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ORIGINAL RESEARCH

Serum Metabolomic Signatures of Dyslipidemia in Narcolepsy Type 1: A Multi-Center Cross-Sectional Study

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Study Objectives: We aimed to explore the candidate metabolic pathways involved in narcolepsy type 1 (NT1)-related dyslipidemia.

Methods: Forty-four patients with NT1, and 44 controls were included in this multicenter metabolomics study. All participants included underwent an overnight polysomnography and multiple sleep latency test. Fasting blood samples were collected to assess lipid levels, including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG). Serum metabolomics was used to explore characteristic metabolites in the discovery and validation sets.

Results: Patients with NT1 had higher values for TC (5.13 ± 0.14 vs 4.63 ± 0.14 mmol/L, $P = 0.024$), and LDL-C (3.01±0.13 vs 2.59 ± 0.13 mmol/L, $P = 0.038$), but a lower HDL-C (1.32 ± 0.07 vs 1.53 ± 0.07 mmol/L, $P = 0.039$) compared to controls after adjusting for potential confounders. Enrichment analysis suggested that arginine biosynthesis and arginine and proline metabolism were different between patients with NT1 and controls. Four NT1-related metabolites were identified, glutamate ($\beta = -0.319$, $P = 0.049$) correlated negatively with HDL-C, after adjusting for potential confounders.

Conclusion: Increased serum glutamate level is associated with decreased level of HDL-C in patients with NT1. Arginine metabolism dysfunction may contribute to dyslipidemia in NT1.

Keywords: narcolepsy type 1, dyslipidemia, serum metabolomics, arginine metabolism, high-density lipoprotein cholesterol

Introduction

Narcolepsy type 1 (NT1) is a chronic sleep disorder with a prevalence of about 0.02–0.05% of the population.[1] NT1 is characterized by excessive daytime sleepiness (EDS), cataplexy, hallucinations, sleep paralysis, and sleep-wake disturbances.[2,3] NT1 typically onsets during adolescence and is often accompanied by psychiatric comorbidities, particularly mood and anxiety disorders.[4–6] Patients with NT1 also exhibit a higher prevalence of cardiovascular and cardiometabolic comorbidities and face an increased risk of future cardiovascular events.[7,8] Given these comorbidities, patients with NT1 tend to have reduced quality of life, greater healthcare utilization, and higher mortality risk compared with the general population.[4,9]

Increased evidence has shown that metabolic changes, including obesity and dyslipidemia, are highly prevalent in patients with NT1.[10,11] Early studies of children and adolescents with NT1 suggest that NT1 occurring during prepubertal age is frequently accompanied by precocious puberty, overweight/obesity, hypertriglyceridemia and metabolic syndrome (MetS).[7,8,12,13] Furthermore, lower high-density lipoprotein cholesterol (HDL-C) levels are found in children and adolescents with NT1 compared with obese controls.[12,13] The pathophysiological mechanisms of impaired lipid metabolism in NT1 are not clear. Some studies have proposed that deficiency of hypocretin may play a role in impaired lipid metabolism in NT1.[14] However, other studies did not show consistent findings.[15–17]

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Metabolomics has been applied to identify biomarkers for pathological changes in NT1.[18,19] So far, three metabolomics studies have been conducted in patients with NT1 to explore the underlying pathophysiology.[18–20] For example, an untargeted metabolomic analysis of cerebrospinal fluid samples from 14 patients with NT1 and 17 controls suggested that glycogenesis is enhanced in NT1 as a compensatory mechanism for fatty acid metabolism.[19] A target metabolomics study of plasma samples from 117 patients with NT1 and 116 body mass index (BMI)-matched controls showed significant differences, in plasma metabolite profiles between patients with NT1 and BMI-matched controls, mainly in glycine and serine metabolism, arachidonic acid metabolism, and tryptophan metabolism.[18] However, the sample sizes of serum studies was too small,[21] and the targeted metabolomics method adopted in the third study may lead to the retention of some important small molecule metabolites. Furthermore, there have been no multicenter studies that use serum metabolomics to explore the candidate metabolic pathways involved in NT1-related dyslipidemia. We hypothesize that NT1-related dyslipidemia is associated with distinct dysregulations in metabolic pathways, and that systematic exploration of these pathways will identify key metabolites mediating the link between NT1 and dysregulated lipid metabolism. We aim to explore the candidate metabolic pathways involved in NT1-related dyslipidemia through a multicenter metabolomics study.

Materials and Methods

Study Design and Population

We conducted a multi-center cross-sectional study to explore the candidate metabolic pathways involved in NT1-related dyslipidemia. All participants included were interviewed by a physician with questionnaires including demographic characteristics, history of sleep complaints, general health, medical history, medication use and mood status. Patients with NT1 met the diagnostic criteria based on the International Classification of Sleep Disorders Third Edition.[22] Control participants were consecutively recruited through advertisement in the community. The control participants did not have any sleep complaints, and had no major medical or psychiatric conditions based on their medical history and physical examination. We excluded patients with NT1 and control participants who had: (1) a current major psychiatric condition (eg, major depression), substance/alcohol abuse, or other serious organic brain diseases (eg, epilepsy), (2) use of any psychiatric drugs or lipid-lowering drugs currently or within the past month, (3) evidence of sleep apnea based on a polysomnographic-measured apnea-hypopnea index (AHI) $\geq$ 15 events per hr, or (4) any other comorbid sleep disorder as per sleep interview.

