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RESEARCH LETTER

Gut Microbiota and Host Cometabolism Are Altered by Patiromer-Induced Changes in Serum and Stool Potassium

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Hyperkalemia is a common and serious electrolyte abnormality in advanced CKD patients.[1] Adaptive increase in colonic potassium (K^+) excretion in patients with end-stage renal disease (ESRD) partially compensates for the loss of K^+ excretion by the kidney.[2] Patiromer is a nonabsorbed polymer that binds K^+ throughout the gastrointestinal (GI) tract and leads to lowering serum K^+ levels.[3] Interestingly, K^+ is essential for the growth, colonization, and virulence of some bacteria.[4,5] Thus, ambient K^+ could have a significant effect on gut microbiota and their metabolic function. Previously, we have examined the effect to K^+ binder patiromer treatment on serum and stool electrolytes in hemodialysis (HD) patients.[6] To our knowledge, there have been no previous reports regarding the impact of K^+ binder–induced changes in intestinal K^+ content on the gut microbiota and microbiota-related metabolites in HD patients. In this clinical trial, we investigated the effect of lowering serum K^+ by patiromer on the gut microbial community composition, their functional capacity, and host-cometabolism in HD patients.

RESULTS

Clinical Parameters

This is an ancillary to a parent study that examined the effect to patiromer treatment on serum and stool electrolytes in HD patients.[6] Characteristics of the study participants (20 controls, 21 HD patients) are shown in Table 1. Blood urea nitrogen, creatinine, and K^+ were significantly elevated in HD patients. Dialysis prescription was unchanged during the study and dialysate K^+ was 2 mEq/l throughout the study period. The dietary intake estimated by food frequency questionnaire showed that there was no significant change in the dietary intake of K^+, protein, carbohydrate, or fat intake during the study phases (Table S1). Patiromer treatment resulted in a significant decrease in serum K^+, which was accompanied increase in stool K^+ (Figure 1b and 1c, Tables S2 and S3). Circulating level of soluble CD14 (Scd14) was lower in control subjects without kidney disease compared to HD patients. None of the inflammatory markers were altered by patiromer treatment (Table S4). The information about dialysis and medication was summarized in Table S5.

Association of Gut Microbiota With K^+

Alpha diversity estimated by Shannon index was negatively correlated with K^+ in stool and positively correlated with K^+ in serum (Figure 2a). A total of 18 sensitive bacterial species were identified, with 10 and 9 significantly correlated with stool and serum K^+, respectively (Figure 2b). Nine microbial species positively associated with stool K^+, including *Streptococcus gordonii*, *Alistipes senegalensis*, *Alistipes finegoldii*, *Clostridium scindens*, *Ruminococcus obeum*, *Parabacteroides merdae*, and *Lachnospiraceae bacterium* 2-1-58FAA, 3-1-46FAA, and 1-1-57FAA (Figure 2c). *Escherichia coli* was negatively associated with stool K^+ but positively correlated with serum K^+ (Figure 2d). Three additional microbial species were positively

Table 1. Subject characteristics at baseline

Patient variable	Control (n = 20)	ESRD/dialysis (n = 21)	P value
Sex female, n (%)	11 (55)	14 (52)	0.99
Age	60 ± 10	57 ± 11	0.22
Race, n (%)			0.12
Asian	3 (15)	0 (0)	
Black	11 (55)	20 (74)	
Caucasian	5 (25)	4 (15)	
Other	1 (5)	3 (11)	
BMI	31.4 ± 7.2	32.7 ± 6.7	0.53
DM, n (%)	8 (40)	11 (41)	0.99
HIV or Hep, n (%)	0 (0)	6 (22)	**0.03**
Glucose	131 ± 82	123 ± 55	0.68
BUN	12 ± 5	61 ± 16	**<0.0001**
Creatinine	0.8 ± 0.2	10.2 ± 2.0	**<0.0001**
Sodium	142 ± 3	138 ± 3	**0.0013**
Potassium	4.3 ± 0.4	5.6 ± 0.6	**<0.0001**
Chloride	103 ± 3	96 ± 4	**<0.0001**
CO_2	24.8 ± 2.6	20.2 ± 2.9	**<0.0001**
Ca	9.5 ± 0.4	8.9 ± 0.7	**0.004**
SBP	140 ± 20	145 ± 23	0.58
DBP	79 ± 10	77 ± 11	0.72

BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; DM, diabetes mellitus; ESRD, end-stage renal disease; Hep, hepatitis; SBP, systolic blood pressure.
Unless otherwise noted, values are mean ± SD. We compared hemodialysis patients' laboratory parameters at baseline (week 2) with controls. Values in bold indicate statistical significance.

associated with serum K^+, including *Parabacteroides unclassified*, *Pyramidobacter piscolens*, and *Bacteroides vulgatus*. Five microbial species were negatively correlated with serum K^+, including *Bacteroides caccae*, *Streptococcus salivarius*, *Streptococcus parasanguinis*, *Eggerthella unclassified*, and *Granulicatella unclassified* (Figure 2c).

