Divergent indicator changes in different exercise states in non-hypertensive individuals and patients with hypertension

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Research Article
Abstract

Background

Hypertension is a kind of clinical syndrome, characterized by the increase of systemic arterial pressure. There is a lack of identifiable triggers and predictors of hypertensive disease in response to treatment at rest and during exercise. In this study, a mathematical model was used to screen and compare the indicators and related changes at rest and during exercise between normotensive and hypertensive individuals.

Methods

Blood pressure and ultrasound-related indicators, blood biochemical indicators and metabolic compounds were collected and logistic regression model and Principal component analysis (PCA) were used to explore the differences of indexes at rest and in different exercise states in healthy and hypertensive patients. An indicator change map for hypertension is established.

Results

The results reveal that hypertension is not only related to oxidative stress, inflammatory reaction and fatty acid oxidation, but also involves various amino acid metabolism. The defined mathematical models and indicators changes during exercise might be helpful for early screening of hypertension and future studies are needed to explore their value on prevention and control of hypertension.

Conclusion

The research shows that the main regulation indicators at different exercise states differ significantly in the normal group and the hypertensive group. The key indicators of the normal group are blood pressure and ultrasound related indicators, while those of the hypertensive group are metabolites related to lactic acid metabolism, glycolysis, aerobic oxidation and lipid metabolism.

1 Introduction

Hypertension is a common chronic disease, which leads to a variety of chronic diseases including cardiovascular disease, stroke, or cancer. According to the World Health Organization, at present, more than 30% of adults in the world suffer from hypertension, and no less than 970 million people suffer from cardiovascular complications related to hypertension[1, 2]. At present, China is facing the double pressures of aging population and prevalence of metabolic risk factors, and the incidence and mortality of hypertension are increasing continuously, which brings a huge burden of diseases to the society. A large national representative survey in 2015 estimated that 244.5 million people (23.2% of the adult population in China) had hypertension[3]. Meta-analysis of the combined prevalence of hypertension among adults in China from 1959 to 2018 shows that the prevalence of hypertension among adults in China is 24.3%[4]. The prevalence of hypertension among adults in China continues to rise, but the level
of awareness, treatment, and control of hypertension in China is low. Although the theory of Renin-Angiotensin-Aldosterone system activation is generally accepted, it still cannot fully explain the pathological process of hypertension[5]. In the process of treatment and monitoring of hypertensive, there is a lack of identifiable trigger factors and predictors of hypertension. Therefore, there is an urgent need to construct a more comprehensive indicator change map in hypertensive, increase the relationship between indicator and pathology, and design effective intervention measures.

Uncontrolled hypertension could lead to molecular mechanisms such as oxidative stress, endoplasmic reticulum stress, inflammation, and metabolic transformation, thus causing damage to target organs such as heart, brain and kidney[6–8]. Understanding the changes of specific indicators in these molecular mechanisms is very important for further strengthening the pathophysiology research of hypertension, and it provide new strategies and new targets for preventing hypertension. Blood pressure and ultrasound indicators are commonly used to judge hypertension[9]. Blood biochemical indicators are commonly used in clinical diagnosis of diseases. They can be used to assess the functioning of specific internal organs, metabolic characteristics, hematopoietic system and trace element levels. Untargeted proteomics from plasma is an analytical study of the entire proteome without specifying a specific protein, allowing more proteins to be measured and new proteins to be discovered. This study comprehensively analyzed the molecular mechanisms in hypertensive through blood pressure and ultrasound, blood biochemical markers, and non-targeted proteomics.

At present, the diagnosis and evaluation of hypertension in clinical practice is mainly conducted through multiple measurements of blood pressure in resting state, while the understanding and attention to hypertension in exercise state are often ignored[10]. However, the risk indicator of hypertension during exercise can monitor cardiorespiratory health more accurately[11]. Cardiopulmonary health is a powerful predictor of morbidity and mortality of cardiovascular diseases, and it has been proved that it can improve the classification of cardiovascular events[12]. Understanding the biochemical indicators during exercise could make the diagnosis of disease status more accurate and comprehensive, and reveal the real changes inside the body of patients with disease, so as to provide information reference for further monitoring of related indicators of hypertension.

The control of blood pressure is a complex process influenced by many molecular mechanisms, so it is particularly important to scientifically reveal the factors that control blood pressure. Logistic regression model is a generalized linear regression analysis model, which has good prediction effect and applicability, and is widely used in disease prediction models. Ni et al. constructed the prediction model of early recurrence of liver cancer after radiofrequency ablation by logistic regression analysis, and screened the risk factors leading to early recurrence of liver cancer after radiofrequency ablation[13]. Jawa et al. used logistic regression analysis model to determine the influence of family isolation on individual psychological stability during COVID-19 and its relationship with various factors[14]. According to the characteristics of specific diseases and data, it is particularly important to explore appropriate methods to build prediction models[15]. Logistic regression model is simple to implement, and can conveniently observe the probability scores of samples, but it cannot handle a large number of features or variables.
Principal component analysis (PCA) is a dimensionality reduction algorithm, which can convert multiple indicators into a few principal components and reflect most of the information of the original data. When the research problem involves multiple variables and there is a strong correlation between the variables, principal component analysis could simplify the data to remove noise and unimportant features, thus achieving the purpose of improving the data processing speed. In this study, combining various biomarkers, logistic regression analysis model and principal component analysis were used to construct a prediction model of hypertension, explore the corresponding molecular mechanism of warning indicators and construct the indicator change map, which was helpful to predict and monitor the risk of hypertension.

