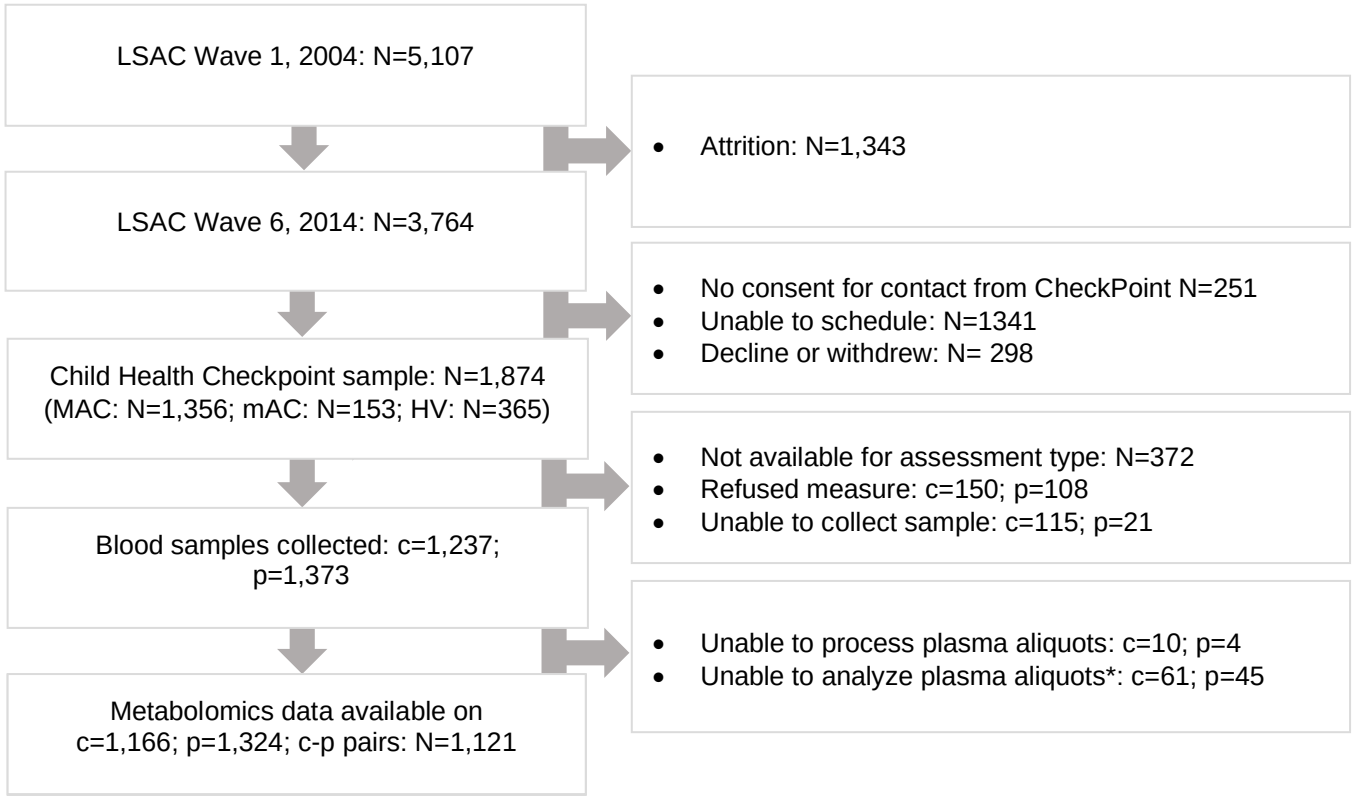
Plasma Trimethylamine N-Oxide (TMAO) and its precursors: Population epidemiology, parent-child concordance, and associations with reported dietary intake in 11-12-year-old children and their parents – Stephanie Andraos - Online Supporting Material



