

Supplementary Materials

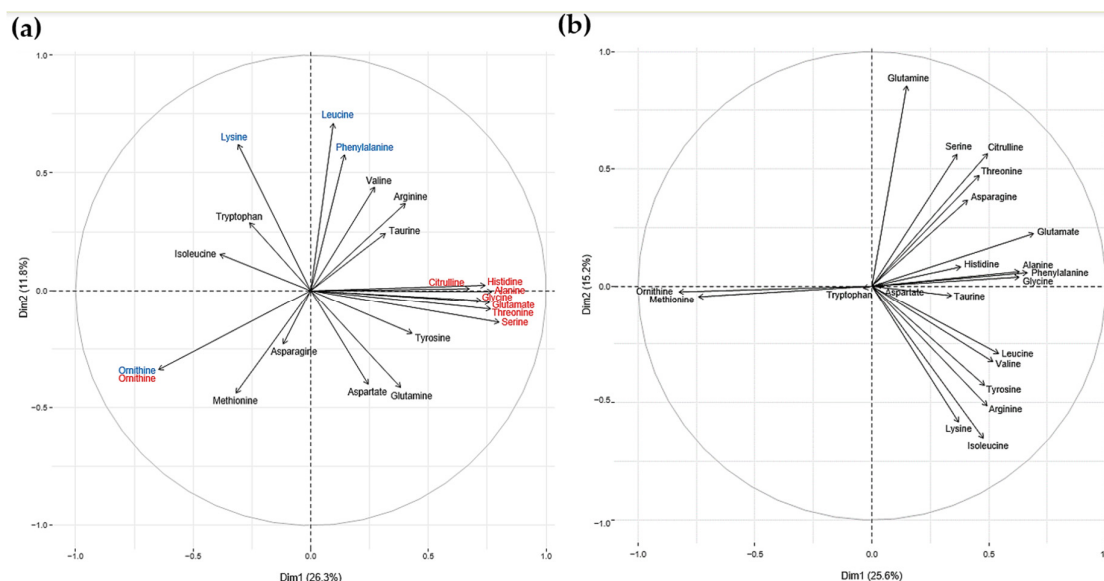


Figure S1. Compositional biplots from principal component analysis of free amino acid (FAA) levels in 6-week human milk (HM) (a) and 6-month HM (b). Potential patterns of FAAs as revealed by factor analyses are colored (red or blue).

Table S1. Free amino acid patterns in 6-week human milk obtained from factor analyses.

FAA	Factors loadings	
	Factor 1	Factor 2
EAAs		
Histidine	0.64	-
Isoleucine	-	-
Leucine	-	0.75
Lysine	-	0.51
Methionine	-	-
Phenylalanine	-	0.59
Threonine	0.76	-
Tryptophan	-	-
Valine	-	-
NEAAs/conditionally EAAs		
Alanine	0.66	-
Arginine	-	-
Asparagine	-	-
Aspartate	-	-

Glutamate	0.68	-
Glutamine	-	-
Glycine	0.66	-
Serine	0.83	-
Tyrosine	-	-
Non-coded AAs		
Citrulline	0.58	-
Ornithine	-0.54	-0.56
Taurine	-	-

FAA: free amino acid; EAA: essential amino acid; NEAA: non-essential amino acid; AA: amino acid; HM: human milk. Values are factor loadings from principal component analysis. Only loadings < -0.50 and > 0.50 are shown.

Table S2A. Levels of free amino acids in human milk for boys and girls.

FAA (μmol/L)	6 weeks Mean (SD)		6 months Mean (SD)		Analysis of differences at 6 weeks (<i>p</i> -value)	Analysis of differences at 6 months (<i>p</i> -value)
	HM for boys	HM for girls	HM for boys	HM for girls		
	(n = 354)	(n = 317)	(n = 232)	(n = 209)		
EAAs						
Histidine	28.4 (9.3)	28.1 (8.9)	29.6 (14.6)	29.9 (13.2)	0.661	0.397
Isoleucine	16.7 (10.5)	16.2 (10.1)	12.6 (6.4)	12.9 (5.3)	0.624	0.136
Leucine	33.7 (16.6)	34.2 (19.1)	34.0 (12.0)	35.8 (10.8)	0.930	0.038
Lysine	35.1 (25.2)	34.6 (22.8)	29.9 (16.3)	30.5 (13.6)	0.708	0.104
Methionine	27.7 (23.9)	28.0 (24.6)	33.8 (26.9)	35.5 (27.6)	0.875	0.588
Phenylalanine	21.7 (10.0)	21.0 (10.2)	16.5 (6.1)	16.4 (5.0)	0.283	0.829
Threonine	71.2 (29.7)	70.9 (29.0)	94.8 (38.0)	99.6 (36.3)	0.863	0.081
Tryptophan	14.3 (7.4)	14.9 (7.6)	28.6 (11.8)	29.5 (11.8)	0.381	0.227
Valine	110.4 (58.1)	109.0 (58.4)	65.4 (26.0)	68.1 (28.9)	0.710	0.277
NEAAs/conditionally EAAs						
Alanine	233.8 (65.1)	229.3 (64.5)	241.2 (75.0)	244.5 (69.0)	0.348	0.391
Arginine	17.0 (12.0)	17.1 (10.5)	16.9 (6.9)	17.2 (5.8)	0.468	0.169
Asparagine	25.6 (14.9)	24.3 (15.0)	17.4 (7.7)	18.0 (9.5)	0.153	0.998
Aspartate	31.1 (18.0)	30.6 (18.4)	63.8 (37.3)	66.9 (40.9)	0.551	0.537
Glutamate	1326.8 (415.7)	1284.9 (403.7)	1498.3 (320.6)	1565.3 (334.3)	0.196	0.023
Glutamine	244.0 (160.2)	233.5 (162.0)	547.9 (258.4)	549.2 (280.0)	0.244	0.713
Glycine	138.9 (41.3)	139.3 (39.5)	143.8 (49.9)	149.1 (47.0)	0.792	0.072

All TAAs (mmol/L)	74.8 (12.2)	73.5 (11.3)	57.7 (8.7)	58.6 (9.2)	0.129	0.435
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TAA: total amino acid; Glx: glutamate + glutamine; Asx: aspartate + asparagine; HM: human milk. ‡
 $p < 0.01$. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/16 = 0.003$.

Table S3A. Spearman correlation coefficients between free amino acids in human milk and maternal anthropometrics.

