

SUPPLEMENTAL MATERIAL

Supplement Table 1. Correlation between baseline circulating BCAA metabolites and cardiometabolic biomarkers in WHS.

	BCAA	Isoleucine	Leucine	Valine	Hba1c%	LPIR	Triglycerides	LDL-C	GlycA
BCAA	1	0.74 <.0001	0.86 <.0001	0.93 <.0001	0.23 <.0001	0.31 <.0001	0.28 <.0001	0.11 <.0001	0.30 <.0001
Isoleucine		1	0.51 <.0001	0.60 <.0001	0.18 <.0001	0.31 <.0001	0.28 <.0001	0.07 <.0001	0.20 <.0001
Leucine			1	0.68 <.0001	0.16 <.0001	0.18 <.0001	0.14 <.0001	0.12 <.0001	0.26 <.0001
Valine				1	0.24 <.0001	0.32 <.0001	0.30 <.0001	0.09 <.0001	0.27 <.0001
Hba1c%					1	0.17 <.0001	0.18 <.0001	0.11 <.0001	0.20 <.0001
LPIR						1	0.71 <.0001	0.13 <.0001	0.46 <.0001
Triglycerides							1	0.29 <.0001	0.46 <.0001
LDL-C								1	0.17 <.0001

Metabolite values are ln-transformed and standardized; other biomarkers are ln-transformed for analysis.

BCAAs=branched-chain amino acids, Hba1c=glycated haemoglobin, LPIR score=lipoprotein insulin resistance score that reflects lipoprotein derangements of insulin resistance, LDL-C=low density lipoprotein cholesterol, GlycA=glycoprotein N-acetyl methyl groups

Supplement Table 2. Prospective association between baseline circulating total BCAAs in relation to incident total cardiovascular disease, MI, stroke, and revascularization risk in 27,401 US women, with further adjustment for individual ln-transformed biomarkers of CVD risk.

	Total CVD
	HR per SD
	(95% CI)
Model 3*	
Total BCAAs	1.13 (1.08, 1.18)
+ Hba1c%	1.07 (1.02, 1.12)
+ triglycerides	1.09 (1.04, 1.14)
+ LPIR Score	1.10 (1.05, 1.15)
+ GlycA	1.10 (1.05, 1.15)
+ hsCRP	1.11 (1.06, 1.16)
+ LDL cholesterol	1.12 (1.08, 1.18)
+ HDL cholesterol	1.12 (1.08, 1.18)
+ Hba1c%, triglycerides, LPIR Score, and GlycA	1.02 (0.98, 1.07)

*Multivariable adjusted model 3 includes the following: age (continuous), randomized treatment assignments (ASA, BC, VE), fasting status at blood draw, menopausal status (pre, post, uncertain, missing), current hormone therapy use, family history of MI, Caucasian race/ethnicity, smoking status (never, past, current <15 c/d, current 15+ c/d), AHEI diet quality score (quintiles), alcohol intake (4 categories), total physical activity MET-hrs/wk (quintiles), history of high cholesterol, history of hypertension, and BMI (10 categories), plus the biomarkers as indicated above.

BCAAs=branched-chain amino acids, Hba1c=glycated haemoglobin, LPIR score=lipoprotein insulin resistance score that reflects lipoprotein derangements of insulin resistance, GlycA=glycoprotein N-acetyl methyl groups, hsCRP=high sensitivity C-reactive protein, LDL-C=low density lipoprotein cholesterol, HR=hazard ratio, CI=confidence interval, SD=standard deviation

Supplement Table 3. Cox proportional hazards regression models of quintiles of baseline circulating BCAAs in relation to incident total cardiovascular disease (MI, stroke, revascularization) risk in 27,401 US women.

