Characteristics of Menstrual Cycle Disorder and Saliva Metabolomics of Young Women in High-temperature Environment

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Abstract

High-temperature environments induce menstrual disorders, which can seriously damage women's reproductive health and workability. The regulation mechanism underlying the induction of these disorders at high temperature is yet to be elucidated. Saliva is an information-rich biological fluid that can reflect systemic diseases. Here, we investigated the characteristics of menstrual cycle disorders and saliva metabolomics. Women from high and normal temperature areas of China were selected and divided into two groups—high-temperature (H group) and control (C group). A questionnaire survey was conducted in summer (July) to investigate the incidence rate of menstrual disorders, characteristics of the disorders, and factors influencing the risk of these disorders in different regions. Metabolomics was applied to analyze the characteristics of the salivary metabolites and neurotransmitters in the two groups of women with menstrual disorders.The incidence rate of menstrual disorders was significantly higher in the H group than in the C group ($P < 0.05$). High-temperature environment, anxiety, pressure, and sleep quality were identified as critical factors associated with menstrual disorders. Non-targeted saliva metabolomics identified 64 significantly different metabolites, which mainly enriched in metabolic pathways such as carbohydrate metabolism, membrane transport, digestive system, and nucleotide metabolism($P < 0.05$). Targeted metabolomics of neurotransmitters revealed increased expression of histamine (HA) and glutamine and decreased expression of 5-hydroxyindole acetic acid (5-HIAA) ($P < 0.05$). Therefore, the incidence rate of menstrual disorder was increased in the H group, which is significantly related to factors such as anxiety and pressure. Changes in metabolites and neurotransmitters indicated that besides mood, endocrine regulation mechanism, inflammatory reactions that affect the formation of the follicular, cell membrane might contribute to the development of menstrual disorders.

1 Introduction

High-temperature, dust, and noise have been identified as significant risks of the occupational environment(Matsuda et al. 1997). In particular, high-temperature environments are inevitable in specific industries, manifested by high temperature, high humidity, and strong radiation. Women have been reported to exhibit lower tolerance to high-temperature environments than men (Marsh and Jenkins 2002; Yu et al. 2010). Because of their unique physiological structure, women are troubled by different reproductive health concerns. When women are exposed to different environments, menstrual abnormalities often appear first, before their physiological functions change, mainly manifested as short, prolonged, or irregular menstrual cycles and bleeding during menstruation (Harlow and Campbell 2000; Rowland et al. 2002). The duration and patterns of the menstrual cycle are essential indices of reproductive health in women (Lisabeth et al. 2004). There are several factors besides high-temperature environments that may cause menstrual disorders. In fact, menstrual disorders are related to neuroendocrine and organic pathologies of the body, such as neuroendocrine disorders, cysts, and tumors of reproductive organs (Adams Hillard and Deitch 2005). It is also related to psychological factors such as long-term stress, depression, and anxiety (Rafique and Al-Sheikh 2018). Moreover, long-term poor

lifestyle habits such as smoking and drinking, following an irregular diet, and poor sleep pattern can also lead to menstrual disorders (Bae et al. 2018; Vyver et al. 2008). In addition, environmental changes such as long-term exposure to high concentrations of nanoparticles (Peng et al. 2020; Wang et al. 2018; Yuan Pan 2020) and the long-term use of hormones and antibiotic drugs can also lead to the development of neuroendocrine disorders because of interference with neuroendocrinology, which in turn causes menstrual disorders (Seif et al. 2015). Past studies have demonstrated that when women are exposed to high temperatures, heat exposure (HE) can result in menstrual and endocrine disorders and ovarian dysfunction in women (Dickson et al. 2018; Zheng et al. 2019); this impacts women's quality of life, increases industrial costs, and reduces operational capacity (Axmon et al. 2006; Labyak et al. 2002; Small et al. 2006). A previous study reported that approximately 13% of female soldiers had menstrual abnormalities, which affected their daily military tasks. Among the factors that cause menstrual irregularities, the outdoor thermal environment is a critical one (Powell-Dunford et al. 2003). The incidence rate of menstrual disorders in women working in a hot and humid environment, for example, in textile factories, reached nearly 40% in a study (Yao et al. 2009). Animal experiments reported that long-term HE significantly increased the rates of estrous cycle disorders in female rats; the organ coefficient of the uterus increased, local cell proliferation occurred, and reproductive function was damaged (An et al. 2020a). Roth reported that heat stress in summer reduced the pregnancy rate of cows and affected their reproductive ability (Roth 2017). These results indicate increased risk of menstrual disorders in women in high-temperature environment, thereby affecting their reproductive health.

The prediction results of the United Nations Intergovernmental Panel on Climate Change indicated that the frequency and intensity of heat stress in summer will continue to increase (Izrael et al. 2007). Therefore, issues related to female reproductive health among outdoor workers and women working in special industries (such as female military and working in steel mills, etc.) are important to be addressed. However, the mechanism of high temperatures resulting in menstrual disorders has been rarely reported. Saliva is an information-rich biological fluid that can reflect systemic diseases, can screen various diseases, and can be collected non-invasively, conveniently, safely, and economically (Kaczor-Urbanowicz et al. 2017; Ueda et al. 2020; Zhang et al. 2012). Specific changes in saliva metabolism may lead to the development of periodontal diseases and oral cancer. Importantly, metabolites in the blood can enter the saliva through extracellular, intracellular, or paracellular pathways, including passive diffusion or active transport in the salivary glands or gingival sulcus (Spielmann and Wong 2011). Therefore, saliva metabolites may provide a window for the use of other parts of the body in the early detection of human diseases.

For this purpose, this study aimed to conduct a cross-sectional survey on menstrual conditions of women working in the same occupational category, classified into the high-temperature (H group) and control (C group) groups to clarify the degree of influence that high-temperature environments has on women's menstruation cycle, characteristics of menstrual disorders, and factors affecting the disorders. Metabolomics was performed to explore the effects of high-temperature environment on women's saliva metabolites and neurotransmitters, screen candidate markers, and analyze the regulatory mechanism behind high temperature resulting in menstrual disorders. Our study will construct a non-invasive and

convenient menstrual disorder monitoring and early warning technology, as well as provide a theoretical basis to obtain deeper understanding of the mechanism of women's menstrual disorders caused by high temperature.

2 Materials And Methods

2.1 Study subjects

In this study, 125 women (aged 22 ± 3 years) with a body mass index (20.83 ± 2.6 kg/m^2) working in the same occupation who had lived in the local area for more than half a year were recruited from two regions of China. The recruited women had no smoking or drinking habits and all had the same daily working hours, exercise intensity, and diet structure. The northern region of China is located at 43.88 ºN latitude, which is in the northern temperate zone, and the southern region is located at 23.05 ºN latitude, which belongs to the tropical zone. Women from the northern region (average annual temperature 0–10°C, summer temperature 15–25°C) were included in the C group (n = 80), and those from the southern region (average annual temperature 20–28°C, summer temperature 28–38°C) were included in the H group (n = 45). The inclusion criteria for subjects were ability to complete the survey correctly; patients with history of pregnancy, hysterectomy, and ovariectomy and without menarche were excluded.

