Original Article

Lactobacillus casei modulates inflammatory cytokines and metabolites during tuberculosis treatment: A post hoc randomized controlled trial

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Background and Objectives: Inflammatory cytokines and metabolic abnormalities are common in patients with tuberculosis. Observational studies have indicated that probiotics modulate inflammatory cytokines and metabolites; however, clinical evidence of the effect of probiotics on patients with tuberculosis is lacking. This study investigated the effects of *Lactobacillus casei* on inflammatory cytokines and metabolites during tuberculosis treatment. **Methods and Study Design:** A randomized controlled trial was conducted. A total of 47 inpatients were included and randomly assigned to receive standard antituberculosis therapy only (control group) or that treatment together with 1×10^{10} colony-forming units per day of *Lactobacillus casei* (low-dose group) or 2×10^{10} colony-forming units per day of *Lactobacillus casei* (high-dose group) for 4 weeks of intensive treatment during hospitalization. Plasma samples were analyzed for inflammatory cytokines and metabolomics with ELISA kits and ultrahigh performance liquid chromatography quadrupole time-of-flight mass spectrometry. **Results:** Daily *Lactobacillus casei* supplementation of up to 2×10^{10} colony-forming units significantly lowered the concentrations of tumor necrosis factor-α, interleukin-6, interleukin-10, and interleukin-12 (p=0.007, p=0.042, p=0.002, p<0.001, respectively) in patients with tuberculosis. Compared with the control and low-dose groups, the plasma metabolites of phosphatidylserine, maresin 1, phosphatidylcholine, L-saccharopine, and pyridoxamine were significantly upregulated, and N-acetylmethionine, L-tryptophan, phosphatidylethanolamine, and phenylalanine were downregulated in the high-dose group. Strong correlations were observed between metabolites and inflammatory cytokines. **Conclusions:** *Lactobacillus casei* supplementation during the intensive phase of tuberculosis treatment can significantly modulate inflammatory cytokines and metabolites. Decreased inflammatory cytokines may be related to metabolite changes.

Key Words: probiotics, tuberculosis, inflammatory cytokines, metabolites, randomized controlled trial

INTRODUCTION

Pulmonary tuberculosis (TB) is a disease caused by the bacterium *Mycobacterium tuberculosis*. The global TB burden was approximately 10 million, and the mortality was approximately 1.2 million in 2019.[1] China also has a high TB burden; in 2019, 833,000 people were diagnosed with TB, and 31,000 patients died of it.[1]

Currently, a combination of four first-line antimycobacterial drugs (isoniazid, rifampicin, ethambutol, and pyrazinamide) are used to treat TB clinically.[2] All four drugs are prescribed to patients during the intensive phase—the first 2 months of TB treatment.[3] Clinical treatment generally produces favorable therapeutic effects. However, inflammatory cytokines and an individual's metabolic profile can be altered after an infection of *M. tuberculosis*.[4,5] Studies have indicated that compared with healthy individuals, those with TB exhibit significantly higher mRNA expressions of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6).[6] Additionally, studies on systemic metabolites have indicated that the abundance of tryptophan, alanine, phosphatidylcholine (PC), and phosphatidylethanolamine (PE) are altered in patients with TB, compared with healthy individuals.[5]

Nutritional approaches are available to improve health.[7,8] Probiotics are defined as "live microorganisms that when administered in adequate amounts, confer a health benefit on the host."[8] Several studies have indicated that the consumption of probiotics can regulate metabolites.[9,10] Supplementation with probiotics, including *Lactobacillus casei* (*L. casei*; 7×10^9 colony-forming units [CFU] per day), can regulate an individual's plasma metabolic profile and increase total plasma glutathione.[11]

Additionally, probiotics can reduce the concentrations

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of anti-inflammatory cytokines.[12] Studies have indicated that metabolites are related to inflammatory cytokines.[13] However, an extensive literature search indicated that no studies have focused on the effects of probiotics on the plasma inflammatory cytokines and metabolites of patients receiving TB treatment.

L. casei is a safe, well-understood probiotic species with the approval and recognition of the United States Food and Drug Administration. *L. casei* provides health-promoting effects, including improving gastrointestinal dysfunction, preventing colorectal tumors, and suppressing cholestasis-related liver indices.[14-16] This study involved a post hoc randomized controlled trial (RCT) to examine the effects of *L. casei* on the plasma inflammatory cytokines and metabolites of patients with TB during intensive treatment. Correlations between inflammatory cytokines and metabolites were also explored.

METHODS

Study design and participants

This RCT was conducted with hospitalized adult patients at a chest hospital in Shandong, China, from December 2017 to January 2019. A total of 429 patients with TB were enrolled in the trial; 10 patients withdrew, 9 patients presented adverse gastrointestinal symptoms, and 13 patients were lost to follow-up (Figure 1). Because of the low availability of plasma samples, inflammatory cytokines and metabolomics were measured in 47 patients, who were simple-randomly allocated to three groups. A total of 16 patients were included in the low-dose *L. casei* group (1×10^{10} CFU daily), 16 in the high-dose *L. casei* group (2×10^{10} CFU daily), and 15 in the control group (without *L. casei* intervention). All patients received TB treatment during the 4 weeks of supplementation.

The trial was performed in accordance with the Declaration of Helsinki, approved by the Ethics Committee of Qingdao Center of Disease Control and Prevention (201703), and registered at the China Clinical Trial Registry Center (ChiCTR-IOR-17013210). All participants provided written informed consent and permission to use their blood samples for this study.

Diagnostic criteria

The inclusion criteria were age 18–65 years, agreement to participate and provide written consent, and a diagnosis of pulmonary TB, which was based on compatible clinical symptoms (e.g., cough, hemoptysis, weight loss, fever, and night sweat) with a computed tomography scan and sputum smear test, as recommended by the WHO,[17] at clinical examination. The exclusion criteria were a diagnosis of extrapulmonary TB (e.g., enterophthisis and bone TB); self-reported cardiovascular disease, diabetes, hematological disease, gastrointestinal disease, liver malfunction, tumor, severe mental or psychological illness, or cognitive impairment; the use of probiotic supplementation within the previous 2 months; or incomplete information.

Randomization and intervention

All participants were inpatients during the first 4 weeks of supplementation. They received standard TB treatment with a combination of four antibiotics (isoniazid, rifampicin, ethambutol, pyrazinamide) and were randomly allocated to three groups. The allocation sequence was generated by an independent investigator using an online randomization generator (http://www.randomization.com). The study was an open-label randomized controlled study, and allocation was unmasked.

