九州大学学術情報リポジトリ
Kyushu University Institutional Repository

Metabolomics profile of Japanese female patients with restricting-type anorexia nervosa

宮田, 典幸

https://hdl.handle.net/2324/4784497

出版情報：Kyushu University, 2021, 博士（医学）, 課程博士
バージョン：
権利関係：(c)2020 The Authors. Published by Elsevier Inc.

KYUSHU UNIVERSITY

Physiology & Behavior 228 (2021) 113204

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Metabolomics profile of Japanese female patients with restricting-type anorexia nervosa

Noriyuki Miyata [a], Tomokazu Hata [a], Shu Takakura [a], Kazufumi Yoshihara [a], Chihiro Morita [a], Katsunaka Mikami [b], Koji Nomoto [c], Kouji Miyazaki [d], Hirokazu Tsuji [d], Nobuyuki Sudo [a,*]

[a] Department of Psychosomatic Medicine, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[b] Department of Psychiatry, Tokai University School of Medicine, Isehara, Japan
[c] Tokyo University of Agriculture, Faculty of Life Sciences, Department of Molecular Microbiology, Japan
[d] Yakult Central Institute, Tokyo, Japan

ARTICLE INFO

Keywords:
Amino acids
Anorexia nervosa
Metabolome
Uremic toxin

ABSTRACT

In this study, the serum metabolic profiles of 10 female patients with restricting type anorexia nervosa (ANR) were compared to those of 10 age-matched healthy female controls. While the levels of amino acids were lower among the patients than among the controls, the levels of uremic toxins, including p-cresyl sulfate (PCS), indole-3-acetic acid, and phenyl sulfate, were higher in ANR patients. The serum PCS levels correlated positively with the abundance of the *Clostridium coccoides* group or the *C. leptum* subgroup in the feces of patients, but not in those of controls. Collectively, these results indicate that the serum metabolic profiles of patients with ANR differ from those of healthy women in terms of both decreased amino acid levels and increased uremic toxins. Gut microbes including *C. coccoides* or *C. leptum* may be involved in such an increase in uremic toxins.

1. Introduction

Anorexia nervosa (AN) is characterized by extreme weight loss and fear of weight gain [1,2], and affects 1 to 4% of all women [3]. AN is a psychiatric condition with one of the highest mortality rates [4] and is divided into the following two sub-types: restricting-type AN (ANR) and binge/purge-type AN (ANBP). Patients with ANR severely restrict their food intake and show physical and psychiatric symptoms due to severe emaciation. Besides such severe restriction of dietary intake, patients with ANBP regularly show frequent binge-eating or purging behaviors, such as self-induced vomiting.

Psychosocial factors have been thought to play important roles in the development and progression of eating disorders [5–7]; however, biological factors are also presumed to exert a substantial effect on AN pathology [8,9]. A recent large-scale genome-wide association study [10] demonstrated that the genetic origins of AN are both metabolic and psychiatric, and not purely psychiatric as thought. Therefore, genetically vulnerable women are considered to develop AN when exposed to certain environmental factors, including psychosocial elements. Therefore, the gut microbiota has emerged as a potential environmental factor that can influence the pathological process of AN [11]. Independent research groups, including our own, have demonstrated the existence of gut dysbiosis in patients with AN [12–15]. Our recent report suggests that such dysbiosis contributed to poor weight gain and anxiety-like behavior in an ANR mouse model involving gut microbiota transplant from patients with ANR [16]. However, how and to what extent such changes in gut microbes can contribute to the development and clinical course of AN are largely unknown.

Recently, metabolomics, one of the new 'omics' approaches, has been recognized as a useful method for exploring potential biomarkers in various diseases [17]. Metabolites are the end products of cellular regulatory processes and include low-molecular-weight organic and inorganic chemicals [18]. As it is conceivable that long-term starvation and low body weight might exert a considerable impact on the physiology and metabolism of patients with AN, obtaining a comprehensive metabolomic view of patients with anorexia may be useful for unraveling AN-specific pathologies that remain to be identified.

Therefore, we hypothesized that patients with anorexia might have an altered profile of serum metabolites derived from gut microbes that could be linked with some anorexia-specific pathologies. To test this hypothesis, we compared the metabolic profiles of female patients with anorexia with those of age-matched healthy female control subjects.

* Corresponding author.
E-mail address: sudo.nobuyuki.935@m.kyushu-u.ac.jp (N. Sudo).

https://doi.org/10.1016/j.physbeh.2020.113204
Received 3 August 2020; Received in revised form 7 October 2020; Accepted 9 October 2020
Available online 11 October 2020
0031-9384/© 2020 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2. Materials & methods

2.1. Subjects

We enrolled Japanese patients with AN who either were admitted to our department or who visited our outpatient section at Kyushu University Hospital between 2010 and 2015, and 10 female patients with ANR agreed to participate in this study. We also enrolled 10 age-matched, healthy female volunteers. Volunteers with a history of digestive disease such as inflammatory-bowel disease and irritable-bowel syndrome were excluded. We also excluded participants with these conditions from the study: severe physical diseases (such as renal failure and infectious diseases), or a history of using antibiotics or regularly consuming yogurt or probiotics within 3 months before the study began.

Patients with AN underwent a structured interview and their AN phenotypes were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders-IV-TR criteria. Patients with binge/purge-type AN were not included in this study, considering the substantial effects of binge-eating and purging behaviors (e.g., self-induced vomiting) on serum metabolomes. The patients with ANR enrolled in this study were not on psychotropic medication, but seven in 10 patients were consuming magnesium oxide at the time of blood sampling. No significant difference was found in the average number of defecations between patients with ANR and healthy participants (ANR 10 ± 5.3 per week, healthy participants 10 ± 6.4 per week). The study protocol was approved by the Institutional Review Boards of Kyushu University Hospital and written informed consent was obtained from all of the participants before enrollment in the study.

2.2. Biochemical assays and self-reported questionnaires

Blood samples were drawn in the morning after an overnight fast for biochemical assays and metabolome analysis. Blood samples were also used to determine the serum levels of albumin, blood urea nitrogen (BUN), creatinine, electrolytes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glucose. All participants completed a battery of self-reported questionnaires. Depression and anxiety levels were evaluated using the Japanese version of the Center for Epidemiologic Studies-Depression Scale [19] and the Japanese version of the State-Trait Anxiety Inventory [20], respectively. Psychopathology related to eating disorders was assessed as per the Eating Disorder Inventory (EDI), as described [21].

2.3. Dietary assessment

Nutrition and food intakes were assessed using the food-frequency questionnaire based on food groups (FFQg) [22]. The FFQg includes food in 29 groups and 10 kinds of cookery and provides information regarding the average intake per week of each food or food group in commonly used units or portion sizes. The Japanese version of the FFQg was validated by comparing weighed dietary records for seven continuous days with 66 subjects, aged 19–60 years [23].

