Limited reduction in uremic solute concentrations with increased dialysis frequency and time in the Frequent Hemodialysis Network Daily Trial

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The Frequent Hemodialysis Network Daily Trial compared conventional three-times weekly treatment to more frequent treatment with a longer weekly treatment time in patients receiving in-center hemodialysis. Evaluation at one year showed favorable effects of more intensive treatment on left ventricular mass, blood pressure, and phosphate control, but modest or no effects on physical or cognitive performance. The current study compared plasma concentrations of uremic solutes in stored samples from 53 trial patients who received three-times weekly in-center hemodialysis for an average weekly time of 10.9 hours and 30 trial patients who received six-times weekly in-center hemodialysis for an average of 14.6 hours. Metabolomic analysis revealed that increased treatment frequency and time resulted in an average reduction of only 15 percent in the levels of 107 uremic solutes. Quantitative assays confirmed that increased treatment did not significantly reduce levels of the putative uremic toxins p-cresol sulfate or indoxyl sulfate. Kinetic modeling suggested that our ability to lower solute concentrations by increasing hemodialysis frequency and duration may be limited by the presence of non-dialytic solute clearances and/or changes in solute production. Thus, failure to achieve larger reductions in uremic solute concentrations may account, in part, for the limited benefits observed with increasing frequency and weekly treatment time in Frequent Hemodialysis Daily Trial participants.

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Conventional 3-times weekly hemodialysis generally sustains life but does not restore health. The Frequent Hemodialysis Network (FHN) Daily Trial tested whether an increase in the frequency of treatment, combined with an increase in total weekly treatment time, would improve an array of objective and patient-reported outcomes. Results showed that frequent treatment reduced left ventricular mass and improved self-reported physical function.[1] Frequent treatment also lowered average predialysis systolic blood pressure and serum phosphate concentrations.[1,2] Frequent treatment did not, however, significantly improve mood, nutritional status, body composition, or objectively measured physical and cognitive performance.[1,3,4]

The reduction in LV mass achieved by frequent treatment in the FHN can reasonably be ascribed to better control of extracellular fluid volume and blood pressure, and improved control of hyperphosphatemia can be explained by the increase in frequency and treatment time.[5] Accounting for the failure of frequent hemodialysis to provide benefits in other objective and subjective measures of physical and mental health has been more difficult. While the residual illness suffered by dialysis patients is complex, we presume that inadequate removal of waste solutes contributes importantly. An important goal of increasing hemodialysis frequency in the FHN was to reduce the plasma concentrations of uremic solutes. The combination of increased frequency and weekly treatment time employed in the FHN Daily Trial reduced pretreatment blood urea nitrogen by approximately 20%.[1] The current study was performed to assess the effects of increasing frequency and dialysis weekly time on concentrations of solutes other than urea. We employed a standard metabolomic platform to assess levels of a large number of uremic solutes and quantitative mass spectrometry with isotopic dilution to measure concentrations of selected solutes.

RESULTS

Demographic and dialytic parameters for FHN Daily Trial subjects who remained on 3-times weekly hemodialysis or were receiving 6-times weekly treatment at the end of 12 months are summarized in Table 1. The size of the 6-times weekly treatment group was limited by the exclusion of

Table 1 | Demographic and dialysis parameters

	3 Rx to 3 Rx n = 53		3 Rx to 6 Rx n = 30	
Age (yr)	51 ± 13		48 ± 12	
Vintage (yr)	4.2 ± 4.4		6.4 ± 4.5	
Female (%)	38		27	
Diabetic (%)	42		43	
	Baseline	**12 months**	**Baseline**	**12 months**
Hours per session	3.6 ± 0.4	3.6 ± 0.4	3.7 ± 0.5	2.4 ± 0.3
Hours per week	10.9 ± 1.3	10.9 ± 1.3	11.0 ± 1.4	14.6 ± 1.7
Blood flow (ml/min)	407 ± 46	410 ± 53	405 ± 58	403 ± 40
Dialysate flow (ml/min)	728 ± 100	725 ± 97	724 ± 103	722 ± 107
stdKt/V	2.67 ± 0.44	2.60 ± 0.39	2.52 ± 0.30	4.01 ± 0.42
URR (%)	73 ± 5	73 ± 6	72 ± 5	62 ± 5*†
nPCR (g/kg/d)	1.11 ± 0.37	1.06 ± 0.26	1.12 ± 0.28	1.33 ± 0.42*†
Baseline residual function (% of subjects)	45%	–	27%	–
Kru in subjects with baseline residual function (ml/min)	1.1 ± 0.7	0.7 ± 0.7†	1.2 ± 0.6	0.6 ± 1.0
Repository day of cycle	15/33/3	18/34/1	10/14/6	9/2/9/9/1/0

