



OPEN Serum metabolomic analysis in patients with Hashimoto's thyroiditis positive for TgAb or TPOAb: a preliminary study

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Hashimoto's thyroiditis (HT) is a prevalent autoimmune disorder, yet the metabolic abnormalities associated with HT and their relationship to antibody positivity remain poorly understood. This study aimed to characterize the distinct metabolic profiles associated with thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) positivity in female patients with HT. Serum metabolomic analysis was performed on 14 TPOAb-positive patients, 4 TgAb-positive patients, and 14 sex-matched healthy controls, evaluating a total of 225 metabolites. Partial least squares discriminant analysis (PLS-DA) revealed significant metabolic differences among the groups, identifying 36 key metabolites. Of these, 13 metabolites showed significant differences between the TPOAb-positive group and healthy controls, while 23 metabolites exhibited marked differences between the TgAb-positive group and controls. Further correlation analysis revealed a moderate positive association between TgAb and phenylacetyl-L-glutamine, while TPOAb was strongly correlated with LPC 16:0 sn-1. Additionally, metabolic pathway analysis showed significant activation of glycine, serine, and threonine metabolism in the TPOAb-positive group, whereas the TgAb-positive group exhibited enhanced activity in galactose metabolism. These findings suggest that TPOAb and TgAb positivity are associated with distinct metabolic profiles, reflecting their differential roles in metabolic pathways linked to Hashimoto's thyroiditis. This study provides valuable exploratory evidence of metabolic abnormalities in HT under different antibody-positive states, laying the foundation for future large-scale investigations to elucidate the underlying mechanisms.

Keywords Hashimoto's thyroiditis, Metabolomics, TPOAb, TgAb

Abbreviations

HT	Hashimoto's thyroiditis
AIT	Autoimmune thyroiditis
TPOAb	Thyroid peroxidase antibody
TgAb	Thyroglobulin antibody
GD	Grave's disease
SPM	Spermene
PC	Phosphatidylcholine
SM	Sphingomyelin
LPC	Lysophosphatidylcholine
GDCA	Glucuronide
NSAIDs	Non-steroidal anti-inflammatory drugs
LC-MS	Liquid Chromatograph Mass Spectrometer
MS	Mass spectrometry
SD	Standard deviation
ANOVA	Analysis of variance
PLS-DA	Partial Least Squares-Discriminant Analysis
OPLS-DA	Orthogonal projections to latent structures discriminant analysis

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VIP	Variable influences on projection
ROC	Receiver operating characteristic
TSH	Thyroid stimulating hormone
FT3	Free triiodothyronine
FT4	Free thyroxine
LPE	Lyso Phosphatidyl Ethanolamine
FFA	Free fatty acid
KEGG	Kyoto Encyclopedia of Genes and Genomes
BMI	Body mass index
WC	Waist circumference
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
OGTT2Hpg	2-hour plasma glucose
IFG	Impaired fasting glucose regulation
TH	Thyroid hormones
DOCA	Deoxycholic acid
CDA	Chenodeoxycholic acid
LCA	Lithocholic acid
CDA	Chenodeoxycholic acid
ADCC	Antibody-dependent cell-mediated cytotoxicity
ROS	Reactive oxygen species
TFC	Thyroid follicular cells

Hashimoto's thyroiditis (HT) is the most common form of autoimmune thyroiditis (AIT), characterized by the presence of thyroid autoantibodies in the serum and extensive lymphocytic infiltration of thyroid tissue. Thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb) are key contributors to the pathogenesis and progression of HT¹. Epidemiological studies have shown that HT is significantly more prevalent in women than in men², with the majority of affected individuals presenting with hypothyroidism³. Notably, approximately 50% of women of reproductive age with HT develop postpartum thyroiditis, which poses significant risks to maternal and fetal health. These include impaired fetal neurodevelopment, increased risk of miscarriage, and preterm delivery during pregnancy^{2,4,5}.

TPOAb is a widely recognized diagnostic marker for autoimmune thyroiditis, detectable in 80–90% of AIT patients and 50–60% of Graves' disease (GD) patients². Another antibody relevant to AIT diagnosis is TgAb, although its sensitivity is lower compared to TPOAb. Numerous studies have established a strong association between thyroid autoimmunity and metabolic disorders⁶. However, most research has predominantly focused on the effects of thyroid hormones on glucose and lipid metabolism, leaving the relationship between thyroid autoantibody positivity and metabolic disturbances largely unexplored.

A cross-sectional study of euthyroid adults revealed a significant positive correlation between TPOAb and/or TgAb levels and HbA1c, obesity, and dyslipidemia, particularly among women⁷. Moreover, elevated TPOAb titers were identified as a risk factor for metabolic syndrome and were positively associated with the severity of fatty liver disease, a relationship that was also more pronounced in women⁸. Another large-scale cross-sectional study demonstrated that isolated TgAb positivity could suppress hypertriglyceridemia in women and lower the risk of impaired fasting glucose (IFG) in men. This suggests that TgAb may act as an independent protective factor, potentially mitigating the risk of glucose and lipid metabolic disorders⁹. Additionally, TPOAb (but not TgAb) has been recognized as a predictive marker for the progression from subclinical hypothyroidism to overt hypothyroidism^{10,11}. These findings highlight the significant association between thyroid autoantibody expression and systemic metabolic disturbances. However, the specific mechanisms underlying the distinct roles of TPOAb and TgAb remain unclear and require further investigation.

