Extended Duration Nocturnal Hemodialysis and Changes in Plasma Metabolite Profiles

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Abstract

Background and objectives In-center, extended duration nocturnal hemodialysis has been associated with variable clinical benefits, but the effect of extended duration hemodialysis on many established uremic solutes and other components of the metabolome is unknown. We determined the magnitude of change in metabolite profiles for patients on extended duration nocturnal hemodialysis.

Design, setting, participants, & measurements In a 52-week prospective, observational study, we followed 33 patients receiving conventional thrice weekly hemodialysis who converted to nocturnal hemodialysis (7–8 hours per session, three times per week). A separate group of 20 patients who remained on conventional hemodialysis (3–4 hours per session, three times per week) served as a control group. For both groups, we applied liquid chromatography-mass spectrometry–based metabolite profiling on stored plasma samples collected from all participants at baseline and after 1 year. We examined longitudinal changes in 164 metabolites among those who remained on conventional hemodialysis and those who converted to nocturnal hemodialysis using Wilcoxon rank sum tests adjusted for multiple comparisons (false discovery rate <0.05).

Results On average, the nocturnal group had 9.6 hours more dialysis per week than the conventional group. Among 164 metabolites, none changed significantly from baseline to study end in the conventional group. Twenty-nine metabolites changed in the nocturnal group, 21 of which increased from baseline to study end (including all branched-chain amino acids). Eight metabolites decreased after conversion to nocturnal dialysis, including L-carnitine and acetylcarnitine. By contrast, several established uremic retention solutes, including *p*-cresol sulfate, indoxyl sulfate, and trimethylamine *N*-oxide, did not change with extended dialysis.

Conclusions Across a wide array of metabolites examined, extended duration hemodialysis was associated with modest changes in the plasma metabolome, with most differences relating to metabolite increases, despite increased dialysis time. Few metabolites showed reduction with more dialysis, and no change in several established uremic toxins was observed.

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Introduction

Patients with ESRD receiving maintenance hemodialysis (HD) experience suboptimal outcomes, with mortality rates up to eight times those of the age-matched general population (1). Although the burden of illness that these patients experience is complex, the inadequate removal of metabolic waste products (or "uremic solutes") is widely viewed as an important limitation of current dialytic approaches (2,3). Enhancing extracorporeal removal of harmful compounds poses an attractive therapeutic strategy as a means to potentially improve outcomes among patients with ESRD. However, large randomized trials targeting increased urea clearance, using higher-flux dialyzers, and increasing the frequency of HD have shown variable results without consistent benefits compared with conventional HD prescriptions (4–7).

Recent studies using liquid chromatography-mass spectrometry (LC-MS) to measure a broad array of uremic solutes have shown important insights into the spectrum of solute removal observed with more intensive HD strategies. First, Meyer *et al.* (8) measured eight uremic solutes in blood samples obtained from 1281 individuals ≥3 months after randomization in the Hemodialysis (HEMO) Study. Compared with individuals achieving a single pool Kt/V_{urea} of approximately 1.3, individuals who achieved a Kt/V_{urea} of approximately 1.7 had only modest reductions of the select uremic solutes, similar to or even less than the observed 9% reduction in urea, such that solute levels remained markedly elevated compared with in individuals with normal kidney function. In a second study, Sirich *et al.* (9) applied LC-MS–based metabolomics to samples from 53 individuals who received standard thrice weekly dialysis and 30 individuals who received six times per week dialysis as part of the Frequent Hemodialysis Network (FHN) Daily Trial. These authors found that increased treatment

frequency resulted only in a mean 15% reduction in uremic solute levels (urea fell by 22%), and they observed no change in putative toxins, such as *p*-cresol sulfate and indoxyl sulfate.

Building on these important observations, the aim of this study was to characterize changes in plasma metabolites, including several established uremic retention solutes, among individuals converting from a conventional (3–4 hours per session) to an extended duration (7–8 hours per session) thrice weekly facility-based HD regimen. Uniquely, our study could better isolate the influence of HD session duration from many of the other relevant HD prescription variables that can influence solute clearance. For example, in the HEMO Study, increased Kt/V_{urea} was achieved in part by increased duration (approximately 1.4 h/wk) but also, by increases in blood flow, dialysate flow, and membrane size. The increase in treatment time was even greater in the FHN Daily Trial, with a net separation of approximately 3.7 h/wk between groups, but the effect of this change is difficult to disentangle from the effect of increased treatment frequency. We thus evaluated the association between conversion to in-center extended duration nocturnal HD (three sessions per week, 7–8 hours per session) and changes in metabolite concentrations. We hypothesized that the substantial increase in total dialysis time conferred by this approach would have a significant effect on the concentration of an array of metabolites implicated in the toxicity of ESRD.

Materials and Methods

Study Population

This was a *post hoc* study designed to test the effect of extended duration nocturnal HD on a wide array of plasma metabolites. The primary study, conducted between July of 2008 and December of 2012, was a 52-week prospective observational study of 37 patients who had previously received standard duration conventional HD (3–4 hours per session, three times per week) for a minimum of 90 days before recruitment and who elected to convert to in-center extended duration nocturnal HD (7–8 hours per session, three times per week; NCT00718848) (10). A separate group of 30 patients who were also eligible for conversion to nocturnal HD but elected to remain on conventional HD were recruited as a nonrandomized control group. From this group, 33 patients who converted to nocturnal HD and 20 patients who remained on conventional HD had the available blood samples and complete datasets across 52 weeks to be included in this study (Table 1). Among the initial 37 patients on nocturnal HD, one died, and two were transplanted; among the initial 30 controls, none died, and two were transplanted. The remaining exclusions were because of incomplete data/sample availability primarily caused by patient withdrawal from the protocol.

