Mechanisms of Peritoneal Water and Solute transport - Computational Modelling and Clinical Implications

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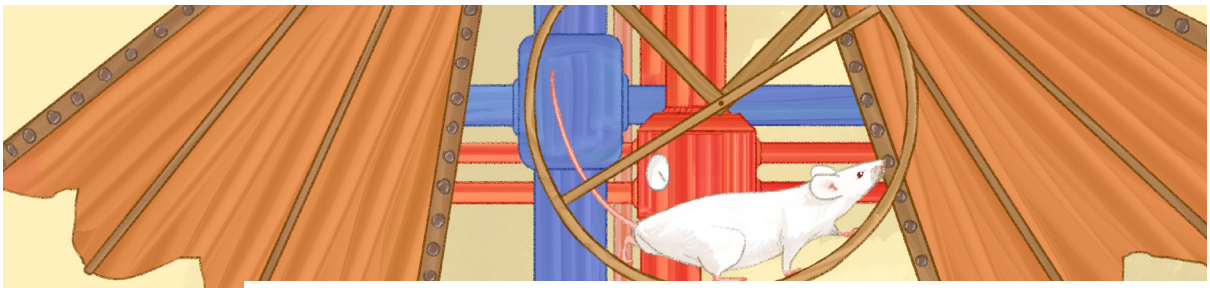

Mechanisms of peritoneal water and solute transport

Computational modelling and clinical implications

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UNIVERSITY

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In study 1, we investigate the effects of very high ultrafiltration rates on blood plasma volume. Experimental peritoneal dialysis was performed on rats using dialysis fluid with a very high glucose content, and plasma volumes were measured using albumin with a radioactive tracer technique. Plasma volumes appeared unchanged despite high ultrafiltration rates.

The aim of study 2 was to validate and test a new mathematical model for estimating ultrafiltration rate in peritoneal dialysis from the phenomenon known as the sodium dip. The model was tested experimentally on rats, by estimating ultrafiltration rates during peritoneal dialysis using the new model and a reference method. Then, the same analysis was performed on a cohort of patient data. Results showed excellent agreement between ultrafiltration estimates obtained with the new method and the reference method in experimental data, and moderate agreement in clinical data. The same was true for osmotic conductance to glucose in clinical data.

Finally, in study 3, the well-established three-pore model for peritoneal transport was modified by using the Nernst-Planck equation to describe electrolyte transport. The model was fitted to experimental data from peritoneal dialysis performed on rats, by using the kinetics of sodium to estimate hydraulic conductance and area-to-diffusion length ratio. After incorporating the parameters, the model independently predicted the transport of water and solute species other than sodium.

Key words: Peritoneal dialysis, computational model, sodium, ultrafiltration, electrolyte

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Abstract

Peritoneal dialysis is a widely used, cost-effective and accessible renal replacement therapy. The primary aim of this thesis is to study and measure the mechanisms that govern fluid and electrolyte transport in peritoneal dialysis by combining theoretical models with experiments on rats and analysis of clinical datasets. Despite the apparent simplicity of the treatment, the physiological mechanisms that govern the transport of solutes and water are highly complex.

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Popular scientific summary

The kidneys are an important organ in the human body. Two of their main functions are to filter the blood to get rid of excess water and waste from the human body, expelling it as urine. Due to a number of diseases, the kidneys can stop functioning, which is called kidney failure. If the kidneys fail completely, the body can start accumulating waste products and excess water, which in turn leads to illness and eventually death. Patients with severe kidney failure therefore must be treated. Using technology, it is possible to filter human blood, partly replacing the functions of the kidney. This is called dialysis. While there are different forms of dialysis, we will focus on one form of dialysis that is called peritoneal dialysis.

The abdominal cavity (the space between the vital organs within the belly) is lined with a thin membranous tissue called the peritoneum. The peritoneum normally functions as a support, holding organs in place and forming structure for blood vessels that are supplying the organs. In peritoneal dialysis, the abdominal cavity is filled with a solution rich in sugar. By osmosis, the sugar then attracts water and other particles from the surrounding blood vessels within the peritoneum. This makes the peritoneum effectively works as a filter, which is similar to the filter in the kidney, albeit lacking many of the control functions of the kidney. The glucose-rich fluid is typically left in the abdomen for a few hours and then drained, effectively removing the toxins from the body. While the principle may sound simple and does not require advanced machines, the fact is that we have a poor understanding of how and why the filter works, meaning how it retains and filters out relevant substances. This makes it difficult to predict how a patient will respond to the treatment.

Another related issue is that the filter is a part of the patient's own body and therefore its properties can vary between different people and over time. Because of this, it is difficult to know how the patient will respond to different types of dialysis fluid. Another aspect is that the peritoneum will change over time because of inflammatory processes caused by the constant exposure to fluid that is foreign to the body, ultimately affecting the efficiency of the peritoneal filter. Today, failure of the peritoneum is often discovered when the dialysis is failing to remove water and toxins from the body.

To tailor the dialysis to the needs of each individual patient, this research broadly focused on the development of new methods for assessment of the filter in each patient. These new methods make it possible to design new products and treatment methods. In the process we learn about the peritoneum itself and what actually happens during peritoneal dialysis.

The kidney has many built in control systems that regulate how much water gets removed from the body. In dialysis treatment, this has to be regulated "manually" by the provider of the treatment. Drawing too much water is known to cause fall in blood pressure in dialysis patients, and the common explanation for this is that the dialysis draws out fluid from the blood, decreasing the blood volume and causing a subsequent drop in pressure. We studied this phenomenon by performing dialysis on rats with much stronger dialysis fluids than the ones used in patients, which caused the fluid extraction to be extremely high, far beyond today's safety requirements. During this procedure, we repeatedly measured the volume of fluid in the bloodstream. To our surprise, the fluid volume in the blood stream did not appear to at all. While this may be an effect of rat physiology, the results indicate that the circulating blood is effectively kept at the same volume during peritoneal dialysis and the fluid is either replaced or recruited from elsewhere within the body.

In the following studies we wanted to better describe the transport of water and dissolved substances using mathematics. To do this, we built upon existing mathematical descriptions of dialysis and tested our models in rats and in clinical data from treatments in patients. It is established that sodium (Na^+) and water often have a connection within the human body. Where water flows, sodium follows and vice versa. This is also true for peritoneal dialysis. In peritoneal dialysis, there is also a suspected relationship between the transport of sodium and the transport of other substances, this means that if you know how the sodium behaves you can draw conclusions about how water and other substances behave. We just had to figure out the relationship. Therefore, we investigated and described what happens with sodium inside the abdomen during peritoneal dialysis. The peritoneum is a complex membrane, which is why this has been difficult to describe. Based on knowledge from the earlier study and research by other researchers on the peritoneal membrane, we could construct a new mathematical model that accurately described how sodium is transported in peritoneal dialysis. The new design performed well on tests in both animal and human experimental data. Using the new models, we could accurately and independently predict both the transport of water and other substances from just measuring sodium.

Hopefully, the new methods will make it easier for patients and doctors to measure the effect of a dialysis treatment and make better adjustments to the dialysis. Also, researchers might benefit from the deepened knowledge of the mechanisms behind peritoneal dialysis.

Populärvetenskaplig sammanfattning

Njurarna är ett viktigt organ i människokroppen. Två av njurarnas huvudfunktioner är att filtrera blodet för att bli av med överskott av vatten och restprodukter från människokroppen och utstöta det som urin. På grund av många olika sjukdomar kan njurarna sluta fungera, vilket kallas njursvikt. Om njursvikten blir mycket allvarlig kan njurarna sluta att fungera helt. Kroppen börjar då ackumulera restprodukter och vätska, vilket i sin tur leder till sjukdom och så småningom död. Patienter med allvarlig njursvikt måste därför behandlas. Med hjälp av medicinsk teknik är det möjligt att filtrera patientens blod, vilket delvis ersätter njurens funktioner. Detta kallas dialys, och förekommer i olika former. Denna avhandling fokuserar på en dialysform som kallas peritonealdialys, eller bukhinnedialys.

Bukhålan (utrymmet mellan de vitala organen i magen) är beklädd med en tunn hinna som kallas bukhinnan. Bukhinnan fungerar normalt som ett stöd som håller organen på plats. Bukhinnan innehåller även de blodkärl som försörjer bukens organ. Vid bukhinnedialys fyller man bukhålan med en lösning rik på socker (glukos), som genom osmos attraherar vatten och andra partiklar från de omgivande blodkärlen i bukhinnan. Detta gör att bukhinnan i praktiken fungerar som ett filter liknande det i njuren, men som tyvärr saknar många av njurens ”automatiska” kontrollfunktioner. Den glukosrika vätskan lämnas vanligtvis kvar i buken i några timmar och dräneras sedan, vilket effektivt avlägsnar både överflödigt vatten och slaggprodukter från kroppen. Även om principen kan låta enkel och inte kräver avancerade maskiner, så har vi en dålig förståelse för hur filtret fungerar, det vill säga hur det behåller nyttiga ämnen och filtrerar bort skadliga ämnen. Detta gör det svårt att förutsäga hur en patient kommer att reagera på behandlingen och att förskriva en optimal behandling.

En viktig aspekt är att filtret är en del av patientens kropp, och därför kan dess egenskaper variera mellan olika personer och över tid. På grund av detta är det svårt att veta hur patienten kommer att reagera på olika typer av dialysvätska. Dessutom kan bukhinnan förändras över tid på grund av inflammatoriska processer orsakade av konstant exponering för vätska som är främmande för kroppen, vilket i slutändan påverkar filtrets effektivitet. Idag upptäcks försämringar i bukhinnans filtreringsförmåga när dialysen inte länge lyckas avlägsna vatten och slaggprodukter från kroppen.

För att skräddarsy dialysen till varje enskild patients behov har forskningen i denna avhandling varit inriktad på utveckling av nya metoder för bedömning av bukhinnans filterfunktion hos varje patient. Dessa metoder skulle kunna göra det möjligt att designa nya produkter och behandlingsmetoder som är bättre anpassade för individen. Dessutom har vi under utvecklingsprocessen lärt oss nya saker om bukhinnan och mekanismerna bakom bukhinnedialysen.

Njuren har många inbyggda kontrollsystem som reglerar hur mycket vatten som avlägsnas från kroppen. Vid dialysbehandling måste detta regleras "manuellt" av förskrivande läkare. Att avlägsna för mycket vatten kan orsaka blodtrycksfall hos dialyspatienter. Förklaringen till detta är att dialysen drar ut vätska från blodet, vilket minskar blodvolymen och orsakar ett efterföljande blodtrycksfall. Vi har studerat detta fenomen genom att utföra dialys på råttor med mycket starkare dialysvätskor än de som används hos patienter, vilket orsakade extremt hög vätskeutvinning, långt över dagens säkerhetskrav. Under denna procedur mätte vi upprepade gånger vätskevolymen i blodomloppet. Förvånande nog verkade vätskevolymen i blodomloppet inte förändras alls. Även om detta kan vara en konsekvens av råttans fysiologi pekar resultaten på att det cirkulerande blodet i praktiken hålls kvar vid samma volym under bukhinnedialys, och att blodvolymen ersätts av vätska från andra delar av kroppen.

I de följande studierna beskrivs transporten av vatten och lösta ämnen under peritonealdialys med hjälp av matematik, där vi byggt vidare på befintliga matematiska beskrivningar av dialys. Modellerna testades på råttor och på kliniska data från behandlingar av patienter. Det är sedan länge fastställt att natrium (Na+) och vatten ofta följs åt inom människokroppen. Dit vatten flödar följer natrium med, och vice versa. Detta gäller även för bukhinnedialys. Vid bukhinnedialys finns det också ett förmodat samband mellan transporten av natrium och transporten av andra ämnen, vilket innebär att om man kan beskriva hur natrium beter sig så kan man också dra slutsatser om hur vatten och andra ämnen beter sig. För att förstå sambandet har vi undersökt och beskrivit vad som händer med natrium inuti buken under bukhinnedialys. Baserat på kunskap från den föregående studien och tidigare forskning om bukhinnan kunde vi konstruera en ny matematisk modell som korrekt beskrev hur natrium transporteras vid bukhinnedialys. Den nya designen presterade bra i tester på både djurmodeller och på mänskliga experimentella data. Med hjälp av de nya modellerna kunde vi förutsäga både transporten av vatten och andra ämnen enbart genom att mäta natrium.