This study was conducted according to the principle of the Declaration of Helsinki. All operational procedures were performed according to ethical principles of the Institute of Environmental and Operational Medicine and approved by the ethics review committee. The subjects were informed about the study’s objective and voluntary participation, and all signed the informed consent form.

2.2 Research questionnaire

A total of three questionnaires, including the Menstrual Status Questionnaire, the Influence Factors of Menstrual Questionnaire, and the Symptom Checklist-90 (SCL-90), were administered (Zhang and Zhang 2013). The Menstrual Status Questionnaire inquires about menstrual characteristics and premenstrual symptoms (the coefficient of internal consistency Cronbach’s α = 0.879). The Influence Factors of Menstrual Questionnaire inquires about emotional and psychological status, family history of the disease, medication history, diet, sleep, and environmental conditions (the coefficient of internal consistency Cronbach’s α = 0.703). The SCL-90 consists of nine subscale dimensions, namely, Somatization (SOM), Obsessive Compulsive (OC), Interpersonal-Sensitivity (INT), Depression (DEP), Anxiety (ANX), Hostility (HOS), Phobic-Anxiety (PHOB), Paranoid Ideation (PAR), and Psychoticism (PSY). This study was conducted during summer. All necessary information was collected from the medical records of participants through direct interviews and questionnaires from the last 6 months.

2.3 Sample collection and preparation

On the day before saliva collection, all subjects were allowed to only drink water after 21:00 (UTC + 8). Subjects' saliva was collected from 09:00 AM to 11:00 AM according to the saliva collection method specified by Asai (Asai et al. 2018) et al. Subjects were not allowed to drink water, smoke, brush their

teeth, perform energetic exercise, or apply lipstick before saliva collection. Participants mouth were rinsed gently with clean water during saliva collection, and straws were used to assist in collecting unstimulated saliva. The collected saliva was stored at -80°C until further use. Furthermore, 5 mL of venous blood was collected on an empty stomach, and serum was collected and stored at -80°C.

2.4 Non-targeted saliva metabolomics

2.4.1 Sample pretreatment and analysis

The saliva samples were thawed at 4°C and centrifuged (7000 *g*, 5 min) to obtain the supernatant. Then, 100 μL of the saliva samples were mixed with 400 μL of precooled methanol acetonitrile solution (1:1, v/v) to remove protein, and then vortex-mixed for 20 min (4°C, 14000 *g*). The supernatant was freeze-dried and stored at -80°C. For mass spectrometry, 100 μL of acetonitrile aqueous solution (acetonitrile: water = 1:1, v/v) was added to re-dissolution, vortexed, and centrifuged (4°C, 14000 *g*, 15 min), and the supernatant was collected for analysis.

The procedure was performed in the Agilent 1290 Infinity LC Ultra High-Performance Liquid Chromatography System (UHPLC) Hydrophilic Interaction Liquid Chromatography (HILIC) column. The column temperature was 25°C, flow rate was 0.3 mL/min, and injection volume was 2 μL. The mobile phase comprised A (water + 25 mM ammonium acetate + 25 mM ammonia) and B (acetonitrile). The gradient elution procedure was as follows: 0–1 min, 95% B; 1–14 min, 95%-65% B; 14–16 min, 65%-40% B; 16–18 min, 40% B; 18–18.1 min, 40%-95% B; and 18.1–23 min, 95% B. The samples were placed in an autosampler at 4°C during the entire analysis.

Electrospray ionization (ESI) experiments were executed on the Triple TOF 5600 mass spectrometer (MS) (AB SCIEX) in positive and negative ion modes. The ESI source conditions after HILIC chromatographic separation were set as follows: ion source Gas 1, 60 psi; ion source Gas 2, 60 psi; curtain gas, 30 psi; source temperature, 600°C; ion spray voltage floating ± 5500 V; TOF MS scan m/z range, 60–1000 Da; production scan m/z range, 25–1000 Da; TOF MS scan accumulation time, 0.20 s/spectra; and production scan accumulation time, 0.05 s/spectra. The secondary mass spectrum was acquired using information dependent acquisition (IDA) and adopting the high sensitivity mode. Declustering potential: ± 60 V; collision energy, 35 ± 15 eV; IDA was set as Exclude isotopes within 4 Da, candidate ions to monitor per cycle: 6.

2.4.2 Data analysis

The raw data were converted into mzXML format using Proteo Wizard. The XCMS program (http://xcmsonline.scripps.edu) was adopted for peak alignment, retention time correction, and peak area extraction. mzXML is a file format published by the Institute for Systems Biology, Insilicos, and other companies for the exchange of mass spectrometry data. mzXML offers the advantages of openness, scalability, and flexibility, and it is particularly suitable for storing and exchanging mass spectrometry data. XCMS is a mass spectrometry data analysis software for endogenous metabolites, which provides a complete metabolomics workflow, including signature detection, retention time correction, alignment,

annotation, and statistical analysis. The software SIMCA-P 14.1 (Umetrics, Umea, Sweden) was used for pattern recognition, and data were reprocessed by Pareto-scaling; then, multi-dimensional statistical analysis was performed. One-dimensional statistical analysis included Student's t-test and fold change analysis. Data were visualized using R software. Metabolites with significant differences between the groups (variable importance for the projection (VIP), VIP > 1; Wilcoxon rank-sum test $P < 0.05$) were screened to perform qualitative analysis.

2.5 Targeted metabolomics of serum neurotransmitters

2.5.1 Sample pretreatment and analysis

The saliva samples (100 μL) were mixed with 400 μL of precooled pure acetonitrile containing 1% FA, vortex-mixed, and ultrasonicated in ice bath for 20 min. The protein was precipitated ultrasonically at -20°C for 1 h in an ice bath, centrifuged (4°C, 14000 *g*, 20 min), and taken dry with the supernatant vacuum. For mass spectrometry, 100 μL of ACN/water (1:1, v/v) with 1% FA was added to re-dissolution and centrifuged (4°C, 14000 *g*, 20 min), and the supernatant was collected for analysis.

Sample separation was performed using the Agilent 1290 Infinity LC UHPLC system. The mobile phase comprised liquid A (0.1% FA 25 mM ammonium formate aqueous solution) and B (0.1% FA acetonitrile). The sample was placed in an automatic sampler at 4°C, column temperature was 45°C, flow rate was 300μl/min, and injection volume was 2 μL. The relevant liquid phase gradients were as follows: 0–18 min, 90%-40% B; 18-18.1 min, 40%-90% B; and 18.1–23 min, 90% B. The 5500 QTRAP mass spectrometer (AB SCIEX) was used for mass spectrometry in the negative ion mode. The 5500 QTRAP ESI source conditions were set as follows: source temperature, 550°C; ion source Gas 1, 60 psi; ion source Gas 2, 60 psi; curtain gas, 35 psi; and ion spray voltage floating, 5000 V. The multiple reaction monitoring (MRM) mode was applied to detect the ion pair.