Dialysis was performed at two tertiary care hospitals (St. Michael's Hospital, Toronto, Canada and St. Paul's Hospital, Vancouver, Canada). In the absence of clinical practice guidelines to inform indications for extended duration HD, primary reasons for conversion to the nocturnal program included intradialytic hypotension on conventional HD limiting volume removal, labile BP, and preservation of employment opportunities. The patient and the treating nephrologist made the decision to convert jointly. The primary outcome of the parent study was change in left ventricular mass measured by cardiac magnetic resonance imaging (MRI) (10). Exclusion criteria were serious comorbidity with life expectancy <1 year, planned kidney transplant from a live donor in the coming year, contraindications to MRI, and confirmed pregnancy. The research ethics boards of each site approved the study, and all study participants provided written informed consent.

Administration of HD Therapies

Nocturnal HD was administered three times per week for 7–8 hours per session. Dialysis machines (Phoenix; Gambro, Richmond Hill, ON, Canada at St. Michael's Hospital and Dialog$^+$; B. Braun, Bethlehem, PA at St. Paul's Hospital) and dialyzers (Xenium 210 [KoA Urea 1614]; Baxter Healthcare Corp., McGraw Park, IL at St. Michael's Hospital and Rexeed 21S [KoA Urea 1683]; Asahi, Memphis, TN at St. Paul's Hospital) were not changed throughout the study period. Per the routine practice of participating centers, dialysate flow was generally set at 1.5–2 times the blood flow rate (provided in Table 2).

Study Follow-Up and Data Collection

Each patient was followed for 52 weeks, which commenced on the date of the first nocturnal HD session or the date of MRI for those remaining on conventional HD. The 52-week follow-up period was preceded by a 12-week "baseline period," during which all patients were on conventional HD. During this time, comorbidities were characterized, and medications and erythropoietin regimens was recorded. Pretreatment blood was drawn, and plasma was isolated and frozen at −80°C at baseline and study end for research purposes.

Study Outcomes and Metabolite Profiling

The primary outcomes for this study were the change in any of the 164 metabolites measured using an established metabolomic platform at baseline and after 1 year (a complete list of metabolites measured can be found in Supplemental Table 1) (11). We applied two distinct LC-MS–based methods to distinct plasma aliquots for each study subject's baseline and 1-year samples. Amino acids, amino acid derivatives, urea cycle intermediates, nucleotides, and other positively charged polar metabolites were profiled as previously described (12). Briefly, 10 μl of plasma was extracted with 90 μl of 74.9:24.9:0.2 vol/vol per volume acetonitrile/methanol/formic acid containing valine-d8 (Sigma-Aldrich, St. Louis, MO). After centrifugation, supernatants underwent hydrophobic interaction chromatography using a 150×2.1-mm Atlantis hydrophobic interaction chromatography column (Waters, Milford, MA), and mass spectrometry data were acquired on an Exactive Plus Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA) using electrospray ionization in the positive ion mode. Organic acids, sugars, bile acids, and other negatively charged polar metabolites were profiled as previously described (13). Briefly, 30 μl of plasma was extracted with the addition of four volumes of 80:20 vol/vol methanol/water containing isotope-labeled inosine-15N4,

Table 1. Baseline characteristics of the nocturnal and conventional hemodialysis groups

Variable	Nocturnal HD, *n*=33	Conventional HD, *n*=20
Demographics		
Age, yr	58±11	54±13
Men, %	17 (52)	12 (60)
Ethnicity, %		
White	13 (41)	4 (20)
Black	6 (19)	5 (25)
Asian	4 (13)	4 (20)
Other	9 (28)	7 (35)
Cause of ESRD diabetes, %	16 (49)	4 (20)
Dialysis vintage, mo	19 (8, 46)	43 (18, 67)
Body mass index, kg/m^2	28.4±6.1	25.5±5.0
Medical history		
Coronary artery disease, %	10 (30)	2 (10)
Cancer, %	4 (13)	3 (15)
Diabetes mellitus, %	19 (58)	6 (30)
Hemoglobin A1c, %	6 (6, 8)	6 (5, 7)
Peripheral vascular disease, %	5 (15)	3 (15)
Medications		
Erythropoietin use, %	32 (97)	17 (85)
Statin use, %	19 (51)	12 (40)

The table presents subjects' characteristics as mean±SD, median (quartile 1, quartile 3), or *n* (percent). Nocturnal HD, in-center extended duration nocturnal hemodialysis; conventional HD, standard duration conventional hemodialysis.

supernatants underwent chromatography on a 150×2.0-mm Luna NH2 column (Phenomenex, Torrance, CA), and mass spectrometry data were acquired using a 5500 QTRAP triple quadrupole mass spectrometer (AB SCIEX, Foster City, CA) using electrospray ionization and multiple reaction monitoring in the negative ion mode.

