

Supplemental Table 1: Characteristics of study participants according to tertile categories of trimethylamine N-oxide (TMAO), choline, or L-carnitine levels at the baseline examination.

	TMAO _{baseline}				Choline _{baseline}				L-carnitine _{baseline}			
	Tertile (T) 1	T2	T3	<i>P</i>	T1	T2	T3	<i>P</i>	T2	T2	T3	<i>P</i>
N	166	169	169	-	168	170	166	-	167	168	169	-
TMAO, μM	1.6 (1.3, 1.8)	2.7 (2.4, 3.0)	4.8 (3.8, 6.9)	-	2.5 (1.7, 3.5)	2.7 (1.8, 3.8)	3.1 (2.3, 4.6)	-	2.5 (1.6, 3.4)	2.8 (2.0, 3.9)	3.0 (2.2, 4.2)	-
L-carnitine, μM	33.6 (28.1, 37.1)	35.3 (30.1, 39.5)	35.5 (32.1, 41.3)	-	32.7 (28.1, 36.5)	34.6 (30.2, 38.9)	36.9 (33.3, 42.5)	-	27.9 (25.6, 30.0)	34.5 (33.4, 36)	41.7 (39.3, 45.2)	-
Choline, μM	8.3 (7.2, 9.6)	8.7 (7.5, 10.2)	9.1 (7.8, 10.7)	-	7.0 (6.4, 7.4)	8.6 (8.2, 9.2)	11.1 (10.3, 12.1)	-	8.0 (6.9, 9.3)	8.7 (7.4, 10.1)	9.5 (8.1, 10.9)	-
Age, y	49.3 (9.3)	52.4 (8.0)	53.0 (9.3)	0.002	50.1 (8.4)	50.8 (9.1)	53.9 (9.1)	<.0001	49.2 (8.7)	52.0 (9.2)	53.6 (8.7)	<.0001
Male sex	55 [33.1%]	68 [40.2%]	63 [37.3%]	0.4	37 [22.0%]	65 [38.2%]	84 [50.6%]	<.0001	39 [23.4%]	58 [34.5%]	89 [52.7%]	<.0001
Whites	124 [74.7%]	136 [80.5%]	148 [87.6%]	0.01	127 [75.6%]	138 [81.2%]	143 [86.1%]	0.049	130 [77.8%]	136 [81.0%]	142 [84.0%]	0.35
Low-carbohydrate/High-fat diet, yes	79 [47.6%]	79 [46.7%]	80 [47.3%]	0.99	77 [45.8%]	80 [47.1%]	81 [48.8%]	0.86	76 [45.5%]	80 [47.6%]	82 [48.5%]	0.85
High-protein diet, yes	80 [48.2%]	75 [44.4%]	80 [47.3%]	0.76	77 [45.8%]	86 [50.6%]	72 [43.4%]	0.4	82 [49.1%]	80 [47.6%]	73 [43.2%]	0.53
Parental history of diabetes	51 [30.7%]	54 [32.0%]	38 [22.5%]	0.11	42 [25.0%]	54 [31.8%]	47 [28.3%]	0.39	53 [31.7%]	46 [27.4%]	44 [26.0%]	0.48
BMI, kg/m ²	32.7 (3.6)	32.6 (3.7)	32.6 (4.0)	0.83	32.2 (3.9)	32.6 (3.8)	33.1 (3.5)	0.009	32.2 (3.7)	32.7 (3.8)	33.1 (3.7)	0.006
Fasting glucose, mmol/L	5.0 (0.7)	5.1 (0.6)	5.2 (0.8)	0.07	5.1 (0.7)	5.1 (0.6)	5.2 (0.8)	0.1	5.0 (0.5)	5.2 (0.8)	5.2 (0.7)	0.0004
HbA1c, %	5.4 (0.4)	5.4 (0.4)	5.5 (0.4)	0.47	5.4 (0.4)	5.4 (0.4)	5.4 (0.4)	0.72	5.4 (0.4)	5.4 (0.5)	5.5 (0.4)	0.007
HbA1c, mmol/mol	35 (4)	36 (4)	36 (5)		36 (5)	36 (4)	36 (5)		35 (4)	36 (5)	36 (5)	
Fasting insulin, pmol/L	83.1 (59.8)	85.3 (50.1)	90.8 (55.1)	0.54	83.7 (49.4)	86.2 (65.6)	89.5 (48.5)	0.19	79 (46.8)	89.3 (63.3)	90.9 (53.5)	0.018
HOMA-IR	2.8 (2.2)	2.8 (1.8)	3.1 (2.1)	0.35	2.8 (1.9)	2.9 (2.4)	3.0 (1.8)	0.13	2.6 (1.7)	3.0 (2.3)	3.1 (2.0)	0.004
Dietary intake per day*												
Total energy (E) intake, kcals	1988 (585)	2014 (614)	2007 (583)	0.62	1996 (540)	2004 (678)	2010 (549)	0.9	1983 (576)	2006 (585)	2018 (619)	0.43
Carbohydrate, %E	46.1 (7.6)	45.3 (6.8)	43.2 (8.2)	0.007	45.6 (7.6)	44.5 (7.7)	44.5 (7.6)	0.19	44.8 (7.4)	45.3 (7.2)	44.4 (8.2)	0.46
Fat, %E	36.1 (5.9)	37.4 (6.0)	37.1 (5.9)	0.2	36.6 (6)	36.6 (5.6)	37.4 (6.3)	0.19	36.6 (5.6)	36.9 (5.9)	37.1 (6.3)	0.34
Protein, %E	18.0 (2.9)	17.8 (3.2)	18.5 (3.4)	0.18	17.8 (2.7)	18.6 (3.7)	17.8 (2.9)	0.43	18.5 (3.5)	18.0 (3.0)	17.9 (3.0)	0.59