Ultimately, we included 44 patients with NT1 and 44 normal sleep control participants. Among these 44 patients with NT1, 24 patients were consecutively recruited from March 2018 to July 2024 from the Sleep Medicine Center of Shantou University Medical College and 20 patients were randomly selected from the Sleep Medicine Center of Peking University People's Hospital between October 2013 to February 2021. This study was approved by the Research Ethics Board of Mental Health Center of Shantou University (202360) and Ethical Committee of Peking University People's Hospital (2022PHB089). Informed consent was obtained from each participant.

Nighttime Sleep Measurement

All enrolled participants completed overnight polysomnography (PSG) in a controlled sleep laboratory environment designed to attenuate sound and regulate light/temperature. Nighttime sleep parameters were assessed using PSG monitoring, encompassing electroencephalography, bilateral electrooculography, electromyography, and electrocardiography. Respiratory activity was tracked via nasal-oral thermocouples, nasal pressure cannulas, and thoracic respiratory effort belts. Peripheral capillary oxygen saturation was continuously recorded using pulse oximetry.[23]

Daytime Sleep Measurement

The multiple sleep latency test (MSLT) was performed on the day following nocturnal PSG recordings to assess objective daytime sleepiness, with 5 naps scheduled every 2 hours, starting 2 hours after initial morning awakening. To assess the presence of REM sleep, testing was continued for ≥15 min after sleep onset. If REM sleep was present, the latency of REM sleep was also recorded. The mean sleep latency (MSL) and REM sleep latency (REM-SL) of the 5 naps were

calculated. Lower values of the MSL of MSLT indicate more objective daytime sleepiness. Sleep latency on MSLT was defined as the time from lights off to the first epoch of any sleep period. Self-reported daytime sleepiness was assessed with the Epworth Sleepiness Scale (ESS) which was conducted on the same day as the MSLT. Higher ESS scores are associated with increased self-reported daytime sleepiness.[24]

Lipid Metabolism Measurements

Fasting blood samples were collected in the morning after the PSG recording to assess lipid metabolism indices, including total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG). BMI was calculated based on measured height (cm) and weight (kg) during the participants' sleep laboratory visit.

Sample Collection and Preparation

Following overnight PSG, fasting venous blood was drawn into 5 mL serum separation tubes. Specimens underwent immediate centrifugation (1500 × g, 4°C, 15 min). Separated serum aliquots were cryopreserved at −80°C pending non-targeted metabolomic profiling.

Nontargeted Metabolomics

Serum metabolic profiles of patients with NT1 and controls were compared via ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Analyses employed an Agilent 1290 Infinity LC UHPLC system interfaced with an AB Sciex TripleTOF 6600 quadrupole time-of-flight mass spectrometer. High-sensitivity mode information-dependent acquisition generated production scans. Quality control (QC) samples, prepared from pooled aliquots, were randomized within analytical batches to monitor system stability. Raw mass spectrometer (MS) data (wiff.scan files) underwent conversion to mzXML format using ProteoWizard msConvert prior to processing in the open-source XCMS software. Metabolite identification utilized MS/MS spectral matching against an in-house database validated with authentic standards. Details of the above are provided in the Supplementary Materials.

Statistical Analysis

Demographic, clinical, and sleep characteristics were presented as means ± standard deviations for continuous variables, while categorical variables were expressed as percentages. Multiple imputation was employed to address missing blood lipid values. Between group comparisons were performed with independent samples *t*-tests for normally distributed continuous variables, and Mann–Whitney *U*-tests for non-normally distributed data, as assessed by Shapiro–Wilk normality testing. Categorical variables were analyzed using Fisher's exact test. To minimize the effects of potential confounding factors on the levels of lipid between patients with NT1 and controls, we conducted analysis of covariance (ANCOVA) with adjustment for age, gender, BMI and AHI to further compare the differences in blood lipid levels between the two groups. Furthermore, to determine whether the sample size was sufficient, we conducted post hoc power analyses of the ANCOVA of blood lipid levels between groups, and the results from power calculation with the G*Power 3.1.9.2 program[25] showed that there was 86.71%, 90.86% and 83.48% power to reject the null hypothesis and detect a significant between-group difference of TC, HDL-C and LDL-C, respectively, in the current study.

