Free water transport in children on peritoneal dialysis is higher with more biocompatible dialysis solutions, higher with older age and declines with time

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Abstract

Background. Water transport in peritoneal dialysis occurs through small pores and aquaporins. Free water transport (FTW) occurs through aquaporins only and gives a reflection of peritoneal aquaporin function. In this study, FWT in children was calculated for the first time in different settings.

Methods. A prospective cohort study was performed; 87 peritoneal equilibrium tests (PETs) were analysed in 65 patients. Three subgroups were analysed: patients with their first PET; patients in their second year on dialysis; patients in their third year on dialysis or thereafter. Patients using 3.86% glucose solution with low pH/high glucose degradation products (GDP) were compared to patients using 3.86% glucose solution with neutral pH/low GDP. Sixteen patients using neutral pH/low GDP solution were followed longitudinally. FWT was calculated using the dialysate/plasma ratio of sodium.

Results. The proportional contribution of FWT was significantly higher in patients using dialysis solution with neutral pH/low GDP solution compared to patients using solutions with low pH/high GDP (50 versus 40%). Transcapillary ultrafiltration (TCUF) showed the same trend but was not statistically significant. Total FWT was higher as well. Higher FWT was observed with older age. In the longitudinal group, TCUF and water transport through small pores declined, while FWT remained stable in the first 1.5 years. The contribution of FWT increased in this period (48–61%), then slowly declined again to baseline level during the third year.

Conclusions. Total FWT and relative contribution of FWT were significantly higher with neutral pH/low GDP solution. This can reflect a better preservation of aquaporins. The decline in the contribution of FWT in long-term
dialysis could hypothetically implicate aquaporin dysfunction or different trafficking of aquaporins.

Keywords: aquaporin function; free water transport; paediatric; peritoneal dialysis

Introduction

Fluid transport in peritoneal dialysis (PD) is dependent on the hydrostatic pressure gradient, the colloid osmotic pressure gradient and the crystalloid osmotic pressure gradient [1]. Solute-coupled transport of water occurs by convection through the small pores. Free water transport (FWT) induced by crystallloid osmosis occurs exclusively through the ultra small pores or aquaporin-1 channels, being impermeable to solutes.

The presence of water-exclusive pathways means that when an osmotic gradient is created across the membrane (by hypertonic glucose) relatively more water than solute is transported, resulting in sieving. The theoretical principles of FWT are based on the three-pore model of Rippe [2, 3], whereby the heteroporous capillary wall is the main barrier. Despite reflection coefficients (sigma) of almost zero, sieving coefficients of small solutes do not approach 1 (1-sigma) because of FWT. The transendothelial water channel have not been established. Specific aquaporin-1 expression was demonstrated in endothelium lining peritoneal capillaries and venules in human peritoneum, in both apical as well as basolateral membranes, using immunostaining with aquaporin-1 antiserum [6, 7]. Yang et al. and Ni et al. proved in aquaporin-1 knockout mice that FWT cannot occur without aquaporin-1 channels [8, 9].

Several indirect methods have been applied to assess FWT, based on the principle that highly osmotic active glucose 3.86% solutions will induce FWT, while less osmotic active solutions like 1.36% glucose or 7.5% icodextrin will induce little FWT. The difference in net ultrafiltration (UF) between these different osmotic active solutions gives an indication of FWT [10–12].

A direct way to calculate FWT is by using the magnitude of the dip in the dialysate to plasma ratio of sodium (D/Psodium). A dilution of dialysate sodium occurs because of FWT from the circulation to the peritoneal cavity in the initial phase of a 3.86% glucose dwell [2, 13], causing a dip in D/Psodium. Direct quantification of FWT in adults has been performed in this way [14, 15]. Direct quantification of FWT in children has never been performed.

The aim of this study was to quantify FWT in children in different settings, as a reflection of the function of the aquaporins in the peritoneal membrane. Different dialysis solutions were compared as well as different age groups and the time course of FWT was investigated. Transcapillary ultrafiltration (TCUF), the contribution of FWT to the TCUF, fluid transport through small pores (SPT), marker clearance (MC), glucose absorption and mass transfer area coefficient (MTACs) of small solutes were assessed as well.