FAA	Maternal anthropometrics							
	Age		Weight		Height		BMI	
	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)
EAAs								
Histidine	-0.02 (-0.09; 0.06)	0.00 (-0.09; 0.10)	-0.03 (-0.10; 0.05)	-0.04 (-0.13; 0.06)	0.02 (-0.06; 0.09)	-0.02 (-0.11; 0.07)	-0.03 (-0.11; 0.04)	-0.03 (-0.12; 0.07)
Isoleucine	-0.11 (-0.18; -0.03)‡	-0.02 (-0.11; 0.08)	0.01 (-0.07; 0.09)	0.09 (-0.01; 0.18)	-0.05 (-0.12; 0.03)	-0.05 (-0.14; 0.05)	0.08 (-0.01; 0.16)	0.12 (0.03; 0.21)‡
Leucine	-0.04 (-0.12; 0.03)	0.01 (-0.08; 0.10)	0.03 (-0.04; 0.11)	0.06 (-0.04; 0.15)	-0.09 (-0.17; -0.02)	-0.01 (-0.10; 0.09)	0.11 (0.04; 0.19)‡	0.09 (-0.01; 0.18)
Lysine	-0.03 (-0.11; 0.05)	0.06 (-0.04; 0.15)	-0.04 (-0.12; 0.03)	0.06 (-0.03; 0.15)	-0.11 (-0.18; -0.04)‡	-0.02 (-0.12; 0.07)	0.01 (-0.07; 0.09)	0.05 (-0.03; 0.13)
Methionine	0.02 (-0.06; 0.09)	0.09 (0.00; 0.19)	-0.03 (-0.10; 0.05)	0.00 (-0.09; 0.10)	-0.03 (-0.11; 0.04)	0.00 (-0.09; 0.10)	-0.01 (-0.09; 0.07)	0.01 (-0.08; 0.11)
Phenylalanine	0.03 (-0.04; 0.11)	0.06 (-0.03; 0.15)	0.03 (-0.05; 0.10)	-0.06 (-0.15; 0.04)	-0.05 (-0.12; 0.03)	0.04 (-0.05; 0.14)	0.05 (-0.02; 0.13)	-0.07 (-0.16; 0.02)
Threonine	0.01 (-0.07; 0.08)	0.14 (0.04; 0.23)‡	-0.05 (-0.13; 0.02)	-0.11 (-0.20; -0.01)	-0.01 (-0.09; 0.07)	0.02 (-0.08; 0.11)	-0.06 (-0.13; 0.02)	-0.12 (-0.21; -0.03)
Tryptophan	0.07 (-0.01; 0.15)	0.16 (0.07; 0.25)*	0.01 (-0.07; 0.08)	0.03 (-0.07; 0.12)	0.05 (-0.03; 0.12)	0.01 (-0.08; 0.10)	0.01 (-0.07; 0.08)	0.02 (-0.08; 0.11)
Valine	0.03 (-0.05; 0.10)	0.06 (-0.04; 0.15)	0.03 (-0.04; 0.10)	0.05 (-0.02; 0.12)	0.00 (-0.08; 0.07)	-0.07 (-0.16; 0.03)	0.08 (-0.01; 0.17)	0.09 (-0.02; 0.19)
NEAAs/Conditionally EAAs								
Alanine	-0.03 (-0.11; 0.04)	0.04 (-0.06; 0.13)	0.04 (-0.04; 0.12)	0.07 (-0.02; 0.16)	-0.06 (-0.13; 0.02)	-0.05 (-0.14; 0.04)	0.07 (-0.01; 0.14)	0.08 (-0.02; 0.17)
Arginine	-0.08 (-0.16; -0.01)	0.04 (-0.06; 0.13)	0.02 (-0.06; 0.09)	0.13 (0.03; 0.22)‡	-0.09 (-0.16; -0.01)	-0.10 (-0.19; 0.00)	0.07 (-0.01; 0.14)	0.16 (0.06; 0.24)‡
Asparagine	0.00 (-0.08; 0.07)	0.04 (-0.06; 0.13)	-0.12 (-0.20; -0.05)*	-0.28 (-0.36; -0.19)*	0.01 (-0.07; 0.08)	0.03 (-0.06; 0.13)	-0.14 (-0.22; -0.07)*	-0.33 (-0.41; -0.24)*
Aspartate	0.00 (-0.08; 0.07)	-0.01 (-0.08; 0.07)	-0.05 (-0.12; 0.02)	-0.06 (-0.14; 0.02)	0.05 (-0.01; 0.11)	0.06 (0.00; 0.12)	-0.08 (-0.17; 0.02)	-0.10 (-0.20; 0.01)
Glutamate	0.01 (-0.07; 0.08)	0.00 (-0.09; 0.10)	-0.04 (-0.10; 0.02)	-0.05 (-0.12; 0.02)	0.01 (-0.04; 0.06)	0.04 (-0.03; 0.11)	-0.05 (-0.12; 0.03)	-0.08 (-0.18; 0.02)
Glutamine	0.05 (-0.03; 0.13)	0.06 (-0.04; 0.15)	-0.12 (-0.19; -0.04)*	-0.18 (-0.27; -0.09)*	0.04 (-0.03; 0.12)	0.09 (-0.01; 0.18)	-0.15 (-0.22; -0.08)*	-0.24 (-0.32; -0.15)*
Glycine	-0.06 (-0.14; 0.01)	0.06 (-0.04; 0.15)	-0.11 (-0.19; -0.04)‡	-0.02 (-0.11; 0.08)	-0.01 (-0.09; 0.06)	0.03 (-0.06; 0.12)	-0.13 (-0.20; -0.05)*	-0.05 (-0.14; 0.05)
Serine	-0.03 (-0.11; 0.04)	0.07 (-0.03; 0.16)	-0.11 (-0.19; -0.04)‡	-0.12 (-0.21; -0.03)‡	-0.01 (-0.09; 0.06)	-0.03 (-0.12; 0.07)	-0.13 (-0.20; -0.06)*	-0.13 (-0.22; -0.04)‡
Tyrosine	-0.02 (-0.09; 0.06)	0.07 (-0.02; 0.17)	0.03 (-0.05; 0.11)	0.12 (0.03; 0.21)	-0.03 (-0.10; 0.05)	-0.01 (-0.1; 0.08)	0.04 (-0.03; 0.12)	0.14 (0.05; 0.23)‡
Non-coded AAs								
Citrulline	0.03 (-0.04; 0.11)	0.13 (0.03; 0.22)‡	-0.02 (-0.09; 0.06)	-0.07 (-0.16; 0.02)	0.03 (-0.05; 0.10)	0.04 (-0.06; 0.13)	-0.03 (-0.11; 0.04)	-0.11 (-0.20; -0.01)
Ornithine	0.02 (-0.06; 0.09)	0.01 (-0.08; 0.11)	-0.02 (-0.09; 0.06)	-0.01 (-0.11; 0.08)	0.04 (-0.03; 0.12)	-0.05 (-0.14; 0.04)	-0.04 (-0.12; 0.03)	0.01 (-0.09; 0.10)
Taurine	0.05 (-0.02; 0.13)	0.11 (0.02; 0.20)	0.06 (-0.01; 0.14)	0.05 (-0.05; 0.14)	0.06 (-0.02; 0.14)	0.03 (-0.07; 0.12)	0.05 (-0.02; 0.13)	0.04 (-0.06; 0.13)
Sum								
All FAAs	0.01 (-0.06; 0.09)	0.05 (-0.05; 0.14)	-0.05 (-0.12; 0.03)	-0.07 (-0.17; 0.03)	-0.02 (-0.08; 0.05)	0.03 (-0.05; 0.10)	-0.06 (-0.14; 0.03)	-0.09 (-0.19; 0.02)

FAA: free amino acid; EAA: essential amino acid; NEAA: non-essential amino acid; BMI: body mass index. ‡ $p < 0.01$, * p -value remains statistically significant following Bonferroni adjustment. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/22 = 0.002$. P -values < 0.002 are indicated in bold.

Table S3B. Spearman correlation coefficients between total amino acids in human milk and maternal anthropometrics.