Association of Microbial Pathways With K^+

A total of 132 microbial pathways were significantly correlated with stool K^+, and 75 were correlated with serum K^+ (Figure 3a). Notably, 48 microbial pathways were correlated with K^+ in both serum and stool (Figure 3a). Among 48 microbial pathways, 17 microbial pathways were positively associated with stool K^+ and negatively associated with serum K^+. They belonged to 6 functional categories (Figure 3b and d). The rest 31 microbial pathways were negatively associated with stool K^+ and positively associated with serum K^+, which belonged to 11 functional categories (Figure 3c and e).

Association of Plasma Metabolome With Serum K^+

A total of 66 plasma metabolites were significantly associated with serum K^+, chemical enrichment analysis of which revealed that 5 compound clusters were enriched (Figure 4a). Pathway enrichment analysis showed that 2 pathways were enriched, aminoacyl-Trna biosynthesis and ascorbate and aldarate metabolism (Figure 4b). Among 66 plasma metabolites, 27 were negatively correlated with serum K^+ (Figure 4c). Meanwhile, 39 of 66 plasma metabolites were positively correlated with serum K^+ (Figure 4d).

Association of Stool Metabolome With Stool K^+

A total of 26 stool metabolites were associated with stool K^+; chemical enrichment analysis showed that 1 compound cluster was enriched (Figure 5a and b). One of 26 metabolites, pipecolinic acid, was significantly

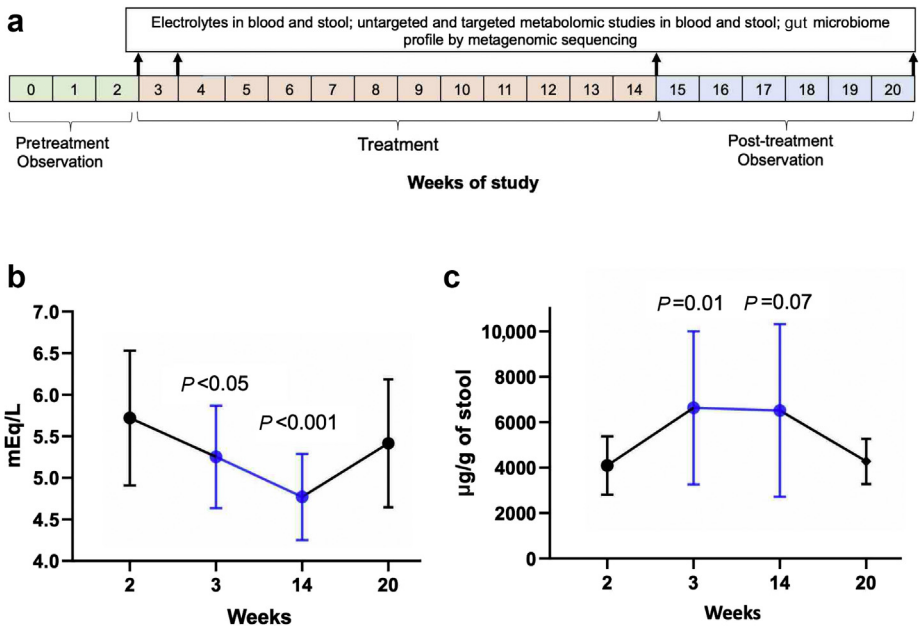

Figure 1. Study design. (a) study design. (b) Serum K^+ in hemodialysis patients. (c) Stool K^+ in hemodialysis patients.