Förhoppningsvis kommer de nya metoderna att göra det enklare för både patienter och läkare att mäta effekten av en dialysbehandling och göra bättre anpassningar av dialysen. Förhoppningsvis kommer även forskare kunna dra nytta av den nyfunna kunskapen om mekanismerna bakom bukhinnedialys.

List of papers

Study 1

Helman, J., & M Öberg, C. (2023). High versus low ultrafiltration rates during experimental peritoneal dialysis in rats: Acute effects on plasma volume and systemic haemodynamics. Peritoneal Dialysis International, 43(1), 84-91.

Original article

Author's contributions to paper: Conceptualisation, formal analysis, investigation, software, writing.

Study 2

Helman, J., Wahlgren, H., Andersson, L., Morelle, J., & Öberg, C. M. (2024). Quantifying Ultrafiltration in Peritoneal Dialysis Using the Sodium Dip. Kidney360, 5(2), 195-204.

Original article

Author's contributions to paper: Conceptualisation, formal analysis, investigation, software, writing, ethical approval application.

Study 3

Helman J. & M Öberg C. (2025) Estimating Transport in Peritoneal Dialysis Using Sodium Kinetics: Combining machine learning with traditional methods.

Draft manuscript

Author's contributions to paper: Formal analysis, investigation, software, writing.

Introduction and background

Renal physiology and basic concepts

The kidney is a vital organ responsible for filtering the blood to excrete water and soluble waste products, regulating acid–base balance through metabolic processes, and performing several important endocrine functions. Blood from the renal artery flows into the capillary bed of the renal cortex which contains unique capillary structures called glomeruli. The glomeruli have a special, fenestrated, vascular endothelium that acts as a semi-permeable filter, allowing water and small molecules to pass through when subjected to a hydrostatic pressure gradient while retaining large molecules. (1)

The smallest functional unit of the kidney is called the nephron. Blood flows into the nephron through an afferent arteriola into the glomerulus. The glomerulus is a tight coil of capillaries with fenestrated endothelial walls. Surrounding the capillaries is the glomerular basal membrane and specialised cells called podocytes. These structures allow blood to be filtered in the glomerulus, retaining cells and large proteins within the bloodstream and allowing smaller molecules to pass through. Hydrostatic pressure drives plasma through the filtration barrier into Bowman's capsule. The filtrate, called primary urine, passes into the proximal tubule, where water and sodium, along with glucose, amino acids, and bicarbonate, are reabsorbed. The next part of the nephron is the loop of Henle and extends into the renal medulla which is hyperosmotic due to the presence of urea. The descending part has a high permeability to water and low permeability to solutes, while the more distal, ascending part has a low permeability to water and high permeability to solutes. This causes the urine to become highly concentrated in the descending part, shifting the concentration gradient in favour of the absorption of solutes (instead of water) in the ascending part. After the loop of Henle comes the distal tubule. The cells in the tubule can secrete hydrogen ions into the urine to regulate acid-base balance according to the current acid-base status. The most distal part of the nephron is the collecting duct, where more water absorption occurs, regulated by antidiuretic hormone, allowing water excretion to be regulated according to hydration status. In addition to its blood purifying role, the kidney also has endocrine functions, consisting mainly of the renin-angiotensin-aldosterone system and hydroxylation of vitamin D. Dialysis is a treatment that replaces the water and solute removal

functions of the kidney. As we can see, the filtration system of the kidney is sophisticated, so replacing it is not a straightforward thing to do. (1)

Dialysis and kidney disease

Around 10 percent of the world's population suffers from chronic kidney disease. Kidney disease can stem from many different causes and have degrees of severity. Therefore, kidney disease patients are very broad group of people. Chronic kidney disease is often classified into stages 1-5 depending on the glomerular filtration rate of the patient. (2, 3)

As stated above, two of the main functions of the kidney are to remove water and solutes from the circulation, regulating the balance of water and soluble compounds within the body. Just as in the kidney, this is done by filtering the blood through a membrane. There are two main dialysis modalities, peritoneal dialysis and haemodialysis. In haemodialysis, blood is led outside the body and pumped through a filter by a machine. The filter consists of a semipermeable membrane, with blood on one side and dialysis fluid on the other, which is a solution of many different electrolytes and small molecules. The filtration works through counter current exchange, maintaining a concentration gradient across the membrane. Often, in clinical situations, the composition of dialysis fluid is adapted to the needs of the patient, for example with respect to electrolyte levels. Also, parameters such as hydrostatic pressure and flow rate can be tightly controlled, allowing for control of water removal. (4)

Peritoneal dialysis

In peritoneal dialysis, the peritoneal membrane of the patient is utilised as a dialysis membrane. Dialysis fluid with a high glucose concentration is infused into the abdominal cavity through a surgically implanted catheter. The high glucose concentration causes an osmotic gradient to arise, leading to the attraction of water and solutes from the surrounding tissue and blood vessels and causing the dialysis fluid in the abdomen to expand. After a predetermined time interval, the fluid is drained and discarded, and new fluid is instilled into the abdomen.

Peritoneal dialysis has many advantages for the patients. It is less expensive than haemodialysis and there are indications that it leads to better quality of life. (5) The choice of dialysis modality depends on many factors, and there is a variation between dialysis centre if peritoneal dialysis is the first choice compared to haemodialysis. (6) In contrast to haemodialysis, peritoneal dialysis can often be

performed at home by the patients themselves, which can be favoured depending on preference. (7) Also, between fluid changes, the patient can carry on with their normal activities. (8) Studies have indicated that home-based peritoneal dialysis treatments lead to better outcomes for patients. (8, 9) The goal of a peritoneal dialysis treatment is often highly individual. Management of a dialysis patient includes blood chemistry combined with recurrent clinical check-ups and occasional medical imaging. (10-12)

Fluid management is central in all dialysis treatment and fluid overload has been linked to various issues, such as cardiovascular disease and death. (13) At the same time, dialysis-induced hypovolemia can lead to adverse events such as hypotension and falls, and also increased discomfort due to thirst. (14) Water transport in peritoneal dialysis is highly individual, and membrane efficiency varies between patients and decreases over time. (15, 16) Ultrafiltration failure is an issue in peritoneal dialysis patients and can lead to a shift of treatment to haemodialysis. (17) The decrease in ultrafiltration capacity has been closely tied to the development of fibrosis in the peritoneum, neo-vascularization and structural alterations in the vascular wall. Glucose in the dialysate has been identified as the main contributor to peritoneal fibrosis, by inducing metabolic biochemical changes. (18-20)

Peritoneal transport and sodium

Transport in peritoneal dialysis occurs due the presence of a hyperosmotic agent (most often glucose) in the dialysis fluid, which will attract water by crystalloid osmosis. There are also iso-/hypoosmotic fluids available that attract water by colloid osmosis. Solutes are transported by both diffusion and convection. (21, 22) Over the course of many years, transport in peritoneal dialysis has been modelled in many ways. (23) In the 1990s. Bengt Rippe developed a mathematical model of the transport of water through the peritoneum, where transport is described as a flux through three types of cylindrical pores. (24, 25) The three pores consist of (i) a large pore that governs the transport of proteins and large molecules, (ii) the small pore through which water and small pores flow and finally (iii) the ultrasmall pore which is only permeable to water. (22) The pores themselves are a theoretical concept but reflect true phenomena. In reality, the large and small pore could represent paracellular transport pathways, while the ultrasmall pores represent trans-cellular water flow through aquaporins (water channels) on the surface of endothelial cells in the peritoneum. (26, 27)

Sodium is the most abundant electrolyte in peritoneal dialysis fluid. Dialytic sodium removal is dependent on both convective and diffusive transport across the peritoneal membrane and closely related to membrane function. (28, 29) Also, sodium overload has been linked to overhydration, which results in a higher risk of

cardiovascular disease and death in peritoneal dialysis patients. (30) Being a small ion, sodium will be transported by both diffusion and convection. (22, 31) The transport of sodium during peritoneal dialysis is closely linked to the transport of water and generally, sodium transport will occur in the same direction as the water transport. (28, 32) One important thing to note, is that solute free water will be transported through aquaporins (ultrasmall pore) parallel to the paracellular flow of sodium (small pore), and this will cause sodium within the dialysate to be diluted by solute free water when the ultrafiltration rate is high. (25, 26)

Peritoneal equilibration testing

Peritoneal equilibration testing (PET) is the main mode of testing the peritoneal membrane function today. In the morning the patient arrives at the dialysis centre, and any initial solution is drained after which a testing solution is instilled (commonly 2.3% glucose-based dialysate). Samples are typically taken from the dialysate directly after instillation, 2h after instillation and finally at 4h after instillation, after which the test is concluded. The samples are analysed for creatinine, urea and glucose, which allow calculation of important metrics of peritoneal membrane function to determine ultrafiltration capacity and small solute transport capacity. (10, 33)

Because the PET procedure is a long and complicated process, different versions of the peritoneal equilibration test have been suggested by researchers to minimize the time needed to perform a test. The modified PET test is a shorter version of the regular PET test, using a dwell time of 2 h that allows for calculation of more parameters by adding dextran to the dialysate. (34) La Milia et al have developed a shorter version of the test called mini-PET and the double mini-PET, which uses stronger glucose solutions and a shorter total dwell time of only 1 h and 2h respectively (10, 35, 36). Generally, PET-tests require time and effort to conduct. Therefore, there is a call for easier assessment methods.

The importance of a correct measurement of ultrafiltration

To assess and understand the fluid balance of the patient, it is crucial to have a good estimation of the ultrafiltration. Therefore, guidelines have for a long time stated that ultrafiltration is an important goal of dialysis. (10, 37, 38) In the clinic, this is typically done by measuring the drained volume after dialysis and comparing it to the instilled volume. This way of measuring ultrafiltration is often imprecise due to

many factors, such as variation in volumes of dialysis bags, inconsistent drainage due to catheter position and varying residual volume. (39, 40) Peritoneal membrane characteristics are often measured using peritoneal equilibration testing, which requires a precise measurement of ultrafiltration. (41) Also, a varying residual volume will cause a variation in certain peritoneal transport properties, because a higher volume increases the surface area available for diffusion. (42, 43) A precise measurement of ultrafiltration is also important when estimating osmotic conductance to glucose, which is an important membrane property that reflects the ability of the peritoneum to transport water. (40, 44-46) Measuring the kinetics of sodium has been proposed as a tool for estimation of the free water transport and ultrafiltration, and there is still a need for methods that can estimate the water transport with greater accuracy. (25, 47)

Introduction to transport theory and pore theory

Important definitions

Clearance

In pharmacology, *clearance* is defined as the volume of plasma (in most cases) which is completely cleared of a substance per unit of time. In contrast to clearance, *removal* is defined as the amount of a substance that leaves the body due per unit of time and is described by the elimination equation below, where V_d is the volume of distribution, C is the extracellular concentration, K is the clearance(1) and $\dot{m}$ a source or sink of substance mass.

$$V_d \frac{dC}{dt} = -KC + \dot{m}$$

Flux

Flux is defined as the amount of substance that passes an area in a certain direction per unit of time. It is often annotated J. Note that flux can be expressed per unit area and for any area. (48)

Diffusion

Diffusion is the transfer of mass that occurs when molecules move spontaneously due to thermal energy. This phenomenon occurs in liquids, gases and solids and is driven by the tendency of systems to minimize concentration gradients, causing particles to move from regions of higher concentration to regions of lower concentration. (49)

Convection

Convection is transport that occurs due to the movement of bulk solute causing solved substances to be dragged along. In transport of mass, the following equation is used to describe the change in mass m with respect to time t due to convective transport

$$\frac{dm}{dt} = c\,\overline{v}$$

Here, c is the concentration of the solute and v is the volume flow of the bulk solution. The equation above describes the transfer of mass when a bulk solution with a certain concentration moves at a certain velocity. (48)

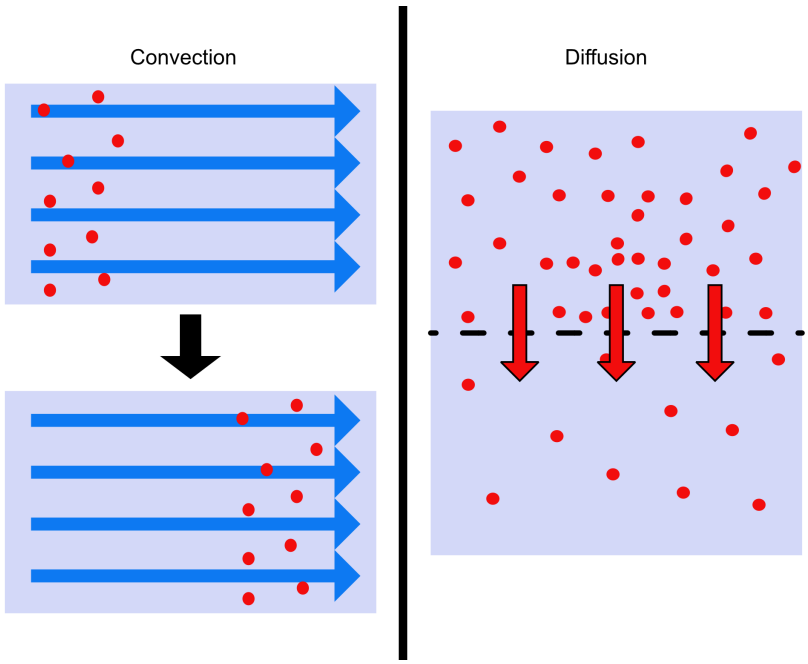

Figure 1: Principles of diffusion and convection. Convection (left panel) happens when a solute (red dots) is dragged along with the movement of a bulk solution. The solute will move in the direction of the bulk flow. Diffusion (right panel) happens when solutes spontaneously wander from an area of higher concentration to an area of lower concentration.