In order to avoid the random discovery of NT1-related metabolites, the total sample was divided into two parts: the 24 patients with NT1 from the Sleep Medicine Center of Shantou University Medical college and 24 controls were set as the discovery set, and the 20 patients with NT1 from the Sleep Medicine Center of Peking University People's Hospital and 20 controls were set as the validation set. Differential metabolites between groups were screened in the discovery set and the validation set. The relative quantitative data of metabolites, annotated in the human metabolome database (HMDB) (https://hmdb.ca), was used for the following analyses. After excluding drug-related metabolites, sum normalization and pareto scaling of the relative quantitative data was performed for further analyses. Orthogonal partial least-squares discriminant analysis (OPLS-DA) was used to compare the differences in metabolic profiles between groups. The variable importance in the projection (VIP) value of each variable in the OPLS-DA model was calculated to indicate its contribution to the classification. To minimize the effects of potential confounders on identifying differential metabolites between NT1 patients and controls, fold change analysis and ANCOVA with adjustment for age, gender,

BMI and AHI were applied to analyze and determine the significance of metabolite differences between two groups. In addition, to decrease false discovery rates (FDR), the Benjamini and Hochberg procedure was used to correct the original p-values from ANCOVA. Metabolites with a $P < 0.01$ and FDR < 0.01 in the ANCOVA were considered as differential metabolites. Differential metabolites of the discovery and validation sets were further used for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Metabolic pathways with an FDR < 0.01 were considered as differential metabolic pathways. The differential metabolic pathways that overlapped in both the discovery set and validation set were determined as the NT1-related metabolic pathways. Finally, the metabolite hits with concordant directional changes in NT1-related metabolic pathways were considered as NT1-related metabolites. Furthermore, to define the metabolites that associate with NT1-related dyslipidemia, multivariable adjusted linear regression was performed with lipid metabolism indices as dependent variables and each NT1-related metabolite as an independent variable. In the linear regression models, when the dependent variable was blood lipids, we took age, gender, BMI and AHI as covariates. Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA), Python version 3.12.7 (https://www.python.org/downloads/) and MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/).

Results

Demographic, Sleep and Clinical Characteristics

The demographic, clinical and sleep characteristics of all participants in total are presented in Table 1. Compared to controls, patients with NT1 had a higher BMI (25.40 ± 5.06 kg/m^2 vs 21.48 ± 3.36 kg/m^2, $P < 0.001$), longer total sleep time (453.09 ± 58.37 min vs 423.72±41.32 min, $P = 0.008$), shorter sleep onset latency (2.97±3.31 min vs 16.25±18.73 min, $P < 0.001$) and REM-SL (60.93±82.47 min vs 104.51±49.15 min, $P = 0.004$), higher percentage of non rapid eye movement sleep stage

Table 1 Demographic, Clinical, and Sleep Characteristics

	NT1 (n=44)	Control (n=44)	*P*
Age, years	24.31±10.8	27.87±6.73	0.068
Female, n (%)	17(38.64)	17(38.64)	>0.999
BMI, kg/m^2	25.40±5.06	21.48±3.36	**<0.001**
TC, mmol/L	5.18±1.07	4.57±0.78	**0.004**
TG, mmol/L	1.59±0.85	1.07±0.67	**0.001**
HDL-C, mmol/L	1.29±0.45	1.56±0.37	**0.001**
LDL-C, mmol/L	3.10±1.07	2.49±0.67	**0.009**
TST, min	453.09±58.37	423.72±41.32	**0.008**
SOL, min	2.97±3.31	16.25±18.73	**<0.001**
REM-SL, min	60.93±82.47	104.51±49.15	**0.004**
SE, %	88.29±9.11	87.38±7.38	0.278
WASO, min	56.69±46.61	45.52±32.58	0.361
NREM 1 (%)	15.55±10.97	9.82±4.64	**0.002**
NREM 2 (%)	44.54±8.42	46.78±6.88	0.185
NREM 3 (%)	19.08±8.48	23.02±5.91	**0.013**
REM (%)	21.14±6.11	20.36±4.65	0.940
AHI, events per hour	3.85±4.37	5.18±5.80	0.637
MSL, min	2.32±1.88	10.23±4.34	**<0.001**
ESS, score	15.59±4.52	6.52±3.46	**<0.001**

Notes: Values in bold indicate a $P < 0.05$. *P*, *p*-values are for NT1 vs controls in total.
Abbreviations: AHI, apnea–hypopnea index; BMI, body mass index; ESS, Epworth Sleepiness Scale; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MSL, mean sleep latency; NREM 1%, percentage of non rapid eye movement sleep stage 1; NREM 2%, percentage of non rapid eye movement sleep stage 2; NREM 3%, percentage of non rapid eye movement sleep stage 3; NT1, narcolepsy type 1; PLMI, periodic limb movement index; PSG, polysomnography; REM%, percentage of rapid eye movement sleep stage; REMSL, rapid eye movement sleep latency; SE, sleep efficiency; SOL, sleep onset latency; TC, total cholesterol; TG, triglycerides; TST, total sleep time; WASO, wake time after sleep onset.