TAA	Maternal anthropometrics							
	Age		Weight		Height		BMI	
	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)
EAAs								
Histidine	0.02 (-0.06; 0.09)	0.11 (0.02; 0.20)	0.02 (-0.05; 0.10)	0.13 (0.04; 0.22)†	0.04 (-0.04; 0.11)	-0.06 (-0.15; 0.04)	0.00 (-0.08; 0.07)	0.13 (0.04; 0.22)†
Isoleucine	0.08 (0.01; 0.16)	0.12 (0.02; 0.21)	0.02 (-0.05; 0.10)	0.12 (0.03; 0.21)	0.06 (-0.02; 0.14)	-0.02 (-0.11; 0.07)	0.00 (-0.08; 0.07)	0.10 (0.00; 0.19)
Leucine	0.05 (-0.02; 0.13)	0.12 (0.02; 0.21)	0.02 (-0.06; 0.10)	0.15 (0.06; 0.24)*	0.04 (-0.03; 0.12)	-0.03 (-0.13; 0.06)	0.00 (-0.08; 0.08)	0.14 (0.05; 0.23)*
Lysine	0.04 (-0.04; 0.12)	0.12 (0.02; 0.21)	-0.02 (-0.09; 0.06)	0.11 (0.02; 0.21)	0.02 (-0.05; 0.10)	-0.04 (-0.14; 0.05)	-0.03 (-0.11; 0.04)	0.11 (0.02; 0.20)
Methionine	0.03 (-0.05; 0.11)	0.14 (0.04; 0.23)†	0.09 (0.01; 0.17)	0.09 (0.00; 0.18)	0.06 (-0.02; 0.13)	0.00 (-0.09; 0.09)	0.06 (-0.01; 0.14)	0.07 (-0.02; 0.16)
Phenylalanine	0.00 (-0.07; 0.08)	0.10 (0.01; 0.19)	0.04 (-0.03; 0.12)	0.17 (0.08; 0.26)*	0.03 (-0.05; 0.10)	-0.04 (-0.13; 0.06)	0.03 (-0.05; 0.10)	0.17 (0.07; 0.26)*
Threonine	0.02 (-0.06; 0.10)	0.10 (0.01; 0.19)	0.07 (-0.01; 0.14)	0.16 (0.07; 0.25)*	0.00 (-0.07; 0.08)	-0.05 (-0.15; 0.04)	0.07 (-0.01; 0.15)	0.16 (0.07; 0.25)*
Valine	0.03 (-0.05; 0.10)	0.10 (0.01; 0.19)	0.09 (0.01; 0.16)	0.19 (0.10; 0.28)*	0.02 (-0.06; 0.09)	-0.02 (-0.11; 0.08)	0.08 (0.00; 0.15)†	0.18 (0.09; 0.27)*
NEAAs/conditionally EAAs								
Alanine	0.00 (-0.07; 0.08)	0.06 (-0.03; 0.16)	-0.01 (-0.09; 0.06)	0.18 (0.08; 0.27)*	-0.04 (-0.11; 0.04)	-0.05 (-0.15; 0.04)	-0.01 (-0.08; 0.07)	0.18 (0.09; 0.27)*
Arginine	0.00 (-0.08; 0.08)	0.00 (-0.09; 0.10)	0.04 (-0.04; 0.12)	0.07 (-0.03; 0.16)	-0.02 (-0.09; 0.06)	-0.07 (-0.17; 0.02)	0.05 (-0.03; 0.12)	0.10 (0.00; 0.19)
Asx	0.02 (-0.06; 0.09)	0.09 (0.00; 0.19)	0.01 (-0.07; 0.08)	0.16 (0.07; 0.25)*	0.01 (-0.06; 0.09)	-0.06 (-0.15; 0.03)	0.00 (-0.08; 0.07)	0.16 (0.07; 0.25)*
Glx	0.06 (-0.01; 0.14)	0.12 (0.03; 0.21)	0.01 (-0.06; 0.09)	0.10 (0.00; 0.19)	0.05 (-0.03; 0.12)	-0.04 (-0.13; 0.06)	-0.01 (-0.09; 0.06)	0.08 (-0.01; 0.17)
Glycine	-0.01 (-0.08; 0.07)	0.08 (-0.02; 0.17)	0.02 (-0.06; 0.09)	0.16 (0.07; 0.25)*	-0.01 (-0.09; 0.07)	-0.04 (-0.14; 0.05)	0.02 (-0.05; 0.10)	0.16 (0.07; 0.25)*
Serine	0.01 (-0.07; 0.09)	0.09 (0.00; 0.18)	0.03 (-0.04; 0.11)	0.17 (0.08; 0.26)*	0.01 (-0.07; 0.08)	-0.06 (-0.16; 0.03)	0.03 (-0.04; 0.11)	0.18 (0.09; 0.27)*

Tyrosine	0.03 (-0.04; 0.11)	0.10 (0.00; 0.19)	0.04 (-0.04; 0.11)	0.19 (0.10; 0.28)*	0.04 (-0.04; 0.11)	-0.02 (-0.11; 0.08)	0.02 (-0.06; 0.09)	0.17 (0.08; 0.26)*
Sum								
All TAAs	0.03 (-0.04; 0.11)	0.11 (0.02; 0.20)	0.03 (-0.05; 0.11)	0.15 (0.05; 0.24)*	0.03 (-0.05; 0.10)	-0.05 (-0.15; 0.04)	0.01 (-0.06; 0.09)	0.14 (0.05; 0.23)*

TAA: total amino acid; EAA: essential amino acid; NEAA: non-essential amino acid; Glx: glutamate + glutamine; Asx: aspartate + asparagine; BMI: body mass index. ‡ $p < 0.01$, * p -value remains statistically significant following Bonferroni adjustment. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/16 = 0.003$. P -values < 0.003 are indicated in bold.

Table S3C. Spearman correlation coefficients between free amino acids in human milk and infant anthropometrics.