Statistical Analyses

To show the relative magnitude of change in metabolites observed, we calculated the final-to-baseline metabolite ratio per subject. These ratios are presented as mean fold change for a given metabolite per treatment arm (Tables 3–5). To assign statistical significance for these changes, we compared metabolite level at baseline versus 1 year (two sided) using Wilcoxon rank sum tests, and *P* values were adjusted for multiple comparisons using the Benjamini–Hochberg method to control false discovery rate (FDR) <5% (Figure 1, Tables 3–5) (14). All *P* values reported for metabolite changes represent the FDR-adjusted *P* value. FDR-adjusted two-tailed *P* values of <0.05 were considered to indicate statistical significance. For clinical variables that were compared from baseline to study end within a treatment group, paired comparisons were used (Table 2). Correlations between percentage metabolite change for all study participants and average treatment time across the study duration (Figure 2) used Spearman rank order correlation. Statistical analyses were conducted with the use of SAS software, version 9.4 (SAS Institute).

Results

Baseline characteristics of study participants are shown in Table 1. Compared with patients who remained on conventional HD, a greater proportion of patients converting to nocturnal HD had diabetes mellitus as their cause of kidney disease. The nocturnal HD group also had a shorter dialysis vintage. Select laboratory and dialytic parameters are shown in Table 2. At baseline, the nocturnal HD recipients had lower serum albumin and a trend for higher phosphate levels. Patients on nocturnal HD also had lower urea reduction ratios at baseline but no difference in predialysis urea concentration or time-averaged concentrations of urea. Conversion to nocturnal HD resulted in a mean±SD increase in dialysis time per treatment from 3.8±0.36 to 7.0±0.5 hours, resulting in a net increase of 9.6±1.6 h/wk (*P*<0.001). The conventional group had no change in dialysis time per treatment from baseline to study end (3.8±0.3 to 3.8±0.6 hours). At the 52-week follow-up, conversion to nocturnal HD resulted in a 17% increase in the urea reduction ratio (*P*<0.001) and a 22% reduction in phosphate concentration (*P*<0.001) compared with baseline. There was no significant change in serum albumin or normalized protein catabolic rate in either the nocturnal or control group.

Table 2. Average change in select clinical variables for the nocturnal and conventional hemodialysis groups

Variable	Nocturnal HD, *n*=33				Conventional HD, *n*=20			
	Baseline	Study End	Change	*P* Value	Baseline	Study End	Change	*P* Value
Dialysis time, h/wk	11.4±1.1	21.1±1.6	9.6±1.6	<0.001	11.4±0.9	11.5±1.8	0.1±1.3	0.80
Blood flow rate, ml/min	333±26[a]	283±22	−51±33	<0.001	354±27[a]	354±30	2±14	0.48
Urea nitrogen, mg/dl	68±16	61±17	−8±18	0.07	68±16	73±19	6±15	0.30
Urea reduction ratio	72±9[a]	84±8	12±21	<0.001	78±5[a]	77±6	−5±30	0.54
TAC urea, mg/dl	43.9±11.5	35.4±10.4	−9.0±12.3	0.17	41.7±10.0	44.7±11.9	3.7±8.6	0.42
Phosphate, mg/dl	5.5±1.8	4.3±1.2	−1.3±1.7	<0.001	5.1±1.0	4.8±1.6	−0.4±1.4	0.39
Albumin, g/dl	3.4±0.4[a]	3.5±0.4	0.1±0.4	0.48	3.6±0.5[a]	3.6±0.4	−0.1±0.4	0.63
nPCR	1.0±0.2	1.1±0.2	0.1±0.2	0.64	1.1±0.2	1.1±0.3	0.1±0.2	0.40
Postdialysis weight, kg	80±20[a]	81±20	1±4	0.95	69±17[a]	68±17	−1±3	0.77

The table presents values as mean±SD. Nocturnal HD, in-center extended duration nocturnal hemodialysis; conventional HD, standard duration conventional hemodialysis; TAC, time-averaged calculated; nPCR, normalized protein catabolic rate.
[a]Denotes *P* value <0.05 for comparison of the baseline variable between nocturnal and conventional groups.

Table 3. Metabolites that significantly decreased after 1 year of extended duration nocturnal hemodialysis

Metabolite	Nocturnal HD Final-to-Baseline Metabolite Ratio ±SD	*P* Value	Conventional HD Final-to-Baseline Metabolite Ratio ±SD	*P* Value
L-carnitine	0.82±0.27	0.003	1.02±0.22	0.99
Acetylcarnitine	0.75±0.30	0.003	1.10±0.31	0.92
α-Glycerophosphate	0.78±0.50	<0.01	1.32±0.82	0.80
Phosphocreatine	0.95±1.73	0.003	2.24±2.68	0.75
Pentose monophosphate	0.81±0.85	0.01	1.40±1.53	0.96
Octanoylcarnitine	0.84±0.43	0.02	1.11±0.53	0.96
Hexanoylcarnitine	0.87±0.53	0.03	1.06±0.53	0.88
Creatinine	0.95±0.17	0.04	0.98±0.22	0.98

Final-to-baseline metabolite ratio denotes the mean final 1-year metabolite level-to-initial baseline metabolite level ratio. *P* values are calculated using the Wilcoxon rank sum test comparing metabolite level at baseline versus 1 year after multiple testing corrections (false discovery rate <0.05). Nocturnal HD, in-center extended duration nocturnal hemodialysis; conventional HD, standard duration conventional hemodialysis.

