Clinical, Experimental and Theoretical Studies of Solute and Water Removal in Peritoneal Dialysis

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Peritoneal dialysis (PD) is a cost-effective, home-based kidney replacement therapy that enhances patient autonomy. Nevertheless, the clinical use of PD is limited by chronic fluid overload and excessive glucose absorption, which can lead to complications, such as cardiovascular disease, metabolic disturbances, and deterioration of the peritoneal membrane.

The aims of this thesis were to examine glucose transport mechanisms across the peritoneum and develop a robust, clinically applicable test for assessing membrane transport properties for both solutes and water. This would enable personalized treatment strategies and facilitate the long-term monitoring of membrane changes, potentially improving patient outcomes and extending the viability of PD as a treatment modality.

Study I showed that acute inhibition of SGLT2 transporters caused well-known effects, such as glycosuria and reduced plasma glucose levels, but did not significantly affect glucose absorption or osmotic water transport across the peritoneum during experimental PD.

Study II demonstrated that phloretin, a non-selective blocker of facilitative glucose channels (GLUTs), and ritonavir, a GLUT1/GLUT4 blocker, reduced the diffusion of the glucose analog, [^{18}F]-deoxyglucose, and improved ultrafiltration. However, selective GLUT1 and GLUT4 blockers had no significant effect on [^{18}F]-deoxyglucose or glucose transport. Reduced [^{18}F]-deoxyglucose clearance during GLUT inhibition suggests that glucose absorption during PD occurs transcellularly across the peritoneal cells.

Study III demonstrated that residual volume significantly impacts measurement of osmotic conductance to glucose (OCG) and the reliability of these results. A new and more accurate OCG measurement method was developed.

Study IV successfully improved and validated a combined peritoneal equilibration (CombiPET) test for measuring OCG and peritoneal solute transport properties in patients with PD, showing accurate and reproducible results across repeated tests.

In conclusion, the new, simple, and reliable CombiPET method for assessing water and solute transport across the peritoneal membrane will facilitate clinical decision making and save time for patients and healthcare professionals. Although SGLT2 inhibitors, such as empagliflozin, did not affect glucose absorption or ultrafiltration, facilitative glucose channels seem to play a significant role in glucose transport during PD.

Key words: Peritoneal dialysis, glucose, osmotic conductance to glucose, solute transport, GLUT, SGLT

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MADE IN SWEDEN

To all who believed, and to all who did not

Table of Contents

Abstract

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Abbreviations

$A_0/\Delta x$	Area parameter (unrestricted pore area over diffusion distance)
AQP	Aquaporin
AGP	Advanced glycation end-product
APD	Automated peritoneal dialysis
BBB	Blood-brain barrier
CAPD	Continuous ambulatory peritoneal dialysis
CKD	Chronic kidney disease
DMSO	Dimethyl sulfoxide
Dt/D0	Dialysate glucose ratio
D/P	Dialysate to plasma ratio
ECM	Extracellular matrix
EMT	Epithelial-to-mesenchymal transition
EPS	Encapsulating peritoneal sclerosis
[^{18}F]-DG	^{18}F-deoxyglucose
FWT	Free-water transport
GDP	Glucose degradation product
GLUT	Facilitative glucose transporter
G6P	Glucose-6-phosphate
HD	Hemodialysis
IC_{50}	Half-maximal inhibitory concentration
IL	Interleukin
IPV	Intraperitoneal volume
K_m	Michaelis constant
L_pS	Ultrafiltration capacity
MMT	Mesothelial-to-mesenchymal transition
MTAC	Mass-transfer area coefficient
OCG	Osmotic conductance to glucose
OCT	Organic cation transporter

PD	Peritoneal dialysis
PET	Peritoneal equilibration test
PMC	Peritoneal membrane cell
PS	Permeability surface area-product
PSTR	Peritoneal solute transport rate
ROS	Reactive oxygen species
RKF	Residual kidney function
KRT	Kidney replacement therapy
RV	Residual volume
SGLT	Sodium glucose linked transporter
TGF-β	Transforming growth factor beta
TPM	Three-Pore Model
UF	Ultrafiltration
UFI	Ultrafiltration insufficiency (previously known as UF failure)
UFR	Ultrafiltration rate
UT-B	Urea transporter B

List of publications

This thesis is based on the following publications or manuscripts referred to in the text by their Roman numerals:

Study I

SGLT2 inhibition does not reduce glucose absorption during experimental peritoneal dialysis

Giedre Martus, Karin Bergling, Javier de Arteaga, Carl M Öberg

Peritoneal Dialysis International. 2021 Jul;41(4):373–380

Study II

Transcellular transport of 18F-deoxyglucose via Facilitative Glucose Channels in Experimental Peritoneal Dialysis

Giedre Martus, Premkumar Siddhuraj, Jonas S Erjefält, András Kádár, Martin Lindström, Karin Bergling, Carl M Öberg

Peritoneal Dialysis International. Published online Dec 5, 2024

Study III

Novel Method for Osmotic Conductance to Glucose in Peritoneal Dialysis

Giedre Martus, Karin Bergling, Ole Simonsen, Eric Goffin, Johann Morelle and Carl M Öberg

Kidney International Reports. 2020 Sep 19;5(11):1974-1981

Study IV

Novel Combined Function Test for Patients on Peritoneal Dialysis

Giedre Martus, Eric Goffin, Johann Morelle, Carl M. Öberg

Draft manuscript

Background

Science can never solve one problem

without raising ten more problems

– George Bernard Shaw

Introduction

Chronic kidney disease (CKD) is a significant public health challenge affecting approximately 10% of the global population with substantial implications for both individual health outcomes and healthcare systems.

The condition is progressive and associated with multiple complications frequently resulting in reduced life expectancy, particularly when left unmanaged or detected late.

As kidney function becomes severely impaired, symptoms, such as decreased appetite, nausea or vomiting, fatigue, muscle weakness or cramps, pruritus, edema, and high blood pressure, may occur. Blood tests show elevated levels of creatinine and urea, low hemoglobin levels, imbalances in electrolytes, and acid-base status. CKD typically progresses over time, and when kidney function reaches a critically low level, it can result in death unless kidney replacement therapy, such as kidney transplantation or dialysis, is initiated to supplement or partially replace kidney function.

Dialysis is a life-sustaining treatment that removes waste products and excess fluid from the blood when the kidneys can no longer adequately perform these tasks. There are two main forms of dialysis: hemodialysis and peritoneal dialysis.

Peritoneal dialysis (PD) is a form of renal replacement therapy that utilizes the peritoneum—the lining of the abdominal cavity—as a natural semipermeable membrane. By instilling a special dialysis solution into the abdominal cavity, uremic toxins and excess water are removed from the blood, restoring the electrolyte and acid-base balance.

This thesis focuses on peritoneal dialysis.

Historic perspectives

The medical terminology of peritoneal dialysis has deep etymological roots in ancient Greek. The term "peritoneum" derives from the Greek word "peritonaion," combining two elements: "peri-" meaning "around," and "teinein" meaning "to stretch" [1]. This etymological composition perfectly describes the peritoneum's anatomical function as a membrane that stretches around and envelops abdominal organs.

Similarly, "dialysis" originates from the Greek word "dialusis," which breaks down into two components: "dia-" meaning "through," and "lysis" meaning "loosening" or "separation" [2]. The term's evolution is particularly interesting, as it initially served in the domains of logic and grammar to describe the process of separating and analyzing concepts. Its application later expanded to chemistry, where it describes the physical process of separating solutes through a semipermeable membrane [2].

The medical application of the term "dialysis" is relatively recent, emerging only in the early 20th century [2]. In medicine, it specifically describes the therapeutic process of removing waste products from the blood, either through the peritoneal membrane or through an artificial membrane, during hemodialysis. Modern medical usage maintains the original Greek concept of separation while applying it to life-saving medical procedures.

Development of peritoneal dialysis

Ancient knowledge of the peritoneum dates to Egyptian embalmers, who first documented the existence of the peritoneum [3]. The Ebers Papyrus (circa 1550 BC) contains some of the earliest written references to the peritoneal cavity [4, 5], though detailed understanding of its structure and function would not emerge for millennia.

Significant advances in knowledge of the peritoneum occurred alongside surgical developments in the 18th and 19th centuries.

In 1743, English surgeon Christopher Warrick pioneered intra-abdominal fluid exchange by treating ascites through drainage and injection of a Bristol water and Bordeaux wine mixture [6, 7]. Stephen Hales enhanced this technique by introducing a dual-trocar system for improved fluid removal [3, 7]. Although these early procedures were not intended to address kidney failure, they laid basic ideas for future dialysis techniques.

The scientific foundation for peritoneal dialysis emerged through several key discoveries. Henri Dutrochet's 1826 discovery of osmosis—the movement of solvents through semipermeable membranes—provided crucial insights into biological fluid balance [5, 8]. Thomas Graham's mid-19th century work on

diffusion and osmotic forces further advanced understanding of fluid separation principles [8-11]. Gustav Wegner's 1877 experimental studies in animals demonstrated the role of the peritoneum in regulating fluid transport [5, 10-12]. Ernest Starling's and Arthur H. Tubby's late 19th-century research revealed bidirectional fluid and solute movement across peritoneal and pleural membranes [5, 11, 13-15].

The modern era of peritoneal dialysis began with Georg Ganter's 1923 pioneering work in both animal models and humans [5, 11]. He induced kidney failure in animals by ligating the ureters and used saline for intraperitoneal dialysis, which was later applied to treat a patient with urinary tract obstruction and kidney failure, marking a pivotal moment in the development of peritoneal dialysis. In 1927 Heinrich Heusser introduced glucose as an osmotic agent, which became standard practice for creating the necessary osmotic pressure to remove excess fluid [10, 16]. In 1938 Jonathan E. Rhoads added lactate to correct metabolic acidosis marking another significant therapeutic advance [16].

From the 1930s to the 1960s, several medical teams performed peritoneal dialysis procedures, demonstrating that this technique could temporarily replace kidney function. However, its widespread adoption was limited by infectious complications and the lack of a reliable method for accessing the abdominal cavity until Henry Tenckhoff developed his eponymous catheter in the late 1960s, enabling reliable long-term peritoneal access [5, 10, 11, 17, 18].

In 1976, Robert Popovich and Jack Moncrief introduced Continuous Ambulatory Peritoneal Dialysis (CAPD), a continuous alternative to hemodialysis that gained widespread popularity [5, 10, 11, 17].

Subsequent innovations, including disposable PVC bags (Polyvinyl chloride bags), the Y-set and double-chamber bags, as well as automated peritoneal dialysis (APD), have significantly enhanced treatment safety, efficiency, and patient comfort [5, 10, 17]. These developments paved the way for the transformation of peritoneal dialysis from an experimental procedure into mainstream kidney replacement therapy.

From Starling to the Three-Pore Model

The understanding of fluid transport across biological membranes began with Ernest Starling's groundbreaking work in 1896. Starling discovered that while isotonic saline injected into a dog's interstitial space is absorbed into the bloodstream, protein-rich serum remains in the tissues [15]. This led to his theory of "Starling's forces," which established that capillary walls function as semipermeable membranes that regulate fluid movement based on molecular size [19]. This movement is governed by two primary forces: hydrostatic pressure pushing fluid out of the capillaries, and oncotic pressure drawing it back in.

A significant advance came in 1940 when Robert Chambers and Benjamin W. Zweifach proposed the concept of an "intercellular cement" between endothelial cells acting as a size-selective filtration barrier [20]. This pioneering attempt to explain endothelial control of molecular permeability was further developed in the 1950s by John R. Pappenheimer et al. suggesting the presence of small pores in the intercellular cement or between endothelial cells [21].

Gunnar Grotte's 1956 discovery that high molecular weight dextrans can access the lymphatic system from the circulation introduced the concept of large pores in the vascular endothelium [22]. In 1963, Clifford Patlak's team further advanced the understanding of the molecular permeability of the peritoneal membrane with their two-membrane model, developing mathematical equations to predict solute movement across capillary walls [23]. The solution to the convection-diffusion equation with known boundary concentrations is sometimes referred to as the Patlak equation and is widely used in the study of biological transport phenomena [24].

Later, in 1969, Nolph et al. observed in clinical studies that peritoneal dialysis fluid was hyponatric, most likely due to the greater removal of extracellular water relative to sodium [25]. A few decades later, this "sodium sieving" phenomenon was attributed to the presence of ultrasmall pores, later identified as aquaporins [26, 27].

By the late 1980s, research had shown that solute and water transport through the peritoneal membrane is more complex than previously had been perceived, revealing that small solutes and large molecules travel at different rates, thus showing that the peritoneal membrane was not an iso-selective membrane (i.e., having only one selective pathway).

The culmination of this research led to Bengt Rippe's Three-Pore Model (TPM) in 1991, which comprehensively describes solute and fluid movement across the peritoneal membrane during dialysis [28]. The TPM describes the capillary wall as the primary transport barrier, featuring three different kinds of porous pathways across the peritoneal membrane: small pores, large pores, and ultrasmall pores for water transport [29]. Rippe's model predicted the existence of ultrasmall water-only pores and sodium sieving before the discovery of aquaporins, which was later validated through studies on aquaporin-deficient animals [30].

The anatomical correspondence to small pores is thought to be clefts between endothelial cells in the peritoneal vascular walls. Thus, the peritoneal vascular endothelium is widely regarded as the primary exchange barrier between blood and dialysate [31]. The bi-selective nature of peritoneal solute transfer was confirmed in the mid-1990s, demonstrating that both small and large pores are required to explain the transperitoneal protein transport [32]. In **Figure 1**, the peritoneal clearances of several plasma proteins and creatinine are plotted along the three-pore model (solid line).

