SUPPLEMENTAL MATERIAL

Table S1. Characteristics of the choline pathway metabolites						
Metabolite	**HMDB ID**	**Metabolic pathways**	**Superclass/class/Subclass**	**Origin**	**Disease**	**Biofluid**
Trimethylamine-N-oxide (TMAO)	HMDB00925	Betaine metabolism	Organonitrogen compounds/Amines/Amine oxides and derivatives	Drug metabolite Endogenous Microbial	Kidney disease CVD Obesity T2D	Blood Feces Saliva Urine
Betaine	HMDB00043	Betaine metabolism Glycine and serine metabolism Methionine metabolism	Organic acids and derivatives/Carboxylic acids and derivatives/Amino acids	Endogenous Diet	Kidney disease Lung cancer CVD T2D	Blood Breast Milk Saliva Urine
Choline	HMDB00097	Betaine metabolism Methionine metabolism Phospholipid biosynthesis	Organonitrogen compounds/quaternary ammonium salts/cholines	Drug Food Plant	Lung cancer Alzheimer CVD Pre-diabetes	Blood Breast Milk Cerebrospinal Fluid Feces Saliva Urine
Phosphocholine	HMDB00284	Phospholipid biosynthesis Sphingolipid metabolism	Organonitrogen compound/quaternary ammonium salts/cholines	Endogenous	Alzheimer CVD	Blood Breast Milk Cerebrospinal Fluid Saliva Urine
Alphaglycerophosphocholine	HMDB00086	Glycerophospholipid metabolism	Lipids/Glycerophospholipids/glycerophosphocholine	Endogenous Food	Alzheimer CVD	Blood Breast Milk Cerebrospinal Fluid Saliva

Abbreviations: CVD, cardiovascular disease; TMAO, Trimethylamine-N-oxide; T2D, type 2 diabetes mellitus.

Table S2. Means and SD of baseline plasma concentration of metabolites (micromole per liter) in the total population				
	Total population	**Cases (229)**	**Non-cases (751)**	**P value**
Trimethylamine-N-oxide	5.88 (6.48)	5.84 (6.28)	5.89 (6.55)	0.92
Betaine	61.00 (17.60)	61.17 (16.66)	60.95 (17.90)	0.86
Choline	23.75 (5.09)	25.04 (5.10)	23.36 (5.03)	<0.001
Phosphocholine	0.68 (0.66)	0.75 (0.81)	0.65 (0.61)	0.09
Alphaglycerophosphocholine	3.47 (2.64)	3.98 (3.15)	3.31 (2.44)	0.57

Cases: composite of cardiovascular disease events: myocardial infarction, stroke and cardiovascular death.

Table S3. Means and SD of plasma concentration of metabolites (micromole per liter) per intervention group at baseline and one year of intervention			
	Control (303)	**Mediterranean Diet + extra-virgin olive oil (363)**	**Mediterranean Diet + Nuts (314)**
BASELINE			
Trimethylamine-N-oxide	6.21 (7.22)	5.61 (5.99)	5.86 (6.28)
Betaine	60.70 (16.12)	60.44 (18.11)	61.95 (18.40)
Choline	23.14 (4.72)	23.99 (5.01)	24.07 (5.49)
Phosphocholine	0.59 (0.54)	0.72 (0.68)	0.71 (0.74)
Alphaglycerophosphocholine	3.31 (2.21)	3.44 (2.19)	3.65 (3.38)
1-YEAR			
Trimethylamine-N-oxide	6.55 (7.75)	7.14 (9.73)	5.80 (7.10)
Betaine	60.62 (15.92)	60.70 (18.21)	62.35 (18.35)
Choline	24.08 (5.11)	24.42 (5.96)	24.46 (5.14)
Phosphocholine	0.61 (0.55)	0.62 (0.53)	0.65 (0.50)
Alphaglycerophosphocholine	3.86 (3.91)	3.50 (2.73)	4.26 (5.67)

Table S4. Joint associations of the scores and intervention group with risk of cardiovascular disease and stroke