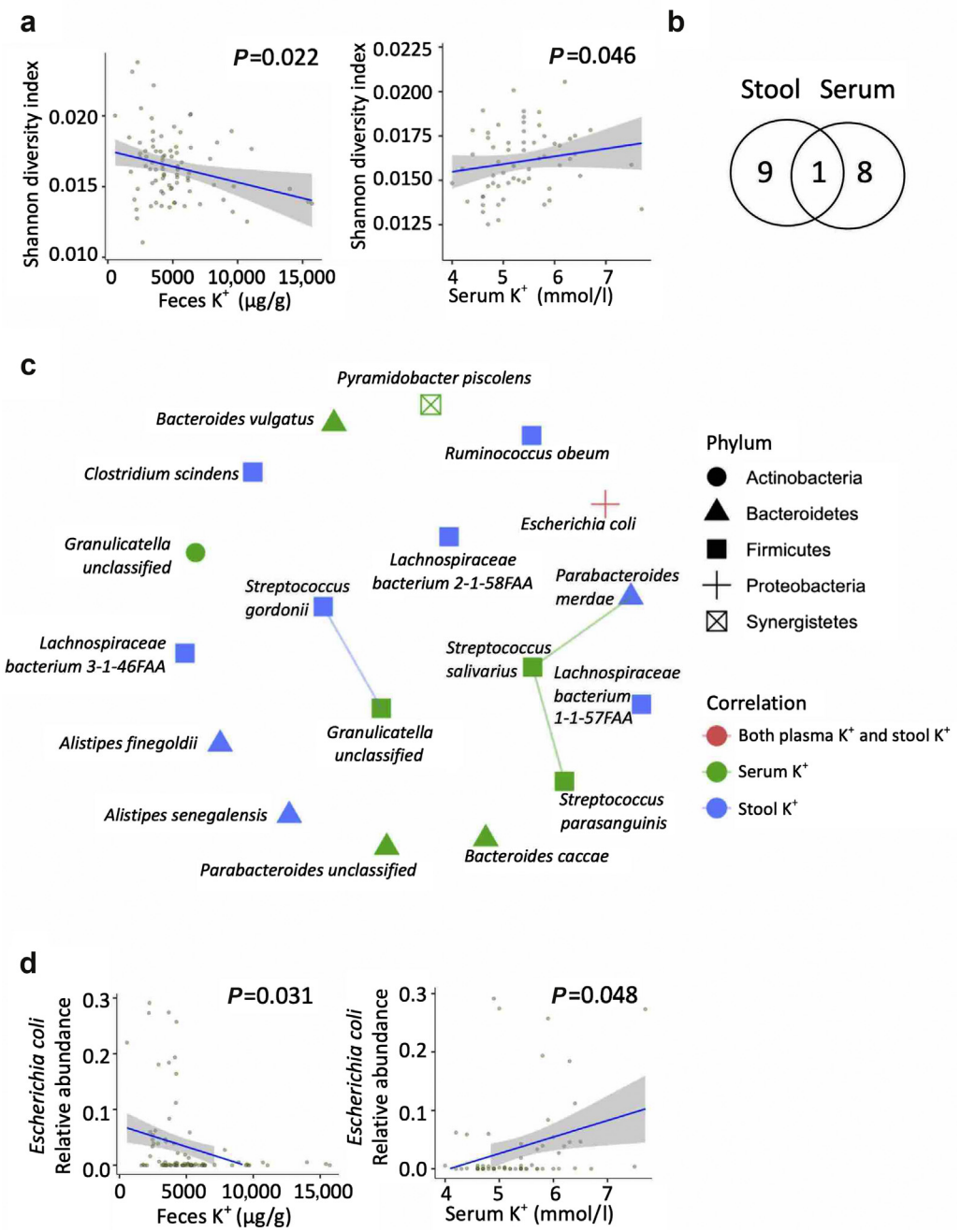

Figure 2. Association of gut microbiota with serum and stool potassium. (a) Correlation between Shannon index and K^+ in stool (left) and serum (right). (b) Venn diagram of microbial species associated with stool and serum K^+. (c) Correlation network of 18 sensitive microbial species. Green: microbial species correlated with serum K^+; blue: microbial species correlated with stool K^+; and red: microbial species correlated with both serum and stool K^+. (d) Correlation between *Escherichia coli* and K^+ in stool (left) and serum (right).

associated with both serum and stool K^+ (Figure 5a). Among 26 stool metabolites, 23 were positively correlated with stool K^+; meanwhile, 3 were negatively correlated with stool K^+ (Figure 5c).

Integrative Analysis

Further, we examined whether any microbial pathway and its related metabolites were correlated with serum and stool K^+. Plasma level of methionine was negatively correlated with serum K^+, as well as microbial pathways such as L-methionine biosynthesis I, L-methionine biosynthesis III, L-homoserine and L-methionine biosynthesis, superpathway of L-methionine biosynthesis transsulfuration, and superpathway of *S*-adenosyl-L-methionine biosynthesis, which were negatively correlated with serum K^+ and positively correlated with stool K^+ (Figure 6a and b). In addition, plasma level of serine was also negatively correlated