2.5.2 Data analysis

The chromatographic peak area and retention time were measured using the Multiquant software. The neurotransmitter standard was used to correct the retention time and identify the metabolites.

2.6 Statistical analysis

SPSS24.0 software was used for statistical analysis. The quality parameters are presented as percentages, and the quality parameters between the two groups were compared using chi-square test. The influencing factors were analyzed using logistic regression analysis. Wilcoxon rank-sum test was used for comparisons between the two groups. Values with $P < 0.05$ were considered statistically significant.

3 Results

3.1 Menstrual disorders

The results of the Menstrual Status Questionnaire revealed that the incidence rate of menstrual disorders in women in the H group was 75.56%, which was significantly higher than the 57.5% noted in women in the C group ($P < 0.05$) (Fig. 1A). Thus, high-temperature environment may lead to increased incidence of menstrual disorders in women. Based on the results of the survey, we analyzed the characteristics of menstrual disorders and noted that the proportion of women with heavy menstruation was significantly higher in the H group than in the C group ($P < 0.05$) (Fig. 1B). Further, we divided the premenstrual symptoms into "emotional and social function" (Fig. 1C), "physical pain" (Fig. 1D), "endocrine" (Fig. 1E), and "others" (Fig. 1F). Analysis using the chi-square test revealed no significant difference in premenstrual symptoms between the two groups of women ($P > 0.05$).

3.2 Influencing factors of menstrual disorders analysis

The Influence Factors of Menstrual Questionnaire was administered to determine the factors influencing menstrual disturbances in the subjects. The single factor logistic regression analysis used the occurrence of menstrual disorder as the dependent variable while the influence factors as independent variables(Table 1). Statistically significant variables in the single-factor analysis served as independent variables and menstrual disorders served as dependent variables. Multifactorial logistic regression analysis was adopted, which revealed nervousness and anxiety, pressure, medication history, sleep quality, and resident temperature as the risk factors for menstrual disorders in women ($P < 0.05$) (Table 2). As can be seen in Tables 1 and 2, B is the regression coefficient and intercept (a constant term), which is statistically significant at $P < 0.05$; SE is the standard error, indicating the mean error of the estimate; Wald is the test statistic; OR is the odds ratio, which is the rate of change in the occurrence of the event. In addition, 95% CI indicates a 95% probability that the OR value falls in this range, and the values in parentheses indicate the lower and upper limits of the range, respectively.

Table 1
Single factor logistic regression analysis of menstrual disorders predictors

Item	B	S.E.	Wald	P	OR	95% CI
Anxiety	1.796	0.454	15.677	0.000*	6.026	(2.477,14.662)
Depression	1.561	0.498	9.806	0.002*	4.762	(1.793,12.649)
Pressure	1.232	0.419	8.642	0.003*	3.427	(1.508,7.791)
Family history of menstrual disorders	1.555	0.671	5.378	0.020*	4.737	(1.272,17.636)
Medication history	1.168	0.698	2.802	0.094*	3.214	(0.819,12.612)
Cold drinks and food	1.000	0.532	3.539	0.060*	0.368	(0.130,1.043)
Sleep time	0.961	0.543	3.131	0.077*	0.383	(0.132,1.109)
Sleep quality	1.386	0.416	11.089	0.001*	3.997	(1.768,9.035)
The time to fall asleep	2.669	0.583	20.966	0.000*	14.419	(4.601,45.187)
Temperature feeling	1.292	0.415	9.711	0.002*	3.641	(1.615,8.207)

*$P < 0.1$ was considered statistically significant, only meaningful predictive variables are shown in the table.

Table 2
Multivariate logistic regression analysis of menstrual disorders predictors

Item	B	S.E.	Wald	P	OR	95% CI
Anxiety	2.076	0.757	7.527	0.006*	7.976	(1.810,35.161)
Depression	0.108	0.827	0.017	0.896	1.115	(0.220,5.641)
Pressure	1.762	0.839	4.408	0.036*	5.823	(1.124,30.156)
Family history of menstrual disorders	0.387	0.978	0.156	0.692	1.472	(0.217,10.000)
Medication history	1.121	1.089	1.060	0.303	3.069	(0.363,25.931)
Cold drinks and food	-0.634	0.790	0.645	0.422	0.530	(0.113,2.493)
Sleep time	-1.194	1.043	1.311	0.252	0.303	(0.039,2.340)
Sleep quality	2.384	0.790	9.105	0.003*	10.844	(2.306,50.998)
The time to fall asleep	-0.097	0.700	0.019	0.889	0.907	(0.230,3.577)
Temperature feeling	2.222	0.795	7.812	0.005*	9.225	(1.942,43.815)
Constant	-13.267	5.009	7.014	0.008*	0.000	

*$P < 0.05$, was considered statistically significant

3.3 SCL-90 analysis

To evaluate the recent emotional status of the two groups of women, the SCL-90 was administered, which distinguishes people who already have psychiatric symptoms from those who do not. A higher total score on the SCL-90 indicates a more urgent need for individual intervention. The results of the questionnaire survey and analysis by t-test indicated differences in the mental and psychological statuses between the two groups of women; the scores of anxiety and depression were significantly higher in the H group than in the C group ($P < 0.05$). These findings suggested that women living in high-temperature environments are more likely to have negative emotions, such as anxiety and depression (Table 3).

Table 3
The SCL-90 scores of women in both the groups after long-term heat exposure (Mean ± SD)

	H group	C group	T	Sig.
SUM-SCL	129.44 ± 33.87	113.58 ± 24.19	2.47	0.017*
SOM	1.44 ± 0.56	1.22 ± 0.42	2.05	0.045*
OC	1.56 ± 0.61	1.42 ± 0.52	1.26	0.09
INT	1.44 ± 0.61	1.31 ± 0.52	1.14	0.26
DEP	1.50 ± 0.66	1.19 ± 0.43	2.47	0.018*
ANX	1.32 ± 0.47	1.13 ± 0.37	2.11	0.04*
HOS	1.38 ± 0.55	1.21 ± 0.47	1.61	0.11
PHOB	1.24 ± 0.55	1.04 ± 0.19	2.01	0.05
PAR	1.32 ± 0.47	1.10 ± 0.35	2.43	0.019*
PSY	1.26 ± 0.45	1.10 ± 0.35	1.86	0.07
Compared with the C group, *$P < 0.05$				

3.4 Salivary metabolic profile

Thirty-four saliva samples from the H group menstrual abnormalities and 21 saliva samples from the C group abnormalities were collected. Based on the LC-MS method, the quality control samples were tightly clustered using principal component analysis (PCA) model, which showed positive and negative ion patterns, indicating good reproducibility of this project (Fig. 2A). The orthogonal partial least squares discriminant analysis (OPLS-DA) model showed good clustering and clear differentiation between the groups (Fig. 2B). Commonly used univariate analysis methods such as fold change (FC) analysis, t-test, and volcano plot combining the first two analysis methods revealed significantly different metabolites between the two groups (Fig. 2C). VIP obtained from the OPLS-DA model was used to measure the strength and explanatory power of the expression patterns of each metabolite on the classification of each group of samples. Hierarchical clustering of samples using qualitatively significantly different metabolite expressions revealed that metabolites that were clustered together had similar expression patterns and were in closer reaction steps in the process of metabolism (Fig. 2D).