Data are mean (SD), median (25th, 75th), or N [%].

Supplemental Table 2A: Differences in serum amino acid levels according to tertile (T) categories of trimethylamine N-oxide (TMAO) at the baseline examination.

	TMAO _{baseline}			<i>P</i> *	<i>P</i> **
	T1	T2	T3		
Gluconeogenesis substrates					
Alanine	251.1 (210.7, 294.0)	250 (217.8, 289.2)	254.7 (219.1, 300.8)	0.79	0.93
Glycine	178.1 (145.7, 220.1)	174 (148.1, 231.8)	177.3 (146.2, 215.8)	0.88	0.35
Glutamine	0.7 (0.5, 1.0)	0.6 (0.4, 0.8)	0.6 (0.5, 0.8)	0.24	0.28
Citrulline	33.9 (28.7, 38.3)	35.0 (30.0, 41.2)	36.8 (29.9, 41.7)	0.03	0.03
Branched-chain amino acids (BCAAs)					
Valine	168.8 (148.6, 191.7)	170.5 (152.9, 192.7)	169.6 (152.2, 188)	0.88	0.72
Leucine, Isoleucine	131.0 (113.0, 147.7)	129.6 (114.9, 149.8)	132.8 (118.3, 154.1)	0.7	0.22
Aromatic amino acids (AAAs)					
Phenylalanine	55.5 (48.9, 63.4)	56.0 (51.2, 62.9)	56.5 (50.5, 63.6)	0.85	0.54
Tyrosine	56.4 (48.8, 67.3)	58.3 (51.6, 68.2)	58.6 (49.9, 68.8)	0.5	0.71
Other amino acids					
Glutamic acid	223.2 (198.0, 272.8)	229.3 (202.0, 268.7)	233.8 (201.6, 266.1)	0.71	0.34
Taurine	1.6 (1.3, 2.0)	1.7 (1.2, 2.0)	1.7 (1.4, 2.0)	0.34	0.1
Tryptophan	25.4 (20.5, 30.9)	24.3 (20.3, 29.1)	26.6 (21.6, 31.6)	0.02	0.19

Data are median (27th, 75th).

*P** values by median test across tertile categories.

*P*** values by general linear model including the outcomes and exposures as continuous variables after log-transformation.

Supplemental Table 2B: Differences in serum amino acid levels according to tertile (T) categories of choline at the baseline examination.

