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Changes in Arginine are Inversely Associated with Type 2 Diabetes: A Case-Cohort Study in the PREDIMED Trial

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Abstract

Background: The associations between arginine-based metabolites and incident type 2 diabetes (T2D) are unknown.

Methods: We employed a case-cohort design nested within the PREDIMED trial to examine six plasma metabolites (arginine, citrulline, ornithine, asymmetric dimethylarginine [ADMA], symmetric dimethylarginine [SDMA], and N-monomethyl-l-arginine [NMMA]) among 892 individuals (251 cases) for associations with incident T2D and insulin resistance. Weighted Cox models with robust variance were used.

Results: 1-year changes in arginine (Adjusted HR per SD = 0.68, 95% CI = 0.49, 0.95; Q4 vs. Q1 = 0.46, 95% CI = 0.21, 1.04, p-trend = 0.02) and arginine/ADMA ratio (Adjusted HR per SD = 0.73, 95% CI = 0.51, 1.04; Q4 vs. Q1 = 0.52, 95% CI = 0.22, 1.25, p-trend = 0.04) were associated with lower risk of T2D. Positive changes of citrulline and ornithine, and negative changes in SDMA and arginine/(ornithine+citrulline) were associated with concurrent 1-year changes in HOMA-IR. Individuals in the low fat diet group experienced higher risk of T2D for 1-year changes in NMMA than individuals in Mediterranean diet groups (p-interaction = 0.02).

Conclusions: Arginine bioavailability is important in T2D pathophysiology.

INTRODUCTION

Arginine is a key amino acid that is at the nexus of several important pathways hypothesized to be involved in T2D development[1]. Derivatives of arginine include urea cycle metabolites, arginine, citrulline, ornithine, and methylarginines, including N-monomethyl-l-arginine (NMMA), asymmetric dimethylarginine (ADMA), symmetrical dimethylarginine (SDMA), and N-monomethyl-l-arginine (NMMA). Interventions involving these metabolites may potentially delay or prevent type 2 diabetes onset[2].

The Prevención con Dieta Mediterránea (PREDIMED) trial was a large multicenter primary prevention trial in Spain that compared the effect of a Mediterranean diet (MedDiet) with extra virgin olive oil (EVOO) or nuts to the effect of advising participants to adhere to a low-fat control diet. The previously reported finding was that a hazard ratio (HR) of 0.70 (95% CI, 0.54 – 0.92) of T2D incidence for the two MedDiet groups combined versus the control group was observed[3]. However, the biological mechanisms by which a MedDiet can reduce T2D risk, and whether a MedDiet may offset an unfavorable metabolite profile remain unsettled.

The present study is a case-cohort study of participants free of diabetes at baseline, nested within the PREDIMED trial. Our objectives were to address: a) whether 1-year changes in arginine-based metabolites were associated with T2D; b) whether 1-year changes in these metabolites were associated with concurrent changes in insulin resistance; c) whether a

MedDiet intervention could counteract the harmful consequences of an unfavorable metabolite profile.

METHODS

Study design and population

We employed a case-cohort design nested within the PREDIMED study (www.predimed.es). Detailed methods and design of the PREDIMED trial are reported elsewhere[4]. Briefly, 7,447 cardiovascular disease (CVD) free participants (ages 55 – 80) at high CVD risk were allocated to 3 arms: 1) A MedDiet with EVOO; 2) A MedDiet with nuts; or 3) a low fat control diet. At baseline, 3,541 participants did not have T2D. From 2003 to 2010, 273 cases of incident T2D were reported[3].

The study population comprises a random subcohort from the PREDIMED trial who were free of diabetes at baseline and had available plasma samples, and all incident cases of T2D that occurred in the full trial. Each Institutional Review Board of the recruitment centers approved the study protocol, and participants provided written informed consent.

Covariate assessment

At baseline and at yearly follow-up visits, a questionnaire about lifestyle variables (i.e. diet, physical activity, smoking), history of illnesses, medication use, and family history of disease was administered. Trained personnel ascertained anthropometric and blood pressure measurements. Hypercholesterolemia and hypertension were based on physician diagnosis or medication use. The MedDiet score was based on a validated 14-item screener (0 – no adherence, 14 – maximum adherence)[3].

