**Table S1**.

Significantly increased serum metabolites of TAI patients compared with HC in early pregnancy.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Metabolites(n=50) | Formula | KEGG ID | HMDB ID | Lipid Maps ID | VIP | FC | *P* |
| 6-Methylquinoline | C10 H9 N | -- | HMDB0033115 | -- | 2.55  | 1.55  | < 0.001  |
| D-Serine | C3 H7 N O3 | cpd:C00740 | HMDB0003406 | -- | 2.63  | 1.57  | < 0.001  |
| D-Erythrose 4-phosphate | C4 H9 O7 P | cpd:C00279 | HMDB0001321 | -- | 2.57  | 1.79  | < 0.001  |
| PC (16:2e/16:0) | C40 H78 N O7 P | -- | -- | -- | 2.43  | 1.70  | < 0.001  |
| Stearoyl Ethanolamide | C20 H41 N O2 | -- | HMDB0013078 | -- | 2.25  | 1.35  | < 0.001  |
| N3,N4-Dimethyl-L-arginine | C8 H18 N4 O2 | -- | -- | -- | 2.21  | 1.28  | < 0.001  |
| 5-chloro-6-(trifluoromethyl)-1,3-dihydro-2H-benzimidazole-2-thione | C8 H4 Cl F3 N2 S | -- | -- | -- | 2.19  | 1.32  | < 0.001  |
| Asparagine | C4 H8 N2 O3 | cpd:C16438 | HMDB0000168 | -- | 2.20  | 1.41  | < 0.001  |
| PC (16:0e/16:0) | C40 H82 N O7 P | -- | -- | -- | 2.01  | 1.52  | < 0.001  |
| L-Glutamic acid | C5 H9 N O4 | cpd:C00025 | HMDB0000148 | -- | 1.92  | 1.41  | < 0.001  |
| L-(-)-Methionine | C5 H11 N O2 S | -- | HMDB0000696 | -- | 1.78  | 1.43  | 0.001  |
| D-(-)-Glutamine | C5 H10 N2 O3 | -- | -- | -- | 1.70  | 1.41  | 0.001  |
| Acipimox | C6 H6 N2 O3 | -- | -- | -- | 1.68  | 1.31  | 0.001  |
| PC (14:0e/18:0) | C40 H82 N O7 P | -- | -- | -- | 1.67  | 1.49  | 0.002  |
| Cnidioside A | C17 H20 O9 | -- | -- | -- | 1.60  | 1.38  | 0.004  |
| Gly-Phe | C11 H14 N2 O3 | -- | HMDB0028848 | -- | 1.57  | 1.30  | 0.004  |
| methyl 3,4,5-trihydroxycyclohex-1-ene-1-carboxylate | C8 H12 O5 | -- | -- | -- | 1.56  | 1.28  | 0.004  |
| FPK | C20 H30 N4 O4 | -- | -- | -- | 1.58  | 1.21  | 0.004  |
| Propionylcarnitine | C10 H19 N O4 | -- | HMDB0000824 | LMFA07070105 | 1.57  | 1.36  | 0.006  |
| SM (d14:3/28:2) | C47 H87 N2 O6 P | -- | -- | -- | 1.37  | 1.28  | 0.010  |
| methyl 4-methyl-2-oxo-2H-pyran-6-carboxylate | C8 H8 O4 | -- | -- | -- | 1.45  | 1.96  | 0.010  |
| 6beta-Naltrexol-d3 | C20 H22 [2]H3 N O4 | -- | -- | -- | 1.49  | 1.22  | 0.011  |
| Lysopc 20:4 | C28 H50 N O7 P | -- | -- | -- | 1.39  | 1.46  | 0.016  |