The *L. casei* was prepared in liquid through a commercial probiotic drink from Yakult Corporation (Tokyo,

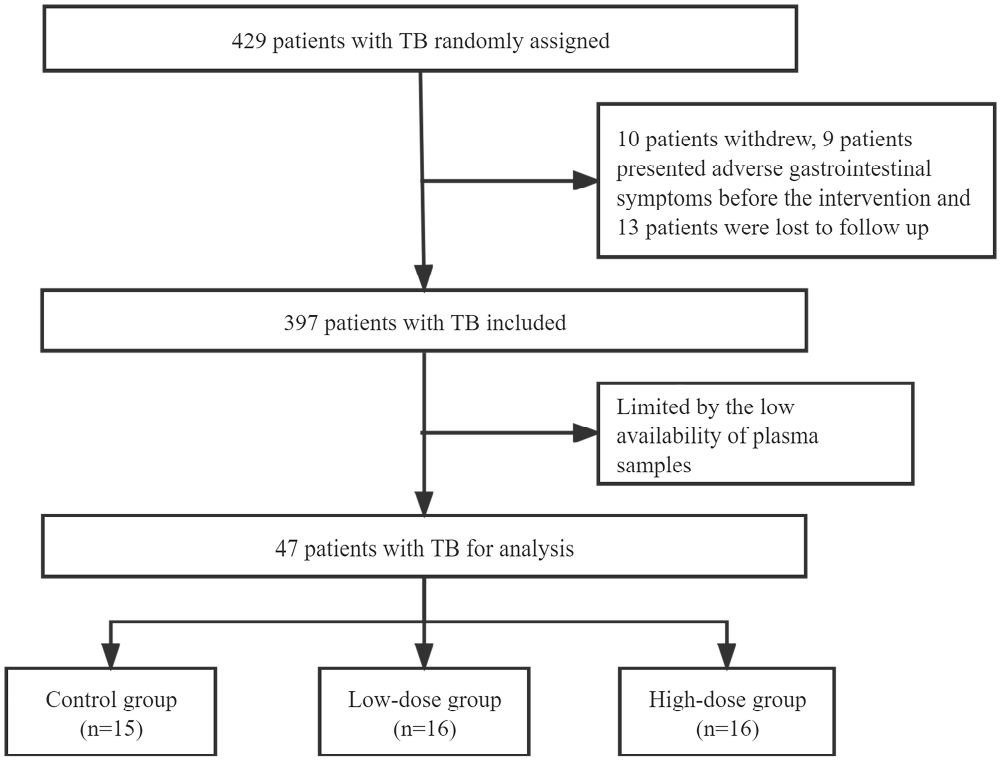

Figure 1. The trial flow chart.

Japan). The probiotic drink contained the *L. casei* strain Shirota, filtered water, skimmed milk powder, glucose, and sucrose. Each bottle (100 mL) provided approximately 10 billion CFU of *L. casei* Shirota, 68.5 kcal of energy, 1.2 g of protein, and 15.7 g of carbohydrates. The probiotic drinks were allocated to patients twice per month. Patients were instructed to shake the bottles before consumption and consume the *L. casei* 30–60 min after meals. The first-month intervention was conducted during the patients' hospitalization period. Compliance was assessed through personal interviews and returned empty bottles.

Data collection and plasma treatment

In the baseline clinical assessment, participants' demographic information was collected, their clinical symptoms were assessed, and they underwent chest radiography; moreover, sputum and blood samples were collected. The blood samples were used to determine the white blood cell differential count (Medical Record) and cytokines concentrations (ABclonal Technology's ELISA kits; Wuhan, China). Symptoms and signs were recorded using a standard questionnaire to calculate TBscores before antituberculosis therapy. TBscore can be used to quantify the severity of TB and the included symptoms and signs were cough, hemoptysis, sputum production, dyspnea, chest pain, night sweat, fatigue, loss of appetite, fever and BMI.[18] Presence of each of the first 9 symptoms and signs scored 1 point. A BMI of less than 16 kg/m^2 scored 2 points, 16-18 kg/m^2 scored 1 point, more than 18 kg/m^2 scored 0 point. The range of TBscore was 0–11 points.

For the ultrahigh performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC Q-TOF LC/MS) analysis, 50 μL of the plasma sample from each patient was transferred to an EP tube and mixed with 250 μL of prechilled acetonitrile. The mixture was then vortexed for 1 min, incubated on ice for 15 min, and centrifuged at 15,000 rpm for 15 min at 4°C. A total of 100 μL of supernatant was removed and filtered with a 0.22-μm organic filter membrane for UHPLC Q-TOF LC/MS analysis.

UHPLC Q-TOF LC/MS

UHPLC Q-TOF LC/MS analysis was performed on the metabolites in the plasma samples using Agilent 1290 Infinity II—UHPLC (Agilent, USA) coupled with Agilent 6530 Q-TOF LC/MS (Agilent, USA). The ACQUITY UPLC BEH C18 column (100 × 2.1 mm^2, 1.7 μm) was the model of chromatographic separation, and the column temperature was set at 20°C, with an injection volume of 2 μL. Separation was performed at a flow rate of 0.4 mL/min under a gradient program in which mobile phase A was composed of water containing 0.1% formic acid (v/v), and mobile phase B was composed of acetonitrile. The elution gradient was set as follows: 0 min, 95% B; 3 min, 80% B; 6.5 min, 50% B; 12.5 min, 15% B; and 17.5 min, 0% B. The stop time was 23 min. The mass spectrometry conditions were as follows: the electrospray ion source was detected using positive ion mode, sheath and auxiliary gases were both nitrogen, mass scanning range was 50–1,000 m/z with a scan time of 0.2 s and scan rate of 1 spectra/s, and capillary and sampling cone voltages were 3 kV and 40 V, respectively.

Statistical analyses

The chi-square test and Kruskal–Wallis H test were adopted for an analysis of baseline characteristics of the study population. Inflammatory cytokine data were log-transformed and analyzed with ANOVA. Peak intensities of metabolites were analyzed with a nonparametric test (p<0.05) with a Dunn's multiple comparisons test conducted between groups. The Spearman nonparametric test was used to analyze the correlations between inflammatory cytokines and metabolites. Mass spectrometry data were further processed through normalization, scaling, filtering, and statistical analysis using MetaboAnalyst 5.0 (http://www.metaboanalyst.ca). The orthogonal partial least squares discrimination analysis (OPLS-DA) model was used to perform analysis between groups with a permutation test to assess the risk of overfitting the model. A fold change (FC) of >1.2 or <1/1.2 and a false discovery rate (FDR) of <0.05 were used to evaluate differential metabolites. Inflammatory cytokine concentrations, shared metabolites peak intensity, and a correlation heat map were illustrated using Graphpad Prism 8.0.2 software. Venn diagrams were constructed by http://bioinformatics.psb.ugent.be/webtools/Venn/. The chi-square test, Kruskal–Wallis H test, and ANOVA were conducted with SPSS 26.0.