2.4. Serum metabolic profiling analysis using capillary electrophoresis-time-of-flight mass spectrometry (CE-TOFMS) and liquid chromatography-time-of-flight mass spectrometry (LC-TOFMS)

Serum samples were separated from whole blood and stored at −80 °C until analysis. Samples taken for metabolic analysis were prepared according to a described method [24,25]. For CE-TOFMS analysis, 50 μl of each serum sample was added to 450 μl of methanol containing an internal standard solution (Solution ID: H3304–1002, Human Metabolome Technologies; HMT, Inc., Tsuruoka, Japan), on ice. Each solution was then mixed thoroughly with 500 μl of chloroform and 200 μl of Milli-Q water, and the resulting mixtures were centrifuged at 2300 × *g* for 5 min at 4 °C. Each upper aqueous layer was centrifuged through a Millipore 5-kDa cut-off filter (Ultrafree-MC PLHCC) at 9100 × *g* for 120 min at 4 °C to remove macromolecules. Each filtrate was then centrifugally concentrated and reconstituted in 25 μl Milli-Q water before CE-TOFMS analysis.

For LC-TOFMS analysis [26,27], 500 μL of each plasma sample was added to 1500 μL of 1% formic acid/acetonitrile containing an internal standard solution (Solution ID: H3304–1002, HMT, Inc.) at 0 °C to inactivate the enzymes. Each solution was mixed and centrifuged at 2300 × g and 4 °C for 5 min. The supernatants were filtrated using a HybridSPE phospholipid filter (55,261-U, Supelco, Bellefonte, PA, USA) to remove phospholipids. The filtrates were desiccated and then dissolved in 100 μL of isopropanol/Milli-Q for LC-TOFMS analysis.

Peaks were generated using MasterHands automatic-integration software (Keio University, Tsuruoka, Japan) to obtain peak information including the mass-to-charge (m/z) ratio, the migration time (MT) for each CE-TOFMS measurement, the retention time (RT) for each LC-TOFMS measurement, and the peak area. Signal peaks corresponding to isotopomers, adduct ions, and other product ions of known metabolites were excluded and the remaining peaks were annotated as putative metabolites from the HMT metabolite database, based on their MT/RT ratios and m/z values, determined using TOFMS. The tolerance range for the peak annotation was configured at ± 0.5 min for the MT and at ± 10 ppm for the m/z ratio. In addition, the peak areas were normalized against those of the internal standards, and the resultant relative-area values were further normalized based on the sample quantity used.

Besides semi-quantitative analysis, the absolute quantities of 48 metabolites, including 35 cations and 13 anions, were calculated based on the peak areas of the internal controls for each metabolite.

2.5. Quantitative measurements of uremic toxins

Serum levels of p-cresyl sulfate (PCS), indoxyl sulfate, indole-3-acetic acid (IAA), phenyl sulfate (PhS), phenyl acetic acid (PAA), and hippuric acid were quantified using selected-reaction monitoring of liquid chromatography/electrospray ionization-mass spectrometry/mass spectrometry (LC/ESI-MS/MS) at Kureha Corporation, as reported [28–30]. The selected-reaction monitoring method of LC/ESI-MS/MS was conducted using a triple quadrupole mass spectrometer (APl4000, AB SCIEX, Framingham, MA), equipped with an electrospray ionization (ESI) source. The mass-ion source parameters were: ion source temperature of 700 °C, ESI voltage of −4.0 kV, curtain gas of 10 pounds per square inch (psi), ion source gas 1 of 60 psi, ion source gas 2 of 80 psi, collision gas of 4 psi, and the interface heater turned on. Data acquisition and processing were conducted using Analyst software, version 1.5.1.

2.6. Determination of bacterial counts via 16S rRNA-targeted reverse transcription-quantitative polymerase chain reaction (qPCR) analysis

The composition of gut bacteria in fecal samples of participants was performed using 16S rRNA-targeted RT-qPCR with a Yakult Intestinal Flora-SCAN (YIF-SCAN) system [13,31–33].

2.7. Statistical analysis

All continuous data are expressed as the mean ± standard deviation or in box plots with the median. All analyses were performed using the JMP Pro-v.14.2.0 software package for Windows (SAS Institute, Japan). Blood-chemistry data, psychological-testing scores, and diet-related parameters between the two groups were evaluated using an unpaired, two-tailed Student's *t*-test.

Regarding serum metabolite analyses, differences in serum compounds between both groups were analyzed using an unpaired, two-tailed Student's *t*-test, followed by the false-discovery rate (FDR) correction for multiple testing ($p < 0.05$). Principal component analysis (PCoA) and hierarchical clustering analysis (dendrograms) were

performed using the web-based metabolomic data processing tool MetaboAnalyst 4.0 (http://www.metaboanalyst.ca, McGill University, Montreal, Canada) [34–36]. Following PCoA, permutational multivariate analysis of variance (PERMANOVA) [37,38] was conducted to evaluate differences in serum metabolomes between the control and ANR groups. The PERMANOVAs were performed using the Adonis function in the vegan package of R software. Pathway analysis was also conducted using MetaboAnalyst 4.0, with which metabolite data were annotated based on data sources such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Human Metabolome Database. Metabolic pathways with FDR-corrected p values of < 0.05 were considered significantly enriched. Topological pathway analysis was performed based on the relative betweenness centrality, and the pathway impact value threshold was set to > 0.1. Pathway impact values closer to 1.0 indicated a more perturbed pathway.

Differences in uremic toxins levels between the two groups were analyzed using an unpaired, two-tailed Student's *t*-test followed by Bonferroni's correction. Pearson's correlation coefficients were used to assess relationships between the number of the *Clostridium leptum* group or the *C. coccoides* subgroup in the feces, and the serum PCS levels.

3. Results

3.1. Participants profiles: demographic features, blood chemistry, psychological assessments, and specific dietary intake features in patients with anorexia

Table 1 shows the body weights and body-mass index (BMI) values of patients with ANR and healthy women. The ANR group showed lower glucose levels and higher AST and ALT levels compared to the control group; however, the blood-chemistry data exhibited no other significant differences between both groups.

In terms of the psychological aspects evaluated using the self-administered questionnaires, depression and anxiety levels were higher in the AN group than in the control group (Supplementary Table 1). In subscales of the EDI questionnaire, interoceptive awareness and interpersonal distrust were also higher in the AN group than in the control group.

As summarized in Supplementary Table 2, patients in the ANR group had a significantly lower total caloric intake than healthy women in the control group ($t_{(17)} = 3.113$, $p = 0.0063$). Caloric intake in terms of fat and protein was also lower in patients with ANR than in the healthy controls. In contrast, dietary fiber consumption did not significantly differ between the control and AN groups.

Table 1
Comparison of the characteristics and blood chemistry between control subjects (CON) and patients with restricting-type anorexia nervosa (ANR).

	CON ($n = 10$)	ANR ($n = 10$)
Age (years)	33.7 ± 8.5	30.3 ± 10.8
Height (cm)	158.3 ± 4.9	154.7 ± 5.0
Weight (kg)	53.5 ± 5.6	30.7 ± 4.6*
BMI (kg/m^2)	21.3 ± 2.1	12.8 ± 1.53*
Albumin (g/dL)	4.5 ± 0.2	4.2 ± 0.6
BUN (mg/dL)	12.5 ± 4.2	11.9 ± 3.2
Crea (mg/dL)	0.61 ± 0.06	0.55 ± 0.09
Na (mEq/L)	141.4 ± 1.4	142.7 ± 1.4
K (mEq/L)	4.1 ± 0.2	4.2 ± 0.4
Cl (mEq/L)	105.2 ± 1.5	105.6 ± 1.8
AST (IU/L)	17.4 ± 5.7	37.8 ± 19.1**
ALT (IU/L)	13.0 ± 3.9	43.1 ± 35.3*
T-Chol (mg/dL)	190.7 ± 30.7	170.2 ± 21.1
TG (mg/dL)	59.6 ± 15.9	76.1 ± 29.5
Glucose (mg/dL)	82.7 ± 4.7	76.3 ± 6.8*

BMI, body-mass index; BUN, blood urea nitrogen; Crea, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Chol, total cholesterol; TG, triglycerides; CRP, C-reactive protein. ** $p < 0.01$ and * $p < 0.05$ indicate a significant difference between the ANR and control group, as determined by performing an unpaired, two-tailed Student's *t*-test.