Among the 164 polar metabolites measured by our platform, none changed significantly from baseline to study end in the control group (Figure 1). By contrast, 29 metabolite levels changed after conversion to extended duration nocturnal HD (Figure 1). Surprisingly, only a minority of these changes represented a decrease in metabolite level after changing to nocturnal HD (Table 3). Predialysis creatinine levels fell significantly, albeit by only 5%, as did seven other metabolites, with a notable cluster of short-chain carnitine derivatives, including L-carnitine (−18%), acetylcarnitine (−25%), octanoylcarnitine (−16%), and hexanoylcarnitine (−13%). A total of 21 metabolites increased significantly from baseline to follow-up in the nocturnal group (Table 4). Of these metabolites, seven were amino acids, including all three branched-chain amino acids valine (+29%), isoleucine (+36%), and leucine (+32%), as well as alanine (+24%), proline (+22%), glutamine (+13%), and histidine (+12%).

Because of specific interest in how increased HD time affects the burden of uremic solutes, we summarized results for the established uremic solutes (http://www.uremic-toxins.org) (15) that were measured by our LC-MS platform as well as long-chain acylcarnitines (Table 5) (11); of note, because plasma proteins are precipitated out of solution before analysis, these measurements reflect total and not free uremic solute levels. None of these metabolites

Table 4. Metabolites that significantly increased after 1 year of extended duration nocturnal hemodialysis

Metabolite	Nocturnal HD Final-to-Baseline Metabolite Ratio ±SD	*P* Value	Conventional HD Final-to-Baseline Metabolite Ratio ±SD	*P* Value
Alanine	1.23±0.26	0.003	1.01±0.37	0.95
S-adenosylmethionine	1.28±0.30	0.003	0.87±0.27	0.73
Valine	1.29±0.40	<0.01	1.01±0.29	0.83
Heptanoylcarnitine	1.16±2.18	<0.01	1.00±0.48	0.88
Phosphocholine	1.37±3.17	<0.01	1.86±2.00	0.88
2-Aminoadipate	1.52±0.80	<0.01	1.17±0.56	0.95
Dimethylglycine	1.19±0.28	0.01	0.90±0.23	0.73
Methylthioadenosine	1.24±0.37	0.02	1.01±0.37	0.99
Isoleucine	1.35±0.50	0.02	1.05±0.36	0.92
Methionine sulfoxide	1.25±0.38	0.02	1.04±0.39	0.92
Fumarate/maleate	1.34±0.55	0.02	1.03±0.40	0.91
Proline	1.22±0.31	0.02	1.03±0.33	0.92
Malate	1.31±0.48	0.03	1.06±0.40	0.93
Arachidoyl carnitine	1.60±1.34	0.03	1.41±1.54	0.88
Leucine	1.32±0.48	0.03	1.02±0.32	0.84
Pyroglutamic acid	1.12±0.23	0.03	1.01±0.23	0.93
β-Alanine	1.54±0.73	0.04	1.16±0.74	0.97
Glutamine	1.13±0.23	0.04	1.01±0.22	0.92
Betaine	1.11±0.20	0.04	0.93±0.18	0.73
Histidine	1.12±0.28	0.04	1.05±0.20	0.80
Ornithine	1.32±0.47	0.04	0.98±0.34	0.79

Final-to-baseline metabolite ratio denotes the mean final 1-year metabolite level-to-initial baseline metabolite level ratio. *P* values are calculated using the Wilcoxon rank sum test comparing metabolite level at baseline versus 1 year after multiple testing corrections (false discovery rate <0.05). Nocturnal HD, in-center extended duration nocturnal hemodialysis; conventional HD, standard duration conventional hemodialysis.

Table 5. Extended duration nocturnal hemodialysis and change in select established uremic retention solutes

Metabolite	Nocturnal HD Final-to-Baseline Metabolite Ratio ±SD	*P* Value	Conventional HD Final-to-Baseline Metabolite Ratio ±SD	*P* Value
p-Cresol sulfate	1.02±0.12	0.35	1.14±0.66	0.84
Indoxyl sulfate	1.10±0.43	0.90	1.16±0.73	0.99
Trimethylamine *N*-oxide	1.07±0.55	0.71	1.13±0.49	0.82
Symmetric dimethylarginine	1.28±0.30	0.23	0.87±0.27	0.99
Asymmetric dimethylarginine	1.08±0.19	0.94	0.96±0.16	0.73
Uridine	0.98±0.41	0.29	1.01±0.48	0.87
Sorbitol	1.45±1.34	0.59	1.56±1.21	0.84
Orotate	1.05±0.36	0.68	0.90±0.38	0.73
Uracil	1.03±0.72	0.23	1.40±1.12	0.87
Cytidine	1.27±1.01	0.99	1.26±0.85	0.84
Kynurenic acid	1.24±0.56	0.19	1.07±0.44	0.92
Hypoxanthine	1.07±0.50	0.90	1.05±0.50	0.93
Thymine	1.02±0.32	0.50	0.98±0.25	0.91
Anthranilic acid	1.11±0.46	0.90	1.21±0.73	0.91
Urate	0.99±0.15	0.45	1.00±0.16	0.99
Hippurate	2.52±6.38	0.46	1.63±1.97	0.92
Xanthine	1.07±0.53	0.07	1.02±0.51	0.73
Kynurenine	1.07±0.34	0.44	0.93±0.31	0.73
Quinolinate	0.99±0.36	0.63	1.02±0.52	0.84
Oxalate	1.17±0.46	0.27	0.93±0.34	0.73
Xanthosine	1.35±0.68	0.07	1.08±0.28	0.73
Oleoylcarnitine	1.04±0.60	0.28	1.10±0.47	0.99
Linoleylcarnitine	1.00±0.67	014	1.00±0.32	0.92
Palmitoylcarnitine	1.01±0.44	0.28	1.01±0.31	0.93
Stearoylcarnitine	1.27±0.85	0.88	0.92±0.38	0.92