Moreover, studies suggest that thyroid antibodies influence the levels of various metabolites, including polyamines. Polyamines, essential polycations present in all living cells, play critical roles in thyroid growth and the regulation of nucleic acid metabolism, making them a key focus of metabolomics research¹². Song et al. reported a significant negative correlation between spermine (SPM) levels and serum TgAb and TPOAb levels, independent of thyroid hormone concentrations. Additionally, low arginine levels have been linked to thyroid autoimmunity¹³. Conversely, a study by Ying Wu et al. demonstrated that, after controlling for thyroid hormone effects, thyroid antibody positivity was positively associated with obesity, elevated LDL-C, and hyperuricemia. Furthermore, increased thyroid antibody levels were found to contribute to distinct metabolic disorders in men and women¹⁴. While these findings underscore the relationship between thyroid antibodies and metabolic disturbances, the specific association between isolated TgAb or TPOAb positivity and small-molecule metabolites remains unclear, necessitating further investigation.

Metabolomics is a discipline focused on analyzing dynamic changes in metabolite profiles within biological systems in response to exogenous stimuli. It enables the qualitative and quantitative assessment of small-molecule metabolites and has wide-ranging applications in endocrinology and metabolic diseases, particularly thyroid disorders¹⁵. For instance, Liu et al. conducted metabolomic analyses on serum samples from hyperthyroid and hypothyroid patients, identifying abnormal levels of lipids and amino acids, including phosphatidylcholines (PC), lysophosphatidylcholines (LPC), sphingomyelins (SM), and glycodeoxycholic acid (GDCA)¹⁶. Similarly, Song et al. reported significant differences in serum polyamine metabolite levels between patients with thyroid diseases—such as Graves' disease, papillary thyroid carcinoma, Hashimoto's thyroiditis, and thyroid storm—and healthy controls¹³. In addition, studies have shown that serum metabolite concentrations are significantly altered by treatment, further highlighting the interplay between metabolite expression and disease states^{17,18}.

These findings underscore the potential of metabolite profiling in distinguishing disease states and offer valuable insights for clinical diagnosis and disease management.

However, studies exploring the relationship between small-molecule metabolites and isolated TgAb or TPOAb positivity in female patients with Hashimoto's thyroiditis remain scarce. To address this gap, our study employs serum metabolomic profiling to preliminarily investigate abnormalities in serum metabolites associated with different antibody states in HT and their correlations with thyroid antibodies. This work aims to provide foundational data for future large-scale longitudinal studies that will further elucidate the underlying mechanisms. Ultimately, we seek to validate and clarify the distinct roles of TPOAb and TgAb in the progression of Hashimoto's thyroiditis.

Materials and methods

Samples

The study protocol was approved by the Ethics Committee of the Second Hospital of Dalian Medical University (Approval Number: 2021 NO.073). All procedures were performed in accordance with the principles outlined in the Declaration of Helsinki. A total of thirty-two samples, matched for age and sex, were included and divided into three groups for the study. The experimental group consisted of eighteen patients with Hashimoto's thyroiditis from the Department of Endocrinology at Dalian Medical University. Among them, fourteen patients were assigned to the HT group with positive thyroid peroxidase antibodies (TPOAb (+)), and four were assigned to the HT group with positive thyroglobulin antibodies (TgAb (+)). Fourteen healthy individuals were recruited from the medical examination center of the Second Hospital of Dalian Medical University and assigned to the control group. The inclusion and exclusion criteria were as follows:

Inclusion criteria

The TPOAb (+) group met the following criteria: (a) Female gender. (b) Diffuse swelling of the thyroid gland with surface roughness and nodularity, as determined by ultrasound. (c) Positive thyroid peroxidase antibodies (TPOAb) and normal thyroglobulin antibodies (TgAb). (d) No use of medications or drugs.

The TgAb (+) group met the following criteria: (a) Female gender. (b) Diffuse swelling of the thyroid gland with surface roughness and nodularity, as determined by ultrasound. (c) Positive thyroglobulin antibodies (TgAb) and normal thyroid peroxidase antibodies (TPOAb). (d) No use of medications or drugs.

Exclusion criteria

Any participant who meets any of the following criteria will be excluded: (a) Patients with other autoimmune diseases, such as diabetes mellitus, lupus, or inflammatory bowel disease. (b) Patients with concurrent acute or chronic infectious diseases, such as acute or chronic hepatitis and pneumonia. (c) Patients taking both non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids. (d) Patients with concurrent malignant tumors or immune deficiency. (e) Pregnant or lactating women.

Thyroid function

Under sterile conditions and in a calm environment, 2 to 3 mL of peripheral blood is drawn from the antecubital fossa of the study participants and promptly sent to the central laboratory for analysis. Thyroid function is assessed using the Siemens ADVIA Centaur XP fully automated chemiluminescent immunoassay system.

Metabolic profiling -LC-MS full-component data acquisition

Metabolites were detected using the LC-MS full-component data system, with chromatographic separations performed in both positive and negative ion modes.

Positive ion mode

Chromatographic column: Waters BEH C8 column (50 mm × 2.1 mm, 1.7 μm) (Waters, Milford, MA). Column temperature: 60 °C. Flow rate: 0.4 mL/min. Mobile phases: water with 0.1% formic acid and acetonitrile with 0.1% formic acid. Gradient: The initial gradient was set to 5% B, maintained for 0.5 min, followed by a linear increase to 40% B within 1.5 min. Subsequently, a further linear increase to 100% B occurred over 6 min, maintained for 2 min, then returned to the initial gradient of 5% B at 10.1 min. Finally, the system was equilibrated for 2 min.

Negative ion mode

Chromatographic column: ACQUITY UPLC HSS T3 (50 mm × 2.1 mm, 1.8 μm) (Waters, Milford, MA). Column temperature: 60 °C. Flow rate: 0.4 mL/min. Mobile phase: Phase A consisted of water with 6.5 mM NH₄HCO₃, while Phase B comprised an aqueous solution containing 95% methanol and 6.5 mM NH₄HCO₃. Gradient: The initial gradient was set to 2% B, maintained for 0.5 min, increased to 40% B within 2 min, followed by a linear increase to 100% B within 6 min, maintained for 2 min, and then returned to the initial gradient of 2% B at 10.1 min. Finally, the system was equilibrated for 1.9 min.