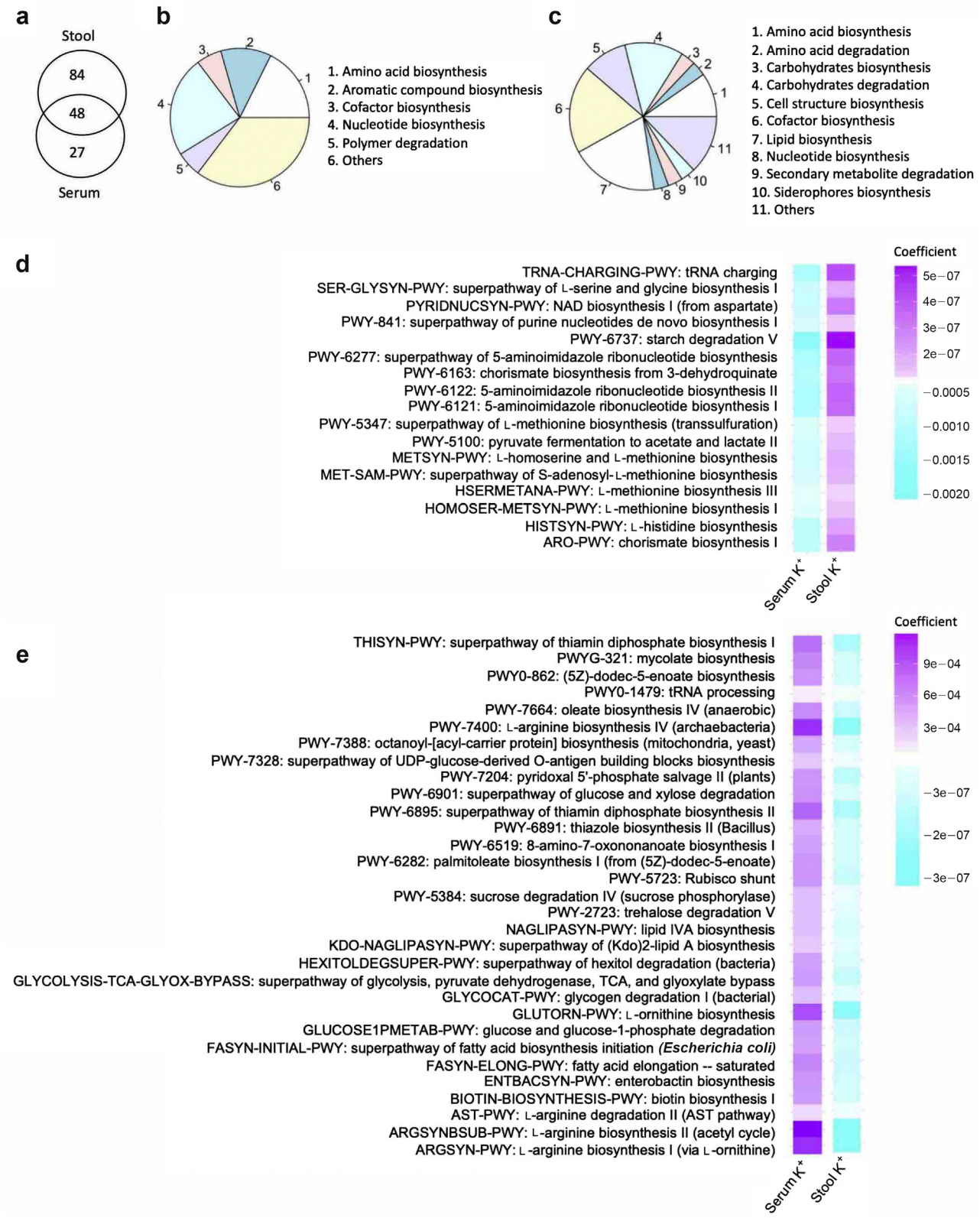

Figure 3. Association of microbial pathways with serum and stool potassium. (a) Venn diagram of microbial pathways associated with stool and serum K^+. (b) Classification of microbial pathway positively associated with K^+ in stool and negatively associated with K^+ in serum. (c) Classification of microbial pathway negatively associated with K^+ in stool and positively associated with K^+ in serum. (d) Microbial pathway positively associated with K^+ in stool and negatively associated with K^+ in serum. (e) Microbial pathway negatively associated with K^+ in stool and positively associated with K^+ in serum.

