

Supplementary Materials for

**The choline metabolite TMAO inhibits NETosis and promotes placental
development in GDM of humans and mice**

Xiaojing Lin, Yunqi Zhang, Xiaoling He, Yan Chen, Nan Chen, Juncheng Liu, Miaoran Wang,
Yue Li, Hong Yang, Lei Fan, Hongbin Ni, Yi Hou, Jibin Li, Chaodong Wu, Hongbo Qi, Hua
Zhang, Xiaoqiu Xiao

Corresponding author: Xiaoqiu Xiao, 203679@cqu.edu.cn

Supplementary Table 1. Characteristics of case-control studies included in the meta-analysis

Author	Year	Location	Cases	Controls	Exposure details	$\bar{x} \pm s$ (μM)		Quality
						Case	Control	
Lever et al	2014	New Zealand	79	396	TMAO Betaine	8 ± 5.8 44.3 ± 13.0	5.6 ± 4.5 47.0 ± 16.9	8
Mueller et al	2015	Austria	137	35	TMAO Choline Betaine	2.9 ± 3.4 11.0 ± 2.8 39.8 ± 15.8	1.1 ± 1.1 10.1 ± 2.9 39.2 ± 11.8	7
Dullaart et al	2016	Netherlands	65	55	Betaine	24.5 ± 10.3	26.7 ± 9.0	6
Svingen et al	2016	Norway	446 449 449	3610 3621 3621	TMAO Choline Betaine	7.9 ± 5.9 10.2 ± 2.8 36.3 ± 12.4	6.2 ± 4.2 9.7 ± 2.4 40 ± 11.6	6
Obeid et al	2016	Germany	98	185	TMAO Choline Betaine	8.6 ± 12.2 10.9 ± 3.0 35.6 ± 12.7	5.4 ± 5.2 9.9 ± 3.9 36.7 ± 11.2	8
Shan et al	2017	China	1346	1348	TMAO	1.9 ± 1.3	1.5 ± 1.0	8
Sanchez-Alcoholado et al	2017	Spain	16	16	TMAO	0.4 ± 0.1	0.2 ± 0.1	7
Tang et al	2017	The United States	1216	300	TMAO Choline Betaine	5.0 ± 3.6 10.8 ± 3.8 38.5 ± 13.4	3.8 ± 2.6 8.3 ± 2.0 39.8 ± 141	8
Schugar et al	2017	The United States	102	333	TMAO Choline Betaine	5.3 ± 3.3 8.0 ± 2.4 35.4 ± 13.5	3.5 ± 2.1 7.3 ± 2 41.0 ± 15.9	6
Dong et al	2018	China	175	243	TMAO	7.6 ± 1.0	3.1 ± 0.1	7
Li et al	2018	China	433	433	TMAO	1.2 ± 0.6	1.1 ± 0.5	8
Li et al	2018	China	276	552	TMAO	1 ± 0.6	0.8 ± 0.5	8
Barzilay et al	2018	Canada	16 16 18	278 278 296	TMAO Choline Betaine	3.2 ± 5.0 10.6 ± 1.9 12.1 ± 2.4	2.4 ± 3.2 10.7 ± 3.2 13.4 ± 4.1	7

Table Legend: The quality of the included studies was evaluated using the Newcastle-Ottawa Scale (Full score is 9), and this scale consists 3 parts: selection, comparability and exposure. TMAO, trimethylamine N-oxide; $\bar{x} \pm s$, mean \pm standard deviation; μM , $\mu mol/L$.

Supplementary Table 2. Characteristics among women with GDM and their age-matched controls at 24th-28th week of gestation

Characteristics	GDM (n=20)	Non-GDM (n=20)	P
Age, y	31.90±3.95	30.70±3.56	0.319
Prepregnancy BMI, kg/m ²	24.43±3.87	20.93±3.32	0.005**
SBP, mmHg	113.07±19.85	108.75±8.82	0.435
DBP, mmHg	68.87±15.11	66.19±10.36	0.567
OGTT, mmol/L			
FBG	5.38±0.43	4.34±0.16	0.000***
1h	10.69±1.68	7.14±1.31	0.000***
2h	10.02±1.43	6.57±0.88	0.000***

Table Legend: Values represents mean ± SD. GDM, gestational diabetes mellitus; BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; OGTT, oral glucose tolerance test.