	Choline _{baseline}			<i>P</i> *	<i>P</i> **
	T1	T2	T3		
Gluconeogenesis substrates					
Alanine	238.1 (208.0, 282.7)	255.5 (217.8, 299.1)	263.2 (219.1, 308.4)	0.01	0.09
Glycine	181.1 (150.6, 235.2)	173.7 (148.6, 212.4)	173.4 (142.3, 218.3)	0.22	0.07
Glutamine	0.6 (0.5, 0.8)	0.6 (0.4, 0.9)	0.6 (0.4, 0.9)	0.53	0.89
Citrulline	32.6 (28.4, 38)	35.6 (29.7, 41.1)	36.7 (31.3, 42.2)	0.008	0.0004
Branched-chain amino acids (BCAAs)					
Valine	164.7 (150.5, 187.5)	169.9 (149.4, 194.1)	174.1 (156.4, 192.7)	0.09	0.03
Leucine, Isoleucine	125.3 (112.4, 141.8)	132.4 (115.6, 152.1)	137.6 (119.1, 154.0)	0.001	0.006
Aromatic amino acids (AAAs)					
Phenylalanine	54.7 (50.2, 61.7)	56.8 (49.4, 63.2)	58.1 (52.4, 64.4)	0.02	0.01
Tyrosine	56.5 (49.0, 65.9)	58.4 (50.0, 67.8)	59.3 (50.4, 69.3)	0.2	0.3
Other amino acids					
Glutamic acid	228.8 (197.9, 265.8)	228.2 (199.1, 261)	233.8 (203.3, 280.7)	0.91	0.43
Taurine	1.7 (1.3, 2.0)	1.6 (1.3, 1.9)	1.7 (1.3, 1.9)	0.31	0.6
Tryptophan	25.4 (20.6, 30.2)	25.0 (20.4, 30.3)	25.4 (20.9, 31.0)	0.75	0.93

Data are median (27th, 75th).

*P** values by median test across tertile categories.

*P*** values by general linear model including the outcomes and exposures as continuous variables after log-transformation.

Supplemental Table 2C: Differences in serum amino acid levels according to tertile (T) categories of L-carnitine at the baseline examination.

	L-carnitine _{baseline}			<i>P</i> *	<i>P</i> **
	T1	T2	T3		
Gluconeogenesis substrates					
Alanine	248.6 (212.8, 298.0)	250.2 (215.7, 289.4)	263.5 (220.3, 308.6)	0.2	0.007
Glycine	187.4 (154.7, 239.1)	178.1 (144.8, 224.0)	162.2 (142.1, 203.6)	0.001	0.14
Glutamine	0.6 (0.5, 0.9)	0.6 (0.4, 0.8)	0.6 (0.5, 0.9)	0.41	0.28
Citrulline	34.6 (29.4, 39.9)	35.1 (30.1, 40.7)	35.5 (29.1, 41.8)	0.45	0.13
Branched-chain amino acids (BCAAs)					
Valine	166.6 (150.5, 191.2)	167.9 (148.5, 190.6)	173.8 (156.7, 192.5)	0.12	0.02
Leucine, Isoleucine	131.1 (113.1, 149.3)	129 (113.9, 147.6)	134.4 (119.1, 154.4)	0.26	0.01
Aromatic amino acids (AAAs)					
Phenylalanine	57.0 (50.7, 63.9)	55.8 (49.5, 62.8)	55.0 (51.8, 63.5)	0.37	0.21
Tyrosine	58.2 (48.8, 69.6)	56.5 (49.4, 66.0)	58.6 (51.5, 69.8)	0.5	0.03
Other amino acids					
Glutamic acid	226.6 (198.7, 274.0)	229.1 (194.8, 264.7)	234.6 (206.9, 271.1)	0.79	0.17
Taurine	1.6 (1.3, 2.0)	1.6 (1.2, 2.0)	1.7 (1.4, 1.9)	0.58	0.11
Tryptophan	25.6 (20.8, 30.8)	25.7 (21.4, 31.1)	24.8 (20.5, 29.5)	0.36	0.53

Data are median (27th, 75th).

*P** values by median test across tertile categories.

*P*** values by general linear model including the outcomes and exposures as continuous variables after log-transformation.

Supplemental Table 3: Long-term (2-year) changes (Δ) in glycemic and insulin measurements according to initial (6-month) decreases in trimethylamine N-oxide (TMAO), choline and L-carnitine levels during the intervention

Outcomes	Δ TMAO		Δ Choline		Δ L-carnitine	
	β (SE)	<i>P</i>	β (SE)	<i>P</i>	β (SE)	<i>P</i>
Model 1						
Δ Fasting glucose, mmol/L	0.02 (0.04)	0.68	-0.32 (0.11)	0.004	-0.22 (0.14)	0.1
Δ HbA1c, %	-0.01 (0.04)	0.87	-0.02 (0.11)	0.85	-0.1 (0.14)	0.46
Δ Log-transformed fasting insulin	0.03 (0.04)	0.4	-0.33 (0.1)	<0.001	-0.19 (0.13)	0.14
Δ Log-transformed HOMA-IR	0.03 (0.04)	0.38	-0.38 (0.11)	<0.001	-0.23 (0.14)	0.11
Model 2						
Δ Fasting glucose, mmol/L	0.01 (0.04)	0.77	-0.30 (0.11)	0.007	-0.20 (0.14)	0.14
Δ HbA1c, %	0 (0.04)	0.97	-0.01 (0.11)	0.91	-0.08 (0.14)	0.56
Δ Log-transformed fasting insulin	0.02 (0.04)	0.49	-0.32 (0.1)	0.002	-0.17 (0.13)	0.18
Δ Log-transformed HOMA-IR	0.03 (0.04)	0.47	-0.37 (0.11)	<0.001	-0.20 (0.14)	0.15

