SUPPLEMENTARY DATA

Supplementary Table S1. Characteristics of total study participants at the baseline examination

Variables	N	Baseline value
Trimethylamine N-oxide (TMAO), μM	510	3.6 (3.3)
median (25th, 75th) μM		2.7 (1.8, 3.8)
Choline, μM	510	9.0 (2.1)
median (25th, 75th) μM		8.6 (7.4, 10.3)
L-carnitine, μM	510	34.9 (7.1)
median (25th, 75th) μM		34.5 (30.0, 39.3)
Age, y	510	51.5 (9.0)
Female	510	324 [63.5%]
White	510	413 [81.0%]
Parental history of diabetes	510	144 [28.2%]
Current smoker	510	13 [2.6%]
Educational level	510	
High school or less	-	51 [10.0%]
Some college	-	104 [20.4%]
College graduate or beyond	-	355 [69.6%]
Marital status	510	
Married	-	366 [71.8%]
Divorced or separated	-	76 [14.9%]
Widowed	-	10 [2.0%]
Never married	-	58 [11.4%]
Diet group	510	
High-protein diet	-	238 [46.7%]
Low-fat diet	-	268 [52.5%]
Body mass index, kg/m^2	510	32.6 (3.8)
Body weight, kg	510	92.6 (15.3)
Waist circumference, cm	510	103 (13)
Resting energy expenditure	509	1543 (295)
Body composition		
Trunk fat %	264	37.9 (5.8)
Whole body total fat mass %	264	37.1 (6.8)
Body fat distribution		
Deep subcutaneous adipose tissue mass	121	5.6 (1.5)
Superficial adipose tissue mass	104	10.7 (2.4)
Visceral adipose tissue mass	121	5.4 (2.5)
Total adipose tissue mass	104	16.1 (3.8)
Fasting glucose, mmol/L	510	5.1 (0.7)
HbA1c, %	509	5.4 (0.4)
HbA1c, mmol/mol	509	36 (5)
Hyperglycemia*, yes	510	159 [31.2%]
Fasting insulin, pmol/L	507	73.6 (48.6, 108.3)
HOMA-IR	507	2.9 (2.0)
Dietary intake per day		
Energy, kcal	269	1998 (591)
Carbohydrate, %**	269	44.9 (7.6)
Fat, %**	269	36.8 (5.9)
Protein, %**	269	18.1 (3.2)

N, number of study participants. Data are mean (SD), median (25th, 75th), or N [% of the number of study participants]. *Hyperglycemia was indicated by either fasting glucose ≥5.6 mmol/L or HbA1c ≥5.7%.
** % of total energy intake.

SUPPLEMENTARY DATA

Supplementary Table S2. Changes in trimethylamine N-oxide (TMAO), choline and L-carnitine across different diets

	ΔTMAO		ΔCholine		ΔL-carnitine	
	Mean (SD)	Median (25th, 75th)	Mean (SD)	Median (25th, 75th)	Mean (SD)	Median (25th, 75th)
1) Low-fat diet group	−0.1 (4.4)	0.1 (−1.2, 1.5)	−0.2 (1.9)	−0.1 (−1.2, 1.1)	0 (5.9)	0.5 (−3.2, 3.7)
High-fat diet group	−0.1 (4.2)	−0.2 (−1.2, 0.9)	−0.2 (2.4)	−0.2 (−1.5, 0.8)	−0.2 (5.8)	0 (−3.5, 3.0)
2) Low-protein diet group	−0.2 (4.1)	0 (−1.2, 1.0)	−0.3 (2.0)	−0.2 (−1.4, 1.0)	−0.2 (6.0)	0.2 (−3.8, 3.3)
High-protein diet group	0 (4.6)	0 (−1.1, 1.5)	−0.1 (2.4)	−0.1 (−1.3, 1.1)	−0.1 (5.6)	0.3 (−2.9, 3.2)
3) Lowest-carbohydrate diet group	0 (4.6)	−0.2 (−1.1, 0.9)	0.1 (2.9)	−0.1 (−1.5, 1.0)	0 (5.3)	−0.1 (−3.2, 3.3)
Highest-carbohydrate diet group	−0.2 (4.3)	0.1 (−1.4, 1.3)	−0.2 (2.1)	0 (−1.4, 1.1)	0.1 (5.9)	0.2 (−3.5, 4.0)