| gamma-Glutamylleucine | C11 H20 N2 O5 | -- | HMDB0011171 | -- | 1.33  | 1.42  | 0.023  |
| 4-Hydroxyisoleucine | C6 H13 N O3 | -- | -- | -- | 1.28  | 1.35  | 0.023  |
| Terephthalic Acid | C8 H6 O4 | cpd:C06337 | HMDB0002428 | -- | 1.15  | 1.36  | 0.033  |
| 1-(2-furyl)pentane-1,4-dione | C9 H10 O3 | -- | -- | -- | 1.26  | 1.23  | 0.034  |
| PC (20:3e/18:0) | C46 H88 N O7 P | -- | -- | -- | 1.17  | 1.21  | 0.036  |
| ACar 16:1 | C23 H44 N O4 | -- | -- | -- | 1.23  | 1.42  | 0.039  |
| 2,3-dihydroxypropyl 12-methyltridecanoate | C17 H34 O4 | -- | -- | -- | 1.15  | 1.26  | 0.042  |
| Lysopc 18:2 | C26 H50 N O7 P | -- | -- | LMGP01050137 | 1.14  | 1.52  | 0.042  |
| ACar 14:0 | C21 H42 N O4 | -- | -- | -- | 1.22  | 1.63  | 0.043  |
| ANH | C13 H20 N6 O5 | -- | -- | -- | 1.31  | 1.74  | 0.044  |
| Irganox 259 | C40 H62 O6 | -- | -- | -- | 1.16  | 1.29  | 0.047  |
| 3-hydroxy-3,4-bis[(4-hydroxy-3-methoxyphenyl)methyl]oxolan-2-one | C20 H22 O7 | -- | -- | -- | 1.11  | 1.33  | 0.048  |
| PA (11:0/18:3) | C32 H57 O8 P | -- | -- | -- | 2.74  | 1.40  | < 0.001  |
| 3-Phosphoglyceric acid | C3 H8 Na O6 P | -- | HMDB0000807 | -- | 2.31  | 1.27  | 0.001  |
| LPI 16:0 | C25 H49 O12 P | -- | -- | LMGP06050002 | 2.36  | 1.35  | 0.002  |
| GM3 d34:1; [M-H]- | C57 H104 N2 O21 | -- | -- | -- | 2.06  | 1.32  | 0.007  |
| Octanedioic acid | C8 H14 O4 | cpd:C08278 | HMDB0000893 | -- | 2.19  | 1.59  | 0.007  |
| cis-Aconitic acid | C6 H6 O6 | cpd:C00417 | HMDB0000072 | -- | 2.04  | 1.21  | 0.008  |
| Methylmalonic acid | C4 H6 O4 | cpd:C02170 | HMDB0000202 | LMFA01170118 | 2.08  | 1.63  | 0.008  |
| PA (18:1/18:2) | C39 H71 O8 P | -- | -- | -- | 1.90  | 1.21  | 0.012  |
| LPC 22:4 | C30 H54 N O7 P | -- | -- | LMGP01050124 | 1.77  | 1.27  | 0.017  |
| L-Threonic acid | C4 H8 O5 | -- | -- | -- | 1.74  | 1.26  | 0.020  |
| PC (16:0/20:3) | C44 H82 N O8 P | -- | -- | LMGP01010627 | 1.63  | 1.27  | 0.032  |
| (S)-2-Hydroxybutanoicacid | C4 H8 O3 | -- | -- | -- | 1.66  | 1.25  | 0.038  |
| PA (11:0/18:0) | C32 H63 O8 P | -- | -- | -- | 1.75  | 1.29  | 0.039  |
| cis-2-Decenoic acid | C10 H18 O2 | -- | HMDB0010726 | -- | 1.57  | 1.56  | 0.043  |
| Cannabidiolic acid | C22 H30 O4 | cpd:C10784 | -- | LMPK13120003 | 1.59  | 1.36  | 0.049  |