β (SE) represents changes in the outcomes per 1 log-transformed decrease in metabolite levels during the intervention.

Model 1: data after adjusted for age, sex, ethnicity, parental history of diabetes, diet group, BMI at baseline, value for the respective outcome traits at the baseline examination, and either TMAO, choline, or L-carnitine at baseline.

Model 2: data after adjusted for covariates in the model 1 and 6-month changes in alanine, branched-chain amino acids, aromatic amino acids, and glutamic acid.

Supplemental Table 4: Changes in trimethylamine N-oxide (TMAO) and improvements of glycemic and insulin measurements at 6 months according to low- or high-fat diet intervention

Outcomes	Low-fat diet		High-fat diet		$P_{interaction}^*$
	β (SE)	P	β (SE)	P	
Model 1					
Δ Fasting glucose, mmol/L	0 (0.04)	0.95	-0.08 (0.05)	0.12	0.01
Δ HbA1c, %	0.05 (0.03)	0.11	-0.03 (0.03)	0.39	0.07
Δ log-transformed fasting insulin	0.01 (0.05)	0.87	-0.08 (0.05)	0.09	0.02
Δ log-transformed HOMA-IR	0.01 (0.05)	0.8	-0.1 (0.05)	0.07	0.008
Model 2					
Δ Fasting glucose, mmol/L	-0.01 (0.04)	0.87	-0.08 (0.05)	0.13	0.02
Δ HbA1c, %	0.04 (0.03)	0.18	-0.04 (0.03)	0.31	0.08
Δ log-transformed fasting insulin	-0.02 (0.04)	0.57	-0.09 (0.04)	0.03	0.04
Δ log-transformed HOMA-IR	-0.02 (0.04)	0.66	-0.1 (0.05)	0.03	0.02

Homeostasis model assessment-of-insulin resistance, HOMA-IR.

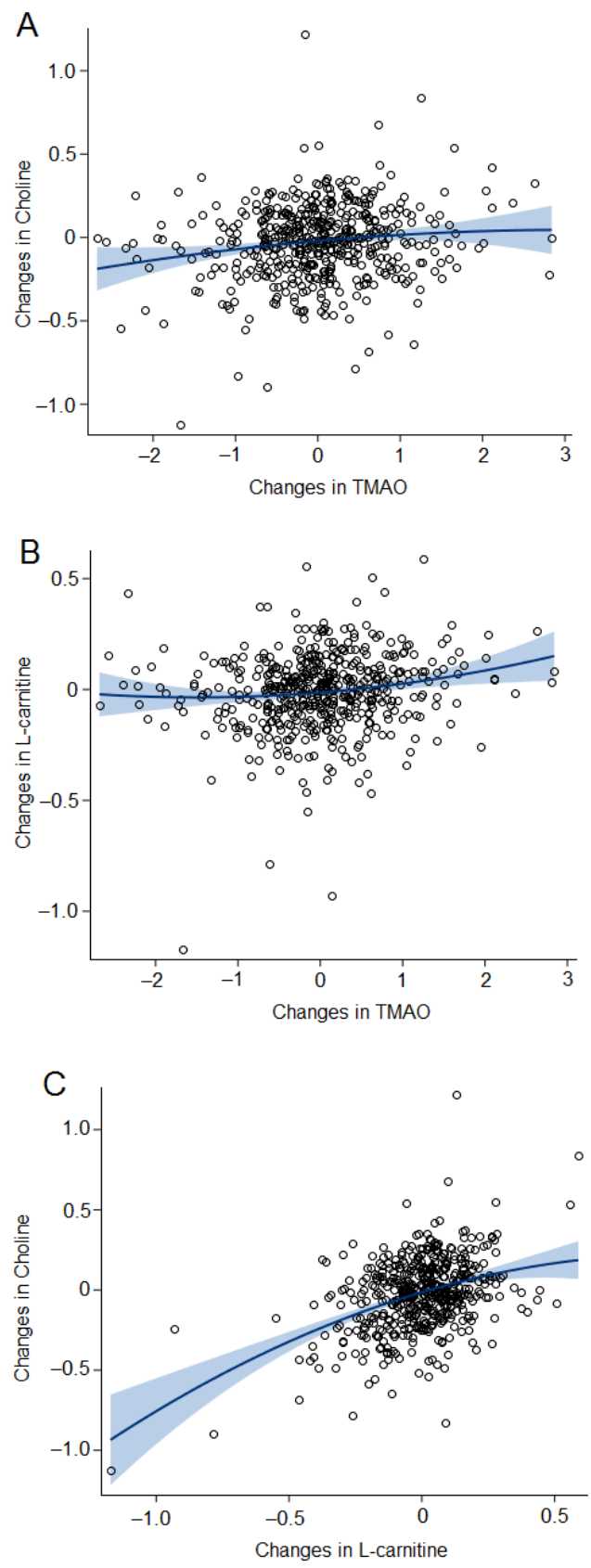
β (SE) represents effect for outcomes by 1 log-transformed decrease in TMAO from baseline to 6 months.