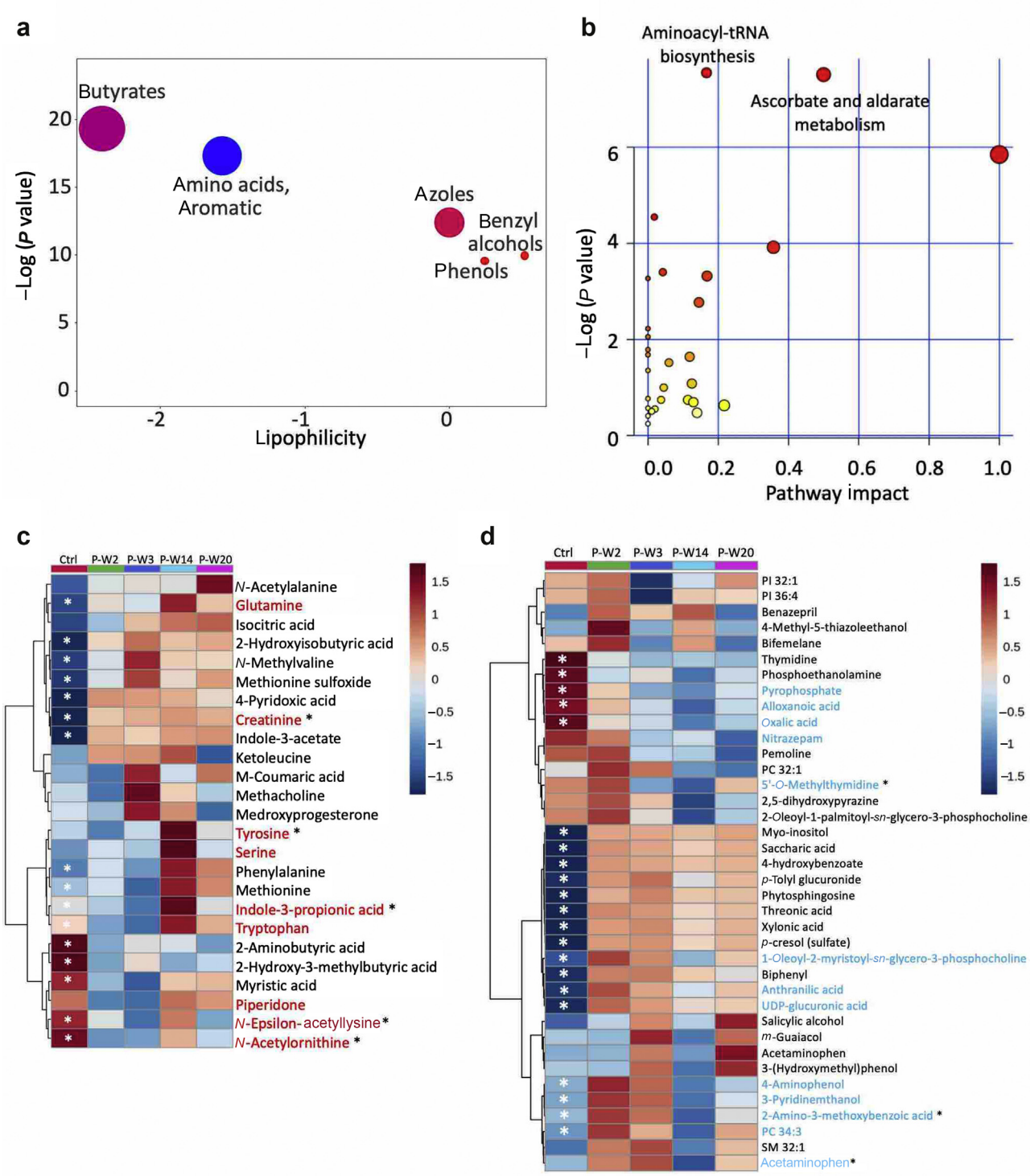

Figure 4. Association of plasma metabolites with serum potassium. (a) Chemical enrichment analysis of plasma metabolites correlated with serum K^+. (b) Pathway enrichment analysis of plasma metabolites correlated with serum K^+. (c) Plasma metabolites negatively correlated with serum K^+. (d) Plasma metabolites positively correlated with serum K^+. * in heatmap: significantly altered between patients at week 2 and control subjects. Text in red: significantly increased metabolites in patients at week 14 compared to week 2. Text in blue: significantly decreased metabolites in patients at week 14 compared to week 2. Text with * on top right: significantly altered metabolites in patients at week 20 compared to week 14.

with serum K^+, as well as the microbial pathway, superpathway of L-serine and glycine biosynthesis I, which was negatively correlated with serum K^+ and positively correlated with stool K^+ (Figure 6c and d).

Comparison Host-Cometabolism in Controls Versus HD Patients

We compared the microbiome and metabolome profile in HD patients to control subjects. Shannon index was