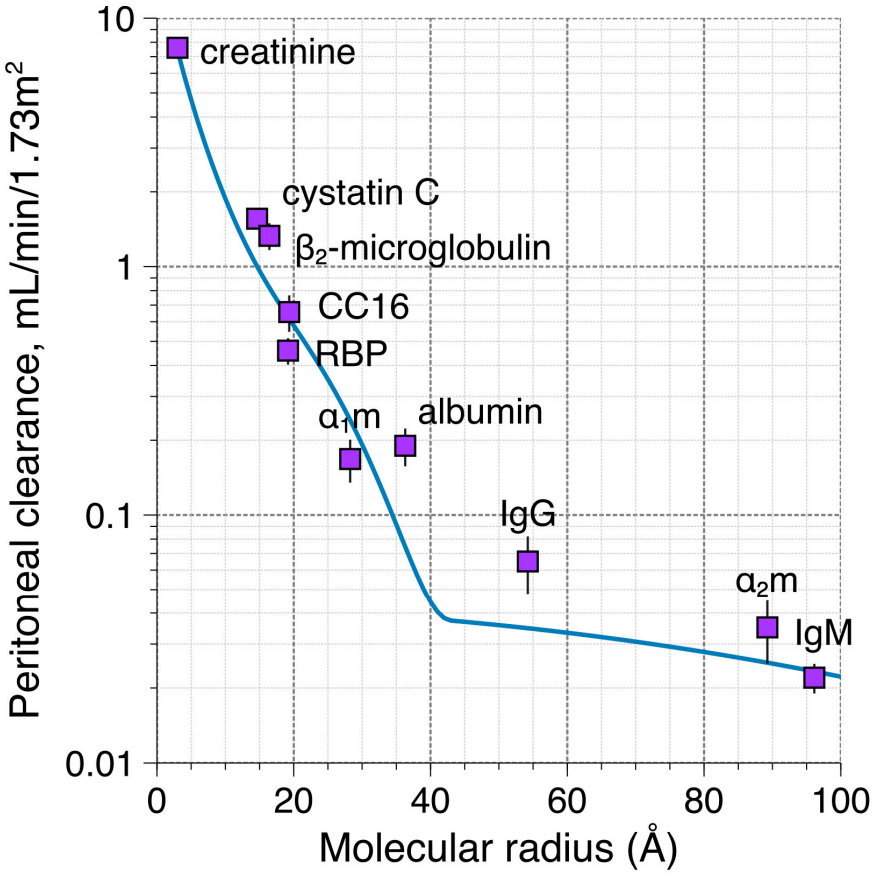

Figure 1. Peritoneal clearance of creatinine and several other proteins (adapted from Kabanda et al. [32]). The three-pore model is plotted with a solid blue line. CC16, clear cell protein; RBP, retinol-binding protein; α1m, alpha-1-microglobulin; IgG, immunoglobulin G; α2m, alpha-2-macroglobulin; IgM, immunoglobulin M.

The development of the TPM was based on data from patients published in 1990 [33], which utilized direct volumetry (*i.e.,* drained volume) to assess intraperitoneal volumes. To align the volume data with the TPM, the diffusion capacity for glucose needed to be approximately 50% higher than that predicted by pore theory [29]. It was also necessary to restrict the diffusion capacity of sodium (and also calcium [34]) to a third of that predicted by pore theory. An updated version of the TPM was published in 2017, extending the model to include the fill and drain phases of PD, thereby enabling the simulation of automated PD [35] and continuous PD [36]. A brief timeline of key events in the development of peritoneal dialysis treatment is presented in **Figure 2**.

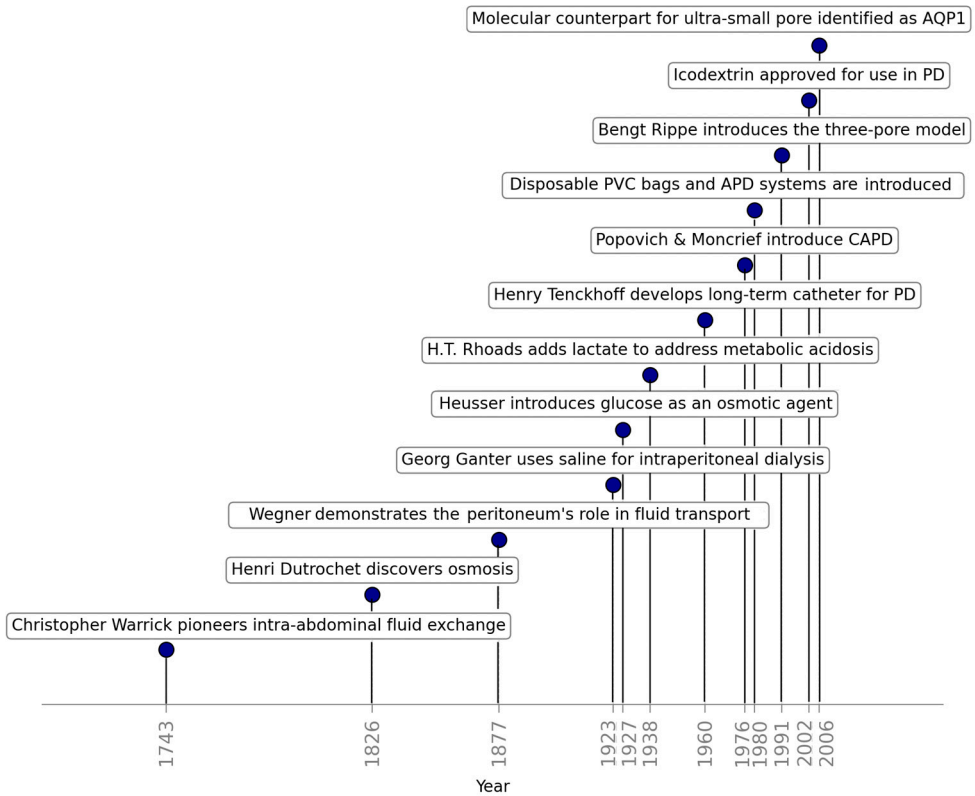

Figure 2. Timeline of key events in the evolution of peritoneal dialysis treatment.

Peritoneal dialysis epidemiology

Global demographic trends show increasing life expectancy and improved healthcare access, accompanied by a rising prevalence of risk factors, including obesity, diabetes, physical inactivity, hypertension, and cardiovascular disease [37-40]. While advances in lifestyle interventions, early diagnosis, and modern treatments help mitigate these health challenges and slow the progression of chronic kidney disease [37, 41], estimates indicate that by 2050, CKD stages 3-5 will affect over 10% of the population in many countries, primarily due to aging demographics [38, 42].

Currently, an estimated 850 million people worldwide experience kidney disease, including acute or chronic kidney failure, or require kidney replacement therapy (KRT) [43].

The escalating global burden of CKD drives a rapidly increasing demand for KRT [37, 38]. From 2.6 million KRT recipients worldwide in 2010, calculations indicate that this number will exceed 5.4 million by 2030 [38]. However, the actual requirements of KRT probably surpass these figures by at least 50%, highlighting the critical need for an enhanced global healthcare infrastructure [37, 38, 44-46].

Peritoneal dialysis, a home-based kidney replacement therapy, represents 11% of all dialysis and 9% of total KRT globally [40, 46, 47]. While available in approximately 80% of countries, PD remains inaccessible in 30 nations, including 20 in Africa [45-47]. Regional disparities in the utilization of peritoneal dialysis are substantial. Hong Kong, implementing a "PD-first" policy, leads globally with 75-80% of patients on dialysis utilizing PD, while Japan's PD utilization rate remains below 5% [40, 48, 49]. These regional variations reflect differences in healthcare policies, priorities, hemodialysis infrastructure availability, and economic considerations [40, 46, 48]. PD is often a cost-effective option, requiring less infrastructure and specialized personnel [49]. Treatment selection depends also on healthcare provider expertise, patient health status, lifestyle preferences, and the availability of home support [40, 47, 48, 50]. One of the greatest future challenges for peritoneal dialysis lies in the inequity of access to the treatment. As shown in **Figure 3**, the global variation in PD utilization varies from only a few percent in countries such as Japan and Poland to > 50% in Hong Kong and Mexico [51].

PD offers distinct advantages over hemodialysis: simpler equipment requirements, a home-based treatment option, and reduced dependence on complex healthcare facilities [49]. These benefits prove particularly valuable in remote areas or during crises, limiting access to treatment centers [52]. PD provides greater lifestyle flexibility, enabling patients to maintain employment, travel, or study with minimal disruption [49, 53]. It typically imposes fewer dietary restrictions and helps to preserve residual kidney function [53-57]. PD is particularly suitable for pediatric patients, avoiding the challenges of creating a vascular access that are common in young children [58, 59].

However, PD has some limitations, including insufficient removal of low-molecular weight toxins, salts, and water, particularly when residual kidney function is absent, as well as potential mechanical and infectious complications [60, 61]. Continuous exposure to peritoneal dialysis solutions also leads to progressive deterioration of the peritoneal membrane [62-66]. Additional challenges include metabolic complications such as insulin resistance, the development of diabetes mellitus type 2, impaired glycemic control in diabetic patients, undesirable weight gain, and cardiovascular disease [67, 68]. These challenges underscore the ongoing need for treatment optimization and innovation in kidney replacement therapy.

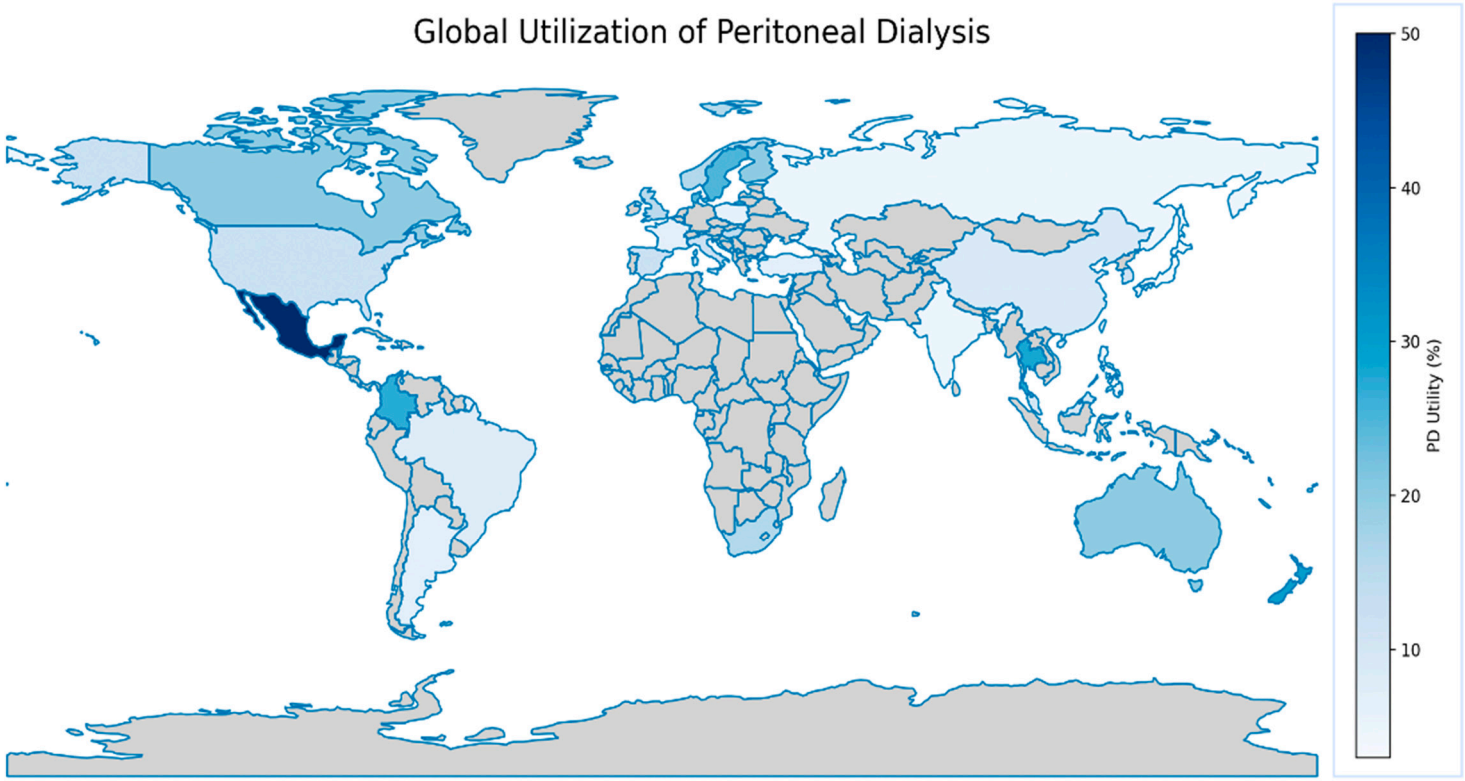

Figure 3. Global utilization of Peritoneal Dialysis (adapted from Briggs et al. **[51]**). Rendered using GeoPandas version 0.14.4.

Anatomy of the peritoneum

The peritoneum is a thin membrane that envelops the abdominal organs and lines the peritoneal cavity [69, 70]. The surface area of an adult peritoneum varies from 0.5 to 2 m², according to different measurement techniques [70-76].

The peritoneum is divided into two parts: the visceral peritoneum, which covers the abdominal organs, the mesentery, the omentum, and the parietal peritoneum, which lines the abdominal wall and diaphragm [70, 76]. Although the parietal peritoneum accounts for only approximately 40% of the entire peritoneal surface area, it plays a crucial role during peritoneal dialysis. This is because only a small portion of the visceral peritoneum is in contact with the dialysis fluid [74].

The peritoneum has multiple essential functions. It acts as a protective barrier and provides organ stabilization while secreting peritoneal fluid, which reduces friction during digestive processes, breathing, and body movements [69, 70, 76]. Furthermore, it facilitates fluid and immune cell transport within the abdominal cavity, produces growth factors and extracellular matrix components, and assists in fibrin breakdown to prevent adhesion [70, 76, 77].

The vascular supply to the peritoneum exhibits a complex organizational structure. The parietal peritoneum receives blood from the iliac, lumbar, epigastric, and intercostal arteries, with subsequent drainage into the inferior vena cava [70, 78]. The visceral part of the peritoneum is supplied by the superior and inferior mesenteric arteries, with drainage occurring through the portal vein. Peritoneal blood flow typically ranges from 50-150 mL/min [74, 79], and solute transport during peritoneal dialysis appears to be only slightly limited by blood flow [80], in contrast to hemodialysis. The primary lymph drainage of the peritoneal cavity is managed by the subdiaphragmatic lymphatic system, which drains into the right lymphatic duct [69, 70, 76, 81].

Peritoneal membrane structure

The peritoneal membrane consists of three main layers: a monolayer of mesothelial cells, interstitial layer, and an extensive network of blood and lymphatic vessels [76, 82].

The mesothelial cell monolayer lining the basement membrane is the first layer facing the peritoneal cavity [76, 77]. Despite their mesodermal origin, mesothelial cells share several key features with epithelial cells. These similarities encompass their polygonal configuration, existence of microvilli that enhance surface area, and manifestation of cell polarity [76, 77, 83]. Well-developed intercellular junctions

between mesothelial cells maintain tissue integrity and regulate permeability [76, 77]. Loss of these intercellular junctions can lead to mesothelial denudation (**Figure 4**), a condition often observed in patients undergoing peritoneal dialysis [84]. The progression of peritoneal membrane fibrosis during extended peritoneal dialysis is influenced by this alteration [77, 83, 85, 86].