FAA	Infant anthropometrics							
	Birth length		Birth weight		Gain in length		Gain in weight	
	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)
EAA								
Histidine	0.00 (-0.08; 0.08)	-0.10 (-0.20; -0.01)	0.04 (-0.04; 0.12)	-0.02 (-0.12; 0.08)	0.01 (-0.07; 0.09)	0.02 (-0.08; 0.11)	0.08 (0.00; 0.16)	0.03 (-0.07; 0.12)
Isoleucine	-0.04 (-0.12; 0.04)	-0.02 (-0.11; 0.08)	-0.08 (-0.16; 0.00)	0.03 (-0.06; 0.13)	-0.04 (-0.12; 0.04)	0.01 (-0.09; 0.10)	-0.01 (-0.09; 0.07)	-0.01 (-0.1; 0.09)
Leucine	0.01 (-0.07; 0.08)	-0.04 (-0.13; 0.06)	-0.01 (-0.09; 0.07)	0.03 (-0.07; 0.12)	0.00 (-0.08; 0.08)	0.02 (-0.08; 0.12)	0.03 (-0.05; 0.11)	-0.01 (-0.11; 0.08)
Lysine	-0.03 (-0.11; 0.05)	-0.06 (-0.16; 0.03)	-0.01 (-0.09; 0.07)	-0.02 (-0.11; 0.08)	-0.07 (-0.15; 0.01)	0.04 (-0.06; 0.13)	-0.03 (-0.11; 0.05)	-0.01 (-0.11; 0.08)
Methionine	-0.02 (-0.10; 0.06)	-0.05 (-0.14; 0.05)	-0.07 (-0.14; 0.01)	-0.03 (-0.12; 0.07)	0.01 (-0.07; 0.09)	-0.03 (-0.12; 0.07)	-0.03 (-0.11; 0.05)	0.03 (-0.07; 0.12)
Phenylalanine	0.02 (-0.06; 0.10)	-0.05 (-0.15; 0.05)	0.01 (-0.07; 0.09)	0.02 (-0.08; 0.11)	-0.05 (-0.13; 0.03)	0.08 (-0.02; 0.17)	-0.04 (-0.12; 0.04)	0.08 (-0.01; 0.17)
Threonine	-0.03 (-0.11; 0.05)	-0.08 (-0.18; 0.01)	0.02 (-0.06; 0.10)	-0.02 (-0.11; 0.08)	0.04 (-0.04; 0.12)	0.00 (-0.10; 0.09)	0.11 (0.03; 0.19)†	-0.02 (-0.12; 0.07)
Tryptophan	0.04 (-0.04; 0.12)	0.11 (0.01; 0.20)	-0.03 (-0.11; 0.05)	0.05 (-0.05; 0.14)	-0.05 (-0.13; 0.03)	0.04 (-0.06; 0.13)	0.03 (-0.05; 0.11)	0.01 (-0.08; 0.11)
Valine	0.00 (-0.08; 0.08)	-0.07 (-0.17; 0.02)	0.03 (-0.05; 0.11)	-0.04 (-0.14; 0.05)	-0.07 (-0.15; 0.01)	-0.02 (-0.11; 0.08)	-0.05 (-0.13; 0.03)	0.00 (-0.10; 0.09)
NEAAs/conditionally EAAs								
Alanine	0.00 (-0.08; 0.08)	-0.01 (-0.11; 0.08)	0.02 (-0.06; 0.09)	-0.03 (-0.12; 0.07)	0.02 (-0.06; 0.10)	0.00 (-0.10; 0.10)	0.03 (-0.05; 0.11)	-0.05 (-0.15; 0.04)
Arginine	-0.08 (-0.16; 0.00)	0.01 (-0.08; 0.11)	-0.07 (-0.14; 0.02)	-0.06 (-0.16; 0.03)	-0.02 (-0.10; 0.06)	-0.01 (-0.10; 0.09)	-0.02 (-0.10; 0.06)	-0.01 (-0.10; 0.09)
Asparagine	-0.01 (-0.09; 0.07)	-0.08 (-0.17; 0.02)	-0.06 (-0.14; 0.02)	-0.06 (-0.16; 0.03)	-0.02 (-0.10; 0.06)	0.03 (-0.06; 0.13)	0.06 (-0.02; 0.14)	0.01 (-0.09; 0.10)
Aspartate	0.05 (-0.03; 0.13)	0.01 (-0.08; 0.11)	-0.01 (-0.09; 0.07)	0.03 (-0.07; 0.12)	-0.09 (-0.17; -0.01)	-0.01 (-0.1; 0.09)	-0.02 (-0.10; 0.06)	-0.07 (-0.16; 0.03)
Glutamate	0.01 (-0.07; 0.09)	-0.02 (-0.12; 0.07)	0.02 (-0.06; 0.10)	0.01 (-0.09; 0.10)	0.00 (-0.08; 0.08)	-0.02 (-0.11; 0.08)	0.12 (0.04; 0.20)*	-0.04 (-0.13; 0.06)

Glutamine	0.01 (-0.07; 0.09)	-0.03 (-0.12; 0.07)	0.00 (-0.08; 0.08)	0.00 (-0.10; 0.09)	0.10 (0.05; 0.15)‡	0.04 (-0.06; 0.13)	0.22 (0.14; 0.29)*	0.06 (-0.03; 0.16)
Glycine	-0.01 (-0.09; 0.07)	-0.04 (-0.13; 0.06)	-0.01 (-0.09; 0.07)	0.01 (-0.08; 0.11)	0.01 (-0.07; 0.09)	0.06 (-0.03; 0.16)	0.05 (-0.03; 0.13)	0.03 (-0.06; 0.13)
Serine	0.02 (-0.07; 0.09)	-0.08 (-0.17; 0.02)	0.00 (-0.08; 0.08)	-0.03 (-0.13; 0.06)	0.06 (-0.03; 0.13)	0.08 (-0.02; 0.17)	0.19 (0.11; 0.27)*	0.08 (-0.02; 0.17)
Tyrosine	0.02 (-0.06; 0.10)	-0.01 (-0.11; 0.08)	-0.01 (-0.08; 0.08)	0.08 (-0.02; 0.17)	-0.07 (-0.15; 0.01)	0.05 (-0.05; 0.15)	0.04 (-0.04; 0.12)	0.09 (-0.01; 0.18)
Non-coded AAs								
Citrulline	0.04 (-0.04; 0.12)	-0.05 (-0.15; 0.05)	0.02 (-0.06; 0.10)	-0.03 (-0.12; 0.07)	0.01 (-0.07; 0.09)	0.04 (-0.06; 0.13)	0.09 (0.01; 0.17)	0.02 (-0.08; 0.11)
Ornithine	0.00 (-0.08; 0.08)	-0.02 (-0.12; 0.07)	-0.03 (-0.11; 0.05)	-0.03 (-0.13; 0.06)	-0.09 (-0.17; -0.01)	0.01 (-0.09; 0.10)	-0.03 (-0.11; 0.05)	0.05 (-0.05; 0.14)
Taurine	0.07 (-0.01; 0.15)	0.09 (-0.01; 0.18)	0.04 (-0.04; 0.12)	0.08 (-0.02; 0.17)	0.06 (-0.02; 0.14)	0.04 (-0.05; 0.14)	0.05 (-0.03; 0.13)	-0.03 (-0.12; 0.07)
Sum								
All FAAs	0.02 (-0.06; 0.10)	-0.03 (-0.12; 0.07)	0.02 (-0.06; 0.10)	0.01 (-0.09; 0.10)	0.00 (-0.08; 0.08)	0.04 (-0.06; 0.13)	0.16 (0.08; 0.24)*	0.00 (-0.09; 0.10)

FAA: free amino acid; EAA: essential amino acid; NEAA: non-essential amino acid. ‡ $p < 0.01$, * p -value remains statistically significant following Bonferroni adjustment. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/22 = 0.002$. P -values < 0.002 are indicated in bold.

Table S3D. Spearman correlation coefficients between total amino acids in human milk and infant anthropometrics.