A total of 64 significantly different metabolites (VIP > 1 and $P < 0.05$) were screened (Table 4), of which the expression levels of 34 salivary metabolites were significantly higher in women in the H group than in those in the C group: D-proline, Pro-Arg, and phosphorylcholine (PC) expression was 3.54-, 3.53-, and 3.38-times higher in the H group than in the C group, respectively ($P < 0.05$). The expression levels of another

30 salivary metabolites were significantly lower in women in the H group: mevalonic acid (MVA), phthalic acid mono-2-ethylhexyl ester, and diethyltoluamide expression was 0.29, 0.52 and 0.55 times higher than in the C group, respectively ($P < 0.05$). Correlation analysis of these metabolites identified possible interactions between these metabolites, such as a positive correlation noted between myo-inositol (MYO) and D-sorbitol and a negative correlation noted between l-glutamine and 3-aminosalicylic acid ($P < 0.05$) (Fig. 2E). Taken together, the salivary metabolomic results suggested a crucial role that these dysregulated metabolites play in the development of menstrual disorders in women at high temperatures.

3.5 Enrichment of the differential metabolites of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway

The Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.kegg.jp/) is a commonly used database for pathway studies, based on the KEGG pathway in the context of a metabolic pathway in which the species or a closely related species are involved. The Fisher's exact test analyzes and calculates the significance level of metabolite enrichment for each pathway to identify the metabolic and signal transduction pathways that are significantly affected. The KEGG pathway enrichment results showed that the differential metabolites were mainly enriched in eight pathways, namely, ABC transporters, fructose and mannose metabolism, protein digestion and absorption, amino sugar and nucleotide sugar metabolism, pyrimidine metabolism, galactose metabolism, ascorbate and alternate metabolism, and pentose phosphate pathway (Fig. 2F).

Table 4
Significant alterations in saliva metabolites of women in 2 groups

name	description	VIP	Fold change	p-value
M116T585	D-Proline	1.720	3.545	0.003
M272T867	Pro-Arg	1.918	3.533	0.001
M184T932	Phosphorylcholine	1.039	3.386	0.028
M164T427	DL-3-Aminoisobutyric acid	1.818	3.150	0.006
M308T725	N-Acetylneuraminic acid	1.589	3.062	0.037
M70T585	2-Amino-2-methyl-1,3-propanediol	1.738	3.054	0.003
M173T731	Glycylproline	1.607	3.025	0.023
M114T586	D-Proline	1.517	2.997	0.046
M223T374	D-Quinovose	2.035	2.960	0.002
M163T376	L-Rhamnose	1.815	2.956	0.016
M163T428	L-Fucose	1.748	2.900	0.022
M130T544	D-Pipecolinic acid	2.598	2.762	0.000
M242T895	Phosphorylcholine	1.366	2.366	0.027
M325T785	Maltitol	1.259	2.214	0.047
M155T449	Orotate	1.513	2.151	0.006
M139T548	Allantoin	1.599	2.044	0.007
M179T750	myo-Inositol	1.598	2.002	0.025
M163T198	D-Sorbitol	1.711	1.921	0.042
M111T449	Uracil	1.556	1.903	0.006
M244T701	Pro-Gln	2.040	1.849	0.003
M177T873	Acetyl phosphate	1.753	1.829	0.003
M376T498	(-)-Tylocrebrine	1.358	1.822	0.050
M183T493	L-Glutamine	1.632	1.764	0.011
M129T83	ketoisocaproic acid	1.354	1.733	0.041
M213T817	2-Deoxyribose 5-phosphate	1.551	1.631	0.024
M297T122	Nname,cis-9,10-Epoxystearic acid	1.871	1.611	0.041
M174T765	N-Acetyl-L-aspartic acid	1.706	1.602	0.036

name	description	VIP	Fold change	p-value
M195T78	4-Hydroxybutanoic acid lactone	1.297	1.576	0.017
M151T468	Xylitol	1.409	1.556	0.029
M259T923	D-Mannose 1-phosphate	1.339	1.520	0.048
M215T494	Val-Pro	1.768	1.477	0.043
M303T512	Phe-His	1.519	1.474	0.037
M261T272	Val-Val	1.659	1.453	0.022
M180T390	Acamprosate	1.616	1.273	0.020
M201T641	Sebacic acid	1.025	0.789	0.042
M157T127	D-Glucuronolactone	1.167	0.775	0.042
M231T520	DL-a-Hydroxybutyric acid	1.789	0.759	0.014
M161T133	D-Mannose	1.282	0.758	0.019
M177T748	D-gluconate	1.405	0.755	0.019
M117T475	2-Hydroxy-3-methylbutyric acid	1.443	0.754	0.031
M133T122	Ethyl 3-hydroxybutyrate	1.419	0.742	0.024
M189T741	N.alpha.-Acetyl-L-lysine	1.201	0.728	0.046
M161T737	L-Gulonic gamma-lactone	1.613	0.724	0.008
M108T138	Nitrosobenzene	1.560	0.722	0.005
M131T405	Hydroxyisocaproic acid	1.447	0.720	0.025
M96T92	2(1H)-Pyridinone	1.737	0.714	0.000
M162T671	L-Carnitine	1.420	0.711	0.041
M152T82	3-Aminosalicylic acid	1.511	0.709	0.002
M161T534	3-Hydorxy-3-methylglutaric acid	1.396	0.686	0.002
M143T146	D-Threitol	1.202	0.678	0.021
M168T701	L-Cysteic acid	1.262	0.663	0.049
M134T452	Oxindole	1.785	0.658	0.004
M125T144	Thymine	2.992	0.640	0.029
M261T429	Ile-Glu	1.576	0.624	0.022
M319T126	12-oxo-ETE	1.315	0.602	0.026

name	description	VIP	Fold change	p-value
M130T499	N-Acetyl-L-alanine	2.037	0.580	0.005
M149T156	3-Methylphenylacetic acid	6.647	0.575	0.000
M321T88	20-Hydroxyarachidonic acid	1.625	0.572	0.006
M120T272	Tyramine	1.705	0.570	0.000
M85T172	Isovaleric acid	2.127	0.566	0.000
M137T75	2-(4-Hydroxyphenyl) ethanol	1.279	0.561	0.026
M192T65	Diethyltoluamide	1.376	0.551	0.005
M277T792	Phthalic acid Mono-2-ethylhexyl Ester	1.990	0.529	0.000
M147T555	Mevalonic acid	2.437	0.299	0.000