Transport theory

Compartment models

Water constitutes more than half of the weight of the human body and this water is spread out through most of the body. A large part of it is within the cells, some of it is in the space between cells and a part of can be found within enclosed spaces, such as the gastrointestinal tract. The human body consists of different organs and structures that perform different functions. There are clear borders between the different organs and looking into them, the composition of solutes will differ between organs depending on its structure and function. This allows for viewing the human body as a system of compartments with different properties, that communicate with each other in different ways. In the case of peritoneal dialysis, it is natural to view the fluid-filled abdominal cavity as a compartment, this compartment can then be assumed to communicate with other compartments, such as the intravascular fluid compartment, this is called a *compartment model*. Compartment models have been extensively used to study peritoneal dialysis and physiology in general. (50, 51)

Fick's law of diffusion

We have established that the transport in peritoneal dialysis can be described by two processes – diffusion and convection. One of the most well-established models of diffusive transport is Fick's first law, which describes the diffusive flow J as a function of the concentration gradient across the membrane *dc/dx*. (48, 52)

$$J = -D\frac{dc}{dx}$$

Where D is the diffusion coefficient, dependent on temperature, viscosity and size of the particles, according to the Stokes-Einstein equation, under the assumption that the solute consists of spherical particles. (53)

$$D = \frac{k_B T}{6\pi\eta r}$$

Here k_B is Boltzmann constant, T denotes the temperature, η denotes the viscosity and r denotes the radius of the solute sphere.

Integrating Ficks law over the total membrane area will yield the total transfer of mass in the system. Here, the gradient *dc/dx* is approximated as the difference between solute concentrations on both sides of the membrane, divided by the membrane thickness.

$$J_S = \int_A -D\frac{dc}{dx}\,\mathrm{dA} = -DA\frac{dc}{dx} = -\frac{DA}{\Delta x}(c_2 - c_1)$$

The continuity equation

In this thesis we will not go through derivations of all equations used, but most models are derived from the continuity equation. Most theories in transport stem from the continuity equation.

$$\frac{\partial c}{\partial t} + \nabla \cdot (cv) = 0$$

In a given volume, the first term represents the change in concentration *c* over time *t* and the second term represents the divergence of mass flux out of the volume (v is a velocity vector in the fluid). This means that a change in concentration equal how much of the solute leaves (or enters) the volume. (48, 54) Steady state is often assumed to simplify calculations, which is equivalent to setting the time derivative $\frac{\partial c}{\partial t} = 0$, which implies that $\nabla(cv) = 0$.

Fluid transport

The Starling equation describes the fluid exchange along a capillary wall.

$$J_v = L_p S\,(\Delta P - \sigma_s \Delta\pi)$$

where the fluid flux J_v is described to depend on the difference in in hydrostatic pressure $\Delta P = (P_{inside} - P_{outside})$ and oncotic pressure $\Delta\pi = (\pi_{inside} - \pi_{outside})$, in addition to some membrane parameters. L_p is the hydraulic conductivity of the vessel wall, S the surface area, and σ_s the osmotic reflection coefficient (1, 48). The reasoning behind this equation is that the intravascular hydraulic pressure will force fluid across the vessel wall, while osmotic pressure from proteins will attract water into the capillary. If the membrane parameters are regarded as constants, this will mean that a high hydrostatic pressure within the capillary will mean a large outflow of fluid, while a large osmotic pressure will lead to a decreased outflow of fluid.

Solute transport

The starling equation describes the transport of fluid. It was modified by Kedem and Katchalsky to incorporate both convective and diffusive transport of solutes. (23, 48, 55)

$$J_s = J_v(1-\sigma)C_s + PS\Delta C$$

Here, σ is the reflection coefficient, C_s is the average concentration within the membrane and PS is the product of the permeability coefficient and the surface. Reflection coefficients can be calculated from diffusive and convective hindrance factors, where $\sigma = (1 - hindrance\ factor)$. The hindrance factors can be estimated using established methods. (56, 57)

Further, modelling trans-vascular transport, under the assumption that the vessel wall is a porous membrane, Patlak et al proposed an equation that describes the transport.

$$J_s = J_v\big(1-\sigma_f\big)\left(\frac{C_i - C_L e^{Pe}}{1-e^{Pe}}\right)$$

Pe denotes the Péclet number, which is a dimensionless number that reflects the ratio between convective and diffusive transports and is defined by

$$Pe = \frac{J_v\big(1-\sigma_f\big)}{PS} = \frac{transport\ by\ convection}{transport\ by\ diffusion}$$

The Peclet number is a dimensionless number, and such numbers are commonly constructed and used in transport theory (and other fields) to be able to calculate meaningful quantities from physical variables, without necessarily knowing the equation. (48) When the Péclet number is large, convective transport dominates and when it is small, diffusive transport dominates. The Patlak equation has been adapted to dialysis by inserting the plasma and dialysate concentrations c_p and c_D. (23)

$$J_s = J_v(1-\sigma)\frac{c_p - c_D e^{-Pe}}{1-e^{-Pe}}$$

The three pore model

The three-pore model is based on a modification of the Starling equation. It was developed by Rippe and describes the flow of fluid and solutes as a flux across three pores: ultrasmall pores, small pores and large pores. Fluid transport in the three-pore model is commonly described by an adaptation of the Starling equation. Two factors are added to the Starling equation; a_f which is an area factor that is the fraction of the peritoneal surface area in contact with the dialysis fluid and α which is the fractional content of the respective pore, ($\alpha_C + \alpha_S + \alpha_L = 1$). (23, 24, 58)

$$J_{vC} = L_p S(\Delta P - \pi) a_f \alpha_C$$

$$J_{vS} = L_p S(\Delta P - \sigma_S \pi) a_f \alpha_S$$

$$J_{vL} = L_p S(\Delta P - \sigma_L \pi) a_f \alpha_L$$

To describe the solute transport across the membrane, a solute transfer model, such as the Patlak equation, can be applied to each of the three pores to describe the transfer of solutes across the membrane. Applying the Patlak equation to pores, here using the subscript *S* for small pore and *L* for large pore. (23, 59)

$$J_{s,S} = J_{vS}(1 - \sigma_S)\frac{c_p - c_D e^{-Pe_S}}{1 - e^{-Pe_S}}$$

$$J_{s,L} = J_{vL}(1 - \sigma_L)\frac{c_p - c_D e^{-Pe_L}}{1 - e^{-Pe_L}}$$

Note that the ultrasmall is impermeable to solutes and is therefore excluded here.

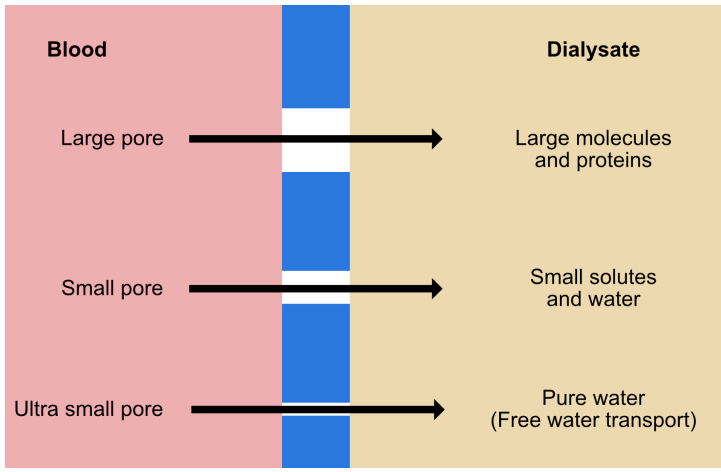

Figure 2: Thr three pore model of peritoneal transport. The peritoneum (blue) is assumed to be a porous membrane containing three different types of pores. The pores account for different types of transport. The solute and water transport is described by the equations above.

Permeability surface area product

When describing the exchange of solute species during dialysis, one important measure is the permeability-surface area product. It is an entity that describes the ability of the peritoneum to transport a solute. In literature, it is often called *mass transfer area coefficient*, or MTAC as it is usually abbreviated in literature. It is defined as the product of $\frac{A_0}{\Delta x}$, which is the ratio between the total available area for diffusion and the diffusion length across the membrane; the diffusive restrictive function $\frac{A}{A_0}$; and the diffusion coefficient D. (60)

$$PS = \frac{A_0}{\Delta x} \cdot \frac{A}{A_0} \cdot D$$

The mass transfer area coefficient incorporates important parameters of the membrane. The overall parameter $\frac{A_0}{\Delta x}$ contains crucial information about the membrane function. (61) The diffusion coefficient and restriction factor are also membrane parameters. Often, estimation of the mass transfer area coefficient requires quantifying the underlying factors. The mass transfer area coefficient is defined as the maximum diffusive clearance across the membrane. To develop an intuition, from the equation above we can reason that the PS will increase with a larger area (A), decrease with a thicker membrane (Δx) and increase with a higher diffusion capacity (D). For example, a thinner membrane with high permeability

and a large area should let more solute through. In haemodialysis the corresponding parameter is called K_0A.

Charged solute transport with electrostatic effects

Electrical fields

An electric field is a region in space around an electrically charged object where other charged objects experience a force. The strength of an electrical field is defined by the force that affects a positive test charge when placed in such field. (62)

$$F = qE$$

Where F is the force affecting the small test-charge q in the field E. The thing to keep in mind from the definition is that a charged particle, when placed in an electric field, will be subjected to a force and subsequently be pushed in the direction of the force. While the simple equation above can aid in grasping the concept of an electric field, more complex equations, such as Maxwells equations are needed to fully describe the field. Of these, Gauss's law will be necessary to understand some of the assumptions made later. (63)

$$\nabla \cdot \vec{E} = \frac{\rho}{\varepsilon_0}$$

$\nabla \cdot \vec{E}$ being the divergence of the electric field, ρ the charge density and ε_0 the vacuum permittivity. Assuming the permittivity is constant, this means that the electrical field E will depend on the charge density. In turn, this implies that a constant charge density will mean that the field is linear, and zero charge density (electroneutrality, in other words) will mean that the field is constant. These distinctions will be important further on.