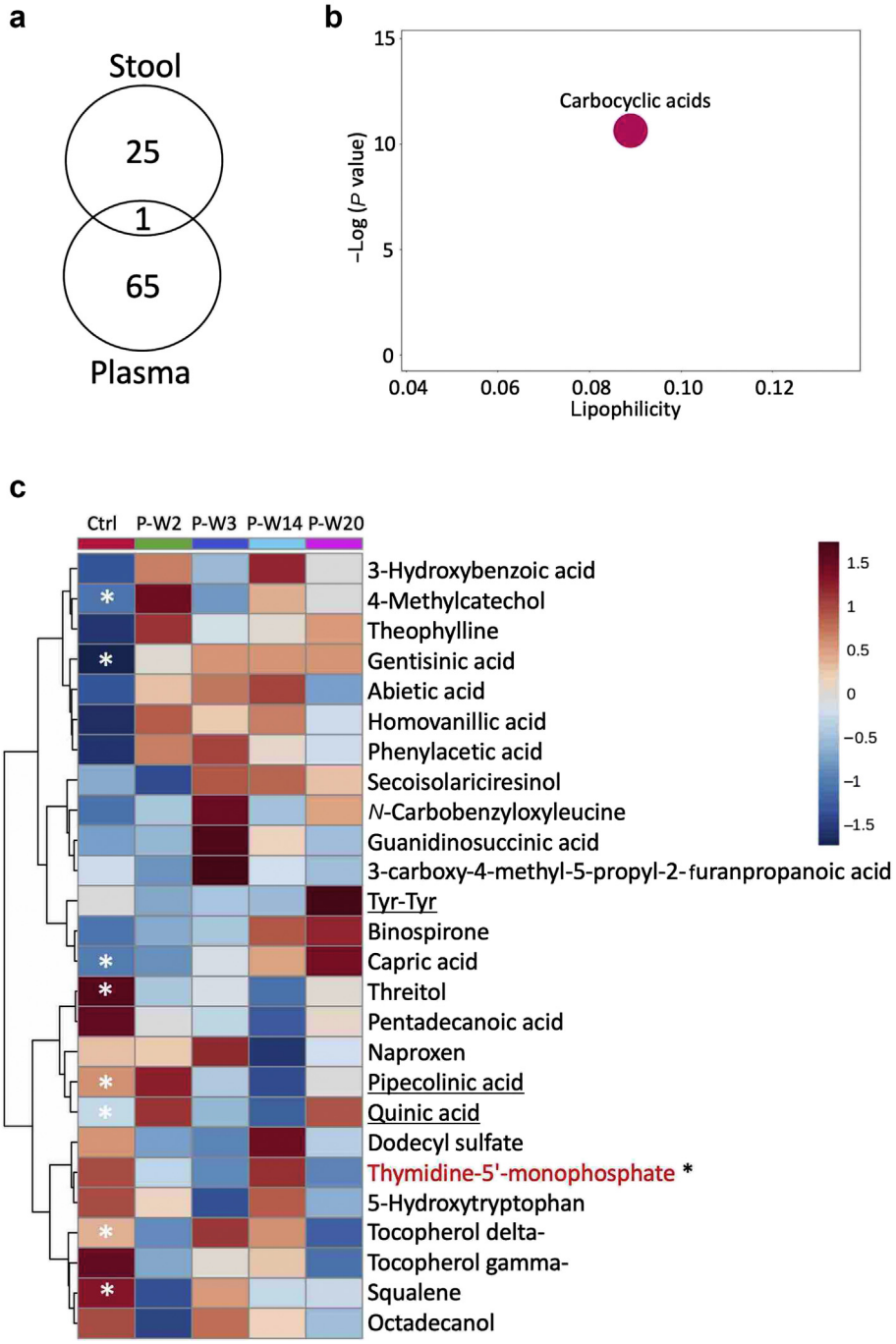

Figure 5. Association of stool metabolites with stool potassium. (a) Venn diagram of metabolites associated with stool and serum K^+. (b) Chemical enrichment analysis of stool metabolites correlated with stool K^+. (c) Stool metabolites correlated with stool K^+. Text with underscore: negative correlation. Text without underscore: positive correlation. * in heatmap: significantly altered between patients at week 2 and control subjects. Text in Red: significantly increased metabolites in patients at week 14 compared to week 2. Text with * on top right: significantly altered metabolites in patients at week 20 compared to week 14.

increased in HD patients compared with controls (Figure S1A). However, the 18 K^+-sensitive microbial species were not significantly different in HD patients at baseline compared with control subjects. Among the 48 K^+-associated microbial pathways identified in HD patients, Trna processing was significantly decreased in HD patients at week 2 compared with controls. Among the 27 plasma metabolites negatively associated with serum K^+ in HD patients, 16 were significantly different between HD patients at week 2 and control subjects (Figure 4c). We noted that 21 of the 39 plasma metabolites positively associated with serum K^+ in HD patients were significantly different control subjects (Figure 4d). Among 26 stool metabolites that were associated with stool K^+ in HD patients, 8 were significantly different between the HD patients at week 2 and control subjects, with half increased and half decreased (Figure 5c).

