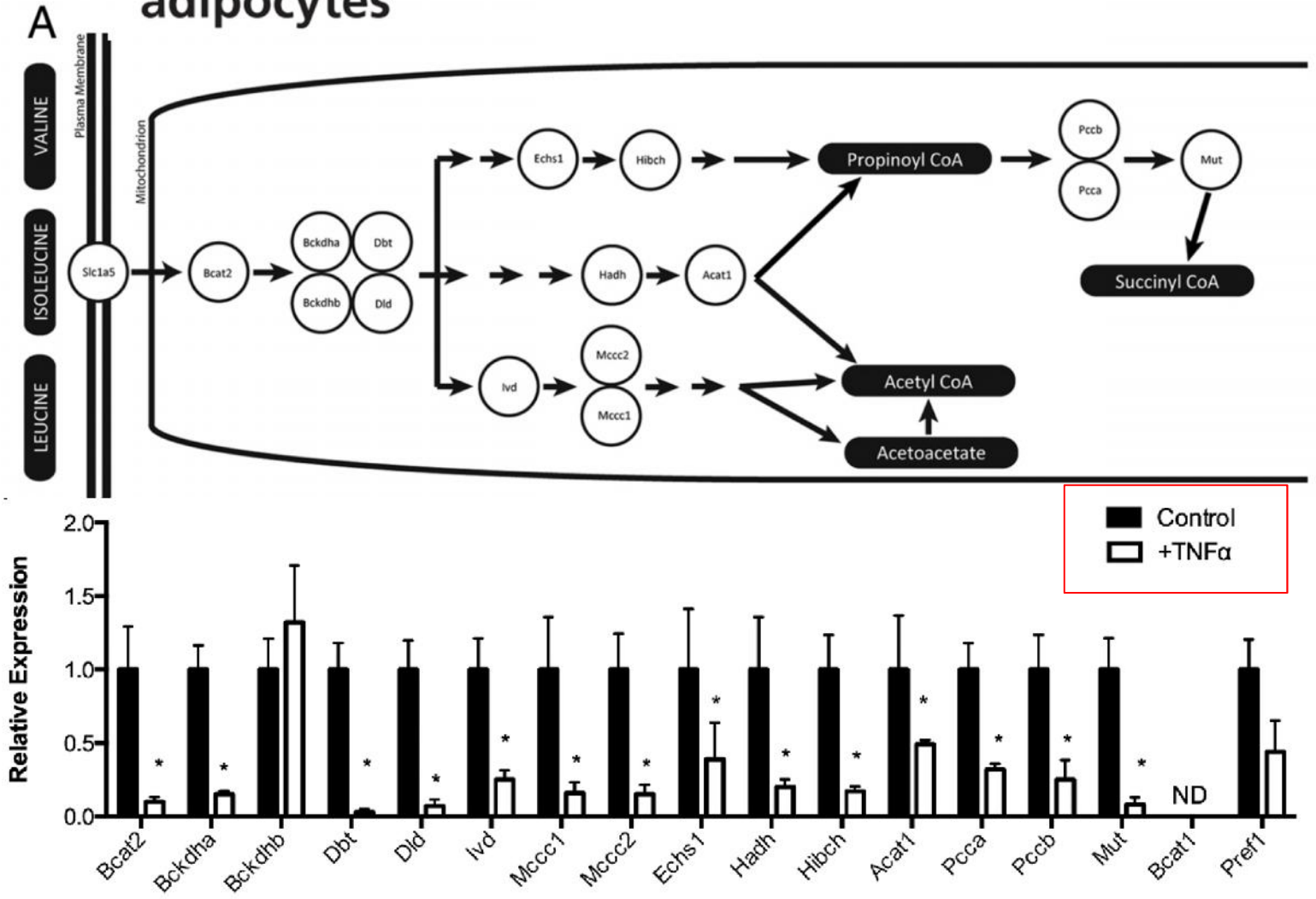
# Obesity and inflammation down-regulate expression of genes linked to BCAA metabolism selectively in adipose tissue and cultured adipocytes




(*Molecular Endocrinology* 29: 411–420, 2015)

Background → Methods → Results → Discussion/Conclusion

# Obesity and inflammation down-regulate expression of genes linked to BCAA metabolism selectively in adipose tissue and cultured adipocytes