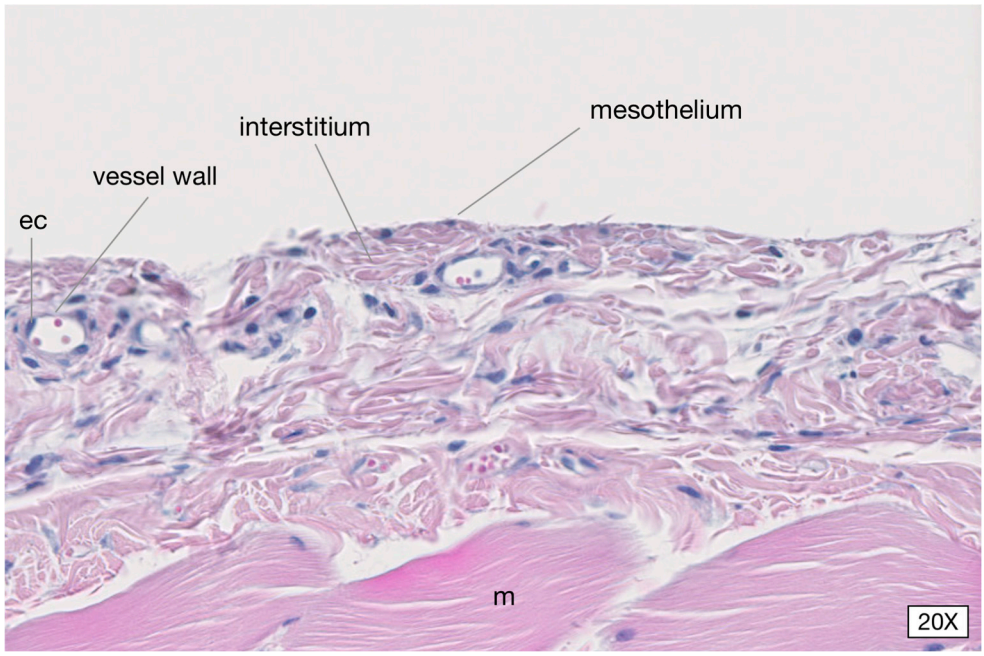

Figure 4. Anatomy of the rat parietal peritoneum after acute peritoneal dialysis (from *Study II*) with partly denuded mesothelium. ec=endothelial cell; m=muscle tissue.

The mesothelium acts as a slippery, non-adhesive layer that facilitates smooth movement of abdominal organs. Additionally, it plays an active role in maintaining homeostasis and structural integrity of the peritoneum, regulating fluid and solute transport, and modulating immune responses [76, 77, 82]. Mesothelial cells synthesize various molecules including cytokines, growth factors, and matrix proteins. These substances play crucial roles in the regulation of inflammatory processes and contribute to tissue repair and remodeling [77]. Under certain conditions, mesothelial cells can acquire mesenchymal properties, transforming into fibroblast-like cells through a process known as mesothelial-to-mesenchymal transition or epithelial-to-mesenchymal transition [76, 84, 87].

The role of the mesothelium in peritoneal transport during peritoneal dialysis remains a subject of scientific discussion. However, the prevailing view is that the

mesothelium plays a minimal role in the transport of fluids and solutes during dialysis [88].

Beneath the mesothelial layer lies the submesothelial compact zone, also known as the interstitial layer. This layer contains components of the extracellular matrix such as collagen and elastin fibers, proteoglycans, and glycosaminoglycans [76, 82]. It also houses various cells, including fibroblasts, myofibroblasts, mesenchymal cells, and adipocytes [76].

Fibroblasts, the most common cells in peritoneal interstitial tissue, are responsible for producing and maintaining the extracellular matrix [76]. During long-term peritoneal dialysis, these fibroblasts are believed to contribute to the development of fibrosis and thickening of the peritoneal membrane. Over time, during peritoneal dialysis, the concentration of myofibroblasts derived from fibroblasts increases, exacerbating fibrosis and thickening of the membrane. These cells contribute to alteration of the peritoneal membrane structure by secreting excessive amounts of collagen and other extracellular matrix elements. Additionally, they generate profibrotic and angiogenic factors that promote the progression of fibrosis. Furthermore, myofibroblasts express elevated levels of facilitative glucose transporter 1 (GLUT-1), potentially initiating a vicious cycle of increased glucose uptake and pseudohypoxia, thereby exacerbating fibrosis [66].

Mesenchymal cells, which can differentiate into various cell types such as fibroblasts and adipocytes, are also present in the interstitial tissue [84].

The submesothelial compact zone typically measures 20-40 μm in thickness [84]. However, in patients receiving peritoneal dialysis over an extended period, the interstitial layer may become substantially thicker, up to tenfold in some cases [74, 84].

Beneath the interstitial layer lies a dense network of arterioles, venules, and capillaries lined with a vascular continuous endothelium [76]. Endothelial cells lining capillaries and venules, which rest on the basement membrane, are thought to play a key role in peritoneal dialysis [29]. The interendothelial clefts between these cells are believed to form a semipermeable barrier, allowing the passage of various molecules up to a radius of approximately 4.0 nm (40 Å), which is roughly the size of human serum albumin [82, 89].

During long-term peritoneal dialysis, several changes are observed in peritoneal blood vessels, including subendothelial hyalinosis, obliteration of the vascular lumen, neovascularization (formation of new blood vessels), interstitial layer thickening, and fibrosis [84]. Interestingly, subendothelial hyalinization begins as uremia progresses even before the initiation of dialysis treatment. However, the introduction of peritoneal dialysis (PD) significantly accelerates this pathophysiological process [84]. These alterations affect the rate of solute and water transport through the peritoneal membrane [64, 66, 90, 91].

It is widely accepted that the transport processes occurring during peritoneal dialysis are best described by the three-pore model [29]. According to this model, small solutes and water pass through the vascular endothelium via three types of pores: large, small, and ultrasmall. The interstitial tissue and mesothelium play relatively minor roles in the transport process [29, 92]. In the three-pore model, large pores measuring 200–300 Å (20–30 nm) are formed at sites where the three endothelial cells meet. These pores represent less than 0.01% of the total pore area and are the main pathway for large molecules, such as serum proteins, to cross the peritoneal membrane. Small pores measuring 40–50 Å (4–5 nm) are located between two endothelial cells and allow the transport of smaller molecules and water. These pores constitute more than 99% of the total pore area. Ultrasmall pores, or aquaporin-1 (AQP-1) channels, have a radius of approximately 2.5–3.0 Å (0.25–0.3 nm) and function as transcellular channels, whose only function is to facilitate the movement of water [29, 93].

Other models, such as the distributed model, also simulate the movement of solutes and water across the peritoneal membrane [94]. This model recognizes that the peritoneal membrane is not uniform and that different regions of the peritoneal cavity vary in thickness, blood flow, and capillary density. Thus implying that not only the blood vessels and endothelium but also the characteristics of the interstitial tissue influence the transport properties of the peritoneal membrane [95].

Tight junctions

The separation of functional compartments by the epithelial or endothelial layers is critical for maintaining homeostasis and enabling efficient physiological processes. These layers act as selective barriers that regulate the movement of substances between different areas of the body [96]. In various organs and tissues, these barrier layers exhibit different permeability levels depending on their specific functions [97]. For example, the skin epidermis and urinary tract epithelium are nearly impermeable, which prevents fluid loss and protects against pathogens, whereas the intestinal epithelial layer is quite permeable, which facilitates the absorption of nutrients from the intestine [98, 99].

Although transport of water and solutes across intercellular spaces might appear to be a straightforward process, these spaces are complex structures composed of tight junctions, gap junctions, desmosomes, and adherens junctions [97, 100, 101] (**Figure 5**).

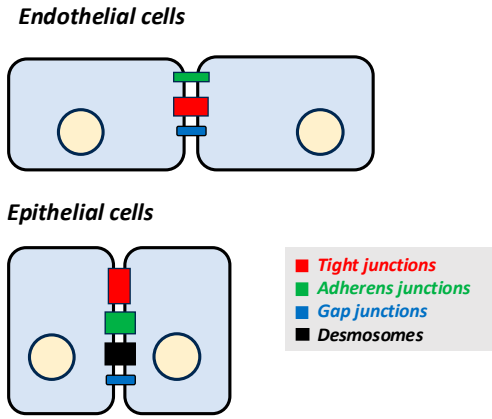

Figure 5. Schematic representation of main intercellular junctions in epithelia and endothelia.

These intercellular structures help maintain tissue integrity by connecting the actin cytoskeletons of neighboring cells. They allow for direct communication between adjacent cells and permit the passage of small molecules, ions, and water.

Tight junctions are believed to have the most significant impact on paracellular transport. They seal the space between endothelial and epithelial cells and regulate and maintain their selective barrier function [101, 102].

Tight junctions are complex assemblies of transmembrane and cytoplasmic proteins [101, 102]. Tight junctions can be divided into bicellular and tricellular. Bicellular tight junctions are formed between two adjacent cells, creating a seal along the lateral interfaces of neighboring cells. In contrast, tricellular tight junctions are specialized junctions located at the vertices where three or more cells meet [99].

The main components of tight junctions are claudins, occludin-related MARVEL domain proteins (occludin, tricellulin, and MARVELD3), and their adaptor and scaffolding proteins, such as junctional adhesion molecules (JAMs), cingulin, zonula occludens (ZO-1, ZO-2, and ZO-3), and MUPP1 [103, 104] (**Figure 6**).

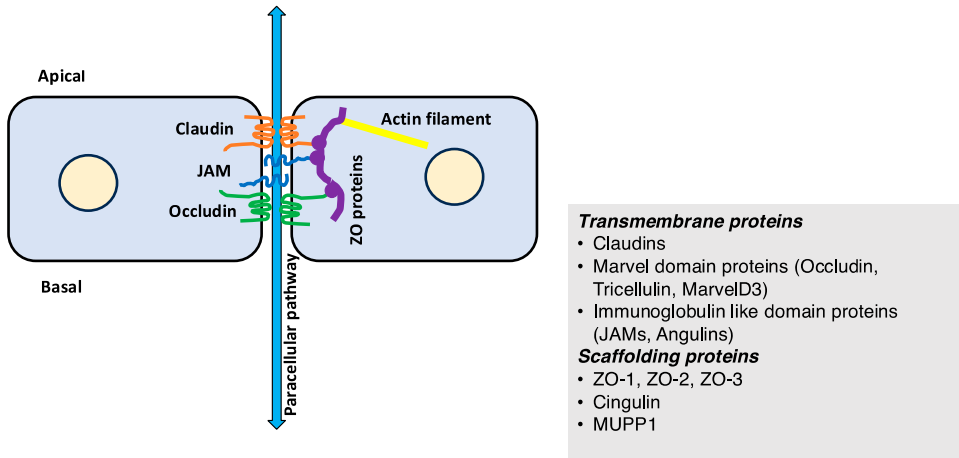

Figure 6. Tight junction structure.

Claudins are a large family of transmembrane proteins—currently, 27 are known—that are critical for tight junction formation and paracellular permeability [97, 104]. Some claudins form pores that allow the passage of small ions and molecules; for example, claudin-2, claudin-10b, and claudin-15 form cation-selective channels, whereas claudin-10a and claudin-17 form anion-selective channels [99]. Paracellular water channels consist of the cation channels claudin-2 and claudin-15 [99]. Other claudins, such as claudin-1, -4, -5, -8, -11, -14, and -19, seal junctions to reduce paracellular permeability [99].

Occludin is a transmembrane protein that contributes to tight junction stability and barrier function by interacting with other tight junction proteins and the actin cytoskeleton [97]. JAM proteins facilitate cell-cell adhesion and also contribute to the formation of tight junctions [97, 103].

Tricellulin and angulins are mainly found at junctions where three cells meet and are essential to maintain barrier function at tricellular contacts, preventing leakage at these critical points [99].

Cytoplasmic scaffolding proteins known as Zonula occludens (ZO) proteins serve as a link between the actin cytoskeleton and transmembrane tight junction proteins.[97, 104]. They are essential for the structural integrity and regulation of tight junctions. Cingulin and MUPP1 are cytoplasmic proteins that interact with tight junction components and contribute to their assembly and regulation [99].

Several claudins, such as claudin-1, -2, -3, -4, -5, -8, and -15, as well as occludin, ZO-1, and tricellulin, have been detected in the peritoneal membrane [105-108]. In a recent study, Levai et al. investigated the alterations in tight junctions associated with peritoneal dialysis treatment. Their findings revealed significant changes in claudin expression patterns within the peritoneum of patients with PD compared

with healthy controls. Levai et al. showed an increase in mesothelial and arteriolar claudin-1 and claudin-2, along with a decrease in mesothelial and arteriolar claudin-3 in the peritoneum of patients with PD [105]. This study revealed that alterations in claudin expression were not limited to patients with PD. Interestingly, changes in claudin were associated with age and chronic kidney disease even before the initiation of dialysis.

The integrity and function of tight junctions, especially in the peritoneal membrane, can be affected by a variety of factors, such as cytokines and inflammatory mediators, elevated glucose levels, reactive oxygen species (ROS), advanced glycation end products (AGEs), and even physical factors such as increased intra-abdominal pressure [100, 106, 109].

Aquaporins

Aquaporins (AQPs) are specialized transmembrane proteins that primarily regulate the passive bidirectional movement of water across cell membranes [26]. Water movement is governed by osmotic gradients created by active solute transport. Aquaporins are extensively expressed in cells of all living organisms, including bacteria, plants, and animals [26, 110, 111]. In humans, aquaporins are found both in organs and tissues involved in fluid regulation, such as the kidneys, salivary glands, sweat glands, and intestines, and in those not directly associated with fluid transport, such as adipose tissue, muscles, and liver [112]. To date, 13 aquaporin isoforms (AQP0–AQP12) have been identified in humans [111-113]. While their name suggests that they exclusively transport water, it is notable that some aquaporins also facilitate the movement of other small neutral solutes, such as urea and glycerol, as well as gases, such as carbon dioxide and ammonia [112, 113].

Based on their selectivity, aquaporins are generally classified into three subgroups. The first subgroup, known as orthodox or traditional aquaporins, includes AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8. These proteins primarily facilitate water transport across the cell membranes. The second subgroup, termed aquaglyceroporins, comprises AQP3, AQP7, AQP9, and AQP10. These channels are notable for their ability to transport not only water but also glycerol and potentially other small, uncharged solutes. The third and final subgroup, referred to as super- or unconventional aquaporins, consists of AQP11 and AQP12. These aquaporins are the least understood members of this family, with unique structural and functional characteristics that distinguish them from the other two subgroups [113].

Various cells in the body express multiple types of aquaporins, which can interact with each other to regulate cellular water transport. In addition to their primary function in water transport, aquaporins contribute to a range of other cellular processes. These include facilitating cell adhesion, participating in signal

transduction pathways, maintaining osmotic balance, and mediating redox signaling. They also interact with other membrane proteins and play a key role in preserving cell volume [112, 113].

The structural features of traditional aquaporins enable them to function as selective water channels. Each aquaporin monomer is composed of six transmembrane α-helical segments connected by intracellular and extracellular loops that span the cell membrane, forming a stable framework [26, 110]. This configuration creates a narrow pore through the center of the protein, permitting water molecules to pass, while excluding ions and other solutes. The specificity for water is attributed to direct hydrogen bonding within the pores between water molecules and the AQP-specific Asparagine-Proline-Alanine (NPA) motif located at the narrowest point of the pore [26, 112, 113] **(Figure 7)**.