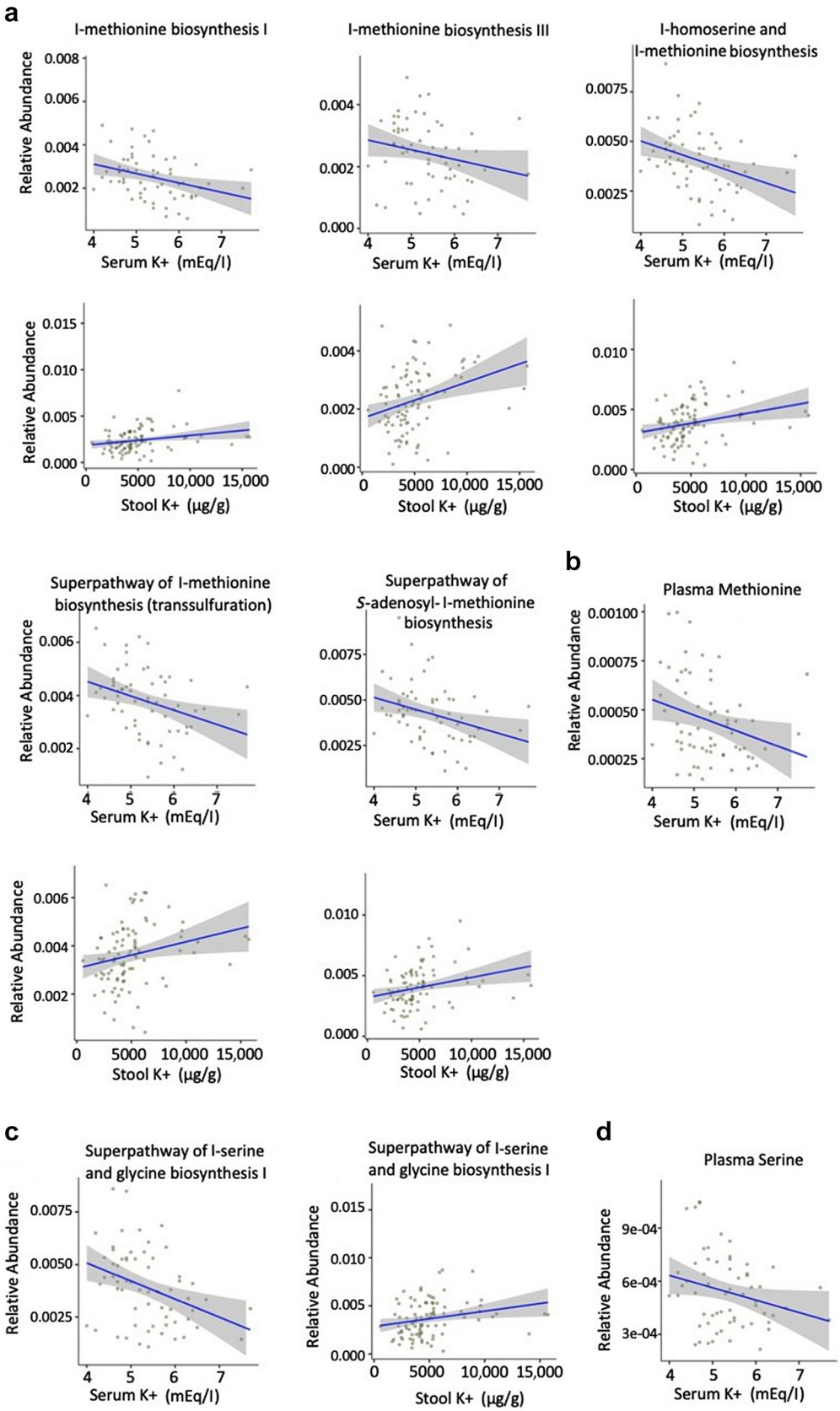

Figure 6. Methionine and serine biosynthesis. (a) Five microbial methionine biosynthesis pathways were negatively associated with serum K^+ and positively associated with stool K^+. (b) Plasma level of methionine was negatively associated with serum K^+. (c) Superpathway of L-serine and glycine biosynthesis was negatively correlated with serum K^+ and positively correlated with stool K^+. (d) Plasma level of serine was negatively correlated with serum K^+.