Final-to-baseline metabolite ratio denotes the mean final 1-year metabolite level-to-initial baseline metabolite level ratio. *P* values are calculated using the Wilcoxon rank sum test comparing metabolite level at baseline versus 1 year after multiple testing corrections (false discovery rate <0.05). Nocturnal HD, in-center extended duration nocturnal hemodialysis; conventional HD, standard duration conventional hemodialysis.

changed significantly in either the nocturnal or control group. In the nocturnal group, there was no trend for decreased levels of the putative toxins, including *p*-cresol sulfate (+2%), indoxyl sulfate (+11%), trimethylamine-*N*-oxide (+7%), and asymmetric dimethylarginine (+6%).

Figure 2 depicts the percentage change for select metabolites from baseline to follow-up plotted against average dialysis session duration across the entire study for all participants. The relative change in each branched-chain amino acid had a significant correlation with treatment duration (Figure 2, A–C), whereas there was no correlation for the uremic solutes trimethylamine-*N*-oxide, indoxyl sulfate, and *p*-cresol sulfate (Figure 2, D–F). For all metabolites, there was a wide distribution of percentage change across all treatment times. Similar heterogeneity was seen for all other measured metabolites, including other metabolites that changed significantly with nocturnal HD (not shown).

Discussion

This study shows that nearly doubling the standard thrice weekly HD treatment time of 3–4 hours per session to 7–8 hours per session, while maintaining the same frequency of treatment, was only associated with modest changes in the plasma metabolome. We found that only 18% (29 of 164) of measured metabolites changed significantly among individuals who converted to extended duration nocturnal HD, with the majority of these solutes increasing with more HD. None of the metabolites that decreased significantly with nocturnal HD were putative uremic toxins, and no significant changes were observed among any of the more established uremic retention solutes measured by our platform.

Our study builds on the important insights gained from the measurement of uremic solutes in the HEMO Study and the FHN Daily Trial (8,9). Whereas more intensive HD was associated with approximately 1.4- and approximately 3.7-hour increases in weekly dialysis treatment time, respectively, in our study, conversion to nocturnal HD resulted in an average 9.6-hour increase in weekly dialysis time. Although the nocturnal HD prescriptions in our study were not solely directed to maximize solute clearance *per se* (as evidenced by the modest reduction in blood flow rate), the significant increase in HD time had a salutary effect on serum phosphate and urea nitrogen levels. Despite such reductions in phosphate and urea, the increase in HD time had no significant effect on the levels of a wide variety of uremic retention solutes, including putative toxins, such as *p*-cresol sulfate, indoxyl sulfate, trimethylamine-*N*-oxide, and asymmetric dimethylarginine, corroborating the results of the HEMO Study and the FHN Daily Trial analyses. In a fourth study, Camacho *et al.* (16) found that increasing dialyzer size and blood and dialysate flows to maximize the clearance of protein-bound solutes also had minimal effect on *p*-cresol sulfate and indoxyl sulfate levels. Taken together, these studies show the inefficacy of several conventional approaches to HD intensification (*i.e.*,