3 Rx to 3 Rx, maintained on 3-times weekly treatment; 3 Rx to 6 Rx, maintained on 6-times weekly treatment at the end of the 12-month intervention period; Kru, residual urea clearance; nPCR, normalized protein catabolic rate; stdKt/V, standard Kt/V_{urea}; URR, urea reduction ratio.
Repository day of cycle gives the number of repository samples collected at the 1st, 2nd, 3rd, etc. treatment of the week. Values at baseline were not significantly different for the 2 groups. Treatment hours and stdKt/V in the 2 groups at 12 months differed by design.
*$P < 0.05$ between groups at 12 months.
†$P < 0.05$ 12 months to baseline within a group. Values are mean ± SD.

subjects randomized to frequent treatment who received 4 or 5 treatments per week rather than 6. Baseline characteristics of subjects in the 2 groups were similar. Weekly treatment times and weekly standard Kt/V (stdKt/V) values for subjects receiving 6-times weekly hemodialysis were slightly higher than those targeted in the original study design.[6] The higher stdKt/V in the 6-times weekly group was achieved almost entirely by increases in treatment time and frequency because blood and dialysate flow rates were similar and the dialyzers used were not notably different.

Predialysis plasma solute concentrations measured using quantitative assays are summarized in Table 2. We first assessed the effect of increasing time and frequency by comparing average solute concentrations at the end of the 12-month intervention period in the 2 groups. We also compared the change in solute concentrations from baseline before randomization to 12 months, as further described below. As expected, results were overall similar, with slight differences ascribable to imperfect balance of solute concentrations at baseline.

As compared with subjects maintained on 3-times weekly treatment, subjects maintained on 6-times weekly treatment had 18% lower predialysis blood urea nitrogen concentrations at the end of 12 months. Modeling predicted that if urea production were equal, measured plasma urea concentrations would have been approximately 30% lower in subjects dialyzed 6 times weekly than in those dialyzed 3 times weekly, as shown in Supplementary Table S1. As shown in Table 1, however, the average normalized protein catabolic rate increased significantly from baseline to 12 months in the 6-times weekly group and was greater at 12 months in the 6-times weekly group than in the 3-times weekly group. Increased urea production could thus account for the limited reduction in plasma urea concentrations observed in the 6-times weekly group. A consideration in comparing urea concentrations in the 2 treatment groups is the day of the cycle on which samples were collected. Disproportionate collection of repository samples early in the week tended to limit the observed reduction in pretreatment urea concentrations in subjects receiving 6-times weekly treatment, as further shown in Supplementary Table S1.

Table 2 also summarizes the pretreatment plasma concentrations measured by quantitative assays of 4 organic acids that accumulate to high concentrations in patients receiving hemodialysis.[7] As compared with the 3-times weekly group, 12-month concentrations in the 6-times weekly group averaged 4% higher for p-cresol sulfate (PCS), 13% lower for indoxyl sulfate (IS), 17% lower for hippurate (HIPP), and 25% lower for phenylacetylglutamine. Comparing the change in solute concentrations from baseline to 12 months yielded similar results, except that the reduction in HIPP concentrations was larger when higher baseline concentrations in the 6-times weekly groups were taken into account. As was the case for urea, the observed differences in solute concentrations achieved with a combination of increased frequency and treatment time were less than would be predicted presuming equal solute production and no nondialytic solute clearance (Supplementary Table S1).

Metabolomic analysis compared pretreatment concentrations of 107 solutes previously identified as uremic.[8] Metabolomic analysis does not provide absolute concentration values, so concentrations for these solutes are expressed as a fraction of the average mass spectrometric peak areas in baseline samples from all subjects. The average effect of increased frequency and treatment time was remarkably modest. At 12 months, solute concentrations were lowered by an average of only 15% in FHN subjects maintained on 6-times weekly hemodialysis compared with those maintained on 3-times weekly hemodialysis. The difference was identified as significant for 32 solutes, as summarized in Table 3. Assessment of the effect of increased time and frequency based on comparison of solute concentrations at 12 months to baseline yielded generally similar results. Results for the entire set of 107 uremic solutes are summarized in Supplementary Table S2.