3.6 Analysis of targeted neurotransmitters

For a more comprehensive exploration of metabolite changes in women with menstrual disorders caused by high-temperature environment, serum was collected from the subjects and analyzed. Based on the MRM approach, targeted metabolomic analysis was performed on serum neurotransmitters in the H and control groups. the expression levels of three neurotransmitters were found to be significantly altered, with increased expression of histamine (HA) and glutamine, which were 2.077- and 1.366-times higher in the H group than in the C group, respectively ($P < 0.05$), and decreased expression of 5-hydroxyindole acetic acid (5-HIAA) in the H group, which was 0.701-times higher than that in the C group ($P < 0.05$) (Fig. 3). This finding indicated that neurotransmitters related to inflammatory response and mood are altered at high-temperature environments.

4 Discussion

4.1 High-temperature environment and menstrual disorders

High temperatures can result in heat stress (Horowitz 2002; Horowitz 2007), which is the sum of nonspecific responses occurring in humans or animals when an organism is exposed to excessive temperature stimuli exceeding its thermoregulatory capacity in a high-temperature environment. It is a stress factor that special operating populations have to guard against in high-temperature environments (Kovats and Hajat 2008). During heat stress, the normal thermal homeostasis system of the body may be disrupted, leading to the disruption of digestive somatic functions, impaired blood circulation, and disruption of the neuroendocrine functions, which together pose a severe threat to the development of reproductive health concerns in women working under high-temperature environments (Fan et al. 2019; Wang et al. 2016). HE can increase the risk of menstrual disorders and impact the reproductive function

in women (An et al. 2020b). The characteristics of changes and regulatory mechanisms of menstrual disorders that appear at high temperatures remain unclear. We selected two regions with significant differences in their mean temperature and used a random, whole-group sampling method to conduct a cross-sectional survey of young women belonging to the same work category. In this study, the confounding factors were controlled accordingly. Only young migrant females rather than native youth were from both regions in this study. In addition, other variables including age, BMI, work intensity, and dietary habits were not significantly different between the two groups. Altitude and environmental temperature were also analyzed as climate variables. It was found that both regions were in the plains, while there were significant differences in environmental temperature. The control group was located at 43.88 ºN latitude, which belongs to the northern temperate zone (average annual temperature 0–10°C, summer temperature 15–25°C) and the high temperature group was located at 23.05 ºN latitude, belonging to the tropics (average annual temperature 20–28°C, summer temperature 28–38°C). Based on the analysis of population characteristics and environmental variables, we consider that it is some representative of these people in this study: young women who were migrant to both areas with the same work intensity.

This study found a significantly higher rate of menstrual cycle disorders in young women working in hot environments. These findings indicate that high-temperature environments are more likely to result in menstrual disorders and increase menstrual volume but has no significant effect on premenstrual syndromes. Anxiety, pressure, history of medication, low sleep quality, and high temperature were noted as influencing factors that affect menstruation and increase the risk of menstrual disorders in women. Hot environment as a stressor also had specific effects on the psychology of women working in such environments, mainly in the form of increased negative emotions such as tension, depression, anxiety, fear, and anger. Common menstrual problems are closely related to increased psycho-emotional changes, anxiety, and excessive psychological stress, which are critical factors affecting menstrual disorders and resulting in amenorrhea (Allsworth et al. 2007; Rafique and Al-Sheikh 2018). At the same time, emotions result in the release of hormones from the pituitary and hypothalamus (Matsuda et al. 1997). Menstruation occurs when there is a change in the regularity of hormones secreted by the ovaries and acting on the endometrium, which is controlled by hormones released by the hypothalamus (Shufelt et al. 2017). In addition, sleep deprivation, short sleep duration, low sleep quality, and altered circadian rhythms inhibit melatonin secretion, affecting ovarian function and reducing menstrual rate, thereby leading to menstrual disorders or dysmenorrhea in women (Czajkowska et al. 2019; Meers and Nowakowski 2020; Najafi et al. 2018). In summary, HE may affect women's emotions and alter hormone levels in them, which can interfere with the hypothalamic-pituitary-ovarian (HPO) axis and affect the normal appearance of menstruation. These changes induce ovulation and disruption of the menstrual cycle, thereby resulting in menstrual disorders.

4.2 High-temperature environment and salivary metabolites

Saliva is an information-rich biofluid that can noninvasively respond to human diseases. In this study, the expression levels of a total of 64 saliva metabolites were significantly altered in women with menstrual

disorders in the H and C groups; of these, the expression of 34 metabolites was upregulated, whereas that of 30 was downregulated. These included amino acids, peptides, and carbohydrates, which can involve inflammatory response, immune regulation, amino acid metabolism, and membrane production in organisms.

Among the metabolites with upregulated expression levels, the levels of PC and N-acetylneuraminic acid were 3.39- and 3.06-times higher in the H group than in the C group, suggesting that HE induces inflammatory and immune response modulation. PC is a structural component of various prokaryotic and eukaryotic pathogens, with a surprising range of immunomodulatory properties that can benefit the infected host by targeting innate and adaptive immune responses. However, its broad immunomodulatory properties can harm the host through immunomodulation (Harnett and Harnett 1999). N-acetylneuraminic acid, a derivative of neuraminic acid collectively known as sialic acid (SA), is the major SA found in mammalian cells (Varki 1992). Changes in SA levels can trigger the development of various diseases, including inflammation, cardiovascular disease, neurological disorders, and endocrine disorders (Reuter and Gabius 1996). It has been suggested that elevated SA levels reflect acute phase response in the inflammatory process; that a positive correlation exists between tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which are essential mediators of the acute phase response; and that elevated inflammatory factors affect loops such as the hypothalamic–pituitary–adrenal (HPA) axis, which in turn affect reproductive function(Demir et al. 2018). Therefore, HE may induce inflammatory and immune response modulation, leading to menstrual disorders by affecting the HPA axis loop.