2 Materials And Methods

2.1 Normal and hypertension groups

A total of 32 samples were collected from Putuo District Central Hospital, including 12 cases in hypertension group and 20 cases in normal group. Hypertensive group received antihypertensive drugs within two weeks. The inclusion criteria of the research: 1) age ≥ 18 years old; 2) ability to tolerate the acute exercise required for running on a treadmill at a speed of 7km/h for 2 minutes. Exclusion criteria: 1) daily habits including eating disorders, excessive drinking, pregnancy and breastfeeding; 2) diseases including previous obesity surgery, acute or severe systemic infection, severe cardiovascular diseases, malignant tumors and mental disorders.

Before obtaining written consent, the subjects were informed of possible risks and discomfort. This study was approved by the Ethics Committee of Putuo District Center Hospital (PTEC-A-2019-31-1), and 32 subjects signed the informed consent form. The demographics and general characteristics of the participants were shown in Supplemental Table S1. Chi-square test was used for categorical variables and T test for quantitative variables. The results showed that there was no significant difference between the study groups (p < 0.05), that is, the influence of baseline characteristics on the discrimination of hypertension was excluded.

2.2 Cardiopulmonary exercise test

Introducing the research concept of individual quasi-medicine, taking the accurate prediction of cardiovascular disease progress as the key point of research, and designing the cardiopulmonary exercise test method (Fig. 1). The subjects ran on the treadmill at 7 km/h for 2 minutes to break the body's own steady state. Five time points, before the cardiopulmonary exercise test (rest state), 0 h (immediately after the test), 0.5h (after the test), 1h (after the test) and 2h (after the test), were selected for indicator detection. The indicators include Blood pressure and ultrasound indicators, Blood biochemical indicators and Metabolic compound. Subjects who fasted all night arrived at Putuo District Central Hospital at 8:00 am. Before the exercise test, basic personal information was collected, including age, height, weight, general conditions (smoking, drinking, exercise, insomnia, anxiety), medical history, medication history, surgical history, and family history. Subjects need to rest for more than 30 minutes to...
collect samples in the resting state. During the test, the environment should be quiet and comfortable, the
subjects should keep a stable state of mind, and the samples should be collected at the same time.

2.3 Detection of indicators

2.3.1 Blood pressure and ultrasound indicators

Transthoracic echocardiography was performed by a commercial cardiac ultrasound system (EPIQ 7,
Philips, USA). Liver portal and renal blood flow was measured using the M-Turbo ultrasound system
(SONOSITE, USA). The above indicators should be tested within 2 minutes of each time point as far as
possible.

2.3.2 Blood biochemical indicators

At five time points during the cardiopulmonary exercise test, venous blood samples were taken from the
participants' upper forearms. Blood samples are placed on ice immediately after collection to avoid
spoilage. The blood samples were then sent to Adicon Clinical Laboratories, LTD (Shanghai, China) for
biochemical testing.

2.3.3 Metabolic compound

The blood samples collected at different time points were sent to Metabo-Profile
Biotechonology(Shanghai)Co.Ltd for non-targeted proteomic analysis. All metabolites-targeting
standards were obtained from Sigma-Aldrich (St. Louis, MO, USA), Steraloids Inc. (Newport, RI, USA) and
TRC Chemicals (Toronto, ON, Canada). All target metabolites in the project were quantified using an ultra-
high performance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS) system
(ACQUITY UPLC-Xevo TQ-S, Milford Waters, MA, USA).

2.4 Statistic analysis

Statistical analysis of the data was performed by Excel, Chi-square test was used for specific variables,
and T-test was used for quantitative variables. P < 0.05 was considered statistically significant. The
univariate logistic regression analysis was used to construct three groups of models, with the presence or
absence of hypertension at rest (Model-1), different exercise states of the normal group (Model-2) and
different exercise states of the hypertension group (Model-3) as dependent variables, and the indicators
of significant changes between the hypertension group and the normal group were screened. At the same
time, principal component analysis is used to reduce the dimension of the selected indicator data and
eliminate collinearity. All models were constructed using R 4.2.1

2.4.1 logistic regression model

Logistic regression model is a probabilistic model, which takes the probability of the occurrence of an
event as the dependent variable and the factors affecting probability as the independent variable.
2.4.1.1 Binary univariate logistic regression analysis

In the comparison data set between the hypertension group and the normal group, whether there is hypertension was regarded as a binary dependent variable (yes = 1, no = 0), and indicators were considered as independent variables. The significance P < 0.05 of "Hosmer-Lemeshaw test" indicated that the research variables fit well with the Logistic model, otherwise, the research variables should be removed from the model. The model formulated as follow[21]:

\[
\left( \frac{p}{1-p} \right) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n
\]

where \( \left( \frac{p}{1-p} \right) \) followed the binary logistic distribution and represents the probability of the event occurring, \( 1-p \) represents the probability of the event not occurring and \( \beta_0 \) was a constant term (intercept). \( \beta_1, \beta_2, \cdots, \beta_n \) was the regression coefficient, and \( X_1, X_2, \cdots, X_n \) was the independent variables respectively.