Materials and methods

Setting

A prospective cohort study was performed in two academic paediatric hospitals in Utrecht and Nijmegen in the Netherlands. Patients were enrolled from 1993 until 2007. The total cohort consisted of 65 patients, with 87 peritoneal equilibrium tests (PETs) performed. In 37 patients, 3.86% glucose solution with low pH and high glucose degradation products (GDP) was used (Dianeal®) and in 28 patients, a 3.86% glucose solution with neutral pH/low GDP was used (Physioneal®). For Dianeal®, the PD1 solution was used as a standard, containing 35 mmol/L lactate. For Physioneal®, the Physioneal 35 was used as a standard, containing a concentration of 25 mmol/L bicarbonate and 10 mmol/L lactate. Physioneal 40 (with 25 mmol/L bicarbonate, 15 mmol/L lactate and a lower calcium content) has been used in individual cases, to reduce the calcium load. Cross-sectional groups and longitudinal groups were both analysed. In the cross-sectional group (Group 1), all 65 patients were analysed as one group with a PET performed after a median of 1 year on PD. Subsequently, subgroups were analysed: Group 1A (n = 31) consisted of the first PETs performed in patients, Group 1B (n = 31) consisted of PETs performed in the second year on dialysis and the PETs in Group 1C (n = 25) were performed in the third year on dialysis or thereafter. Patients could be analysed in more than one subgroup with a different PET. Patients using low pH/high GDP solution were compared to patients using neutral pH/low GDP solution. Longitudinal (Group 2): 16 patients were prospectively followed for at least 3 years, with 50 PETs performed with neutral pH/low GDP solution.

Subjects

Baseline characteristics of the total cohort and subgroups are shown in Tables 1 and 2. The total cohort consisted of 42 male patients and 23 female patients. Underlying renal diseases were ureteral valves/reflux nephropathy (25%), dysplastic kidneys (25%), nephrotic syndrome (11%), glomerulonephritis (5%), haemolytic uraemic syndrome (5%), focal segmental glomerulosclerosis (5%), unknown (5%) and other diseases (19%), nephrolithiasis, hereditary tubulonephritis, Denys–Drash syndrome, ischaemic cortical necrosis, diffuse mesangial sclerosis, hyperoxaluria, autosomal recessive polycystic kidney disease). The proteinuria rate in the total patient cohort was 0.9 episodes/patient-year; no difference was found between the low pH/high GDP and the neutral pH/low GDP group. The majority of the patients used 1.36% glucose, three patients (5%) used a mixture of 1.36 and 2.27% glucose solution and six patients (9%) used 2.27% glucose solution. These patients were equally divided among the low pH/high GDP group and the neutral pH/low GDP group. Two patients in the neutral pH/low GDP group used icodextrin in daytime.

Procedure and measurements

All PETs were performed during 4 h dwells as described previously [16, 17]. An intraperitoneal volume (IPV) of 1200 mL/m² body surface area (BSA) was used in all tests. Dialysate samples were taken before inflow (time-point zero), at 5, 30, 60, 120, 180 min and at the end of the dwell at 240 min with measurements of sodium, urea, creatinine, glucose and dextran. Dextran 70 4 g/L (Hyskon; Medisan pharmaceuticals AB, Uppsala, Sweden or Macronex; NPBI, Emmercompascuum, The Netherlands) was added as volume marker to calculate the time course of TCUF and marker clearance.

| Table 1. Baseline characteristics Group 1: total patient cohorta |
|-----------------|-----------------|-----------------|
| PD solution (n) |       |       |
| Low pH/high GDP (n = 37) | Neutral pH/low GDP (n = 28) |
| Time on PD (months) | 11 (1–68) | 12.5 (4–58) |
| Age at start (months) | 77 (0–201) | 48 (0–171) |
| BSA (m²) | 0.79 (0.24–1.79) | 0.68 (0.35–1.68) |

aMedians and ranges are given.
Blood samples were taken halfway at time-point 120 min, with measurement of sodium, urea, creatinine and glucose. None of the patients suffered from peritonitis at the moment of the PET or in the 4 weeks preceding the PET. None of the patients had UF failure.