MYO and D-sorbitol were mainly enriched in the membrane transport pathway. MYO has high biological importance and is widely distributed in nature; it belongs to the glycan family. Its derivatives are important components of the cell membrane structural phospholipids and are precursors of second messengers of metabolic pathways (Milewska et al. 2016). The results suggested that menstrual cycle disorders are associated with endocrine disruption and maintenance of cell membrane stability. MYO deficiency and impairment of the MYO-dependent pathways might play crucial roles in the pathogenesis of insulin resistance and hypothyroidism. Insulin and thyroxine are important components of the HPO axis. In a previous study, the kinetic cycle was disturbed and insulin levels were reduced in female rats after prolonged heat stress (An et al. 2020a). Abnormalities in insulin metabolism also underlie several clinical diseases (MacFarlane and Di Fiore 2018). Heat stress can downregulate the expression of inositol-requiring enzyme 1α (IRE1α), leading to termination of the IRE1α signaling pathway, which causes an unfolded protein response in cells and affect the production of cell membrane proteins (Homma and Fujii 2016). The expression of D-sorbitol, a metabolite that was positively correlated with MYO, was also increased. Appropriate D-sorbitol supplementation can significantly reduce the formation of blastocysts and increase the apoptosis index (Lin et al. 2015). Therefore, long-term exposure to high temperatures and HE may result in an imbalance in the metabolism of insulin and thyroxine. Cell membrane stability is reduced, which may increase apoptosis and cause menstrual disorders.

Among the metabolites with reduced expression in this study, the expression levels of MVA and tyramine were 0.3 and 0.57 times higher in the H group than in the C group. Reduced expression of MVA affects the

MVA pathway, which is an essential metabolic pathway for the organism and is critical for cell survival and function (Ermini et al. 2017; Mullen et al. 2016). MVA biosynthetic intermediates are crucial regulators of intrinsic immunity in bovine endometritis (Mullen et al. 2016). Tyramine is a biological trace amine that is generated through decarboxylation of the amino acid tyrosine, and substantial evidence suggest that tyramine is a neuroactive chemical exhibiting multiple physiological effects (Lange 2009); it can affect various physiological mechanisms, exhibits neuromodulatory properties and cardiovascular and immunological effects (Andersen et al. 2019), stimulates the insulin-IGF-1 signaling (IIS) pathway, and blocks the induction of stress response genes by activating adrenergic-like receptors in the intestine. Tyrosine can directly or indirectly act on the ovaries to inhibit luteal function, thereby affecting reproduction. Therefore, changes in N-acetylneuraminic acid levels, MYO, and tyramine in salivary metabolites at high-temperature environment may affect the menstrual cycle through inflammatory responses, influence membrane production, and participate in immune regulation, leading to the development of menstrual disorders.

In this study, KEGG pathway enrichment revealed the involvement of several metabolites in carbohydrate metabolism, mainly fructose and mannose, galactose, and amino and nucleotide sugar metabolism. Glucose metabolism is a core component of energy metabolism, essential in the maintenance of normal physiological functions of the body. Abnormal glucose metabolism is closely associated with metabolic syndrome and diseases such as cancer (Andersen et al. 2013). Additionally, abnormal glucose metabolism is associated with several gynecological disorders (Ferreira and Motta 2018). Insulin resistance, as a pathogenic base of glucose metabolism abnormalities, affects the action of sex hormones on the ovaries and endometrium through insulin-like growth factor-1 receptor (IGF-1R), leading to anovulation or endometrial lesions (Li et al. 2012). Therefore, abnormal glucose metabolism triggers gynecologically related diseases and also induce menstrual disorders.

4.3 High-temperature environment and serum neurotransmitters

Neurotransmitter is a chemical "messenger" molecule that transmits signals between synapses. The secretion of neurotransmitters promotes the balance of amino acid metabolism in the body, regulate body's immune functions and cardiovascular activities, and mediate smooth muscle contraction. In this study, serum targeted metabolomics revealed increased expression of HA in the H group, which was 2.077 times higher than that in the C group. HA is present in the mammalian myocardium, mast cells, basophils, skin, gastrointestinal tract, and lungs, as well as in the central nervous system. Central HA, as a central neurotransmitter, is related to obesity, diabetes, and endocrine (Watanabe and Yanai 2001). Plasma HA is mainly used as an inflammatory mediator and immune substance. Studies have confirmed that HA released by mast cells can stimulate histamine type 2 receptor (H2R) in the rat kidney as an inflammatory mediator. The release of renin and under stress conditions resulted in increased HA levels in the hypothalamus and periphery in mice(He et al. 2009). This is consistent with the results of our saliva metabolomics analysis, in which inflammatory reactions occurred under high-temperature environments. In contrast, the expression of 5-HIAA, which is a primary end product of 5-hydroxy tryptamine (5-HT)

metabolism and plays an emotional regulation role, decreased under high-temperature environment (Elghozi and Laude 1989). Dysfunction of 5-HT can cause different mental diseases, including depression, impulsive aggression, and anxiety, which is also consistent with our previous questionnaire results. Thus, prolonged hot environments may increase anxiety in humans and affect the endocrine system. Therefore, changes in serum neurotransmitter levels provide better corroboration for our salivary metabolomics results.

5 Conclusion

Our findings revealed that the rate of menstrual disorders increased in women who were exposed to long-term heat environment, and pressure and anxiety were identified as the main influencing factors. The changes in the metabolite levels, such as the levels of N-acetylneuraminic acid, MYO, and tyramine may be candidate markers for early diagnosis. The elevation of N-Acetylneuraminic acid level could respond to the acute-phase response during an inflammatory process, affecting the reproductive system by influencing the HPA axis loop. myo-Inositol causes termination of the IRE1α signaling pathway by inducing a downregulation of IRE1α protein, which is required for inositol, in response to heat stress, thereby causing the formation of an unfolded protein in the cell reaction, which in turn affects oocyte membrane production. Moreover, decreased tyramine, with changes in the complexing concentrations, can act directly or indirectly on the ovary to inhibit the luteal functions, which in turn affects reproduction. These changes in the differential metabolites may be closely related to the occurrence of menstrual disorders in women. The effect of high temperature as a stressor on mood is closely related to the occurrence of menstrual cycle disorders. Therefore, it is suggested that high temperature environment and mood control may reduce the risk of female reproductive health.

Abbreviations

H group, high-temperature group; C group, control group; 5-HIAA,5-hydroxyindole acetic acid; HE, heat exposure; SCL-90, Symptom Checklist-90; SOM, Somatization; OC, Obsessive Compulsive; INT, Interpersonal-Sensitivity, DEP, Depression; ANX, Anxiety; HOS, Hostility; PHOB, Phobic-Anxiety; PAR, Paranoid Ideation; UHPLC, Ultra High-Performance Liquid Chromatography; HILIC, Hydrophilic Interaction Liquid Chromatography; ESI, electrospray ionization; MS, mass-spectrometer; IDA, information dependent acquisition; VIP ,variable importance for the projection, MRM, multiple reaction monitoring; QC, quality control; PCA, principal component analysis; OPLS-DA, Orthogonal partial least squares discriminant analysis; FC, fold change; MVA , mevalonic acid; MYO, myo-inositol; FET, Fisher's Exact Test; KEGG, Kyoto Encyclopedia of Genes and Genomes; HA ,histamine; HPO, Hypothalamus-pituitary-ovaries; SA, sialic acid; TNF-α, tumor necrosis factor-α; IL-6,interleukin-6; HPA, hypothalamic–pituitary–adrenal; IRE1α, inositol-requiring enzyme 1α; UPR, unfolded protein response; IIS, insulin-IGF-1 signaling; IR Insulin resistance; IGF-1R, insulin-like growth factor-1 receptor; H2R, histamine type 2 receptor; 5-HT, 5-hydroxy tryptamine.