**Table S2**

Significantly decreased serum metabolites of TAI patients compared with HC in early pregnancy.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Metabolites(n=42) | Formula | KEGG ID | HMDB ID | Lipid Maps ID | VIP | FC | FDR |
| Methyl palmitate | C17 H34 O2 | cpd:C16995 | HMDB0061859 | -- | 3.06  | 0.23  | < 0.001  |
| N-Desmethyltramadol | C15 H23 N O2 | -- | HMDB0061007 | -- | 2.20  | 0.68  | < 0.001  |
| S-(Methyl)Glutathione | C11 H19 N3 O6 S | -- | -- | -- | 1.94  | 0.70  | < 0.001  |
| Tiglic acid | C5 H8 O2 | cpd:C08279 | HMDB0001470 | LMFA01020030 | 1.89  | 0.63  | 0.001  |
| Kinetin | C10 H9 N5 O | cpd:C08272 | HMDB0012245 | -- | 2.00  | 0.53  | 0.001  |
| Camptothecin | C20 H16 N2 O4 | cpd:C01897 | -- | -- | 1.90  | 0.65  | 0.002  |
| Methionine sulfoxide | C5 H11 N O3 S | -- | HMDB0002005 | -- | 2.01  | 0.78  | 0.002  |
| 3-Methoxybenzaldehyde | C8 H8 O2 | -- | HMDB0031459 | -- | 1.85  | 0.80  | 0.004  |
| 2-((Dimethylamino)methyl)phenol | C9 H13 N O | -- | -- | -- | 1.65  | 0.71  | 0.005  |
| Glycochenodeoxycholic acid sodium salt | C26 H43 N O5 Na | -- | -- | -- | 1.58  | 0.59  | 0.006  |
| PC (18:2e/16:0) | C42 H82 N O7 P | -- | -- | -- | 1.57  | 0.37  | 0.006  |
| Sulfoacetic acid | C2 H4 O5 S | cpd:C14179 | -- | -- | 1.65  | 0.73  | 0.007  |
| PC (20:2/20:2) | C48 H88 N O8 P | -- | -- | LMGP01011856 | 1.54  | 0.77  | 0.008  |
| SM (d24:2/18:0) | C47 H93 N2 O6 P | -- | -- | -- | 1.52  | 0.63  | 0.010  |
| Ecdysterone | C27 H44 O7 | cpd:C02633 | HMDB0030180 | -- | 1.32  | 0.76  | 0.019  |
| CDP | C9 H15 N3 O11 P2 | cpd:C00112 | HMDB0001546 | -- | 1.46  | 0.83  | 0.022  |
| Citraconic acid | C5 H6 O4 | cpd:C02226 | HMDB0000634 | LMFA01170099 | 1.43  | 0.69  | 0.022  |
| Hexadecanamide | C16 H33 N O | -- | HMDB0012273 | LMFA08010009 | 1.59  | 0.83  | 0.023  |
| PC (14:1e/24:4) | C46 H84 N O7 P | -- | -- | -- | 1.42  | 0.78  | 0.023  |
| SM (d22:2/12:0) | C39 H77 N2 O6 P | -- | -- | -- | 1.52  | 0.82  | 0.029  |
| PC (22:5e/16:3) | C46 H78 N O7 P | -- | -- | -- | 1.37  | 0.79  | 0.032  |
| Alloxan | C4 H4 N2 O5 | -- | HMDB0002818 | -- | 1.35  | 0.77  | 0.038  |
| 5,8-dihydroxy-10-methyl-5,8,9,10-tetrahydro-2H-oxecin-2-one | C10 H14 O4 | -- | -- | -- | 1.16  | 0.65  | 0.041  |
| N-(1H-benzo[d]imidazol-2-yl)guanidine | C8 H9 N5 | -- | -- | -- | 1.35  | 0.65  | 0.049  |
| NSI-189 | C22 H30 N4 O | -- | -- | -- | 3.21  | 0.79  | < 0.001  |
| 3-(1-cyano-1,2-dihydroisoquinolin-2-yl)-3-oxopropyl propionate | C16 H16 N2 O3 | -- | -- | -- | 3.16  | 0.72  | < 0.001  |
| Orsellinic acid ethyl ester | C10 H12 O4 | -- | -- | -- | 2.79  | 0.77  | < 0.001  |
| FAHFA (17:0/18:0) | C35 H68 O4 | -- | -- | -- | 2.84  | 0.53  | < 0.001  |
| Methionine | C5 H11 N O2 S | cpd:C01733 | HMDB0000696 | -- | 2.44  | 0.80  | 0.001  |
| 2-Furoic acid | C5 H4 O3 | cpd:C01546 | HMDB0000617 | -- | 2.35  | 0.81  | 0.002  |
| (+/-)-CP 47,497-C7-Hydroxy metabolite | C21 H34 O3 | -- | -- | -- | 1.86  | 0.73  | 0.013  |
| D-Ribose-1-phosphate | C5 H11 O8 P | -- | HMDB0001489 | -- | 1.77  | 0.67  | 0.016  |
| 5,6-dimethyl-3-[5-(trifluoromethyl)pyridin-2-yl]-1,2,4-triazine | C11 H9 F3 N4 | -- | -- | -- | 1.76  | 0.60  | 0.023  |
| P-Aminohippuric Acid | C9 H10 N2 O3 | -- | HMDB0001867 | -- | 1.64  | 0.68  | 0.025  |
| PC (17:1/18:2) | C43 H80 N O8 P | -- | -- | -- | 1.69  | 0.83  | 0.027  |
| MGMG (18:2) | C27 H48 O9 | -- | -- | -- | 1.78  | 0.60  | 0.030  |
| PC (15:0/18:2) | C41 H78 N O8 P | -- | -- | LMGP01010543 | 1.84  | 0.79  | 0.032  |
| PE (18:2e/20:4) | C43 H76 N O7 P | -- | -- | -- | 1.60  | 0.73  | 0.033  |
| 1,4-Cyclohexanedicarboxylic acid | C8 H12 O4 | -- | -- | -- | 1.78  | 0.73  | 0.038  |
| 2,4-Dichlorophenol | C6 H4 Cl2 O | cpd:C02625 | HMDB0004811 | -- | 1.51  | 0.50  | 0.039  |
| PC (17:0/22:6) | C47 H82 N O8 P | -- | -- | LMGP01010720 | 1.54  | 0.78  | 0.045  |
| alpha-Ketoglutaric acid | C5 H6 O5 | cpd:C00026 | HMDB0000208 | -- | 1.52  | 0.82  | 0.050  |

