

SUPPLEMENTARY DATA

Supplementary Table 1. Islet autoantibody status in study children and samples

Age at first sample (years)	HLA DRB1	HLA DQB1	AB status in first sample	AB status overall	Age at T1D manifestation (years)	Methionin level (μM)
0.72	03/04	02/0302	IAA	IAA, GADA, IA-2A, ZnT8A	6.9	16.47
0.80	03/04	0302/0302	IAA	IAA		10.88
0.85	03/04	02/0302	IAA	IAA		15.03
1.03	04/04	0301/0302	IAA	IAA, GADA, ZnT8A		16.45
1.06	03/04	02/0302	GADA	GADA		19.37
1.26	03/04	02/0302	IAA, GADA	IAA, GADA, IA-2A, ZnT8A	6.0	27.77
1.51	03/07	02/02	IAA	IAA, GADA		17.45
1.53	03/03	02/02	GADA	IAA, GADA, ZnT8A	6.2	16.75
1.54	03/04	02/0304	IAA	IAA, GADA, ZnT8A		13.75
1.62	04/04	0302/0302	ZnT8A	IAA, GADA, IA-2A, ZnT8A		29.09
2.14	03/04	02/0302	IAA, GADA	IAA, GADA		18.04
2.55	03/04	02/0302	IAA,	IAA, GADA, IA-2A, ZnT8A		17.71
3.02	03/03	02/02	GADA	GADA		23.26
7.98	03/04	0302/0302	GADA	GADA		20.37
8.03	03/07	02/02	IAA	IAA		28.63
8.03	03/03	02/0301	IAA	IAA		18.67
8.07	04/07	0302/02	IAA	IAA		34.66
8.10	12/08	0301/0402	GADA	GADA		34.02
8.17	03/04	02/0302	IAA, IA2, ZnT8A	IAA, GADA, IA-2A, ZnT8A	10.0	27.46
8.18	04/04	0302/0302	IAA	IAA		30.17
8.32	03/04	02/0302	IAA	IAA, GADA, ZnT8A		31.66
8.34	04/04	0302/0301	GADA	GADA	16.3	29.54
8.50	01/03	0501/02	IAA	IAA, GADA		30.80
8.98	01/03	0501/02	GADA	GADA		30.60
9.18	12/12	0301/0301	GADA	GADA		31.05
9.32	04/07	0302/02	GADA	GADA		24.87
11.05	03/11	02/0301	IAA	IAA, GADA		35.95
11.06	03/04	02/0302	IAA	IAA		37.30
11.08	01/04	0501/0302	IAA, GADA, ZnT8A	IAA, GADA, IA-2A, ZnT8A		29.91
13.13	03/04	02/0305	IA-2A	IA-2A		27.86
13.85	03/03	02/02	GADA, ZnT8A	IAA, GADA, ZnT8A	16.5	37.06
13.87	01/04	0501/0302	IAA, GADA	IAA, GADA, IA-2A, ZnT8A	16.7	25.89
14.03	16/15	0502/0602	IAA	IAA		37.67
14.17	03/13	02/0604	GADA	IAA, GADA		38.11
14.27	16/04	0302/0502	IAA	IAA		49.82

Abbreviations: AB, autoantibody; T1D, type 1 diabetes.

SUPPLEMENTARY DATA

Supplementary Table 2. Description of lipid clusters obtained from lipidomics platform.

Cluster name	Cluster size	Description	Representative abundant lipids
		(dominant lipid classes)	
LC1	55	Diverse, mainly different phospholipids	PC(38:4), PE(36:6e), PE(36:3)
LC2	27	lysoPCs	lysoPC(16:0), lysoPC(20:4)
LC3	83	Major phospholipids, mainly PCs	PC(36:2), PC(34:2), PE(38:2)
LC4	72	Sphingomyelins	SM(d18:1/24:1), SM(d18:1/16:0)
LC5	23	PUFA-containing PCs and TGs	PC(38:6), PC(40:6), TG(56:6), TG(58:9)
LC6	59	PEs	PE(38:4), PE(40:4), PE(38:4e)
LC7	39	MUFA containing TGs	TG(16:0/18:2/18:1), TG(18:1/18:1/18:1), TG(18:1/18:2/18:1)
LC8	60	Diverse, PUFA containing PCs, odd-chain TGs	PC(40:7), PC(38:5), PC(36:4), TG(55:2), TG(55:4), TG(49:3)
LC9	37	SFA and MUFA containing TGs	TG(16:0/18:1/18:1), TG(14:0/18:1/18:1), TG(17:0/18:1/18:1)
LC10	19	Short-chain TGs	TG(42:0), TG(44:0), TG(48:2)
LC11	12	Odd-chain SFA containing TGs	TG(49:1), TG(51:1), TG(45:1)
LC12	24	SFA containing TGs	TG(16:0/18:0/18:1), TG(14:0/16:0/18:0)

Abbreviations: lysoPC, lysophosphatidylcholine; MUFA, monounsaturated fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SM, sphingomyelin; TG, triglyceride.