P >0.05 for mean values using t test

P >0.05 for median values using median test

Supplementary Table S3. Nutrient intake and biomarkers of adherence according to tertile categories of changes (Δ) in trimethylamine N-oxide (TMAO), choline and L-carnitine

	At baseline						At 6 months					
	Energy, kcal	Carbohydrate, %	Fat, %	Protein, %	Respiratory quotient	Urinary nitrogen (mg/day)	Energy, kcal	Carbohydrate, %	Fat, %	Protein, %	Respiratory quotient	Urinary nitrogen (mg/day)
ΔTMAO												
Tertile (T) 1	1943 (589)	44.7 (7.3)	36.3 (5.4)	18.4 (3.3)	0.84 (0.04)	12.2 (4.0)	1583 (461)	49.9 (10.7)	31.4 (8.0)	19.9 (4.4)	0.84 (0.04)	11.0 (4.2)
T2	2008 (591)	45.7 (7.6)	36.3 (5.6)	18.0 (3.2)	0.84 (0.04)	11.9 (4.4)	1597 (480)	52.0 (11.2)	28.8 (8.7)	19.6 (4.6)	0.84 (0.04)	11.1 (5.1)
T3	2039 (596)	44.4 (7.9)	37.7 (6.5)	17.9 (3.0)	0.84 (0.04)	12.0 (4.2)	1639 (469)	52.0 (9.3)	29.6 (8.3)	19.5 (3.8)	0.84 (0.04)	11.7 (4.3)
P-trend	0.15	0.69	0.09	0.28	0.07	0.71	0.52	0.22	0.23	0.51	0.87	0.08
ΔCholine												
T1	1935 (604)	44.9 (7.4)	37.0 (5.9)	18.3 (3.3)	0.84 (0.04)	11.7 (4.1)	1584 (475)	50.5 (10.3)	31.3 (8.7)	19.8 (4.2)	0.84 (0.05)	10.9 (4.5)
T2	2015 (552)	44.5 (7.2)	37.3 (6.0)	17.9 (2.8)	0.84 (0.04)	12.2 (4.4)	1598 (496)	52.1 (11.1)	29.1 (8.9)	19.8 (4.8)	0.84 (0.04)	11.1 (4.4)
T3	2052 (612)	45.3 (8.1)	36.3 (5.9)	18.0 (3.4)	0.84 (0.04)	12.2 (4.1)	1642 (439)	51.6 (9.9)	29.1 (7.3)	19.3 (3.8)	0.83 (0.04)	12.0 (4.7)
P-trend	0.28	0.52	0.38	0.34	0.88	0.14	0.58	0.48	0.08	0.43	0.27	0.02

ΔL-carnitine												
T1	2005 (636)	44.2 (7.3)	37.4 (5.7)	18.2 (3.2)	0.84 (0.05)	12.1 (4.6)	1565 (431)	50.7 (11.0)	30.5 (8.7)	19.5 (4.5)	0.84 (0.05)	10.9 (4.1)
T2	1991 (535)	44.9 (7.3)	36.5 (6.0)	18.4 (3.3)	0.84 (0.04)	12.1 (4.0)	1548 (447)	51.5 (10.7)	29.7 (8.4)	20.0 (4.0)	0.84 (0.04)	11.1 (4.4)
T3	1996 (604)	45.7 (8.1)	36.5 (6.1)	17.7 (2.9)	0.84 (0.04)	11.9 (4.0)	1713 (517)	51.9 (9.3)	29.5 (8.0)	19.6 (4.2)	0.84 (0.04)	12.0 (5.0)
P-trend	0.97	0.25	0.38	0.31	0.39	>0.99	0.01	0.45	0.44	0.85	0.82	0.007

Data are mean (SD) values. P-trend values were adjusted for age, sex, and ethnicity.