Mass spectrometry data acquisition parameters

The mass spectrometry (MS) full scan range for positive ions was set from m/z 80 to 1200 with a spray voltage of 3.50 kV, while for negative ions, the range was also from 80 to 1200 with a spray voltage of 3.00 kV. The capillary temperature was maintained at 300 °C, and the auxiliary heating gas temperature was set to 350 °C. The flow rates for sheath gas and auxiliary gas were 45 and 10 (arbitrary units), respectively. The resolution was set to 7e4. The internal standard for serum sample analysis is provided in Table S1.

Statistical analysis

The statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA). Normally distributed data were expressed as mean ± standard deviation (SD), whereas non-normally distributed data were presented as median with interquartile range. For comparisons among three or more groups, analysis of variance (ANOVA) or Kruskal-Wallis tests were employed, followed by appropriate post-hoc tests. To correct for multiple comparisons, the significance threshold was adjusted using the Bonferroni method, with the adjusted p-value threshold set at 0.017 (0.05/3). A p-value of less than 0.017 was considered statistically significant.

Furthermore, Partial Least Squares Discriminant Analysis (PLS-DA) and Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) were performed using SIMCA version 14.1 (Umetrics AB, Umea, Sweden). Serum metabolites with a Variable Importance in Projection (VIP) score greater than 1.5 in the OPLS-DA model were subjected to statistical significance evaluation using either a t-test or a non-parametric test.

The data were normalized using MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca/>) to minimize systematic bias and enhance consistency. Features with more than 25% missing values were excluded, and any remaining missing values were imputed with the mean of the original data. The data were normalized by sum, mean-centered, and scaled by dividing each variable by the square root of its standard deviation. Enrichment analysis and heatmap generation of differential metabolites were conducted using MetaboAnalyst. Correlation analysis was performed using Origin 2021. The receiver operating characteristic (ROC) curve was generated using SPSS, and metabolite point plotting was carried out using GraphPad Prism 9.0.

Results

Baseline parameters

The baseline parameters of the control, TPOAb (+), and TgAb (+) groups are presented in Table 1. Significant differences were observed in the levels of thyroid peroxidase antibody (TPOAb) between the control and TPOAb (+) groups. However, no statistically significant differences were found in the levels of thyroglobulin antibody (TgAb), thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), or free thyroxine (FT4). Between the control and TgAb (+) groups, significant differences were observed only in TgAb levels. Both TPOAb and TgAb levels exhibited significant differences between the TPOAb (+) and TgAb (+) groups, with no other significant differences in the remaining parameters.

Metabolomic analysis

A total of 225 differential metabolites were assessed in each group (Table S2). Partial Least Squares Discriminant Analysis (PLS-DA) was employed to visualize the differences between the Control, TPOAb (+), and TgAb (+) groups (R2X = 0.195, R2Y = 0.44, Q2 = 0.281, Fig. 1a). Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) revealed distinct separations between the Control (yellow dots) and TPOAb (+) (blue dots) groups (R2X = 0.262, R2Y = 0.756, Q2 = 0.641, Fig. 1b), as well as between the Control (yellow dots) and TgAb (+) (purple dots) groups (R2X = 0.219, R2Y = 0.718, Q2 = 0.570, Fig. 1c).

Based on the criteria of VIP > 1.5 and P < 0.05, a total of 36 differential metabolites were identified. Thirteen differential metabolites were identified in the comparison between the Control and TPOAb (+) groups, while 23 differential metabolites were identified in the comparison between the Control and TgAb (+) groups (Table 2).

Comparison between the Control and TPOAb (+) groups revealed elevated levels of Glycoursodeoxycholic acid, Glycocholate, LPE 16:1, Glycocholic acid, Indoxyl sulfate, and Phenylacetyl-L-glutamine, as well as reduced levels of LPC 16:0 sn-1, PC 36:3, Hydrocortisone, LPC 18:2 sn-1, SM 36:3, PC 16:0/18:2, and D(-)-Arginine in the TPOAb (+) group compared to the Control (Fig. 2a). The heatmap of differential metabolites is depicted in Fig. 3a. In the TgAb (+) group, concentrations of Dehydroepiandrosterone Sulfate, Androsterone sulfate-4, LPC 16:0 sn-1, Oxypurinol, Androsterone sulfate-3, Androsterone sulfate-1, Decenoylcarnitine, LPC 16:0 sn-2, LPC 15:0 sn-1, FFA 20:5*2, FFA 22:1, 2-Ketohexanoic acid, FFA 18:2, PC 36:3, FFA 18:0, Lactic acid, FFA

	Group			P-value	G1vs.G2 comparison	G1vs.G3 comparison	G2vs.G3 comparison
	Control (G1)	TPOAb (+) (G2)	TgAb (+) (G3)		P-value	P-value	P-value
Sex, M/F	0/14	0/14	0/4	–	–	–	–
Age, y	38.65 ± 2.80	38.43 ± 2.67	44.25 ± 5.00	0.583	–	–	–
TSH, mIU/L	2.14 ± 0.23	14.14 ± 10.48	13.91 ± 6.90	0.462	–	–	–
FT3, pmol/l	4.97 ± 0.13	4.37 ± 0.30	4.20 ± 0.11	0.103	–	–	–
FT4, pmol/l	14.95 ± 0.49	13.48 ± 1.08	12.15 ± 1.12	0.211	–	–	–
TgAb, U/mL	15.00 (15.00,15.00)	19.99 (15.00,33.47)	267.74 (180.40,308.50)	<0.0001	0.897	<0.0001	<0.0001
TPOAb, U/mL	29.15 (28.00,34.61)	1300.00 (768.39,1300.00)	31.34 (28.00,45.08)	<0.0001	<0.0001	1.000	<0.0001