Calculations

All calculations were performed as described previously [18, 19]. In brief, TCUF was calculated from the dilution of the volume marker by subtraction of the initial IPV from the theoretical IPV (when fluid absorption and sampling are not taken into account) at any time-point. For the measurements of effective lymphatic absorption rate, the marker clearance from the peritoneal cavity was used, calculated as the convective loss of dextran during the dwell [20]. The MTAC, the maximal theoretical diffusive clearance of a solute at time-point zero, was calculated for urea and creatinine according to the Waniewski model [21, 22], with a correction of serum solute concentration for plasma water.

FWT at 60 min was calculated by subtracting total sodium transport from the total fluid transport, using the magnitude of the dip in D/P sodium as previously described by La Milia et al. [14, 15]. The maximum dip in D/P sodium was calculated by subtracting the smallest dialsate-to-plasma (D/P) at any of the time-points from the D/P sodium at time-point zero. According to the three-pore model, water transport will occur through small pores and aquaporins. Transport of water through the small pores is coupled to convective transport of sodium (no sieving of sodium), while water transport through the aquaporins (FWT) will occur with complete sieving of sodium. This dissociation of water transport from sodium transport is used to calculate FWT. To correct for the diffusion of sodium from the circulation to the dialysate, a diffusion correction was performed using the MTAC of creatinine as was validated by Zweers et al. [23]. Glucose absorption at 60 min and at 240 min was assessed by calculating the ratio of the glucose concentrations at these time-points corrected for BSA according to the Mosteller formula [26] and expressed per 1.73m2.

Statistical analysis

Results are expressed as median values and ranges for cross-sectional data. For comparison of different dialysate fluids (low pH/high GDP solution versus neutral pH/low GDP solution), the Mann–Whitney U-test was used. For longitudinal data, the linear mixed model procedure was used to test whether a general model for the time course exists for the transport parameters. The linear mixed model examines the average changes in subjects, taking the association between variables into account in every individual subject, measured at different time-points. Results of the longitudinal data are expressed as means and SEM.

Results

Baseline characteristics of the total group and the subgroups were not different with respect to dialysis fluid used, except for sex distribution in the total group, with significantly more male patients in the low pH/high GDP group compared to the normal pH/low GDP group. The patients who were on PD for the longest time and the longitudinal patients tended to be younger at start, but these differences were not significant.

Results of the cross-sectional study

A significantly higher contribution of FWT (P = 0.007) and a higher total FWT at 60 min (P = 0.007) and 240 min (P = 0.014) was found with the neutral pH/low GDP solution compared to the low pH/high GDP solution in the whole cross-sectional group (Table 3). The same trend was seen in the subgroups but significance was not reached in each separate subgroup (Table 4). The trend was most pronounced in Group 1A (first PET) and Group 1B (second year on PD) with significantly higher FWT at 60 and 240 min in the neutral pH/low GDP group versus the low pH/high GDP group. In Group 1B, a significantly higher TCUF was seen in the neutral pH/low GDP group compared to the low pH/high GDP group. In the other subgroups and the...
total group, TCUF tended to be higher in the neutral pH/low GDP groups in comparison with the low pH/high GDP groups as well, without statistical significance being achieved. The maximum dip in D/P sodium did not differ significantly between the low pH/high GDP group and the neutral pH/low GDP group. In the neutral pH/low GDP group, a smaller median D/P ratio was found at the individual time-points in the whole group (Figure 1) as well as in Subgroup 1A, giving a higher dip of D/P sodium at each time-point, including at baseline. The area under the curve (AUC) was calculated for both the neutral pH/low GDP group and for the low pH/high GDP group, with a higher AUC for the neutral pH/low GDP group, independently of the D/P sodium at baseline (21.9 versus 17.9). This gives an indication that an overall higher dip in D/P sodium is present in the neutral pH/low GDP group, although the AUC can overestimate the true difference between the two groups.

The calculations of FWT were not influenced by the difference in the D/P sodium at baseline. The D_60/D_0 (glucose) and the D_240/D_0 (glucose) in the total group were 0.61 and 0.29, respectively. There were no differences between the low pH/high GDP group and the neutral pH/low GDP group.