Supplementary Table 3. Characteristics among women with GDM and their matched controls at delivery

Characteristics	GDM (n=16)	Non-GDM (n=17)	P
Age, y	31.31±2.82	29.59±3.06	0.103
Gestational week at blood sample collection, wk	38.44±1.50	39.12±0.60	0.108
Prenatal BMI, kg/m ²	30.23±4.38	26.08±2.29	0.005**
SBP, mmHg	119.75±12.40	110.65±9.70	0.025*
DBP, mmHg	76.81±10.94	71.65±10.64	0.179
Placenta thickness, mm	42.13±3.98	39.24±2.31	0.136
Newborn weight, g	3614.06±459.17	3298.24±319.72	0.028*

Table Legend: Values represents mean ± SD. GDM, gestational diabetes mellitus; BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Supplementary Table 4. Analysis for the correlation between TMAO and placental thickness

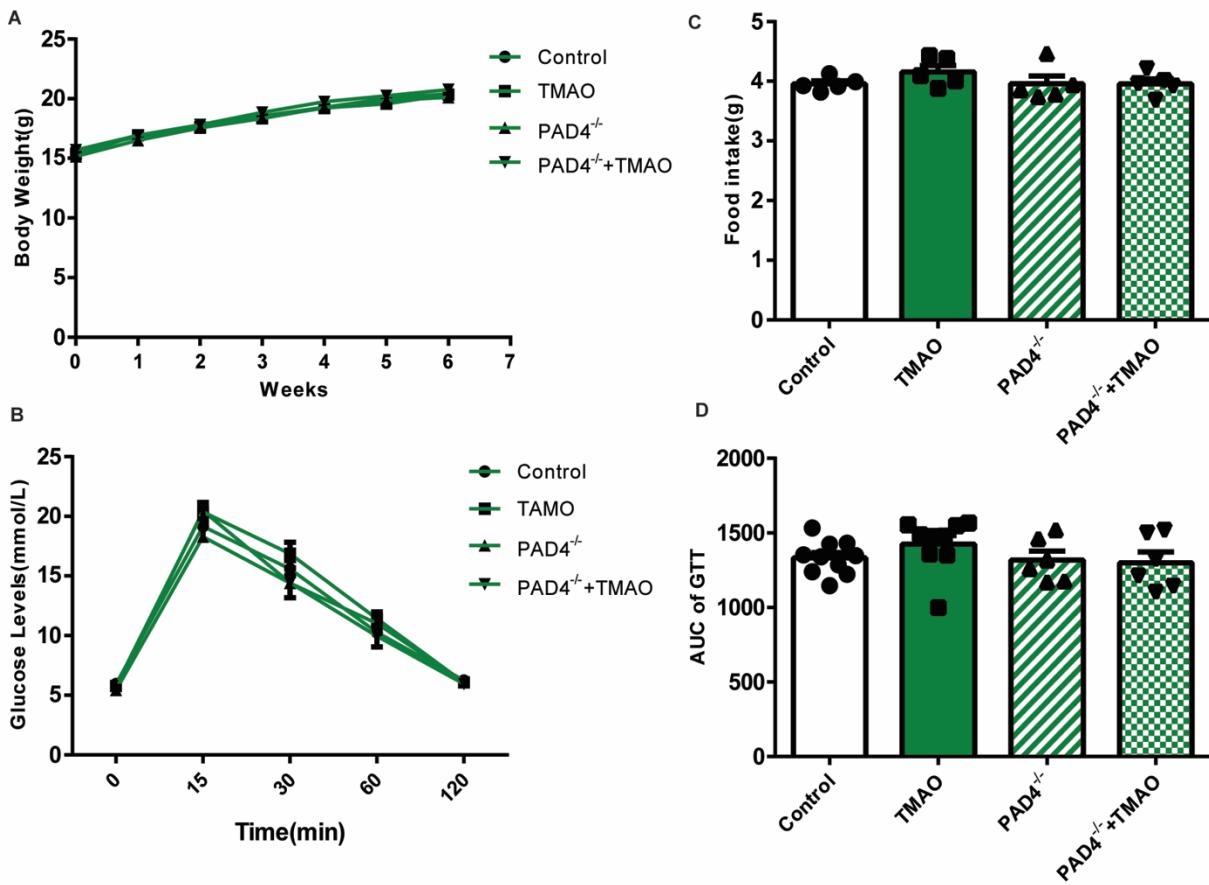
Variable entered	Standard β	P
TMAO in peripheral blood	0.429	0.026*
TMAO in cord blood	0.631	0.083

Table Legend: Multiple linear regression analysis, adjusted for Body mass index, age, fasting blood glucose, 1-hour postprandial blood glucose, 2-hour postprandial blood glucose.

Supplementary Table 5. Analysis for the correlation between TMAO and newborn weight

Variable entered	Standard β	P
TMAO in peripheral blood	0.348	0.047*
TMAO in cord blood	0.626	0.017*

Table Legend: Multiple linear regression analysis, adjusted for Body mass index, age, fasting blood glucose, 1-hour postprandial blood glucose, 2-hour postprandial blood glucose.



Supplementary Figure S1. The effects of TMAO and NETs on prepregnancy metabolism in wild-type and PAD4^{-/-} mice. (A) Body weight of four groups of mice. (B) Food intake of the four groups of mice. (C) Blood glucose levels of four groups of mice. (D) AUC of GTT of four groups of mice. (n≥3/group)