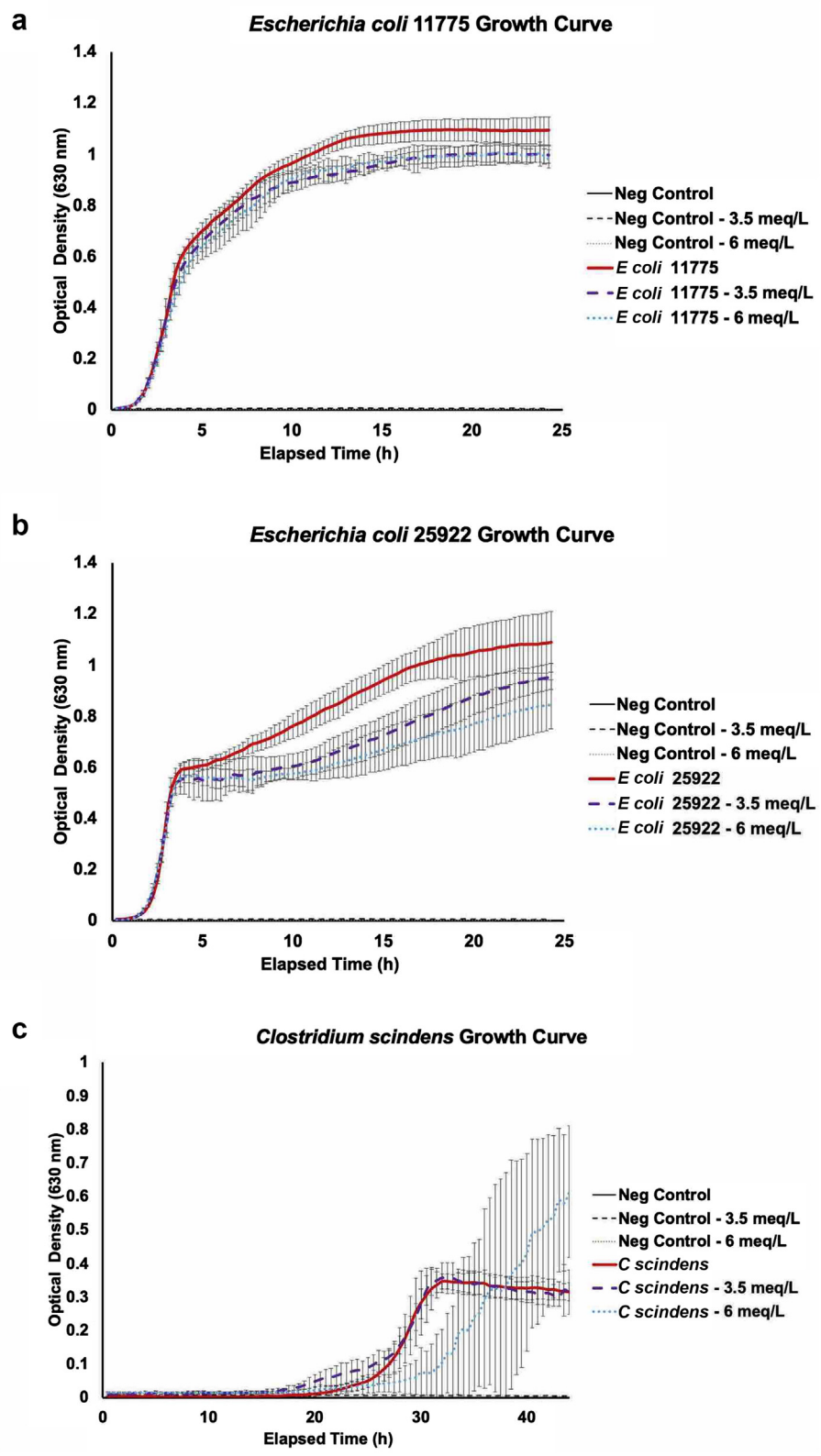

Figure 7. *In vitro* culture. (a) *Escherichia coli* 11775. (b) *E coli* 25922. (c) *Clostridium scindens*.

Impact of K^+ on the Growth of *E coli* and *Clostridium scindens In Vitro*

Maximum density was lower in the culture supplemented with 3.5 and 6.0 mEq/l K^+ compared with control group (0 mEq/l K^+) in 2 *ollie coli* strains, *ollie coli* 11775, and *ollie coli* 25922 (Figure 7a and b). Maximum density of *C scindens* was higher in the culture supplemented with 6 mEq/l K^+ compared with culture supplemented with 3.5 mEq/l K^+ and the control group without K^+ supplementation (Figure 7c).

DISCUSSION

This study has several strengths, including robust study design with rigorous patient selection criteria; controlling for diet, medication use, and dialysis treatment; sequential measurements of K^+ in stool and serum; and integrated microbiome-metabolome analysis. Some of the potential limitations include the control subjects were studied only at 1 time point and the confounding effect of patiromer treatment on the findings could not be totally excluded. There was no controlling for phosphate binder use nor a reporting of either of phosphate binders or the recipient of iron dosing, both of which have the potential to change the gut microbiome. The quality of nutrients can affect gut microbiota variability; however, we have no information on fiber intake and the ratio of animal-vegetal protein intake. Patients using prebiotic or probiotic agents were excluded in this patient cohort. But in real life, this is a rather common occurrence so that the results of this study could not apply to most patients.

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DISCLOSURE

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Dietary pattern during the study period.

Table S2. Serum electrolytes.

Table S3. Stool electrolytes.

Table S4. Cytokine levels.

Table S5. Dialysis and medication information.

Figure S1. Shannon diversity of control subjects and patients at different time points.

Supplementary Methods

STROBE statement.

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