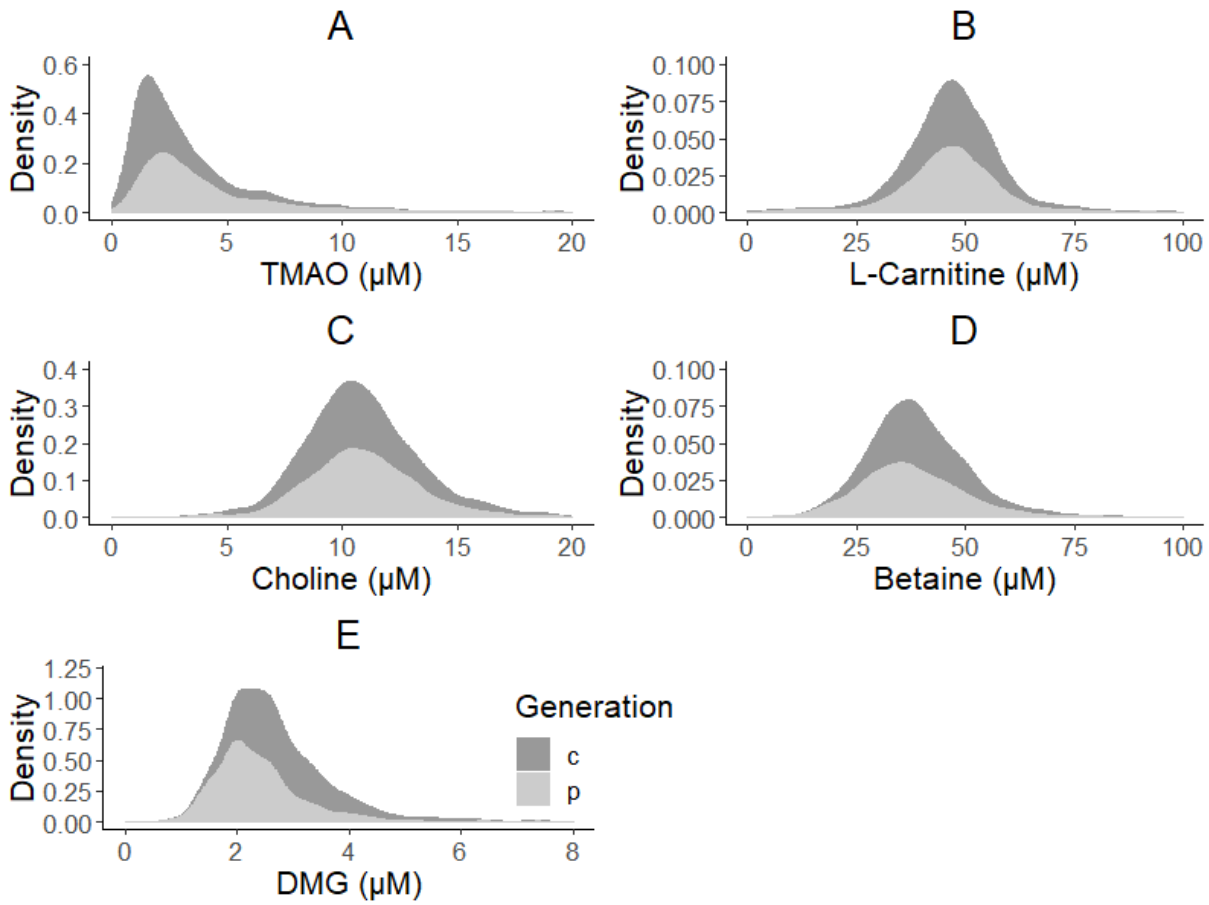
Supplemental Figure 1: Participant flow chart.

*Unable to analyse due to insufficient volume or poor-quality sample. HV: home visit; LSAC: Longitudinal Study of Australian Children; MAC: main assessment centre; mAC: mini assessment centre; N: number of families; p: number of attending adults; c: number of attending children.

Supplemental Table 1: Calibration curve composition for TMAO assay			
Standard	**Solvent**	**Target Concentration (µM)**	**Volume of standard to create the most concentrated calibration standard *(µL)**
Pyridoxal 5'-phosphate monohydrate	HCl 0.1 mol/L	2000	75
Nicotinic Acid	HCl 0.1 mol/L	4500	33.3
4-Pyridoxic Acid	HCl 0.1 mol/L	2000	75
Nicotinamide	HCl 0.1 mol/L	10000	60
FMN Riboflavin 5'-Phosphate	H_2O, Ac.ac 0.12%	1000	60
Pyridoxal	HCl 0.1 mol/L	3000	30
Pyridoxine	HCl 0.1 mol/L	2500	24
Pyridoxamine	HCl 0.1 mol/L	2300	26.1
Riboflavin	H_2O, Ac.ac 0.12%	400	75 (Dilute stock 1:2 first)
Thiamine	HCl 0.1 mol/L	1700	35.3
Biotin	EtOH/water 1:1	2000	30 (Dilute stock 1 :10 first)
Pantothenic Acid	H_2O	4500	133.3
Nicotinuric Acid	HCl 0.1 mol/L	2800	53.6
Folic acid	5% NaOH 0.1mol/L, 20% EtOH in H_2O	1000	150
Trimethylamine N-oxide dihydrate	H_2O	10000	300