* $P_{interaction}$ between changes in TMAO and low- or high-fat diet for the outcomes.

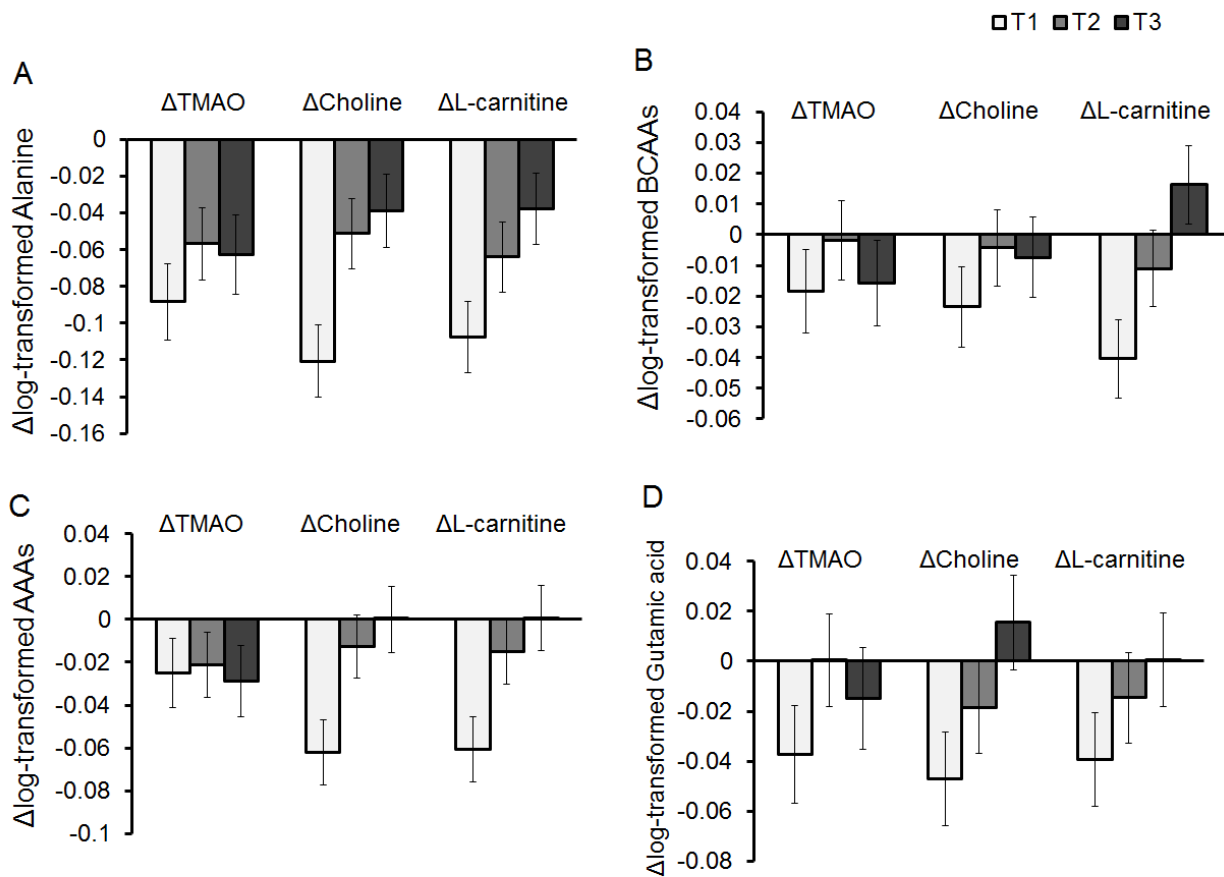
Model 1: data after adjusted for age, sex, ethnicity, parental history of diabetes, BMI at baseline, TMAO at baseline, and respective phenotype at the baseline.