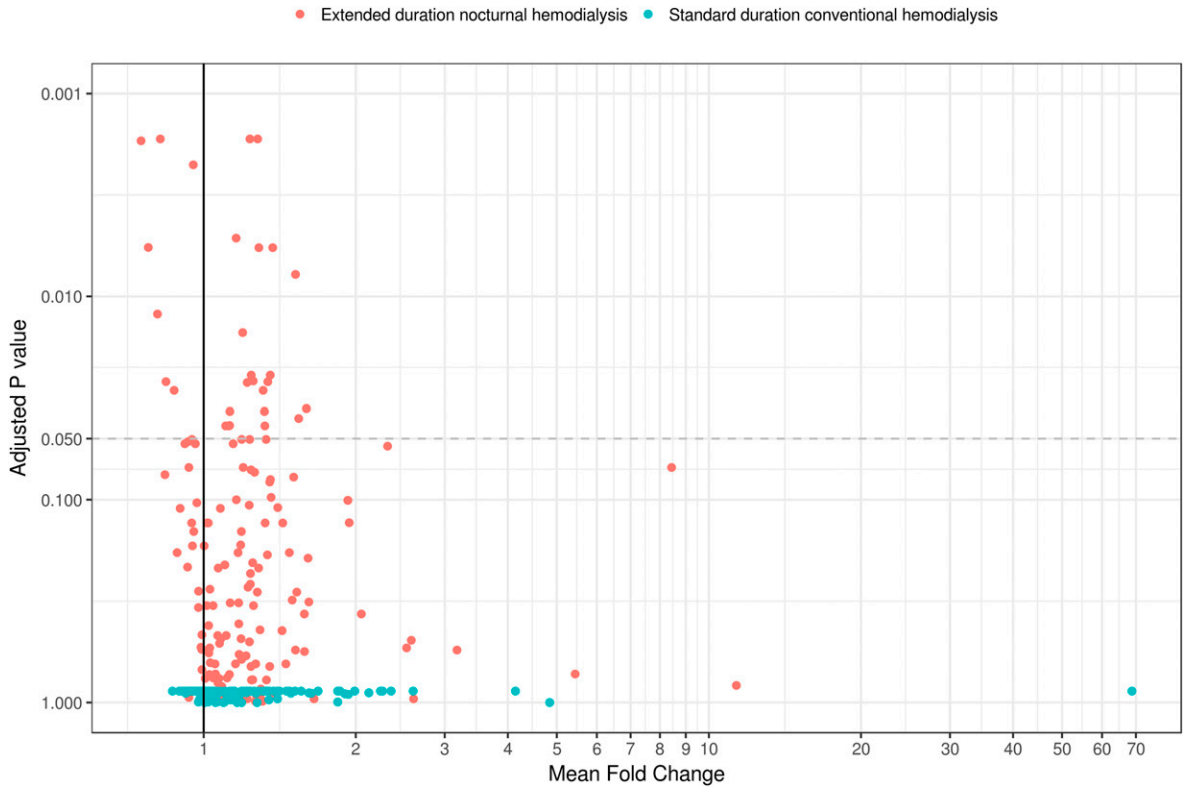

Figure 1. | **Only the extended duration hemodialysis group showed statistically significant changes in metabolites at 1 year.** False discovery rate–adjusted *P* values versus mean fold change for 164 metabolites among those who remained on standard duration conventional hemodialysis (blue dots) and those who converted to in-center extended duration nocturnal hemodialysis (orange dots). False discovery rate–adjusted *P* values were calculated using Wilcoxon rank sum tests comparing metabolite level at baseline versus 1 year after multiple testing corrections (false discovery rate <0.05). The *x* axis represents the mean fold change (1 year per baseline) for each metabolite and is presented on a log10 scale for visualization purposes.

increasing per unit time clearance, increasing time, and increasing treatment frequency) for reducing the burden of select uremic solutes in ESRD.

On the basis of their analyses in the HEMO Study and the FHN Daily Trial, the authors deduce that predominantly nonkidney-based metabolite clearance and/or increased production are likely explanations for the minimal decrease in uremic solute levels, despite increased HD (8,9). In this regard, it is notable that the majority of significant metabolite changes that we observed with extended duration HD were metabolite increases, including several amino acids (the majority of which are highly water soluble and well dialyzed) (17). Indeed, as shown in Figure 2, the increase in branched-chain amino acids (isoleucine, leucine, and valine) was positively correlated to average treatment time across the study duration (whereas change in several uremic toxins showed no significant relationship to treatment time). Because the branched-chain amino acids and histidine are essential amino acids, these increases might reflect increased protein intake on nocturnal HD, resulting in increased free amino acid "production." However, there was no significant change in normalized protein catabolic rate from baseline to follow-up to suggest a corresponding increase in urea production. Alternatively, elevations in branched-chain amino acids can reflect insulin resistance in the general population, but we have previously shown that this association does not persist in ESRD (18); also, nocturnal HD is not known to worsen insulin sensitivity. Whereas increases in amino acids may be a positive nutritional indicator, it is also possible that select metabolite increases could signal harm. In our study, nocturnal HD led to increases in dimethylglycine and betaine levels, both of which have been associated with cardiovascular events in the non-ESRD population (19–21).

Although attempts to increase dialytic clearance are directed toward uremic solutes (*i.e.*, molecules that are significantly elevated in ESRD), they also affect all dialyzable metabolites, regardless of starting concentration. We found that nocturnal HD significantly reduced several nonuremic metabolites, including L-carnitine and several short-chain acylcarnitines. The reduction in L-carnitine (or "free" carnitine) is notable given that L-carnitine is already depleted in patients with ESRD undergoing standard HD. Depletion of L-carnitine, an essential cofactor in fatty acid oxidation, could have deleterious effects on cardiovascular function and could attenuate the other potentially beneficial effects of extended duration nocturnal HD. One prior study has also shown that nocturnal HD reduces L-carnitine levels, but it suggested that this was compensated for by a greater decrease in acylcarnitines, such that the ratio of L-carnitine to acylcarnitines actually seemed more favorable (22). Because our platform does not provide absolute metabolite concentrations, we were unable to gauge the effect of nocturnal HD on this ratio. Moreover,