SUPPLEMENTARY DATA

Supplementary Table 3. Serum lipid concentrations of individual lipids from LC8 in the first antibody-positive samples from islet autoantibody-positive children and in aged-matched samples from autoantibody-negative BABYDIAB children

Lipids	Children	Children	p	Children	Children	p
	Age ≤ 2 years	Age ≤ 2 years		Age ≥ 8 years	Age ≥ 8 years	
	AB-positive	AB-negative		AB-positive	AB-negative	
(μM)	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Cer(d18:1/22:6)	10.2 (9.0-10.6)	7.4 (7.2-7.9)	2×10^{-6}	9.2 (8.0-10.7)	7.9 (7.5-8.3)	0.0002
DG(33:5)	0.3 (0.3-0.4)	0.3 (0.3-0.3)	0.003	0.4 (0.3-0.4)	0.3 (0.3-0.3)	0.001
LysoPC(18:3)	12.4 (10.7-13.5)	10.1 (9.4-11.0)	0.016	13.1 (11.5-15.7)	11.0 (9.9-12.6)	0.001
PC(34:3)	0.4 (0.4-0.4)	0.3 (0.3-0.4)	0.001	0.4 (0.4-0.5)	0.3 (0.3-0.4)	0.0001
PC(36:4)	5.9 (5.7-6.6)	4.8 (4.5-5.4)	5×10^{-5}	6.2 (5.6-6.9)	5.1 (4.7-5.5)	9×10^{-6}
PC(36:5)	6.2 (5.6-7.0)	4.5 (4.1-4.9)	2×10^{-6}	5.8 (5.0-6.7)	4.5 (4.3-4.9)	4×10^{-7}
PC(38:5)	8.0 (7.1-8.4)	5.6 (5.3-5.9)	2×10^{-6}	7.5 (6.7-8.7)	5.8 (5.4-6.4)	2×10^{-7}
PC(40:7)	6.1 (5.3-6.3)	5.0 (4.8-5.5)	0.007	6.7 (6.2-7.6)	5.9 (5.4-6.2)	2×10^{-6}
PE(34:4e)	12.3 (11.1-13.6)	9.4 (8.5-10.5)	0.0003	10.8 (9.7-12.7)	10.1 (9.4-10.6)	0.03
TG(16:0/18:1/16:0)	1.2 (1.0-1.4)	0.7 (0.6-0.9)	0.0001	1.1 (0.9-1.3)	0.8 (0.7-0.8)	2×10^{-5}
TG(49:3)	2.0 (1.9-2.5)	1.8 (1.5-2.0)	0.08	2.5 (2.2-2.9)	2.1 (1.8-2.4)	0.009
TG(50:2)	0.7 (0.6-0.8)	0.5 (0.5-0.6)	0.005	0.8 (0.7-0.9)	0.7 (0.5-0.7)	0.002
TG(55:2)	2.7 (2.1-2.9)	2.0 (1.8-2.4)	0.019	2.4 (2.2-3.1)	2.3 (1.9-2.5)	0.04
TG(55:3)	2.3 (1.9-2.7)	2.0 (1.6-2.1)	0.05	2.4 (2.1-2.8)	2.0 (1.7-2.4)	0.009
TG(55:4)	1.9 (1.6-2.5)	1.7 (1.3-1.8)	0.007	1.8 (1.3-2.1)	1.4 (1.4-1.8)	0.18

Abbreviations: Cer, ceramides; DG, Diglyceride; LysoPC, lysophosphatidylcholine; PC, phosphatidylcholine; TG, triglyceride; PE, phosphatidylethanolamine; AB, autoantibody; IQR, interquartile range.