RESULTS

Clinical characteristics of the study population

In this study, 47 patients with TB were recruited between December 2017 and January 2019. A total of 15 patients were randomly assigned to the control group, 16 to the low-dose probiotics group, and 16 to the high-dose probiotics group. Patients' clinical characteristics are presented in Table 1. Baseline information comprising age, sex and BMI were comparable among the three groups (p>0.05). The signs and symptoms of the patients were recorded and quantified as TBscore.[18] TBscore levels did not significantly differ among the three groups (p>0.05).

Effects of probiotics on white blood cell differential count and inflammatory cytokines

The white blood cell differential count and inflammatory cytokine concentrations after a 4-week follow-up are presented in Table 2. The numbers of neutrophils, lymphocytes, monocytes, and eosinophils or concentrations of interferon-γ (IFN-γ; p=0.912) did not differ significantly among the control, low-dose, and high-dose groups. However, the concentrations of TNF-α, IL-6, interleukin-10 (IL-10), and interleukin-12 (IL-12) in the high-dose group were significantly lower than in the control and low-dose groups (p<0.05; Figure 2). The TNF-α, IL-6, IL-10, and IL-12 concentrations were similar in the control and low-dose groups (Figure 2).

Metabolomic alteration between the control group and probiotic group

The plasma metabolites changed significantly between the control and high-dose groups, but no significant differences were observed between the control and low-dose

Table 1. Baseline characteristics of patients with tuberculosis†

	Control group (n=15)	Low-dose group (n=16)	High-dose group (n=16)	*p*
Age	33.5 (15.2)	26.1 (10.8)	26.1 (10.6)	0.171
Sex (male)	8 (53.3%)	11 (68.8%)	8 (50.0%)	0.521
Body-mass index, kg/m^2	21.2 (3.4)	20.6 (2.8)	20.0 (2.4)	0.581
TBscore	3.00 (1.25, 4.00)	2.00 (1.25, 3.75)	4.00 (1.25, 4.00)	0.525

†Numerical variables are presented as mean ± standard deviation for normally distributed variables or median (interquartile range) for non-normally distributed variables. Categorical variables are presented as number (percentage).

Table 2. The plasma white blood cell differential count and inflammatory cytokine concentrations after a 4-week follow-up†

	Control group (n=15)	Low-dose group (n=16)	High-dose group (n=16)	*p*
White blood cell count (10^9/L)	5.20 (3.86-6.74)	5.70 (5.04-6.85)	6.16 (4.39, 7.51)	0.580
Neutrophil count (10^9/L)	2.95 (2.21-4.22)	3.34 (2.50-4.36)	3.34 (2.47, 4.63)	0.733
Lymphocyte count (10^9/L)	1.42 (0.96-1.87)	1.60 (1.12-1.95)	1.91 (1.42, 2.35)	0.210
Monocyte count (10^9/L)	0.53 (0.46-0.65)	0.62 (0.40-0.77)	0.55 (0.43, 0.67)	0.798
Eosinophil count (109/L)	0.19 (0.11-0.24)	0.19 (0.12-0.36)	0.10 (0.06, 0.23)	0.113
IFN-γ lg (pg/mL)	0.53 (0.22)	0.52 (0.17)	0.50 (0.25)	0.912
TNF-α lg (pg/mL)	0.91 (0.36)	0.88 (0.18)	0.60 (0.36)	0.012
IL-6 lg (pg/mL)	0.51 (0.58)	0.53 (0.46)	0.11 (0.48)	0.057
IL-10 lg (pg/mL)	0.91 (0.36)	0.98 (0.21)	0.42 (0.54)	0.001
IL-12 lg (pg/mL)	1.04 (0.22)	1.07 (0.10)	0.72 (0.29)	<0.001

†Inflammatory cytokine data were log-transformed and analyzed with ANOVA. Plasma white blood cell differential count are presented as median (interquartile range) and inflammatory cytokine concentrations are presented as mean±standard deviation

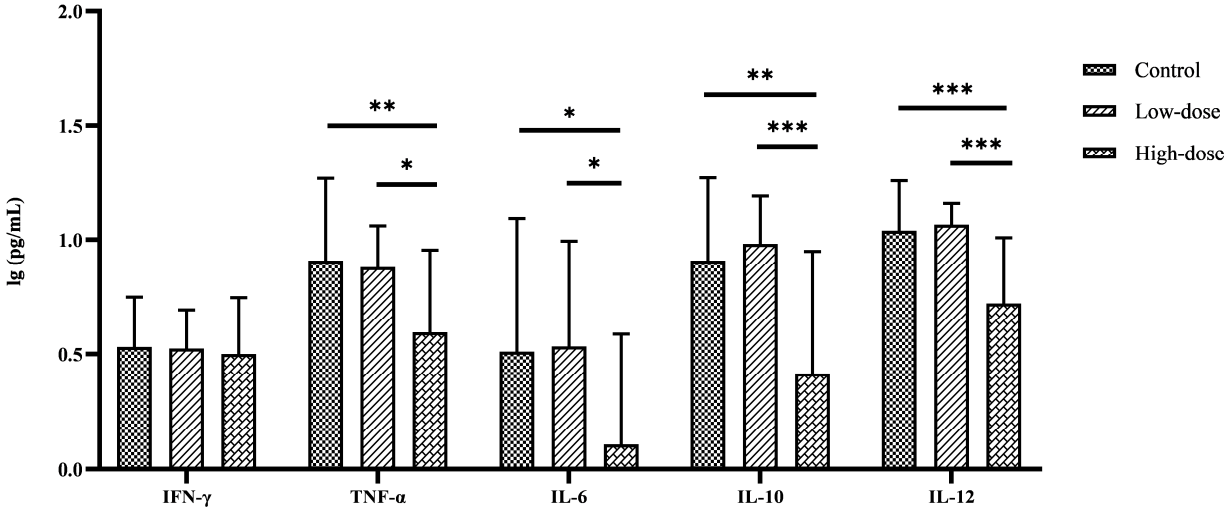

Figure 2. *L. casei* intervention reduced inflammatory cytokine concentrations in patients with tuberculosis. Inflammatory cytokine data were log-transformed and analyzed with ANOVA. $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

groups. The metabolites in the control and high-dose groups were clearly separated by the OPLS-DA model (Figure 3a). One thousand permutation tests yielded an R^2Y value of 0.977 (p=0.049) and a Q^2 value of 0.553 (p<0.001) between the control and high-dose groups (Figure 3b), suggesting model reliability with no evidence of overfitting. Using an FC cutoff value of >1.2 or <1/1.2 and an FDR cutoff value of <0.05, 44 differential metabolites were identified in patients in the high-dose group (in relation to the control group), of which 22 were upregulated and 22 were downregulated (Figure 3c, Supplementary table 1). The primary upregulated metabolites in the high-dose group (in relation to the control group) were pyridoxamine, histidine, N-3-oxo-dodecanoyl-L-Homoserine lactone (3-oxo-C12-HSL), phosphatidylserine (PS), maresin 1 (MaR1), and PC. The primary downregulated metabolites were N-acetylmethionine; 11, 12 epoxyeicosatrienoic acid (11, 12-EET); L-tryptophan; and PE.