The most concentrated standard undergoes a serial dilution to create a calibration curve with a large dynamic range of concentrations

Supplemental Table 2: Internal standard solution composition for TMAO assay			
Internal standard	**Solvent**	**Target Concentration (µM)**	**Volume of internal standard**
Nicotinamide-2, 4, 5, 6-d4	HCl 0.1 mol/L	4000	12.5
Pyridoxine-d2 HCl (5-Hydroxymethyl-d2)	HCl 0.1 mol/L	2500	12.5
Biotin d4	EtOH/water 1:1	2000	12.5
Thiamine hydrochloride $^{13}C3$	HCl 0.1 mol/L	1500	33.3
Riboflavin $^{13}C4$	H_2O, Ac.ac 0.12%	600	41.7
Pyridoxamine d3	HCl 0.1 mol/L	2000	12.5
Pantothenic acid $^{13}C6$ $^{15}N2$	H_2O	1000	100
Folic acid $^{13}C5$	5% NaOH 0.1mol/L, 20% EtOH in H_2O	500	50
Nicotinic acid d4	HCl 0.1 mol/L	2000	12.5
Nicotinuric acid d4	HCl 0.1 mol/L	2000	12.5
Trimethylamine N-oxide $^{13}C3$	H_2O	2000	250



Supplemental Figure 2 : Density plots of TMAO (A), Carnitine (B), Choline (C), Betaine (D), and DMG (E) in children “c” and parents “p”

Supplemental Table 3: Linear models of age-specific differences in the parent subgroup			
Compound	**Estimate in linear model**	**Adjusted R_2 of linear model**	**p value**
TMAO*	0.08	0.002	0.05
Carnitine	0.07	-0.0002	0.40
DMG*	0.003	0.001	0.10
Betaine	0.20	0.006	0.002
Choline	0.02	0.0003	0.23
Log-transformed variable			

Supplemental Table 4: Mean ± SDs of TMAO and precursors' concentrations by menstruation from T-test analysis			
Generation	**Menstruating on blood collection day?**		**p value**
	no	**yes**	
Children			
TMAO*	1.93 ± 2.13	1.67 ± 2.12	0.44
DMG*	2.39 ± 1.26	2.34 ±1.38	0.80
Betaine	37.54 ± 8.34	38.69 ± 9.42	0.62
Choline	10.48 ± 2.33	10.40 ± 2.54	0.89
Carnitine	44.89 ± 12.00	44.00 ± 10.60	0.74
Adults			
TMAO*	3.36 ± 2.19	3.27 ± 2.09	0.70
DMG*	2.16 ± 1.43	2.25 ± 1.40	0.16
Betaine	36.02 ± 11.08	37.82 ± 11.30	0.05
Choline	10.82 ± 2.62	10.96 ± 2.59	0.52
Carnitine	46.26 ± 15.60	48.90 ± 16.20	0.05
***Values back transformed from log transformed calculations**			

Supplemental Table 5: Correlation matrix and p values of TMAO and its precursors					
	TMAO	**DMG**	**Betaine**	**Choline**	**Carnitine**
TMAO* **(p value)**	-	-0.004 (0.86)	0.02 (0.64)	0.08 (<0.001)	0.05 (0.02)
DMG* **(p value)**	-0.004 (0.86)	-	0.45 (<0.0001)	0.37 (<0.0001)	0.11 (<0.0001)
Betaine **(p value)**	0.02 (0.64)	0.45 (<0.0001)	-	0.30 (<0.0001)	0.11 (<0.0001)
Choline **(p value)**	0.08 (<0.001)	0.37 (<0.0001)	0.30 (<0.0001)	-	0.44 (<0.0001)
Carnitine **(p value)**	0.05 (0.02)	0.11 (<0.0001)	0.11 (<0.0001)	0.44 (<0.0001)	-
***Log transformed variable**					