Blood specimens and metabolite profiling

Fasting blood samples were collected at baseline and at yearly intervals thereafter throughout follow-up. Urea cycle metabolites (arginine, citrulline, ornithine), and methylarginines (ADMA, SDMA, NMMA) were analyzed using a targeted approach (Broad Institute). Raw data were obtained and processed using liquid chromatography tandem mass spectrometry as previously described[5]. The coefficient of variation for each metabolite was: arginine, 2.5%; ornithine, 2.4%; citrulline, 2.1%; ADMA, 7.2%; SDMA, 4.5%; NMMA, 5.5%.

Fasting glucose and insulin were also determined from these plasma samples. Insulin resistance was computed by using the homeostasis model assessment (HOMA-IR) method (HOMA-IR = fasting insulin * fasting glucose/22.5, where insulin is in μU/mL and glucose is in mmol/L) at baseline and one year.

Assessment of type 2 diabetes

The adjudication of new-onset cases of T2D during follow-up was performed in a blinded fashion by the Clinical Endpoint Committee of the PREDIMED, an ad hoc panel of physicians[3]. A patient was considered diabetic if they had two confirmations of fasting

plasma glucose ≥7.0 mmol/L or 2-h plasma glucose ≥11.1 mmol/L, after a 75-g oral glucose load.

Statistical analysis

Metabolite values were first normalized using an inverse normal transformation. Our estimates therefore have the interpretation of the hazard ratio of T2D per 1 standard deviation increase in the exposure. For example, a 2.00 HR per 1 SD increase would mean that an individual with a plasma concentration of a given metabolite that is 1 standard deviation above another individual would have twice the relative rate of T2D incidence of that individual. We also considered a pre-hypothesized arginine/ADMA ratio[6], as well as the global arginine bioavailability ratio (GABR) defined as arginine / (citrulline + ornithine) in our primary analysis[7].

We fitted Cox regression models stratified by recruitment center, using inverse probability weights and employing a sandwich variance estimator[8]. Follow-up time was defined as date of enrollment to the date of diagnosis of T2D, date of the last visit, or December 1, 2010, whichever came first. In Model 1, we adjusted for: age (years), sex (male/female), intervention group (control, MedDiet+EVOO, MedDiet+nuts), smoking status (never/current/former), body mass index (kg/m^2), leisure-time physical activity (metabolic equivalent task MET-min/day), hypertension (yes/no) dyslipidemia (yes/no), and baseline plasma glucose (with a quadratic term). In Model 2, we additionally adjusted for inverse normal transformed creatinine (quartiles) as a proxy for kidney function.

We also tested potential effect modification by intervention group on the relationship between 1-year changes in metabolites and T2D using likelihood ratio tests. Finally, we examined the association between 1-year changes in plasma metabolites with concurrent 1-year changes of continuous HOMA-IR among patients with valid insulin and glucose measurements at baseline and 1-year by using generalized estimating equations. Analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Our study population of 892 participants (641 non-cases, 251 cases; median follow-up time was 3.8 years) at baseline and 655 participants (497 non-cases, 158 cases) with available samples after one year. Supplemental Figure 1 depicts the study population flow chart. Supplemental Table 1 details descriptive characteristics of the 694 individuals in the subcohort according to baseline arginine levels.

1-year changes in metabolites and risk of T2D

Table 1 shows the results of the 1-year change analysis of metabolites and ratios with T2D. 1-year changes in arginine were inversely associated with T2D risk (HR per SD = 0.68, 95% CI = 0.49, 0.95). The arginine/ADMA ratio showed a significant trend, with individuals in higher quartiles of change in this ratio demonstrating a lower risk of T2D (p-trend = 0.04).