**Table S3**

The list of metabolic pathways related differential metabolites in the TAI group compared to HC group.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Pathway name | x | y | n | N | *P* value | Meta IDs |
| Taurine and hypotaurine metabolism | 2 | 3 | 7 | 2 | 0.027840909 | L-Glutamic acid, Sulfoacetic acid |
| Citrate cycle (TCA cycle) | 2 | 5 | 4 | 81 | 0.017589888 | cis-Aconitic acid, alpha-Ketoglutaric acid |
| Glyoxylate and dicarboxylate metabolism | 2 | 5 | 4 | 81 | 0.017589888 | cis-Aconitic acid, alpha-Ketoglutaric acid |
| 2-Oxocarboxylic acid metabolism | 2 | 6 | 4 | 81 | 0.025929532 | cis-Aconitic acid, Methionine |
| Valine, leucine and isoleucine degradation | 1 | 1 | 4 | 81 | 0.049382716 | Methylmalonic acid |
| D-Glutamine and D-glutamate metabolism | 1 | 1 | 4 | 81 | 0.049382716 | alpha-Ketoglutaric acid |
| Propanoate metabolism | 1 | 1 | 4 | 81 | 0.049382716 | Methylmalonic acid |
| Antifolate resistance | 1 | 1 | 4 | 81 | 0.049382716 | Methionine |

N represents the total number of metabolites involved in the KEGG pathway, while n refers to the number of differential metabolites among those in N. The variable y denotes the number of metabolites annotated to a certain KEGG pathway, and x represents the number of differential metabolites enriched in that pathway. If the ratio condition x/n > y/N and *P* < 0.05, then the pathway is considered a KEGG pathway that is significantly enriched in differential metabolites. Meta IDs is the list of enriched differential metabolites.