Electric fields in membranes

Knowing the concentrations and a couple of other parameters it is possible to model the potential across the membrane. Assuming that a membrane is only permeable to one ion, the Nernst equation describes the relationship between the potential and the concentrations of the ion. (64)

$$V = \frac{RT}{zF} \ln\left(\frac{[C_{outside}]}{[C_{inside}]}\right)$$

The characteristic length of electrostatic fields in solutions is called the Debye length and described by the equation

$$\kappa^{-1} = \sqrt{\frac{\varepsilon_r \varepsilon_0 k_B T}{2e^2 I}}$$

Where $\varepsilon_0$is the vacuum permittivity, ε_r is the dielectric constant, k_B is the Boltzmann constant and T is the temperature in Kelvin, e is the elementary charge and I is the Ionic strength of the solution. In an electrolyte solution of physiological strength, this results in a very small electric field, only a few Å in length. (65)

The Gibbs Donnan effect

It has been established earlier in this introduction; the electric field is defined as the force per unit charge acting on a charged particle. Also, any local charge imbalance will produce a small electric field. Any system of solutes will converge to the least possible energy for that system, therefore charges will equalize if given time and possibility to do so. In the case of membrane transport, such as in peritoneal dialysis, this process will be more complicated due to the presence of a semipermeable membrane. The peritoneal filter (mainly the vascular endothelial wall) does not permit certain proteins, such as albumin, to cross. Albumin carries a strong negative charge compared to the small solutes. (66) The presence of a strong negative charge on one side of a negative membrane will affect the distribution of small solutes across the membrane when the system is in equilibrium, causing the concentration if solutes close to the membrane, interface, to be slightly different from the concentration in the solution. (67)

The Gibbs-Donnan effect will result in a solute concentration at the plasma-pore interface that is slightly different from the concentration in the bulk solution (plasma in this case). In electrostatics, this is commonly modelled using the Boltzmann distribution

$$\mathrm{C}_{\text{pore interface}} = C_{bulk} e^{\frac{zF}{RT} V_d}$$

We add partition coefficient to account for steric exclusion (exclusion due to presence of other ions). (68)

$$\mathrm{C}_{\text{pore interface}} = C_{bulk} \Phi\, e^{\frac{zF}{RT} V_d}$$

Where Φ is the partition coefficient, x is the distance from the pore, C is the plasma concentration, z is the charge of the ion, F is the Faraday constant, R is the ideal gas constant, T is the temperature in Kelvin and V_d is the Gibbs-Donnan potential. The resulting potential will be the sum of the partial potentials caused by different species of charged particles. The partition coefficient $\Phi = (1 - \lambda)^2$, where λ is the ratio between the stokes-einstein radius of the solute and the pore radius. If we multiply the ions I by respective charges, and add all ions in the system, we get the charge density ρ. (69)

$$\rho = \sum_i \Phi_\mathrm{i} C_{i,bulk} z_i e^{\frac{F z_i}{RT} V_d}$$

Assuming electroneutrality, the above sum should equal zero at a certain voltage – the Gibbs-Donnan potential V_d. The equation above can be solved numerically using Brent's method.

$$c_{pore\ interface} = \sum_i \Phi_\mathrm{i} C_{i,bulk} e^{\frac{Fz}{RT} V_d}$$

where V_d is the Gibbs-Donnan potential at respective pore interfaces.

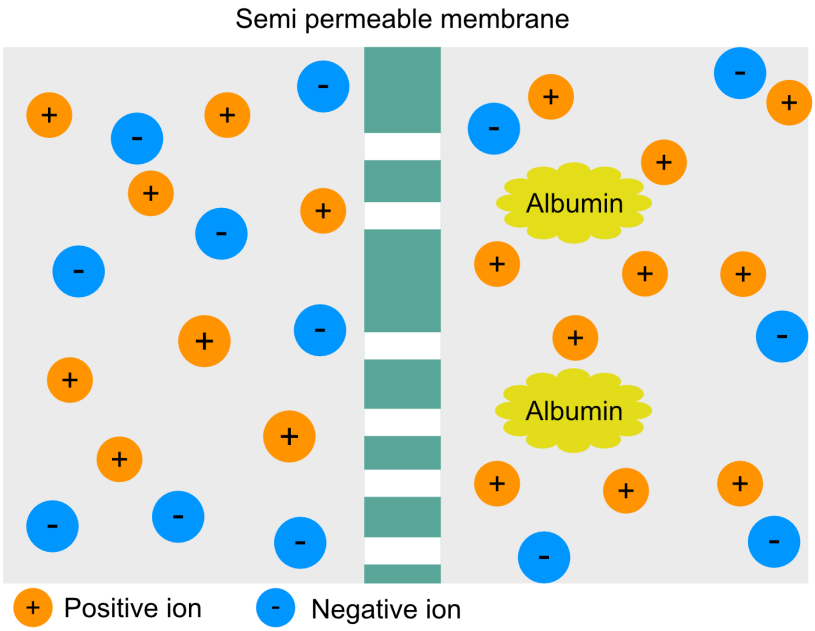

Figure 3: Schematic image of the Gibbs-Donnan effect near a semi permeable membrane. On the left side, there is no albumin, while on the right side albumin is present. There is electroneutrality on both sides, buu close to the membrane, the presence of albumin causes attracts positive ions, causing a slightly different distribution of ions close to the membrane.

Nernst-Planck Equation

One of the weaknesses of the Patlak model is that it does not account for electrical fields in and near the membrane. The Nernst-Planck model describes solute flow, involving convection, diffusion and electric fields. (70)

$$J_{total} = J_{diffusion} + J_{convection} + J_{electric} =$$

$$-D\nabla \mathrm{c} + C\overline{v} - \frac{Dze}{k_B T} cE$$

Note that the above expression consists of three terms, where the first is diffusive ad derived from Fick's law. The reason for the minus sign is that flux will be positive given a negative concentration gradient (a gradient from higher to lower concentration). The second term is convective and is the product of concentration and the flow velocity of the bulk solution. The third term is derived from the Nernst equation and describes the flux of electrolytes in an electrical field.

The isocratic model for peritoneal transport

In 2021, Martus *et al* extended the Henderson-Nolph model for diffusion capacities by assuming a constant ultrafiltration rate. Here the concentration change with respect to time is given by

$$\frac{dc}{dt} = \frac{1}{V_t}\left(\left(\frac{V_t}{V_0}\right)^{\frac{2}{3}} PS\left(C_p - c\right) + \frac{J_v W}{2} \cdot \frac{C_p - c}{2} - J_v c\right)$$

Here, V_t is the intraperitoneal volume as a function of time, V_0 is the initial intraperitoneal volume, W is the hydraulic restriction factor, J_v is the ultrafiltration rate, C_p is the plasma concentration of a solute, c is the solute concentration in the dialysate as a function of time, and PS is the diffusion capacity which is scaled by a volume factor $\left(\frac{V_t}{V_0}\right)^{\frac{2}{3}}$, according to the cube-square law. (43, 71)

Phenomenological model for water transport

In the 1990s, Bengt Rippe and Gunnar Stelin described water transport in peritoneal dialysis using the equation, by describing the dialysate volume as a function of time t. (72)

$$V(t) = V_0 + a_1\left(1 - e^{-kt}\right) - a_2 t$$

Here V_0 is the initial volume, a_1, a_2 and k are phenomenological constants, where a_1 is related to the flow of water due to the glucose gradient and a_2 is directly associated with the osmotic pressure differences in the Starling equilibrium.

Aims and research questions

Study 1

In this study, the purpose was to use a rat model to investigate the mechanisms of blood plasma refilling by measuring the volume of blood plasma during peritoneal dialysis with two different ultrafiltration rates. It was hypothesized that plasma volumes will significantly decrease when ultrafiltration is very high, indicating that the comparatively low ultrafiltration rates observed in peritoneal dialysis are the reason why acute decrements in blood pressure (intradialytic hypotension) is typically not observed during peritoneal dialysis treatments.

Study 2

In study two, a method was developed to estimate the ultrafiltration rate in peritoneal dialysis from the kinetics of sodium. A novel model was developed based on the isocratic transport equation, by (i) correcting the sodium concentration change for free water transport, (ii) including the Gibbs-Donnan effect and (iii) correcting for the plasma water effect. The aim was to develop and test the new method in animal experiments and then on clinical cohort data. An additional aim was to investigate if the corrections for Gibbs-Donnan and plasma water effects are valid in the three-pore model.

Study 3

In the third study, the concept of using sodium as an indicator of peritoneal transport was expanded upon. Building on key findings in the previous studies, the aim was to develop and experimentally test a modified version of the three-pore model that incorporates electrostatic effects through a Nernst–Planck description of electrolyte transport. By introducing electrostatic interactions, the model was designed to more realistically capture the coupled transport of sodium, water, and other solutes across the peritoneal membrane. Can the modified model be used to predict water and solute transfer solely based on sodium kinetics during peritoneal dialysis?

Methods

Experiments

Experimental rat models have been central in all three studies. Rat models have been used in peritoneal dialysis research for a long time for a wide variety of purposes. (73) In our group, very similar setups have been used numerous times. (71, 74) ^{51}Cr-EDTA, (EDTA marked with a radioactive chrome isotope) was generally used as a marker for extracellular fluid and delivered as a continuous infusion. ^{125}I-albumin (albumin marked with a radioactive iodine isotope) was used as a marker for plasma volume or intraperitoneal volume where this was applicable. Specifics for each experiment setup are thoroughly explained in respective papers.

In study 1, dialysis was performed on two groups of rats, one with a very high glucose content in the dialysate and one with low. The purpose of this was to achieve very high ultrafiltration rates in one group and close to zero ultrafiltration rates in the other. The number of animals was chosen with respect to effect size by performing a power calculation, so that there was over 80% power to detect a 5% change in plasma volume. Plasma volume was estimated by injecting a known amount of ^{125}I-albumin, allowing it to distribute in the plasma volume, and then drawing a blood sample of known volume, allowing for estimation of the volume of distribution of the ^{125}I-albumin. The method was validated in the lab, which is explained is a separate section. In all experiments, blood and dialysate chemistry was measured on an iSTAT (Abbott, Abbott Park, IL), and activity measurements for radiopharmaceuticals were measured using a gamma counter (Wizard 1480, LKB-Wallac, Turku, Finland).

In study 2, experiments were designed with the purpose of studying the sodium dip and water transport in relation to sodium. Therefore, six groups of rats were constructed with three different levels of dialysate glucose content (1.5%, 2.3% and 4.25%) and two different dialysis fluid sodium concentrations (125 mmol/L and 134 mmol/L). The purpose of this study design was to vary both ultrafiltration and the sodium gradient, to allow for evaluation of different ultrafiltration rates and different values of the sodium dip.

A similar setup as before was used in study 3. Here the rats were also divided into six groups with three different dialysate glucose contents (1.5%, 2.3% and 4.25%) and two different initial fill volumes (20 mL and 30 mL). In this case the aim was to estimate both water and electrolyte transport. In this study, the objective was to estimate both water and electrolyte transport across the peritoneal membrane. As in previous studies, a range of glucose levels was used to induce a range of water transport rates. At the same time, the two different fill volumes influenced the diffusion capacities, based on geometry (differing diffusion area) and the amount of solvent fluid (keeping osmotic pressures more stable with higher volumes). A large variety of electrolytes was measured in the dialysate at tight intervals during the experiment.

Albumin based estimation of intra-peritoneal volume

Intraperitoneal volume was estimated in the experiments based on the dilution of ^{125}I-albumin, according to a method developed by Zackaria and Rippe. (75) The intraperitoneal volume is calculated from the dilution of ^{125}I-albumin, corrected for the clearance of ^{125}I-albumin from the peritoneal cavity. A first-order elimination of ^{125}I-albumin is assumed.

Validation of ^{125}I-Albumin based plasma volumes

The plasma volume in study 1 was calculated from the dilution of radio iodinated (^{125}I) serum albumin. (76-78) To validate the method, an experiment was performed where plasma volume measurements were performed in a beaker of rat blood. As a reference method, the erythrocyte volume fraction was measured by centrifugation of glass capillaries. The result revealed a systematic error where the radio iodinated albumin-based method was on average 88% of the volumetrically measured. Therefore, a correction factor was applied to all albumin-based plasma volumes (1/0.88 = 1.136).

Clinical cohort

Results derived from animal experiments need to be validated in clinical data to be applicable. Therefore, in study 2, the method derived in animal experiments was tested and validated on a clinical cohort from an earlier study. (44) The cohort was chosen due to its sodium data and thoroughly measured ultrafiltration, including residual volume. In the original study, patient data from 21 peritoneal dialysis patients were collected with the aim of assessing a novel method for estimating osmotic conductance to glucose. Data were anonymised and therefore the risk of

leaking personal data or any other harm to the patients in the cohort was considered low. The potential outcome of the study was assessed as beneficial for peritoneal dialysis patients in general. Ethics are discussed in more detail in the ethical considerations section.