Age-dependent effect

The whole patient cohort as well as the low pH/high GDP group and neutral pH/low GDP group were divided into two different age groups: 0–5 years and >5 years, to evaluate a possible effect of age. The results are shown in Table 5. A significantly higher contribution of FWT was seen in all patients >5 years, with the low pH/high GDP group and neutral pH/low GDP group combined (P = 0.045). Total FWT at 60 min tended to be higher in the neutral pH/low GDP group as well, with a P-value of 0.07. This trend towards a higher FWT was observed in the two groups separated on basis of dialysis solution as well, but these results were not significantly different.

Longitudinal results

The TCUF showed a steady decrease over the years, reflecting the changes in FWT as well as SPT (Figure 2a–h). Total SPT decreased at a slightly larger rate in concordance with TCUF in the first 1.5 years, with a stabilization thereafter. Total FWT remained stable in the first 1.5 years, with a slow decline afterwards. An increase in the contribution of FWT was seen during the first 1.5 years, from 48 to 61%, followed by a decrease after the second year to baseline level (51%) at the end of the third year. The maximum D/P sodium showed an increase followed by a decrease. MTACs of urea

| Table 4. Transport characteristics of Group 1: subgroups |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| PD solution (n) | Low pH/high GDP (n = 17) | Neutral pH/low GDP (n = 14) | Low pH/high GDP (n = 14) | Neutral pH/low GDP (n = 17) |
| FWT: 60 min (mL/1.73m²) | 197 (75–300) | 269*(110–372) | 178 (54–390) | 300**(158–392) |
| FWT% | 43 (17–65) | 46 (23–79) | 40 (23–100) | 56 (37–77) |
| TCUF: 60 min (mL/1.73m²) | 473 (280–639) | 543 (369–812) | 446 (238–567) | 519*(368–712) |
| SPT: 60 min (mL/1.73m²) | 270 (165–469) | 263 (85–524) | 228 (0.50–403) | 207 (92–412) |
| Max dip D/P Na⁺ | 0.12 (0.07–0.16) | 0.09 (0.04–0.16) | 0.10 (0.04–0.16) | 0.13 (0.04–0.17) |
| D/P Na⁺: 60 min | 0.87 | 0.82** | 0.87 | 0.83** |
| MC: 240 min (mL/1.73m²) | 279 (32–780) | 311 (114–1351) | 197 (61–574) | 354 (37–949) |
| MTAC creatinine (mL/min/1.73m²) | 9.4 (4.5–20) | 9.9 (4.7–30) | 11 (3.4–14) | 8.0 (3.5–16) |
| MTAC urea (mL/min/1.73m²) | 19 (12–36) | 19 (0.2–54) | 19 (12–28) | 17 (9.5–31) |

*a Medians and ranges are given. 
*P = 0.05 for neutral pH/low GDP versus low pH/high GDP. 
**P < 0.05 for neutral pH/low GDP versus low pH/high GDP.
and creatinine remained fairly stable, showing a shallow U-shaped curve.

In the group using low pH/high GDP dialysis solution, the number of patients with longitudinal follow-up was too small to allow for a valid linear mixed model.

### Discussion

In this present study, a higher FWT was observed in paediatric patients using a more biocompatible neutral pH/low GDP dialysis solution compared to paediatric patients using a conventional low pH/high GDP dialysis solution. This could be because of better preservation of peritoneal membrane quality with more biocompatible solutions. With the low pH/high GDP solutions, acute and chronic toxicity lead to progressive deterioration of the mesothelial cell layer with increased compact zone thickness giving rise to fibrosis and vasculopathy with progressive subendothelial hyalinization and higher density of blood vessels [27].