Metabolomic alteration between the low-dose group and the high-dose group

Results of OPLS-DA demonstrated that the plasma samples of patients with TB in the low-dose and high-dose groups were clearly separated, suggesting the probiotics caused significant changes in their metabolic profiles (Figure 4a). One thousand permutation tests yielded an R^2Y value of 0.983 (p=0.007) and a Q^2 value of 0.665

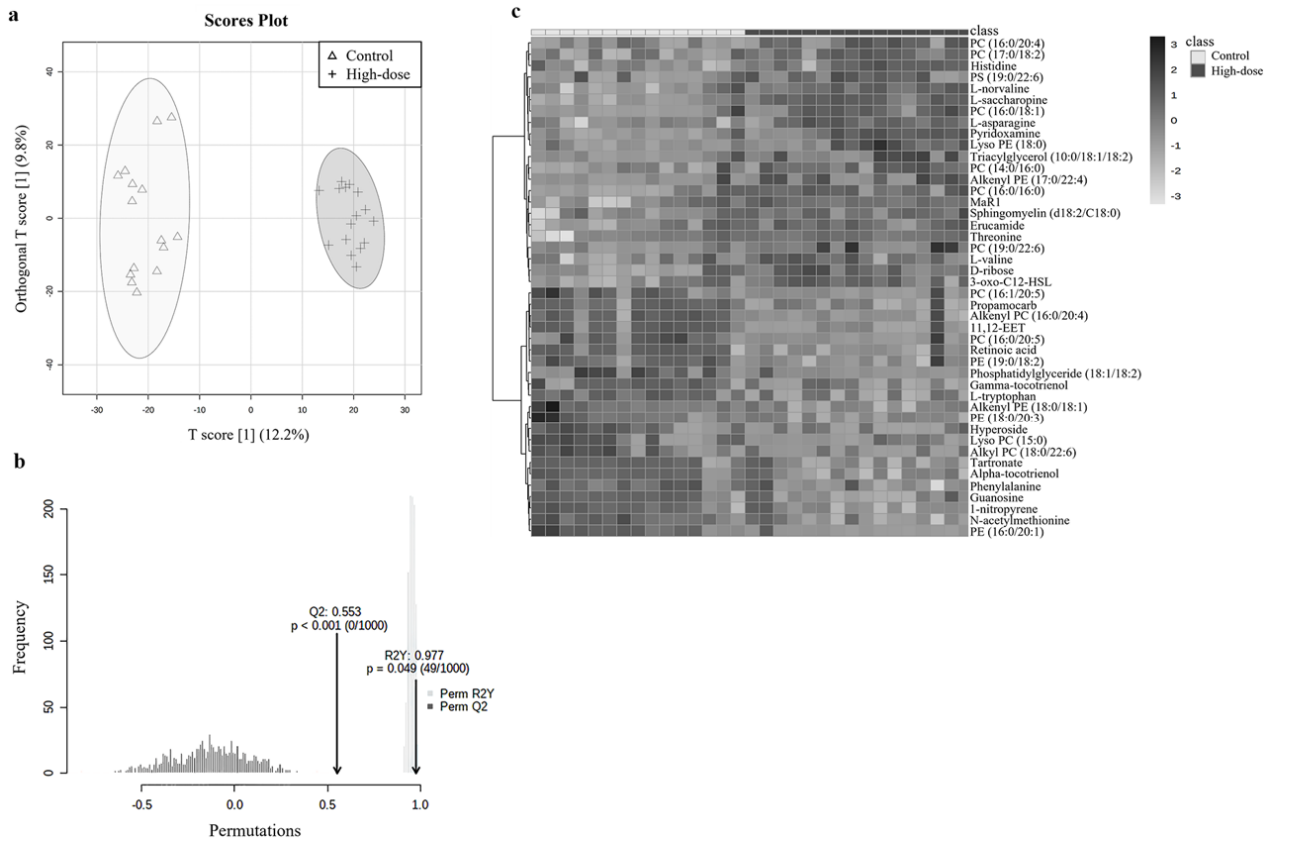

Figure 3. Metabolomic alteration between the control and high-dose groups. (a) The metabolites in the control and high-dose groups were clearly separated by the orthogonal partial least squares discrimination analysis model. (b) The model had no evidence of overfitting. One thousand permutation tests yielded an R^2Y value of 0.977 (p=0.049) and a Q^2 value of 0.553 (p<0.001). (c) Heat map of differential metabolites in the high-dose group compared with the control group. The shades of the color represented metabolites levels (black, and white indicated higher level, and lower level, respectively).

(p<0.001; Figure 4b) indicating no overfitting. With cut-offs of FC >1.2 or <1/1.2 and FDR <0.05, 49 differential metabolites were identified in the high-dose group (in relation to the low-dose group), of which 29 were upregulated and 20 were downregulated (Figure 4c, Supplementary table 2). In patients in the high-dose group, the upregulated metabolites comprised L-valine, linoleic acid, L-asparagine, MaR1, 3-oxo-C12-HSL, pyridoxamine, PC, and PS. The downregulated metabolites comprised PE, L-tryptophan, and N-acetylmethionine.

Changes of key differential metabolites after L.casei suppplementation

According to the FC and FDR, 32 metabolites were commonly identified as differential metabolites through comparison among the control, low-dose, and high-dose groups (Figure 5a). Among the three groups, 11 metabolites exhibited dramatic changes. Compared with the control and low-dose groups, the high-dose group exhibited significant upregulation of pyridoxamine, L-saccharopine, PS (19:0/22:6), MaR1, PC (16:0/20:4), PC (16:0/18:1), and PC (16:0/16:0) (Figure 5b–5h). Phenylalanine, N-acetylmethionine, PE (16:0/20:1), and L-tryptophan, however, were downregulated in the high-dose group (Figure 5i–5l).