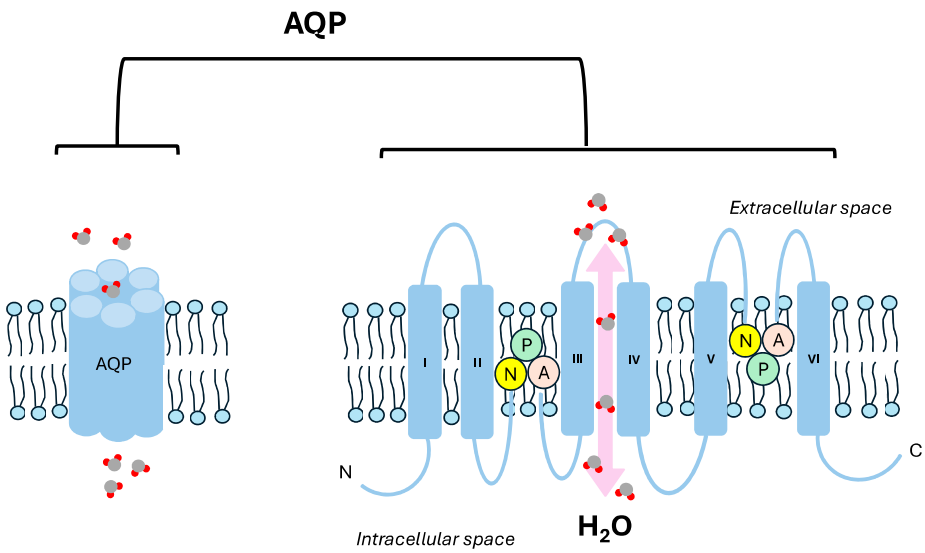

Figure 7. Aquaporin structure. The structure of the aquaporin (AQP) is characterized by six transmembrane α-helices connected by five loops. Two of these loops, the second and fifth, contain conserved NPA (Asparagine-Proline-Alanine) motifs. These motifs converge at the center of the membrane, forming a selective water pore.

Although the NPA motif is considered to be a characteristic feature of aquaporins, some members of the AQP family, known as aquaglyceroporins, exhibit slightly different motifs. Instead of the typical Asparagine-Proline-Alanine (NPA) motif, aquaglyceroporins may contain Asparagine-Alanine-Alanine (NAA) or Asparagine-Proline-Serine (NPS) motifs. These variations allow aquaglyceroporins

to transport not only water but also other small neutral molecules, particularly glycerol [113].

Extensive studies since the discovery of aquaporins have shown that the expression of these channels is highly dynamic and regulated by a variety of hormonal and environmental stimuli, including hypoxia, osmotic changes, mechanical stress, and pathogen exposure [111]. The permeability of AQP channels and their cellular localization, such as their movement from vesicles or the Golgi apparatus to the plasma membrane, can change within seconds to minutes, leading to changes in water permeability. The expression ratios of different AQP isoforms with different permeabilities may also change, which also affects the membrane permeability [114].

AQP1 is predominantly expressed in the endothelial cells of peritoneal capillaries and venules, where it is found in large amounts [115]. However, its presence is not limited to these structures; AQP1 is also found in mesothelial cells [116].

Experimental peritoneal dialysis (PD) studies using AQP1 knockout mice have demonstrated that AQP1 is responsible for approximately 50% of osmotic water transport when hypertonic glucose solutions are used. In contrast, AQP1 does not significantly influence ultrafiltration when icodextrin is used as the osmotic agent [30, 117].

Recently, researchers identified three genotypes of the AQP1 promoter variant rs2075574 that affect AQP1 gene transcription, namely TT, CT, and CC [118]. Patients with the TT genotype were found to have lower daily net ultrafiltration than those with the CC genotype, as well as a higher risk of permanent transition to hemodialysis and an increased risk of death.

Although AQP1 is the predominant aquaporin in the peritoneal membrane, other aquaporin subtypes are also present, albeit in significantly lower quantities. AQP3, AQP5, AQP7, and AQP9 have been detected in peritoneal tissue, but their expression levels are substantially lower than those of AQP1. Consequently, these aquaporins are believed to have a limited impact on water transport during peritoneal dialysis [30, 119].

Solute and water transport across the peritoneal membrane

Two key principles govern the movement of substances across the peritoneal membrane during dialysis: concentration-driven diffusion, and pressure-driven convection facilitated by osmotic or hydrostatic forces. The phenomenon of osmosis

occurs during which water moves through a semi-permeable membrane that is more restrictive to the passage of solutes than water.

Diffusion

When dissolved substances enter a liquid medium such as water, they naturally disperse throughout the available space through diffusion, moving from areas of high to low concentration until they become evenly distributed [120].

During peritoneal dialysis, diffusion is the primary mechanism by which waste products, such as urea and creatinine, move from the blood (where concentrations are high) into the dialysate (where concentrations are low).

The fundamental process of diffusion is based on Brownian motion, where dissolved molecules undergo continuous random movement due to their thermal energy. Although individual molecules move randomly through collisions with the surrounding liquid, their overall movement trends from higher to lower concentration regions. This movement persists until it reaches dynamic equilibrium, at which point the concentration becomes uniform throughout the solution and the rates of molecular movement in all directions balance each other, maintaining stable concentration levels [120].

Diffusion is primarily driven by the concentration gradient, but its rate is also affected by various other factors, including the molecular size, temperature, and solvent viscosity. The relationship between these factors can be described using the Stokes-Einstein equation [121]:

$$D = \frac{kT}{6\pi\eta R} \tag{1}$$

where D is the diffusion coefficient, k is the Boltzmann constant, T is the absolute temperature of the fluid, η is the dynamic viscosity of the fluid, R is the radius of the spherical particle, and π is the mathematical constant. This equation balances thermal energy driving molecular motion (kT) against frictional forces resisting movement ($6\pi\eta R$).

Diffusion becomes more complex when molecules encounter barriers such as membranes. A hindrance factor (H) between 0 and 1 quantifies this effect, with 1 representing unimpeded diffusion, and 0 indicating complete blockage. The restricted diffusion coefficient when considering a barrier becomes HD (restricted diffusion coefficient=HD).

The overall capacity for substances to diffuse across a membrane is described by the mass transfer area coefficient (MTAC) or permeability surface area-product (PS). MTAC indicates the maximum clearance of solutes when the dialysate solute concentration is zero. MTAC or PS can be expressed as:

$$MTAC = PS = \frac{HDA_0}{\Delta x} \quad (2)$$

which considers the unrestricted surface area or pore area (A_0) and the permeability of the solute ($HD/\Delta x$), which is defined as the terminal velocity of the molecule (in cm/min) as it travels across the membrane. It is worth noting that HD, the restricted diffusion coefficient, is a property of the molecule (in relation to a membrane), whereas $A_0/\Delta x$—the diffusive conductance or the unrestricted pore area to diffusion length (Δx)—is entirely a property of the membrane. Thus, the capacity for diffusion for any molecular species across a membrane is simply the product of its restricted diffusion coefficient and the diffusive conductance of the membrane.

Diffusion capacity is useful in the study of transport phenomena because it is a coefficient in Fick's first law of diffusion [122]. Suppose a concentration difference ΔC exists across a semi-permeable membrane between the blood and dialysate, then the diffusive flow of the solute (J_s, mmol/min) is given by:

$$J_s = -PS \cdot \Delta C \quad (3)$$

The concentration difference is defined as $\Delta C = C_d - C_p$, where C_d and C_p represent the dialysate and plasma concentrations, respectively. Thus, flow is, by definition, positive when directed into the peritoneal cavity. For a solute diffusing from blood plasma to dialysate, the plasma-to-dialysate clearance is given by J_s/C_p. Similarly, for a solute diffusing from dialysate to blood plasma, the dialysate-to-plasma clearance is given by J_s/C_d. For example, if the creatinine diffusion capacity is 12 mL/min (a typical value for adult patients [123], corresponding to an $A_0/\Delta x$ of 25,000 cm) and the plasma creatinine concentration is 500 µmol/L (and 0 µmol/L in the dialysate), the diffusive flow is approximately 6 µmol/min. There are several methods to estimate the diffusion capacity during PD, such as the Henderson-Nolph equation [124] or the isocratic model (for details, see the Methods section in *Study I*).

Convection

Convective transport occurs when dissolved substances move across the peritoneal membrane along with the flow of water, which is driven by pressure differences resulting from osmotic or hydrostatic forces across the membrane.

The efficiency of the process can be approximated using a mathematical relationship [125, 126] as follows: $Cl=J_v(1-\sigma)$, where the convective clearance (Cl) measures the amount of plasma cleared of solutes over time in mL/min, the reflection coefficient (σ) measures the membrane's ability to impede solute movement, and the ultrafiltration rate (J_v) indicates the amount of fluid passing through the peritoneal membrane. If only transport across small pores is considered, J_v can be multiplied

by a factor 0.5, roughly compensating for the fact that only approximately half of the UF rate occurs through the interendothelial small pores (and ~50% is free-water transport). The reflection coefficient varies between 0 and 1, with 0 indicating that solutes move without restriction alongside the fluid, 1 indicating complete blockage by the membrane, and intermediate values indicating partial movement restriction. For many small plasma molecules and electrolytes such as urea and sodium, the reflection coefficient is close to zero. Nevertheless, convection is particularly important for the removal of some ions, such as sodium, magnesium, and calcium, where the concentration gradients between blood and dialysate are minimal, and convection becomes the primary pathway for clearance [34]. The clearance of sodium from the plasma to the dialysate is typically proportional to the UF rate [35]. Similarly, for larger molecules, such as albumin and β2-microglobulin, which diffuse poorly due to their size, convective flow is an essential pathway for their removal [36]. If the above equations are combined, the result is a variant of the convection-diffusion equation:

$$J_s = -PS\Delta C + J_v(1-\sigma)\frac{C_d+C_p}{2} \quad (4)$$

Here, $(C_d + C_p)/2$ is an approximate of the average concentration inside the membrane (most accurate for small solutes).

Osmosis

The process of osmosis takes place when molecules of water traverse a semipermeable barrier, moving from an area with a higher concentration of water to one with a lower concentration.[127]. While an ideal semipermeable membrane allows only water molecules to pass while blocking all solutes, the peritoneal membrane, like all biological membranes, contains variously sized pores that permit selective solute passage [127]. This selective permeability is characterized by the osmotic reflection coefficient (σ), where $\sigma = 1$ indicates complete solute reflection, and $\sigma = 0$ represents complete solute permeability. For small solutes, σ approaches 0, indicating a high membrane permeability.

According to the three-pore theory, the peritoneal hydraulic conductance or ultrafiltration coefficient is distributed among different pore sizes: small pores (90%), ultrasmall water-permeable pores or aquaporins (2%), and large pores (5-8%) [33, 93].

Under normal physiological conditions (without dialysis), fluid movement across the peritoneal membrane is governed by the Starling forces, including hydrostatic and oncotic pressure gradients. Approximately 60% of transcapillary fluid flow occurs through small pores, with large pores accounting for the remaining 40% [93].

The addition of osmotic agents to dialysis fluid creates additional osmotic gradients across the peritoneal membrane. The type of osmotic agent significantly influences the osmotic processes occurring during peritoneal dialysis. Glucose promotes water flow through both the aquaporins and small pores almost equally. Approximately 40-50% of glucose-induced ultrafiltration occurs through aquaporins, while 50-60% occurs through small pores. In contrast, high-molecular-weight agents such as icodextrin primarily affect fluid removal through small pores independently of aquaporins [30, 93, 128].

The net transperitoneal volume flow (J_v) during peritoneal dialysis can be expressed mathematically as [126, 129]:

$$\frac{\mathrm{d}V}{\mathrm{d}t} = J_v = L_p S\left(\Delta P - \sigma_{prot}\Delta\pi_{prot} - \sigma_g\Delta\pi_g - \sum\sigma_i\Delta\pi_i\right) - L \quad (5)$$

This equation incorporates several key components. L_pS represents the ultrafiltration coefficient, that is, the product of hydraulic permeability and effective surface area. ΔP denotes the difference in hydrostatic pressure, while $\sigma_{prot}\Delta\pi_{prot}$ represents the protein-related oncotic effect. $\sigma_g\Delta\pi_g$ describes the glucose-related osmotic gradient, and $\sum\sigma_i\Delta\pi_i$ accounts for osmotic pressure differences due to other solutes. Finally, L represents for lymphatic flow back into the bloodstream.

Maintaining optimal fluid balance during peritoneal dialysis is critical because it significantly influences patient survival and treatment outcomes [130-132]. Fluid overload can develop through various mechanisms, such as declining residual kidney function, excessive fluid or salt consumption, peritoneal membrane defects causing fluid leakage, and inadequate ultrafiltration (UF) across the peritoneal membrane, termed true ultrafiltration insufficiency (UFI) [133, 134].

True UFI in PD follows the “rule of four”: insufficient achievement of 400 mL net ultrafiltration during a 4-hour dwell using 4.25% glucose solution [135].

Based on their underlying mechanisms, UFI are classified into four distinct types, each requiring a different management approach [134].

Type 1 UFI, the most prevalent form, manifests as a fast transport state characterized by an enlarged effective peritoneal surface area [123, 134]. This condition leads to accelerated transport of small solutes, including glucose, across the peritoneal membrane. The resulting rapid dissipation of the glucose concentration gradients diminishes the osmotic driving force and reduces the ultrafiltration volume. The prevalence of this high-transport state increases with longer PD duration [64, 90, 91].

Type 2 UFI involves diminished osmotic conductance to glucose (OCG), which typically manifests after 3-4 years of dialysis therapy [64, 134]. This condition, characterized by OCG values below 2 μL/min/mmHg, most likely results from

compromised free-water transport [34, 91]. Reduced sodium sieving (≤ 5 mmol/L) has been suggested as an indicator of this condition [123, 136].

Types 3 and 4 UFI occur less frequently. Type 3 UFI stems from a reduced effective peritoneal surface area, either present at dialysis initiation or developing gradually due to adhesions or sclerosis, limiting fluid exchange capacity. Type 4 UFI, the rarest form, results from enhanced fluid reabsorption into lymphatic vessels and surrounding tissues, diminishing net ultrafiltration [134].

Recognizing these distinct UFI mechanisms enables healthcare providers to implement targeted interventions for optimal fluid balance management in patients with PD, ultimately improving treatment outcomes and well-being. Causes other than UFI should always be considered first (**Figure 8**) in patients with fluid overload. If UF is low in the absence of a fast transport status and a normal OCG (*i.e.,* suspected type 3 or 4 UFI), extraperitoneal leaks should be ruled out.