3.2. Serum metabolome profile of patients with anorexia

We detected 275 metabolites in serum samples from patients with AN and healthy controls (Supplementary Table 3). Of these, 176 and 99 compounds (including 114 cations and 62 anions) were identified using CE-TOFMS and LC-TOFMS, respectively.

Nineteen metabolites showed significantly different levels between the two groups, based on an unpaired, two-tailed Student's *t*-test with FDR-corrected p value of < 0.05 (Table 2). Most metabolites were at lower levels in the AN group than the control group; however, guanidinosuccinic acid and phenylacetylglutamine were present at higher levels in the AN group than in the control group. Fold-change analysis revealed 11 metabolites with either a fold-change of > 2 or < 0.5, when expressed as abundance ratios (AN: control group; Supplementary Table 4). The compounds α-hydroxyisobutyric acid and phenylacetylglutamine displayed > 2-fold increases in serum levels in patients with AN compared with the control women.

Quantitative analysis of serum metabolites based on CE-TOFMS detected 48 compounds, including 20 amino acids. As summarized in Table 3, eight of these 20 amino acids were present in significantly lower serum levels of patients with AN versus the control women (FDR-corrected $p < 0.05$), although cysteine levels were not quantified.

As shown in Fig. 1a, unsupervised PCoA showed that a cluster of the AN group differed from that of the control group. A significant difference between the two groups was also confirmed via PERMANOVA ($f = 3.43$, $p = 0.0029$). Hierarchical cluster-analysis data, presented as a dendrogram (Fig. 1b) or a heatmap (Fig. 1c), also revealed that the AN group was separated from the control group.

3.3. Amino acid-related pathways enriched in patients with ANR

We next conducted pathway enrichment analyses based on metabolic pathways registered in the KEGG.

Twenty-one metabolic pathways that significantly differed between the ANR and control groups (Fig. 2 and Supplementary Table 5). The pathway of "phenylalanine, tyrosine, and tryptophan biosynthesis" was ranked with the highest impact score (impact 1, FDR-corrected $p = 0.028$). We also found that 10 of the 21 pathways were associated with amino acid biosynthesis and metabolism.

Table 2
Compounds with different levels between the ANR and control groups, based on an unpaired, two-tailed Student's *t*-test.

Compound	t.stat	Raw p value	−log10 (p)	FDR
Citric acid	−7.074	1.35E-06	5.8705	0.0002
Betaine	−4.878	0.00017	3.9176	0.0076
cis-Aconitic acid	−4.732	0.00012	3.7795	0.0076
Palmitoylcarnitine	−4.631	0.00021	3.6836	0.0076
Guanidinosuccinic acid	4.202	0.00059	3.2713	0.0122
Asparagine	−4.158	0.00066	3.229	0.0122
Butyrylcarnitine	−4.129	0.00063	3.2152	0.0122
Glycerophosphocholine	−4.108	0.00054	3.1803	0.0122
2-Oxoisovaleric acid	−3.906	0.00103	2.9855	0.0148
O-Acetylcarnitine	−3.892	0.0011	2.9822	0.0148
Octanoylcarnitine	−3.877	0.00107	2.9576	0.0148
N2-Phenylacetylglutamine	3.705	0.00168	2.7912	0.0191
Choline	3.688	0.00162	2.7741	0.0191
Taurine	−3.540	0.00244	2.6316	0.0241
Ethanolamine	−3.520	0.00234	2.6118	0.0241
Tyrosine	−3.358	0.0035	2.4562	0.0313
Methyl-2-oxovaleric acid	−3.331	0.00381	2.4292	0.0313
Alloisoleucine	−3.32	0.00371	2.4192	0.0313
Isoleucine	−3.118	0.00593	2.2269	0.0462

Important compounds that showed significantly different levels between the ANR and control groups were analyzed when the FDR-corrected p value between the groups was < 0.05. FDR: false-discovery rate.

Table 3
Quantitative measurements of serum amino acid concentrations.

	CON (μM)	ANR (μM)	p value	FDR
Asparagine	53 ± 7.5	41 ± 4.5*	0.00058	0.005947
Tyrosine	67 ± 12	50 ± 11*	0.003657	0.021422
Isoleucine	64 ± 14	46 ± 12*	0.006258	0.032074
Alanine	341 ± 62	274 ± 36*	0.008847	0.035969
Histidine	94 ± 8.2	81 ± 11*	0.009163	0.035969
Leucine	118 ± 21	90 ± 23*	0.011405	0.035969
Methionine	16 ± 3.0	12 ± 3.4*	0.010817	0.035969
Proline	149 ± 36	109 ± 27*	0.010294	0.035969
Tryptophan	60 ± 6.9	51 ± 8.8	0.020491	0.055106
Valine	217 ± 26	181 ± 36	0.019756	0.055106
Serine	134 ± 13	119 ± 20	0.056286	0.11328
Phenylalanine	63 ± 7.6	53 ± 15	0.078046	0.14545
Arginine	95 ± 21	84 ± 8.6	0.13129	0.22429
Threonine	128 ± 25	112 ± 30	0.20757	0.30394
Aspartic acid	19 ± 6.9	16 ± 5.6	0.30498	0.39075
Glycine	254 ± 23	280 ± 72	0.29663	0.39075
Glutamate	72 ± 21	62 ± 22	0.32313	0.40147
Lysine	217 ± 36	227 ± 22	0.45873	0.53737
Glutamine	497 ± 71	515 ± 61	0.55064	0.61017
Cysteine	ND	ND		

Serum amino acid levels were quantitatively measured as described in the Methods section. An asterisk indicates a significant difference between the ANR and control groups when the threshold was set at an FDR-corrected p value of < 0.05. ND, not detected; FDR, false-discovery rate.

3.4. Increased uremic toxins in the sera of patients with ANR

Although the patients enrolled in this study showed no apparent markers of renal dysfunction such as elevated creatinine or BUN, the serum levels of guanidinosuccinic acid and *N2*-phenylacetylglutamine, a uremic toxin, were significantly higher in patients with ANR. Therefore, we next quantified and compared the serum concentrations of uremic toxins between the two groups.

As summarized in Table 4, six uremic toxins (PCS, indoxyl sulfate, IAA, PAA, PhS, and hippuric acid) were quantified in serum samples from patients with ANR and healthy participants. The concentrations of PCS, IAA, and PhS were significantly higher in the AN group than in the control group.

3.5. Positive correlation between PCS levels with the abundance of the Clostridium group

Previously, we showed that the gut microbiotas in patients with AN were substantially different from those in age-matched healthy women [13]. Therefore, we next examined whether such increased uremic toxins might correlate with the abundances of certain gut microbes.

The PCS concentrations in patients with AN associated positively with the abundance of the *C. coccoides* group or the *C. leptum* subgroup (Fig. 3a), although such a positive association was not found with the control group (Fig. 3b).