Table 1. Comparison of baseline data among the control, TPOAb (+), and TgAb (+) groups. Data are presented as means ± standard deviations for continuous variables and as n (%) for categorical variables. TgAb and TPOAb levels are expressed as medians (P25–P75). Group differences were assessed using analysis of variance (ANOVA) or the Kruskal-Wallis test. To account for multiple comparisons, the Bonferroni correction was applied, with a significance threshold set at 0.05/3. A p-value of less than 0.017 was considered statistically significant. Normal Ranges: TSH, mIU/L 0.38–4.34; FT3, pmol/L 2.77–6.31; FT4, pmol/L 10.44–24.38; TgAb, U/mL 0–60; TPOAb, U/mL 0–60.

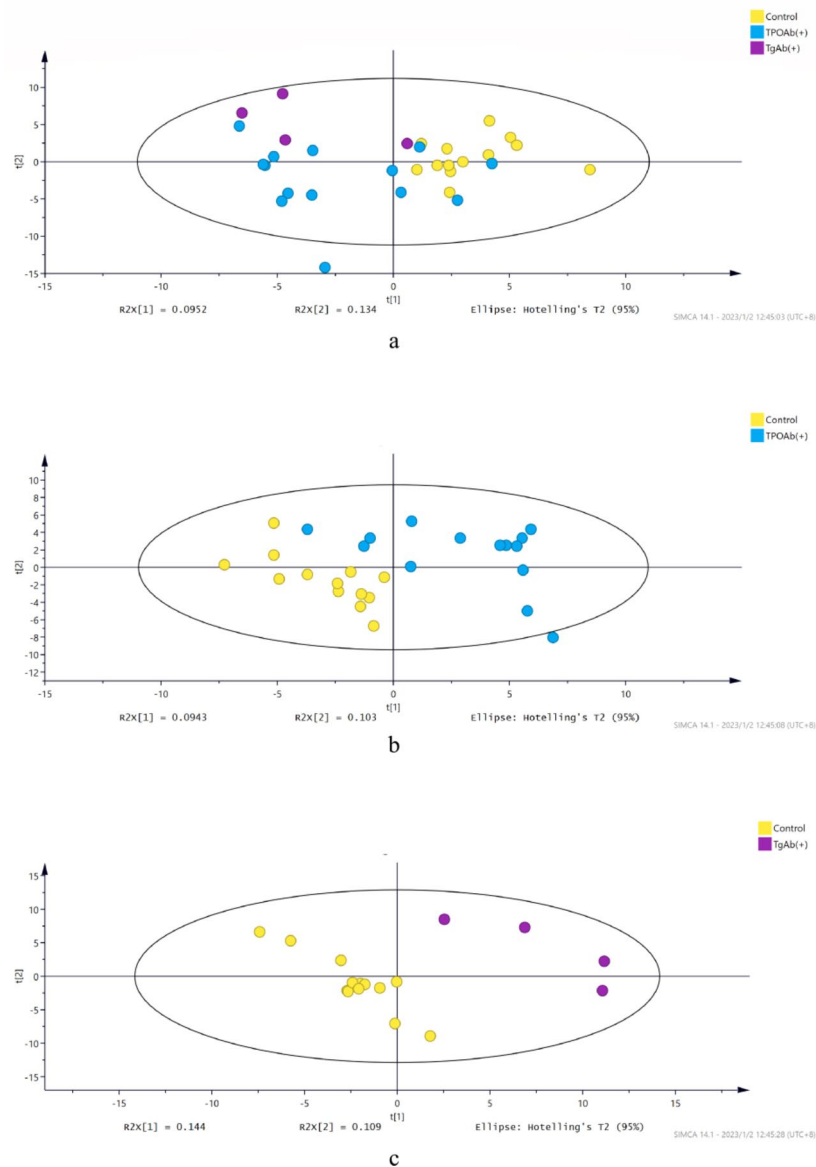


Fig. 1. The PLS-DA score plot comparing the control group to the TPOAb (+) and TgAb (+) groups. **(a)** PLS-DA score plot of three groups ($R^2X=0.195$, $R^2Y=0.44$, $Q^2=0.281$). **(b)** OPLS-DA score plot comparing the Control group to the TPOAb (+) group ($R^2X=0.262$, $R^2Y=0.756$, $Q^2=0.641$). **(c)** OPLS-DA score plot comparing the Control group to the TgAb (+) group ($R^2X=0.203$, $R^2Y=0.56$, $Q^2=0.233$). The $t[1]$ and $t[2]$ values in the figures represent the scores of each sample in principal components 1 and 2, respectively. Each dot on the plot represents a sample in the corresponding group.

16:0, FFA 16:2, and Decadienoylcarnitine were lower than in the Control, while PC 32:2 was elevated, along with Glycoursodeoxycholic acid-pos, Glycochenodeoxycholic acid 3-glucuronide, LPE 20:4 sn-1, and LPE 16:1 (Fig. 2b). The distribution of the measured differential metabolites is depicted in the heatmap (Fig. 3b). These findings suggest distinct metabolic alterations associated with varying antibody profiles, which may provide valuable insights for identifying potential biomarkers for disease monitoring and developing therapeutic strategies.