Differential metabolites correlate with inflammatory cytokines

To examine potential associations between metabolites and inflammatory cytokines, Spearman correlation analysis was performed. A heat map of the scaled correlations was generated between the metabolites and identified inflammatory cytokines (Figure 6). Strong correlations were observed between PS (19:0/22:6) and TNF-α (r=−0.507, p<0.001), IL-10 (r=−0.573, p<0.001), and IL-12 (r=−0.528, p<0.001); between L-tryptophan and IL-12 (r=0.553, p<0.001); between PC (16:0/18:1) and TNF-α (r=−0.403, p=0.005); between PC (16:0/16:0) and IL-12 (r=−0.467, p=0.002); and between PE (16:0/20:1) and TNF-α (r=0.439, p=0.002).

DISCUSSION

The present study is the first RCT to investigate the effects of probiotics on plasma inflammatory cytokines and metabolites in patients with TB. The results indicated that daily *L. casei* supplementation during TB treatment modulated inflammatory cytokines and metabolites in plasma. Spearman correlation analysis revealed strong correlations between several inflammatory cytokines and metabolites.

The authors' previous studies have revealed the beneficial effect of *L. casei* on the composition of gut microbiota[16] and that the circulating metabolites affect gut microbiota composition.[19] The present RCT was conducted on the basis of the aforementioned results and revealed that *L. casei* supplementation regulated the abundance of MaR1, L-tryptophan, N-acetylmethionine, PS, PC and the concentrations of TNF-α, IL-6, IL-10, IL-12.

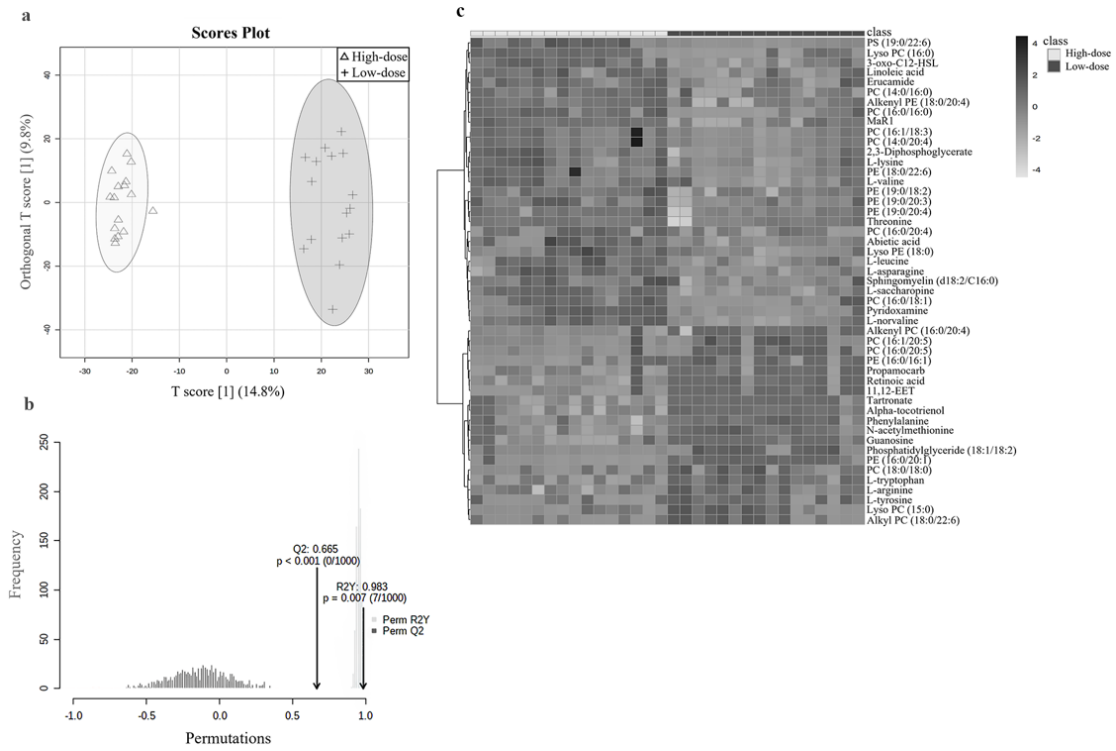

Figure 4. Metabolomic alteration between the low-dose and high-dose groups. (a) The metabolites in the low-dose and high-dose groups were clearly separated by the orthogonal partial least squares discrimination analysis model. (b) The model had no evidence of overfitting. One thousand permutation tests yielded an R^2Y value of 0.983 (p = 0.007) and a Q^2 value of 0.665 (p<0.001). (c) Heat map of differential metabolites in the high-dose group compared with the low-dose group. The shades of the color represented metabolites levels (black, and white indicated higher level, and lower level, respectively).

The current results are consistent with those of studies that have reported that probiotic supplementation can lower the concentration of N-acetylmethionine,[20] a methyl donor.[21] Decreased N-acetylmethionine diminishes the methylation of insulin-like growth factor binding protein 1 and methionine sulfoxide reductase A and reduces the risk of aberrant glucose metabolism.[20] Moreover, studies have indicated that MaR1 can induce bactericidal/permeability-increasing protein expression and Nrf2 nuclear translocation.[22] Therefore, MaR1 can improve the anti-inflammatory and antimicrobial properties of *M. tuberculosis*-infected human macrophages.[23] MaR1 can also reduce *M. tuberculosis*-induced TNF-α production.[22] Additionally, increased indoleamine 2,3 dioxygenase 1 (IDO-1)–mediated tryptophan catabolism may modulate the CD4+ T cell responses of patients with TB, alleviating inflammation and inducing immune tolerance.[24] However, daily probiotic supplementation can limit the drops in tryptophan concentrations,[25] and thus affect body immunity, likely by increasing metabolites.