Supplemental Table 6: ANOVA results (estimates and p values) of reported intakes of animal protein sources and TMAO and precursors' plasma concentrations in children and adults					
Children					
	TMAO* Estimate (p value)	**L-Carnitine Estimate (p value)**	**Choline Estimate (p value)**	**Betaine Estimate (p value)**	**DMG* Estimate (p value)**
Red Meat (*e.g.* beef, lamb)	0.06 (0.01)	0.01 (0.97)	0.01 (0.88)	-0.11 (0.72)	-0.004 (0.67)
Meat products (*e.g.* sausages, chicken nuggets, bacon)	0.01 (0.49)	0.75 (0.06)	-0.02 (0.78)	0.24 (0.40)	-0.003 (0.69)
Chicken	0.02 (0.45)	1.21 (0.02)	0.05 (0.57)	-0.12 (0.75)	0.01 (0.20)
Fish	0.11 (<0.0001)	-0.11 (0.84)	-0.05 (0.56)	0.30 (0.44)	0.003 (0.79)
Fast-food meals and snacks (*e.g.* Pizzas, Burgers, Chips)	-0.08 (0.02)	1.42 (0.03)	0.04 (0.73)	0.002 (0.99)	-0.01 (0.44)
Dairy products (*e.g.* Yogurts, puddings)	0.003 (0.89)	0.01 (0.97)	-0.09 (0.12)	-0.04 (0.88)	-0.007 (0.32)
Cheese	0.01 (0.61)	0.08 (0.81)	-0.05 (0.40)	-0.21 (0.37)	0.004 (0.55)
Adults					
Red Meat (*e.g.* beef, lamb)	0.13 (<0.0001)	1.07 (0.03)	0.09 (0.29)	-0.44 (0.24)	0.02 (0.12)
Meat products (*e.g.* sausages, chicken nuggets, bacon)	0.07 (0.001)	1.44 (0.002)	0.25 (0.001)	-0.18 (0.60)	0.02 (0.01)
Chicken	0.05 (0.04)	0.44 (0.43)	0.04 (0.66)	-0.09 (0.83)	0.01 (0.36)
Fish	0.09 (0.001)	0.73 (0.17)	-0.06 (0.49)	0.18 (0.65)	0.009 (0.46)
Fast-food meals and snacks (*e.g.* Pizzas, Burgers, Chips)	-0.05 (0.12)	0.94 (0.19)	0.26 (0.03)	-0.07 (0.89)	0.03 (0.05)
Dairy products (*e.g.* Yogurts, puddings)	0.02 (0.16)	-0.17 (0.61)	-0.05 (0.36)	-0.39 (0.11)	-0.01 (0.07)
Cheese	0.02 (0.31)	0.27 (0.47)	-0.01 (0.80)	-0.08 (0.78)	-0.01 (0.15)
Log-transformed variable					

Supplemental Table 7: Population distribution by frequency of habitual food intakes in children and adults						
Number of children per subgroup						
	Never	**Less than once a week**	**About 1-2 times a week**	**About 3-4 times a week**	**About 5-6 times a week**	**Everyday**
Red Meat (*e.g.* beef, lamb)	48	172	468	325	83	44
Meat products (*e.g.* sausages, chicken nuggets, bacon, including all steaks, chops, roasts, mince, stir fries and casseroles, excluding pork and chicken)	21	193	442	293	105	85
Chicken	27	238	595	236	38	10
Fish	185	538	349	57	9	2
Fast-food meals and snacks (*e.g.* Pizzas, Burgers, Chips)	128	693	285	37	1	2
Dairy products (*e.g.* Yogurts, puddings)	38	158	371	306	121	153
Cheese	86	204	360	273	113	113
Number of adults per subgroup						
Red Meat (*e.g.* beef, lamb)	58	64	527	568	81	18
Meat products (*e.g.* sausages, chicken nuggets, bacon)	104	501	502	171	32	10
Chicken	47	42	625	544	53	6
Fish	98	464	622	108	19	7
Fast-food meals and snacks (*e.g.* Pizzas, Burgers, Chips)	207	866	226	19	-	-
Dairy products (*e.g.* Yogurts, puddings)	84	354	376	286	108	113
Cheese	34	243	445	369	134	93