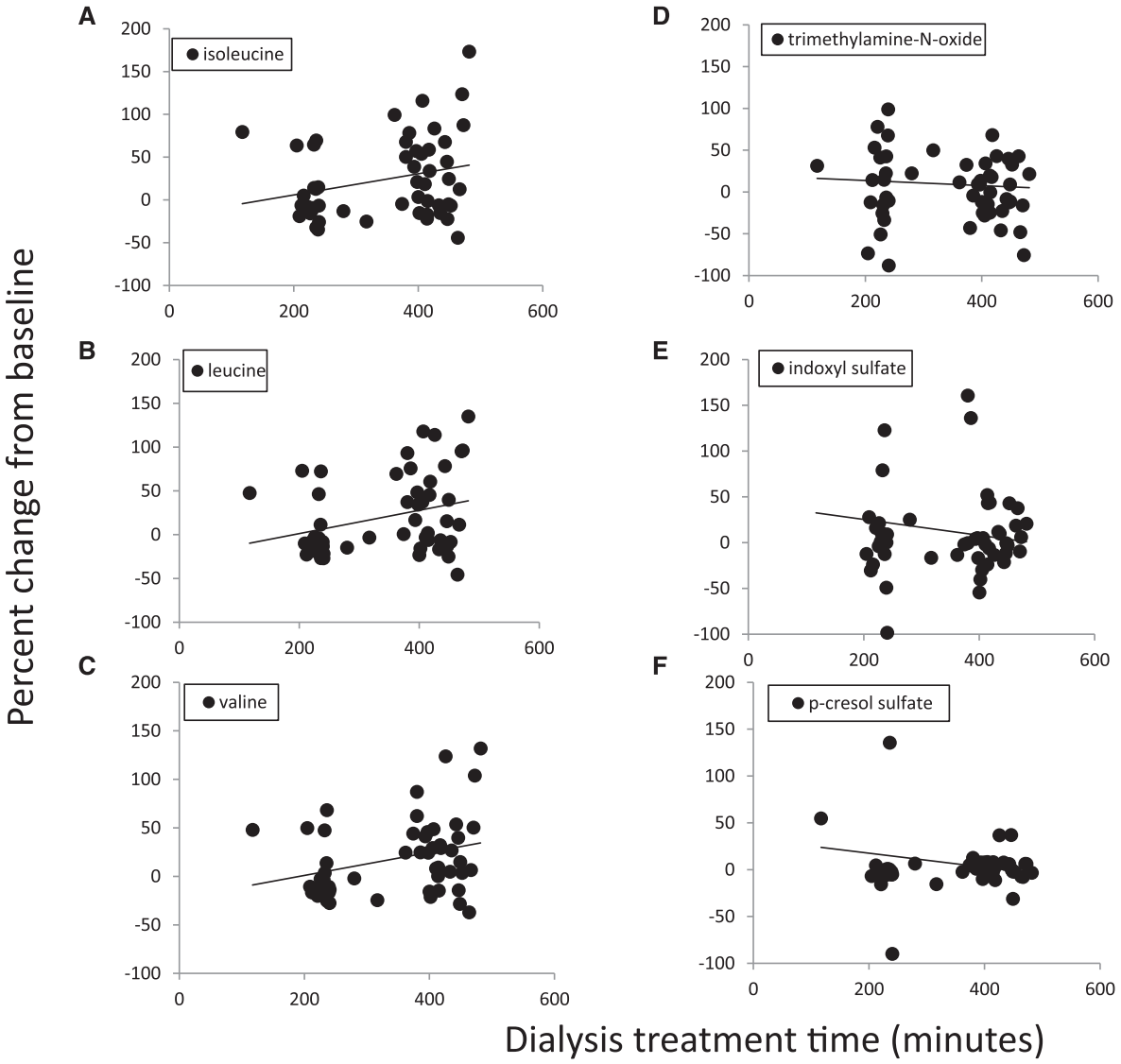

Figure 2. | **Branched chain amino acid levels were positively correlated with dialysis time, while select uremic toxins showed no correlation to treatment time.** Percentage change from baseline of select metabolites in relation to average dialysis session treatment time over 1 year (all study subjects included; n=53). A–C show the three branched-chain amino acids, all with significant positive correlations to treatment time. (A) Isoleucine: Spearman correlation coefficient (r) =0.26; P=0.05. (B) Leucine: r=0.30; P=0.03. (C) Valine: r=0.25; P=0.02. D–F show three select uremic retention solutes, all with no significant correlation to treatment time. (D) Trimethylamine N-oxide: r=−0.01; P=0.99. (E) Indoxyl sulfate: r=−0.18; P=0.18. (F) p-Cresol sulfate: r=0.09; P=0.50.

the importance of carnitine deficiency in ESRD remains unclear, because the benefits of supplementation have yet to be established (23).

Nocturnal HD has been credited with a variety of clinical advantages in observational studies, including regression of left ventricular mass, reduction in hospitalizations, improved BP control, and even reduction in protein carbamylation (10,24,25). These benefits parallel those observed with other forms of intensified HD that have been studied more rigorously (6,26,27). Studies showing an association between nocturnal HD and improved survival have also been reported (28–30), but they must be viewed cautiously given the null results of the majority of the intensive HD randomized trials to date (4,6,27). The results of our study suggest that the benefits of nocturnal HD cannot be attributed to a reduction in the select uremic solute levels measured by our platform. Whether the significant increases and decreases in metabolite levels that we did observe have salutary or deleterious (or both) effects on patient outcomes is unknown as is the effect of changes to the hundreds of metabolites not captured by our platform. More generally, the heterogeneous effect of nocturnal HD on the metabolome reinforces the value of performing unbiased metabolomic screens to generate a more complete understanding of ESRD and its treatment.