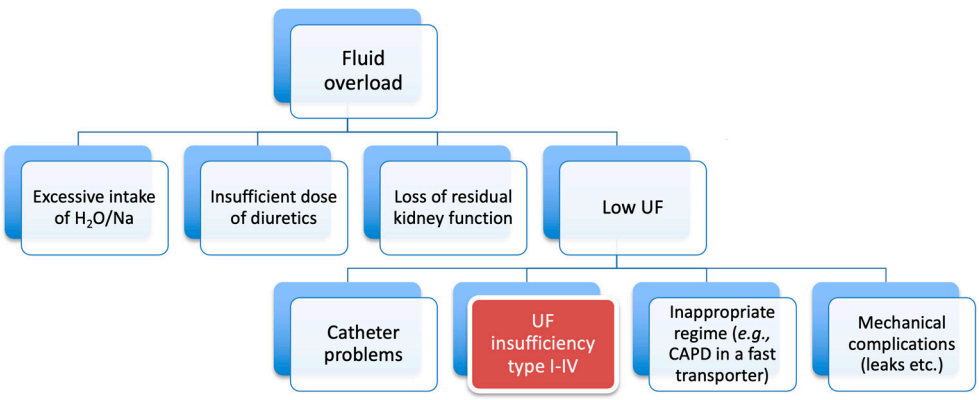

Figure 8. Causes of fluid overload in patients treated with peritoneal dialysis.

Glucose transport and metabolism

Glucose is the primary source of energy and a key metabolic substrate in living cells [137]. Most glucose is derived from the hydrolysis of disaccharides and polysaccharides consumed in the diet. Additionally, glucose is produced in certain organs through glycogen breakdown (glycogenolysis) and gluconeogenesis from substrates, such as lactate, pyruvate, certain amino acids, and glycerol [138]. However, because most mammalian cells lack sufficient levels of glucose-6-phosphatase, a crucial enzyme for the final steps of both glycogenolysis and gluconeogenesis, they cannot produce sufficient free glucose and must rely on glucose from the bloodstream to meet their metabolic needs [139, 140].

Owing to its hydrophilic nature, glucose cannot pass through cell membranes by simple diffusion and must be transported into cells via specific transport proteins [141-143]. Once glucose enters the cell, it becomes part of various metabolic processes essential for cellular function, energy production, and overall homeostasis. Glucose is rapidly phosphorylated into glucose-6-phosphate (G6P) by the enzyme hexokinase (or glucokinase in the liver and pancreatic β-cells). This phosphorylation traps glucose inside the cell, as G6P cannot easily cross the cell membrane and prepares it for further metabolic pathways. G6P can enter glycolysis to produce pyruvate, ATP, and NADH [138].

Hexokinase serves as a key regulator of glucose metabolism by initiating the conversion of glucose into glucose-6-phosphate (G6P) [138]. This phosphorylation step is crucial, as it traps the glucose molecule within the cell, preparing it for subsequent metabolic pathways. Enzymatic activity is intricately controlled through feedback inhibition by G6P [144, 145]. When G6P accumulates, it suppresses the activity of most hexokinase isoforms (I, II, and III), thereby preventing excessive phosphorylation, conserving ATP, and avoiding needless buildup of phosphorylated intermediates. Unphosphorylated glucose can exit the cell through membrane transporters when intracellular glucose concentrations are higher than extracellular levels. One exception is hexokinase IV (also known as glucokinase), which is expressed in the liver, pancreatic beta cells, and enteroendocrine cells. Unlike other isoforms, glucokinase is not inhibited by G6P, allowing it to continue phosphorylating glucose even at high G6P concentrations [144, 145].

Glucose transporters

Glucose transporters are divided into two structurally and functionally distinct groups: sodium-dependent glucose transporters (SGLTs) and sodium-independent glucose transporters (GLUTs) [146-148]. SGLTs mediate the secondary active transport of glucose across cell membranes by utilizing the sodium gradient. This means that glucose transport via SGLTs is independent of the glucose concentration gradient, allowing glucose to be transported against its concentration gradient from areas of lower to higher concentration [146, 148, 149]. In contrast, GLUTs facilitate passive glucose transport through facilitated diffusion, allowing glucose to move down its concentration gradient [146, 148, 150, 151]. A schematic representation of glucose secondary active transport and the facilitated passive diffusion is shown in **Figure 9**.

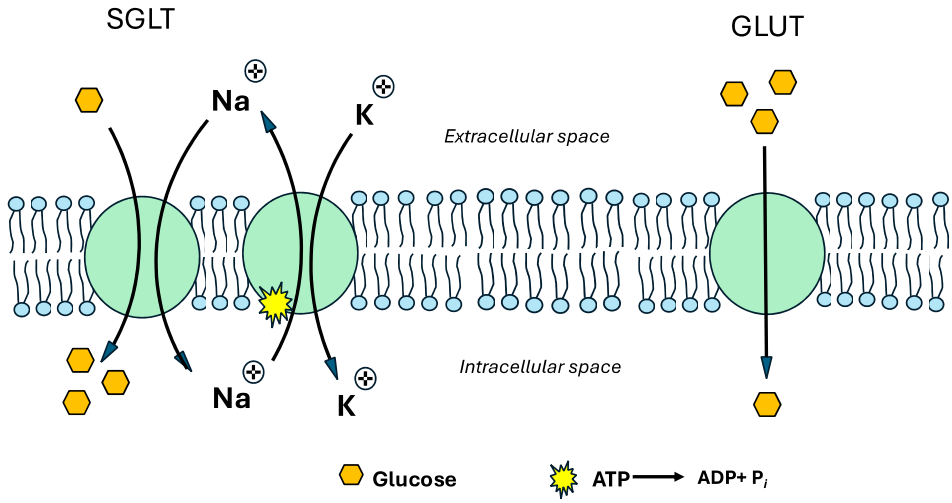

Figure 9. Schematic representation of glucose secondary active transport and facilitated passive diffusion.

Beyond the classical glucose transporter families, recent studies have identified additional glucose transporters such as the SWEET (SLC50) and Spinster (SLC63) protein families [146, 152, 153]. SWEET transporters, an acronym for "Sugars Will Eventually be Exported Transporters," primarily function as sugar efflux transporters and are predominantly found in plants. However, animal homologues are believed to be involved in the release of glucose from glucose-producing cells in the liver, epididymis, and mammary glands. In humans, the SWEET family is represented by a single member, SWEET1 (SLC50A1) [153]. The Spinster1 transporter plays a role in exporting sugars from the lysosomes. Despite these discoveries, the transport mechanisms and biological significance of these transporters have not been fully elucidated [152, 153].

Sodium-dependent glucose transport plays a central role in glucose absorption within the gastrointestinal tract and in its reabsorption in the kidneys. In contrast, glucose homeostasis in most other tissues, such as adipose tissue, skeletal muscle, neurons, and red blood cells, is primarily maintained by facilitated glucose transporters [154].

The substrate affinity of glucose transporters is usually quantified in terms of the Michaelis constant (K_m), which is a key parameter in enzyme and transporter kinetics [155]. K_m represents the substrate concentration (*e.g.,* glucose concentration in mM) at which a transporter operates at half of its maximum transport velocity (V_{max}). The transport velocity is given by:

$$v = \frac{V_{max}c}{K_m + c} \tag{6}$$

A low K_m value indicates a high affinity between the transporter and its substrate (glucose), indicating that the transporter can achieve half-maximal velocity at a lower substrate concentration. In contrast, a high K_m value suggests a lower affinity, requiring a higher substrate concentration to reach half-maximal velocity (**Figure 10**).

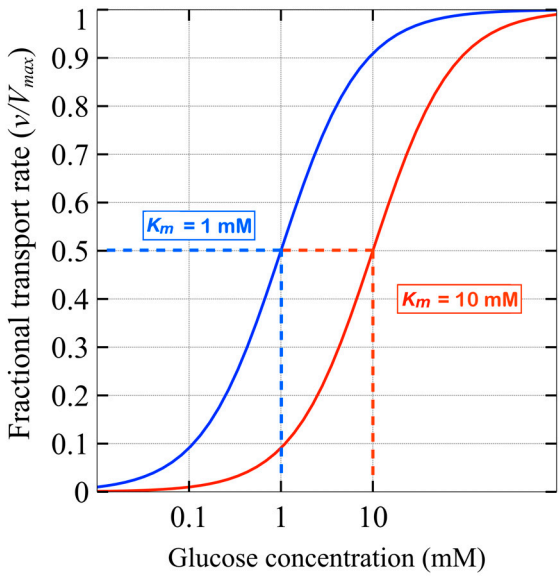

Figure 10. A lower affinity for the substrate (glucose) in terms of the Michaelis constant (K_m) indicates that a higher substrate concentration is required to achieve the same fractional transport rate (v/V_{max}).

Most cells in the body express multiple glucose transporters that facilitate the movement of glucose across cell membranes. Different tissues express specific glucose transporters with varying affinities for glucose tailored to their unique metabolic needs [139, 156].

These differences in affinity are crucial for regulating glucose uptake and directing glucose flow into and out of cells, ensuring efficient glucose absorption, reabsorption, and maintenance of glucose levels within cells and body fluids [139, 157]. For example, GLUT3 has a low K_m value, indicating a high affinity for glucose, which allows it to efficiently transport glucose, even at low concentrations. This is especially important for neurons, which rely heavily on glucose for energy and require continuous glucose uptake despite fluctuations in blood glucose levels [146]. In contrast, GLUT2 has a high K_m value (low affinity) but possesses a high capacity for glucose transport, making it suitable for transporting large amounts of glucose at high concentrations. GLUT2 is expressed in liver cells (hepatocytes), pancreatic β cells, renal tubular cells, and enterocytes of the small intestine [147, 158]. In hepatocytes, it facilitates bidirectional glucose transport, enabling glucose

to enter the cell when blood glucose levels are high (such as after a meal) and exit the cell during gluconeogenesis when glucose is produced and released into the bloodstream [158].

Sodium-glucose transporters

Sodium-glucose transporters (SGLTs) belong to the solute carrier's family, SLC5, which includes 12 different members [159, 160]. These transporters mediate the active transport of various substances, including sugars (such as glucose and galactose), vitamins, amino acids, and small organic ions (such as choline) [159, 160]. SGLTs utilize sodium gradients across the cell membrane to transport these substrates into cells. Among the 12 SLC5 family members, SGLT1-5 are sugar transporters [159, 160] (**Table 1**). SGLT1 and SGLT2 have been the most widely studied.

Table 1. Sodium-glucose transporters (SGLTs)

SLC5	Expression site	Substrate	Glucose affinity (K_m)	Reference
SGLT1/ SLC5A1	Intestinal tract, kidney	Glucose Galactose	0.2-0.5 mM ~0.5 mM	[149, 154, 159, 160]
SGLT2/ SLC5A2	Kidney	Glucose	3-6 mM	[149, 154, 159, 160]
SGLT3/ SLC5A4	Enteric neurons, neuromuscular junctions	Glucose sensor	~20 mM	[149, 159, 160]
SGLT 4/ SLC5A9	Small intestine, kidney	Glucose Mannose	~2 mM ~0.15 mM	[149, 159, 160]
SGLT 5/ SLC5A10	Kidney	Mannose, Fructose	Transfers mainly fructose	[149, 159, 160]
SMIT1/ SLC5A3	Small intestine, brain	Myo-inositol	Transfers mainly myo-inositol	[159, 160]
SMIT2/ SLC5A11	Brain	Myo-inositol	Transfers mainly myo-inositol	[159, 160]
NIS/ SLC5A5	Thyroid gland	Iodide	Not well characterized	[159, 160]
SMCT1/ SLC5A8	Colon, kidney, brain, retina	Lactate, Monocarboxylates, Short chain fatty acids	Transfers mainly monocarboxylates	[159, 160]
SMCT2/ SLC5A12	Intestinal tract, kidney	Lactate, Monocarboxylates, Short chain fatty acids	Transfers mainly monocarboxylates	[159, 160]
CHT1/ SLC5A7	Cholinergic neurons	Choline	Transfers mainly choline	[159, 160]
SMVT/ SLC5A6	Intestinal tract, kidney, placenta	Water-soluble vitamins	Transfers mainly biotin, pantothenic acid (vitamin B5), and lipoic acid	[159, 160]

SGLT1 is mainly found in the small intestine and proximal convoluted tubules of the kidney, where it plays a key role in glucose absorption and reabsorption. SGLT1 facilitates the co-transport of sodium and glucose at a ratio of 2:1, meaning that two sodium ions are transported with each glucose molecule. This transporter has a high affinity for glucose, but a low capacity. SGLT1 is the primary mechanism for absorbing dietary glucose in the intestine, ensuring a steady supply of this essential energy source from the food consumed. In the kidneys, SGLT1 complements the function of SGLT2 during reabsorption of filtered glucose (5-10%) [149, 154, 159].

SGLT2 is mainly found in the kidneys (proximal convoluted tubule, S1/S2 segments). SGLT2 facilitates the co-transport of sodium and glucose at a 1:1 ratio. This transporter has a low affinity for glucose, but high capacity, and is responsible for 90-95% of glucose reabsorption from the glomerular filtrate; the remaining 5-10% is reabsorbed by SGLT1 downstream of the SGLT2 transporters in the distal proximal tubule (S2/S3 segments). This arrangement with a high-capacity/low-affinity transporter followed by a low-capacity/high-affinity transporter ensures that almost no glucose is eliminated in the urine under normal physiological conditions [149, 154, 159].

The specific properties and tissue distribution of SGLT channels are utilized to regulate glucose absorption and are applied in the treatment of various disorders. For example, SGLT2 inhibitors induce glucosuria and improve metabolic control in diabetes patients. SGLT1 inhibitors can be used to treat constipation by inhibiting intestinal glucose absorption [154, 161].

Facilitative glucose transporters

Facilitative glucose transporters (GLUTs) are transmembrane proteins encoded by SLC2 genes that passively transport glucose and other hexoses, such as fructose, mannose, galactose, as well as glucosamine, myo-inositol, and even ascorbic acid, across cell membranes [140, 150, 153]. These GLUT transporters are energy-independent and move glucose and other similar substrates along their concentration gradients. In certain organs such as the liver, the direction of glucose transport can be reversed under specific conditions. For instance, during fasting or exercise, liver cells release glucose derived from glycogenolysis or gluconeogenesis into the blood to maintain systemic glucose levels. This dynamic regulation of glucose transport is essential for maintaining overall glucose homeostasis across different tissues and metabolic states [139, 140, 150]. It has been suggested that the affinity for glucose is asymmetric, insofar that the transport of glucose in the outward direction is lower than that in the inward direction. However, the data supporting this hypothesis were obtained at a low temperature and do not seem to apply to normal physiological conditions [140, 151, 162].

The expression of different GLUT transporters varies depending on the specific cell type and glucose requirement. GLUTs are divided into three classes based on their

amino acid sequence similarity and affinity for glucose transport. Fourteen GLUT channels have been identified, although the functions of some remain unclear [140, 150, 151, 163]. GLUT1-4 are the most extensively studied and well-characterized glucose transporters [139, 140, 147, 151, 163, 164]. The main sites of GLUT channel expression, preferred substrates, and substrate affinity are summarized in **Table 2**.