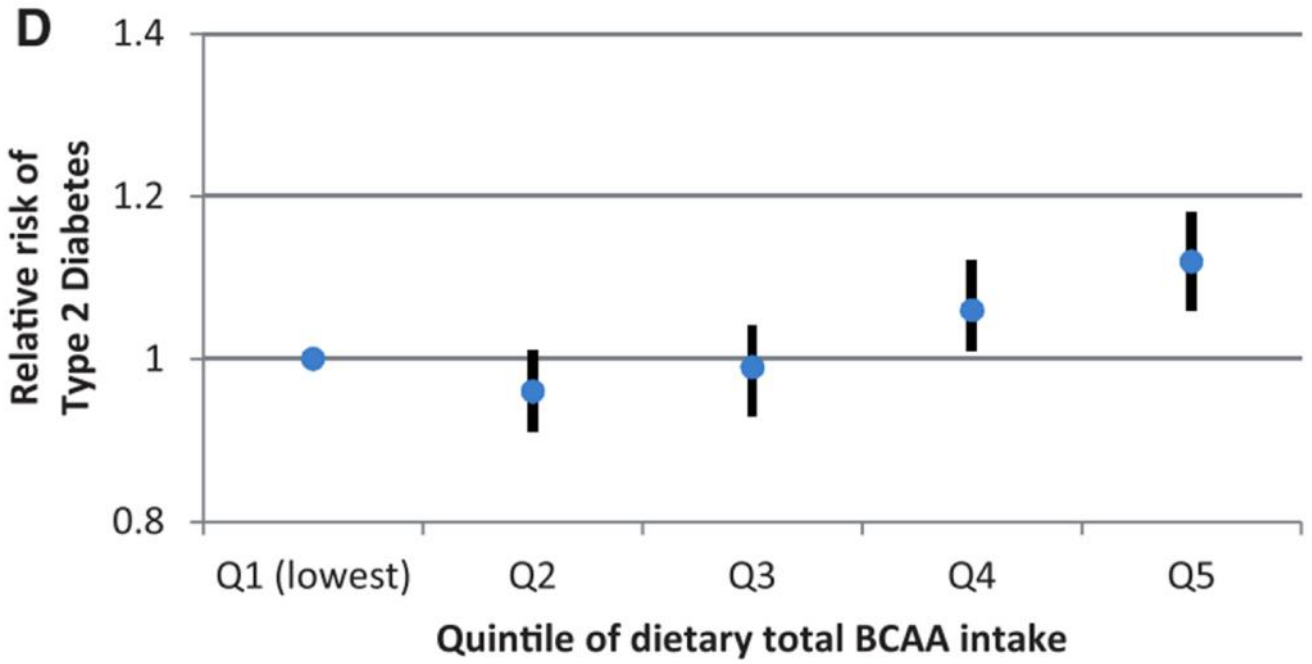

(*Molecular Endocrinology* 29: 411–420, 2015)

Background → Methods → Results → Discussion/Conclusion

Yan Zheng,[1] Yanping Li,[1] Qibin Qi,[2] Adela Hruby,[1] JoAnn E. Manson,[3,4,5]
Walter C. Willett,[1,3,4] Brian M. Wolpin,[6] Frank B. Hu[1,3,4] and Lu Qi[1,7]*

*International Journal of Epidemiology*, 2016, 1–11

# Cumulative consumption of branched-chain amino acids and incidence of type 2 diabetes




Background → Methods → Results → Discussion/Conclusion

BASELINE

YEAR 1

YEARS 2-7

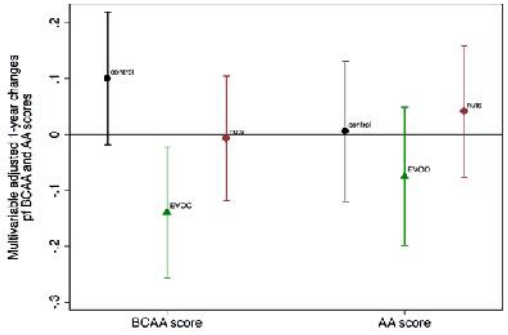
↑ BCAA

MedDiet + EVOO

0.60 (95% CI, 0.43 to 0.85) (Ann Inter Med 2014)

T2D

↓ BCAA




P for interaction < 0.001

Background → Methods → Results → Discussion/Conclusion

# Conclusions

1) Baseline BCAAs and AA scores were *independently* associated with a higher risk of incident T2D after a median follow-up of 3.8 years;

2) One-year changes in BCAAs were associated with a higher risk of subsequently developing T2D (during years 2 to 7 of follow-up) *only in the control group* of the trial, but not in the active intervention groups receiving MedDiets, and this differential association was supported by statistically significant interactions *for each of the 2 active intervention groups*;

3) The intervention with the MedDiet+EVOO was associated with 1-year *significant reductions* in leucine, isoleucine and the overall BCAA score.

# Thank you very much!

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