For the 5 solutes assessed by both means, metabolomic results were on average similar to those obtained by quantitative assays, as summarized in Supplementary Table S3. There were, however, notable differences in values obtained in individual subjects, as shown in Supplementary Figure S1. When expressed as a fraction of the average normalized value obtained using the quantitative and metabolomic assays, the

Table 2 | Solutes assessed by quantitative assays

	3 Rx to 3 Rx n = 53		3 Rx to 6 Rx n = 30		12 months 3 Rx to 6 Rx versus 3 Rx to 3 Rx[b]		Baseline[a] to 12 months 3 Rx to 6 Rx versus 3 Rx to 3 Rx[c]	
	Baseline mg/dl	12 months mg/dl	Baseline mg/dl	12 months mg/dl	% difference	*P* value[d]	% difference	*P* value[d]
Urea nitrogen	52 ± 12	54 ± 14	58 ± 15	45 ± 16	−18 (−31, −4)	0.01	−28 (−15, −67)	0.001
p-cresol sulfate	3.4 ± 1.5	3.2 ± 1.4	3.3 ± 1.7	3.3 ± 1.6	4 (−18, 26)	0.69	16 (−13, 35)	0.27
Indoxyl sulfate	2.6 ± 0.9	2.9 ± 1.1	2.7 ± 1.3	2.5 ± 1.0	−13 (−30, 4)	0.13	−10 (−41, 12)	0.36
Hippurate	5.1 ± 4.2	5.7 ± 4.0	6.5 ± 5.4	4.8 ± 3.3	−17 (−45,12)	0.25	−52 (−227, −31)	0.002
Phenylacetylglutamine	4.2 ± 2.1	4.4 ± 2.3	4.4 ± 2.1	3.3 ± 1.6	−25 (−44, −6)	0.01	−29 (−79, −12)	0.004

3 Rx to 3 Rx, maintained on 3-times weekly treatment; 3 Rx to 6 Rx, maintained on 6-times weekly treatment at the end of the 12-month intervention period.
[a]Concentration before randomization.
[b]Percentage differences in average solute concentrations at 12 months.
[c]Differences in the change in solute concentrations from baseline to 12 months for the 2 groups calculated as described in Materials and Methods.
[d]*P* values are not corrected for multiple comparisons. Values are mean ± SD or mean (95% confidence intervals).

Table 3 | Solutes assessed by metabolomic analysis for which 6-times weekly treatment significantly reduced levels

