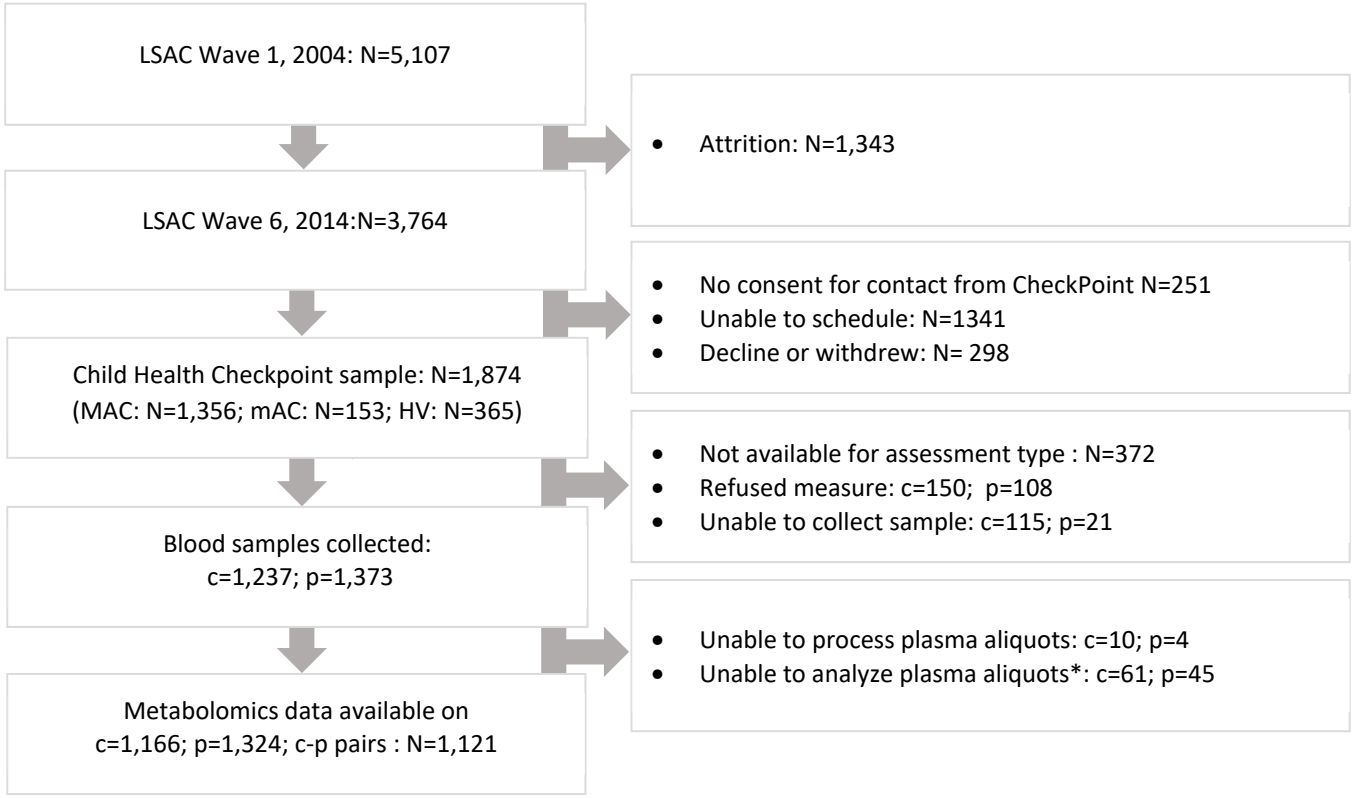
Supplementary material

Supplementary Table S1: List of materials	
Material	**Purchased from**
LC-grade Acetonitrile	LiChrosolv (Germany)
LC-grade acetic acid	EMSURE (Germany)
Heptafluorobutyric acid (HFBA)	Sigma Aldrich
Bovine serum albumin (BSA)	Sigma Aldrich
Phosphate buffer saline (PBS)	Sigma Aldrich
Ascorbic acid	Scharlau Chemie (Barcelona).
Impact protein precipitation 96-well plate	Phenomenex®
96-well collection plate (square 2ml/well)	Phenomenex®
Eppendorf tips (50, 300 and 1000µL)	Eppendorf

Supplementary Table S2: Internal standard solution composition			
Internal standard	**Solvent**	**Target Concentration (µM)**	**Volume of internal standard (µL)**
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 13C3	HcL 0.1 mol/L	1500	33.3
Riboflavin 13C4	H2O, Ac.ac 0.12%	600	41.7
Pyridoxamine d3	HcL 0.1 mol/L	2000	12.5
Pantothenic acid 13C6 15N2	H2O	1000	100
Folic acid 13C5	5%NaOH 0.1mol/L, 20%EtOH in water	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 13C3	H2O	2000	250

Supplementary Table S3: Calibration curve standard solution composition			
Standard	**Solvent**	**Target Con(µM)**	**Volume of most concentrate 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	H2O, 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	H2O, 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	H2O	4500	133.3
Nicotinuric Acid	HcL 0.1 mol/L	2800	53.6
Folic acid	5%NaOH 0.1mol/L, 20%EtOH in H20	1000	150
Trimethylamine N-oxide dihydrate	H2O	10000	300
**The most concentrated standard undergoes a serial dilution to create a calibration curve with a large dynamic range of concentrations*			

Supplementary Table S4: Stock solution water dilutions									
Standard	**S'1**	**S'2**	**S'3**	**S'4**	**S'5**	**S'6**	**S'7**	**S'8**	**S'9**
Volume of H2O µL	160	180	180	190	180	160	120	60	no prep
Volume of Stock µL	40 of S3	20 of S5	20 of S6	10 of S9	20 of S9	40 of S9	80 of S9	140 of S9	



Supplementary Figure S1: 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.

Supplementary Table S5: Linear model results of age-specific differences in B vitamer concentrations in the adult subgroup			
Vitamer*	**Estimate**	**Adjusted R^2 of linear model**	**p value**
Thiamine (B1)	0.01	0.002	0.07
Riboflavin (B2)	0.01	0.004	0.01
FMN (B2)	0.001	-0.0005	0.58
Nicotinamide (B3)	-0.002	-0.0003	0.42
Pantothenic acid (B5)	0.02	0.025	<0.0001
4-Pyridoxic acid (B6)	0.01	0.003	0.02
***Log transformed variables**			