Supplementary Table S4. Changes (Δ) in obesity measurements and energy expenditure at 6 months per 1 log-transformed decreases in trimethylamine N-oxide (TMAO), choline and L-carnitine levels after further adjusted for fasting glucose levels at baseline and parental history of diabetes

	ΔTMAO		ΔCholine		ΔL-carnitine	
Outcomes	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*
ΔBody weight	0.56 (0.41)	0.18	–7.67 (1.12)	<0.0001	–5.47 (1.45)	<0.001
ΔWaist circumference	0.73 (0.44)	0.1	–6.53 (1.22)	<0.0001	–4.56 (1.55)	0.003
ΔWhole body total fat mass %	0.80 (0.31)	0.01	–2.73 (0.85)	0.002	–1.77 (0.98)	0.07
ΔTrunk fat %	1.11 (0.42)	0.01	–3.66 (1.13)	0.001	–2.51 (1.32)	0.06
ΔDeep subcutaneous adipose tissue mass, DSAT	0.25 (0.15)	0.09	–1.04 (0.39)	0.01	–0.82 (0.43)	0.06
ΔSuperficial adipose tissue mass, SAT	0.57 (0.29)	0.06	–1.95 (0.76)	0.01	–0.75 (0.77)	0.34
ΔVisceral adipose tissue mass, VAT	0.22 (0.16)	0.17	–0.98 (0.42)	0.02	–0.70 (0.46)	0.14
ΔTotal adipose tissue mass, TAT	0.96 (0.46)	0.04	–3.28 (1.20)	0.008	–1.54 (1.20)	0.2
ΔResting energy expenditure	–13.8 (10)	0.17	–71.3 (28)	0.01	–35.7 (35.5)	0.32

β (SE) represents changes in the outcomes when the circulating metabolite levels were decreased during the diet intervention.
Data after adjusted for age, sex, diet group, BMI, fasting glucose, parental history of diabetes, value for the respective outcome traits at the baseline examination (except for the outcome Δbody weight), and either TMAO, choline, or L-carnitine at baseline.

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Supplementary Table S5. Changes (Δ) in obesity measurements and energy expenditure at 6 months per 1 log-transformed decreases in trimethylamine N-oxide (TMAO), choline and L-carnitine levels according to the baseline diabetes risk status*

	Normoglycemia*							**Hyperglycemia***								
	N†	ΔTMAO		ΔCholine		ΔL-carnitine		N†	ΔTMAO		ΔCholine		ΔL-carnitine		$P_{interaction(\Delta TMAO*hyperglycemia)‡}$	$P_{interaction(\Delta Choline*hyperglycemia)‡}$	$P_{interaction(\Delta L\text{-}carnitine*hyperglycemia)‡}$
Outcomes		β (SE)	*P*	β (SE)	*P*	β (SE)	*P*		β (SE)	*P*	β (SE)	*P*	β (SE)	*P*			
ΔBody weight	351	0.37 (0.5)	0.46	–8.21 (1.43)	<.0001	–7 (1.82)	0.0001	159	1.15 (0.77)	0.14	–6.75 (1.86)	0.0004	–2.47 (2.41)	0.31	0.6	0.53	0.3
ΔWaist circumference	350	0.69 (0.52)	0.19	–8.25 (1.53)	<.0001	–6.73 (1.94)	0.0006	159	0.95 (0.8)	0.24	–3.58 (2.05)	0.08	–0.33 (2.56)	0.9	0.61	0.06	0.18
ΔWhole body total fat mass %	171	0.6 (0.37)	0.11	–3.51 (1.1)	0.002	–2.54 (1.28)	0.049	62	1.34 (0.53)	0.01	–1.93 (1.33)	0.15	–0.54 (1.47)	0.71	0.98	0.22	0.45
ΔTrunk fat %	171	0.77 (0.5)	0.13	–4.43 (1.46)	0.003	–3.32 (1.72)	0.06	62	2.09 (0.71)	0.005	–3.11 (1.77)	0.08	–1.14 (2.01)	0.57	0.73	0.2	0.45
ΔDeep subcutaneous adipose tissue mass, DSAT	73	0.16 (0.19)	0.4	–1.4 (0.52)	0.01	–0.88 (0.62)	0.17	31	0.24 (0.26)	0.37	–1.29 (0.6)	0.04	–0.33 (0.63)	0.6	0.39	0.16	0.59
ΔSuperficial adipose tissue mass, SAT	57	0.55 (0.3)	0.07	–1.95 (1.15)	0.1	–0.33 (1.16)	0.78	26	0.36 (0.66)	0.59	–2.34 (1.15)	0.06	–0.81 (1.19)	0.5	0.88	0.92	0.59
ΔVisceral adipose tissue mass, VAT	73	0.26 (0.2)	0.19	–0.9 (0.58)	0.13	–0.38 (0.67)	0.57	32	0.17 (0.3)	0.59	–1.82 (0.69)	0.01	–0.6 (0.73)	0.42	0.65	0.96	0.76
ΔTotal adipose tissue mass, TAT	57	1.06 (0.49)	0.04	–2.78 (1.96)	0.16	–0.69 (1.91)	0.72	26	0.61 (1.05)	0.57	–4.21 (1.73)	0.03	–1.4 (1.88)	0.47	0.64	0.98	0.61
ΔResting energy expenditure	331	–12.5 (11.7)	0.29	–67 (34.9)	0.06	–81.4 (43.4)	0.06	146	–6.4 (20)	0.75	–81.5 (49.1)	0.1	48.0 (62.7)	0.44	0.87	0.83	0.18