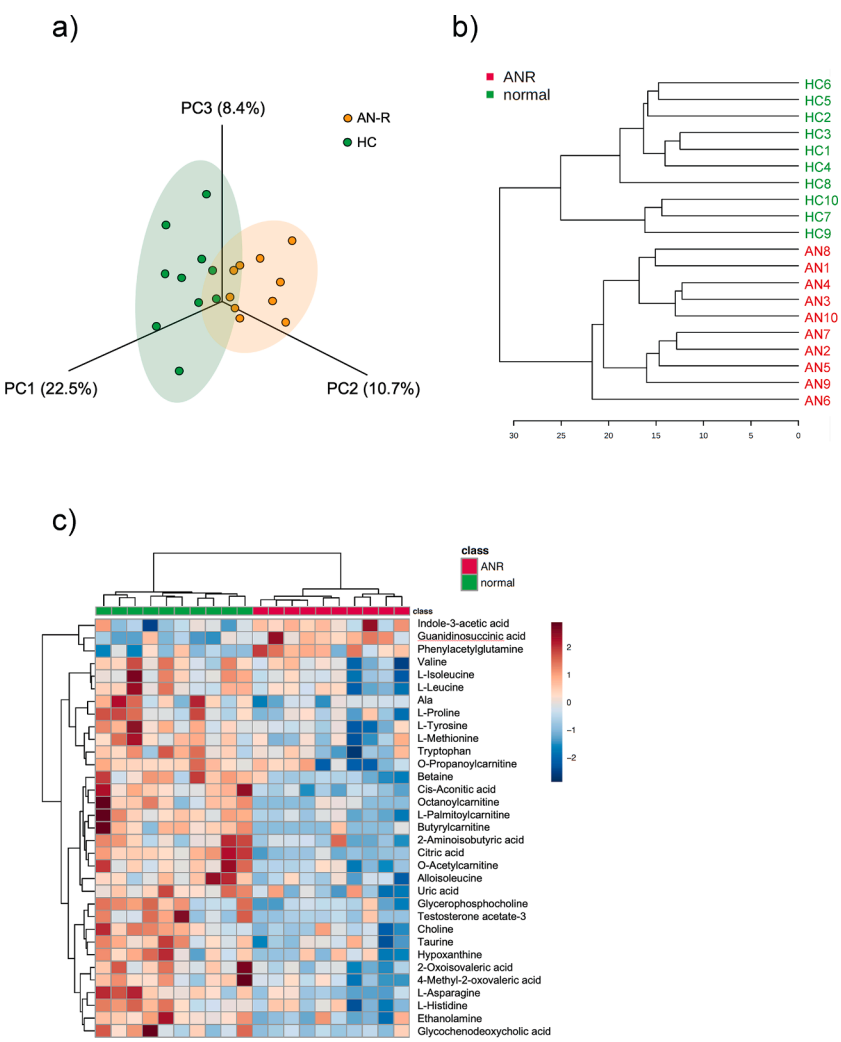
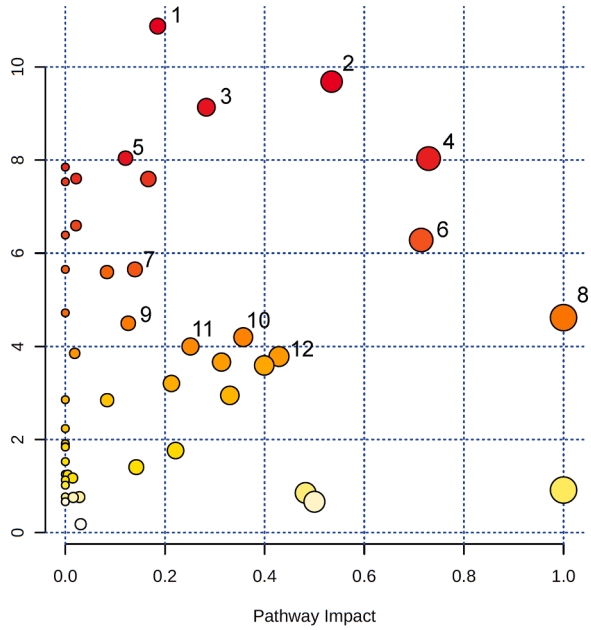


Fig. 1. Serum metabolomics profiles of patients with anorexia and healthy control subjects.
(a) Three-dimensional PCoA between 10 patients with ANR (ANR1–ANR10) and 10 healthy participants (CON1–CON10) was performed, using MetaboAnalyst 4.0. The explained variances are shown in the round brackets. Each colored ellipse covers 95% of the samples belonging to a cluster. (b) Hierarchical clustering is shown in a dendrogram. Euclidean distances and Ward's method were used to measure similarities and for clustering analysis, respectively. (c) The clustering results are exhibited in a heatmap using the top-25 compounds showing the largest differences between patients with ANR and healthy participants.



1. Citrate cycle (TCA cycle)
2. Alanine, aspartate and glutamate metabolism
3. Glyoxylate and dicarboxylate metabolism
4. Glycine, serine and threonine metabolism
5. Aminoacyl-tRNA biosynthesis
6. Taurine and hypotaurine metabolism
7. Tyrosine metabolism
8. Phenylalanine, tyrosine and tryptophan biosynthesis
9. Cysteine and methionine metabolism
10. Phenylalanine metabolism
11. Tryptophan metabolism
12. Arginine and proline metabolism

Fig. 2. Metabolic pathways based on the serum metabolomics profiles of patients with anorexia and healthy controls.
Pathway analysis was conducted using MetaboAnalyst 4.0, as described in the Methods section. The horizontal and vertical axes correspond to topology (pathway impact) and enrichment (–log (p)), respectively. Only pathways with $p < 0.05$ and a pathway impact value > 0.10 were marked. The impact value indicates the cumulative percentage for the matched metabolic nodes.

Table 4
Comparison of serum uremic toxin levels between patients with ANR and control participants.

(mg/dl)	CON ($n = 10$)	ANR ($n = 10$)
p-Cresyl sulfate	0.892 ± 0.461	2.701 ± 0.437*
Indoxyl sulfate	0.092 ± 0.018	0.099 ± 0.017
Indole-3-acetic acid	0.0246 ± 0.002	0.0325 ± 0.002*
Phenyl acetic acid	0.0064 ± 0.002	0.0142 ± 0.002*
Phenyl sulfate	0.0237 ± 0.006	0.0176 ± 0.005
Hippuric acid	0.0367 ± 0.014	0.033 ± 0.014

An asterisk indicates a significant difference between the ANR and control group after the Bonferroni correction, based on the total number of tests (*$p < 0.0083$).

4. Discussion

In this study, patients with ANR had different serum metabolite profiles, such as lower amino acid levels and higher levels of uremic toxin-related compounds, compared to age-matched healthy women. Decreased amino acid levels and increased uremic toxins (such as PCS, IAA, and PhS) in the sera of patients with ANR were also confirmed via quantitative measurements. The serum PCS levels in patients with ANR associated positively with the abundance of the *C. coccoides* group or the *C. leptum* subgroup, whereas such an association was not noted in sera from healthy controls. Taken together, these results indicate that the metabolic profiles of patients with ANR differed from those of healthy women. Whether such a difference may contribute to AN-specific pathological features remains to be clarified in future studies.