TAA	Infant anthropometrics							
	Birth length		Birth weight		Gain in length		Gain in weight	
	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)	6 weeks Rho (95% CI)	6 months Rho (95% CI)
EAAs								
Histidine	0.02 (-0.06; 0.10)	0.10 (0.01; 0.20)	-0.03 (-0.11; 0.05)	0.06 (-0.03; 0.16)	-0.11 (-0.19; -0.04)†	-0.12 (-0.22; -0.03)	-0.16 (-0.23; -0.08)*	-0.06 (-0.15; 0.04)
Isoleucine	-0.01 (-0.09; 0.07)	0.08 (-0.02; 0.17)	0.00 (-0.08; 0.08)	0.06 (-0.04; 0.15)	-0.06 (-0.13; 0.03)	-0.12 (-0.21; -0.03)	-0.12 (-0.20; -0.04)*	-0.08 (-0.17; 0.02)
Leucine	0.03 (-0.05; 0.11)	0.10 (0.00; 0.19)	-0.01 (-0.09; 0.07)	0.07 (-0.02; 0.17)	-0.11 (-0.19; -0.03)†	-0.13 (-0.22; -0.03)†	-0.12 (-0.20; -0.04)*	-0.07 (-0.16; 0.03)
Lysine	0.04 (-0.04; 0.12)	0.10 (0.01; 0.19)	-0.01 (-0.09; 0.07)	0.07 (-0.02; 0.17)	-0.12 (-0.2; -0.04)†	-0.12 (-0.22; -0.03)	-0.13 (-0.20; -0.05)*	-0.07 (-0.16; 0.03)
Methionine	-0.01 (-0.08; 0.07)	0.03 (-0.06; 0.13)	-0.03 (-0.11; 0.05)	0.03 (-0.07; 0.12)	-0.03 (-0.11; 0.05)	-0.08 (-0.18; 0.01)	-0.09 (-0.17; -0.01)	-0.05 (-0.14; 0.05)
Phenylalanine	0.02 (-0.06; 0.10)	0.09 (0.00; 0.18)	-0.03 (-0.11; 0.05)	0.07 (-0.02; 0.17)	-0.12 (-0.20; -0.04)†	-0.11 (-0.20; -0.01)	-0.16 (-0.23; -0.08)*	-0.05 (-0.15; 0.04)
Threonine	0.00 (-0.08; 0.08)	0.09 (-0.01; 0.18)	-0.01 (-0.09; 0.07)	0.06 (-0.03; 0.16)	-0.10 (-0.18; -0.02)	-0.11 (-0.20; -0.01)	-0.16 (-0.23; -0.08)*	-0.06 (-0.16; 0.04)
Valine	0.00 (-0.08; 0.08)	0.04 (-0.06; 0.13)	-0.01 (-0.08; 0.08)	0.05 (-0.05; 0.14)	-0.10 (-0.18; -0.02)	-0.09 (-0.18; 0.00)	-0.16 (-0.24; -0.08)*	-0.04 (-0.14; 0.05)
NEAAs/conditionally EAAs								
Alanine	0.05 (-0.03; 0.13)	0.09 (0.00; 0.19)	0.00 (-0.08; 0.08)	0.06 (-0.04; 0.15)	-0.13 (-0.21; -0.05)*	-0.09 (-0.18; 0.01)	-0.13 (-0.21; -0.06)*	-0.06 (-0.15; 0.04)
Arginine	0.03 (-0.05; 0.11)	0.11 (0.01; 0.20)	0.01 (-0.07; 0.09)	0.04 (-0.05; 0.14)	-0.13 (-0.21; -0.06)*	-0.09 (-0.18; 0.01)	-0.12 (-0.20; -0.04)†	-0.08 (-0.17; 0.02)
Asx	0.03 (-0.05; 0.11)	0.11 (0.01; 0.20)	-0.02 (-0.10; 0.06)	0.07 (-0.03; 0.16)	-0.13 (-0.21; -0.05)*	-0.12 (-0.21; -0.02)	-0.15 (-0.23; -0.07)*	-0.08 (-0.17; 0.02)
Glx	0.02 (-0.06; 0.10)	0.08 (-0.02; 0.17)	-0.01 (-0.09; 0.07)	0.06 (-0.04; 0.15)	-0.09 (-0.17; -0.01)	-0.13 (-0.22; -0.03)†	-0.08 (-0.16; 0.00)	-0.09 (-0.18; 0.01)
Glycine	0.03 (-0.05; 0.11)	0.10 (0.00; 0.19)	-0.02 (-0.10; 0.06)	0.07 (-0.03; 0.16)	-0.14 (-0.21; -0.06)*	-0.08 (-0.18; 0.01)	-0.14 (-0.22; -0.06)*	-0.05 (-0.14; 0.05)
Serine	0.01 (-0.07; 0.09)	0.10 (0.00; 0.19)	-0.02 (-0.10; 0.06)	0.07 (-0.03; 0.16)	-0.12 (-0.20; -0.04)†	-0.09 (-0.19; 0.00)	-0.15 (-0.22; -0.07)*	-0.04 (-0.14; 0.05)

Tyrosine	0.02 (-0.06; 0.10)	0.13 (0.03; 0.22)	-0.01 (-0.09; 0.07)	0.10 (0.00; 0.19)	-0.10 (-0.18; -0.02)	-0.13 (-0.22; -0.03)‡	-0.14 (-0.22; -0.06)*	-0.06 (-0.15; 0.04)
Sum								
Sum of all TAAs	0.03 (-0.06; 0.10)	0.10 (0.01; 0.19)	-0.02 (-0.10; 0.06)	0.07 (-0.03; 0.16)	-0.13 (-0.21; -0.05)*	-0.12 (-0.22; -0.03)	-0.15 (-0.23; -0.07)*	-0.08 (-0.17; 0.02)

TAA: total amino acid; EAA: essential amino acid; NEAA: non-essential amino acid; Glx: glutamate + glutamine; Asx: aspartate + asparagine. ‡ $p < 0.01$, * p -value remains statistically significant following Bonferroni adjustment. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/16 = 0.003$. P -values < 0.003 are indicated in bold.

Table S4. Partial correlation coefficients between amino acids in human milk and infant and maternal factors.

Anthropometric measurement	Sample collection time	Amino acid	Beta estimate (95% CI)	<i>p</i> -value	Putative confounders included
Maternal characteristics					
Pre-pregnancy weight	6 weeks	Free glutamine	-0.676 (-1.556; 0.205)	0.132	Infant gender, maternal age, history in allergic disease
		Free asparagine	-0.089 (-0.171; -0.008)	0.031 [†]	
	6 months	Free glutamine	-2.686 (6.783; -1.410)	0.198	
		Free asparagine	-0.029 (-0.160; 0.102)	0.667	
		Sum of all TAAs	8.340 (-7.430; 24.111)	0.299	
Pre-pregnancy BMI	6 weeks	Free glutamine	-1.301 (-3.752; 1.150)	0.298	Infant gender, maternal age, history in allergic disease
		Free asparagine	-0.167 (-0.396; 0.063)	0.155	
		Free serine	-0.403 (-0.935; 0.128)	0.136	
		Free glycine	-0.513 (-1.135; 0.103)	0.102	
	6 months	Free glutamine	-0.916 (-6.101; 4.270)	0.729	
		Free asparagine	-0.099 (-0.268; 0.069)	0.245	
		Sum of all TAAs	9.938 (-10.177; 30.053)	0.332	
Age	6 months	Free tryptophan	0.298 (0.022; 0.574)	0.034 [†]	Infant gender, maternal pre-pregnancy BMI, history in allergic disease
Infant characteristics					
Gain in weight	6 weeks	Free glutamate	0.032 (-0.016; 0.080)	0.190	Infant gender, maternal age, maternal pre-pregnancy BMI, history in allergic disease
		Free glutamine	0.015 (0.008; 0.023)	0.005 [‡]	
		Free serine	0.004 (0.001; 0.008)	0.046 [†]	
		Sum of all TAAs	-0.212 (-0.364; -0.060)	0.003*	
	6 months	Sum of all TAAs	-0.155 (-0.267; -0.043)	0.007 [†]	
Gain in length	6 weeks	Free glutamine	5.436 (-0.811; 11.683)	0.088	
		Sum of all TAAs	-8.568 (-13.554; -3.582)	<0.001*	

TAAs: total amino acids; BMI: body mass index. [†] $p < 0.05$, [‡] $p < 0.01$, * p -value remains statistically significant following Bonferroni adjustment. Bonferroni-adjusted level of statistical significance for FAAs is $\alpha = 0.05/16 = 0.002$ and for TAAs is $\alpha = 0.05/16 = 0.003$.

Table S5A. Free amino acid levels in human milk of mothers with or without an allergy.