Correlation analysis

Correlation analysis between clinical data and differential metabolites was conducted using Origin 2021 to explore the relationships between specific metabolites and clinical markers. Pearson correlation coefficients between clinical variables and differential metabolites are depicted in Fig. 3c and d, with different colors representing the strength of correlation. A Pearson correlation coefficient greater than 0.5 and a significance threshold of $P < 0.05$ were used as criteria to evaluate the correlations. Distinct correlations between differential metabolites and clinical data were observed across the different comparison groups. In the TPOAb (+) group,

Control vs. TPOAb(+)			Control vs. TgAb(+)		
Metabolites	VIP	P	Metabolites	VIP	P
LPC 16:0 sn-1	2.09743	0.007	Glycoursodeoxycholic acid	2.19109	0.001
Glycoursodeoxycholic acid	2.04194	0.017	Glycochenodeoxycholic acid 3-glucuronide	2.06339	0.015
PC 36:3	1.94119	0.014	Dehydroepiandrosterone Sulfate	2.05961	0.005
Hydrocortisone	1.93085	0.046	LPE 20:4 sn-1	2.04749	0.002
LPC 18:2 sn-1	1.91563	0.015	Androsterone sulfate-4	1.95584	0.006
Glycocholate	1.76544	0.019	LPC 16:0 sn-1	1.93899	0.007
SM 36:3	1.6853	0.035	Oxypurinol	1.88521	0.011
LPE 16:1	1.68272	0.043	Androsterone sulfate-3	1.76495	0.020
Glycocholic acid	1.66577	0.013	Androsterone sulfate-1	1.76307	0.016
PC 16:0/18:2	1.64949	0.024	Decenoylcarnitine	1.73976	0.012
Indoxyl sulfate	1.64882	0.049	LPC 16:0 sn-2	1.72257	0.015
D(-)-Arginine	1.64535	0.043	LPC 15:0 sn-1	1.7061	0.024
Phenylacetyl-L-glutamine	1.55777	0.027	FFA 20:5*2	1.69361	0.021
			FFA 22:1	1.69164	0.022
			2-Ketohexanoic acid	1.66508	0.019
			FFA 18:2	1.66466	0.026
			LPE 16:1	1.61327	0.028
			PC 36:3	1.59937	0.037
			FFA 18:0	1.5923	0.037
			Lactic acid	1.58033	0.046
			FFA 16:0	1.56845	0.041
			FFA 16:2	1.53792	0.037
			Decadienoylcarnitine	1.51107	0.035

Table 2. Differential metabolites identified between the control, TPOAb (+), and TgAb (+) groups.

a moderate correlation was found between TgAb and phenylacetyl-L-glutamine, while a strong correlation was observed between TPOAb and LPC 16:0 sn-1. Conversely, in the TgAb (+) group, a strong correlation was noted between TPOAb and LPE 16:1. The results from each group highlight the specific relationships between metabolites and clinical markers, emphasizing the distinct metabolic profiles associated with different antibody statuses. These findings provide a basis for future research aimed at identifying potential biomarkers for disease monitoring and guiding therapeutic strategies.

Enrichment analysis

Enrichment analysis of metabolic pathways, based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, was conducted using MetaboAnalyst to identify significant changes in metabolic pathways. In the comparison between the control and TPOAb (+) groups, notable changes were observed in glycine, serine, and threonine metabolism; glycerophospholipid metabolism; and primary bile acid biosynthesis (Fig. 4a and b). Similarly, the comparison between the control and TgAb (+) groups revealed key roles for galactose metabolism, ascorbate and alternative metabolism, and inositol phosphate metabolism (Fig. 4c and d). These findings suggest that different antibody statuses affect distinct metabolic pathways. Further investigation of these metabolic alterations could enhance our understanding of disease mechanisms and support the development of targeted therapies.

Discussion

Thyroid peroxidase (TPO) is a critical enzyme in thyroid hormone biosynthesis, and thyroid peroxidase antibodies can impair its function, leading to reduced thyroid hormone production. Research has shown a strong association between TPOAb and adverse pregnancy outcomes, including miscarriage, infertility, fetal intellectual impairment, and Hashimoto's encephalopathy. Anti-thyroglobulin antibodies, which target thyroglobulin, are autoantibodies that serve as biomarkers of thyroid autoimmunity. However, their diagnostic sensitivity for Hashimoto's thyroiditis is lower than that of TPOAb¹⁹.

Wu et al. found significantly higher body mass index (BMI), waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), and 2-hour plasma glucose (OGTT2hPG) in the TPOAb/TgAb-positive group compared to the TPOAb/TgAb-negative group in men. Similarly, in women, BMI, WC, SBP, DBP, total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) were significantly elevated in the TPOAb/TgAb-positive group compared to the TPOAb/TgAb-negative group¹⁴. Wang et al. observed that the severity of fatty liver increased with rising titers of TPOAb, particularly in women⁸. Zhang et al. demonstrated that positive TgAb alone suppressed the development of hypertriglyceridemia in women and reduced the risk of impaired fasting glucose (IFG) in men, suggesting it may serve as an independent protective factor⁹.