	3 Rx to 3 Rx n = 53		3 Rx to 6 Rx n = 30		12 months 3 Rx to 6 Rx versus 3 Rx to 3 Rx[b]	Baseline[a] to 12 months 3 Rx to 6 Rx versus 3 Rx to 3 Rx[c]
	Baseline	12 months	Baseline	12 months	% difference	% difference
Levoinositol	1.02 ± 1.29	1.71 ± 2.09	0.96 ± 1.30	0.72 ± 1.18	−58 (−100, −16)	−58 (−320, −36)
Tartaric acid	0.74 ± 0.98	1.36 ± 1.64	1.45 ± 2.75	0.62 ± 1.03	−54 (−97, −12)	−68 (−566, −46)
N2,N5-diacetylornithine	1.11 ± 0.77	1.06 ± 0.96	0.80 ± 0.48	0.53 ± 0.46	−50 (−82, −18)	−24 (−149, −31)
Scyllitol	0.91 ± 0.57	1.33 ± 1.51	1.16 ± 0.90	0.72 ± 0.59	−46 (−81, −11)	−50 (−183, −39)
Proline betaine	0.98 ± 0.89	1.36 ± 1.16	1.03 ± 0.97	0.78 ± 0.95	−43 (−77, −8)	−50 (−233, −23)
Gamma-CEHC glucuronide[d]	1.01 ± 0.53	1.14 ± 0.82	0.98 ± 0.74	0.68 ± 0.48	−40 (−65, −15)	−29 (−94, −3)
Phenol sulfate	0.92 ± 0.45	1.21 ± 0.70	1.14 ± 0.43	0.76 ± 0.30	−37 (−55, −19)	−45 (−119, −52)
Erythritol	0.97 ± 0.22	1.20 ± 1.25	1.04 ± 0.29	0.76 ± 0.29	−37 (−67, −7)	−34 (−80, −29)
D-Threitol	0.97 ± 0.38	1.07 ± 0.46	1.06 ± 0.43	0.69 ± 0.24	−36 (−50, −22)	−41 (−110, −37)
L-Gulonolactone	0.96 ± 0.38	1.02 ± 0.56	1.07 ± 0.40	0.68 ± 0.29	−33 (−51, −15)	−38 (−105, −27)
L-Xylonate	0.97 ± 0.34	1.01 ± 0.39	1.06 ± 0.35	0.68 ± 0.22	−32 (−45, −19)	−38 (−93, −33)
Galactitol	0.96 ± 0.59	1.09 ± 0.70	1.06 ± 0.48	0.75 ± 0.51	−32 (−56, −7)	−39 (−116, −24)
D-Xylose	1.01 ± 0.73	0.97 ± 0.75	0.99 ± 0.48	0.67 ± 0.28	−31 (−54, −7)	−28 (−73, −13)
Erythronic acid	0.97 ± 0.27	1.01 ± 0.36	1.06 ± 0.29	0.71 ± 0.25	−30 (−44, −17)	−37 (−84, −36)
1-Methylhistidine	0.96 ± 0.63	0.93 ± 0.47	1.07 ± 0.56	0.67 ± 0.32	−28 (−47, −10)	−40 (−121, −25)
3-Methylglutarylcarnitine	0.98 ± 0.46	0.96 ± 0.57	1.04 ± 0.43	0.70 ± 0.33	−28 (−48, −7)	−32 (−86, −17)
C-mannosyltryptophan	0.99 ± 0.27	0.98 ± 0.27	1.02 ± 0.22	0.71 ± 0.18	−28 (−38, −18)	−31 (−65, −26)
N-Acetylneuraminic acid	0.97 ± 0.26	1.00 ± 0.34	1.05 ± 0.33	0.73 ± 0.28	−27 (−41, −13)	−31 (−75, −19)
Glutarylcarnitine	0.99 ± 0.38	1.02 ± 0.56	1.01 ± 0.38	0.75 ± 0.38	−27 (−47, −7)	−29 (−65, −20)
N6-Carbamoyl-L-threonyladenosine	0.99 ± 0.22	1.01 ± 0.32	1.01 ± 0.20	0.74 ± 0.21	−26 (−38, −15)	−27 (−56, −22)
Quinolinic acid	0.95 ± 0.35	0.92 ± 0.33	1.09 ± 0.73	0.68 ± 0.41	−26 (−45, −7)	−35 (−100, −18)
Myoinositol	0.92 ± 0.24	1.00 ± 0.32	1.14 ± 0.42	0.76 ± 0.38	−24 (−41, −8)	−39 (−103, −35)
6-Sialyl-N-acetyllactosamine	1.00 ± 0.26	1.02 ± 0.30	1.00 ± 0.26	0.79 ± 0.25	−23 (−35, −11)	−23 (−50, −13)
L-Arabitol	0.97 ± 0.24	1.00 ± 0.29	1.05 ± 0.27	0.78 ± 0.22	−22 (−33, −11)	−28 (−63, −19)
4-Acetamidobutanoic acid	0.98 ± 0.26	0.98 ± 0.30	1.03 ± 0.29	0.77 ± 0.19	−22 (−33, −11)	−25 (−51, −19)
N-Acetylserine	0.96 ± 0.23	0.99 ± 0.29	1.08 ± 0.39	0.77 ± 0.33	−22 (−36, −7)	−32 (−72, −26)
Pseudouridine	0.99 ± 0.14	1.00 ± 0.17	1.01 ± 0.15	0.80 ± 0.12	−20 (−27, −14)	−21 (−39, −17)
Fumaric acid	1.02 ± 0.32	1.02 ± 0.26	0.97 ± 0.22	0.81 ± 0.19	−20 (−30, −10)	−17 (−36, −6)
Gluconic acid	0.93 ± 0.31	0.94 ± 0.35	1.13 ± 0.55	0.75 ± 0.27	−19 (−34, −5)	−30 (−74, −17)
2-Methoxyphenol sulfate	0.97 ± 0.31	0.92 ± 0.30	1.05 ± 0.41	0.75 ± 0.34	−19 (−35, −3)	−27 (−61, −17)
Urea	0.96 ± 0.19	0.98 ± 0.21	1.07 ± 0.23	0.81 ± 0.28	−17 (−29, −5)	−28 (−67, −17)
Alpha-N-Phenylacetyl-L-glutamine	1.00 ± 0.34	1.03 ± 0.29	1.00 ± 0.30	0.86 ± 0.26	−16 (−28, −4)	−19 (−44, −5)

3 Rx to 3 Rx, maintained on 3-times weekly treatment; 3 Rx to 6 Rx, maintained on 6-times weekly treatment at the end of the 12-month intervention period.
Solute levels are expressed as a fraction of the average peak area in samples from all subjects at baseline. Listed here are the 32 solutes for which the difference in the 12-month values for the 2 groups was different with a false discovery rate <0.05. For all but 1 of these (N2,N5-diacetylornithine), the solutes were detectable in all 53 baseline-end pairs in the 3 Rx to 3 Rx group and all 30 baseline-end pairs in the 3 Rx to 6 Rx group, and the false discovery rate was also <0.05 when comparing differences in solute levels at the beginning and end of the study for the 2 groups.
[a]Solute level before randomization.
[b]Percentage differences in average solute levels at 12 months.
[c]Differences in the change in solute levels from baseline to 12 months for the 2 groups calculated as described in Materials and Methods.
[d]Solute for which a reagent standard was not run but for which identity was considered well-established by tandem mass spectrometry. Values are mean ± SD or mean (95% confidence intervals).

difference between the methods was greatest on average for PCS (0.59 ± 0.46) and least for urea (0.11 ± 0.08). For these solutes and for IS, the discrepancy in results was not different across the range of observed values. For HIPP and phenylacetylglutamine, however, the metabolomic analysis tended to yield higher values at the low end and lower values at the high end of the observed range, as shown in Supplementary Figure S1.