Our study has several limitations. Although the noted effect sizes that we observed were significant after adjusting for multiple comparisons, the number of patients included was relatively small, and the subjects were not randomized. As noted, a small number of participants from

the parent study were excluded due to incomplete data, and there were differences in some baseline characteristics among patients who converted to nocturnal HD compared to those who remained on conventional HD. However, our primary analysis was a comparison of metabolite profiles at baseline and 1-year follow-up, examining the nocturnal and conventional groups separately and using each individual as his or her own baseline. Generalizability of our findings may be limited to patients who are willing to voluntarily undergo extended duration HD sessions as well as the practice patterns of the two centers where the study took place. Also, unrecorded changes to medications and diet (or timing of blood draw relative to last meal) as well as differences in residual kidney function may alter solute levels, and we could not account for these variabilities. Finally, our LC-MS–based metabolite measurements are semiquantitative, such that the fold changes in metabolite levels should be interpreted as estimates. Despite such limitations, this study offers important insights into the isolated influence of HD time on uremic retention solutes, independent of multiple other relevant HD prescription variables.

In summary, HD intensification with extended duration nocturnal HD was associated with modest changes in metabolite profiles. Among the metabolites captured by our platform, few showed reduction with nocturnal HD, and change in several putative uremic solutes was not observed. Such findings may account for the variable clinical benefits observed with HD intensification. The etiology and effect of the various metabolite increases and decreases that we observed are certain to be multifactorial and complex. Nevertheless, they outline the challenge in fully comprehending the risks and benefits of different dialysis modifications and reinforce the limitations of using urea clearance as the sole metric for dialysis adequacy.

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Disclosures

None.

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Supplemental materials

Supplemental Table 1: Metabolites measured within the sample set

1-methylhistamine
1-methylnicotinamide
2,3-phosphoglycerate
2-aminoadipate
2-hydroxyglutarate
3-hydroxyanthranilic acid
3-hydroxykynurenate
3-methyladipate; pimelate
4-hydroxybenzaldehyde
4-hydroxymandelate
4-pyridoxate
5-adenosylhomocysteine
5-aminolevulinic acid
acetylcarnitine (2:0 carnitine)
aconitate
adenine
adenosine
adipate
alanine
allantoin
alpha-glycerophosphate
alpha-glycerophosphocholine
alpha-hydroxybutyrate
aminoisobutyric acid
anserine
anthranilic acid
arachidonyl carnitine (20:4 carnitine)
arginine
asparagine
aspartate
asymmetric dimethyl-arginine (ADMA)
beta-alanine
beta-hydroxybutyrate
betaine
bilirubin
butyrobetaine
butyrylcarnitine (4:0 carnitine)
decadienoyl carnitine (C10:2 carnitine)
dodecenoylcarnitine (C12:1 carnitine)
tetradecenoylcarnitine (C14:1 carnitine)
tetradecadienoylcarnitine (C14:2 carnitine)
palmitoylcarnitine (C16 carnitine)
3-Hydroxypalmitoylcarnitine (C16-OH carnitine)
3-hydroxyoleoylcarnitine (C18:1-OH carnitine)
arachidoyl carnitine, eicosanoyl carnitine (C20 carnitine)
malonylcarnitine (C3-DC carnitine)
C3-DC-CH3 carnitine
3-hydroxy-butyryl carnitine (C4-OH carnitine)
tiglyl carnitine (C5:1 carnitine)
glutaryl carnitine (C5-DC carnitine)
carnitine
choline
citrate
citrulline
cotinine
creatine
creatinine
cystathionine
cytidine
deoxycytidine monophosphate (dCMP)
decanoylcarnitine (10:0 carnitine)
dimethylglycine
deoxyuridine monophosphate (dUMP)
fructose/glucose/galactose
fumarate; maleate 1
fumarate; maleate 2
gamma-aminoisobutyric acid
gentisate
glucose
glucuronate
glutamate
glutamine
glycine
glycocholate
glycodeoxycholate; glycochenodeoxycholate
guanosine
heptanoylcarnitine (7:0 carnitine)
hexacosanoylcarnitine (26:0 carnitine)
hexanoylcarnitine (6:0 carnitine)
hexose monophosphate
hippurate
histamine
histidine
homocysteine
homogentisate
hydroxyphenylacetate
hydroxyproline
hypoxanthine
indole-3-propionate
indolelactate
indoxyl sulfate
inosine
inositol
isocitrate
isoleucine
kynurenic acid
kynurenine
lactate
lactose
lauroylcarnitine (12:0 carnitine)
leucine
linoleylcarnitine (18:2 carnitine)
lithocholate
lysine
malate
methionine
methionine sulfoxide
methylmalonate
methylthioadenosine
myristoylcarnitine (14:0 carnitine)
N-carbamoyl-beta-alanine
niacinamide
NMMA
nonanoylcarnitine (9:0 carnitine)
octanoylcarnitine (8:0 carnitine)
oleoylcarnitine (18:1 carnitine)
ornithine
orotate
oxalate
pantothenate
p-cresol sulfate
pentose monophosphate
phenylalanine
phosphocholine
phosphocreatine
phosphoethanolamine
pipecolic acid
proline
propionate
propionylcarnitine (3:0 carnitine)
putrescine
pyroglutamic acid
quinolinate
s-adenosylmethionine
salicylurate
sarcosine
serine
serotonin
sorbitol
spermidine
stearoylcarnitine (18:0 carnitine)
suberate
succinate
sucrose
symmetric dimethyl-arginine (SDMA)
taurine
taurodeoxycholate; taurochenodeoxycholate
thiamine
threonine
thymine
thyroxine
triiodothyronine
trimethylamine-N-oxide
tryptophan
tyrosine
uracil
urate
uridine
valerylcarnitine (5:0 carnitine)
valine
vanillylmandelic acid (VMA)
xanthine
xanthosine
xanthurena