Computational models

Compartment model for fluid exchange

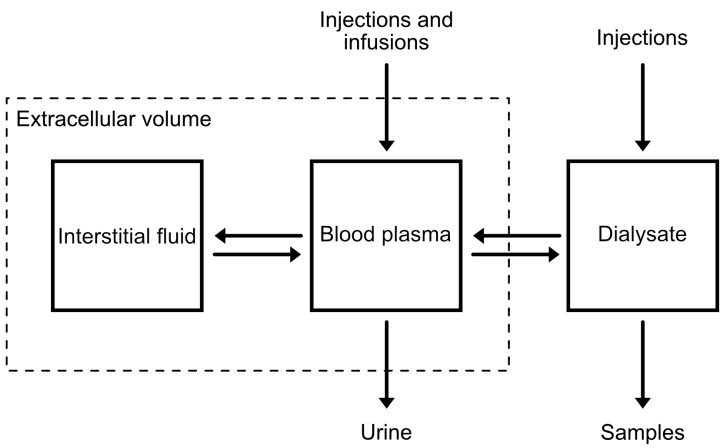

Figure 4: Illustration of the compartment model in study 1. Water is recruited from the blood plasma due to the steep glucose gradient, in turn there is being refilled from the interstitial fluid compartment. Together, the interstitial fluid compartment and blood plasma compartment constitute the extracellular fluid compartment.

In study 1, a compartment model was created with three fluid compartments, the interstitial volume V_I, plasma volume V_P, urine volume V_U and dialysis volume V_D. The extracellular fluid volume is $V_{ECV} = V_I + V_D$, which is assumed equal to the

volume of distribution of Cr-EDTA. Before the the start of dialysis the above diagram can be simplified.

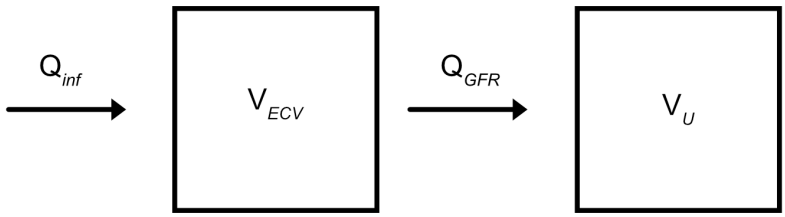

Figure 5: Before dialysis, a more simple model is used to calculate the extracellular volume. ^{51}Cr-EDTA distributes in the extracellular volume and is eliminated through the kidneys. Q_{inf} and Q_{GFR} denote the inflow due to infusions and outflow due to urine production (in this case equivalent to glomerular filtration rate).

In the figure above, Q_{inf} and Q_{GFR} denote the inflow due to infusions and outflow due to renal removal of water. This was used before the start of dialysis to estimate the extracellular volume in the rat.

$$V_{ECV}\frac{dC}{dt} = Q_{inf}C_{inf} - Q_{GFR}C(t) - K_{ex}C(t)$$

Here concentrations are denoted C, fluxes Q and volumes V. Essentially, this equation states that the change in the amount of Cr-EDTA over time will be the sum of influx through infusions $Q_{inf}C_{inf}$ (regarded as a source), renal losses $Q_{GFR}C(t)$ and extra-renal losses $K_{ex}C(t)$ such as radioactive decay (regarded as a sink in this case). Solving the equation makes it possible to estimate the extracellular volume V_{ECV}.

After dialysis was started, ^{125}I-albumin was used to measure the plasma volume multiple times. Plasma volume was calculated as $V_P = \frac{A_{RISA}}{C_{RISA}} - V_{exogenous}$, where A_{RISA} is the activity of ^{125}I-albumin and C_{RISA} is the activity per unit volume in the blood sample. $V_{exogenous}$ is the amount added through injections and infusions.

Modified isocratic model

In study 2, the isocratic model for diffusion capacities was used to model the sodium concentration in dialysis fluid, while accounting for fractional free water transport, here denoted fFWT. (71) The model keeps the assumption of a constant ultrafiltration rate, meaning that the intraperitoneal volume V as a function of time t is $V(t) = J_v t + V_0$.

$$\frac{dc}{dt} = \frac{1}{J_v t + V_0}\left(\left(\frac{J_v t + V_0}{V_0}\right)^{\frac{2}{3}} PS\left(C_p - c\right) + J_v(1 - \mathrm{f}FWT) \cdot \frac{C_p - c}{2} - J_v c\right)$$

Free water transport was set to 50% based on literature values (79-82). It is worth to note that it has been shown that free water transport contributes more to total ultrafiltration in rats than in humans (83). Here, plasma concentrations Cp were corrected for plasma volume fraction and the Gibbs-Donnan effect by multiplying by the factor $q = 0.96^z/0.93$, where z is the valence of the ion in question. (67, 84)

There are many assumptions in this method that allow for the system to be solved as a simple ordinary differential equation, using a shooting algorithm to find the ultrafiltration rate J_v as the unknown variable (using a root-finding algorithm). The downsides are that the assumptions introduce a rigidity to the system, meaning that the result may be less reliable if the assumptions are not fulfilled, leading to systematic deviations between model predictions and physiological reality. In a situation such as peritoneal dialysis, it is possible for local changes to occur in the peritoneal capillaries affecting plasma water and ion concentrations. On the other hand, a model that accounts for these effects would be much more complicated and possibly lacking a unique solution.

Three pore model and electrostatics

For study 2 and study 3, the three-pore model was modified in different ways, then simulations were performed to estimate peritoneal transport parameters. Mainly, the models for solute transport have been modified to test the respective hypothesises. In this section there will be a brief explanation the general reasoning and biggest differences behind each of the models and some discussion concerning the model choices.

Study 2

In study 2, the purpose of the three-pore analysis was to validate the Gibbs-Donnan/Plasma water factor $q = 0.96^z/0.93$ for the experimental model. To achieve this, the classic three pore model was modified by multiplying plasma concentrations with the factor q. Diffusion capacities were set as regression parameters and fitted to experimental data for each solute in each animal using a non-linear least squares method. This was done for both the unmodified and modified models.

Study 3

The purpose in study 3 was to further develop the three-pore model so that it corrects for the Gibbs-Donnan effect, plasma water concentration and incorporates electrostatic phenomena within the membrane. In this case, the transport of solutes was instead modelled using the Nernst-Planck equation. This expression consists of three terms.

$$j_i = -H_i D_i A_0 \frac{dc_i}{dx} - H_i D_i A_0 \frac{Fz_i}{RT}\frac{d\psi}{dx} c_i + J_{v,pore}(1-\sigma_i)c_i$$

The first term is derived from Fick's law and describes diffusive flux for a single ion in the system. Here $H_i = A/A_0$ is the diffusive restriction factor, D_i is the diffusion coefficient and A_0 is the total unrestricted area of diffusive exchange. The second term describes the movement of ions due to the electric field that arises within the membrane. Finally, the third term describes solute transport due to convection. The electrostatic field that arises within the peritoneal membrane can be modelled in different ways. In the chosen model, the electric field was assumed to be constant (meaning a linear potential field) $\frac{d\psi}{dx} = -V_m/\Delta x$, where V_m is the potential across the membrane (interface-to-interface) that results in a zero net current. This is a simple model that allows for faster computations. As an alternative, a Teorell-Meyer-Sievers field was utilized, which is a more complex field that arises under the assumption of zero net current and pointwise electroneutrality. It is explained in the next section.

The pore interface concentrations were given by the earlier mentioned relationship

$$c_{pore\ interface} = \sum_i \Phi_i C_{i,bulk} e^{\frac{Fz}{RT} V_d}$$

And charge density

$$\rho = \sum_i \Phi_i C_{i,bulk} z_i e^{\frac{Fz_i}{RT} V_d} = 0$$

Where V_d is the potential at respective pore interfaces. Assuming electroneutrality is equivalent to assuming a charge density of zero. The equation above can be solved numerically using Brent's method. Knowing V_d, it is possible to calculate the individual ion concentrations of each ion at the pore interface. The potential at the plasma-pore and dialysate-pore interfaces were used as boundary conditions for the field within the membrane V_m. The figure below shows an illustration of the three electrostatic fields; plasma- and dialysate-side Gibbs-Donnan fields and the field within the pore.

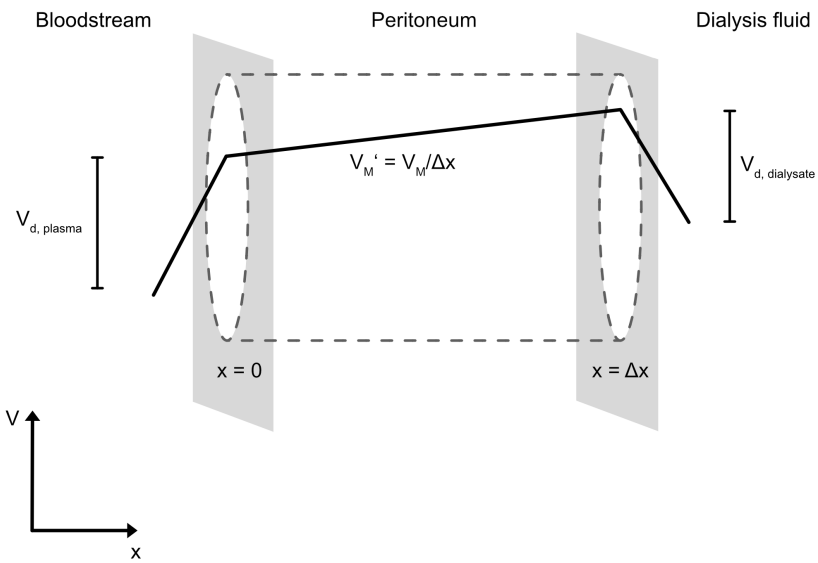

Figure 6: Illustration of the electric potential across the peritoneum due to the Gibbs-Donnan effect and electrostatic effects, assuming a linear field. The initial increase in voltage is the plasma side Gibbs-Donnan, which arises due to redistribution of plasma proteins. On the dialysate side, there is a corresponding Gibbs-Donan potential. Across the pore itself, there is a linear increase in electric potential.

$$J_{v,pore} = \alpha_{pore} L_p S(\Delta P - \Delta\pi)$$
$$= \alpha_{pore} L_p S\left(\Delta P - RT\sum_{i=1}^{N} \varphi_i \sigma_{pore,i}\left(C_{p,i} - C_{D,i}\right)\right)$$

Note that for the ultrasmall pore σ_{pore} equals zero. Here the osmotic pressure difference is estimated from the solute concentrations on each side of the membrane from a modified van't Hoff's Law, using the concentration C_s for each solute s and the respective osmotic coefficient φ_s. (85)

$$\pi = RT\sum_s \varphi_s C_s$$

R and *T* are the gas constant and body temperature in Kelvin, respectively. This approach was chosen because of the great range of solute data made it possible to make a reasonable estimation of osmotic pressure.

Solving the Nernst-Planch equation with the Teorell-Meyer-Sievers field assumptions

According to the 1D Nernst-Planck equation, solute flux is the sum of diffusive, electro-diffusive and convective solute flux – all described as an ordinary differential equation, as follows:

$$J_i = J_v W_i c_i(x) - H_i D_i A_0 \left(\frac{\mathrm{d}c_i(x)}{\mathrm{d}x} - \frac{F z_i}{RT} c_i(x) \frac{\mathrm{d}\psi(x)}{\mathrm{d}x}\right)$$

The concentration function for each solute species *i* is described by an unknown function $c_i(x)$. The single dimension *x* is here in the direction of transport. Importantly, if the solute species is non-neutral ($z_i \neq 0$), it is under the influence of an unknown electrostatic field $\mathrm{d}\psi(x)/\mathrm{d}x$ resulting in an electric current $I_i = Fz_iJ_i$. The Teorell-Meyer-Sievers field arises from two assumptions: (*i*) local electroneutrality and (*ii*) zero net current. This is a realistic assumption in biological systems where there is no net accumulation of charge on a macroscopic scale. First the ordinary differential equation above is rearranged separately for the electrostatic field $\frac{\mathrm{d}\psi(x)}{\mathrm{d}x}$ for both assumptions.