The serum levels of both essential amino acids (isoleucine, leucine, and methionine) and non-essential amino acids (asparagine, tyrosine, alanine, histidine, and proline) were significantly lower in the ANR group than in the control group. Several reports have shown that prolonged starvation or undernutrition can elicit decreased serum amino acid levels in humans [39,40]. For example, 30 days of undernutrition caused decreased serum alanine, tyrosine, and tryptophan levels in American soldiers [39]. Since both tyrosine and tryptophan are neurotransmitter precursors, the decrease in amino acids that mainly originate from a reduction in dietary intake may contribute to some psychiatric symptoms, like anxiety and depression [41].

In this study, patients with ANR exhibited higher serum levels of uremic toxin-related compounds than healthy controls. Such toxins are generated by proteins and peptides that enter the colon without being absorbed by the intestines [42]. Since these uremic toxins are produced by gut microbes, the current finding that patients with ANR had increased uremic toxin levels regardless of reduced oral protein intake raises the possibility that gut microorganisms may promote the elevation of such uremic toxins. This possibility was supported by our current results showing that serum PCS levels in the ANR group (but not the control group) correlated positively with the abundance of the *C. coccoides* group or the *C. leptum* subgroup. Interestingly, in another cohort of patients with ANR [16], the relative abundances of members of the *Blautia* genus were significantly higher in patients with ANR than in age-matched healthy women. Previously, it was reported that the *Blautia* genus is the most abundant subgroup in human intestinal *C. coccoides* group populations identified using the YIF-SCAN system [43]. In addition, Saito and colleagues [44] showed that *Blautia hydrogenotrophica* was the highest p-cresol producer. Taken together, these findings suggest that increased populations of certain bacteria belonging to the *Blautia* genus may help elevate the levels of serum uremic toxins in patients with ANR. Further studies are needed to identify a bacterium or some bacterial groups that contribute to the increased uremic toxin levels in patients with ANR.

Quantitative measurements obtained using LC/ESI-MS/MS showed increased PCS, IAA, and PhS levels in patients with AN. Semi-quantitative analysis using CE-TOFMS also revealed that patients with ANR had higher serum levels of three uremic toxins (guanidinosuccinic acid, phenylacetylglutamine, and IAA) than control participants. These compounds are reported to exert a significant effect on the pathological process of either renal [45,46] and cardiovascular diseases [47,48], or neuropsychiatric disorders [49,50]. However, whether these uremic toxins can promote ANR development or progression remains unclear.

This study had some limitations. First, the number of participants was small; this is because our inclusion criteria were strictly set to exclude patients with AN either with episodes of binge-eating and vomiting or with a history of taking psychotropic medication. However, considering the small number of patients with AN enrolled, we should have included patients with AN with a history of bulimia or vomiting. Furthermore, our current results should be validated in other cohort studies with a larger population of patients with AN. In addition, it is critically important to investigate whether AN-specific metabolite features, such as decreased amino acid levels and increased uremic toxin levels, could contribute to AN pathologies (i.e., poor weight gain and psychiatric symptoms).

Most of our results cannot be related to mechanisms of development and maintenance of AN; however, these comprehensive descriptions will

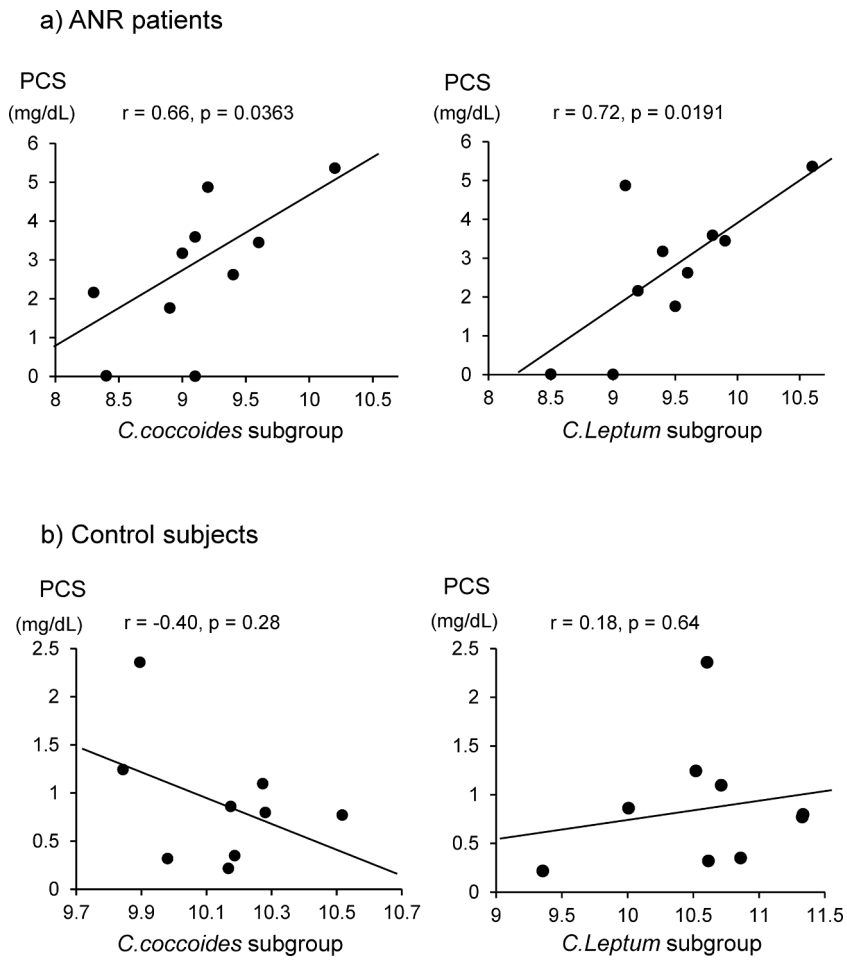


Fig. 3. The abundance of the *Clostridium coccoides* subgroup or *C. leptum* group positively correlated with the serum PCS levels.
The relationships between the serum PCS levels and the abundances of *C. leptum* and *C. coccoides* bacteria in a) patients with ANR and b) healthy control subject were analyzed using Pearson's correlation coefficient. The horizontal axis indicates the number of bacteria ($\log_{10}$ cells/g feces).

generate testable hypotheses and fuel interest among the community of eating disorders researchers, as the Russell's [51] or Kaye's [52,53] pioneering works raised the possibility that amino acids may have a role in the development and maintenance of AN. While the present results provide a more comprehensive metabolomic description of the AN phenotype than that in previous works, cause-effect studies based on realistic mechanistic hypotheses remain to be undertaken.

Funding information

This work was supported by a KAKENHI Grants-in-Aid for Scientific Research on Innovative Areas "Will dynamics" (JP 16H06404: NS), Scientific Research (B) (JP 16H05278 and JP 20H04106: NS), and Exploratory Research (JP 16K15413: NS), and by a Grants-in-Aid from Smoking Research Foundation (NS).

Declaration of Competing Interest

The authors declare no conflicts of interest associated with this manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2020.113204.