Model 2: data after adjusted for covariates in model 1 + 6-month changes in body weight, 6-month changes in alanine, branched-chain amino acids, aromatic amino acids, and glutamic acid.

Supplemental Figure 1: Correlations among changes in trimethylamine N-oxide (TMAO), choline, and L-carnitine.



Supplemental Figure 2: Changes (Δ) in serum amino acids at 6 months according to tertile (T) categories of changes in trimethylamine N-oxide (TMAO), choline, and L-carnitine



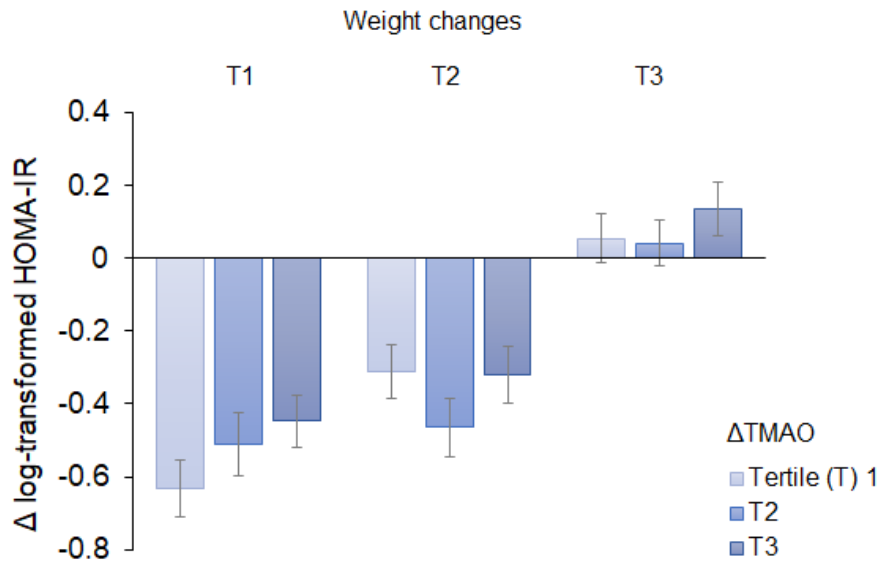
Branched-chain amino acids, BCAAs; aromatic amino acids, AAAs. Data are means \pm SE values after adjusted for age, sex, ethnicity, parental history of diabetes, body mass index, baseline value for the respective amino acids at the baseline examination, and baseline value of either TMAO, choline, or L-carnitine.

For Δ TMAO, median (25th, 75th) values were T1 (white): -2.0 ($-3.5, -1.2$) μM , T2 (gray): 0 ($-0.3, 0.3$) μM , and T3 (black): 1.9 ($1.3, 4.0$) μM , respectively.

For Δ Choline, median (25th, 75th) values were T1: -1.9 ($-2.9, -1.4$) μM ; T2: -0.2 ($-0.5, 0.2$) μM ; T3: 1.6 ($1.0, 2.1$) μM , respectively.

For Δ L-carnitine, median (25th, 75th) values were T1: -5.0 ($-7.5, -3.4$) μM , T2: 0.2 ($-0.9, 1.1$) μM , and T3: 4.6 ($3.2, 6.7$) μM , respectively.

Supplemental Figure 3: Changes (Δ) in log-transformed homeostasis model assessment-of-insulin resistance (HOMA-IR) according to a combination of changes in TMAO and changes in body weight



Data are means \pm SE values after adjusted for age, sex, ethnicity, parental history of diabetes.

For Δ TMAO, median (25th, 75th) values were T1: -2.0 ($-3.5, -1.2$) μM , T2: 0 ($-0.3, 0.3$) μM , and T3: 1.9 ($1.3, 4.0$) μM , respectively. For weight changes, mean (SD) values were T1: -12.8 (4) kg, T2: -6.0 (1.2) kg, and T3: -0.9 (2.2) kg, respectively.