	Q1	Q2	Q3	Q4	Q5	
		HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	p for trend*
Total BCAAs						
CVD Events	358	395	421	454	579	
Model 1: Age, treatment	[ref]	1.07 (0.93, 1.24)	1.09 (0.95, 1.26)	1.24 (1.08, 1.42)	1.67 (1.47, 1.91)	<0.0001
Model 2: Multivariable	[ref]	1.07 (0.93, 1.24)	1.06 (0.92, 1.22)	1.13 (0.99, 1.30)	1.43 (1.25, 1.64)	<0.0001
Model 3: + BMI	[ref]	1.05 (0.91, 1.21)	1.01 (0.88, 1.17)	1.06 (0.92, 1.23)	1.31 (1.14, 1.50)	0.0001
Isoleucine						
CVD Events	400	352	415	449	591	
Model 1: Age, treatment	[ref]	0.99 (0.85, 1.14)	1.02 (0.89, 1.17)	1.16 (1.01, 1.33)	1.74 (1.53, 1.98)	<0.0001
Model 2: Multivariable	[ref]	0.95 (0.83, 1.10)	0.97 (0.84, 1.11)	1.06 (0.93, 1.22)	1.47 (1.29, 1.67)	<0.0001
Model 3: + BMI	[ref]	0.95 (0.82, 1.09)	0.94 (0.82, 1.08)	1.02 (0.89, 1.17)	1.37 (1.20, 1.57)	<0.0001
Leucine						
CVD Events	399	417	444	403	544	
Model 1: Age, treatment	[ref]	1.09 (0.95, 1.25)	1.10 (0.96, 1.26)	1.09 (0.95, 1.25)	1.43 (1.26, 1.63)	<0.0001
Model 2: Multivariable	[ref]	1.10 (0.96, 1.26)	1.08 (0.94, 1.23)	1.03 (0.90, 1.18)	1.26 (1.10, 1.43)	0.003
Model 3: + BMI	[ref]	1.09 (0.95, 1.25)	1.05 (0.92, 1.21)	0.98 (0.86, 1.13)	1.18 (1.03, 1.34)	0.07

Valine

CVD Events	357	387	440	454	569	
Model 1: Age, treatment	[ref]	1.07 (0.92, 1.23)	1.17 (1.01, 1.34)	1.28 (1.11, 1.47)	1.62 (1.42, 1.85)	<0.0001
Model 2: Multivariable	[ref]	1.08 (0.93, 1.25)	1.13 (0.99, 1.30)	1.19 (1.03, 1.36)	1.42 (1.24, 1.63)	<0.0001
Model 3: + BMI	[ref]	1.05 (0.91, 1.22)	1.08 (0.94, 1.25)	1.11 (0.96, 1.28)	1.29 (1.12, 1.49)	0.0002

BCAAs=branched chain amino acids, BMI=body mass index, Q=quintile, HR=hazard ratio, CI=confidence interval

Model 2 includes the following: age (continuous), randomized treatment assignments, fasting status at blood draw, menopausal status (pre, post, uncertain, missing), current hormone therapy use, family history of MI, Caucasian race/ethnicity, smoking status (never, past, current <15 c/d, current 15+ c/d), AHEI diet quality score (quintiles), alcohol intake (4 categories), total physical activity MET-hrs/wk (quintiles), history of high cholesterol, history of hypertension; Model 3 includes the covariates adjusted for in Model 2 plus BMI (adjusted for in 10 categories).

*P-values for tests for trend were estimated by modeling the median values across quintiles as a continuous variable.

Supplement Table 4. Prospective association between baseline circulating total BCAAs (continuous, per SD) in relation to incident total cardiovascular disease, MI, stroke, and revascularization risk in 27,401 US women, stratified by risk factors at time of baseline blood draw.

		Total CVD
		HR per SD*
Total BCAAs	N events	(95% CI)
BMI, kg/m²		
<25	920	1.08 (1.01, 1.16)
≥25	1,227	1.15 (1.09, 1.22)
<i>P</i> -interaction		0.14
Menopausal status		
Pre	668	1.13 (1.04, 1.23)
Post	1,539	1.12 (1.07, 1.19)
<i>P</i> -interaction		0.32
Current hormone therapy use		
No	1,222	1.15 (1.08, 1.22)
Yes	985	1.11 (1.03, 1.18)
<i>P</i> -interaction		0.19
Metabolic syndrome		
No	1,251	1.07 (1.01, 1.14)
Yes	902	1.12 (1.05, 1.20)
<i>P</i> -interaction		0.14
Baseline HbA1c		
<5.7%	1,836	1.08 (1.02, 1.13)
≥5.7%	359	1.17 (1.06, 1.30)
<i>P</i> -interaction		0.023

Fasting at blood draw

No	471	1.11 (1.02, 1.20)
Yes	1,611	1.14 (1.07, 1.20)
<i>P-interaction</i>		0.70

Follow-up time, years

<10	951	1.18 (1.10, 1.26)
≥10	1,056	1.11 (1.04, 1.18)
<i>P-interaction</i>		0.24

BCAAs=branched-chain amino acids, HR=hazard ratio, CI=confidence interval, SD=standard deviation
*Multivariable adjusted model includes the following: age (continuous), randomized treatment assignments (ASA, BC, VE), fasting status at blood draw, menopausal status (pre, post, uncertain, missing), current hormone therapy use, family history of MI, Caucasian race/ethnicity, smoking status (never, past, current <15 c/d, current 15+ c/d), AHEI diet quality score (quintiles), alcohol intake (4 categories), total physical activity MET-hrs/wk (quintiles), history of high cholesterol, history of hypertension, and BMI (10 categories).