GDP’s are involved in stimulation of vascular endothelial growth factor and transforming growth factor-beta release and induce advanced glycosylation end-product (AGE) formation and up-regulation of the AGE receptor with augmentation of chronic inflammation, neoangiogenesis and endothelial cell dysfunction. This all leads to progressive peritoneal damage [28, 29]. More biocompatible fluids show less AGE formation and better preservation of peritoneal integrity [30]. Paediatric patient studies performed with a low GDP bicarbonate-buffered solution (Bicavera®) support the hypothesis of better membrane preservation with less AGE formation and an increase of mesothelial cell mass measured by higher carcinoma antigen 125 values [31, 32]. Differences in overall solute transport were not observed in short-term studies, comparing conventional versus more biocompatible solutions during two consecutive PETs in both children and adults [33, 34]. An explanation lays in the fact that only acute effects of different solutions can be seen in this way, instead of longer term effects. Vasodilatation plays a more prominent role in low pH/high GDP solutions [35, 36], resulting in a transient increase of peritoneal surface area in the early phase of a dwell, which affects the capillary pressure gradient and thus water and solute transport as well. FWT can be a sensitive parameter in the chronic effect of different dialysis solutions on the peritoneal membrane. A specific role of aquaporin functioning in chronic PD was shown in the comparison of transport characteristics in patients with peritonitis to patients with increased surface area on long-term PD [37]. These findings lead to the suggestion that peritonitis patients have only vascular changes, with a temporary increased number of perfused peritoneal capillaries, without disturbed osmotic conductance, while in chronic PD, with an increased number of peritoneal microvessels, impaired FWT was regarded as a contributing factor, because of a different shape of the curve and a smaller D/P sodium dip. This supports the hypothesis that the differences found in FWT between conventional and more biocompatible solutions in our chronic population are aquaporin-1 mediated. Lower UF because of hyperpermeability could also play a role in our patients, with the trend of a higher TCUF in our patients on neutral pH/low GDP solution compared to low pH/high GDP solution.

The calculation of FWT is made by subtracting the SPT from the TCUF. Both the maximum D/P sodium and the TCUF showed a higher trend but were not significantly higher in this group. The time course of D/P sodium indicated an overall larger dip in D/P sodium in the neutral pH/low GDP group.
but the calculated AUCs can overestimate the true difference between the two groups. The fact that the D/P sodium was statistically significant at baseline was an unexpected finding. Hypothetically in the neutral pH/low GDP group, the total residual IPV at start could be slightly different or the residual intraperitoneal fluid could be more diluted because