	MedDiet interventions	Control	P interaction
CARDIOVASCULAR DISEASE			
Choline metabolite score			
Q1	Ref.	1.74 (0.77, 3.89)	0.09
Q2-Q4	1.70 (0.99, 2.91)	2.53 (1.41, 4.53)	
Betaine to choline ratio			
Q1	0.67 (0.45, 0.99)	Ref.	0.91
Q2-Q4	0.43 (0.25, 0.77)	0.74 (0.38,1.44)	
STROKE			
Choline metabolite score			
Q1	Ref.	2.48 (0.94, 6.53)	0.06
Q2-Q4	1.76 (0.85, 3.63)	2.66 (1.22, 5.80)	
Betaine to choline ratio			
Q1	0.60 (0.37, 0.96)	Ref.	0.76
Q2-Q4	0.48 (0.23, 0.99)	0.77 (0.32, 1.84)	

An inverse normal transformation was applied to raw baseline values. To build the score, we applied a weighted sum of concentrations of five metabolites in the choline pathway (trimethylamine-N-oxide (TMAO), betaine, choline, phosphocholine and alphaglycerophosphocholine). The ratio between betaine and choline was calculated by dividing the raw values and then applying inverse normal transformations. Models were adjusted for age, sex, body mass index, family history of premature heart disease, smoking, physical activity (metabolic equivalent task units in min/d), hypertension, dyslipidemia, and diabetes. Abbreviations: TMAO, trimethylamine N oxide. P for interaction were derived from Cox models adjusted as above, including an interaction term between the intervention group (MedDiet vs. control) and the score as a continuous variable. MedDiet, Mediterranean diet.

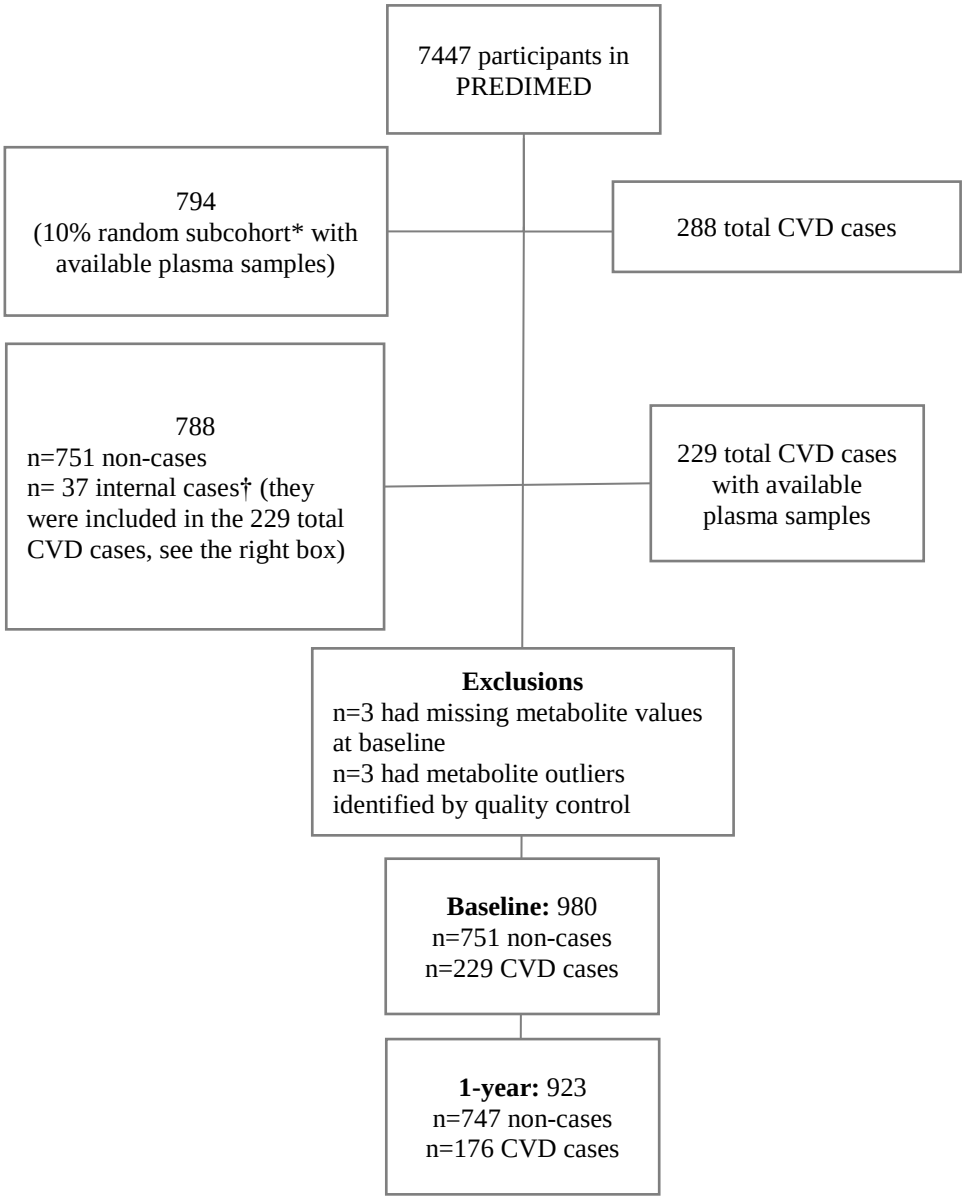
Table S5. Risk of cardiovascular disease and stroke by 1-year changes in choline pathway metabolites in the PREDIMED Study