(NREM) 1 (15.55±10.97% vs 9.82±4.64%, $P = 0.002$) and lower percentage of NREM 3 (19.08±8.48% vs 23.02±5.91%, $P = 0.013$) during nighttime sleep. Furthermore, patients with NT1 had shorter MSL (2.32±1.88 min vs 10.23±4.34 min, $P < 0.001$) and higher values of ESS (15.59±4.52 vs 6.52±3.46, $P < 0.001$) compared to controls. Moreover, patients with NT1 had elevated levels of TC (5.18 ± 1.07 mmol/L vs 4.57 ± 0.78 mmol/L, $P = 0.004$), TG (1.59 ± 0.85 mmol/L vs 1.07 ± 0.67 mmol/L, $P = 0.001$), LDL-C (3.10 ± 1.07 mmol/L vs 2.49 ± 0.67 mmol/L, $P = 0.009$) and lower HDL-C (1.29 ± 0.45 mmol/L vs 1.56 ± 0.37 mmol/L, $P=0.001$) compared to controls. The demographic, sleep and clinical characteristics of participants from the discovery set and validation set are presented in Supplementary Table 1.

As shown in Figure 1, ANCOVA with adjustment for age, gender, BMI and AHI showed that patients with NT1 had an elevated levels of TC (5.13 ± 0.14 vs 4.63 ± 0.14 mmol/L, $P = 0.024$), LDL-C (3.01±0.13 vs 2.59 ± 0.13 mmol/L, $P = 0.038$) and lower HDL-C (1.32 ± 0.07 vs 1.53 ± 0.07 mmol/L, $P = 0.039$) compared to controls. However, no significantly different level of TG (1.36 ± 0.09 mmol/L vs 1.30 ± 0.09 mmol/L, $P = 0.631$) between the two groups was observed.

Metabolic Profiling and Differential Metabolites

In the discovery set, 497 metabolites could be annotated to HMDB, with 276 metabolites in positive ion mode (ES^+) and 221 metabolites in negative ion mode (ES^-), and were included in the analyses. OPLS-DA demonstrated clear separation between patients with NT1 and controls (Figure 2A and B). After adjusting for age, gender, BMI and AHI, we found 68 metabolites in ES^+ and 69 metabolites in ES^- that were different between patients with NT1 and controls (all $P < 0.01$ and FDR < 0.01). Details of the 137 differential metabolites in the discovery set are presented in Supplementary Table 2.

In the validation set, 549 metabolites that could be annotated to HMDB were identified, including 304 metabolites in ES^+ and 245 metabolites in ES^-, and were included in the analyses. OPLS-DA demonstrated clear separation between patients with NT1 and controls (Figure 2C, D and Supplementary Table 7). After adjusting for age, gender, BMI and AHI,

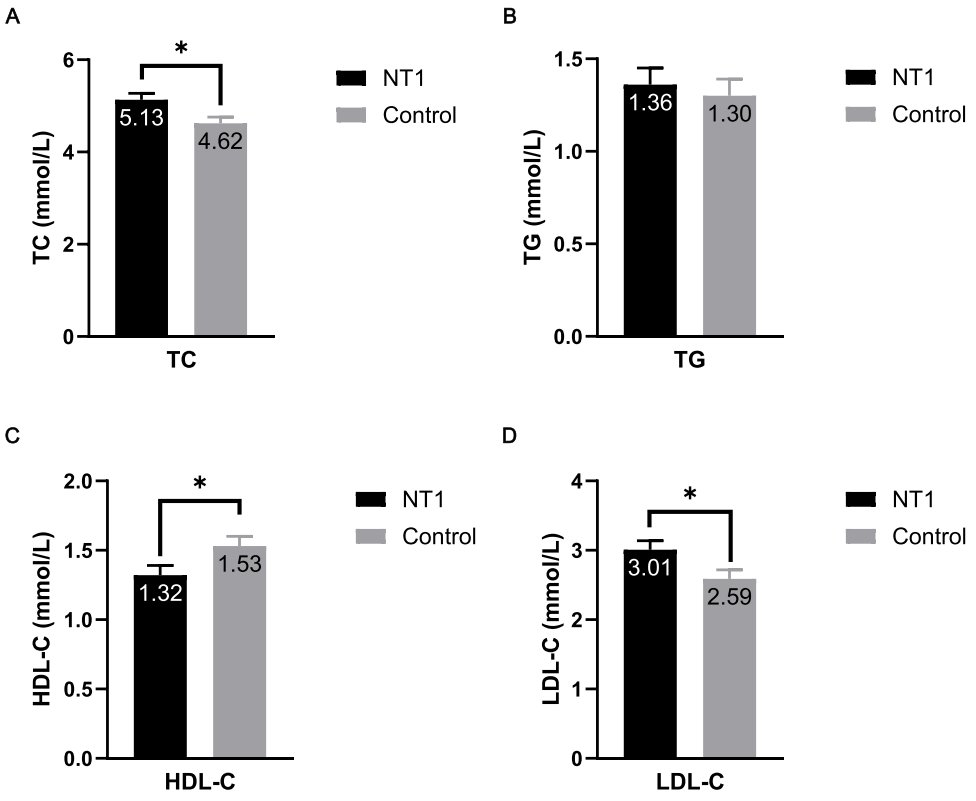

Figure 1 Covariance analysis of lipid levels between patients with NT1 and controls.
Notes: (**A**) Comparison of TC between NT1 and controls. (**B**) Comparison of TG between NT1 and controls. (**C**) Comparison of HDL-C between NT1 and controls. (**D**) Comparison of LDL-C between NT1 and controls. * indicates P <0.05. Error bars indicate standard errors.
Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NT1, narcolepsy type 1; TC, total cholesterol; TG, triglycerides.