For the electroneutrality assumption:

$$\frac{\mathrm{d}\psi(x)}{\mathrm{d}x} = \frac{\sum_{i=1}^{N} z_i H_i D_i A_0 \frac{\mathrm{d}c_i(x)}{\mathrm{d}x} - J_v \sum_{i=1}^{N} z_i W_i c_i(x)}{\sum_{i=1}^{N} z_i^2 H_i D_i A_0 c_i(x)} \cdot \frac{RT}{F}$$

For the zero current assumption:

$$\frac{\mathrm{d}\psi(x)}{\mathrm{d}x} = \frac{\sum_{i=1}^{N} z_i \frac{J_i - J_v W_i c_i(x)}{H_i D_i A_0}}{\sum_{i=1}^{N} z_i^2 c_i(x)} \cdot \frac{RT}{F}$$

This forms a coupled system of differential equations. A shooting algorithm was used to find the derivatives $\frac{\mathrm{d}c_i(x)}{\mathrm{d}x}$. To achieve this, the initial Nernst-Planck equation was differentiated.

$$\frac{d^2 c_i(x)}{dx^2} = \frac{J_v W_i}{H_i D_i A_0} \frac{\mathrm{d}c_i(x)}{\mathrm{d}x} + \frac{F z_i}{RT} \left[\frac{\mathrm{d}\psi(x)}{\mathrm{d}x} \frac{\mathrm{d}c_i(x)}{\mathrm{d}x} + \frac{\mathrm{d}^2\psi(x)}{\mathrm{d}x^2} c_i(x) \right]$$

The 2nd order differential of the electrostatic field is also needed.

$$\frac{\mathrm{d}^2\psi(x)}{\mathrm{d}x^2} = \left[\frac{\sum_{i=1}^{N} z_i \frac{J_v W_i c_i(x) - J_i}{H_i D_i A_0}}{\left(\sum_{i=1}^{N} z_i^2 c_i(x)\right)^2} \sum_{i=1}^{N} z_i^2 \frac{\mathrm{d}c_i(x)}{\mathrm{d}x} - \frac{\sum_{i=1}^{N} z_i \frac{J_v W_i}{H_i D_i A_0} \frac{\mathrm{d}c_i(x)}{\mathrm{d}x}}{\sum_{i=1}^{N} z_i^2 c_i(x)} \right] \frac{RT}{F}$$

Here it is evident that $\frac{\mathrm{d}^2\psi(x)}{\mathrm{d}x^2} \neq 0$ despite the assumption of local electroneutrality which may seem to be a contradiction according to Gauss law since local electroneutrality $\rho=0$ implies Poisson's equation reduces to the Laplace equation in the bulk, i.e. $\frac{\mathrm{d}^2\psi(x)}{\mathrm{d}x^2} = 0$. However, all dimensions involved here greatly exceed the Debye length and local electroneutrality is an acceptable approximation. Stated differently, ψ is not being solved as an electrostatic potential from Poisson's equation, but as a *constraint field* ensuring transport constraints (zero net current + local electroneutrality).

To solve the coupled system numerically, it was rewritten as a second-order boundary value problem. The electric field $E(x) = \frac{\mathrm{d}\psi}{\mathrm{d}x}$ was then computed at each x from the equation that satisfies the zero-current assumption, whereas its derivative E'(x) is obtained from differentiating the equation that satisfies the electroneutrality relation.

Phenomenological model

The phenomenological model for water transport developed by Rippe and Stelin was used to model sodium concentration $C(t)$ (instead of intra-peritoneal volume) (72)

$$C(t) = \mathrm{C}(0) + a_1\left(1 - e^{-kt}\right) - a_2 t$$

Given the earlier established relation between water and sodium, this equation could possibly be used to describe the kinetics of sodium.

Statistical analysis and software

The R programming language was used for all calculations (versions are specified in the papers), together with a variety of packages. (86-99) Linear regression and Bland-Altman plots were used for method comparison. Both these methods assume normally distributed, independent samples.

In the animal experiments, data was not assumed to be normally distributed due to the small groups. For group comparisons in study 2 and 3 an aligned rank transform was applied (R-package "ARTool"), and then hypothesis tests were performed using a factorial 2x2 analysis of variance (ANOVA) to account for both, in which case the ANOVA becomes non-parametric, as opposed to an ANOVA on non-transformed data. (100) It is worth to note that the aligned rank transform is not without criticism and has been found to inflate type 1 errors in interactions (finding an interaction when there is no true interaction). (101)

Non-linear least squares algorithms were used for parameter estimations in the three-pore model. Calculations were performed using the *minpack.LM* package and the *stats* package, that implement the Levenberg-Marquardt and port algorithms, respectively. (102, 103) A non-linear regression algorithm can adapt well to complex data, however one major downside is that there usually is more than one valid solution, as opposed to linear regression. Also, a non-linear regression model is prone to overfitting, which can lead to inaccurate predictions.

For solving ordinary differential equations, the *deSolve* package was used. The second order ordinary differential equation system for the Teorell-Meyer-Sievers field was solved using the *bvpSolve* package. (91, 92)

AI usage

No generative AI was used to write the text of this thesis. Microsoft Copilot was used to search and summarise documentation used when writing the code in studies 2 and 3.

Results

Study 1

This is an experimental study that investigates the effect of very high ultrafiltration rates on plasma volume in Sprague-Dawley rats. Rats were divided into two groups, one with high ultrafiltration rate and one with very low ultrafiltration rate.

Key findings

- No significant difference in plasma volume was observed after 30 minutes of peritoneal dialysis, even though ultrafiltration rate was extremely high in the group with high ultrafiltration rate. Mean ultrafiltration rates were 56,4 ml/kg/h (SD: 9) in the high ultrafiltration group and -7.8 ml/kg/h (SD: 3.6) in the low ultrafiltration group. Over the course of the entire dwell, this corresponds to an ultrafiltration volume of 8.0 in the high ultrafiltration group and a volume of -1.2 in the low ultrafiltration group.
- Heart rate, mean arterial pressure and central venous pressure was not significantly changed after 30 minutes of dialysis ($p=0.30$, $p= 0.60$ and $p=0.86$, respectively). From visual assessment of the plots of each parameter, there is a slight tendency of a decrease in central venous pressure.
- Extracellular volume decreased 11% (SD: 3.9%) in rats with subjected to high ultrafiltration rates but increased 3% (SD: 1.4%) in the group with low ultrafiltration rate after 30 minutes of dialysis ($p<0.01$ for difference between groups).
- Validation of ^{125}I-albumin based plasma volume measurements showed a bias when compared volumes measured using centrifugation of glass capillaries. The volumes from the albumin-based method corresponded on average to 88% (SD: 0.03%) of the volumes from the centrifugation method.

Study 2

In this study, a mathematical model was developed to estimate the ultrafiltration rate from the sodium dip. The model was tested experimentally in rats and then in humans. A modified three pore model was used on the same data to validate correction factors for the Gibbs-Donnan and plasma water effects.

Key findings

- Visual analysis of the intraperitoneal volume curve showed the steepest increase in volume happened at the beginning of the dwell. This increase is most pronounced in the groups with high dialysate glucose content and is equivalent with the ultrafiltration rate being the highest at the beginning of the dwell.
- Sodium dip-based estimation of ultrafiltration rate was highly correlated with volumetrically measured ultrafiltration rate ($R^2 = 0.76$) in rats. Bland-Altman analysis showed a high degree of agreement with mean difference of -1.7µl /min.
- In a separate analysis on clinical data from a previous study, the albumin dilution-based ultrafiltration rate correlated moderately (R^2=0.35) with the sodium dip-based estimation of ultrafiltration rate. Mean difference was 2 ml/min (95% CI: -401 – 405 ml/min) in Bland-Altman analysis.
- In the same patient cohort, osmotic conductance to glucose calculated based on ultrafiltration rates from the sodium dip method correlated well (Pearson's $r = 0.77$) with osmotic conductance to glucose calculated using a volumetric reference method. Bland-Altman analysis showed low bias with a mean difference of −0.008 L min−1 mm Hg−1 (95% CI: -2.94 – 2.92).
- Three-pore model simulations with corrected concentrations for Gibbs-Donnan and plasma water effects fit experimental data closely, based on visual assessment, indicating that the theoretical factors are in line with experimental data.
- Dialysate to plasma clearance of ^{125}I-albumin was lower than the disappearance rate from the dialysate and neither was associated with either sodium content or glucose strength. The discrepancy means that all ^{125}I-albumin that leaves the abdominal cavity does not appear in the plasma.

Study 3

In this study, a modified version of the three-pore model was developed that incorporated electrostatic effects and was used to predict transport in experimental peritoneal dialysis in rats.

Key findings

- Ultrafiltration and ultrafiltration rate both varied significantly with fill volume ($p<0.001$) and glucose concentration ($p<0.01$).
- Dialysate sodium kinetics were well described by a phenomenological model when assessed visually. The initial slope of the model, which should correspond to the ability of the peritoneum to transport water, varied significantly with both fill volumes ($p<0.001$) and dialysate glucose concentration ($p<0.001$). The initial slope was steeper in groups with higher glucose content. Final slopes only varied significantly with fill volume ($p<0.05$).
- The sodium dip occurred later in groups with larger fill volume ($p<0.05$) and the sodium dip itself was larger with higher glucose concentrations $p<0.001$). The median sodium dip was more than double in the groups with high ultrafiltration rate.
- From visual assessment, a modified version of the three-pore model accurately predicted water and small solute transport after being fitted to individual sodium data to determine the water and solute permeabilities (parameters L_pS and $A_0/\Delta x$).
- Water permeability was higher in groups with lower glucose strength ($p<0.001$), but there was no significant association between solute permeability and glucose strength or fill volume ($p=0.41$ and $p=0.18$, respectively).
- There was a correlation between the magnitude of the sodium dip and osmotic conductance to glucose (Spearman rho = 0.82), and an inverse correlation between sodium dip and the surface area parameter (Spearman rho = -0.42).
- After performing parameter estimation using a linear field model the simulations utilizing a more complex Teorell-Meyer-Sievers field to simulate transport in peritoneal dialysis differed only slightly from the simulations using the linear field within the pore (Figure 7).

- Similarly to study 2, dialysate clearance of ^{125}I-albumin was lower than the expected appearance rate in blood, but it corresponded well to the fluid exchange predicted by the three-pore model. The clearance was not associated with either glucose strength or fill volume.

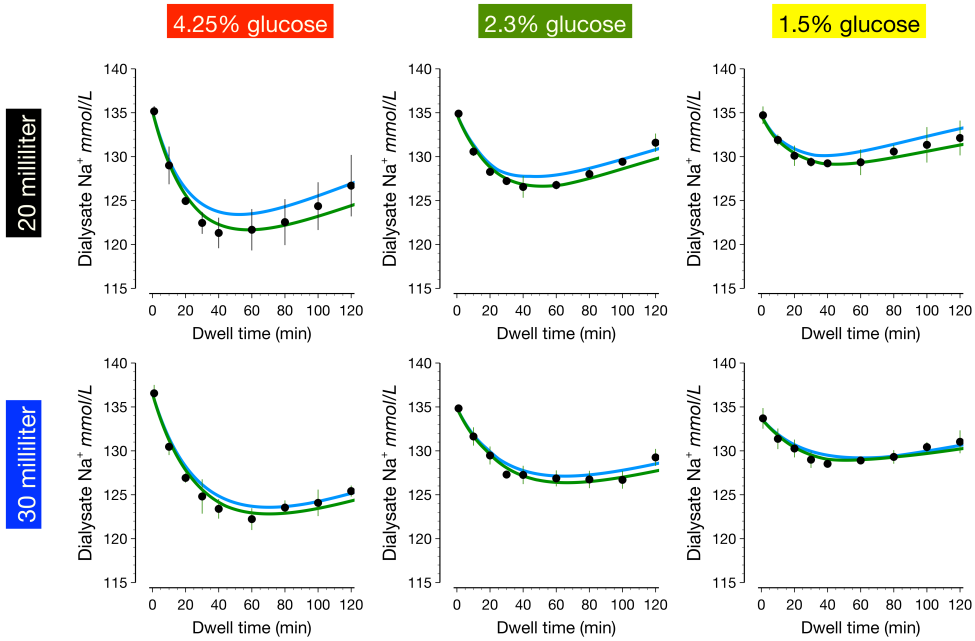

Figure 7: Model fit to experimentally measured dialysate sodium concentrations in six different groups of sprague dawley rats. The predicted sodium concentrations are represented by the lines and were conducted after parameter estimation using either the linear field (green line) or the Teorell-Meyer-Sievers field (blue line).