We also tested potential effect modification by intervention group on the association between 1-year changes in metabolites and T2D risk. For 1-year changes in NMMA, the

control group showed significantly higher HRs for T2D (HR per SD = 1.65, 95% CI = 1.00, 2.73) compared to either the MedDiet+EVOO (HR per SD = 0.90, 95% CI = 0.46, 1.76) or the MedDiet+nuts (HR per SD = 0.71, 95% CI = 0.31, 1.63) arms (p-interaction = 0.02) (full data available upon request).

Changes in metabolites with concurrent changes in HOMA-IR

From participants with available data on fasting glucose and insulin at baseline and 1-year (n=223), we analyzed changes in metabolites with changes in HOMA-IR in Figure 1. In fully adjusted models, baseline ornithine (1-year change in HOMA-IR per 1 SD increase in 1-year change in metabolite value = +0.22, 95% CI = +0.06, +0.39), citrulline (change in HOMA-IR per 1 SD increase = +0.38, 95% CI = +0.20, +0.54), SDMA (change in HOMA-IR per 1 SD increase = −0.26, 95% CI = −0.47, −0.07), and GABR (change in HOMA-IR per 1 SD increase = −0.21, 95% CI = −0.36, −0.06) were associated with significant concurrent changes in HOMA-IR. Supplemental Table 2 shows the associations of baseline metabolites and their ratios with future risk of T2D, indicating a confirmatory positive association of baseline ornithine with T2D (HR per SD = 1.31, 95% CI = 1.04, 1.66).

DISCUSSION

In the present prospective case-cohort study of 892 participants nested in the PREDIMED trial, we found a negative association for 1-year changes in arginine and arginine/ADMA ratio with the risk of T2D in individuals at high cardiovascular risk. Furthermore, positive changes in ornithine and citrulline, and negative changes in SDMA and GABR were associated with concurrent changes in HOMA-IR. We found significant effect modification by intervention on the association between 1-year changes in NMMA and T2D risk.

Our results for incident T2D and HOMA-IR are largely consistent with previous reports, although none of them had assessed an intervention or used repeated measurements. A large body of literature supports an inverse association of arginine with cardiometabolic disease[9], although the underlying mechanisms are not fully elucidated.

One putative mechanism is the formation of nitric oxide (NO) from l-arginine by NO synthase in pancreatic β-cells[10]. Arginine is required for the production of NO via catalysis to citrulline by nitric oxide synthase (NOS)[11], and the prevailing hypothesis is that greater arginine bioavailability may stimulate NO production and insulin sensitivity[12,13]. Thus, disturbances in the arginine-NO pathway may underlie both CVD and T2D pathogenesis through similar mechanisms such as chronic inflammation, mitochondrial dysfunction, and oxidative stress[14–16]. Nevertheless, a potential role for arginine in the primary prevention of diabetes is still unsettled.

In our analysis of HOMA-IR, we found two metabolites associated with positive changes in HOMA-IR: ornithine and citrulline, and two metabolites associated with negative changes in HOMA-IR: SDMA and GABR. Ornithine may be considered a marker of low arginine bioavailability as substrate of NOS[17]. On the other hand, our finding that SDMA was inversely associated with HOMA-IR is surprising given the established role of SDMA in inhibiting NOS and its positive association with CVD[18]. Since low SDMA concentration is a

validated marker of high GFR[19], significant inverse associations of SDMA with T2D may be a marker for supranormal kidney filtration characteristic of prediabetes[20].

We lastly found significant effect modification by a MedDiet intervention on the relationship between 1-year changes in NMMA and T2D, suggesting that harmful changes in plasma NMMA may be offset by consuming a MedDiet. Taking into consideration results of numerous primary studies, we continue to recommend a MedDiet supplemented with either EVOO or nuts against a low-fat control diet for the prevention of cardiometabolic disease[3].

Our study has several strengths. We used an efficient case-cohort design to evaluate associations of metabolites with incident T2D. We were able to adjust for multiple confounders, as well as test for several potential effect modifiers with sufficient power. We also note several limitations of the present study. First, T2D was initially specified as a secondary outcome. Second, we cannot rule out the possibility of residual and unmeasured confounding. Third, there could be possible selection bias if the distribution of metabolites was unequal among missing and non-missing blood specimens. Finally, our analysis among individuals of predominately white individuals at high risk of CVD may not be generalizable to other populations.