**Table S4**

A review of serum metabolomics studies on autoimmune thyroiditis during pregnancy and non-pregnancy.

|  |  |  |  |
| --- | --- | --- | --- |
| References | Research methods | Subjects | Main findings |
| Liu et al. [1] | UFLC-MS/MS analysis | Three groups①43 GD patients with hyperthyroidism②45 HT patients with hypothyroidism③52 HC | ①Both the GD and HT groups had higher levels of glutamine, PC (16:0/22:4), PC (18:2/20:4), and SM (d20:1/22:4) and lower levels of L-glutamic acid, L-citrulline, taurine, and GDCA than HC group. The GD patients had higher levels of L-valine, SM (d18:1/24:1), PC (18:0/22:6), and PC (18:2/20:1) and lower levels of glycine, L-serine, L-ornithine, L-arginine, CDCA, DCA, PC (16:0/18:2), ceramide (d18:1/25:0), LPC 16:0, and SM (d16:0/18:1) than HC group.②Pathway analysis showed that hyperthyroidism had a significant impact on arginine and proline metabolism and aminoacyl-tRNA biosynthesis, while hypothyroidism had a significant impact on alanine, aspartate, and glutamate metabolism. |
| Song et al.[2] | UFLC-MS/MS analysis | Four groups①36 GD patients with clinical hyperthyroidism or subclinical hyperthyroidism②33 HT patients with clinical hypothyroidism or subclinical hypothyroidism③29 TAI patients④38 HC | ①Both GD and HT patients had higher L-arginine, L-ornithine, lysine and agmatine levels and lower putrescine, 1,3-diaminopropane, spermine, N-acetylputrescine levels than HC group. GD patients had significantly higher spermidine, N-acetylspermidine and γ-aminobutyric acid and lower cadaverine than HC group, whereas HT patients had significantly decreased N-acetylspermine than HC group. Only spermine and N-acetylspermine were significantly lower in TAI patients than HC group. ②Spermine was negatively correlated with thyroid-specific antibodies grade.③N-acetylspermidine might be a risk factor for TAI progression to overt hypothyroidism. |
| Struja T et al.[3] | High-throughput proton NMR metabolomics | Four groups①87 GD patients ②17 HT patients ③5 thyroiditis patients④11 toxic goiter patients | No metabolomic biomarker combination was found to distinguish the four disease types. |
| Cai et al.[4] | Nontargeted LC-MS analysis | Two groups①27 pregnant women with hypothyroidism②28 normal pregnant women (HC) | ①A panel consisting of SM (d36:1), PC (38:4), PE (36:4), PC (36:2), PC (16:1/18:1), LPC (18:0), LPC (16:0), PC (40:7), SM (d42:6), SM (d42:7) were identified as characteristic metabolites for patients with hypothyroidism during pregnancy.②Pathway analysis showed that patients with hypothyroidism during pregnancy had a significant impact on autophagy, glycerophospholipid metabolism. |
| [Han et al.](https://www.metaboanalyst.ca/MetaboAnalyst/Secure/pathway/PathResultView.xhtml) [5] | 1H-NMR metabolomics on cord blood | Two groups①11 patients with euthyroid status but anti-TPO antibodies positivity in early pregnancy②11 normal pregnant women (HC) | ①D-Glucose, L-Glutamine, 3-Hydroxybutyric acid, Myo-Inositol, Creatinine were up-regulated and L-Leucine, L-Lysine, L-Glutamic acid, L-Tyrosine, and L-Phenylalanine were down-regulated in the anti-TPO antibodies positivity group. ②Pathway analysis suggested that amino acid metabolism pathways (especially the phenylalanine metabolism) were associated with anti-TPO antibodies positivity. |
| Zhao et al.[6] | 1H-NMR metabolomics on cord blood | Two groups①18 patients with GHT in early pregnancy②18 normal pregnant women (HC) | ①Creatinine and O-Phosphocholine were up-regulated and Carnitine, L-alpha-Aminobutyric acid, Citric acid, Sarcosine, 3-Methyl-2-oxovaleric acid, Tyrosine were down-regulated in the GHT group. The eight differential metabolites were correlated with the GHT related thyroid hormones. ②Pathway analysis suggested that tyrosine metabolism and phenylalanine, tyrosine and tryptophan biosynthesis were significantly altered in GHT. |
| Li et al.[7] | Nontargeted lipidomics | Two groups①30 patients with SCH in late pregnancy②30 normal pregnant women (HC) | ①30 differentially expressed lipid metabolites are potential biomarkers.②Pathway analysis showed that response to pathogenic Escherichia coli infection, regulation of lipolysis in adipocytes, metabolic pathways, glycerophospholipid metabolism, and fat digestion and absorption pathways were significantly altered in SCH group. ③Correlation analyses revealed SM and PC positively correlate to TNF-α, CRP, and IL-6, while PG, and PI negatively correlate with them. In addition, PG positively correlates to birth weight. |
| The current study | Nontargeted LC-MS analysis | Two groups①26 patients with TAI in early pregnancy②30 normal pregnant women (HC) | ①A panel consisting of 15 metabolites (6-Methylquinoline, D-Erythrose 4-phosphate, 4-Hydroxyisoleucine, PC (16:2e/16:0), N3,N4-Dimethyl-L-arginine, N-Desmethyltramadol, 3-Methoxybenzaldehyde, SM (d14:3/28:2), gamma-Glutamylleucine, NSI-189, 3-(1-cyano-1,2-dihydroisoquinolin-2-yl)-3-oxopropyl propionate, LPI 16:0, cis-Aconitic acid, PA (18:1/18:2) and FAHFA (17:0/18:0)) were identified as characteristic metabolites for patients with TAI in early pregnancy.②Pathway analysis showed that taurine and hypotaurine metabolism, citrate cycle (TCA cycle), glyoxylate and dicarboxylate metabolism and 2-oxocarboxylic acid metabolism were significantly altered in TAI group. ③Correlation analyses showed that 6-ethylquinoline and 4-Hydroxyisoleucine had a negative correlation with birth weight. |