SUPPLEMENTARY DATA

Supplementary Table 4. Serum concentrations of methionine and LC8 lipids in the first antibody-positive samples from islet autoantibody-positive children and in aged-matched samples from autoantibody-negative BABYDIAB children

Children	n	Methionine	p*	LC8	p*
		[μ M]		[rel. av. lipid conc.]	
		Median (IQR)		Median (IQR)	
AB-positive	35	27.9 (18.0-31.7)	0.005	0.48 (-0.07-0.99)	<0.0001
IAA+	22	27.6 (17.0-33.2)	0.009	0.54 (0.14-0.96)	<0.0001
GADA+	15	27.8 (20.4-31.1)	0.02	0.45 (-0.16-1.26)	<0.0001
Single AB+	29	28.6 (17.6-32.8)	0.007	0.48 (-0.18-1.00)	<0.0001
Multiple ABs+	6	27.6 (22.0-33.5)	0.16	0.54 (0.35-1.43)	0.0002
AB-negative	35	33.7 (26.9-37.2)		-0.51 (-0.81- -0.31)	

*comparison with AB-negative children, Mann-Whitney-U test;
Abbreviations: AB, autoantibody; IQR, interquartile range.

SUPPLEMENTARY DATA

Supplementary Table 5. Correlation of amino acid metabolite concentrations between first and second serum samples obtained 1 year apart.

Metabolites	r_s	p
Hydroxyproline	0.4	0.005
Histidine	0.2	0.1
Asparagine	0.4	0.001
3-Methylhistidine	-0.03	0.9
Taurine	0.3	0.008
1-Methylhistidine	0.5	<0.001
Serine	0.05	0.7
Glutamine	0.5	<0.001
Carnitine	0.4	0.002
Arginine	0.6	<0.001
Glycine	0.2	0.09
Ethanolamine	0.3	0.007
Aspartic acid	0.2	0.1
Glutamic acid	0.3	0.009
Citric acid	0.3	0.04
Beta-Alanine	0.5	<0.001
Threonine	0.3	0.01
Alanine	0.3	0.03
Proline	0.3	0.006
Ornithine	0.5	<0.001
ABBA	0.4	0.002
Lysine	0.4	0.001
Tyrosine	0.2	0.1
Methionine	0.6	<0.001
Valine	0.4	0.001
Isoleucine	0.3	0.02
Leucine	0.3	0.03
Phenylalanine	0.2	0.06
Tryptophan	0.3	0.04

SUPPLEMENTARY DATA

Supplementary Table 6. Correlation of relative concentrations of lipid clusters between first and second serum samples obtained 1 year apart.

Lipid cluster	r_s	p
LC1	0.2	0.1
LC2	0.4	0.005
LC3	0.4	<0.001
LC4	0.3	0.01
LC5	0.7	<0.001
LC6	0.4	0.001
LC7	0.8	<0.001
LC8	0.8	<0.001
LC9	0.6	<0.001
LC10	0.4	0.001
LC11	0.5	<0.001
LC12	0.4	0.002

SUPPLEMENTARY DATA

Supplementary Table 7. Serum concentrations of metabolites from the amino acid metabolism in the first antibody-positive samples from islet autoantibody-positive children and in aged-matched samples from autoantibody-negative BABYDIAB children