2.4.1.2 Multinomial univariate logistic regression analysis

Multinomial univariate logistic regression analysis was used to analyze results containing more than two categories of data. In multinomial logistic regression, one of the categories of response variables was selected as the reference category to fit the logistic regression model of the remaining categories relative to the reference category. Let the response variable \( Y \) be a disordered classification variable containing \( g \) categories (the value of \( Y \) is \( 1, 2, \cdots, g \)), and there were \( n \) independent variables \( (X_1, X_1, \cdots, X_n) \) affecting the value of \( Y \). Given the \( g \) group as the reference group, the model formulated as follow:

\[
\left( \frac{P(Y = j/X)}{P(Y = g/X)} \right) = \beta_{0j} + \beta_{1j} X_1 + \beta_{2j} X_2 + \cdots + \beta_{nj} X_n
\]

Where \( j = 1, 2, \cdots, g - 1 \), the above equation contained \( g - 1 \) regression equations. \( \beta_{0j} \) was constant term of equation, \( \beta_{1j}, \beta_{2j}, \cdots, \beta_{nj} \) was the regression coefficient of regression equation.

2.4.2 Principal Component Analysis

Principal Component Analysis (PCA) was a mathematical dimensionality reduction method that uses orthogonal transformation to convert a series of possibly linearly correlated variables into a group of linearly unrelated new variables[22]. Thus, new variables can be used to display the characteristics of data in a smaller dimension. In this method, the original \( n \) features were replaced by a smaller number of \( k \) features. The new \( k \) features were guaranteed to maximize the sample variance and to be independent
of each other. New features were linear combinations of old features, providing a new framework for analyzing and solving practical problems.

The basic steps of PCA were as follows:

1) The original data were formed into a p×n dimensional matrix X by columns.

2) The data was normalized by subtracting the mean of the row.

3) The covariance matrix of the normalized data set was calculated. The formula was as follows:

\[ C = \frac{1}{n} X X^T \] (3)

4) The eigenvalues and eigenvectors of the covariance matrix were calculated.

5) The most important k features were retained (k ≤ n).

6) Eigenvectors(loadings) corresponding to k eigenvalues were found.

7) The principal component score was obtained by multiplying the normalized matrix by eigenvectors

8) PCA score plots were drawn.

3 Results And Analysis

According to the different detection methods, the indexes were mainly divided into Blood pressure and ultrasound indicators (19), Blood biochemical indicators (89), Metabolic compound (166), among which Blood biochemical indicators were divided into Routine blood indicators (26), Biochemical indicators of blood lipid (9), Biochemical indicators of organs(24) and Biochemical indicators of function(30) according to different detection purposes. The 274 indicators were used to construct three sets of logistic regression models (Supplemental Table S2), aiming to screen the relevant influencing factors of hypertension in resting state (Model-1), the influencing factors related to normal group in resting and different exercise states (Model-2), and the influencing factors related to hypertension group in resting and different exercise states (Model-3). Since some indicators cannot be measured in time during exercise, the missing data in this process are not compared and discussed. Depending on the patient's situation, the missing data will be removed during the comparison of different models.

3.1 Construction of the model of influencing factors of hypertension in resting state

Each quantitative variable was counted by using a T-test, and the variables with statistical significance were initially seen. The study took the presence or absence of hypertension as the dependent variable, and univariate logistic regression analysis was performed for each independent variable and the
categorical dependent variable of hypertension to determine whether the exposure factor was a risk factor and protective factor for the disease. The indicators with significant influence in logistic regression analysis were further used by principal component analysis to check the classification effect of indicators, and at the same time, the main factors causing hypertension were identified by loading matrix.

### 3.1.1 T Test

Data were input into Excel software. The study conducted statistical analysis of quantitative data through independent sample T test to test whether there were significant differences between the measurement indicators in the resting state between the hypertension group and the normal group. The statistical results are shown in Supplemental Table S3.

T-test is used to analyze the contrast between the quantitative data of paired hypertension group and normal group. The results show that there are statistically significant differences in 37 indicators (P < 0.05). Except for international normalized ratio, NADPH/NADP⁺ (plasma), palmitelaidic acid, NADPH/NADP⁺ (erythrocyte), L-Histidine and interleukin - 8, other indexes in hypertension group are higher than those of normal group.

### 3.1.2 Determination of the related factors causing hypertension in resting state

The binary logistic regression model was constructed to find the univariate that could distinguish the hypertension group from the normal group (P < 0.05) (Supplemental Table S4). In this model, the incidence of hypertension was taken as the dependent variable and 274 possible influencing variables were taken as the independent variables to carry out logistic regression (Fig. 2). The Odds Ratio (OR) was adopted to reflect the correlation strength between certain exposure factors and dependent variables, so as to reduce the risk of misdiagnosis of hypertension.