Table 2. Main expression sites of different GLUT transporters, their preferred substrates, and substrate affinity

Class	GLUT	Expression site	Substrate	Substrate affinity, K_m	Reference
I	GLUT-1	Blood-brain and -tissue barrier, erythrocytes, fetal tissue	Glc, Gal, Man, GlcN	Glc 1-3 mM	[140, 150, 151, 158]
	GLUT-2	Liver, pancreas, small intestine, kidney, brain	Glc, Gal, Fru, Man, GlcN	Glc 15-20 mM GlcN ~0.8 mM Fru ~76 mM Gal ~92 mM Man ~125 mM	[140, 150, 151, 158]
	GLUT-3	Brain, testis	Glc, Gal, Man, Xyl	Glc 1-2 mM	[140, 150, 151, 158]
	GLUT-4	Adipose tissue, skeletal and cardiac muscle	Glc, GlcN	Glc ~5 mM	[140, 150, 151, 158]
	GLUT-14	Testis	Unknown	Not known	[140, 150]
II	GLUT-5	Small intestine, kidney	Fru	Fru 6-13 mM	[140, 150, 151, 158]
	GLUT-7	Testis, prostate, small intestine and colon	Fru, Glc	Glc ~0,3 mM Fru ~0,06 mM	[140, 150, 151]
	GLUT-9	Kidney, liver, lung, small intestine, placenta	Urate (Glc, Fru)	Not known for urate Glc ~0,61 mM Fru ~0.42 mM	[140, 150, 151, 158]
	GLUT-11	Heart, skeletal muscle	Fru, Glc	Fru ~0.06 mM Glc ~0.2 mM	[140, 150, 151, 158]
	GLUT-6	Brain, spleen, leukocytes	Glc	Glc ~5 mM	[140, 150, 151, 158]
III	GLUT-8	Testis, brain, adipose tissue, adrenal gland, liver, lung	Glc, Fru, Gal	Glc ~2 mM	[140, 150, 151, 158]
	GLUT-10	Heart, lung, brain, liver, pancreas, skeletal muscle, kidney	Glc, Gal	Glc ~0.3 mM Gal ~0.3 mM	[140, 150, 151, 158]
	GLUT-12	Heart, prostate, skeletal muscle, placenta	Glc	Glc ~0.3 mM	[140, 150, 151, 158]
	GLUT-13	Brain, adipose tissue	Myo-inositol	Myo-inositol ~100 mM	[140, 150, 151]

Glc, glucose; Gal, galactose; Man, mannose; Xyl, xylose; GlcN, glucosamine; Fru, fructose; Affinity K_m, Michaelis-Menten constant

Glucose transporters differ in their affinity for glucose. GLUT1, GLUT3, and GLUT4 have high affinity, allowing rapid glucose uptake into cells. In contrast, GLUT2, primarily found in the liver and pancreas, has a lower affinity; which explains why, glucose transport varies with changes in concentration, such as during meals or fasting [140, 150, 165]. GLUT4 is mainly found in insulin-sensitive tissues such as muscle and fat. After insulin release, GLUT4 quickly moves to the cell membrane, increasing glucose absorption in these tissues. This mechanism allows insulin to precisely regulate glucose uptake in adipose and muscle cells [156, 166].

Several GLUT inhibitors originating from natural, semi-natural, or synthetic sources have been identified [167, 168]. Inhibition of GLUT is a promising strategy for treating malignant tumors, as many cancer cells depend on increased glucose uptake. Additionally, GLUT inhibitors are widely used in cell metabolism research to explore glucose transport mechanisms and their roles in various diseases including cancer [164, 167-169].

Glucose transport across different types of endothelia

A layer of capillary endothelial cells acts as a barrier that separates blood and its components from surrounding tissues [170]. This barrier is essential to prevent uncontrolled penetration of fluids, molecules of various sizes, and blood cells into adjacent tissues. The permeability of capillaries varies significantly depending on their location and function within the body [170]. For example, the blood-brain barrier (BBB) is composed of brain-specific endothelial cells that differ markedly from microvascular endothelial cells found elsewhere [170, 171]. The endothelial monolayer of the BBB forms the strongest endothelial barrier in the body. In addition to endothelial cells, the BBB includes a basement membrane, astrocytic end-foot projections, and numerous embedded pericytes, creating extremely tight intercellular junctions with gaps as small as 1.4–1.8 nm [170]. This structure effectively prevents even paracellular glucose transport under physiological conditions. Consequently, glucose entry into the brain relies heavily on glucose transporters located in endothelial cell membranes. GLUT1 is the primary transporter in endothelial and glial cells of the BBB, whereas neurons predominantly express GLUT3. A small subset of neurons also express GLUT2 and/or GLUT4. The presence of transporters with different affinities for glucose in endothelial cells and neurons ensures that the glucose requirements of nervous tissue are met [146, 170, 172].

Although endothelial cells of peripheral capillaries form relatively tight intercellular junctions, they allow limited bidirectional transport of nutrients and molecules. Peripheral endothelial cells express GLUT1, GLUT3, and GLUT4 but do not express the low-affinity glucose-fructose transporter GLUT2 [170].

In contrast to GLUT transporters, knowledge about the presence and function of SGLT channels in capillaries from various sites is limited, although their presence

in certain capillaries has been documented. For example, the expression of SGLT1, has been demonstrated in brain capillaries. This transporter may contribute to the removal of glucose from the brain interstitium, which is particularly important for maintaining the glucose concentration gradient between the blood and brain interstitium [172]. Although SGLT2 has also been detected in specific brain regions such as the amygdala and hypothalamus, SGLT2 expression in the brain is significantly lower than that of SGLT1 [172, 173]. SGLT2 protein expression has been observed in cultured human umbilical vein, coronary arteries, and aortic endothelial cells [174]. However, the specific distribution and function of SGLT2 in all types of capillaries are not fully established.

Glucose transporters and the peritoneal membrane

The role of glucose transporters, GLUTs, and SGLTs in peritoneal dialysis is complex and incompletely understood, despite their identification in the peritoneal membrane. Studies over the past few decades have provided valuable insights into the expression and function of these transporters in the context of PD; however, many questions remain unanswered.

Early research by Kruse et al. demonstrated that interleukin-1 beta and the non-selective GLUT inhibitor cytochalasin B influence the transport of radiolabeled deoxy-(^{3}H)-glucose, a glucose analog, across human peritoneal mesothelial cell membranes [175]. This study underscores the potential significance of glucose transporters in facilitating glucose movement across the peritoneal membrane. Later, Schröppel et al. identified GLUT1, GLUT3, and SGLT1 in cultured human peritoneal mesothelial cells, providing further evidence of the presence of multiple glucose transporter types in the peritoneum [176].

Debray-García et al. were the first to identify SGLT2 in peritoneal mesothelial cells [177]. They also reported the expression of SGLT1, SGLT2, GLUT1, and GLUT2 in mesothelial cells of diabetic animals, both with and without exposure to peritoneal dialysis solutions. The mesothelial changes observed in diabetic animals closely resembled those induced by the PD solutions.

Balzer et al. conducted a comprehensive study demonstrating the expression of SGLT1 and SGLT2 in mouse and human peritoneal membranes [178]. Using immunofluorescence in both mouse and human peritoneal membranes, they showed that both SGLT1 and SGLT2 are predominately expressed in a single layer of mesothelial cells but can also be found in the submesothelial zone. Their research in a mouse model also revealed that chronic exposure to glucose-based PD fluid led to the upregulation of GLUT1 and GLUT3, while GLUT4 was downregulated.

Schricker et al. conducted an analysis of peritoneal membrane biopsies from various patient groups, comprising individuals without kidney failure, those undergoing peritoneal dialysis, and long-term PD patients who had developed encapsulating

peritoneal sclerosis (EPS) [179]. SGLT2, GLUT1, and GLUT3 were consistently expressed in the human peritoneum across all patient groups. Notably, they observed significant upregulation of SGLT2 expression in patients with EPS. This finding suggests a potential association between increased SGLT2 expression and pathological changes occurring in the peritoneal membrane during the development EPS. Schricker et al. observed that glucose channels are predominantly, but not exclusively, located near the vessel walls, and that most mesothelial cells do not express SGLT-2.

Glucose sparing strategies

Since their commercial introduction there have been significant advancements in the development of peritoneal dialysis solutions, yet the quest continues for an ideal solution that combines therapeutic effectiveness with minimal local and systemic complications. Although glucose-based solutions prevail owing to their cost-effectiveness, availability, and general safety profile, their high glucose concentrations contribute to both peritoneal membrane deterioration and systemic complications, including hyperglycemia, insulin resistance, diabetes, and cardiovascular diseases [67, 68, 180, 181].

Currently, peritoneal dialysis fluids are available in five different anhydrous glucose concentrations: 76 mM (1.36% anhydrous glucose), 83 mM (1.5% anhydrous glucose), 126 mM (2.3% anhydrous glucose), 214 mM (3.86% anhydrous glucose), and 236 mM (4.25% anhydrous glucose). The 1.36% and 1.5% solutions are typically labelled with a yellow color, the 2.3% solution with green, and the two highest glucose concentrations are labelled red (**Table 3**).

Although several alternative osmotic agents have been tested, only icodextrins and amino acids are currently available as alternatives to glucose in clinical practice. However, these solutions cannot completely replace glucose-based solutions [180].

Table 3. Commercially available dialysis fluids for PD

	Physioneal	Extraneal	Nutrineal	Balance	BicaVera
Glucose	75.5 126.0 214.0	0	0	83.2 126.1 235.8	83.3 126.1 235.9
Na	132	133	132	134	134
Cl	101	96	105	100.5	103.5
Ca	1.25/1.75	1.25	1.25	1.25/1.75[c]	1.25/1.75
Mg	0.25	0.25	0.25	0.50	0.50
HCO3	25	0	0	0	34
Lactate	15	40	40	35	0
Icodextrin	0	12[a]	0	0	0
Amino acids	0	0	87.2[b]	0	0

All concentrations are given in mmol/L.

[a] 12 mmol/L ≈ 75 g/L icodextrin (7.5%), see also Morelle et al. [181]
[b] 87.2 mmol/L ≈ 11 g/L amino acids (1.1%)
[c] 1.75 mmol/L Ca is not available for the 4.25% glucose fluid.

FRESENIUS	VANTIVE (Baxter)
4.25% = 236 mM	3.86% = 214 mM
2.3% = 126 mM	2.27% = 126 mM
1.5% = 83 mM	1.36% = 76 mM

Icodextrin is an isoosmolar solution composed of high-molecular-weight glucose polymers derived from corn starch [182]. It provides sustained ultrafiltration, making it particularly useful for patients requiring long dwell times or who have highly permeable peritoneal membranes [128]. Unlike glucose, icodextrin is absorbed more slowly from the peritoneal cavity, thus maintaining a colloid osmotic pressure gradient over an extended period [183]. Sustained ultrafiltration can help improve fluid balance [184] and blood pressure control [185]. Moreover, replacing part of the glucose-containing peritoneal dialysis solutions with icodextrin can reduce unwanted weight gain, improve dyslipidemia, and decrease insulin resistance [180, 186]. Additionally, using icodextrin instead of glucose reduces the total amount of glucose degradation products (GDP), which enhances peritoneal health [187-189]. Icodextrin's isosmolar properties also have positive effects on the membrane by reducing osmotic stress. To date a neutral-pH icodextrin solution is not available on the market, which could further enhance its positive properties. A concern associated with the use of icodextrin is the absence of maltase, an enzyme that breaks down maltose into glucose in human blood, leading to increased accumulation of maltose [188]. Although pharmacokinetic studies show that maltose is efficiently cleared by the kidneys in people with preserved renal function and is removed during subsequent dialysis exchanges in patients on dialysis [182, 188], the use of icodextrin should in general not exceed once daily.

Amino acid-based peritoneal dialysis solutions offer an alternative approach that may help reduce glucose exposure during PD. These solutions have shown potential benefits, particularly for malnourished patients, by providing a source of amino

acids and promoting protein synthesis. However, their use has been limited by several factors. The impact of amino acid-based solutions on peritoneal membrane function over extended periods remains unclear, and some patients experience difficulties tolerating these solutions. The use of amino acid-based solutions can result in elevated blood urea nitrogen levels, potentially exacerbating uremic symptoms. Current practice generally restricts the use of amino acid-based solutions to no more than once daily [185].

Other proposed alternatives, including glycerol, xylitol, and L-carnitine, are still experimental [190, 191]. Their low molecular weights lead to rapid osmotic gradient loss and potential systemic accumulation, particularly in patients without residual renal function [192].

Beyond the search for new osmotic agents, novel strategies aim to reduce glucose absorption and/or enhance ultrafiltration. These approaches include bimodal treatment strategies such as combining icodextrin with glucose solutions [183, 193] or alternating between hypertonic glucose dwell and ultralow glucose dwell periods [194, 195].

Although there is still limited evidence that regulating the entry of glucose into the endothelium and other peritoneal membrane cells through glucose channels or transporters could reduce the negative effects of glucose or enhance its effectiveness as an osmotic agent during dialysis, initial experimental studies are promising.

Bergling et al. demonstrated that blocking GLUT channels with phloretin, a non-selective GLUT channel inhibitor, significantly reduced both glucose absorption and ultrafiltration in an experimental peritoneal dialysis rat model [196]. In contrast, the selective SGLT1 inhibitor mizagliflozin had no significant effect on glucose absorption or ultrafiltration [196]. An earlier study with phlorizin, a dual SGLT channel blocker, also demonstrated a reduction in glucose diffusion across the peritoneal membrane [197], possibly due to the rapid conversion of phlorizin to phloretin, a non-selective GLUT blocker.

Animal studies, research on human mesothelial cells, and peritoneal membrane biopsies have shown the presence of at least four GLUT channels in the peritoneal membrane [176-179]. However, studies confirming their functional role, rather than just their expression, in the context of peritoneal dialysis are lacking. While there are more studies on SGLT2 inhibitors and several clinical trial results with various SGLT2 inhibitors are in the pipeline, the findings to date are quite inconsistent, and there is no strong evidence supporting the benefits of SGLT2 inhibitors in the context of peritoneal dialysis [198-202].

In a non-uremic peritoneal dialysis rat model, Zhou et al. showed that intragastric administration of empagliflozin, a selective SGLT2 inhibitor, reduced glucose absorption and improved ultrafiltration [203]. In an earlier study (available only in Chinese), Hong et al. demonstrated that phlorizin, a dual SGLT1/SGLT2 inhibitor,

and phloretin both improved ultrafiltration and reduced the D/P creatinine ratio in a chronic rat model of peritoneal dialysis [204]. In a 2018 patent application, Vorobiov et al. reported using a single subcutaneous injection of phlorizin in an acute mouse model, as well as intraperitoneal administration in rats, for peritoneal dialysis. They observed higher dialysate glucose concentrations compared with SHAM animals [205]. The patent application was subsequently abandoned due to the discovery of the article by Hong et al.