Discussion

Despite being investigated for several decades, peritoneal transport mechanisms are still not fully understood. So far, it is known that water is transported by paracellular, transcellular, and lymphatic pathways. Transcellular water transport occurs through aquaporins and is therefore free from solutes, a phenomenon that is well described in literature. For sodium transfer, convection dominates in the beginning of a dialysis dwell (lowering dialysate sodium), while diffusion dominates in later stages (increasing dialysate sodium). A central finding of this thesis is that the capacities for convective water transport and solute diffusion can be characterized using sodium kinetics in the dialysate alone.

Sodium kinetics, ultrafiltration and solute transport

The phenomenological model for fluid transport, initially developed by Rippe and Stelin describes the kinetics of dialysate sodium well. There is an initial steep decline in sodium concentration until it reaches a minimum, and then the concentration increases again. An explanation for this is that the steep glucose gradient in the beginning of the dwell draws water very effectively, making water driven convection (paracellular) and sieving (transcellular) dominating over diffusive processes. In study 2 we hypothesize that the sodium dip will reflect water transport during peritoneal dialysis, if corrected for the influx of sodium due to convective paracellular transport. The results, where we see a strong correlation between sodium-dip based estimates of ultrafiltration rate and volumetric measurements are well in line with the hypothesis. Results from study 3 also indicate that convection is the dominating transport process for sodium, which is in line with earlier research on its correlation to water transport and osmotic conductance to glucose. Together, the studies present not only solid indications that the sodium dip reflects the water transport, but also a viable method to estimate it.

There are a couple of factors that can complicate the estimation of water transport in peritoneal dialysis. In studies 2 and 3 there is a discrepancy between the appearance of ^{125}I-albumin in dialysate and the disappearance of the molecule from the peritoneal cavity. This means that the fluid absorption rate is not only due to the lymphatic return to the bloodstream, but also due to a parallel absorption elsewhere, for example into the tissue surrounding the peritoneum. The good fit with the three-pore model in turn supports that the measured disappearance rate of albumin from

the abdominal is a good marker for the fluid absorption rate. Since the 1990's, dextran has been commonly used to estimate the lymphatic reabsorption from the peritoneal cavity. (104) In many ways, albumin can be preferable because it exists naturally in the body and generally foreign substances have the potential to cause adverse effects.

Simulations using the Gibbs-Donnan and electrostatic effects are more successful in predicting the solute transport in peritoneal dialysis than models that do not incorporate these phenomena. For many years, electrostatic effects within and near the membrane due to the presence of multiple ion species have been hypothesized to be important in the description of transport. (67, 105) The good fit to data in these studies add further evidence to these claims that the electrostatic phenomena can be the key to understanding the transport. Despite this, there are limitations. Both the fixed factor-based approach from study 2 and the Nernst equation-based approach for estimating the Gibbs-Donnan potential from study 3 were static models which makes it less adaptable to changes in local physiological conditions during the dialysis. To account for this, the protein charge, and by extension the Gibbs-Donnan equilibrium, was assumed to be varied with glucose strength to achieve a better fit for chloride. This could reflect changes in the local physiological conditions that lead to slight alterations in the electrical fields which are unaccounted for by the model. For example, haemoconcentration due to local depletion of fluid within the peritoneal capillaries could affect local ion concentrations, in turn shifting the electrostatic potential. Also, from such phenomenon we would expect a local increase in the osmolality, which would reduce the membrane conductivity of water and small solutes. This could explain the decreased solute conductivity in groups with stronger glucose. The relationship between ultrafiltration and the strength of the Gibbs-Donnan potential can be modelled, but further investigation would be needed to validate such model in peritoneal dialysis. Under the influence of an electric field, ions around charged surfaces arrange in electrical double layers. (106, 107) Including this arrangement of ions in a model could be a first step in the development of a dynamic model of the Gibbs-Donnan equilibrium.

In simulations using the Teorell-Meyer-Sievers field, there were only small differences compared to the linear field, which can be interpreted in multiple ways. One possibility is that the field could have had only a small impact on the end result. Alternatively, the model is an inaccurate representation of reality, or both models are equally good representations of the electrostatic effects. Another reason for the apparent lack of impact in using the Teorell-Meyer-Sievers field is that intra-membrane electrostatic effects are small compared to those occurring at the interface between blood plasma and membrane. A different aspect is that the water and solute permeabilities were estimated by fitting the model to sodium data using the linear field in both cases. This was done mainly due to computational limitations, where the solvers failed to converge and/or were very slow. This issue stems from

the nature of the non-linear regression, where there is not one single solution, but many. A way of overcoming the issue with the regression is to construct a custom solver that is adapted to this application, although this comes with the downside of being less applicable to other data.

The model in study 3 fails to predict the transport of potassium, clearly underestimating dialysate concentrations. The interesting thing about potassium in peritoneal dialysis is that fresh dialysis fluid contains no potassium. Hence, the concentration gradient of potassium is relatively high, meaning that diffusive transport should be near maximum capacity according to Fick's law. However, the transport of ^{51}Cr-EDTA was accurately predicted, even though it is also not present in the dialysis fluid from the start. One possible difference is that potassium is abundant within cells and could be released following the destruction of cell membranes. Regardless, further development of the electrostatic models is needed.

Model assumptions

The three studies feature four different approaches to modelling water transport. The models that are presented in this thesis are dependent on assumptions to make calculations possible. In the compartment model in the first study, ultrafiltration is calculated as a simple difference between instilled and drained volume, compensated for an assumed residual volume of 3 ml. The resulting ultrafiltration volume will be influenced by variations in residual volume and the completeness of the final drainage (which is done by opening the abdomen and swabbing out fluid). One of the limitations here is that the study cannot itself discern how the refilling of the plasma volume happens. Also, taken into perspective, the rat receives an infusion of ^{51}Cr-EDTA of 0.05 mL/min, which over the course of 30 minutes accounts for 10-20 % of its plasma volume, which for a human would be considered a significant amount.

The three-pore model is a central part of studies 2 and 3, and it comes with an underlying set of assumptions. Firstly, the electric field is assumed to be a constraint field (rather than a Gaussian field) assuming that there is local electroneutrality and local net zero current and thus no net transport of charges (ions in this case) across the membrane. It should be noted that this does not mean that there is no transfer of ions, but that all such transfer (both using the non-linear constraint field and the linear field) occurs while maintaining global electroneutrality. For the case of the constraint field this was extended to local electroneutrality. On the other hand, at the interface between blood-plasma and membrane, we used Nernst's equation to model the Gibbs-Donnan effect. Nernst's equation assumes no flux of ions and zero convective transport. This, of course, is not true because we know that charged solutes are transferred to the dialysate, and that there is substantial convective

transport. However, the Nernst equation is not used to model transport but to impart a boundary condition for the transport equations.

A study by Galach et al, highlighted the fact that it is important to consider multiple ions in the solution through computer simulations. (105) In study 3 we have done just that, yet there are still improvements to be made to the models of the electric fields. Apart from the earlier mentioned proposition of testing a dynamic approach to the Gibbs-Donnan effect, more aspects of ion-ion interactions could be considered. Here we include steric exclusion at the pore interface based purely on the size of the ion, and one limitation of this is that it does not account for the charge of the ions, which could cause local electric fields to arise that are much more complex. Even small electrical fields can have an impact on the bigger picture, which is indicated by the results of study 3. A different example where small fields have a large impact is one well known effect that has been known to interfere with biosensors that is called the *Debye screening effect*. (108) In peritoneal dialysis the effect mostly applies to albumin. In a saline solution, the highly negatively charged solute will become surrounded with smaller counter-ions. The albumin molecule itself is charged, and so are the ions surrounding it. Therefore, at the nanoscale (within and around the molecule), small electric fields and differences in charges will arise within the Debye length (the characteristic length of an electrostatic field). On the other hand, on a micrometre scale, such a solution would appear completely electroneutral. At the same time there will be a local displacement of ions within the solution to preserve electroneutrality. In any case, our modelling of electrostatic effects at the interface is a gross simplification of the real process. More complicated models of electrostatic fields could be a way forward.

Rat models – strengths, limitations and alternatives

All experiments were performed on rats that were peritoneal dialysis naïve and healthy. This limits the generalizability of the results, when put in contrast with dialysis patients that often have advanced kidney failure in combination with other chronic illnesses. Rat physiology differs from human physiology in several ways. Rats have been commonly used to study physiology in peritoneal dialysis, which is due to several advantages, such as affordability and availability. (109) When comparing humans and rats, the most apparent aspect is the size difference. One could suspect that small changes could have a large impact on the vital parameters of the rat, which could impact generalizability to humans. On the other hand, earlier studies have found water transport comparable in humans and rats, with the exception that the intraperitoneal volume in rats increases quicker and earlier than in humans. (110) This is very important in study 2, where the model assumes a constant ultrafiltration rate, which is equivalent to a linear increase in intraperitoneal volume. The steepness of the early part of the intraperitoneal volume curve fits well with a linear model, while the latter part does not. In humans, the curve could be

expected to be flatter and therefore less linear than in rats. However, in peritoneal dialysis situations, it is often of greater clinical value to know the ultrafiltration rate during the dwell in general, rather than the ultrafiltration rate at a very specific timepoint during the dwell. Using the mean value theorem for integrals, it is possible to show that calculating the ultrafiltration rate using the isocratic model will reflect the mean ultrafiltration rate from the start of the dwell to the time of sodium sampling. As such, the model could be useful even if the assumption of linearity is not fulfilled.

In study 2, the assumption of 50% free water transport fraction is a central part of the model and is in line with earlier studies. (111) The same model performed well when applied to patients even though the fractional free water transport has been shown to be somewhat lower in human subjects. (112) The reason for this could be that the difference between humans and rats in the diluting effect of the free water transport could still be regarded as small. Patients also have a higher biological variation compared to laboratory animals; therefore, it is possible that the assumption is closer to the truth for some parts of the patient cohort and further from the truth for other patients.

Apart from differences in water transport, rats also have been found to have higher ratio of peritoneal surface area. This could cause diffusion capacities to be higher in rats than in humans. (109) The three pore model from study 3 is fitted to sodium data to determine area to diffusion length ratio, and therefore, any difference in area ratio should be accounted for in the model. Regardless, this aspect of rat physiology should be kept in mind when evaluating such results. Similarly, the somewhat unexpected result of study 1, where there seems to be a large transcapillary refilling flow from the interstitial volume into the bloodstream, could also be a consequence of rat physiology. Normally, a comparable loss of blood volume in a human would have had noticeable effects, where a loss of around 15% can cause measurable physiological responses. In the rats, the plasma volume loss corresponded to almost 100% of plasma volume in the high ultrafiltration group. (113) Another aspect and possible source of error that is worth to note is the effect of anaesthesia on rats. There was no effect on blood pressure and heart rate despite a significant decrease in extracellular volume. In a study from 2014, Yang et al noted a significant effect of isoflurane on the cardiovascular system of rats during anaesthesia already at concentrations of 3%, where gas anaesthesia caused a decrease in heart rate, as well as arterial and venous pressures. (114) This could be an important aspect to have in mind when interpreting results, because it could mask cardiovascular effects of the dialysis. At the same time, the unaffected pressures and heart rate that were observed in study 1, in combination with near constant plasma volumes, all point toward the original explanation of a balancing transcapillary refilling rate.

There are alternatives to rats when it comes to animal models, but each comes with its advantages and disadvantages. Larger animals are closer to humans in size, but are often expensive and require advanced facilities. Smaller animals such as mice are often easier to modify genetically but are very hard to work with from a technical perspective due to the size, even though the group has performed dialysis in rats. (109) Regardless of the animal of choice, there will always be differences between animals and humans, which highlights the need for clinical evaluations of any new method in peritoneal dialysis.