Declarations

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Authors' contributions

QM, DFY and JW conceived, designed, and supervised the project. MFW and GHA executed the project and prepared the manuscript. LJF and XWC performed statistical analysis and drew the figures. CL and JJC was responsible for collecting data. All authors have read and approved the final manuscript.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All operational procedures were carried out according to the Institute of Environmental and Operational Medicine ethical principles and approved by the ethics review committee. All subjects were informed about the aim of the study, signed the informed consent form.

Consent for publication

All authors consented to publication.

Competing interests

The authors declare that they have no competing interests.

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Figures

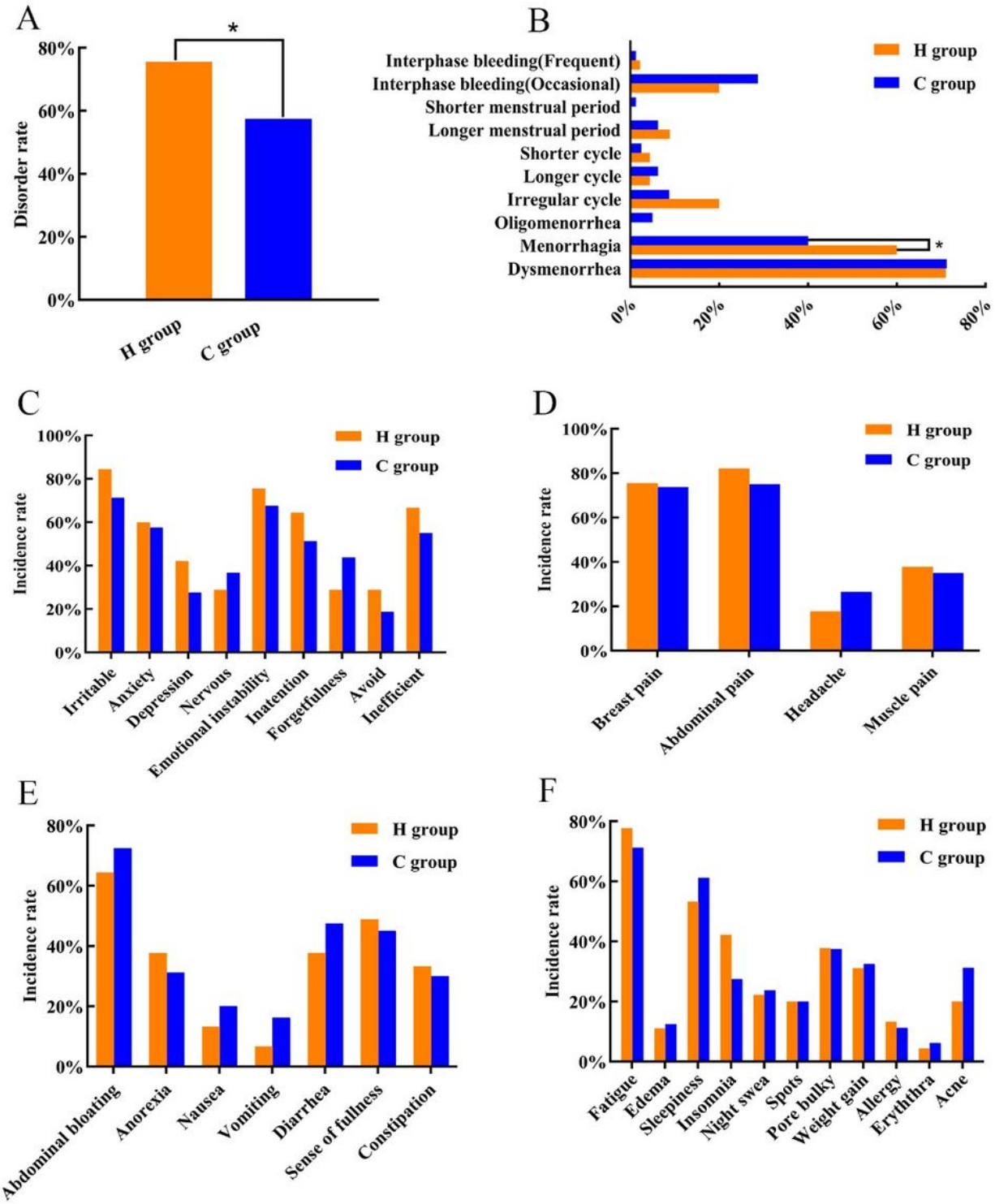

Figure 1

Menstrual disorder status in the 2 groups of women. (A) Menstrual disorder rate. (B) Menstrual disorder characteristics. (C) Premenstrual symptoms “mood and social functioning.” (D) Premenstrual symptoms “physical pain.” (E) Premenstrual symptoms “endocrine.” (F) Premenstrual symptoms “others.” *P < 0.05 compared with the C group