	Quartiles of metabolite concentration				P_{trend}	HR per 1 SD increment	*P* value
	Q1	Q2	Q3	Q4			
CARDIOVASCULAR DISEASE							
TMAO							
Cases	39	35	47	55			
Multivariable Model 1	Ref.	0.98 (0.44, 2.14)	1.34 (0.64, 2.81)	1.39 (0.71, 2.74)	0.13	1.06 (0.88, 1.27)	0.54
Multivariable Model 2	Ref.	1.08 (0.47, 2.50)	1.44 (0.65, 3.18)	1.42 (0.69, 2.92)	0.18	1.04 (0.85, 1.25)	0.72
Betaine							
Cases	56	38	44	38			
Multivariable Model 1	Ref.	0.61 (0.36, 1.02)	0.59 (0.35, 0.99)	0.60 (0.35, 1.02)	0.08	0.80 (0.66, 0.98)	0.03
Multivariable Model 2	Ref.	0.58 (0.34, 0.98)	0.63 (0.37, 1.06)	0.61 (0.35, 1.04)	0.11	0.83 (0.68, 1.01)	0.07
Choline							
Cases	59	40	42	35			
Multivariable Model 1	Ref.	0.72 (0.43, 1.21)	0.81 (0.47, 1.39)	0.65 (0.36, 1.16)	0.20	0.89 (0.73, 1.09)	0.27
Multivariable Model 2	Ref.	0.71 (0.41, 1.20)	0.81 (0.46, 1.42)	0.68 (0.38, 1.23)	0.28	0.92 (0.75, 1.14)	0.46
Phosphocholine							
Cases	42	49	36	49			
Multivariable Model 1	Ref.	0.97 (0.58, 1.62)	0.94 (0.55, 1.63)	1.20 (0.71, 2.00)	0.47	1.15 (0.94, 1.41)	0.15
Multivariable Model 2	Ref.	0.95 (0.57, 1.60)	1.02 (0.58, 1.79)	1.21 (0.70, 2.06)	0.44	1.15 (0.93, 1.42)	0.18
Alphaglycerophosphocholine							
Cases	47	52	29	47			
Multivariable Model 1	Ref.	1.12 (0.63, 1.99)	0.77 (0.41, 1.44)	1.02 (0.58, 1.81)	0.79	1.10 (0.91, 1.33)	0.30
Multivariable Model 2	Ref.	1.07 (0.59, 1.92)	0.78 (0.41, 1.49)	1.05 (0.59, 1.88)	0.92	1.14 (0.94, 1.38)	0.17
STROKE							
TMAO							
Cases	21	20	24	30			
Multivariable Model 1	Ref.	1.04 (0.35, 3.03)	1.24 (0.44, 3.54)	1.29 (0.51, 3.26)	0.42	1.02 (0.80, 1.28)	0.90
Multivariable Model 2	Ref.	1.16 (0.36, 3.65)	1.35 (0.45, 4.09)	1.34 (0.50, 3.59)	0.47	0.99 (0.78, 1.26)	0.82
Betaine							
Cases	31	17	25	22			
Multivariable Model 1	Ref.	0.47 (0.23, 0.96)	0.60 (0.31, 1.14)	0.63 (0.32, 1.23)	0.29	0.84 (0.64, 1.09)	0.20