In patients with TB, the concentrations of IFN-γ, TNF-α, IL-10, and IL-12 may increase.[26,27] In the present trial, *L. casei* supplementation led to a significant reduction of TNF-α, IL-6, IL-10, and IL-12 concentrations. Studies have consistently reported that *L. casei* supplementation lowered TNF-α, IL-6, and IL-12 concentrations.[28,29] The IL-10 concentration also decreased in this study, possibly because of the immune-stimulatory effects of IL-10. IL-10 exerts these effects by inducing bal-2 protein expression to inhibit peripheral T cell apoptosis or by promoting the proliferation and differentiation of B lymphocytes into plasmocytes.[30,31]

Correlation analysis may explain the regulating effect of metabolites on inflammatory cytokines. Studies have demonstrated that PS treatment reduces the concentrations of TNF-α and IL-6.[32,33] Apoptosis occurs during the fight against *M. tuberculosis*, and PS inhibits the phagocytosis of apoptotic cells and induces an anti-inflammatory state.[34] This study indicated a strong correlation between PS (19:0/22:6) and IL-12, likely because IL-12 is anti-inflammatory. Tryptophan can be catabolized by IDO-1 in splenic macrophages. Vitro experiments displayed that expression of IDO-1 significantly suppresses IL-12 production in splenic macrophages through a primary downstream effector, metabolic-stress sensing protein kinase General Control Non-depressible 2.[35] PC is the precursor of lysophosphatidylcholine, a chemoattractant for T lymphocytes,[36] and PC lowered the concentrations of TNF-α and IL-6 in rat experiments.[37,38] Overall, the present results indicated that *L. casei* may regulate the plasma metabolic profile and inflammatory cytokine concentrations of patients with TB, and inflammatory cytokine changes may partly cause the changes in the metabolites.

This study has several strengths. First, this is the first clinical trial to investigate the effect of probiotics on plasma inflammatory cytokines and metabolites in patients with TB, and the results indicated that *L. casei* supplementation modulates inflammatory cytokines and metabolites in patients with TB. Second, the potential correlations between the abundance of plasma metabolites and the concentrations of inflammatory cytokines were examined. The effect of *L. casei* supplementation on inflammatory cytokines may be related to the metabolite changes.

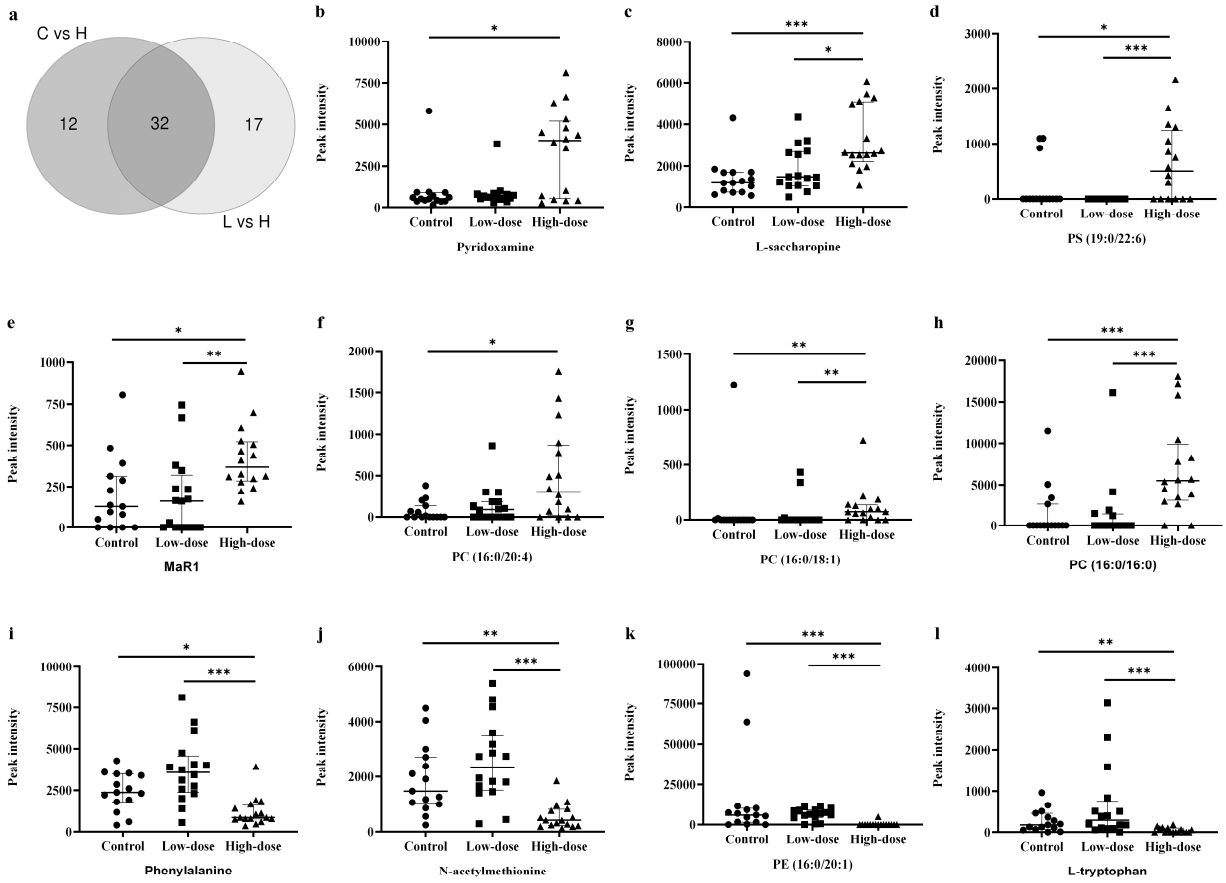

Figure 5. (a) Venn diagram displayed the number of differential metabolites in the control group vs high-dose group, low-dose group vs high-dose group. The values for shared differential metabolites: (b-l) Peak intensity of 11 differential metabolites in the control, low-dose and high-dose groups. Data were displayed as scatter plots with median and interquartile range, with each dot representing one individual. Peak intensities of metabolites were analyzed with a nonparametric test with a Dunn's multiple comparisons test conducted between groups. *p<0.05, **p<0.01, ***p<0.001.

Third, patients with TB were all inpatients during the first 4 weeks of supplementation and shared similar living and dietary habits, which could have increased the authenticity of the results.

Limitations of the study should also be acknowledged. The follow-up duration of 1 month prevented an investigation on the long-term effects of *L. casei* during TB treatment on patients' metabolic profiles and immunity conditions, which had been altered when patients were infected with *M. tuberculosis*. The results demonstrated that a 1-month intervention resulted in significant improvements. Second, the present study only employed two supplementation dosages; therefore, elucidating the dosage of *L. casei* supplementation for modulating metabolites and the concentrations of inflammatory cytokines is difficult.

In conclusion, daily *L. casei* supplementation of up to 2 × 10^{10} CFU during the intensive phase of TB treatment modulates metabolites and inflammatory cytokines. Decreased concentrations of inflammatory cytokines may be related to the metabolite changes. Future work can investigate the effect of long-term probiotic interventions in patients with TB.