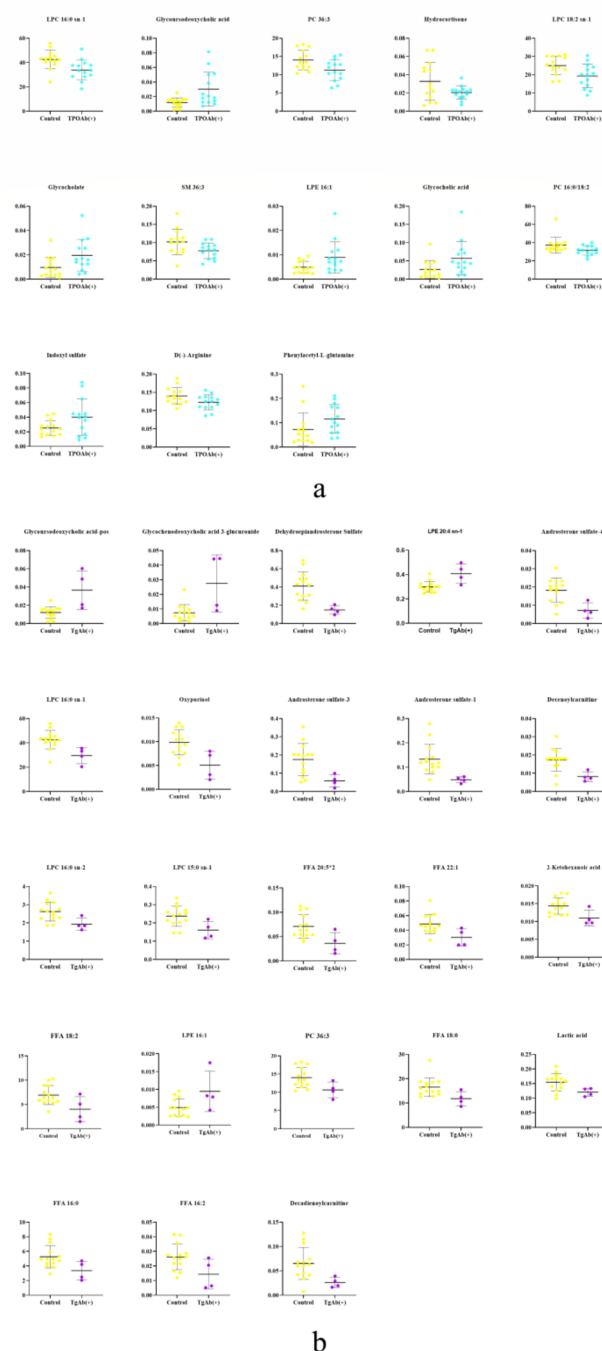


Fig. 2. (a) Differential metabolites from the comparison between the Control group and the TPOAb (+) group. (b) Differential metabolites from the comparison between the Control group and the TgAb (+) group.

Our study revealed that female patients with HT in the TPOAb-positive group exhibited significantly elevated levels of glyoursodeoxycholic acid, glycocholate, and glycocholic acid compared to the Control group. Enrichment analysis identified the primary bile acid biosynthesis pathway as a key affected pathway, indicating distinct alterations in bile acid metabolism associated with different antibody statuses in HT. Cholic acid, a product of bile acid metabolism, has been implicated in metabolic disorders²⁰. Studies have shown that bile acid metabolism can influence the function and differentiation of Foxp3 + Treg cells²¹.

Thyroid hormones (TH) have been shown to enhance bile acid metabolism²², while simultaneously suppressing the rate-limiting enzymes (CYP8B1, CYP27A1) involved in bile acid metabolism^{23–25}. Kosuge T et al. suggested that deoxycholic acid (DCA or DOCA) levels are elevated in patients with hypothyroidism, while chenodeoxycholic acid (CDA) levels are increased in those with hyperthyroidism²⁶. The ratio of deoxycholic acid to total bile acids and lithocholic acid (LCA) to chenodeoxycholic acid (CDA) has been proposed as a marker of thyroid function. Antibody-dependent cell-mediated cytotoxicity (ADCC) induced by TPOAb accelerates