Balzer et al. also showed that intraperitoneal SGLT2 inhibition with dapagliflozin reduced the structural and functional changes associated with PD fluid-induced peritoneal fibrosis, thus improving ultrafiltration capacity [178]. This finding suggests a potential therapeutic role of SGLT2 inhibitors in preserving peritoneal membrane function during long-term PD. However, their study also revealed a paradoxical effect: intraperitoneal administration of dapagliflozin without PD fluid tended to cause peritoneal membrane thickening and impair ultrafiltration efficiency, albeit to a lesser extent than administration of PD solutions alone.

The studies and their results involving GLUT and SGLT channels are summarized in **Table 4**.

Table 4. Summary of evidence on the role of SGLT or GLUT inhibitors in peritoneal dialysis

Study	Year	Subjects	PD duration	SGLT inhibitors	GLUT inhibitors	Key findings
Kruse M et al [175]	1996	Human PMCs in vitro			Cytochalasin B	Glucose transport was almost completely inhibited by cytochalasin B.
Schröppel B et al [176]	1998	Human PMCs in vitro		Phlorizin (SGLT1/2)	Cytochalasin B Phloretin	MsC express GLUT1, GLUT3, and SGLT1. Glucose uptake is mediated by SGLT1 and GLUTs. Phlorizin reduced glucose uptake by 30 to 45%. Cytochalasin B and phloretin inhibited glucose uptake almost completely.
Debray-García Y et al [177]	2016	Peritoneal samples from diabetic rats and rat PMCs in vitro				SGLT-1, SGLT-2 and GLUT-2 were increased while GLUT-1 was decreased in diabetes. In diabetes and peritoneal fluid group expression of SGLT2 decreased but there were no changes in other transporters. Diabetes induced changes in glucose transporters. EMT was present in diabetic rats, even before initiation of uremia or dialysis.
Hong M et al [204]	2016	Chronic PD model in uremic rats (5/6-nephrectomy) and studies of HPECs in vitro	Chronic PD	Phlorizin (SGLT1/2)	Phloretin	Higher ultrafiltration, lower D/P-creatinine, higher D/D0 glucose and less peritoneal thickening in phloretin/phlorizin treated animals. Upregulation of GLUT1 and SGLT1 in chronic PD that was alleviated in phlorizin- and phloretin treated animals.
Zhou Y et al [203]	2019	PD model in nonuremic rats and Human PMCs in vitro	Acute PD	Empagliflozin (SGLT2)		Empagliflozin reduced glucose uptake and increased ultrafiltration (240min PET). Empagliflozin decreased both glucose consumption and uptake of human

						PMCs in high glucose concentration conditions. Expression of SGLT-2 was also increased.
Balzer M et al [178]	2020	Chronic PD model in nonuremic mice and mice and human peritoneal samples	5 weeks	Dapagliflozin (SGLT2)		Dapagliflozin reduced effluent TGF-β, peritoneal thickening, fibrosis, microvessel density and improved UF (120min PET), but did not affect development of high-glucose transporter status. Upregulation of SGLT2 expression in mice receiving high-glucose peritoneal dialysis fluids, was completely abrogated by dapagliflozin. GLUT1 and 3 was upregulated and GLUT4 downregulated in response to chronic exposure to peritoneal dialysis fluids. GLUT1, 3, 4 expression was unaffected by dapagliflozin. SGLT1 and SGLT2 expression in mesothelial cell layer and SGLT1around capillaries in human peritoneal biopsies.
Martus G et al [206]	2021	PD model in nonuremic rat	Acute PD	Empagliflozin (SGLT2)		No significant treatment effect on glucose or osmotic water transport.
Shentu Y et al [207]	2021	Chronic PD model in nonuremic mice and human PMCs in vitro	4 weeks	Empagliflozin (SGLT2)		Protective effect on peritoneal fibrosis by suppressing TGF-β/Smad signaling- associated proteins and inflammatory cytokines (TNF-α, IL-1β, IL-6). D30/D0 and D/Purea ratios were significantly improved in the empagliflozin treatment group.
Martus G et al [197]	2022	PD model in nonuremic rat	Acute PD	Phlorizin (SGLT1/SGLT2)		Glucose diffusion capacity during the initial part of the PD dwell was reduced. No significant treatment effect on UF rate and sodium clearance.

Schrincker S et al [179]	2022	Human peritoneal biopsies				SGLT-2, GLUT1 and GLUT3 were expressed in the peritoneal membrane, mainly near the peritoneal membrane vessel walls. SGLT-2 protein expression increases with the duration of PD, especially in EPS patients.
Shi P et al [208]	2022	Chronic PD model in nonuremic rat and human PMCs in vitro	4 weeks	Empagliflozin (SGLT2)		Reduction of peritoneum thickness. Inhibition of high glucose- induced EMT and oxidative stress by activating the Nrf2/HO-1 signaling pathway. Improvement of peritoneal function (Net UF, D/P of creatinine and D240/D0).
Alhwiesh A et al [209]	2022	PD patients (50 patients)	6 months	Dapagliflozin (SGLT2)		Dapagliflozin was not associated with change of the peritoneal transport status. Dapagliflozin increased urine and ultrafiltration volume.
Bergling K et al [196]	2022	PD model in nonuremic rat	Acute PD	Mizagliflozin (SGLT1)	Phloretin	Phloretin reduced glucose absorption (>30%) and improved UF efficiency (>50%). No effect on glucose transport–related parameters or osmotic water transport was observed using mizagliflozin.
Wang J et al [210]	2023	Chronic PD model in nonuremic rat and human PMCs in vitro	5 weeks	Canagliflozin (SGLT2)		SGLT2 inhibition improved peritoneal fibrosis by ameliorating peritoneal hypoxia and inhibiting the HIF-1α/TGF-β/p-Smad3 signaling pathway. High glucose peritoneal dialysate increased the expression of GLUT1, GLUT3 and SGLT2, all of which were inhibited by Canagliflozin. Canagliflozin inhibited glucose uptake and creatinine hypertransportation and increased UF.

Hamdan Z et al [200]	2024	PD patients (20 patients)	1 month	Dapagliflozin (SGLT2)		No significant treatment effect on glucose absorption (D240/D0)
Lai Jia-Wen et al [199]	2024	PD patients (11patients)	31 months	Empagliflozin or Dapagliflozin (SGLT2)		SGLT2 inhibition increased ultrafiltration volume and higher hemoglobin levels.
Moral Berrio E et al [211]	2024	PD patients (12 patients)	6 months	Empagliflozin or Dapagliflozin (SGLT2)		SGLT2 inhibitors are safe and effective to preserve RKF.
Vorobiov M et al [205]	2024	PD model in anuric mice and rat	Acute PD	Dapagliflozin (SGLT2) Empagliflozin (SGLT2) Sotagliflozin (SGLT1/2) Phlorizin (SGLT1/2)		Glucose diffusion from the dialysis fluid into the blood was sodium-dependent. Dual SGLT blockade with phlorizin and sotagliflozin reduced the increase in blood glucose and fluid absorption from the peritoneal cavity. Selective SGLT2 inhibitors failed to reduce glucose and fluid absorption from the peritoneal cavity.
Martus G et al [212]	2024	PD model in nonuremic rat	Acute PD		Phloretin Ritonavir Indinavir BAY-876	Non-selective GLUT channel blockade with phloretin and ritonavir, which inhibit both GLUT1 and GLUT4 channels, significantly reduced the diffusion capacity of [^{18}F]-DG and improved the ultrafiltration rate. Selective GLUT1 channel blockade with BAY-876 and selective blockade of GLUT4 channels with indinavir did not result in significant changes in [^{18}F]-DG and glucose diffusion or water transport.

Dt/D0, dialysate glucose ratio, t=min; D/P, dialysate plasma ratio; EMT, epithelial to mesenchymal transformation; EPS, encapsulating peritoneal sclerosis; [^{18}F]-DG, fluorodeoxyglucose; GLUT, facilitate glucose transporter; HIF-1α, hypoxia-inducible factor 1-alpha; HPEC, human peritoneal endothelial cells; IL, interleukin; PD, peritoneal dialysis; PET, peritoneal equilibration test; PMCs, peritoneal membrane cells; p-Smad3, phosphorylated Smad3; RKF, residual kidney function; SGLT, sodium-glucose cotransporter; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; UF, ultrafiltration

Peritoneal membrane aging

Changes in the peritoneal membrane during peritoneal dialysis are the result of a complex interplay between various factors. These include mechanical stress from increased intraperitoneal volume, catheter irritation, osmotic stress from hypertonic solutions, peritonitis episodes, and the uremic state itself [213].

The composition of PD solutions plays a crucial role in peritoneal membrane health. Traditional PD solutions containing lactate as a buffer and having a low pH can damage mesothelial cells and induce mesothelial-to-mesenchymal transition [62, 187, 191]. Additionally, heat sterilization of glucose-based peritoneal dialysis solutions results in the production of glucose degradation products (GDPs). GDPs are highly toxic to cells, inducing oxidative stress and causing membrane damage [62, 214].

In recent years, the development of dual-chamber PD fluid bags has led to the development of more biocompatible solutions with neutral pH and lower GDP concentrations. These bags separate glucose from the buffer and electrolytes until use, thereby reducing GDP formation during sterilization [187, 215, 216]. However, access to these improved solutions varies globally, with limited availability in low-income and middle-income countries.

While there is no definitive evidence that biocompatible PD fluids improve overall patient or technique-specific survival [180, 217], current evidence suggests fewer adverse effects on the peritoneal membrane over time compared with conventional PD fluids [187, 218]. Furthermore, biocompatible solutions have been associated with better preservation of residual kidney function [215, 217].

Glucose remains the most widely used osmotic agent in peritoneal dialysis solutions, despite its well-documented adverse effects. This is due to its cost-effectiveness, safety, availability, and efficacy in achieving desired dialysis outcomes [191]. However, long-term use of glucose-based PD solutions leads to significant alterations in the peritoneal membrane structure and function [66, 219-221].

During PD, the peritoneal cavity is exposed to dialysate glucose concentrations that substantially exceed normal plasma levels. This creates a persistent hyperglycemic environment within the peritoneal membrane, affecting both diabetic and non-diabetic patients. Long-term PD induces several structural changes in the peritoneal membrane, such as the accumulation of advanced glycation end-products (AGEs) in subendothelial and perivascular areas, subendothelial hyalinosis, vascular narrowing or occlusion, neoangiogenesis, thickening of the interstitial layer, and alterations of mesothelial cells [66, 123, 213, 222]. These changes are manifested within months of initiating PD and progressively worsen over time [213, 222].

High glucose levels in PD solutions trigger various molecular pathways that contribute to peritoneal membrane damage. The TGF-β signaling pathway plays a crucial role, with increased TGF-β expression in mesothelial cells leading to activation of Smad2/3, which forms a complex with Smad4 [62, 191, 213]. This complex translocates to the nucleus and regulates transcription of fibrosis-related genes. TGF-β also promotes mesothelial-to-mesenchymal transition (MMT) [62, 223] and activates connective tissue growth factor (CTGF) [62, 191]. Moreover, elevated production of interleukin IL-1β, IL-6, and IL-17 plays an important role in the development of peritoneal fibrosis. [62, 191].

The hypoxia-inducible factor 1α (HIF-1α) pathway is another important mechanism underlying glucose-induced peritoneal damage [65, 191]. Hyperglycemia enhances glycolysis, inhibits the pyruvate dehydrogenase complex, and leads to lactic acid accumulation, increased collagen synthesis, and decreased extracellular matrix (ECM) degradation [191]. Furthermore, chronic hyperglycemia induces a state of cellular pseudohypoxia by impairing the oxidation of NADH to NAD^+, resulting in an increased NADH/NAD^+ ratio [65, 66]. This pseudohypoxic state stabilizes and activates HIF-1α, enhancing the expression of its downstream target genes, such as VEGF, GLUT1, TGF-β, and connective tissue growth factor (CTGF) [65, 66, 191].

Progressive functional changes in the peritoneal membrane include increased small solute transport and decreased ultrafiltration efficiency. Approximately three to four years after PD initiation, ultrafiltration through small pores and aquaporins decrease more than would be predicted by the increased small solute transport alone. These functional alterations are attributed to vascular hyalinosis, changes in vascular lumen, thickened interstitial layer, increased GLUT1 channel expression, and enhanced glucose uptake by myofibroblasts due to cellular pseudohypoxia [65, 224].

To date, there is no convincing evidence that significant changes in aquaporin-1 (AQP1) expression occur in peritoneal capillaries, even during the development of encapsulating peritoneal sclerosis (EPS), despite a marked reduction in free-water transport and sodium sieving. This hypothesis is supported by computer simulations [225] and studies on AQP1 expression in peritoneal biopsies [63]. When comparing AQP1 expression between EPS patients and controls, Morelle et al. found no significant difference in AQP1 levels; however, their study comprised only seven patients with EPS [63]. Similarly, Devuyst et al. examined AQP1 expression in peritoneal membrane biopsies and found relatively stable AQP1 expression in healthy individuals, patients with renal failure, and those with hemodialysis or peritoneal dialysis. Notably, this study included only four biopsies from patients with PD, with a median PD duration of 13 months [226].

Chen and colleagues recently proposed an alternative hypothesis to explain the microvascular complications of diabetes [227]. They compared changes in retinal microvascular endothelial cells and aquaporin-1 (AQP1) expression between

patients with diabetes and healthy controls. They also observed changes in cultured human retinal endothelial cells and a zebrafish model under hyperglycemic conditions. Their study showed that both AQP1 expression and retinal microvessel diameter and perfusion were reduced in patients with diabetes. Similarly, an experimental zebrafish model of hyperglycemia showed reduced expression of Aqp1a.1 and Aqp8a.1, the zebrafish counterparts of human AQP1 and AQP8. These changes in aquaporin expression were associated with altered endothelial cell vacuolization, which regulates microvessel diameter. Overexpression of these aquaporins reduces microvascular complications, in part, by affecting the regulation of vessel diameter. Although studies in isolated retinal cells and an experimental zebrafish model cannot directly be extrapolated to changes in the peritoneal membrane during dialysis, these results are interesting. However, these findings in microvascular structure and aquaporin expression under hyperglycemic conditions, as well as the role of aquaporins in regulating vascular lumen size, may elucidate the changes in the peritoneal membrane that occur during long-term peritoneal dialysis.

Peritoneal membrane testing

The thin peritoneal membrane lining the abdominal cavity plays a crucial role in peritoneal dialysis by allowing passage of water and dissolved substances. This process aims to remove excess fluid and toxic metabolic waste from the bloodstream.