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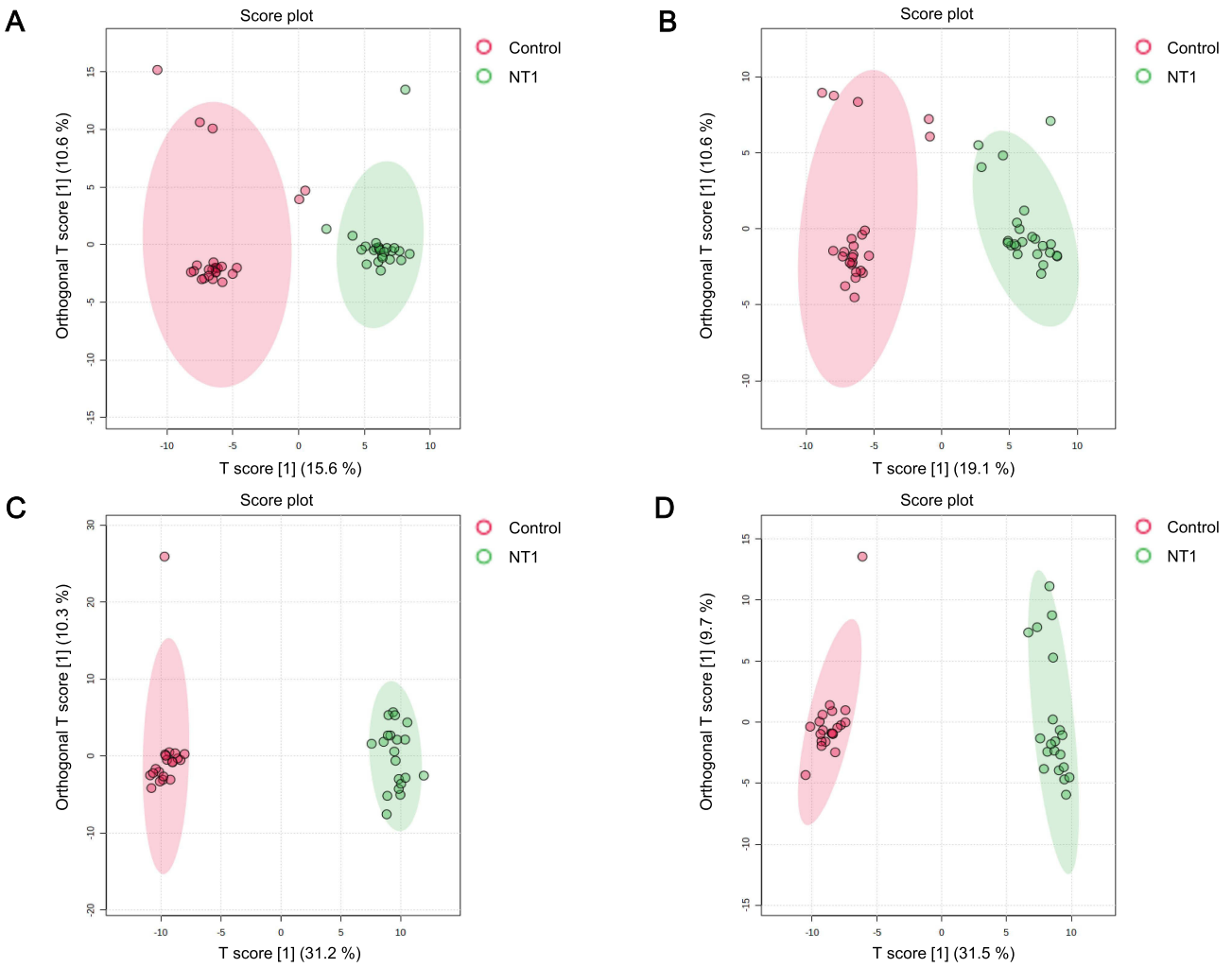

Figure 2 OPLS-DA score plots between NT1 and controls.
Notes: (**A**) Positive ion mode OPLS-DA analysis of NT1 and controls in the discovery set. (**B**) Negative ion mode OPLS-DA analysis of NT1 and controls in the discovery set. (**C**) Positive ion mode OPLS-DA analysis of NT1 and controls in the validation set. (**D**) Negative ion mode OPLS-DA analysis of NT1 and controls in the validation set.
Abbreviations: NT1, narcolepsy type 1; OPLS-DA, orthogonal partial least-squares discriminant analysis.

we found 153 metabolites in ES^+ and 126 metabolites in ES^- that were different between NT1 and controls (all $P < 0.01$ and FDR < 0.01). Details of the 279 NT1-related metabolites in the validation set are provided in Supplementary Table 3.