DISCUSSION

This study found that the combination of increased frequency and weekly treatment time employed in the FHN achieved only a small average reduction in concentrations of a large number of uremic solutes. Metabolomic analysis assessed the concentrations of 107 solutes that we have previously identified as elevated in the plasma of patients undergoing hemodialysis.[8] The average concentrations of these solutes were only 15% lower in pretreatment samples from FHN subjects receiving 6-times weekly treatment than in pretreatment samples from subjects remaining on conventional 3-times weekly treatment. Quantitative measurements confirmed that concentrations of IS and PCS, 2 of the uremic solutes that have been most widely considered toxic, were not significantly reduced.

Given the effort required to increase treatment frequency and time, the observed reductions in solute concentrations are disappointing. Theoretical analysis would lead us to expect more pronounced reductions in solute concentrations. Increasing treatment frequency is expected to be particularly effective in lowering concentrations of solutes that exhibit a large fall in concentration during a single conventional treatment.[9] Increasing weekly treatment time can lower concentrations of solutes that are not much affected by increasing frequency alone because their concentrations are reduced only modestly during a single conventional hemodialysis session. The combined increase in frequency and time in FHN subjects receiving 6-times weekly treatment might thus be expected to reduce average pretreatment concentrations of uremic solutes by more than 30%, as depicted in Figure 1.

Several factors may have limited the reduction in predialysis solute concentrations we observed in FHN subjects receiving 6-times weekly treatment. One potential contributor is increased solute production. Increased urea production reflected by an increase in normalized protein catabolic rate presumably accounted for the failure of urea concentrations to fall as much as predicted in FHN subjects receiving 6-times weekly treatment. Urea is the only solute whose production is routinely estimated, and we indeed know very little about the production of most other solutes. For PCS, however, there is evidence that production changes in response to the dialysis prescription. We and others have found that plasma PCS concentrations are not higher in patients undergoing peritoneal dialysis, which provides a very low PCS clearance, than in patients undergoing conventional hemodialysis.[10,11] We have also found that "high-dose" hemodialysis did not lower plasma PCS concentrations in HEMO study subjects and that doubling the PCS clearance did not lower plasma PCS concentrations in patients undergoing in-center nocturnal hemodialysis.[12,13] The similarity of plasma PCS concentrations across a wide range of hemodialysis prescriptions, as summarized in Supplementary Table S4, suggests that PCS production increases as its removal by dialysis increases. We cannot, however, provide a biochemical explanation for the presumed variation in PCS production.

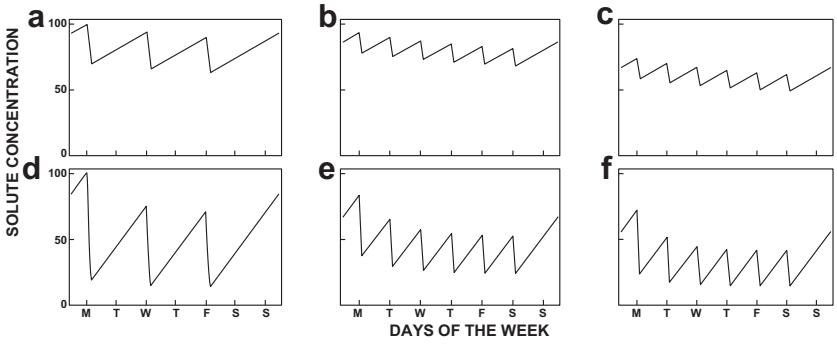

Figure 1 | The modeled effect of increasing treatment frequency and time in Frequent Hemodialysis Network (FHN) subjects. Solute levels are presented in arbitrary units on the *y* axis, with the days of the week on the *x* axis. The upper panels show values for a solute whose levels are reduced by 30% during a single conventional 3-times weekly treatment, while the lower panels show values for a solute whose levels are reduced by 80% during a single conventional treatment. (**a,d**) Three-times weekly treatment for the average total time of 10.9 hours prescribed for FHN subjects undergoing conventional treatment who were included in the current study. (**b,e**) The predicted effect of increasing treatment frequency to 6-times weekly without any increase in total treatment time. (**c,f**) The combined effect of increasing frequency and increasing weekly treatment time to the average total time of 14.6 hours prescribed for FHN subjects undergoing frequent treatment who were included in the current study. The reduction ratios of 30% and 80% during single conventional treatments encompass the range of values reported for most known uremic solutes. The solute profiles shown are predicted assuming solute production does not change with the dialysis prescription and that solute clearance occurs only by dialysis. The profiles shown here also assume that solutes are cleared from a single compartment. As shown in Supplementary Table S5, however, the predicted magnitude of changes in solute levels with increased frequency and treatment time is not much different if 2 compartments are assumed.