A clinical chemistry perspective

Measurements of dialysis fluids often pose a challenge due to matrix effects that can be hard to tackle and can skew results. In peritoneal dialysis fluid, glucose concentrations often range around 150-300 mmol/L, in contrast to a normal plasma glucose that is 4-6 mmol/L. To account for the matrix effects of glucose, the measurement methods for electrolytes and glucose were validated and correction formulas were applied where needed (CV: 0.8% for Na^+, 1.7% for Cl^-,7.1% for HCO_3^- and 5-6% for glucose). This makes results more applicable, but at the same time it can add error when correction factors incorporate other measured parameters in correction formulas. When studying a population, one can get an understanding for analytical error by assessing the spread of values, but clinical implementations often rely on one single measurement, which leads to more strict requirements on analytic performance. Also, laboratory animals have significantly less biological variation than patients. From this point of view, the small experimental groups can be an issue because they may not have enough statistical power to accurately reflect analytical error.

A strength of study 1 was the validation of the ^{125}I-albumin dilution-based plasma volume estimations. Apart from the systematic error, the method appears to be very precise within the measured interval. The measured plasma volumes in the rat are of the same magnitude as the volumes measured in the validation dataset. To our knowledge, this type of validation has not been performed in rats before. Ideally, a validation of a method such as this should be expanded to include different levels of plasma volume to investigate aspects such as coefficient of variation (commonly referred to as CV), linearity and limits of quantification. Because the plasma volumes in the validation only cover a very limited range (around 7-8 mL), it is difficult to draw conclusions about how the method performs when plasma volumes become smaller or larger than the ones in the validation, even though such volumes could be of interest in the study.

Reflections on study design

When testing new methods (such as the models in this thesis), it is important test one variable at a time. This was done in all experiments in this thesis in different ways depending on the research questions in mind. The validation of plasma volumes in study 1, was performed on a beaker of rat blood to remove all physiological variation that stems from the body of the animal responding to external stimuli. This is essential when assessing the internal validity of the method, and in this case the only source of variance was the method itself, which minimises bias. When assessing the physiological effects themselves, animal experiments are robust because of the very low biological variation, which decreases the risk of biological factors other than the intervention influencing the results of the study. An additional strength of the studies in this thesis is that they were all prospective, which decreases the risks of many common biases such as confounding or recall bias. (115) The clinical cohort study was also prospective, and this type of study is generally considered as strong evidence. Generally, these types of studies can prove causality in a way that, for example, observational studies cannot. An example of this from the world of peritoneal dialysis is the relationship between Kt/V and clinical outcomes. Several observational studies had suggested that Kt/V is associated with higher mortality, and the inverse associated with better clinical outcomes. Later, a randomised controlled trial could not confirm the same association. (116, 117)

A weakness in the experiments is that the animals were not randomized into groups, which could introduce bias. Even though animals are genetically identical, the phenotype will still vary. Factors such as cage placement, personnel and other environmental factors can influence how animals respond to treatments. (118) The endpoints are measured very soon after exposure, before the rats wake up from anaesthesia and this decreases the risks of animals adapting differently post exposure, simply due to the timeframe. The animals in these studies were not randomised to treatments, which could introduce a risk of bias due to aforementioned effects causing a difference between batches. Also, the lab technician performing the experiments was aware of what treatment was given, which could give rise to observer bias and leading to systematic error, for example registering higher ultrafiltration rates in groups with higher levels of dialysate glucose. On the other hand, the lab technician was not informed of the hypothesizes or aims of the studies until after the experiments, which decreases the risk of observer bias. To further minimize these risks, the animals should be randomized into groups, for example using a block randomisation approach with blinding which is widely considered a robust design and highly applicable to the experiments in this thesis due to the clear division into groups. (119)

Choosing the right model for the right application

In physiology, and in mathematical modelling in general, one of the greatest challenges is choosing a model that is useful. In this thesis, different approaches to modelling of water and solute transport are presented that incorporate different levels of detail and account for different physical phenomena. Generally, detailed mechanistic aspects of certain phenomena are often less relevant in clinical situations than high-level descriptions of the effects. Regardless of the level of detail, a mathematical model will always be an approximation or a simplified image of reality, and therefore, the model needs to be adapted to its intended purpose. The three pore model from study 3 could, for example, be applied to data in clinical situations, but this would require rigorous collection of data from the patient and careful interpretation of the results because of its many pitfalls, making it impractical. Also, it introduces many assumptions which would need to be validated for it to be implemented in clinical practice. Conversely, it is useful in research situations, when there is a need to simulate physiological effects due to a new treatment or altered physiological conditions. Another pitfall is that the three pore models used in the studies of this thesis rely on statistical approximations of some parameters. This means that the same input might not lead to the same output every time, which can reduce the reproducibility.

In contrast to the three-pore model, the isocratic model from study 2 is much simpler in its construction, and assumptions are fewer and easier to understand because it has one input parameter leading to a defined output. This means that the method will be reproducible, in contrast to a non-deterministic model where different inputs could generate different outputs. This can be crucial in clinical situations, where it is important for the physician to understand the connection between the biology of the patient and measurements. Therefore, a simpler model such as the isocratic model could be much more easily adapted to clinical situations and converted into a method that can be used in the clinic. Another benefit of a simpler model is that it usually requires less computational power to use. At the same time, the isocratic model is more rigid due to its relative simplicity and assumption of constant ultrafiltration rate. Therefore, it may be of limited value when investigating new situations in a research environment, because it may be harder to adapt to severely altered conditions. Also, in clinical practice, the overall ability of the peritoneum to transport water may be much more relevant to the physician than small mechanistic effects, calling for more general models that ignore small mechanistic effects and, instead, focus on the big picture.

To summarize, when putting a model to use, it is important to understand the underlying assumptions and complexity in relation to the task. In 1976, statistician George Box wrote that “all models are wrong, but some are useful”, which is as relevant today as it was then.

Ethical considerations

Animal research is always problematic from an ethical point of view because animals are harmed and cannot consent to experiments. Our experiments involve surgical interventions in rats and exposing to peritoneal dialysis treatment and radiation. To study the models in detail, very precise measurements are needed. These measurements are harmful to the subjects because of the use of surgery and radioactive markers. Therefore, the experiments are conducted in animals rather than in humans. As stated earlier, the potential outcomes can bring substantial benefits to peritoneal dialysis patients. In many cases peritoneal dialysis is the favorable modality and can improve clinical outcomes as well as bring more autonomy to the patient. The aims of these studies are designed with clinical question in mind and always aim to solve a clinical problem.

The principles *replace (I), reduce (II)*, *refine (III)*, are a cornerstone of ethical animal research and were a key part of ethical decisions. The experiments would be unsuitable to perform in humans due to several factors. (120) (I) Firstly, experimental treatments were used, such as dialysis fluids with altered glucose and sodium concentrations. Secondly, the use of radiopharmaceuticals can be harmful to humans and would not be acceptable at this stage. Finally, invasive monitoring and measurements of physiological parameters would not be suitable in human subjects. At this stage, pure *in silico* experiments do not fit the aims of the studies which require more precise experimental data, and to my knowledge this has not been available before. (II) Animal experiments often lead to small datasets, but at the same time there is (in theory) no genetic variation and measurements can be made more precisely due to the access to methods unavailable in humans. In study 1, power analysis was conducted to ensure that we use no more animals that are needed, while having enough statistical power to assess the effects. (III) The animal experiments were performed under total anesthesia, to minimize suffering. Also, the animals were healthy until the experiments and sacrificed before waking up when the experiment was finished, as a humane endpoint. The experiments were designed with scientific rigor in mind, which is discussed elsewhere in this thesis.

Patient data was analyzed in study 2. The data that were analyzed were already collected and no additional patient participation was needed and therefore no more physical risks to patients could be identified. Despite this, there are some considerations to be made. The original collection of the data was conducted using modern ethical principles and was compliant to the *Declaration of Helsinki*, which

could be problematic if that were not the case. (44, 121) Patients had given consent that their data could be used for research that was very similar to the research in study 2. Finally, there is always a risk of health data being compromised, exposing sensitive information about patients which can cause harm the trust between patients and researchers. All data was anonymized to minimize the risk, and researchers did not know the identities of the patients. Also, there were substantial benefits in testing the new model in a clinical population, given the promising results of the animal trial. In this case, the benefits were assessed to outweigh the risks.

Future perspective and applications

With this research, we are hopefully one step closer to uncovering the mechanisms of the peritoneal membrane. Due to its level of detail, the three-pore model may prove useful in future research and can be used to further study transport mechanisms within the human body. The relationships between sodium transport, water transport and the transport of other solutes that are presented in this research could open new uses for already collected data, where there is information on sodium and/or water transport. To my knowledge, similar methods of using a simple sodium sample for drawing conclusions about the kinetics of the peritoneum have not been used before. Even though the models in these studies have been partially successful, the possibilities for the future development of mathematical models in peritoneal dialysis are near endless, given the complexity of physiological processes. Other researchers have suggested everything from accounting for cellular metabolism of solutes to creating virtual patients that can be used for clinical testing *in silico*. (23)

Today, there is a clinical study in progress, where the isocratic model for estimations of ultrafiltration rate is being put to use to develop a new peritoneal function test. (122) Preliminary results of the study were published last year by Martus et al and showed promising results with strong reproducibility for osmotic conductance to glucose and area to diffusion length ratio. (123) A logical continuation of these studies would be to incorporate peritoneal transport parameters with clinical parameters and clinical outcomes by applying them to both existing and new data. This could make it possible to create new products and tailor treatments that better correspond to the needs of each patient. In the world of peritoneal dialysis it is well known that the environmental impacts of transporting dialysis fluid are substantial and there has been a push towards point of care generation of dialysis fluid. (124) If technological challenges of generating home-based dialysis fluids are overcome, it could create a unique possibility to tailor the contents of the fluid to the needs of each patient before each dialysis dwell. In turn, this requires simple and reliable peritoneal function tests which could be made possible using the research from this thesis.

Conclusions

Study 1

Rats subjected to dialysis with extreme ultrafiltration rates experienced no change in blood plasma volume or vital parameters compared to a null ultrafiltration sham group. A physiological explanation for this could be that there is sufficient transcapillary refilling of fluid into the bloodstream to compensate for high ultrafiltration rates during peritoneal dialysis, possibly due to a shift in osmotic pressure in the peritoneal capillaries.

The dilution of the radio pharmaceutical ^{125}I-albumin proves to be a reliable method for estimating plasma volumes in rats.

Study 2

Ultrafiltration rates in both rats and humans were successfully assessed from the sodium dip using a new model for the sodium kinetics in the peritoneum, which accounts for free water transport, the Gibbs-Donnan effect and the plasma water effect using a correction factor. If provided with sodium data, the model can precisely estimate ultrafiltration rate during peritoneal dialysis. The value for the Gibbs-Donnan/plasma water factor was successfully validated using a three-pore model.

The ultrafiltration rate can, in turn, be used to precisely and reliably estimate the osmotic conductance to glucose in patients, and is it is fully possible to apply the model to clinical studies.

Study 3

Peritoneal transport of water and small solutes can be determined from sodium kinetics alone by fitting a modified three pore model to sodium data and accounting for electrostatic effects within and around the membrane. This is a novel finding that further indicates that electrostatic membrane phenomena play an important role in the transport of water and solutes during peritoneal dialysis. Also, sodium kinetics were accurately described by a phenomenological model originally developed for water transport, highlighting the connection between water and sodium transport.

Final remarks

To summarise, the research in this thesis has explored water and solute transport during peritoneal dialysis using an interdisciplinary approach, incorporating mathematical models, animal experiments and clinical data. The connection of sodium and water transport has been indicated in multiple ways by the studies through both experimental animal models and the analysis of clinical data. The impacts of molecular-scale electrostatic phenomena on the macroscopic phenomenon of ultrafiltration have been explored. Most importantly, new methods for the estimation peritoneal transport kinetics have been proposed and further clinical testing is already in progress. Mathematical models and data offer increasing advantages in our everyday lives, which we all have seen in this age of artificial intelligence. Hopefully, these new findings could help change lives for the better.

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