Enrichment analysis of the 137 differential metabolites in the discovery set was conducted, identifying enrichment in two specific pathways (Figure 3A and Supplementary Table 4): arginine biosynthesis ($P < 0.001$ and FDR = 0.004) and arginine and proline metabolism ($P < 0.001$ and FDR = 0.006). Enrichment analysis of the 279 differential metabolites in the validation set was conducted and found to be enriched in four specific pathways (Figure 3B and Supplementary Table 5): glycine, serine and threonine metabolism ($P < 0.001$ and FDR = 0.002), valine, leucine and isoleucine biosynthesis ($P < 0.001$ and FDR = 0.002), arginine biosynthesis ($P < 0.001$ and FDR = 0.003), and arginine and proline metabolism ($P < 0.001$ and FDR = 0.006). Finally, arginine biosynthesis, arginine and proline metabolism overlapped in the discovery and validation sets, and were considered as the NT1-related metabolic pathways. In NT1-related metabolic pathways, four hit metabolites (glutamate, L-arginine, urea and pyruvate) with concordant directional changes were considered as NT1-related metabolites (Supplementary Table 6). Among them, glutamate and urea were up-regulated, while L-arginine and pyruvate were down-regulated in patients with NT1.

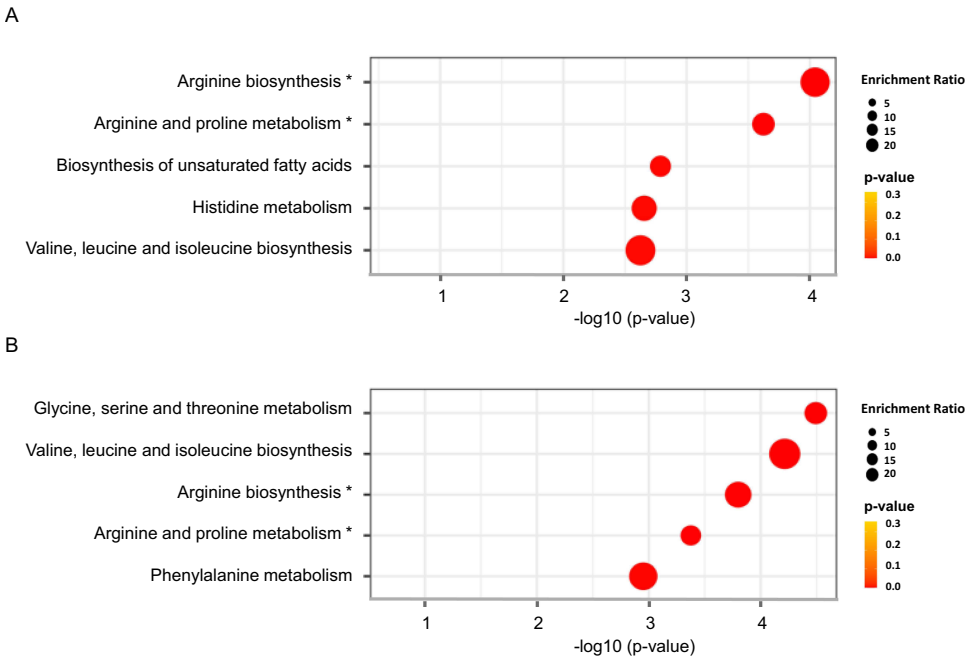

Figure 3 Enrichment analysis of differential metabolites in the discovery set and validation set.
Notes: (**A**) Overview of differential metabolite-enriched metabolite pathways in the discovery set (Top 5). (**B**) Overview of differential metabolite-enriched metabolite pathways in the validation set (Top 5). * indicates overlapping metabolic pathways in the discovery set and validation set with FDR<0.01.
Abbreviation: FDR, false discovery rate.

Correlation Between NT1-Related Metabolites and Lipid Metabolism

To further investigate the relationship between NT1-related metabolites (glutamate, L-arginine, urea and pyruvate) and TC, HDL-C, as well as LDL-C, multivariable adjusted linear regression was conducted. As shown in Table 2, after adjusting for potential confounders, glutamate was found to be negatively associated with HDL-C ($\beta = -0.319$, $P = 0.049$).

Discussion

To our knowledge, this is the first multicenter serum-metabolomics study in NT1 that links pathway-level changes to quantified lipid profiles. Through exploring the characteristics of lipid metabolism and metabolites in patients with NT1, our findings show an increased glutamate level in the arginine metabolic pathway is associated with decreased levels of HDL-C. It is suggested that dysfunction of arginine metabolism may be the candidate metabolic pathways linking NT1 and risk of dyslipidemia.