Interventricular septum, posterior wall thickness, D-Ribulose, Oxoadipic acid, absolute lymphocyte(LYM), erythrocyte count, D-fructose, apolipoprotein E (ApoE), D-xylulose, glycosylated hemoglobin (HbA1c), Monocyte absolute value(MONO)(p < 0.05, OR > 3) were not shown in Fig. 2, indicating that these variables have a high effect on hypertension. The model judged whether the variable was a risk or protective factor for hypertension according to the OR (Fig. 2). Studies show that portal vein velocity, left atrial diameter, L-Alpha-aminobutyric acid and isocitric acid are risk factors for hypertension, and NADPH/NADP⁺ (plasma) are protective factors for hypertension.

### 3.1.3 Cluster analysis of hypertension group and normal group

In this study, the disease control indicators selected from logistic regression analysis were further used to construct a cluster model by principal component analysis. According to the standard that the characteristic value was greater than 1, the first 8 principal components were selected, and their
cumulative contribution rate reached 79.74%, including 29 indicators (Supplemental Table S5). Therefore, these 8 principal components (PC) can be selected as the criteria for evaluating hypertension. 

Since retaining the original data features, 8 new comprehensive indicators (PC1-PC8) were used to replace the input hypertension discriminators (Supplemental Table S5). The loading value reflects the importance of each variable in the principal component. The positive and negative loading values do not indicate practical significance, but rather the degree of relative to the average, with positive values indicating above average and negative values indicating below average. NADPH/NADP+ (plasma), international normalized ratio, NADPH/NADP+ (erythrocyte) and palmitelaidic acid are all positive numbers, that is, these variables are the main parts of PC1 and should be focused on. The original data standardization matrix was multiplied by the loading matrix to obtain the final dimensionality reduction principal component score, and the first two principal component scores were used to draw the scatter plot (Fig. 3). The study showed that the model had good classification results and could clearly distinguish the hypertension group from the normal group.

3.2 Analysis of the relevant influencing factors of the sample population in different exercise states

Exercise status (rest state 0h 0.5h 1h 2h) was used as the dependent variable, and univariate logistic regression analysis was conducted for each independent variable and the classification of exercise status to determine whether the exposure factor had significant differences in different exercise status. The principal component analysis was used to construct the cluster model for the selected indicators. While studying the classification effect of indexes, the main influencing factors of different Exercise states were identified by loading matrix.

3.2.1 Multivariate univariate logistic regression model

Multivariate univariate logistic regression model was constructed to screen relevant indicators that could distinguish different exercise states of the population (p < 0.05) (Supplemental Table S6 and Table S7).

In this study, indicators (Group1, Group2 and Group3) (Supplemental Table S4, Table S6 and Table S7) screened by logistic regression model (Model-1, Model-2 and Model-3) were used to draw Wayne diagram (Fig. 4). The intersection of the three groups was systolic blood pressure. The study examined the systolic blood pressure of the hypertensive group and the normal group under different exercise conditions (Supplemental Figure S1) and found that the data of the two groups overlapped more at different time points, that is, exercise affected the judgment of hypertension symptoms. We suggest adding biochemical indicators on the basis of systolic blood pressure and diastolic blood pressure to judge the symptoms of hypertension. Group 2 and group 3 share 17 indicators (Fig. 4). In addition to systolic blood pressure, there were 16 indicators, including 7 organic acids (pyruvic acid, fumaric acid, succinic acid, maleic acid, cis and trans-acetic acid, L-Malic acid and L-Lactic acid), 3 fatty Acids (oleic acid, palmitoleic acid and methylmalonic acid), 1 Carbohydrates (N-Acetyleneuraminic acid), 1 Phenylpropanoids (3,4-
dihydroxyhydrocinnamic acid), 2 blood pressure and ultrasound indicators (heart rate, right renal artery flow rate) and 2 immune examination indicators (CD3 + CD4+, CD3-CD16 + CD56+).

The number of indicators in Group2 was higher than that in Group3. It was concluded that after hypertension, 19 indicators including blood pressure and ultrasonic-related indicators and organic compound indicators (excluding the unmeasured indicators in the hypertension group) could not be regulated normally during exercise. Except for the left renal artery flow rate and plasma aldosterone, all of them were organic compounds such as amino acids, carbohydrates, organic acids and fatty acids. They were myristelaidic acid, pyroglutamic acid, glucose-6-phosphate, petroselinic acid, 5-aminolevulinic acid, octanoic acid, citric acid, octanoylcarnitine, 5-dodecenoic acid, 2-hydroxybutyric acid, 10(Z)-heptadecenoic acid, formic acid, ketoleucine, 3-methyl-2-oxovaleric acid, adenosine monophosphate, D-glucuronolactone and sarcosine. It was found that renin activity, CD3+, nicotinic acid and N-acetylserine in Group3 did not appear in Group2. The reason may be the regulatory mechanism activated in hypertensive patients.