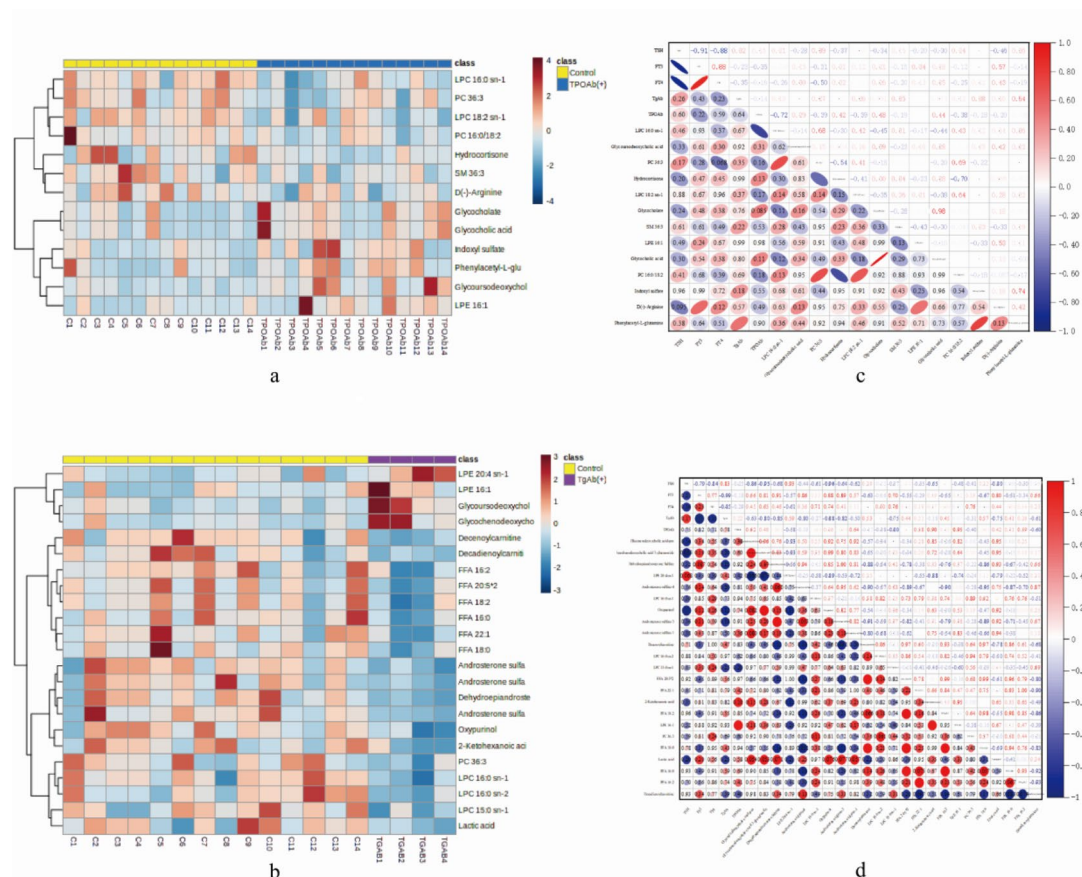


Fig. 3. (a) Heatmap of differential metabolites comparing the Control group and the TPOAb (+) group. (b) Heatmap of differential metabolites comparing the Control group and the TgAb (+) group. (c,d) Pearson correlation coefficients between clinical variables and differential metabolites displayed by Correlograms.

damage to thyroid follicular cells (TFC), resulting in suppressed thyroid hormone synthesis²⁷. Consequently, alterations in serum TPOAb levels in TPOAb-positive patients can impact thyroid hormone levels, modifying bile acid metabolism and its related metabolite products. This, in turn, may affect the ratio of Treg cells, potentially influencing disease progression.

Amino acids are essential components for the body's vital functions. Amino acid metabolism supports various immune cell functions during immune cell activation and plays a crucial role in autoimmune regulation²⁸. In this study, enrichment analysis revealed dysregulated glycine, serine, and threonine metabolism pathways in the TPOAb (+) group compared to the Control group, indicating that amino acid metabolism is influenced by different thyroid antibody statuses. TPOAb may modulate amino acid metabolism through redox signaling pathways. Oxidative stress has been shown to modulate the function of metabolic enzymes via post-translational modifications. For example, reactive oxygen species (ROS) can alter the activity of key enzymes, thereby affecting both the synthesis and degradation of amino acids. Additionally, thyroid hormones are closely linked to amino acid metabolism. Research indicates that thyroid hormones can enhance glutamine breakdown by facilitating glutamate aminotransferase GPT2²⁹, which leads to a decrease in glutamine levels, inversely correlating with THs. When TPOAb reduces TH synthesis by triggering thyroid follicular cell damage via ADCC, glutamine levels are elevated. Additionally, Marta Toral et al. demonstrated that THs promote arginine uptake activation and gene expression of the arginine transporter³⁰. Consequently, as TH levels decrease, the content of arginine also decreases. Thus, the level of TPOAb affects TH levels, subsequently regulating amino acid levels in the body.

Phospholipids are essential for maintaining the structural integrity of cell membranes, facilitating signal transduction, and supporting immune responses in HT. Phospholipids are hydrolyzed by phospholipases into glycerol, fatty acids, phosphoric acid, and a variety of amino alcohols (e.g., choline, ethanolamine, serine)³¹. Glycerol can be converted to dihydroxyacetone phosphate, which participates in glucose metabolism³². Fatty acids undergo β -oxidation, which influence the development and differentiation of Treg cells³³. Phosphoric acid is essential for the metabolism of various substances in the body, while amino alcohols contribute to the resynthesis of phospholipids. The formation and metabolism of phospholipids are closely associated with human physiological functions³⁴. Sphingolipids modulate cellular stress and inflammation³⁵, while lysophospholipids serve as signaling molecules that mediate immunomodulatory functions^{36,37}.