Multivariable Model 2	Ref.	0.47 (0.23, 0.98)	0.64 (0.33, 1.23)	0.67 (0.34, 1.32)	0.40	0.86 (0.66, 1.13)	0.30
Choline							
Cases	34	22	21	18			
Multivariable Model 1	Ref.	0.62 (0.32, 1.20)	0.61 (0.31, 1.21)	0.53 (0.25, 1.09)	0.10	0.89 (0.69, 1.16)	0.40
Multivariable Model 2	Ref.	0.62 (0.32, 1.21)	0.64 (0.32, 1.29)	0.58 (0.28, 1.21)	0.18	0.93 (0.71, 1.22)	0.61
Phosphocholine							
Cases	28	24	21	22			
Multivariable Model 1	Ref.	0.92 (0.49, 1.74)	1.08 (0.54, 2.15)	0.99 (0.50, 1.95)	0.93	1.00 (0.77, 1.30)	0.86
Multivariable Model 2	Ref.	0.90 (0.47, 1.70)	1.17 (0.58, 2.39)	1.00 (0.49, 2.00)	0.89	0.98 (0.74, 1.30)	0.92
Alphaglycerophosphocholine							
Cases	25	28	19	23			
Multivariable Model 1	Ref.	1.03 (0.56, 1.87)	0.84 (0.42, 1.68)	0.88 (0.46, 1.68)	0.57	1.04 (0.81, 1.34)	0.74
Multivariable Model 2	Ref.	1.00 (0.53, 1.88)	0.88 (0.48, 1.77)	0.90 (0.45, 1.77)	0.69	1.08 (0.83, 1.39)	0.56

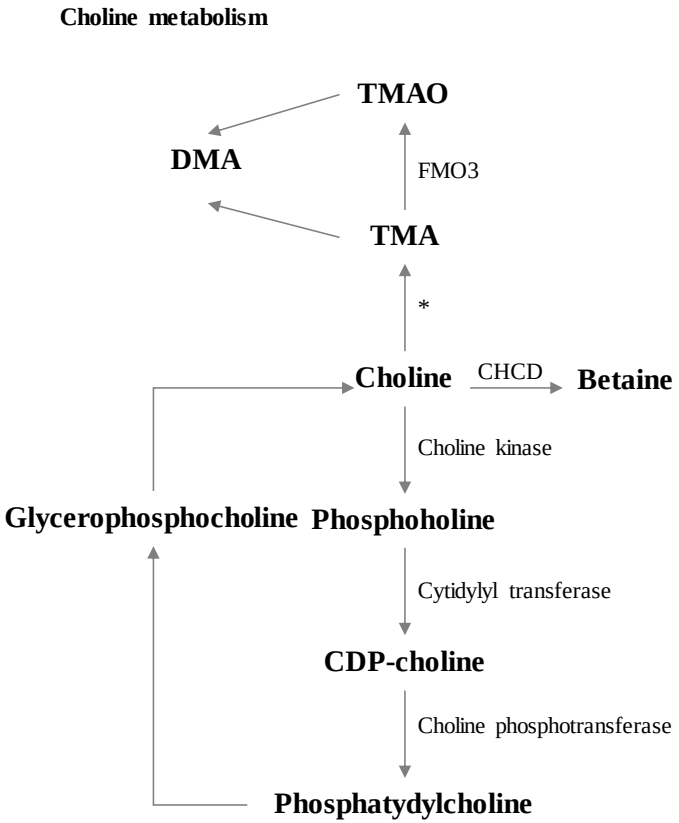
Inverse normal transformation was applied to raw values of changes in metabolite concentration. Model 1 was adjusted for age, sex, BMI, family history of premature heart disease, and smoking and was stratified by intervention group. Model 2 was further adjusted for physical activity, hypertension, dyslipidemia, and diabetes. All models were adjusted for baseline metabolites levels and the interaction between baseline levels and 1-year changes.

Figure S1. Flow chart of study participants



*subcohort: a random, non-stratified sample of approximately 10% of PREDIMED participants at baseline.
†internal cases: incident cases which were subcohort members (37 overlapping cases). They were treated as cases in the analysis (included in the 229)

Figure S2. Diagram of choline pathway



The figure shows key metabolic choline pathway. *Bacterial degration of choline by the gut microbiota. FMO3 indicates, Flavin-containing monoxygenase; CHCD, choline dehydrogenase;

Figure S3. Correlation matrix for plasma metabolite levels

Spearman Correlations coefficients of metabolites in the PREDIMED Study