AUTHOR DISCLOSURES

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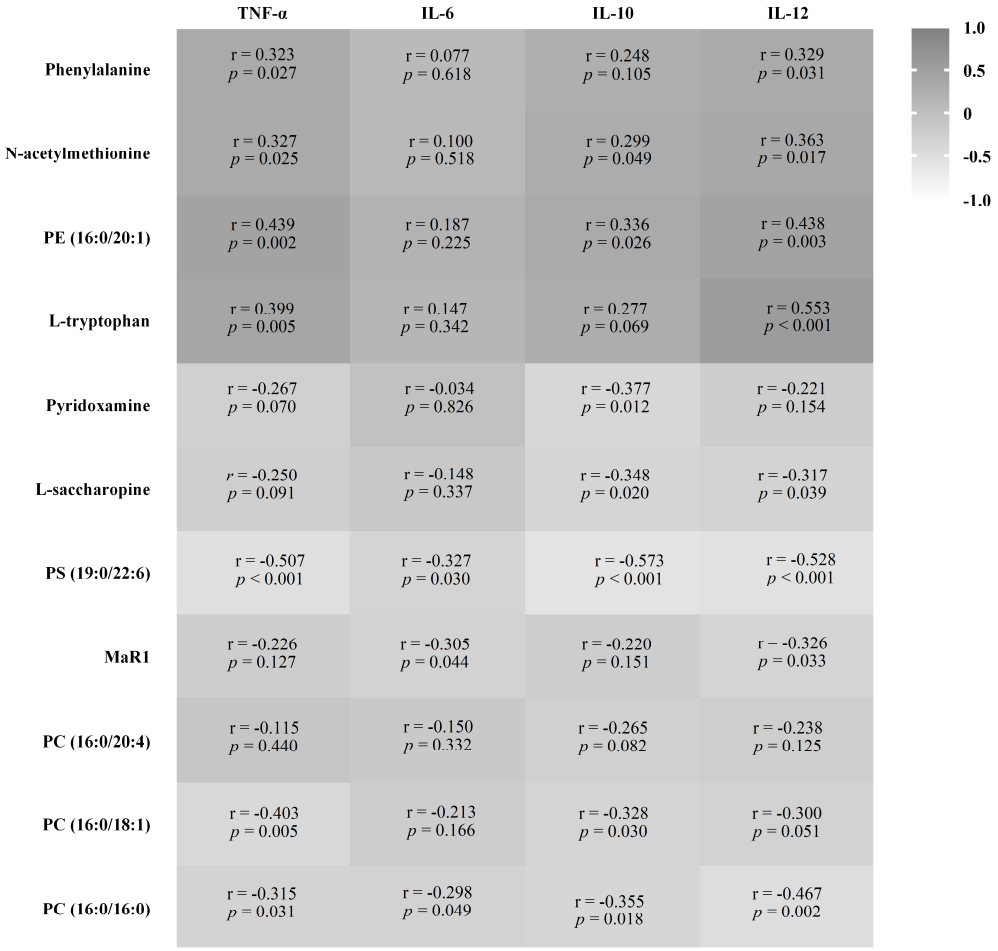

Figure 6. Correlations between inflammatory cytokines and metabolites through Spearman nonparametric test. The shades of the color represented correlations levels (Black, and white indicated positive correlation, and negative correlation, respectively).

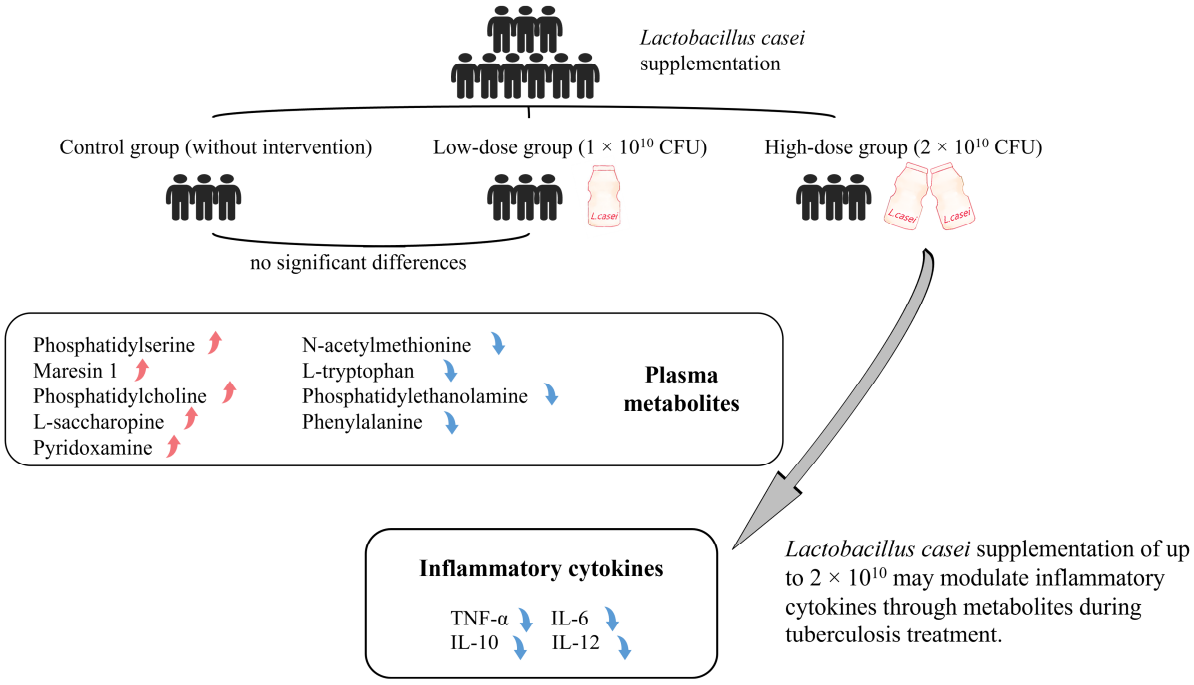

Figure 7. Graphical abstract.