Consistent with previous studies,[13,26–28] our findings show that NT1 is associated with increased risk of impaired lipid metabolism, manifested as increased levels of TC, LDL-C, and TG, and decreased levels of HDL-C. It needs to be noted that these findings did not change even after adjusting for BMI, suggesting that the occurrence of dyslipidemia in NT1

Table 2 Linear Regression Analysis Between NT1-Related Metabolites with Blood Lipids in Patients with NT1

	TC				HDL-C				LDL-C			
	B [95% CI]	**SE**	***β***	***P***	**B [95% CI]**	**SE**	***β***	***P***	**B [95% CI]**	**SE**	***β***	***P***
Glutamate	0.061 [−0.306, 0.429]	0.182	0.052	0.737	−0.126 [−0.251, 0.000]	0.062	−0.319	**0.049**	0.057 [−0.229, 0.343]	0.141	0.064	0.689
L-arginine	0.129 [−0.308, 0.566]	0.216	0.093	0.553	0.141 [−0.009, 0.292]	0.074	0.302	0.065	0.081 [−0.259, 0.421]	0.168	0.077	0.632
Urea	−0.007 [−0.308, 0.566]	0.179	−0.006	0.969	0.062 [−0.066, 0.191]	0.063	0.154	0.330	−0.036 [−0.318, 0.245]	0.139	−0.040	0.795
Pyruvate	−0.153 [−0.422, 0.117]	0.133	−0.178	0.258	0.046 [−0.051, 0.143]	0.048	0.160	0.341	−0.117 [−0.326, 0.093]	0.104	−0.178	0.266

Notes: Linear regression analysis adjusted for age, gender, BMI and AHI. B is the regression coefficient from the linear regression analysis, and β is the standardized regression coefficient from the linear regression analysis. Values in bold indicate a $P < 0.05$.
Abbreviations: AHI, apnea-hypopnea index; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NT1, narcolepsy type 1; TC, total cholesterol.

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may not be solely associated with BMI, but rather involves other mechanisms. Previous case-control studies have suggested that the majority of patients with NT1 are overweight, have MetS (79.3% show elevated levels evaluated by the homeostasis model of insulin resistance) and low HDL-C, are more sensitive to insulin, and have a lower rate of lipolysis.[13,29–31] Although the HDL-C level of patients with NT1 decreased moderately compared with the controls. Previous study suggested that such moderate decrease of HDL-C could still contribute to the risk of MetS in NT1.[13] The specific mechanisms of the above-mentioned metabolic disorders remain unclear, and may involve hypothalamic secretin deficiency, insulin resistance and mitochondrial dysfunction.[29–31]

In the current study, decreased levels of L-arginine and pyruvate, and increased levels of glutamate and urea in the arginine biosynthesis and/or arginine and proline metabolism pathways were observed in patients with NT1 compared with controls. This is consistent with a previous study.[18] L-arginine is a semi-essential amino acid and the key precursor in the synthesis of nitric oxide (NO).[32] Furthermore, L-arginine is a key intermediate in the urea cycle.[32] Decrease of L-arginine may lead to the obstruction of the urea cycle and cause metabolic disorders.[33,34] Also, pyruvate is an important precursor of aspartate, which is a key nitrogen donor in the urea cycle.[35,36] Pyruvate deficiency may restrict aspartate production and suppressed urea cycle.[37] The urea cycle occurs in the liver, which is also the primary site of lipid metabolism.[38,39] Urea cycle disorders may cause liver function damage,[40] which in turn disrupts the synthesis and secretion of lipoproteins, thereby contributing to dyslipidemia.[41] Overall, the down-regulation of L-arginine and pyruvate, and the up-regulation of glutamate and urea in patients with NT1 may indicate the dysregulation of arginine metabolism. And it may affect liver lipid metabolism through reduced arginine synthesis and enhanced catabolism, excessive activation of NOS, and dysfunction of the urea cycle, ultimately leading to dyslipidemia (Supplementary Figure 1).

Furthermore, we found decreased levels of HDL-C are associated with higher levels of glutamate in patients with NT1. The increased release of glutamate has been demonstrated in obesity and type 2 diabetes mellitus,[42,43] indicating an excessive glutamate increase may be involved in lipid accumulation. Furthermore, glutamate has deleterious effects on the liver at higher doses, animal models of obesity and steatosis have found that glutamate causes inflammation and liver fibrosis.[44] The upregulation of glutamate level in patients with NT1 may further affect lipid metabolism by causing damage to the liver. Studies have found that NO as neurotransmitters can regulate the circadian rhythm through the suprachiasmatic nucleus of the hypothalamus or influence the sleep-wake cycle through gamma-aminobutyric acid neurons.[45–47] The reduction of arginine in patients with NT1 may further exacerbate sleep rhythm disorders by reducing NO production. In addition, as the main substrate of NOS, L-arginine participates in the regulation of vascular endothelial function by producing NO.[48,49] L-arginine may cause NT1-related dyslipidemia by affecting the synthesis of NO and the modification of apolipoprotein A1 (apoA1). NO has been suggested to regulate lipid metabolism through the activation of hepatic sterol regulatory element-binding protein-2.[50] The down-regulation of L-arginine may affect the level of HDL-C by reducing the synthesis of the downstream metabolite NO. Furthermore, previous studies have shown that L-arginine is involved in the modification of apoA1, which is the main structural protein of HDL-C.[51,52] With the down-regulation of L-arginine, the binding of apoA1 to phospholipids may be decreased, resulting in a reduction in the formation of HDL-C particles or a deterioration in their stability.[51,52] Taken together, our findings suggest that dysregulation of arginine metabolism might be the candidate metabolic pathways for NT1-related dyslipidemia. Glutamate may be potential biomarkers of dyslipidemia risk for patients with NT1. It is worth noting that in patients with NT1, the association between glutamate and HDL-C is marginal. Further research with a larger sample size is needed to further validate these findings.