### 3.2.2 Cluster analysis of different exercise states of sample population

In this study, the exercise regulation indicators of the sample population screened out in the logistic regression analysis were further used to construct a cluster model by principal component analysis. According to the standard that the eigenvalue was greater than 1, the 12 principal components (Supplemental Table S8) and 6 principal components (Supplemental Table S9) were selected as the criteria to evaluate the different exercise status of the normal group and hypertension group.

Principal component analysis was adopted to reduce the dimensionality of the original data. The 12 integrated principal components obtained reflected the direct relationship between the detection parameters and the motion state, and the cumulative contribution rate reached 79.34%, which greatly reduced the statistical analysis indicators while retaining the original data information (Supplemental Table S8). PC1 mainly includes blood pressure and ultrasound indicators, that is, these indicators play an important role in judging the exercise state of the normal group.

Six principal components were selected in the cluster model, and the accumulative contribution was 78.05% (Supplemental Table S9). L-lactic acid, 3,4-dihydroxyhydrocinnamic acid, succinic acid, methylmalonic acid, pyruvic acid and N-acetylneuraminic acid in PC1 were metabolites of lactic acid and glycolysis-aerobic oxidation. The systolic blood pressure, heart rate and right renal flow rate shared by groups 2 and 3 did not appear in PC1, so it can be seen that the blood pressure and ultrasound related indicators of hypertension group did not change regularly with exercise.

In this study, the first two principal component scores were used to draw scatter plots (Fig. 5). The results showed that PCA cluster analysis did not clearly distinguish the different exercise states between the hypertension group and the normal group in two-dimensional space, but PC1 (horizontal) still had a greater contribution. Analysis of the main indicators of PC1 could reveal the real changes in the body of
patients with diseases, and provide information reference for monitoring the related indicators of hypertension.

4 Discussion

Hypertension is a multifactorial and complex disease that increases the risk of cardiovascular disease, and its causes and molecular mechanisms are not fully understood. In general, biomarkers can be widely used for risk stratification and treatment response assessment of cardiovascular disease. The pathophysiological mechanisms of hypertension require precise and accurate biomarkers, which are essential for effective management and monitoring of the disease course of hypertension. In this study, we designed cardiopulmonary exercise test, integrated the blood pressure and ultrasound-related indexes, blood biochemical indexes and metabolic compounds of the sample population at resting and different exercise states, and used logistic regression analysis and principal component analysis to construct a cluster model to screen biomarkers that judge hypertension, so as to reveal the real changes in patients' bodies. It was found that hypertension was accompanied by oxidative stress, lipid metabolism, glycolysis, aerobic oxidation, pentose phosphate pathway, inflammatory reaction and amino acid metabolism, and the related indicators were summarized in Fig. 6.

The preliminary statistics of the data by T-test showed that 37 indicators (P < 0.05) had statistically significant differences, among which 6 indicators (international normalized ratio, NADPH/NADP+ (plasma), Palmitelaidic acid, NADPH/NADP+(erythrocyte), L-histidine and Interleukin-8) in the hypertension group were lower than those in the normal group. International normalized ratio (INR) for both groups was within the normal range (0.8 to 1.2). The low NADPH/NADP+ in the hypertensive group indicates that oxidative stress was related to the occurrence of hypertension[23]. The low levels of histidine, palmitic acid and interleukin-8 were all attributed to hypertensive patients taking medication. Histidine forms natural aldehydes in liver tissues under the action of ethylamine oxidase, which leads to the failure of tissues and organs to absorb the drug effect. Patients with hypertension would try to eat less food rich in histidine during medication. The accumulation of palmitelaidic acid, the main saturated fatty acid in the blood, causes cardiomyocyte lipid toxicity by inducing oxidative stress and persistent endoplasmic reticulum stress, eventually inducing inflammatory response, cell hypertrophy, cell dysfunction and even cell death[24]. Theoretically, palmitic acid and interleukin-8 in hypertension group were both higher than those in normal group, but the results were opposite. It is speculated that hypertension drugs taken by patients for a long time have the effects of anti-inflammatory and reducing free fatty acids in the body (Felodipine/Valsartan and Amlodipine) [25][26].

In the resting state, the binary logistic regression between groups showed that the effects of 11 indexes were large. Interventricular septum and posterior wall thickness thickening are common symptoms in patients with hypertension[27][28]. In hypertrophic myocardium, glucose utilization and glycolysis process are enhanced, while aerobic oxidation process was relatively weakened, namely "glycolysis-uncoupling of glucose oxidation"[29][30], which led to the upregulation of D-xylulose, D-fructose and oxyadipic acid. At the same time, the hypertrophic ventricle increases the load of the heart, leading to
heart failure, thus causing oxidative stress and inflammation. The decrease of oxygen utilization rate directly reduces the synthesis of ATP, and ribose, as the raw material of nucleotides, is accumulated[31]. In the long-term environment of oxygen deficiency, the erythrocyte would increase and aggregate compensatively[32, 33]. Leukocytes are the most important inflammatory cells mediating inflammatory response, and the leukocytes involved in inflammatory response are mainly neutrophils and lymphocytes[34]. The inflammatory response causes by hypertension leads to the impact of blood pressure on the vascular wall, which could damage the vascular endothelium, lead to coagulation process, smooth muscle cell proliferation, tube wall thickening, stiffness and lumen narrowing, and increase of apolipoprotein E, thus promoting the formation of vascular atherosclerosis[34, 35]. Glycosylated hemoglobin (HbA1c) is the most important regulatory marker in diabetes care, but there is still evidence showing a significant positive correlation between HbA1c and hypertension[36].