FAA (μmol/L)	6 weeks Mean (SD)		6 months Mean (SD)		Analysis of differences at 6 weeks (<i>p</i> -value)	Analysis of differences at 6 months (<i>p</i> -value)
	Mothers without an allergy (n = 443)	Mothers with an allergy (n = 225)	Mothers without an allergy (n = 274)	Mothers with an allergy (n = 166)		
EAAs						
Histidine	28.1 (9.3)	28.6 (8.8)	28.6 (12.1)	31.2 (16.0)	0.456	0.068
Isoleucine	16.8 (10.8)	15.8 (9.1)	12.1 (5.1)	13.6 (6.8)	0.179	0.017
Leucine	34.1 (16.7)	33.6 (19.9)	34.0 (10.8)	36.3 (12.3)	0.740	0.045
Lysine	36.0 (25.6)	32.5 (20.6)	28.9 (12.9)	32.1 (17.6)	0.058	0.043
Methionine	27.9 (23.8)	27.9 (25.2)	33.1 (26.7)	36.8 (27.8)	0.997	0.162
Phenylalanine	21.3 (9.9)	21.3 (10.6)	16.2 (5.2)	16.8 (6.1)	0.972	0.325
Threonine	72.2 (30.7)	69.0 (26.4)	97.6 (38.8)	96.0 (34.7)	0.160	0.644
Tryptophan	14.4 (7.3)	14.9 (7.8)	29.3 (11.9)	28.7 (11.7)	0.444	0.613
Valine	108.6 (59.6)	111.4 (55.7)	65.2 (27.4)	69.0 (27.4)	0.546	0.157
NEAAs/Conditionally EAAs						
Alanine	231.9 (65.1)	230.4 (62.4)	238.4 (70.3)	250.0 (75.0)	0.780	0.108
Arginine	16.9 (12.0)	17.2 (10.0)	16.6 (5.8)	17.7 (7.3)	0.755	0.091
Asparagine	24.1 (14.4)	26.8 (15.8)	18.1 (8.6)	16.9 (8.6)	0.130	0.146
Aspartate	31.1 (18.0)	30.6 (18.6)	62.5 (36.5)	69.6 (42.7)	0.709	0.076
Glutamate	1325.2 (418.8)	1273.5 (391.3)	1531.4 (323.1)	1526.6 (338.6)	0.116	0.882
Glutamine	238.4 (160.6)	241.3 (162.4)	579.4 (268.0)	498.3 (263.4)	0.828	<0.001*
Glycine	140.0 (41.4)	137.3 (38.3)	145.5 (46.5)	146.6 (49.9)	0.403	0.817
Serine	101.6 (35.7)	97.8 (32.3)	137.4 (58.5)	136.9 (63.5)	0.165	0.928
Tyrosine	27.2 (19.8)	28.0 (21.0)	16.7 (7.0)	18.7 (8.9)	0.658	0.014
Non-coded AAs						
Citrulline	10.6 (4.7)	10.9 (4.8)	20.9 (6.2)	20.5 (5.8)	0.410	0.424
Ornithine	14.4 (16.3)	15.6 (17.5)	13.6 (23.1)	18.6 (31.1)	0.370	0.071
Taurine	244.4 (94.3)	242.9 (91.3)	264.6 (103.2)	269.2 (111.3)	0.840	0.668
Sum						
All FAAs	2765.3 (668.7)	2707.3 (593.2)	3390.2 (681.8)	3350.0 (666.6)	0.253	0.543

FAA: free amino acid; EAA: essential amino acid; NEAA: non-essential amino acid. * *p*-value remains statistically significant following Bonferroni adjustment. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/22 = 0.002$. *P*-values < 0.002 are indicated in bold.

Table S5B. Total amino acid levels in human milk of mothers with or without an allergy.

TAA (μmol/L)	6 weeks Mean (SD)		6 months Mean (SD)		Analysis of differences at 6 weeks (<i>p</i> -value)	Analysis of differences at 6 months (<i>p</i> -value)
	Mothers without an allergy (n = 443)	Mothers with an allergy (n = 225)	Mothers without an allergy (n = 274)	Mothers with an allergy (n = 166)		
EAAs						
Histidine	1790.6 (262.2)	1805.2 (270.1)	1322.1 (197.3)	1334.9 (207.3)	0.646	0.221
Isoleucine	4013.1 (575.6)	4077.0 (646.8)	3380.2 (457.9)	3389.2 (464.2)	0.078	0.930
Leucine	8684.2 (1227.8)	8710.3 (1250.2)	6772.1 (902.8)	6833.8 (949.9)	0.759	0.127
Lysine	6367.2 (940.1)	6354.0 (969.2)	3948.8 (607.3)	3993.6 (653.1)	0.386	0.851
Methionine	1009.9 (207.3)	1011.5 (189.1)	749.7 (256.0)	752.5 (144.4)	0.763	0.292
Phenylalanine	2615.1 (439.4)	2605.0 (389.8)	2043.9 (282.3)	2082.9 (337.5)	0.506	0.132
Threonine	3700.5 (581.6)	3722.3 (576.0)	3345.0 (465.8)	3409.0 (567.5)	0.763	0.213
Valine	4654.0 (760.9)	4695.0 (747.8)	4164.4 (603.9)	4259.4 (663.0)	0.797	0.501
NEAAs/Conditionally EAAs						
Alanine	5349.3 (989.4)	5374.2 (990.5)	3938.8 (569.7)	4040.4 (733.9)	0.799	0.503
Arginine	2874.7 (1038.3)	3028.0 (1070.0)	2145.7 (545.6)	2140.4 (653.0)	0.805	0.567
Asx	8571.5 (1329.0)	8543.8 (1319.9)	6219.5 (858.6)	6283.7 (1039.3)	0.876	0.160
Glx	13469.5 (1622.0)	13502.7 (1655.9)	11517.4 (1448.8)	11426.8 (1702.5)	0.505	0.521
Glycine	3913.7 (720.3)	3888.6 (689.1)	2838.8 (451.1)	2921.1 (605.3)	0.662	0.130
Serine	5125.9 (828.5)	5115.5 (799.8)	4062.1 (571.6)	4152.9 (703.3)	0.918	0.884
Tyrosine	1895.6 (296.7)	1903.0 (298.9)	1498.3 (215.0)	1522.1 (238.3)	0.211	0.843
Sum						
All TAAs	74.0 (11.8)	74.3 (11.9)	57.9 (8.4)	58.5 (9.7)	0.867	0.473

TAA: total amino acid; EAA: essential amino acid; NEAA: non-essential amino acid; Glx: glutamate + glutamine; Asx: aspartate + asparagine. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/16 = 0.003$. *P*-values < 0.003 are indicated in bold.

Table S6A. Adjusted model results for associations between free amino acids in human milk and infant wheeze outcomes.