Metabolites	Children	Children	p	Children	Children	p	Children	Children	p
	Age ≤ 2 years	Age ≥ 8 years		Age ≤ 2 years	Age ≤ 2 years		Age ≥ 8 years	Age ≥ 8 years	
				AB-positive	AB-negative		AB-positive	AB-negative	
(μM)	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Hydroxyproline	25.7 (18.9-32.5)	26.0 (21.6-32.3)	0.68	25.5 (18.2-30.6)	26.6 (20.7-32.8)	0.51	24.3 (20.7-28.0)	28.8 (23.9-38.4)	0.03
Histidine	105.4 (95.1-120.5)	113.6 (101.3-127.5)	0.36	102.9 (91.4-134.0)	113.5 (96.2-120.6)	0.51	113.5 (104.9-138.4)	113.8 (92.7-123.7)	0.52
Asparagine	87.3 (80.5-117.6)	92.6 (69.0-117.8)	0.92	85.3 (77.7-109.5)	89.3 (81.4-123.0)	0.58	102.5 (83.1-120.7)	79.6 (64.0-105.5)	0.06
3-Methylhistidine*	10.0 (6.2-43.1)	10.6 (6.3-17.2)	0.70	7.3 (4.6-131.0)	11.4 (6.8-19.1)	0.90	10.3 (6.3-17.2)	11.0 (7.4-23.2)	0.85
Taurine	234.3 (157.8-269.7)	213.5 (108.4-251.5)	0.26	235.5 (146.8-256.5)	233.1 (157.5-290.6)	0.58	224.3 (164.6-249.6)	180.6 (73.8-267.2)	0.20
1-Methylhistidine*	8.1 (5.3-1.6)	5.5 (4.7-7.0)	0.01	6.7 (4.9-7.9)	11.2 (8.0-12.0)	0.04	5.5 (4.7-7.0)	5.5 (4.8-7.0)	0.77
Serine	202.2 (174.2-229.0)	202.7 (170.3-242.0)	0.49	199.3 (164.4-228.6)	202.4 (178.4-230.2)	0.69	208.5 (183.6-265.3)	197.0 (167.9-238.0)	0.37
Glutamine	627.4 (535.2-689.8)	717.0 (635.9-810.7)	0.004	609.7 (545.8-693.9)	644.7 (504.2-702.5)	0.76	696.0 (587.1-790.4)	740.4 (676.7-833.1)	0.26
Carnitine*	7.1 (5.4-9.1)	6.7 (4.6-9.9)	0.55	6.4 (5.3-8.5)	8.0 (5.4-10.7)	0.47	5.6(4.2-10.2)	6.7 (4.6-9.7)	0.36
Arginine	59.5 (42.8-83.9)	100.5 (51.3-160.4)	0.008	59.1 (43.4-80.9)	59.9 (42.7-89.9)	0.84	85.9 (42.9-130.0)	129.1 (62.4-188.8)	0.15
Glycine	167.3 (133.7-201.2)	197.5 (176.6-234.3)	0.0004	170.8 (124.8-201.7)	165.7 (133.7-196.7)	0.92	191.6 (172.6-247.6)	203.3 (177.1-223.7)	0.55
Ethanolamine	23.7 (21.5-30.3)	26.6 (16.9-30.4)	0.93	22.3 (19.3-23.9)	27.4 (23.1-31.0)	0.02	27.4 (19.8-30.7)	22.6 (15.6-30.6)	0.17
Aspartic acid	43.8 (18.1-63.0)	41.4 (29.2-59.4)	0.37	26.0 (16.8-48.3)	52.7 (21.8-63.4)	0.26	52.4 (32.7-62.5)	36.6 (27.8-57.1)	0.14
Glutamic acid	272.3 (236.2-317.6)	283.4 (118.2-346.9)	0.57	243.2 (222.8-272.3)	310.6 (266.6-351.4)	0.03	301.4 (145.6-358.5)	171.2 (107.7-340.7)	0.26
Citric acid	27.0 (24.2-34.5)	32.9 (29.2-38.5)	0.006	27.1 (25.4-34.1)	26.3 (21.9-34.7)	0.51	33.9 (28.9-38.8)	32.4 (29.5-38.3)	0.74
Beta-Alanine*	5.6 (5.0-7.9)	5.3 (4.4-6.3)	0.20	5.8 (4.5-50.6)	5.6 (5.0-7.6)	0.85	5.5 (4.7-6.4)	4.8 (4.2-6.3)	0.25
Threonine	136.8 (126.6-167.8)	148.3 (123.1-168.4)	0.74	132.8 (125.8-159.3)	139.7 (127.5-185.0)	0.65	155.6 (139.1-180.2)	140.8 (112.7-167.1)	0.14
Alanine	424.6 (358.7-523.6)	497.3 (428.5-588.7)	0.02	444.1 (358.1-525.7)	413.9 (347.8-511.7)	0.84	469.1 (428.1-566.7)	519.3 (413.8-631.4)	0.54
Proline	237.3 (187.3-271.6)	210.3 (187.9-243.8)	0.09	217.2 (185.6-269.2)	249.2 (207.6-306.5)	0.39	201.4 (186.6-226.3)	220.0 (191.5-272.7)	0.17
Ornithine	102.9 (82.3-116.3)	118.1 (50.6-146.8)	0.64	103.4 (73.1-116.8)	102.3 (86.4-117.1)	0.65	135.2 (62.0-150.4)	81.4 (46.3-138.3)	0.20
ABBA	17.4 (14.5-23.7)	20.1 (16.2-24.6)	0.21	16.9 (15.1-22.5)	20.5 (13.8-24.9)	0.55	22.2 (17.2-25.7)	19.5 (14.6-23.2)	0.19
Lysine	196.0 (172.7-230.3)	217.5 (175.3-260.0)	0.17	195.7 (166.6-208.9)	228.7 (173.6-248.0)	0.17	229.3 (178.0-264.2)	209.2 (170.3-245.9)	0.13
Tyrosine	87.6 (71.0-123.8)	89.8 (73.1-98.8)	0.56	80.9 (70.2-99.2)	108.4 (72.1-126.0)	0.29	89.7 (75.3-100.0)	89.8 (70.2-99.2)	0.43
Methionine	27.2 (17.3-37.1)	30.7 (26.4-36.1)	0.13	17.4 (15.7-21.3)	36.3 (29.3-41.8)	9x10 ⁻⁶	30.7 (27.8-36.2)	30.4 (24.2-36.2)	0.72
Valine	244.0 (212.4-334.9)	249.3 (226.0-298.0)	0.93	241.2 (210.5-294.7)	274.5 (212.5-390.4)	0.31	273.4 (229.5-302.7)	247.5 (213.4-278.0)	0.21
Isoleucine	78.7 (63.8-116.5)	73.6 (61.7-86.6)	0.21	68.2 (63.1-97.8)	99.2 (61.5-126.0)	0.36	78.0 (63.5-90.3)	70.8 (61.4-80.6)	0.09