Fig. 2. a–h) Longitudinal course of water and solute transport characteristics with neutral pH/low GDP solution. (a) FWT at 60 min. (b) Proportional FWT. (c) TCUF at 60 min. (d) SPT at 60 min. (e) Maximum dip in D/P sodium. (f) Dip in D/P sodium at 60 min. (g) MTAC of urea. (h) MTAC of creatinine. PET numbers: 0 = start PD; 1 = 6 months on PD (n = 8); 2 = 12 months on PD (n = 11); 3 = 18 months on PD (n = 8); 4 = 24 months on PD (n = 9); 5 = 30 months on PD (n = 7); 6 = 36 months on PD (n = 7). Means with standard error of the mean are given. The P-values indicate the significance of the firmness of the observed trend model [(a): P = 0.09; (b) P = 0.16; (c) P = 0.13; (d) P = 0.10; (e) P = 0.04; (f) P = 0.25; (g) P = 0.84; (h) P = 0.48]. min = minutes, mL = millilitre; time interval between PETs = 6 months.
of a slightly higher FWT during the preceding nightly dialysis episode, but this baseline difference could on the other hand reflect systematical differences not attributable to FWT. To be sure that the FWT results were not influenced by this finding, it was tested and confirmed that after correction for this difference in baseline D/P_sodium, the statistically significant difference in FWT between the two groups remained uninfluenced. Our results on FWT in low pH/high GDP solution are in concordance with the results found in adults with conventional low pH/high GDP solution (Dianeal®) [15]. FWT on the longer term has not been compared between conventional acidic solutions and more biocompatible solutions before. The longitudinal results show a decline in TCUF that reflects the trend of a better preservation of FWT compared to TCUF as well as the larger decrease of SPT compared to TCUF that is seen in the first 1.5 years of the patients using neutral pH/low GDP dialysis solution. The same trend of a decrease in long-term TCUF due to the combination of an increase in the contribution of FWT together with a decrease in the contribution of SPT was seen in adult patients that were followed longitudinally by Parikova et al. [33], although these patients used low pH/high GDP solutions as well as neutral pH/low GDP solutions and the follow-up period was longer. In another longitudinal adult study by Coester et al. [37], patients with and without ultrafiltration failure (UFF) on conventional dialysis solution were followed for >4 years. The patients with UFF showed a much lower proportional FWT with a higher proportional SPT than the patients without UFF, suggesting peritoneal damage leading to impaired FWT combined with a fast loss of the osmotic gradient. The relationship between loss of UF and aquaporin-1 expression, however, remains a difficult one. A quantitative loss of aquaporins is unlikely to be responsible for UFF since no differences in aquaporin-1 expression were seen in normal subjects compared to subjects on PD [6] and it was shown in a patient with UFF that the aquaporins were still present [38]. Impairment of FWT may therefore be caused by dysfunctioning of aquaporin-1 due to structural or conformational changes rather than quantitative changes in aquaporin-1 expression. A proposed mechanism of aquaporin-1 dysfunctioning is less effective in the trafficking of aquaporins to the endothelial cell membrane. Furthermore, a loss of osmotic conductance contributes to the decrease in FWT. Inflammation and vasculopathy lead to hyperpermeability for small solutes, so the osmotic gradient disappears fast, explaining a part of the impaired FWT as well [6, 39]. This loss of osmotic conductance with rise in MTAC of creatinine was observed in adult patients on long-term PD by Clerbaux et al. [39]. Another contributing factor in UFF, possibly influencing FWT as well, is the alteration of the glyocalyx that leads to submesothelial thickening and vasculopathy of the peritoneal membrane, leading to an impaired UF coefficient [38, 40]. When a subdivision in the cross-sectional group was made according to age, the contribution of FWT was higher in the patients >5 years compared to children under the age of five. In the separate low pH/high GDP and neutral pH/low GDP groups, the same trend was seen, without statistical significance, likely due to the relative small patient numbers. Time on PD in these groups did not differ significantly, so bias for time on PD is not an explanation. A hypothesis for the higher FWT in children of older age is that maturation of the peritoneal membrane plays a role, with more recruitment of aquaporins to an effective localization in older children. The other transport characteristics revealed no differences with age. Warady et al. [41] investigated the effect of age in children concerning membrane transport function and found higher MTAC values for creatinine and glucose in the youngest children, under 1 year of age, suggesting a higher membrane permeability. The number of patients aged under 1 year in the present study was too small to confirm these findings. The values of MTACs of creatinine and urea in Warady’s overall patient group were similar compared to our patients.

Comparing transport parameters to earlier performed paediatric studies [16, 42], MTACs of creatinine and urea and marker clearances are similar to those in our patients and show no differences with adult data [35]. Values of TCUF in our study are also similar to earlier paediatric PET data performed with 3.86% glucose [16]. A flaw in our study is the fact that the neutral pH/low GDP solution that was used in this study still contains more GDP’s than the newer solutions like Bicavera®. In future investigations, it would be very useful to investigate FWT in these newer, more biocompatible solutions. In this study, we did not distinguish between the different subtypes of the used neutral pH/low GDP solution, with 10 versus 15 mmol/L lactate, as it was not expected that this small difference would have an effect on FWT.

In conclusion, the contribution of FWT is significantly higher when normal pH/low GDP solution is used compared to low pH/high GDP solution, with a trend of a higher TCUF as well. This can reflect a better preservation of aquaporin function as well as less impairment of osmotic conductance. The longitudinal results show a decline of TCUF over time that reflects the decrease in SPT as well as the initial preservation of FWT, followed by a decline in FWT after 2 years. This suggests aquaporin dysfunction or different trafficking of aquaporins as well as loss of osmotic conductance on the long-term. With longer follow-up duration, the course of FWT in time can be investigated more profoundly. To further confirm the hypothesis of aquaporin dysfunction with low pH/high GDP solutions and in time, peritoneal membrane biopsies in these patient groups might be useful to explore aquaporin-1 expression and function in future studies.

Conflict of interest statement. None declared.

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