The risk factors of hypertension are positively correlated with hypertension. Increased left atrial diameter and high portal vein velocity are common symptoms associated with hypertension[37, 38]. L-Alpha-aminobutyric acid and Isocitric acid have not been considered risk markers for hypertension in the past. But Isocitric acid is involved in the tricarboxylic acid cycle. Aminobutyric acid has anti-diabetes, anti-hypertension, liver and kidney protection, sleep promotion and other activities[39, 40]. NADPH/NADP+(plasma) was a protective factor of hypertension, that is, oxidative stress was associated with the occurrence of hypertension. The 29 indicators screened by logistic regression analysis were further constructed by principal component analysis, and the study showed that the first 8 principal components could be used as the criteria for determining hypertension. The scatter plot of the first two principal component scores showed a good classification result of the model, which could clearly distinguish the hypertensive group from the normal group on the horizontal axis. Principal component analysis showed that NADPH/NADP+(plasma), international normalized ratio, NADPH/NADP+(erythrocyte), and palmitelaidic acid were key factors in determining hypertensive disease. The accumulation of major saturated fatty acids(palmitelaidic acid) in the blood may cause cardiomyocyte lipid toxicity by inducing oxidative stress and persistent endoplasmic reticulum stress. At the same time, NADPH/NADP + in plasma and erythrocyte indicate that indicators related to oxidative stress are the key to determine patients with hypertension.

With exercise status as the dependent variable, multiple univariate logistic regression analysis was carried out on the indicators of the hypertension group and the normal group respectively under resting and different exercise status. The study mainly focused on the intersection of the three groups (Group1, Group2 and Group3) of indicators and their unique indicators. Group2 and Group3 had 17 indicators in common, that is, these indicators were regulated in the human body in different exercise states. Pyruvic acid, lactic acid and heart rate are common research indicators during exercise[41, 42]. The metabolic characteristics of athletes in the high-load training stage are as follows: aerobic oxidative metabolism plays the biggest role, glycolysis occupies a large proportion, lactic acid accumulation, active amino acid metabolism, enhanced catabolism of most amino acids, and high oxidative stress level[43]. After exercise, the metabolism of organic acids in the body is obviously enhanced, and lactic acid, fumaric acid, succinic acid, malic acid and aconitic acid all change significantly. The metabolic processes
involved include glycolysis and tricarboxylic acid cycle[43, 44]. Maleic acid has not been reported, but both maleic acid and fumaric acid are isomers of butene dioic acid. The changes in lipids are particularly significant during exercise[10, 45]. During exercise, fatty acid oxidation and degradation of triacylglycerol occurred, and oleic acid, palmitoleic acid, methylmalonic acid were involved in the process(https://hmdb.ca/metabolites). N-acetylneuraminic acid is found in high levels in the brain, adrenal glands and heart. It has not been reported in exercise studies, but it can regulate innate immunity, antiviral, anti-tumor, inhibit leukocyte adhesion and anti-inflammatory. Various derivatives of 3,4-dihydroxyhydrocinnamic acid (caffeic acid) can act as antioxidants in living organisms, helping to reduce the pathogenic effects of free radicals and oxidative compounds and prevent the oxidative stress caused by diseases[46].

The study suggested that 19 indicators unique to Group2 could not be regulated properly during exercise in the hypertensive group. The Human Metabolome Database(https://hmdb.ca/) was used to investigate pathways involved in these compounds. Fatty acids (myristelaidic acid, octanoic acid, 5-dodecenoic acid, 10(Z)-heptadecenoic acid, formic acid and petroselinic acid) oxidize during exercise. Octanoylcarnitine is involved in mitochondrial beta-oxidation of short chain Saturated fatty acids. Carbohydrates (glucose-6-phosphate, D-gluconolactone) participate in the pentose phosphate pathway. Citric acid participates in the tricarboxylic acid cycle. Pyroglutamic acid and sarcosine are involved in glutathione metabolism. 5-aminolevulinic acid is involved in glycine and serine metabolism and porphyrin metabolism. 2-hydroxybutyric acid is involved in glutamic acid metabolism and butanoate metabolism. Ketoleucine and 3-methyl-2-oxovaleric acid participate in the biosynthesis and degradation of valine, leucine and isoleucine. Adenosine monophosphate is involved in metabolism of various fatty acids and amino acids. Sarcosine is involved in glycine and serine metabolism and methionine metabolism.