SUPPLEMENTARY DATA

Leucine	159.6 (133.5-230.6)	168.2 (140.0-192.5)	0.90	145.1 (132.0-178.9)	198.5 (134.6-258.7)	0.14	180.0 (154.7-204.5)	162.8 (138.2-181.2)	0.14
Phenylalanine	96.0 (75.0-118.3)	99.9 (84.7-110.9)	0.54	92.3 (73.7-106.2)	107.8 (77.1-122.0)	0.31	101.1 (84.1-114.6)	96.0 (85.8-110.7)	0.67
Tryptophan	62.7 (52.4-76.6)	60.9 (54.2-69.2)	0.54	60.6 (51.3-75.7)	68.8 (51.5-83.7)	0.65	59.8 (51.1-68.0)	62.1(55.0-70.4)	0.48

*some values below detection limit, Mann-Whitney non-parametric test; Abbreviations: AB, autoantibody; IQR, interquartile range.

SUPPLEMENTARY DATA

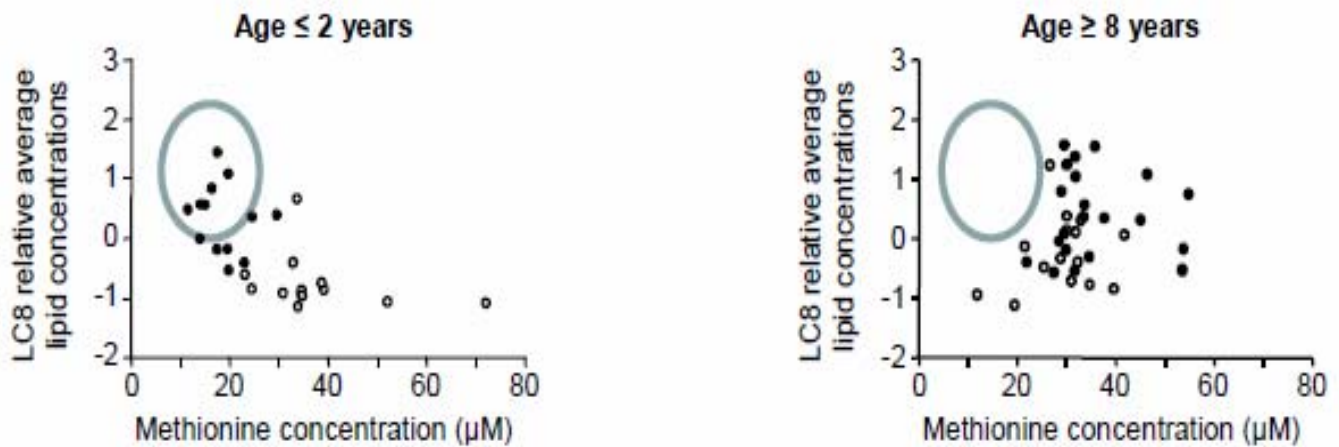
Supplementary Table 8. Relative serum concentrations of lipid clusters in the first antibody-positive samples from islet autoantibody-positive children and in aged-matched samples from autoantibody-negative BABYDIAB children