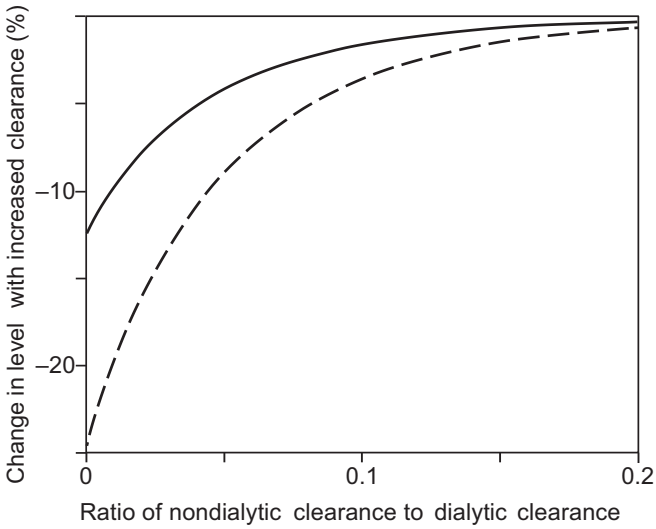

Figure 2| The predicted effect of nondialytic clearance on the reduction in solute levels achieved by increasing frequency and time. A continually operating nondialytic clearance that is only a small fraction of the dialytic clearance blunts the effect of increasing frequency and time. The figure shows this effect for solutes whose levels are reduced by 30% (solid line) and by 80% (dashed line) during a single conventional 3-times weekly treatment in the absence of nondialytic clearance. The predicted reduction in the average pretreatment solute concentration with the Frequent Hemodialysis Network (FHN) Daily Trial's increase in frequency and time is shown on the *y* axis with the ratio of the nondialytic clearance to dialytic clearance on the *x* axis. When there is no nondialytic clearance, the FHN Daily Trial's combination of increased frequency and time is predicted to achieve a greater reduction in the average peak concentrations of solutes whose levels are reduced by a larger fraction during a single treatment, as shown in Figure 1. Dialytic clearance here refers to the clearance rate provided by dialysis during treatment.

The presence of nondialytic solute clearance may also limit the extent to which increasing treatment time and frequency can lower uremic solute concentrations. The extent to which a continuously operating nondialytic clearance limits the effect of intensive dialysis has been shown most clearly for the low-molecular weight protein ß$_2$ microglobulin.[14,15] Nondialytic clearance presumably accounted for the finding that a 10-fold increase in ß$_2$ microglobulin's dialytic clearance lowered plasma ß$_2$ microglobulin concentrations by only about 20% when high-flux was compared to low-flux treatment in the HEMO study.[14] Most subjects in the FHN Daily Trial did not have sufficient residual kidney function to have contributed meaningfully to nondialytic clearance. The question of whether there is nondialytic clearance in other parts of the body for the small solutes assayed in our study has not been addressed to our knowledge. As shown in Figure 2, however, the presence of a nondialytic clearance equal to only a small fraction of the dialytic clearance would greatly reduce the predicted effect on solute concentrations of the FHN's combined increase in treatment frequency and time.

A final potential contributor to the limited reduction in uremic solute concentrations seen in FHN subjects receiving 6-times weekly treatment is solute disequilibrium within the body due to solute distribution across multiple compartments. Increasing frequency avoids continued application of dialysis after an initial period of treatment has lowered solute concentrations. Doubling treatment frequency without increasing total treatment time is thus predicted to lower solute concentrations as depicted in the left-hand panels of Figure 1. Previous analyses have emphasized that the reduction in time-averaged solute concentrations achieved by increasing frequency may be enhanced when there is solute disequilibrium within the body.[16] These analyses have failed to note that a more pronounced reduction in time-averaged solute concentration may be accompanied by a less pronounced reduction in the pre-treatment concentrations. The magnitude of the effect of intercompartmental disequilibrium, however, is not large. We believe that it could have had only a limited effect on solute concentrations in FHN subjects, as shown in Supplementary Table S5.