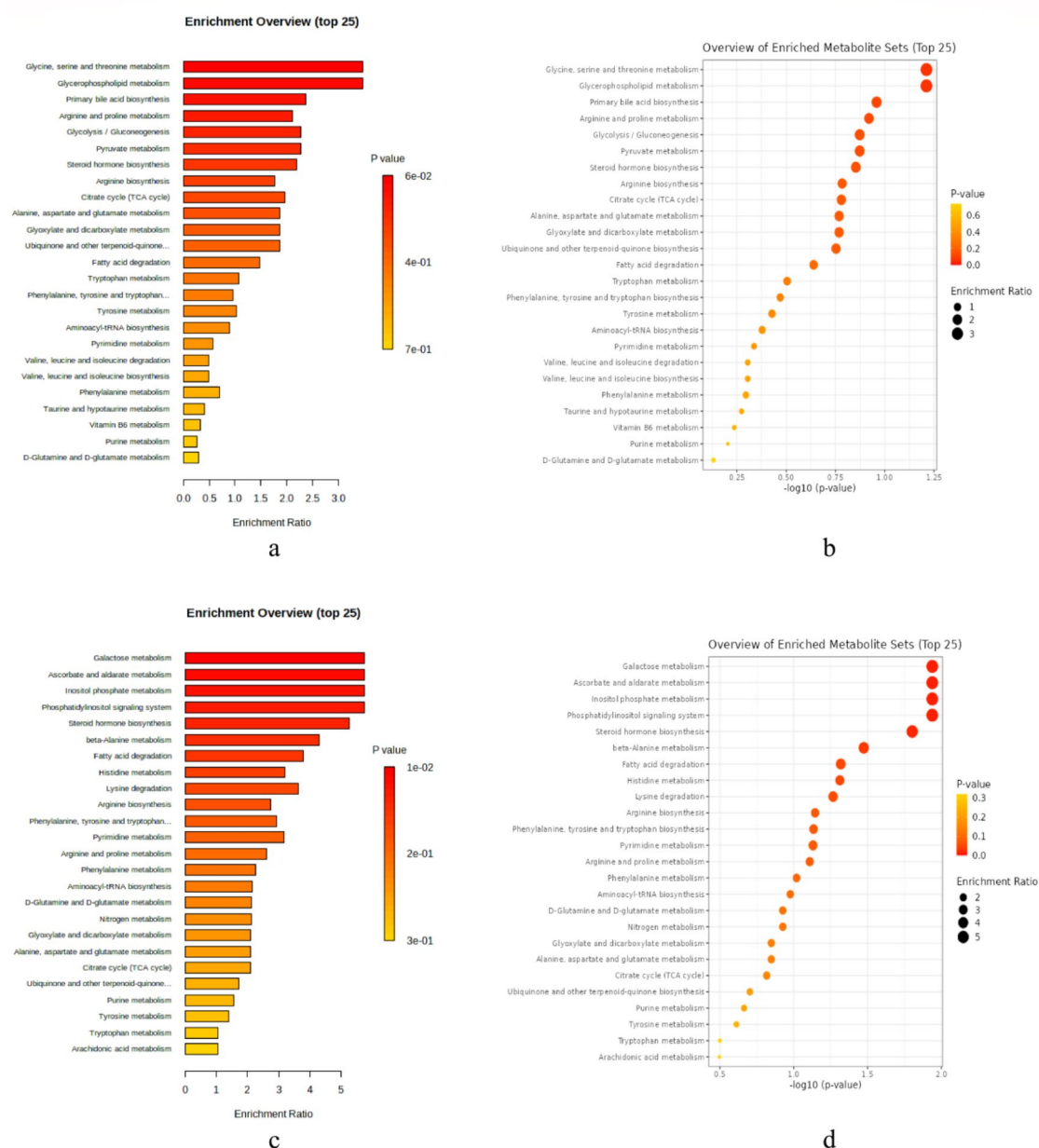


Fig. 4. Enrichment analysis was performed to identify the significantly altered metabolic pathways based on the Kyoto Encyclopedia of Genes and Genomes database through Metaboanalyst. **(a,b)** Comparison between the Control group and the TPOAb (+) group. **(c,d)** Comparison between the Control group and the TgAb (+) group.

Previous research has demonstrated that thyroid hormones can regulate phospholipid metabolism. In a mouse model of hypothyroidism, phospholipid levels were reduced³⁸. Additionally, Rabkin SW showed a positive correlation between thyroid hormones and phospholipid concentrations in the heart, lung, kidney, and skeletal muscle³⁹. In TPOAb-positive patients, abnormal levels of phospholipid metabolites such as SM, LPC, and PC are found in the serum. Correlation analysis suggests a relationship between TPOAb and LPC. Since TPOAb inhibits thyroid hormone synthesis, the overall phospholipid metabolism decreases, leading to changes in phospholipid metabolites. These changes may lead to the abnormal secretion of inflammatory factors and immune disorders, ultimately resulting in inflammatory abnormalities and immune dysfunction.

TgAb is one of the most common autoantibodies found in the thyroid gland and is often used to diagnose and identify autoimmune thyroiditis, albeit with low sensitivity [8]. Previous studies have suggested that TgAb positivity alone suppresses the development of hypertriglyceridemia in women and reduces the risk of impaired fasting glucose regulation (IFG) in men. In our study, we observed decreased levels of LPC, FFA, and PC in female patients in the TgAb (+) group, indicating suppressed lipid metabolism. Additionally, we found a significant correlation between TgAb levels and serum glutamate. However, due to the lack of literature on the

mechanisms underlying the association between serum TgAb levels and suppressed lipid metabolism, further research is needed to elucidate how TgAb influences metabolism.

Conclusion

Our study indicates that female patients with Hashimoto's thyroiditis exhibit abnormal levels of small molecule metabolites depending on TPOAb or TgAb positivity. TPOAb affects metabolic levels by modulating thyroid hormones, while the mechanisms through which TgAb affects metabolism require further investigation. This research provides insights into potential biomarkers for disease monitoring and treatment. However, longitudinal studies involving larger populations are needed to confirm these results, and further research is needed to elucidate the underlying mechanisms.

Limitations

There are several limitations to our study. First, the sample size was relatively small. Second, it did not include metabolomic analysis of male patients with Hashimoto's thyroiditis. Therefore, future research will involve expanding the sample size and adopting more stringent diagnostic criteria, including pathological diagnosis, as inclusion criteria for thyroiditis patients. Additionally, we plan to conduct further studies in larger and more diverse populations to improve statistical power and more robustly validate our findings.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Received: 13 November 2024; Accepted: 13 February 2025

Published online: 22 March 2025

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Acknowledgements

We gratefully acknowledge the approval from the Second Hospital of Dalian Medical University and the support provided by the Dalian Institute of Chemical Physics, Chinese Academy of Sciences.

Author contributions

Conceptualization, Xiao Jiang and Haixia Liu; methodology and validation, Tao Luo and Xinyu Zhao; formal analysis, Tao Luo and Xiao Jiang; data curation, Pengqian Li and Xiaotong Gu; writing—original draft preparation, Tao Luo, Xinyu Zhao and Xiao Jiang; writing—review and editing, Pengqian Li and Xingjie Xie; funding acquisition, Haixia Liu. All authors have reviewed and approved the final version of the manuscript for publication.

Funding

This study was supported by the following grants: the “Xingliao Talent Plan” of Liaoning Province, China (YX-MJ-QN-05); the Dalian Science and Technology Innovation Fund, Dalian Science and Technology Bureau (2022JJ12SN048); and the “1 + X” Program for Clinical Competency Enhancement – Clinical Research Incubation Project, The Second Hospital of Dalian Medical University (2022LCYJZD03).

Declarations

Ethics approval

This study protocol was approved by the Ethics Committee of the Second Hospital of Dalian Medical University (Approval No. 2021NO.073). Informed consent was obtained from all participants. According to national regulations and institutional requirements, written informed consent was not required for this study.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-90467-5>.

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