FAA	Wheeze					
	Transient wheeze		Persistent wheeze		Intermediate wheeze	
	6 weeks (n = 47) RR (95% CI)	6 months (n = 30) RR (95% CI)	6 weeks (n = 31) RR (95% CI)	6 months (n = 18) RR (95% CI)	6 weeks (n = 31) RR (95% CI)	6 months (n = 27) RR (95% CI)
EAAs						
Histidine	0.98 (0.95; 1.02)	1.00 (0.97; 1.03)	1.02 (0.98; 1.06)	1.02 (0.98; 1.07)	0.95 (0.89; 1.02)	1.04 (1.01; 1.06)*
Isoleucine	0.98 (0.94; 1.02)	1.04 (0.97; 1.11)	1.00 (0.97; 1.04)	1.01 (0.91; 1.13)	0.97 (0.92; 1.02)	1.07 (0.99; 1.14)
Leucine	0.98 (0.95; 1.00)	1.02 (0.99; 1.05)	0.99 (0.96; 1.02)	1.02 (0.98; 1.06)	1.02 (1.00; 1.03)	1.04 (1.00; 1.09)
Lysine	0.99 (0.97; 1.00)	1.02 (0.99; 1.05)	0.98 (0.97; 1.00)	1.00 (0.96; 1.03)	1.00 (0.99; 1.03)	1.04 (1.02; 1.06)*
Methionine	1.00 (0.98; 1.01)	1.00 (0.99; 1.02)	1.00 (0.99; 1.02)	1.01 (0.99; 1.02)	0.99 (0.97; 1.01)	1.02 (1.00; 1.03)
Phenylalanine	0.98 (0.93; 1.03)	1.09 (1.03; 1.15)†	1.00 (0.97; 1.04)	1.05 (0.95; 1.16)	0.98 (0.93; 1.02)	1.04 (0.96; 1.12)
Threonine	1.00 (0.98; 1.01)	1.00 (0.99; 1.00)	1.00 (0.99; 1.01)	1.01 (1.00; 1.02)	1.00 (0.98; 1.01)	1.01 (1.01; 1.02)†
Tryptophan	0.96 (0.92; 1.01)	1.01 (0.98; 1.04)	1.02 (0.98; 1.07)	0.99 (0.92; 1.06)	0.98 (0.91; 1.04)	0.99 (0.96; 1.03)
Valine	1.00 (0.99; 1.00)	1.01 (1.00; 1.03)	1.00 (0.99; 1.01)	1.00 (0.98; 1.02)	1.00 (0.99; 1.01)	1.01 (1.00; 1.02)
NEAAs/conditionally EAAs						
Alanine	1.00 (0.99; 1.01)	1.00 (1.00; 1.01)	1.00 (0.99; 1.01)	1.00 (0.97; 1.01)	1.00 (0.99; 1.01)	1.01 (1.00; 1.01)
Arginine	0.96 (0.92; 1.00)	1.06 (1.00; 1.11)	0.97 (0.93; 1.01)	0.93 (0.85; 1.00)	1.02 (0.98; 1.07)	1.04 (0.98; 1.10)
Asparagine	1.00 (0.98; 1.02)	1.00 (0.95; 1.05)	1.00 (0.98; 1.03)	0.96 (0.92; 1.00)	0.95 (0.92; 0.98)*	1.04 (0.99; 1.11)

Aspartate	1.00 (0.98; 1.02)	0.99 (0.97; 1.00)	1.02 (1.00; 1.05)	1.00 (0.99; 1.02)	0.97 (0.95; 0.99)‡	1.00 (0.99; 1.01)
Glutamate	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)
Glutamine	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (0.99; 1.00)	1.00 (1.00; 1.00)
Glycine	1.00 (0.99; 1.01)	1.00 (0.99; 1.01)	1.01 (1.00; 1.02)	1.01 (1.00; 1.02)	0.99 (0.98; 1.00)	1.01 (1.00; 1.02)
Serine	0.99 (0.98; 1.01)	1.00 (0.99; 1.01)	1.00 (1.00; 1.01)	1.01 (1.00; 1.02)	0.98 (0.96; 1.00)‡	1.01 (1.00; 1.01)‡
Tyrosine	1.00 (0.98; 1.02)	1.01 (0.97; 1.06)	1.01 (0.99; 1.03)	1.02 (0.93; 1.10)	0.96 (0.94; 0.99)‡	1.06 (1.00; 1.12)
Non-coded AAs						
Citrulline	0.96 (0.91; 1.02)	1.05 (0.97; 1.12)	1.01 (0.94; 1.09)	0.99 (0.92; 1.08)	0.88 (0.78; 0.99)	1.04 (0.95; 1.14)
Ornithine	0.99 (0.96; 1.02)	0.99 (0.97; 1.01)	1.02 (1.00; 1.04)	1.02 (1.00; 1.03)	0.98 (0.94; 1.02)	1.01 (1.01; 1.02)
Taurine	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (0.99; 1.00)	1.00 (0.99; 1.01)	1.00 (1.00; 1.01)	1.00 (0.99; 1.00)
Sum						
All FAAs	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)

FAA: free amino acid; EAA: essential amino acid; NEAA: non-essential amino acid; RR: risk ratio. ‡ $p < 0.01$, * p -value remains statistically significant following Bonferroni adjustment. Bonferroni adjusted level of statistical significance is $\alpha = 0.05/22 = 0.0023$. P -values < 0.0023 are indicated in bold. Models were adjusted for maternal pre-pregnancy body mass index (BMI), maternal age, infant sex, maternal education and smoking status.

Table S6B. Adjusted model results for associations of free amino acids in human milk and infant atopic disease and lower respiratory tract infections.

FAA	Atopic disease (AD)						Lower respiratory tract infections	
	Parent-reported AD		Paediatrician-reported AD		Both parent- and paediatrician-reported AD			
	6 weeks (n = 44) RR (95% CI)	6 months (n = 27) RR (95% CI)	6 weeks (n = 64) RR (95% CI)	6 months (n = 37) RR (95% CI)	6 weeks (n = 73) RR (95% CI)	6 months (n = 44) RR (95% CI)	6 weeks (n = 151) RR (95% CI)	6 months (n = 108) RR (95% CI)
EAAs								
Histidine	0.98 (0.95; 1.02)	0.98 (0.92; 1.04)	0.98 (0.95; 1.01)	1.01 (0.98; 1.04)	0.97 (0.93; 1.02)	0.98 (0.92; 1.05)	0.99 (0.96; 1.01)	1.01 (1.00; 1.03)
Isoleucine	1.01 (0.98; 1.05)	0.91 (0.78; 1.06)	1.01 (0.98; 1.04)	0.99 (0.91; 1.07)	1.02 (0.98; 1.06)	0.89 (0.72; 1.10)	0.97 (0.95; 0.99)	1.03 (0.99; 1.06)
Leucine	1.01 (0.98; 1.03)	0.93 (0.87; 1.00)	1.00 (0.98; 1.02)	0.97 (0.92; 1.02)	1.00 (0.97; 1.04)	0.92 (0.84; 1.01)	1.00 (0.98; 1.01)	1.01 (0.99; 1.03)
Lysine	1.01 (1.00; 1.02)	0.99 (0.96; 1.02)	1.01 (0.99; 1.02)	1.00 (0.98; 1.03)	1.01 (1.00; 1.03)	0.97 (0.95; 1.00)	1.00 (0.99; 1.01)	1.01 (1.00; 1.02)
Methionine	1.00 (0.98; 1.02)	0.98 (0.96; 1.00)	1.00 (0.98; 1.01)	0.99 (0.98; 1.01)	1.01 (0.99; 1.02)	0.98 (0.95; 1.00)	0.99 (0.98; 1.00)	1.00 (0.99; 1.01)
Phenylalanine	1.00 (0.97; 1.04)	0.95 (0.81; 1.11)	0.99 (0.96; 1.02)	0.96 (0.87; 1.07)	1.00 (0.96; 1.04)	0.95 (0.78; 1.15)	1.00 (0.97; 1.04)	0.98 (0.96; 1.00)
Threonine	1.01 (0.99; 1.02)	0.98 (0.96; 1.01)	1.00 (0.99; 1.02)	1.00 (0.99; 1.01)	1.00 (0.99; 1.02)	0.98 (0.96; 1.01)	0.99 (0.98; 1.00)	1.00 (1.00; 1.01)
Tryptophan	1.04 (1.00; 1.08)	1.00 (0.96; 1.04)	1.02 (0.98; 1.06)	1.00 (0.97; 1.04)	1.04 (0.99; 1.08)	1.00 (0.96; 1.05)	0.99 (0.96; 1.01)	0.99 (0.97; 1.02)
Valine	0.99 (0.99; 1.00)	0.97 (0.95; 1.00)	1.00 (0.99; 1.00)	0.99 (0.98; 1.01)	0.99 (0.98; 1.00)	0.97 (0.94; 1.01)	1.00 (1.00; 1.00)	1.01 (1.00; 1.02)
NEAAs/conditionally EAAs								
Alanine	1.00 (0.99; 1.00)	1.00 (0.99; 1.00)	1.00 (0.99; 1.00)	1.00 (1.00; 1.01)	1.00 (0.99; 1.00)	1.00 (0.99; 1.00)	1.00 (0.99; 1.00)	1.00 (1.00; 1.01)
Arginine	1.04 (1.00; 1.08)	0.95 (0.89; 1.01)	1.01 (0.97; 1.04)	1.00 (0.95; 1.06)	1.03 (0.98; 1.08)	0.93 (0.85; 1.02)	1.00 (0.98; 1.02)	1.03 (1.00; 1.05)
Asparagine	1.00 (0.97; 1.02)	0.94 (0.87; 1.01)	0.99 (0.96; 1.01)	0.96 (0.91; 1.02)	0.99 (0.96; 1.02)	0.93 (0.85; 1.01)	0.98 (0.97; 1.00)	1.02 (0.99; 1.04)
Aspartate	1.01 (0.98; 1.03)	1.00 (0.99; 1.01)	1.00 (0.98; 1.01)	1.00 (0.99; 1.01)	1.01 (0.99; 1.04)	0.99 (0.98; 1.01)	0.99 (0.98; 1.00)	1.00 (1.00; 1.01)
Glutamate	1.00 (1.00; 1.00)	0.99 (0.99; 0.99)†	1.00 (1.00; 1.00)	0.99 (0.99; 0.99)	1.00 (1.00; 1.00)	0.99 (0.99; 0.99)†	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)