We conclude that arginine bioavailability is important in T2D pathophysiology. Specifically, we report inverse associations of 1-year changes in arginine and arginine/ADMA ratio with the risk of incident T2D. Baseline ornithine was also predictive of higher T2D risk. Positive changes in ornithine and citrulline; and negative changes in SDMA and GABR were inversely associated with concurrent changes in HOMA-IR. Lastly, a MedDiet may counteract harmful changes in plasma NMMA with respect to T2D.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

ADMA	asymmetric dimethylarginine
CI	confidence interval
CVD	cardiovascular disease
EVOO	extra virgin olive oil
GABR	global arginine bioavailability ratio

HOMA-IR	homeostatic model assessment of insulin resistance
HR	hazard ratio
MD	mean difference
MedDiet	Mediterranean diet
NMMA	N-monomethyl-l-arginine
SDMA	symmetric dimethylarginine
T2D	type 2 diabetes

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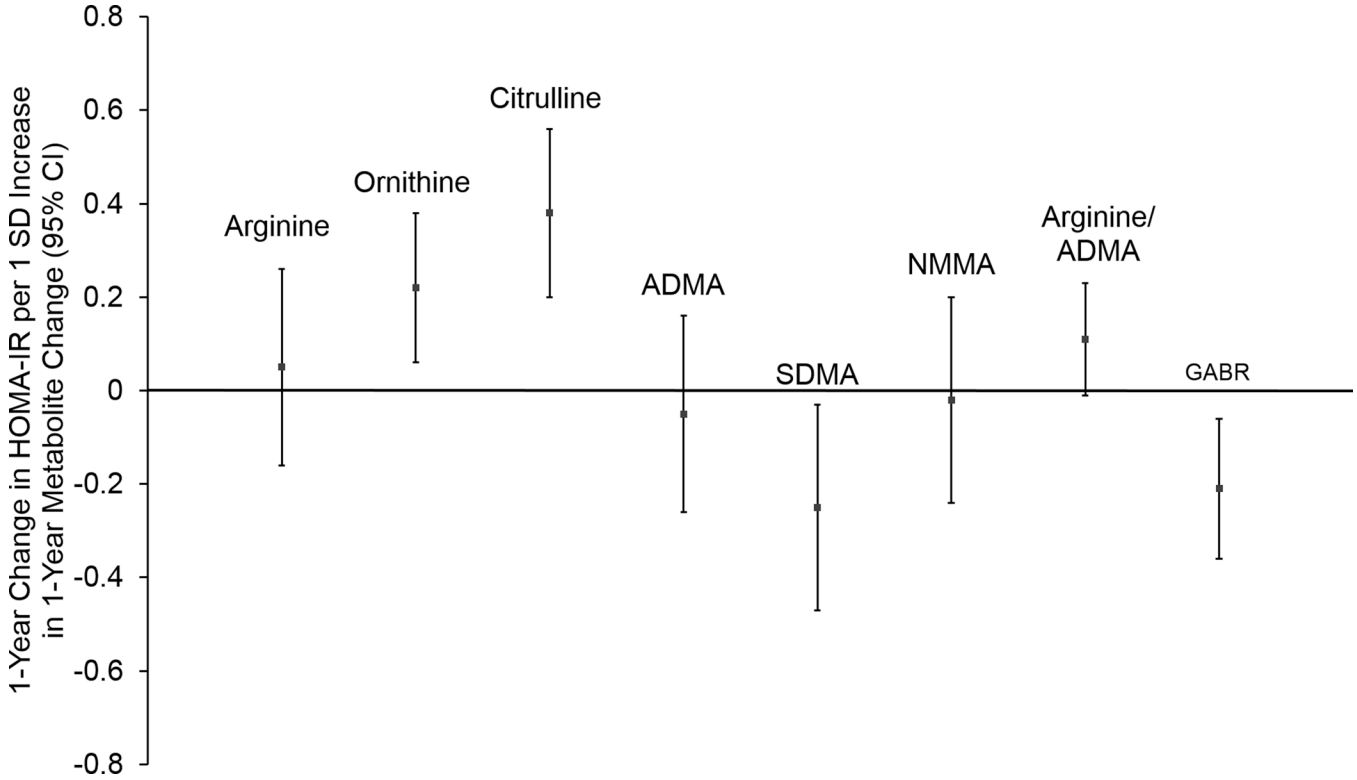

Figure 1.