UFLC-MS/MS, ultra-fast liquid chromatography–tandem mass spectrometry; GD, Graves’ disease; HT, Hashimoto’s thyroiditis; HC, healthy controls; PC, phosphatidylcholine; SM, sphingomyelin; GDCA, glycodeoxycholic acid; DCA, deoxycholic acid; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LC-MS, mass spectrometry; anti-TPO, anti-thyroid peroxidase; GHT, gestational hypothyroidism; SCH, subclinical hypothyroidism; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; IL-6, interleukin-6; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPI, lysophosphatidylinositol; PA, polyamide; FAHFA, fatty acyl esters of hydroxy fatty acid.



**Fig S1.** Group size estimation using MetSizeR with an estimated significant rate of 20%, FDR 0.05, minimal group size of n=10, and 903 spectral bins.

**References**

1. Liu, J., et al., Serum Metabolomic Patterns in Patients with Autoimmune Thyroid Disease. Endocr Pract, 2020. 26(1): p. 82-96.

2. Song, J., et al., Serum polyamine metabolic profile in autoimmune thyroid disease patients. Clin Endocrinol (Oxf), 2019. 90(5): p. 727-736.

3. Struja, T., et al., Metabolomics and Their Ability to Distinguish Thyroid Disorders: A Retrospective Pilot Study. Horm Metab Res, 2019. 51(4): p. 256-260.

4. Cai, Y., et al., Plasma Lipid Profile and Intestinal Microflora in Pregnancy Women With Hypothyroidism and Their Correlation With Pregnancy Outcomes. Front Endocrinol (Lausanne), 2021. 12: p. 792536.

5. Han, L., et al., Cord blood metabolomics reveals gestational metabolic disorder associated with anti-thyroid peroxidase antibodies positivity. BMC Pregnancy Childbirth, 2022. 22(1): p. 244.

6. Zhao, C., et al., 1H-NMR based metabolomic profiling of cord blood in gestational hypothyroidism. Ann Transl Med, 2020. 8(6): p. 296.

7. Li, J., et al., Differential lipids in pregnant women with subclinical hypothyroidism and their correlation to the pregnancy outcomes. Sci Rep, 2021. 11(1): p. 19689.