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Supplementary table 1. The differential metabolites between the control and high-dose groups in plasma

Metabolite name	FC	log2(FC)	*p*. adjusted
Phosphatidylethanolamine (18:0/20:3)	0.01	-6.72	2.84E-02
Phosphatidylethanolamine alkenyl (18:0/18:1)	0.01	-6.19	2.20E-02
Phosphatidylethanolamine (16:0/20:1)	0.04	-4.65	4.82E-04
Phosphatidylcholine alkyl (18:0/22:6)	0.05	-4.35	3.73E-02
Phosphatidylglyceride (18:1/18:2)	0.10	-3.37	2.90E-02
Phosphatidylcholine lyso (15:0)	0.11	-3.17	3.77E-02
Hyperoside	0.15	-2.75	2.97E-02
gamma-tocotrienol	0.22	-2.17	3.35E-02
alpha-tocotrienol	0.23	-2.10	9.52E-03
Retinoic acid	0.24	-2.05	9.76E-03
Tartronate	0.27	-1.88	7.09E-03
L-tryptophan	0.27	-1.87	9.92E-03
1-nitropyrene	0.29	-1.81	2.56E-02
Phosphatidylcholine (16:1/20:5)	0.29	-1.78	2.72E-02
Phosphatidylcholine alkenyl (16:0/20:4)	0.31	-1.69	6.81E-03
11, 12 epoxyeicosatrienoic acid	0.33	-1.61	2.93E-03
Guanosine	0.33	-1.58	1.18E-02
Phosphatidylcholine (16:0/20:5)	0.40	-1.34	3.09E-02
N-acetylmethionine	0.41	-1.28	7.34E-03
Propamocarb	0.46	-1.11	9.40E-03
Phosphatidylethanolamine (19:0/18:2)	0.50	-1.00	2.27E-02
Phenylalanine	0.65	-0.61	3.73E-02
L-valine	1.37	0.46	4.65E-02
L-norvaline	1.60	0.68	7.75E-03
Erucamide	1.70	0.77	3.51E-02
Phosphatidylcholine (16:0/18:1)	1.80	0.84	8.27E-03
Threonine	1.85	0.89	4.06E-02
Sphingomyelin (d18:2/C18:0)	1.94	0.95	3.53E-02
D-ribose	1.94	0.96	1.08E-02
Maresin 1	2.04	1.03	1.28E-02
L-asparagine	2.12	1.08	9.55E-03
Phosphatidylserine (19:0/22:6)	2.55	1.35	4.13E-02
Phosphatidylethanolamine lyso (18:0)	2.62	1.39	9.92E-03
Phosphatidylcholine (14:0/16:0)	2.87	1.52	1.45E-02
L-saccharopine	2.90	1.54	1.34E-04
Phosphatidylethanolamine alkenyl (17:0/22:4)	3.43	1.78	3.02E-02
Triacylglycerol (10:0/18:1/18:2)	3.44	1.78	3.20E-02
N-3-oxo-dodecanoyl-L-Homoserine lactone	3.55	1.83	1.51E-02
Phosphatidylcholine (17:0/18:2)	3.60	1.85	1.24E-02
Phosphatidylcholine (16:0/16:0)	3.73	1.90	5.15E-03
Histidine	4.09	2.03	7.29E-03
Phosphatidylcholine (19:0/22:6)	4.10	2.04	4.49E-02
Pyridoxamine	4.28	2.10	2.99E-03
Phosphatidylcholine (16:0/20:4)	9.17	3.20	2.52E-02

Supplementary table 2. The differential metabolites between the high-dose and low-dose groups in plasma

Metabolite name	FC	log2(FC)	*p*. adjusted
Phosphatidylcholine alkyl (18:0/22:6)	0.04	-4.77	1.96E-02
Phosphatidylcholine lyso (15:0)	0.12	-3.10	2.68E-02
Phosphatidylglyceride (18:1/18:2)	0.13	-2.99	7.78E-03
L-tryptophan	0.15	-2.77	3.20E-03
Phosphatidylethanolamine (16:0/20:1)	0.15	-2.75	1.35E-05
Phosphatidylcholine (18:0/18:0)	0.16	-2.67	3.03E-02
Tartronate	0.22	-2.16	5.96E-04
Retinoic acid	0.25	-2.02	7.04E-04
alpha-tocotrienol	0.25	-1.98	1.17E-03
L-tyrosine	0.29	-1.79	4.46E-02
11, 12 epoxyeicosatrienoic acid	0.31	-1.69	1.87E-04
Phosphatidylcholine alkenyl (16:0/20:4)	0.33	-1.62	1.66E-02
Phosphatidylcholine (16:1/20:5)	0.34	-1.58	3.01E-02
L-arginine	0.34	-1.56	2.35E-02
Guanosine	0.34	-1.54	6.50E-04
N-acetylmethionine	0.37	-1.42	1.52E-03
Propamocarb	0.38	-1.39	7.28E-03
Phosphatidylethanolamine (16:0/16:1)	0.40	-1.33	4.23E-02
Phosphatidylcholine (16:0/20:5)	0.41	-1.28	1.30E-02
Phenylalanine	0.53	-0.91	3.32E-03
L-valine	1.45	0.54	9.68E-03
Abietic acid	1.59	0.66	1.25E-02
Erucamide	1.59	0.67	3.02E-02
Linoleic acid	1.63	0.71	1.16E-02
Threonine	1.68	0.75	1.89E-02
L-leucine	1.77	0.82	4.75E-03
L-asparagine	1.77	0.83	8.53E-03
Phosphatidylcholine (14:0/16:0)	1.79	0.84	2.25E-02
Phosphatidylcholine (14:0/20:4)	1.80	0.85	5.06E-03
Phosphatidylcholine (16:1/18:3)	1.80	0.85	5.06E-03
L-lysine	1.81	0.85	2.75E-02
Phosphatidylcholine (16:0/18:1)	1.89	0.91	5.47E-03
L-norvaline	1.91	0.94	4.48E-05
Phosphatidylethanolamine (18:0/22:6)	1.95	0.96	1.25E-02
2,3-Diphosphoglycerate	2.01	1.01	2.02E-03
Phosphatidylethanolamine alkenyl (18:0/20:4)	2.15	1.10	1.60E-02
Phosphatidylethanolamine lyso (18:0)	2.36	1.24	1.16E-02
Sphingomyelin (d18:2/C16:0)	2.43	1.28	4.95E-02
L-saccharopine	2.66	1.41	6.31E-05
Maresin 1	2.95	1.56	2.53E-03
Phosphatidylethanolamine (19:0/20:4)	3.08	1.62	3.84E-02
Phosphatidylcholine lyso (16:0)	3.50	1.81	1.49E-03
Phosphatidylethanolamine (19:0/20:3)	3.64	1.86	1.77E-02
Phosphatidylcholine (16:0/16:0)	4.74	2.25	5.96E-04
Pyridoxamine	4.75	2.25	4.31E-04
Phosphatidylcholine (16:0/20:4)	5.23	2.39	2.51E-02
Phosphatidylethanolamine (19:0/18:2)	5.38	2.43	2.54E-02
N-3-oxo-dodecanoyl-L-Homoserine lactone	5.70	2.51	6.67E-04
Phosphatidylserine (19:0/22:6)	14.39	3.85	9.68E-05