References

[1] S. Zipfel, B. Löwe, D.L. Reas, H.C. Deter, W. Herzog, Long-term prognosis in anorexia nervosa: lessons from a 21-year follow-up study, Lancet 355 (2000) 721–722. https://doi.org/10.1016/S0140-6736(99)05363-5.
[2] S. Zipfel, K.E. Giel, C.M. Bulik, P. Hay, U. Schmidt, Anorexia nervosa: aetiology, assessment, and treatment, Lancet Psychiatry 2 (2015) 1099–1111. https://doi.org/10.1016/S2215-0366(15)00356-9.
[3] F.R.E. Smink, D. Van Hoeken, H.W. Hoek, Epidemiology of eating disorders: incidence, prevalence and mortality rates, Curr. Psychiatry Rep. 14 (2012) 406–414. https://doi.org/10.1007/s11920-012-0282-y.
[4] J. Arcelus, Mortality rates in patients with anorexia nervosa and other eating disorders, Arch. Gen. Psychiatry 68 (2011) 724–731. https://doi.org/10.1001/archgenpsychiatry.2011.74.
[5] J. Treasure, A.M. Claudino, N. Zucker, Eating disorders, Lancet 375 (2010) 583–593. https://doi.org/10.1016/S0140-6736(09)61748-7.
[6] C.G. Fairburn, P.J. Harrison, Eating disorders, Lancet 361 (2003) 407–416. https://doi.org/10.1016/S0140-6736(03)12378-1.
[7] C.G. Fairburn, Z. Cooper, H.A. Doll, S.L. Welch, Risk factors for anorexia nervosa: three integrated case-control comparisons, Arch. Gen. Psychiatry 56 (1999) 468–476. https://doi.org/10.1001/archpsyc.56.5.468.
[8] J. Couzin-Frankel, Rethinking anorexia: challenging long-standing theories about the eating disorder, new research suggests biology is a powerful driver, Science 368 (2020) 124–127. https://doi.org/10.1126/science.368.6487.124.
[9] M. Schorr, K.K. Miller, The endocrine manifestations of anorexia nervosa: mechanisms and management, Nat. Rev. Endocrinol. 13 (2017) 174–186. https://doi.org/10.1038/nrendo.2016.175.
[10] H.J. Watson, Z. Yilmaz, L.M. Thornton, C. Hübel, J.R.I. Coleman, H.A. Gaspar, J. Bryois, A. Hinney, V.M. Leppä, M. Mattheisen, S.E. Medland, S. Ripke, S. Yao, P. Giusti-Rodríguez, K.B. Hanscombe, K.L. Purves, R.A.H. Adan, L. Alfredsson, T. Ando, O.A. Andreassen, J.H. Baker, W.H. Berrettini, I. Boehm, C. Boni, V. B. Perica, K. Buehren, R. Burghardt, M. Cassina, S. Cichon, M. Clementi, R.D. Cone, P. Courtet, S. Crow, J.J. Crowley, U.N. Danner, O.S.P. Davis, M. de Zwaan, G. Dedoussis, D. Degortes, J.E. DeSocio, D.M. Dick, D. Dikeos, C. Dina, M. Dmitrzak-Weglarz, E. Docampo, L.E. Duncan, K. Egberts, S. Ehrlich,

G. Escaramís, T. Esko, X. Estivill, A. Farmer, A. Favaro, F. Fernández-Aranda, M. M. Fichter, K. Fischer, M. Föcker, L. Foretova, A.J. Forstner, M. Forzan, C. S. Franklin, S. Gallinger, I. Giegling, J. Giuranna, F. Gonidakis, P. Gorwood, M. G. Mayora, S. Guillaume, Y. Guo, H. Hakonarson, K. Hatzikotoulas, J. Hauser, J. Hebebrand, S.G. Helder, S. Herms, B. Herpertz-Dahlmann, W. Herzog, L. M. Huckins, J.I. Hudson, H. Imgart, H. Inoko, V. Janout, S. Jiménez-Murcia, A. Julià, G. Kalsi, D. Kaminská, J. Kaprio, L. Karhunen, A. Karwautz, M.J.H. Kas, J. L. Kennedy, A. Keski-Rahkonen, K. Kiezebrink, Y.R. Kim, L. Klareskog, K.L. Klump, G.P.S. Knudsen, M.C. La Via, S. Le Hellard, R.D. Levitan, D. Li, L. Lilenfeld, B. D. Lin, J. Lissowska, J. Luykx, P.J. Magistretti, M. Maj, K. Mannik, S. Marsal, C. R. Marshall, M. Mattingsdal, S. McDevitt, P. McGuffin, A. Metspalu, I. Meulenbelt, N. Micali, K. Mitchell, A.M. Monteleone, P. Monteleone, M.A. Munn-Chernoff, B. Nacmias, M. Navratilova, I. Ntalla, J.K. O'Toole, R.A. Ophoff, L. Padyukov, A. Palotie, J. Pantel, H. Papezova, D. Pinto, R. Rabionet, A. Raevuori, N. Ramoz, T. Reichborn-Kjennerud, V. Ricca, S. Ripatti, F. Ritschel, M. Roberts, A. Rotondo, D. Rujescu, F. Rybakowski, P. Santonastaso, A. Scherag, S.W. Scherer, U. Schmidt, N.J. Schork, A. Schosser, J. Seitz, L. Slachtova, P.E. Slagboom, M.C.T. Slof-Op 't Landt, A. Slopien, S. Sorbi, B. Świątkowska, J.P. Szatkiewicz, I. Tachmazidou, E. Tenconi, A. Tortorella, F. Tozzi, J. Treasure, A. Tsitsika, M. Tyszkiewicz-Nwafor, K. Tziouvas, A.A. van Elburg, E.F. van Furth, G. Wagner, E. Walton, E. Widen, E. Zeggini, S. Zerwas, S. Zipfel, A.W. Bergen, J.M. Boden, H. Brandt, S. Crawford, K. A. Halmi, L.J. Horwood, C. Johnson, A.S. Kaplan, W.H. Kaye, J.E. Mitchell, C. M. Olsen, J.F. Pearson, N.L. Pedersen, M. Strober, T. Werge, D.C. Whiteman, D. B. Woodside, G.D. Stuber, S. Gordon, J. Grove, A.K. Henders, A. Juréus, K.M. Kirk, J.T. Larsen, R. Parker, L. Petersen, J. Jordan, M. Kennedy, G.W. Montgomery, T. D. Wade, A. Birgegård, P. Lichtenstein, C. Norring, M. Landén, N.G. Martin, P. B. Mortensen, P.F. Sullivan, G. Breen, C.M. Bulik, Genome-wide association study identifies eight risk loci and implicates metabo-psychiatric origins for anorexia nervosa, Nat. Genet 51 (2019) 1207–1214. https://doi.org/10.1038/s41588-019-0439-2.

[11] A. Larroya-García, D. Navas-Carrillo, E. Orenes-Piñero, Impact of gut microbiota on neurological diseases: diet composition and novel treatments, Crit. Rev. Food Sci. Nutr 59 (2019) 3102–3116. https://doi.org/10.1080/10408398.2018.1484340.

[12] F. Armougom, M. Henry, B. Vialettes, D. Raccah, D. Raoult, Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients, PLoS ONE 4 (2009) e7125. https://doi.org/10.1371/journal.pone.0007125.

[13] C. Morita, H. Tsuji, T. Hata, M. Gondo, S. Takakura, K. Kawai, K. Yoshihara, K. Ogata, K. Nomoto, K. Miyazaki, N. Sudo, Gut dysbiosis in patients with anorexia nervosa, PLoS ONE 10 (2015), e0145274.

[14] S.C. Kleiman, H.J. Watson, E.C. Bulik-Sullivan, E.Y. Huh, L.M. Tarantino, C. M. Bulik, I.M. Carroll, The intestinal microbiota in acute anorexia nervosa and during renourishment, Psychosom. Med. 77 (2015) 969–981.