Least squares mean differences of 1-year changes in HOMA-IR (95% CI) with concurrent 1-year changes (per 1 SD) in urea cycle metabolites, methylarginines, arginine/ADMA ratio, and GABR (n=223).

Abbreviations: ADMA, asymmetric dimethylarginine; GABR, global arginine bioavailability ratio (defined as arginine/ornithine+citrulline); NMMA, N-monomethyl-l-arginine; SD, standard deviation; SDMA, symmetric dimethylarginine.

Stratified by recruitment center and adjusted for respective baseline metabolite (continuous), age (years), sex (male, female), intervention group (control, MedDiet+EVOO, MedDiet +nuts), body mass index (kg/m^2), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia, hypertension, baseline HOMA, baseline HOMA*baseline HOMA, and plasma creatinine (quartiles).

Table 1.

Hazard ratios (95% CI) for type 2 diabetes with 1-year changes in urea cycle metabolites, methylarginines, arginine/ADMA ratio, and GABR in the PREDIMED Trial, 2003–2010.

	158 cases, 533 subcohort						
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	**p-trend**	**HR per 1 SD**	**p**
			Model 1				
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)	0.04
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)	0.77
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)	0.29
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)	0.67
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)	0.22
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)	0.70
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)	0.09
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)	0.26
			Model 2				
Arginine	1.00 (Ref)	1.25 (0.60, 2.61)	0.65 (0.31, 1.36)	0.46 (0.21, 1.04)	0.02	0.68 (0.49, 0.95)	0.02
Ornithine	1.00 (Ref)	0.85 (0.40, 1.78)	0.55 (0.26, 1.19)	0.83 (0.38, 1.82)	0.43	0.94 (0.69, 1.29)	0.70
Citrulline	1.00 (Ref)	1.29 (0.59, 2.80)	1.64 (0.77, 3.49)	0.77 (0.35, 1.69)	0.67	0.86 (0.66, 1.12)	0.27
ADMA	1.00 (Ref)	0.98 (0.50, 1.94)	0.72 (0.34, 1.53)	0.74 (0.35, 1.56)	0.30	0.90 (0.68, 1.19)	0.46
SDMA	1.00 (Ref)	0.86 (0.42, 1.75)	0.39 (0.18, 0.87)	0.75 (0.34, 1.66)	0.20	0.82 (0.59, 1.14)	0.24
NMMA	1.00 (Ref)	1.10 (0.54, 2.24)	1.05 (0.49, 2.27)	0.98 (0.40, 2.39)	0.94	1.08 (0.78, 1.49)	0.64
Arginine/ADMA	1.00 (Ref)	0.90 (0.43, 1.90)	0.38 (0.16, 0.87)	0.52 (0.22, 1.25)	0.04	0.73 (0.51, 1.04)	0.08
GABR	1.00 (Ref)	0.35 (0.17, 0.73)	0.41 (0.19, 0.88)	0.47 (0.22, 1.01)	0.10	0.79 (0.57, 1.09)	0.15

Abbreviations: ADMA, asymmetric dimethylarginine; EVOO, extra virgin olive oil; GABR, global arginine bioavailability ratio (defined as arginine/ornithine+citrulline); HR, hazard ratio; NMMA, N-monomethyl-l-arginine; SD, standard deviation; SDMA, symmetric dimethylarginine.

All models stratified by recruitment center. Model 1 adjusted for respective baseline metabolite (continuous), age (years), sex (male, female), intervention group (control, MedDiet+EVOO, MedDiet+nuts), body mass index (kg/m^2), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia, hypertension, baseline glucose and baseline glucose*baseline glucose. Model 2 additionally adjusted for plasma creatinine (quartiles).