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*The baseline diabetes risk status was indicated by the presence of hyperglycemia (hyperglycemia: impaired fasting glucose ≥5.5 mmol/L or elevated HbA1c levels ≥5.7%) at the baseline examination.
† N, Number of participants eligible for the analysis
‡ *P* values for interactions between changes in each metabolite (ΔTMAO, ΔCholine, or ΔL-carnitine) and the presence of hyperglycemia for the outcomes

Supplementary Table S6. Long-term changes in obesity measurements based on per 1 log-transformed initial decreases in trimethylamine N-oxide (TMAO), choline and L-carnitine concentrations

	ΔTMAO		ΔCholine		ΔL-carnitine	
Outcomes	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*
At 12 months						
ΔBody weight	0.83 (0.54)	0.13	−9.35 (1.45)	<.0001	−6.64 (1.88)	0.0004
ΔWaist circumference	1.41 (0.57)	0.01	−7.66 (1.57)	<.0001	−5.12 (1.99)	0.01
At 18 months						
ΔBody weight	0.66 (0.57)	0.24	−9.38 (1.55)	<.0001	−6.95 (1.99)	0.0005
ΔWaist circumference	1.00 (0.61)	0.1	−9.11 (1.67)	<.0001	−6.57 (2.13)	0.002
At 24 months						
ΔBody weight	1.00 (0.55)	0.07	−7.14 (1.53)	<.0001	−4.41 (1.94)	0.02
ΔWaist circumference	1.21 (0.57)	0.04	−5.60 (1.62)	0.0006	−3.22 (2.04)	0.11
ΔWhole body total fat mass %	0.24 (0.42)	0.57	−3.49 (1.04)	0.001	−1.88 (1.3)	0.15
ΔTrunk fat %	0.39 (0.54)	0.48	−4.84 (1.32)	0.0003	−2.89 (1.66)	0.08
ΔDeep subcutaneous adipose tissue mass, DSAT	0.09 (0.22)	0.67	−0.55 (0.51)	0.28	−0.73 (0.51)	0.16
ΔSuperficial adipose tissue mass, SAT	0.16 (0.42)	0.7	−1.06 (0.84)	0.21	−0.91 (0.82)	0.27
ΔVisceral adipose tissue mass, VAT	0.28 (0.23)	0.22	−1.12 (0.55)	0.046	−0.40 (0.56)	0.48
ΔTotal adipose tissue mass, TAT	0.53 (0.66)	0.42	−1.90 (1.33)	0.16	−1.40 (1.3)	0.29

β (SE) represents changes in the outcomes when the circulating metabolite levels were decreased during the intervention.
Data after adjusted for age, sex, diet group, BMI, value for the respective outcome traits at the baseline examination (except for the outcome Δbody weight), and either TMAO, choline or L-carnitine at baseline.