Metabolomic analysis allowed us to show that, whatever mechanisms were responsible, limitation in the reduction of plasma solute concentrations achieved with an increase in frequency and weekly dialysis time was not confined to the few solutes for which we have developed quantitative assays. Metabolomic methods are being increasingly employed to investigate kidney disease because they allow simultaneous measurement of numerous solutes in single samples.[17] Important limitations of metabolomic analysis, however, should be acknowledged. First, no metabolomic platform includes all plasma solutes. The current study employed the largest metabolomic platform that is widely available. Our previous studies have shown, however, that this platform includes only about 40% of the uremic solutes that have been identified.[8] It should be emphasized that these solutes have been identified as uremic simply because they accumulate in renal failure, and not because they are known to be toxic. Second, metabolomic methods sacrifice quantitative accuracy while increasing the number of solutes assayed. Because internal standards cannot be employed for each solute, solute concentrations are expressed as relative peak areas and not absolute concentrations. When metabolomic data are adjusted to yield the same average concentration as quantitative assays, there may be considerable differences in the results obtained in individual samples, as seen in Supplementary Figure S1. We were reassured that the results obtained by metabolomic analysis were on average similar to those obtained by quantitative analysis for the 5 solutes measured using both methods in the current study. We cannot be sure that the same would be true for other solutes.

Additional limitations should be acknowledged. Although the FHN Daily Trial performed extensive measurements of physical and cognitive performance, tools for making these measurements are imperfect and may not fully encompass the defects associated with uremia. The limited sample size of the FHN Daily Trial may have also prevented detection of some differences. We excluded FHN subjects receiving fewer than 6-times weekly treatment to more accurately assess the extent to which increasing frequency and weekly dialysis time would

lower solute levels. Because both frequency and time were increased in the FHN, we cannot verify that separate increases in these parameters would have the effects depicted in Figure 1. Fagugli *et al.*,[18] however, have reported that increasing frequency without time affords relatively little reduction in the levels of protein bound solutes. And a recent analysis by Eloot *et al.*[19] indicated that treatment time must be increased to reduce levels of tightly bound solutes, which exhibit low reduction ratios with standard treatment.

In summary, increased treatment frequency and weekly dialysis time achieved only modest average reductions in a large number of uremic solutes in FHN Daily Trial subjects receiving 6-times weekly hemodialysis. Limited reduction in solute concentrations may have accounted for the failure of these patients to exhibit a marked improvement in physical and cognitive performance and other aspects of general health that could be reasonably expected to improve following correction of uremia. The failure of solute concentrations to decline as much as expected can be explained by changes in solute production and/or the presence of nondialytic solute clearance. Different properties, however, likely determine the behavior of individual solutes. More data on solute toxicity is needed. If we knew which solutes were toxic, we could identify factors that prevent their plasma levels from falling. We could then develop treatments that could be combined with dialysis to control toxic solute concentrations.

METHODS

FHN samples and data

We obtained plasma samples from the NIH repository. Samples of pretreatment plasma obtained both before randomization and at 12 months were available for 53 of 120 subjects randomized to remain on 3-times weekly conventional treatment and 53 of 125 subjects randomized to frequent hemodialysis in the FHN Daily Trial. The current study included 30 subjects randomized to frequent hemodialysis who were receiving treatment 6 times weekly at 12 months; 23 subjects receiving treatment fewer than 6 times weekly were excluded.

Assay methods

We quantified plasma concentrations of PCS, IS, HIPP, and phenylacetylglutamine by stable isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a previously described method.[20] Plasma urea concentrations were measured using a standard kit (Infinity; Thermo Fisher Scientific, Waltham, MA). Plasma samples were also shipped to Metabolon, Inc. (Durham, NC) for metabolomic profiling. The methods used by Metabolon for identification and estimation of the relative amounts of individual compounds in biologic samples have been described in detail.[21,22] In brief, all samples are deproteinized with methanol and then aliquoted and dried. At the time the current analysis was conducted, individual aliquots were reconstituted differently for analysis by gas chromatography-tandem mass spectrometry (GC-MS/MS) and to LC-MS/MS in both positive and negative modes. Samples were run with retention time markers to allow correction for variability in retention times over multiple runs. Mass spectrograms were then compared with those in a library that has been prepared by analyzing more than 2400 purified compounds using the same methods. A single "quant-ion" was selected from either the GC-MS/MS, LC-MS/MS–positive, or LC-MS/MS–negative data that was considered to best represent each individual compound in the sample set as a whole. Software rated each compound's identification in individual samples based on retention time and comparison of the ion profiles with those recorded in the library. The mass spectrometric peak area for the quant-ion was automatically recorded as representing the concentration of the compound when the quality of identification was considered high and hand-checked when the quality of identification was considered intermediate. No value was recorded for samples in which identification did not meet a threshold value. For the current analysis, we entered a value halfway between zero and the minimum recorded peak area for the data set as a whole for such samples. Because internal standards cannot be employed when so many compounds are assayed, peak areas provide only an estimate or relative concentration. Peak areas obtained by metabolomic analysis were normalized to provide the same mean value as was obtained by quantitative assays to allow comparison of metabolomic and quantitative assay results. Chemical names used are from the Human Metabolome Database for solutes found in that database and in other cases are those used by Metabolon, Inc,. except that 2-methoxyphenol sulfate replaces the Human Metabolome Database name O-methylcatechol-O-sulfate.