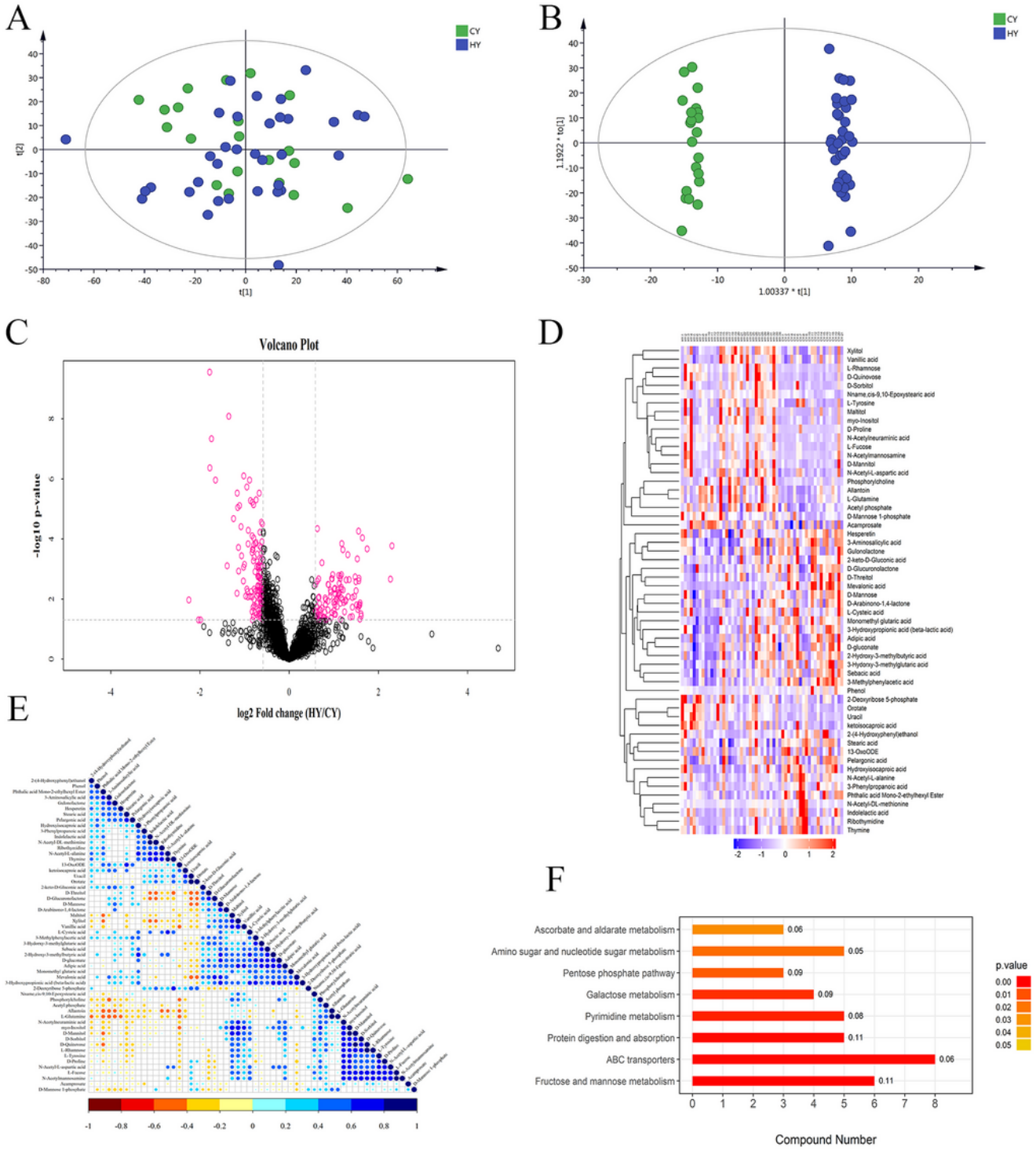

Figure 2

Multivariate statistical analysis, heat map, cluster analysis, and metabolic pathways (take positive ion mode as an example) (A) PCA score chart. (B) OPLS-DA score chart of serum metabolite analysis between the H and C groups. (C) The volcano map of the differential metabolites between the H and C groups. The red spots in the figure are the metabolites with FC > 1.5 and P < 0.05. These metabolites are the differential metabolites screened by univariate statistical analysis. (D) Results of hierarchical

clustering of metabolites that changed significantly in the sample. Red and blue represent higher and lower metabolite concentrations, respectively. (E) The correlation of metabolites of significant difference between the H and C groups. (F) The KEGG pathway enrichment analysis results of differential metabolites, the smaller the P value, statistically more significant is the enrichment of the KEGG pathway ($P < 0.05$).

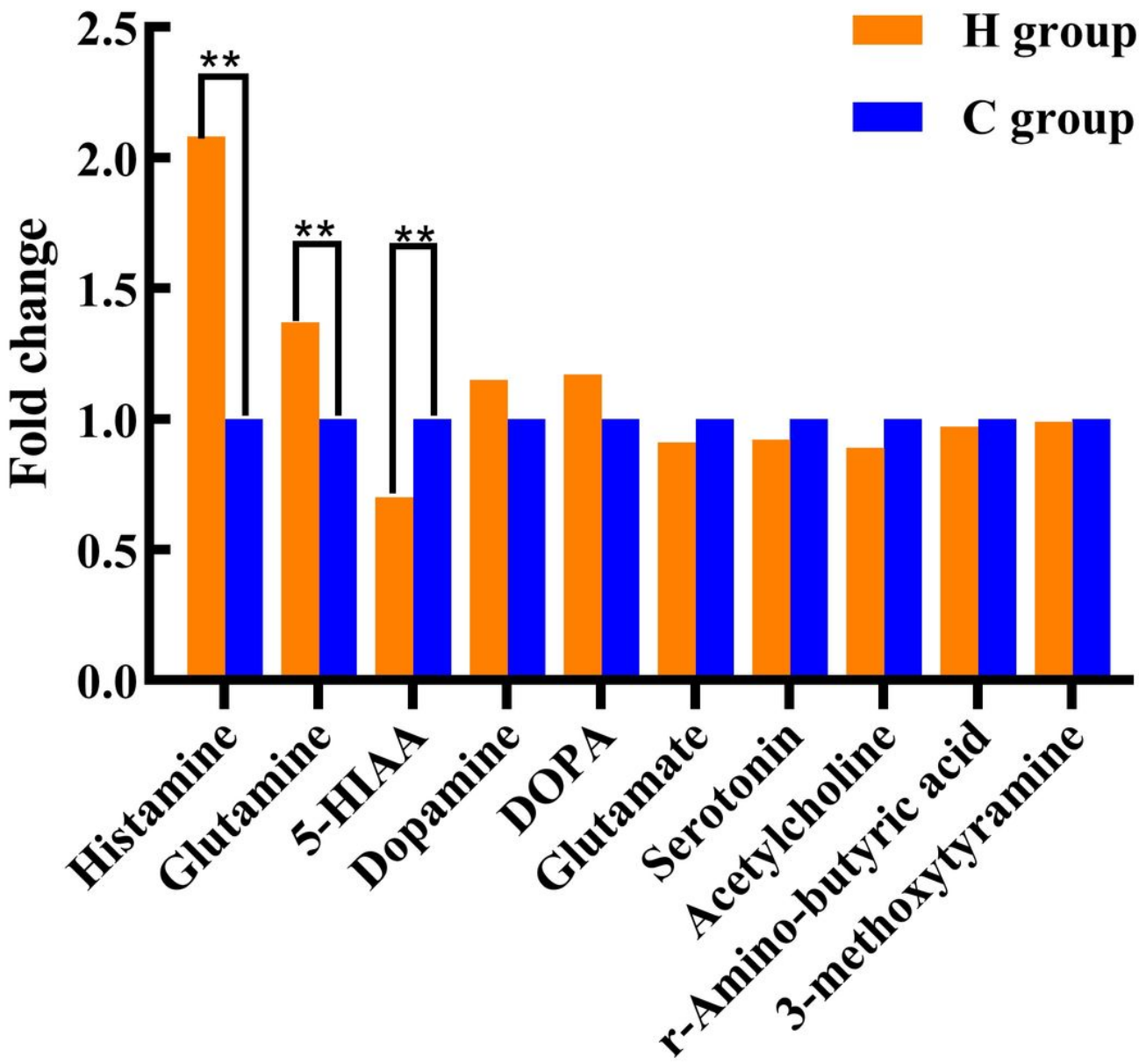

Figure 3

Changes in serum neurotransmitters, as detected by targeted metabolomics analysis. Compared with the C group, **$P < 0.01$.