Glutamine	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)
Glycine	1.00 (0.99; 1.01)	0.99 (0.97; 1.01)	0.99 (0.99; 1.00)	1.00 (0.99; 1.01)	1.00 (0.99; 1.00)	0.99 (0.97; 1.01)	0.99 (0.99; 1.00)	1.00 (1.00; 1.01)
Serine	1.01 (1.00; 1.02)	1.00 (0.99; 1.01)	1.00 (0.99; 1.01)	1.00 (0.99; 1.01)	1.01 (0.99; 1.02)	1.00 (0.99; 1.01)	1.00 (0.99; 1.00)	1.00 (1.00; 1.01)
Tyrosine	1.01 (0.99; 1.03)	0.91 (0.81; 1.02)	1.00 (0.98; 1.02)	0.96 (0.90; 1.03)	1.01 (0.99; 1.03)	0.90 (0.79; 1.03)	0.99 (0.98; 1.00)	1.01 (0.98; 1.03)
Non-coded AAs								
Citrulline	1.03 (0.97; 1.10)	0.96 (0.88; 1.05)	0.99 (0.92; 1.07)	0.97 (0.90; 1.04)	1.05 (0.96; 1.14)	0.99 (0.89; 1.09)	0.97 (0.93; 1.02)	1.00 (0.96; 1.04)
Ornithine	0.99 (0.95; 1.04)	1.00 (0.99; 1.02)	0.98 (0.95; 1.02)	1.01 (1.00; 1.02)	1.00 (0.96; 1.05)	1.00 (0.99; 1.02)	1.00 (0.98; 1.02)	1.01 (1.00; 1.01)
Taurine	1.00 (1.00; 1.00)	1.00 (0.99; 1.01)	1.00 (1.00; 1.01)	1.00 (1.00; 1.01)	1.00 (1.00; 1.01)	1.00 (0.99; 1.01)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)
Sum								
All FAAs	1.00 (1.00; 1.00)	0.99 (0.99; 0.99)†	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	0.99 (0.99; 0.99)†	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)

FAA: free amino acid; EAA: essential amino acid; NEAA: non-essential amino acid; RR: risk ratio. ‡ $p < 0.01$, * p -value remains statistically significant following Bonferroni adjustment. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/22 = 0.002$. P -values < 0.002 are indicated in bold. Models were adjusted for maternal pre-pregnancy body mass index (BMI), maternal age, infant sex, maternal education and smoking status.

Table S6C. Adjusted model results for associations between free amino acids in human milk with infant food allergy.

FAA	Food allergy	
	6 weeks (n = 13) RR (95% CI)	6 months (n = 7) RR (95% CI)
EAA s		
Histidine	1.02 (0.80; 1.29)	1.00 (0.99; 1.01)
Isoleucine	0.99 (0.82; 1.17)	1.00 (0.97; 1.03)
Leucine	0.99 (0.86; 1.14)	1.00 (0.99; 1.01)
Lysine	0.98 (0.83; 1.15)	1.00 (0.99; 1.01)
Methionine	0.93 (0.85; 1.02)	1.00 (0.99; 1.01)
Phenylalanine	1.01 (0.86; 1.18)	1.00 (0.97; 1.03)
Threonine	0.95 (0.89; 1.03)	1.00 (1.00; 1.00)
Tryptophan	0.97 (0.78; 1.22)	1.00 (0.99; 1.01)
Valine	1.00 (0.97; 1.03)	1.00 (0.99; 1.01)
NEAA s/conditionally EAA		
Alanine	0.99 (0.96; 1.02)	1.00 (1.00; 1.00)
Arginine	0.92 (0.86; 0.99)†	1.00 (0.98; 1.02)
Asparagine	0.70 (0.58; 0.84)*	1.00 (0.98; 1.02)
Aspartate	0.97 (0.92; 1.03)	1.00 (1.00; 1.00)
Glutamate	0.94 (0.83; 1.04)	1.00 (1.00; 1.00)
Glutamine	0.85 (0.69; 1.02)	1.00 (1.00; 1.00)
Glycine	0.98 (0.94; 1.02)	1.00 (1.00; 1.00)
Serine	0.97 (0.93; 1.01)	1.00 (1.00; 1.00)
Tyrosine	0.93 (0.80; 1.07)	1.00 (0.98; 1.02)

Non-coded AAs		
Citrulline	0.85 (0.71; 0.98)†	1.00 (0.98; 1.03)
Ornithine	0.98 (0.82; 1.18)	1.00 (0.99; 1.01)
Taurine	0.99 (0.97; 1.02)	1.00 (1.00; 1.00)
Sum		
All FAAs	0.94 (0.84; 1.05)	1.00 (1.00; 1.00)

FAA: free amino acid; EAA: essential amino acid; NEAA: non-essential amino acid; RR: risk ratio. † $p < 0.05$, * p -value remains statistically significant following Bonferroni adjustment. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/23 = 0.002$. P -values < 0.002 are indicated in bold. Models were adjusted for maternal pre-pregnancy body mass index (BMI), maternal age, infant sex, maternal education and smoking status.