Statistical analysis

We compared demographic data and dialysis prescription data from the same point in the study in the 2 groups using the unpaired *t*-test. We compared dialysis data at baseline and at 12 months in each group using the paired *t*-test. We assessed the effect of 6-times weekly compared with 3-times weekly treatment by 2 methods. First, we compared the 12-month values in the 2 groups using the unpaired *t*-test. Second, we compared the change from baseline to 12-month values for 6-times weekly treatment to the change from baseline to 12-month values for 3-times weekly treatment. Values for the natural logarithm of the ratio of the 12-month value to the baseline value for each solute in each subject in the 2 groups were calculated. The significance of the difference between changing from conventional to 6-times weekly treatment and remaining on conventional 3-times weekly treatment was then assessed by comparing these values in the 2 groups using the unpaired *t*-test. Cases in which no peak area was assigned for both the baseline and 12-month value for an individual solute were excluded from analysis. The percentage difference in the effect on solute levels of changing from conventional to 6-times weekly treatment compared with remaining on conventional treatment was calculated from the inverse logarithm of the difference in the group mean logarithms for the ratio of the 12-month values to baseline values and the 95% confidence bounds for the percent difference from the inverse logarithms of the 95% bounds for the difference in the group mean logarithms. Values for the false discovery rate q were obtained from P values using q-value (http://genomics.princeton.edu/storeylab/qvalue/).

Modeling

The predicted effect of 3-times weekly compared with 6-times weekly treatment on solute concentrations was modeled based on standard analyses of solute kinetics.[23] Calculations were made for the average recorded treatment time for each group and assuming there was no nondialytic clearance and that solute production was the same with 3-times weekly and 6-times weekly treatment. A MATLAB (R2015 Mathworks, Natick, MA) routine generated solute concentration profiles, including pretreatment concentrations on each treatment day and time-averaged concentrations from values for

treatment time, dialytic clearances, and volumes of distribution. For comparison with measured solute concentrations, modeled pretreatment values were appropriately weighted for the days in the cycle on which the repository values had been collected. Urea was modeled assuming 2 compartments with a total distribution volume (V) derived from the Watson anthropometric volume as described by Greene *et al.*,[24] an intercompartmental clearance estimated as $0.016 \times V$ as described by Daugirdas *et al.*,[25] and a urea clearance of 270 ml/minute. The volume of the accessible compartment for urea was assumed to be one-third of the total end treatment volume and to be reduced by an amount equivalent to the average pre- and post-treatment weight differences for the groups as summarized in Table 1. PCS, IS, HIPP, and phenylacetylglutamine were initially modeled assuming single compartments. The clearances and volumes of these compartments were estimated from values obtained in patients studied locally by Sirich *et al.*[7] For the current analysis the solute clearances were assumed to have the same relation to urea clearances, and the volumes of distribution the same relation to body weight, as in the subjects studied locally. Values are provided in Supplementary Table S6. Additional analysis was performed to assess the effect of assuming solute distribution in 2 compartments as described in Supplementary Table S4.

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Table S1. Modeled changes in solute levels.
Table S2. Uremic solutes assessed by metabolomic analysis.
Table S3. Results for solutes assessed by both quantitative assay and metabolomic analysis.
Table S4. The effect of different dialysis prescriptions on plasma p-cresol sulfate levels.
Table S5. The potential effect of solute disequilibrium on the reductions in plasma solute levels achieved by increasing treatment frequency and time in the Frequent Hemodialysis Network (FHN) Daily Study.
Table S6. Values used in predicting the effect of increased treatment frequency and time on solute levels.
Figure S1. Comparison of solute levels obtained by metabolomic and quantitative assays. Peak areas obtained by metabolomic analysis were normalized to provide the same mean value as was obtained by quantitative assays to allow comparison of the 2 methods. The left-hand panels depict the correlation of the metabolomic value on the *y* axis against the quantitative value on the *x* axis. The right-hand panels are Bland–Altman plots with the *y* axis being the metabolomic value minus the quantitative value.
Supplementary material is linked to the online version of the paper at www.kidney-international.org.

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