[15] I. Mack, U. Cuntz, C. Grämer, S. Niedermaier, C. Pohl, A. Schwiertz, K. Zimmermann, S. Zipfel, P. Enck, J. Penders, Weight gain in anorexia nervosa does not ameliorate the faecal microbiota, branched chain fatty acid profiles, and gastrointestinal complaints, Sci. Rep. 6 (2016) 26752. https://doi.org/10.1038/srep26752.

[16] T. Hata, N. Miyata, S. Takakura, K. Yoshihara, Y. Asano, T. Kimura-Todani, M. Yamashita, X.T. Zhang, N. Watanabe, K. Mikami, Y. Koga, N. Sudo, The gut microbiome derived from anorexia nervosa patients impairs weight gain and behavioral performance in female mice, Endocrinology 160 (2019) 2441–2452. https://doi.org/10.1210/en.2019-00408.

[17] P. Monteleone, A.M. Monteleone, J. Troisi, R. Dalle Grave, G. Corrivetti, S. Calugi, G. Scala, G. Patriciello, A. Zanetti, M. Maj, Metabolomics signatures of acutely ill and short-term weight recovered women with anorexia nervosa, Mol. Psychiatry (2019). https://doi.org/10.1038/s41380-019-0573-3.

[18] M. Sugimoto, D.T. Wong, A. Hirayama, T. Soga, M. Tomita, Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles, Metabolomics 6 (2010) 78–95. https://doi.org/10.1007/s11306-009-0178-y.

[19] R.E. Roberts, S.W. Vernon, The center for epidemiologic studies depression scale: its use in a community sample, Am. J. Psychiatry 140 (1983) 41–46. https://doi.org/10.1176/ajp.140.1.41.

[20] N. Iwata, N. Mishima, T. Shimizu, T. Mizoue, M. Fukuhara, T. Hidano, C. D. Spielberger, The Japanese adaptation of the STAI form Y in Japanese working adults - The presence or absence of anxiety, Ind. Health 36 (1998) 8–13. https://doi.org/10.2486/indhealth.36.8.

[21] D.M. Garner, M.P. Olmstead, J. Polivy, Development and validation of a multidimensional eating disorder inventory for anorexia nervosa and bulimia, Int. J. Eat. Disord 2 (1983) 15–34. https://doi.org/10.1002/1098-108X(198321)2:2<15::AID-EAT2260020203>3.0.CO;2-6.

[22] C. Horikawa, Y. Yoshimura, C. Kamada, S. Tanaka, S. Tanaka, A. Takahashi, O. Hanyu, A. Araki, H. Ito, A. Tanaka, Y. Ohashi, Y. Akanuma, N. Yamada, H. Sone, Dietary intake in Japanese patients with type 2 diabetes: analysis from Japan Diabetes Complications Study, J. Diabetes Investig. 5 (2014) 176–187. https://doi.org/10.1111/jdi.12146.

[23] S. Tanaka, S. Tanaka, S. Iimuro, H. Yamashita, S. Katayama, Y. Ohashi, Y. Akanuma, N. Yamada, H. Sone, J.D.C.S. Group, Cohort profile: the Japan diabetes complications study: a long-term follow-up of a randomised lifestyle intervention study of type 2 diabetes, Int. J. Epidemiol. 43 (2014) 1054–1062. https://doi.org/10.1093/ije/dyt057 [doi].

[24] S. Koike, M. Bundo, K. Iwamoto, M. Suga, H. Kuwabara, Y. Ohashi, K. Shinoda, Y. Takano, N. Iwashiro, Y. Satomura, T. Nagai, T. Natsubori, M. Tada, H. Yamasue, K. Kasai, A snapshot of plasma metabolites in first-episode schizophrenia: a capillary electrophoresis time-of-flight mass spectrometry study, Transl. Psychiatry. 4 (2014) 1–8. https://doi.org/10.1038/tp.2014.19.

[25] M. Matsumoto, R. Kibe, T. Ooga, Y. Aiba, E. Sawaki, Y. Koga, Y. Benno, Cerebral Low-Molecular Metabolites Influenced by Intestinal Microbiota: a Pilot Study, Front. Syst. Neurosci. 7 (2013) 9. https://doi.org/10.3389/fnsys.2013.00009.

[26] N. Yoshimi, T. Futamura, K. Kakumoto, A.M. Salehi, C.M. Sellgren, J. Holmén-Larsson, J. Jakobsson, E. Pålsson, M. Landén, K. Hashimoto, Blood metabolomics analysis identifies abnormalities in the citric acid cycle, urea cycle, and amino acid metabolism in bipolar disorder, BBA Clin 5 (2016) 151–158. https://doi.org/10.1016/j.bbacli.2016.03.008.

[27] T. Tsuji, M. Matsumoto, M. Nakamura, T. Miyamoto, M. Yagi, N. Fujita, E. Okada, N. Nagoshi, O. Tsuji, K. Watanabe, Metabolite profiling of plasma in patients with ossification of the posterior longitudinal ligament, J. Orthop. Sci. 23 (2018) 878–883. https://doi.org/10.1016/j.jos.2018.07.001.

[28] K. Kikuchi, Y. Itoh, R. Tateoka, A. Ezawa, K. Murakami, T. Niwa, Metabolomic search for uremic toxins as indicators of the effect of an oral sorbent AST-120 by liquid chromatography/tandem mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 878 (2010) 2997–3002. https://doi.org/10.1016/j.jchromb.2010.09.006.

[29] Y. Itoh, A. Ezawa, K. Kikuchi, Y. Tsuruta, T. Niwa, Protein-bound uremic toxins in hemodialysis patients measured by liquid chromatography/tandem mass spectrometry and their effects on endothelial ROS production, in: anal, Bioanal. Chem. (2012) 1841–1850. Springer, https://doi.org/10.1007/s00216-012-5929-3.

[30] M. Kikuchi, M. Ueno, Y. Itoh, W. Suda, M. Hattori, Uremic toxin-producing gut microbiota In rats with chronic kidney disease, Nephron 135 (2017) 51–60. https://doi.org/10.1159/000450619.

[31] K. Matsuda, H. Tsuji, T. Asahara, Y. Kado, K. Nomoto, Sensitive quantitative detection of commensal bacteria by rRNA-targeted reverse transcription-PCR, Appl. Environ. Microbiol. 73 (2007) 32–39, https://doi.org/AEM.01224-06pii].

[32] K. Matsuda, H. Tsuji, T. Asahara, K. Matsumoto, T. Takada, K. Nomoto, Establishment of an analytical system for the human fecal microbiota, based on reverse transcription-quantitative PCR targeting of multicopy rRNA molecules, Appl. Environ. Microbiol. 75 (2009) 1961–1969. https://doi.org/10.1128/AEM.01843-08.

[33] S. Sakaguchi, M. Saito, H. Tsuji, T. Asahara, O. Takata, J. Fujimura, S. Nagata, K. Nomoto, T. Shimizu, Bacterial rRNA-targeted reverse transcription-PCR used to identify pathogens responsible for fever with neutropenia, J. Clin. Microbiol. 48 (2010) 1624–1628. https://doi.org/10.1128/JCM.01724-09.