The four indicators unique to Group3 were renin activity, CD3+, nicotinic acid and N-acetylserine. The renin-angiotensin-aldosterone system (RAAS) plays an important role in regulating blood pressure and maintaining electrolyte balance and internal environment stability. In pathological conditions, RAAS can increase blood pressure by constricting blood vessels, improving sympathetic nerve activity, and increasing water and sodium retention[5]. Nicotinic acid is an effective high-density lipoprotein raising drug involved in nicotinate and nicotinamide metabolism. Studies have shown that nicotinic acid is a new strategy to prevent atherosclerosis. In addition to its beneficial effect on high-density lipoprotein, niacin can also dilate peripheral blood vessels[47] [48]. In the pathogenesis of hypertension, endothelial dysfunction and immune system activation accompany the development of systemic inflammation and the production of inflammatory cytokines, and CD3 + are upregulated[49]. N-acetylserine has been a potential biomarker for severe sarcopenia and has not been reported in biomarker studies for hypertension[50].

In this study, the indexes screened by univariate logistic regression analysis with significant differences in different exercise states between the hypertension group and the normal group were further analyzed by principal component analysis. The variance contribution rate of PC1 was 21.91% (normal group) and 34.90% (hypertension group), respectively. The key indicators in the normal group were blood pressure...
and ultrasound related indicators, while the key indicators in the hypertension group were lactic acid, glycolysis, aerobic oxidation and lipid-related metabolites. Lactic acid becomes a metabolite with obvious changes during exercise in hypertensive patients. It has also been reported in other reference [51] [52]. Lactic acid is involved in pyruvate metabolism, gluconeogenesis and Warburg effect. Lactic acid accumulation can cause liver damage and nerve damage. Various derivatives of caffeic acid can protect against disease-induced oxidative stress [46]. Succinic acid and pyruvic acid participate in glycolysis-uncoupling of glucose oxidation. Methylmalonic acid, a malonic acid derivative, is an important intermediate in lipid metabolism, suggesting that the disease group used more fat for energy supply during exercise.

In this study, the cardiopulmonary exercise experiment was designed, and the indicators related to the risk of hypertension were screened by logistic regression analysis, and the correlation between this factor and hypertension was examined. The indicators were eventually retained in the cluster model constructed by principal component analysis to reduce the risk of misjudgment of hypertension. However, there are some limitations in this study. Due to the insufficient sample size of the included studies, large-scale randomized trials cannot be conducted to verify the classification effect of this biomarker. In the future, in order to further confirm the predictive value of this biomarker for the occurrence of hypertension, it is necessary to further expand the study sample. Moreover, various mathematical models such as K-Nearest Neighbor classification algorithm [53], Support Vector Machine [54] and Radial Basis Function [55] are used to confirm this.

5 Conclusion

This study collected blood pressure and ultrasound indicators, blood biochemical indicators and metabolic compound indicators before and after cardiopulmonary experiment in normal and hypertensive participants. With the presence or absence of hypertension and the exercise status of the sample population as dependent variables, the logistic regression analysis model and principal component analysis method were used to build a classification model. While screening the indicators with significant differences, the corresponding molecular mechanism of warning indicators was discussed, and the change map of hypertension indicators was determined.

The results reveal that hypertension is closely related to oxidative stress, inflammatory reaction, fatty acid oxidation and many amino acid metabolisms. It is helpful to predict and monitor the risk of hypertension, to identify early hypertensive patients and provide reference for clinical prediction and evaluation of adverse risks of hypertensive patients. Future studies are warranted to validate these hypotheses.

Abbreviations

PCA: principal component analysis

SBP: systolic blood pressure
DBP: diastolic blood pressure
OR: odds ratio
LYM: absolute lymphocyte
ApoE: apolipoprotein E
HbA1c: glycosylated hemoglobin
MONO: monocyte absolute value
PC: principal components
INR: international normalized ratio
LAD: left atrial diameter
FBG: fasting blood-glucose
PWT: posterior wall thickness
IVS: interventricular septum

Declarations

Availability of data and materials
The datasets supporting the conclusions of this article are included within the article and its supplementary information files.

Acknowledgements
No.

Funding
The present study was supported by Medical innovation research project of "scientific and technological innovation action plan" (21Y11909600), The Shanghai Key Medical Specialties Construction Project (ZK2019A11), Clinical Advantage Discipline of Health System of Putuo District in Shanghai (2019ysxk01).

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Contributions

LX and ZL: conception and design. HZ and YX and JG: data collection and analysis.

JF, XD, CY, LZ, YL, YX, MW and QL: provision of study materials or patients, collection, and assembly of clinical data. HZ, YX, JG, LX and ZL: results interpretation and manuscript writing. HZ, YX, JG, LX, ZL, JF, XD, CY, LZ, YL, YX, MW and QL: final approval of manuscript. All authors contributed to the article and approved the submitted version. All authors read and approved the final manuscript.

Corresponding author

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Ethics approval and consent to participate

Written informed consent was obtained from each patient.

Consent for publication

All individuals must consented to publication.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References


Vasculature 2022, 43:101147.