Lipid cluster	Children	Children	P	Children	Children	P	Children	Children	P
	Age ≤ 2 years	Age ≥ 8 years		Age ≤ 2 years	Age ≤ 2 years		Age ≥ 8 years	Age ≥ 8 years	
				AB-positive	AB-negative		AB-positive	AB-negative	
(relative average lipid concentration)	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
LC1	-0.03 (-0.2- 0.3)	-0.07 (-0.3-0.1)	0.24	0.40 (-0.1-0.4)	-0.04 (-0.3-0.2)	0.19	0.02 (-0.3-0.2)	-0.10 (-0.3-0.0)	0.06
LC2	-0.65 (-1.0- -0.5)	0.34 (-0.3-1.0)	5x10 ⁻⁹	-0.82 (-1.0- -0.4)	-0.63 (-0.9- -0.4)	0.51	0.19 (-0.2-1.2)	0.54 (-0.3-0.9)	0.77
LC3	-0.18 (-0.5- -0.0)	0.08 (-0.2-0.5)	0.001	-0.2 (-0.5- -0.1)	-0.13 (-0.7-0.1)	0.69	0.28 (-0.1-0.9)	0.01 (-0.3-0.2)	0.08
LC4	-0.29 (-0.60-0.0)	0.07 (-0.2-0.5)	0.002	-0.60 (-0.6- -0.1)	-0.01 (-0.4-0.1)	0.13	0.21 (-0.1-0.7)	0.05 (-0.3-0.5)	0.18
LC5	-0.21 (-0.6-0.6)	0.11(-0.4-0.7)	0.15	-0.32 (-0.5-0.6)	-0.17 (-0.7-0.3)	0.72	0.40 (-0.5-1.3)	0.08 (-0.4-0.6)	0.49
LC6	-0.28 (-0.6- -0.0)	0.09 (-0.4-0.6)	0.004	-0.27 (-0.5-0.0)	-0.29 (-0.8- -0.0)	0.51	0.05 (-0.4-0.8)	0.14 (-0.4-0.5)	0.97
LC7	0.25 (-0.4- 1.2)	-0.20 (-0.6-0.4)	0.06	0.59 (-0.2-1.3)	-0.24 (-0.5-0.7)	0.09	-0.18 (-0.7-0.4)	-0.20 (-0.6-0.3)	0.97
LC8	-0.30 (-0.6-0.7)	-0.18 (-0.5-0.5)	0.53	0.74 (0.05-1.0)	-0.52 (-0.9- -0.4)	3 x 10 ⁻⁵	0.47 (-0.1-1.0)	-0.42 (-0.7--0.3)	2 x 10 ⁻⁶
LC9	0.08 (-0.5-0.8)	-0.35 (-0.7-0.7)	0.32	0.45 (-0.3-0.8)	-0.26 (-0.6-0.8)	0.48	-0.35 (-0.7-0.3)	-0.23 (-0.7-0.9)	0.70
LC10	0.29 (-0.3- 1.7)	-0.44 (-0.7-0.0)	4x10 ⁻⁵	0.22 (-0.3-1-7)	0.57 (-0.3-1.8)	0.80	-0.46 (-0.7- -0.2)	-0.30 (-0.6-0.1)	0.51
LC11	0.01 (-0.5-0.6)	-0.38 (-0.6-0.1)	0.07	0.003 (-0.5-0.5)	0.04 (-0.5-0.6)	0.76	-0.41 (-0.6- -0.1)	-0.33 (-0.6-0.3)	0.59
LC12	0.003 (-0.3-1.2)	-0.27 (-0.7--0.0)	0.005	0.6 (-0.3-1.2)	-0.18 (-0.6-1.0)	0.42	-0.28 (-0.7- -0.1)	-0.24 (-0.8-0.1)	0.90

Abbreviations: AB, autoantibody; IQR, interquartile range.

SUPPLEMENTARY DATA

Supplementary Figure 1. Methionine concentrations are plotted against LC8 concentrations for the follow-up samples from children aged ≤ 2 years (left panel) and children aged ≥ 8 years (right panel). Concentrations are negatively correlated in the younger age group ($r = -0.745$, $p < 0.0001$), but do not correlate in the older age group ($p = 0.3$). Islet autoantibody-positive children (filled circles) and autoantibody-negative children (open circles) are indicated. Circled are children with a low methionine/high LC8 profile.



SUPPLEMENTARY DATA

Supplementary Figure 3. Methionine concentration in sera from 5 adult blood donors. Samples were processed and frozen at -80 °C either immediately after blood drawing or after 1, 5 and 24 hours of exposure to room temperature (19 to 23 °C), respectively.