[34] L.M. Poisson, H. Suhail, J. Singh, I. Datta, A. Deni, K. Labuzek, N. Hoda, A. Shankar, A. Kumar, M. Cerghet, S. Elias, R.P. Mohney, M. Rodriguez, R. Rattan, A.K. Mangalam, S. Giri, Untargeted plasma metabolomics identifies endogenous metabolite with drug-like properties in chronic animal model of multiple sclerosis, J. Biol. Chem. 290 (2015) 30697–30712. https://doi.org/10.1074/jbc.M115.679068.

[35] J. Chong, O. Soufan, C. Li, I. Caraus, S. Li, G. Bourque, D.S. Wishart, J. Xia, MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis, Nucleic Acids Res. 46 (2018) W486–W494. https://doi.org/10.1093/nar/gky310.

[36] J. Chong, D.S. Wishart, J. Xia, Using metaboanalyst 4.0 for comprehensive and integrative metabolomics data analysis, Curr. Protoc. Bioinforma (2019) 68. https://doi.org/10.1002/cpbi.86.

[37] M.J. Anderson, A new method for non-parametric multivariate analysis of variance, Austral Ecol. 26 (2001) 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.

[38] S. Wandro, S. Osborne, C. Enriquez, C. Bixby, A. Arrieta, K. Whiteson, The microbiome and metabolome of preterm infant stool are personalized and not driven by health outcomes, including necrotizing enterocolitis and late-onset sepsis, MSphere (2018) 3. https://doi.org/10.1128/mSphere.00104-18.

[39] H.R. Lieberman, W.E. Askew, R.W. Hoyt, B. Shukitt-Hale, M.A. Sharp, Effects of 30 days of undernutrition on plasma neurotransmitter precursors, other amino acids, and behavior, J. Nutr. Biochem. 8 (1997) 119–126. https://doi.org/10.1016/S0955-2863(97)00008-9.

[40] P. Felig, O.E. Owen, J. Wahren, G.F. Cahill, Amino acid metabolism during prolonged starvation, J. Clin. Invest. 48 (1969) 584–594. https://doi.org/10.1172/JCI106017.

[41] M. Leyton, S.N. Young, R.O. Pihl, S. Etezadi, C. Lauze, P. Blier, G.B. Baker, C. Benkelfat, Effects on mood of acute phenylalanine/tyrosine depletion in healthy women, Neuropsychopharmacology 22 (2000) 52–63. https://doi.org/10.1016/S0893-133X(99)00086-X.

[42] P. Evenepoel, B.K.I. Meijers, B.R.M. Bammens, K. Verbeke, Uremic toxins originating from colonic microbial metabolism, Kidney Int. 76 (2009) S12–S19. https://doi.org/10.1038/ki.2009.402.

[43] T. Kurakawa, K. Ogata, K. Matsuda, H. Tsuji, H. Kubota, T. Takada, Y. Kado, T. Asahara, T. Takahashi, K. Nomoto, Diversity of intestinal Clostridium coccoides group in the Japanese population, as demonstrated by reverse transcription-quantitative PCR, PLoS ONE 10 (2015), e0126226. https://doi.org/10.1371/journal.pone.0126226 [doi].

[44] Y. Saito, T. Sato, K. Nomoto, H. Tsuji, Identification of phenol- and p-cresol-producing intestinal bacteria by using media supplemented with tyrosine and its metabolites, FEMS Microbiol. Ecol. 94 (2018) fly125, https://doi.org/10.1093/femsec/fiy125.

[45] K. Kikuchi, D. Saigusa, Y. Kanemitsu, Y. Matsumoto, P. Thanai, N. Suzuki, K. Mise, H. Yamaguchi, T. Nakamura, K. Asaji, C. Mukawa, H. Tsukamoto, T. Sato, Y. Oikawa, T. Iwasaki, Y. Oe, T. Tsukimi, N.N. Fukuda, H.J. Ho, F. Nanto-Hara, J. Ogura, R. Saito, S. Nagao, Y. Ohsaki, S. Shimada, T. Suzuki, T. Toyohara, E. Mishima, H. Shima, Y. Akiyama, Y. Akiyama, M. Ichijo, T. Matsuhashi,

A. Matsuo, Y. Ogata, C.C. Yang, C. Suzuki, M.C. Breeggemann, J. Heymann, M. Shimizu, S. Ogawa, N. Takahashi, T. Suzuki, Y. Owada, S. Kure, N. Mano, T. Soga, T. Wada, J.B. Kopp, S. Fukuda, A. Hozawa, M. Yamamoto, S. Ito, J. Wada, Y. Tomioka, T. Abe, Gut microbiome-derived phenyl sulfate contributes to albuminuria in diabetic kidney disease, Nat. Commun. (2019) 10. https://doi.org/10.1038/s41467-019-09735-4.

[46] P.P. De Deyn, R.L. Macdonald, Guanidino compounds that are increased in cerebrospinal fluid and brain of uremic patients inhibit GABA and glycine responses on mouse neurons in cell culture, Ann. Neurol. 28 (1990) 627–633. https://doi.org/10.1002/ana.410280505.

[47] I. Nemet, P.P. Saha, N. Gupta, W. Zhu, K.A. Romano, S.M. Skye, T. Cajka, M. L. Mohan, L. Li, Y. Wu, M. Funabashi, A.E. Ramer-Tait, S.V. Naga Prasad, O. Fiehn, F.E. Rey, W.H.W. Tang, M.A. Fischbach, J.A. DiDonato, S.L. Hazen, A cardiovascular disease-linked gut microbial metabolite acts via adrenergic receptors, Cell 180 (2020) 862–877, e22, https://doi.org/10.1016/j.cell.2020.02.016.

[48] R. Poesen, K. Claes, P. Evenepoel, H. De Loor, P. Augustijns, D. Kuypers, B. Meijers, Microbiota-derived phenylacetylglutamine associates with overall mortality and cardiovascular disease in patients with CKD, J. Am. Soc. Nephrol. 27 (2016) 3479–3487. https://doi.org/10.1681/ASN.2015121302.

[49] S. Furukawa, K. Usuda, M. Abe, S. Hayashi, I. Ogawa, Indole-3-acetic acid induces microencephaly in mouse fetuses, Exp. Toxicol. Pathol. 59 (2007) 43–52. https://doi.org/10.1016/j.etp.2006.12.001.

[50] A. Torremans, B. Marescau, D. Van Dam, C. Van Ginneken, F. Van Meir, P.P. Van Bogaert, R. D'Hooge, J. De Vente, P.P. De Deyn, GSA: behavioral, histological, electrophysiological and neurochemical effects, Physiol. Behav. 84 (2005) 251–264. https://doi.org/10.1016/j.physbeh.2004.12.001.

[51] G.F.M. Russell, The nutritional disorder in anorexia nervosa, J. Psychosom. Res. 11 (1967) 141–149. https://doi.org/10.1016/0022-3999(67)90066-9.

[52] W.H. Kaye, N.C. Barbarich, K. Putnam, K.A. Gendall, J. Fernstrom, M. Fernstrom, C.W. McConaha, A. Kishore, Anxiolytic effects of acute tryptophan depletion in anorexia nervosa, Int. J. Eat. Disord. 33 (2003) 257–267. https://doi.org/10.1002/eat.10135.

[53] W.H. Kaye, H.E. Gwirtsman, D.T. George, D.C. Jimerson, M.H. Ebert, CSF 5-HIAA concentrations in anorexia nervosa: reduced values in underweight subjects normalize after weight gain, Biol. Psychiatry. 23 (1988) 102–105. https://doi.org/10.1016/0006-3223(88)90113-8.