Our study has several clinical implications. The observed role of glutamate in NT1-related dyslipidemia provides valuable clues for the development of specific treatments for improving metabolic health and overall well-being in patients with NT1. For example, diet or medications that can regulate glutamate level may provide a direction for improving NT1 lipid metabolism.[53–55] As a sodium salt of glutamate, monosodium glutamate (MSG) has been applied to induce obesity, while some studies of medicinal plants for the treatment of obesity have identified plants and their components to have protective effects against MSG-induced obesity.[56] Furthermore, statins have been suggested to up-regulate intracellular L-arginine levels via activation of the transient receptor potential vanilloid type 1-AMP-activated protein kinase-autophagy-urea cycle pathway in endothelial cells, which ultimately increases NO bioavailability and endothelial function.[57] Future studies are needed to confirm the findings that elevated L-arginine is effective in lowering

risk of dyslipidemia in patients with NT1. Overall, these findings carry clinical relevance, as they suggest targeted assessment of arginine metabolites could inform cardiovascular risk stratification and identify potential biomarkers for dyslipidemia in patients with NT1.

Strengths of the current study include adopting a multi-center design, which mitigated the potential bias caused by specific samples from a single center. However, the limitations of this study should be acknowledged. First, the sample size in this study was relatively small, although post hoc power analyses of the ANCOVA showed the powers were above 80% for blood lipid levels between groups. The limited sample size constrained the analysis of subgroups, including those based on age or centers. Second, this study is a cross-sectional comparative study. It cannot establish the temporal sequence or causal relationship between arginine metabolism pathway and NT1 or its metabolic sequelae. Rigorous designed longitudinal multi-omics studies with larger sample size are needed to further clarify the underlying pathological molecular mechanism of NT1-related dyslipidemia. Third, the control group recruited through advertisement in the community, to a certain extent, restricted in terms of lifestyle factors such as dietary and physical activity, thus limiting the generalizability. Future studies need to fully consider various confounding factors in order to increase the generalizability. And, lifestyle factors such as dietary and physical activity that may influence blood lipid were not available. Future studies should take these factors into consideration to confirm the findings. Additionally, the possible residual effects from prior stimulant or antidepressant use could have lingering metabolic effects. In this study, although individuals with a history of depression were excluded, it still could not be completely ruled out that subjects had used stimulant or antidepressant one month ago. Future studies should meticulously collect the previous medication information of the subjects in order to control the potential influence of drug effects on metabolic findings.

Conclusions

NT1 is associated with significant changes in lipid levels, including higher levels of TC and LDL-C, and lower levels of HDL-C. Glutamate in arginine metabolism correlates with decreased levels of HDL-C in patients with NT1. Dysfunction of arginine metabolism may be a candidate pathway that awaits further study for the association between NT1 and the risk of dyslipidemia.

Abbreviations

AHI, apnea–hypopnea index; ANCOVA, analysis of covariance; BMI, body mass index; EDS, excessive daytime sleepiness; FDR, false discovery rates; ESS, Epworth Sleepiness Scale; HDL-C, high-density lipoprotein cholesterol; HMDB, human metabolome database; KEGG, Kyoto Encyclopedia of Genes and Genomes; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; MS, mass spectrometer; MSG, monosodium glutamate; MSL, mean sleep latency; MSLT, multiple sleep latency test; NO, nitric oxide; NOS, nitric oxide synthase; NREM, non rapid eye movement; NT1, narcolepsy type 1; OPLS-DA, Orthogonal partial least-squares discriminant analysis; PSG, polysomnography; QC, quality control; REM, rapid eye movement; TC, total cholesterol; TG, triglycerides; UPLC-MS, ultra performance liquid chromatography-mass spectrometry; VIP, variable importance in the projection.

Data Sharing Statement

The data will be shared on reasonable request to the corresponding author.

Ethics Approval and Informed Consent

This study was approved by the Research Ethics Board of Mental Health Center of Shantou University (202360) and Ethical Committee of Peking University People's Hospital (2022PHB089) and informed consent was obtained from each participant. This study complied with the Declaration of Helsinki.

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Author Contributions

Jun Wu: Methodology, Writing- Original draft preparation, Visualization, Investigation, Formal analysis; Yanyuan Dai: Data curation, Investigation, Software, Writing - Review & Editing; Liyue Xu: Resources, Data curation, Writing - Review & Editing; Jiansheng Zhang: Data Curation, Writing - Review & Editing; Le Chen: Data Curation, Writing - Review & Editing; Dandan Zheng: Data Curation, Writing - Review & Editing; Baixin Chen: Data Curation, Writing - Review & Editing; Fang Han: Resources, Conceptualization, Writing - Review & Editing; Yun Li: Conceptualization, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. All the authors have reached consensus on the journal this article will be submitted. All authors agree to review all versions of the article prior to submission, during revision, acceptance of the final version for publication, and any significant changes introduced during the proofreading phase, and to be responsible for the content of the article.

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Disclosure

All authors report no potential conflicts of interest for this work.

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