Figures
Figure 1

Design of cardiopulmonary exercise test.
Figure 2

Binary univariate logistic regression analysis of hypertension. OR>1 indicates that exposure factors are risk factors for the disease (positive correlation); OR < 1 indicates that exposure factors are protective factors for the disease (negative correlation). Variables (p<0.05, 0.3<OR<3) are shown in the figure.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>P value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td>0.010</td>
<td>1.139(1.050-1.288)</td>
</tr>
<tr>
<td>NADPH+NADP⁺(erythrocyte)</td>
<td>0.013</td>
<td>1.002(1.000-1.003)</td>
</tr>
<tr>
<td>NADPH/NADP⁺(plasma)</td>
<td>0.016</td>
<td>0.322(0.094-0.639)</td>
</tr>
<tr>
<td>Portal vein velocity</td>
<td>0.016</td>
<td>1.295(1.082-1.669)</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.017</td>
<td>1.001(1.000-1.002)</td>
</tr>
<tr>
<td>Fasting blood-glucose</td>
<td>0.020</td>
<td>1.001(1.000-1.003)</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>0.021</td>
<td>1.001(1.000-1.002)</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>0.022</td>
<td>1.048(1.011-1.098)</td>
</tr>
<tr>
<td>L-Acetylcarnitine</td>
<td>0.024</td>
<td>1.126(1.027-1.269)</td>
</tr>
<tr>
<td>Palmitelaidic acid</td>
<td>0.025</td>
<td>0.861(0.739-0.967)</td>
</tr>
<tr>
<td>NADPH/NADP⁺(erythrocyte)</td>
<td>0.026</td>
<td>0.923(0.849-0.981)</td>
</tr>
<tr>
<td>Left atrial diameter</td>
<td>0.026</td>
<td>1.410(1.068-1.989)</td>
</tr>
<tr>
<td>L-Alpha-aminobutyric acid</td>
<td>0.028</td>
<td>1.401(1.070-1.992)</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>0.033</td>
<td>1.027(1.005-1.057)</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.033</td>
<td>1.084(1.014-1.183)</td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.034</td>
<td>1.028(1.007-1.059)</td>
</tr>
<tr>
<td>Isocitric acid</td>
<td>0.036</td>
<td>2.003(1.151-4.305)</td>
</tr>
</tbody>
</table>
Figure 3

Principal component scores chart for identifying hypertensive diseases.
Figure 4

Venn Diagram. Group1: Indicators related to Model-1 screening (P<0.05); Group2: Indicators related to Model-2 screening (P<0.05); Group3: Indicators related to Model-3 screening (P<0.05).

Figure 5

Normal group

Hypertension group
Principal component scores chart for identifying different motion states.

<table>
<thead>
<tr>
<th></th>
<th>Oxidative stress</th>
<th>Lipid metabolism</th>
<th>Glycolysis /Aerobic oxidation /Pentose phosphate pathway</th>
<th>Inflammation /Immunity</th>
<th>Amino acid metabolism</th>
<th>No reported</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td>NADPH/NADP+ (plasma)</td>
<td>Palmitelaidic acid</td>
<td>D-fructose</td>
<td>LYM</td>
<td></td>
<td>L-Alpha-aminobutyric acid</td>
</tr>
<tr>
<td></td>
<td>NADPH/NADP+ (erythrocyte)</td>
<td></td>
<td>D-xylulose</td>
<td>MONO</td>
<td></td>
<td>HbA1c</td>
</tr>
<tr>
<td></td>
<td>NADPH+ (erythrocyte)</td>
<td></td>
<td>Oxadipic acid</td>
<td>ApoE</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-ribulose</td>
<td></td>
<td>Isocitric acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erythrocyte count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td>Caffeic acid</td>
<td>Oleic acid</td>
<td>Pyruvic acid</td>
<td>CD3+CD4+</td>
<td></td>
<td>Maleic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palmitoleic acid</td>
<td>L-lactic acid</td>
<td>CD3-CD6-CD56+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methylmalonic acid</td>
<td>Fumaric acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Succinic acid</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>L-malic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cis-and trans-aconitic acid</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Group 2' Group 3</strong></td>
<td>Myristelaidic acid</td>
<td>Glucose-6-phosphate</td>
<td>Pyroglutamic acid</td>
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<tr>
<td></td>
<td>myristoleic acid</td>
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<td>5-aminolevulinic acid</td>
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</tr>
<tr>
<td></td>
<td>5-dodecanonic acid</td>
<td>Citric acid</td>
<td>Sarcosine</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>10(Z)-heptadecenoic acid</td>
<td></td>
<td>2-hydroxybutyric acid</td>
<td></td>
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<tr>
<td></td>
<td>formic acid</td>
<td></td>
<td>Ketoconuline</td>
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<tr>
<td></td>
<td>petroselinic acid</td>
<td></td>
<td>3-methyl-2-oxovaleric acid</td>
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<td></td>
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<tr>
<td></td>
<td>octanoylcarnitine</td>
<td></td>
<td>Adenosine monophosphate</td>
<td></td>
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<tr>
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<td>Adenosine monophosphate</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Group 3</strong></td>
<td>Nicotinic acid</td>
<td></td>
<td>CD3+</td>
<td></td>
<td></td>
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</tbody>
</table>

**Figure 6**

Indicator change map of hypertension.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.doc
- GraphicAbstract.pdf