Each person's peritoneal membrane has specific characteristics, that can be challenging when selecting an appropriate treatment regimen or understanding the causes of a patient's declining well-being or dealing with concomitant complications. Although the fundamental concept of peritoneal dialysis may appear straightforward, clinical practice often requires personalized approaches. The assumption that a single treatment regimen is effective in all patients is rarely successful, emphasizing the need for tailored solutions.

To support clinical decision-making, several tests have been developed to evaluate peritoneal membrane function and dialysis efficiency. These tests also enable monitoring of membrane changes over time and facilitating timely adjustments to treatment plans. The most used tests for assessing peritoneal function in scientific research and clinical practice are summarized in **Table 5** and **Figure 11**.

In recent years, the criteria used to evaluate PD efficiency have changed significantly. The focus has shifted from solely removing solutes to managing fluid balance and tailoring therapy to meet each patient's specific goals [123, 130, 228-232].

In clinical practice, the Peritoneal Equilibrium Test (PET) [233] is the most commonly used method to assess the rate of peritoneal solute transfer (PSTR). Owing to its simplicity, PET is widely adopted in many clinics and often serves as the reference method. The standard PET is a 4-hour test using a dialysis solution with a glucose concentration of either 2.3% or 4.25% [123, 133, 233]. This test evaluates the transfer of low molecular weight solutes and measures net ultrafiltration. Two main ratios are calculated: the dialysate-to-plasma creatinine ratio (D_{240}/P-creatinine) measured at the end of the 240 min test, which indicates the rate of solute transfer across the peritoneal membrane, and the dialysate glucose concentration ratio (D_{240}/D_0-glucose), which compares the glucose concentration in the dialysate at 240 min to that at 0 min, reflecting glucose absorption over time. Net ultrafiltration is determined by subtracting the instilled volume from the drained volume. When a 4.25% glucose solution is used, additional evaluations such as sodium dip or sodium sieving, assessed by taking extra samples one hour after the test begins, can be performed. Sodium dip or sieving refers to the initial decrease in the dialysate sodium concentration [234]. A higher glucose concentration also allows for more accurate assessment of net ultrafiltration [133, 235].

Based on the D/P creatinine ratio, the results of the peritoneal equilibration test are often categorized according to solute transfer characteristics as high (H), average (A), or low (L). Therefore, the conventional PET is often described as a semi-quantitative test. However, the primary clinical focus is often on identifying patients with fast solute transfer status [123, 134, 236, 237]. Patients with fast peritoneal membrane solute transfer characteristics often experience fluid retention when using glucose-based dialysis solutions. This issue arises because their membranes absorb glucose quickly, causing a rapid loss of the osmotic gradient required for effective ultrafiltration [134, 236, 238]. Peritoneal equilibration testing not only helps identify these high-risk patients but also guides the selection of treatment regimens tailored to mitigate fluid balance issues [123, 238].

Double Mini-PET involves two consecutive one-hour exchanges with solutions of different concentrations, allowing the assessment of sodium sieving and osmotic conductance to glucose (OCG) [239]. This test is sometimes combined with a 4-hour PET, known as Uni-PET [240].

The Standard Peritoneal Permeability Analysis is a more sophisticated method for evaluating peritoneal membrane function [241]. In this test, dextran 70 is added to a standard PD solution to study the fluid kinetics over a 4-hour period [242]. Other tests, such as the Personal Dialysis Capacity [243] test and Dialysis Adequacy and Transport Test [244], are also available to assess the transport properties of the peritoneal membrane.

The Personal Dialysis Capacity test [243] offers several advantages over the traditional Peritoneal Equilibration Test [233], despite requiring a 24-hour period for completion. This test can be performed by patients in their home environment

following a specific protocol that involves five dialysis exchanges at varying intervals using solutions of different concentrations. The PDC test also incorporates an assessment of residual kidney function and provides comprehensive information about dialysis efficiency parameters, including clearance and Kt/V, along with other peritoneal membrane characteristics that help evaluate the effectiveness of the current therapy [243].

Current evidence provides limited support for routine peritoneal membrane monitoring in patients on peritoneal dialysis, with recommendations for testing frequency varying considerably between guidelines and clinicians. While some practitioners assess membrane function only in response to specific clinical issues, others advocate regular, scheduled testing.

Traditionally, an initial functional assessment has been recommended 4 to 12 weeks after dialysis initiation to establish baseline characteristics and provide a reference point for interpreting future changes [123, 245-247]. However, such testing at the beginning of PD treatment and its benefits is debatable. The latest recommendations advocate a more comprehensive approach when selecting treatment regimens for patients on peritoneal dialysis rather than relying solely on peritoneal transfer measurements. A more patient-centered approach is taking over, emphasizing preservation of residual kidney function (RKF), optimization of quality of life, and addressing individual patient needs [230, 232, 248]. This approach recognizes that preserving RKF can comprise simpler and more flexible dialysis modalities, which can be particularly beneficial for patients still adjusting to a new treatment regimen. Maintaining RKF contributes to better solute clearance, fluid balance, and nutritional status, potentially reducing the need for more intensive dialysis prescriptions [246, 249, 250].

The reliability and clinical utility of peritoneal membrane tests have been the subject of ongoing debate within the nephrological community. Although these tests provide valuable insights into peritoneal membrane function, several factors complicate their interpretation and reproducibility. The protocols for some tests are complex, which can lead to variability in their execution and interpretation across different clinical settings. Moreover, a range of factors can significantly influence test results, potentially leading to erroneous conclusions. These factors include variations in filling volume [240, 251], incomplete drainage of the abdominal cavity prior to testing [252, 253], and differences in the glucose concentrations of the peritoneal solutions [254, 255]. Specific patient health conditions or medications can also influence the results of peritoneal membrane function tests [243]. Especially tests focusing on volume measurements are prone to fluctuations in results and can have problems with reproducibility [253, 256].

These challenges in achieving consistent and reliable results have led some clinicians to question the importance of frequent routine testing. Instead, there is a growing trend towards identifying patients with very high or very low peritoneal

transfer values and reserving detailed testing when clinical problems arise or significant changes in peritoneal function are suspected.

Consequently, there is a definite need for a practical clinical approach applying a brief yet reliable test capable of measuring all the most important properties of the peritoneal membrane in a single assessment. The ideal test should evaluate water transport capacity, osmotic conductance, and solute transfer with reproducible results. It should be adaptable to different fill volumes and glucose concentrations, demonstrate strong correlation with important clinical outcomes, enable personalized treatment planning, and be suitable for patient self-administration. The development of such a comprehensive and practical test would encourage regular monitoring and enhance optimization of individual treatment strategies.

Table 5. Tests for the evaluation of peritoneal membrane function

Test	Study	Year	Solution	Duration	Measurements
Classic Peritoneal Equilibration Test (PET) [233]	Twardowski Z J et al.	1987	2.3%	4h	D/PCrea Dt/D0 glucose UF
Dialysis Adequacy and Transport Test (DATT) [244]	Rocco M V et al	1994	1.5%+1.5%+1.5+2.3% (Four exchanges performed consecutively)	24h (4 exchanges)	D/PCrea Dt/D0 glucose UF Dialysis adequacy measurements
Standard Permeability Analysis (SPA) [241]	Pannekeet M M et al	1995	1.36%+ Dextran 70	4h	D/PCrea Dt/D0 glucose UF MTAC Clpr ELAR
Peritoneal Dialysis Capacity test (PDC) [243]	Haraldsson B	1995	Varying glucose concentration	24h (5 exchanges) 24h urine collection	$A_0/\Delta x$ J_{vL} J_{vAR} Dialysis adequacy measurements
3.86%- Peritoneal Equilibration Test [133]	International Society for Peritoneal Dialysis	2000	3.86%	4h	D/PCrea Dt/D0 glucose UF Na sieving
Modified PET (SPA) [242]	Smit W et al.	2000	3.86%+ Dextran 70	4h	D/PCrea Dt/D0 glucose UF MTAC Clpr ELAR

Test	Study	Year	Solution	Duration	Measurements
Mini-PET [257]	La Milia V et al.	2005	3.86%	1h	D/PCrea Dt/D0 UF Na sieving FWT UFSP
Double Mini-PET [239]	La Milia V et al.	2007	1.36% and 3.86% (Two exchanges performed consecutively)	2h (1h+1h)	D/PCrea Dt/D0 UF Na sieving FWT UFSP OCG
Combined 3.86%-PET [258]	Cnossen T T et al	2009	3.86%	4h (Temporary drainage of dialysate after 1 h)	D/PCrea Dt/D0 UF Na sieving FWT UFSP
Uni-PET [240]	La Milia V	2010	1.36%+3.86% (Two exchanges performed consecutively)	5h (1h+4h) (Temporary drainage of 3.86% dialysate after 1 h)	D/PCrea Dt/D0 UF Na sieving FWT UFSP OCG
Single Dwell test [259]	Martus et al	2020	3.86% or 4.25%	1h	OCG RV Na sieving

$A_0/\Delta x$, diffusive conductance (area parameter); Clpr, protein clearance; D/PCrea, dialysate-to-plasma creatinine ratio; Dt/D0, dialysate glucose ratio; ELAR, effective lymphatic absorption; FWT, free-water transport; J_{vAR}, absorption parameter; J_vL, large pore fluid flux; OCG, osmotic conductance to glucose; UF, ultrafiltration; UFSP, ultrafiltration through small pores.

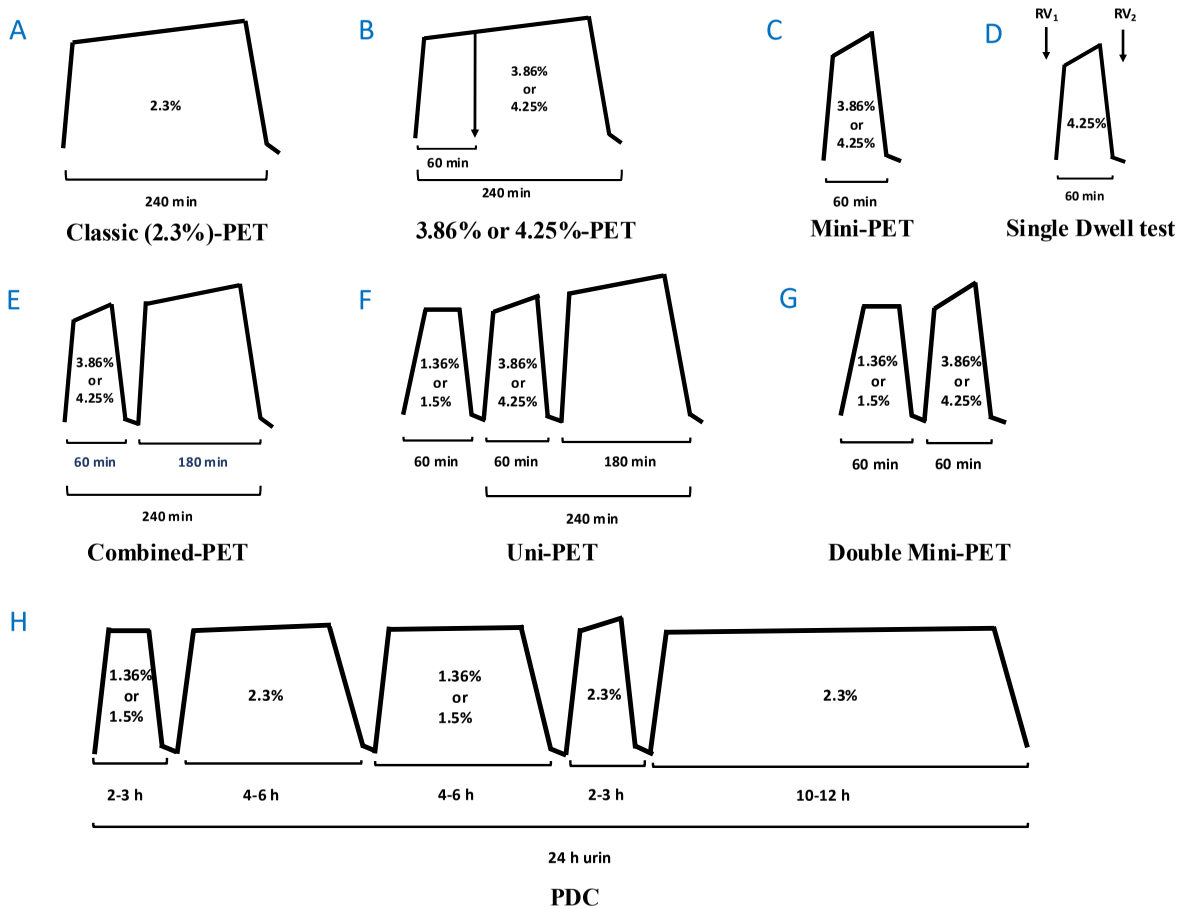

Figure 11. Schematic representation of a test to assess peritoneal membrane function. **A.** Classic (2.3%)-PET. **B.** 3.86% or 4.25%-PET. **C.** Mini-PET. **D.** Single Dwell test. **E.** Combined-PET. **F.** Uni-PET.. **G.** Double Mini-PET. **H.** PDC

Osmotic conductance to glucose

More than 50% of patients undergoing peritoneal dialysis are overhydrated at the start of dialysis treatment [260, 261]. Fluid overload in patients undergoing peritoneal dialysis has a significant impact on cardiovascular health and negatively affects patient prognosis [131, 132].

Water transport across the peritoneal membrane is crucial for patients on peritoneal dialysis, especially for those with insufficient diuresis, to maintain adequate fluid balance.

During PD, the amount of water removed through the peritoneal membrane depends on the characteristics of the membrane itself, such as the ultrafiltration coefficient (L_pS) and reflection coefficient (σ), as well as the properties of the dialysis solution, dwell time, and rate of peritoneal fluid reabsorption [34]. The ability of glucose to induce ultrafiltration through the peritoneal membrane is referred to as osmotic conductance to glucose (OCG), reflecting the efficiency of glucose as an osmotic agent. OCG is expressed as the product of the ultrafiltration coefficient and reflection coefficient ($OCG = L_pS \times \sigma$) [34].

According to the three-pore theory, when glucose-based solutions are used, water is transported across the peritoneal microvascular endothelium via both paracellular (small pores) and transcellular pathways (aquaporins or ultrasmall pores) [34, 183]. Water transported through the paracellular pathways carries small dissolved solutes, whereas free-water transport (FWT) through the aquaporins is without accompanying solutes [29, 34, 92, 117, 183].

The efficiency of the glucose-induced osmotic flow through the small pores is relatively low, with a reflection coefficient of approximately 0.05. This is due to the small size of the glucose molecules compared to the diameter of the small pores, as well as the large number of small pores in the peritoneal membrane. In contrast, aquaporins, ultrasmall pores, are completely impermeable to glucose (reflection coefficient of 1), resulting in 100% efficiency of glucose-induced osmotic flow through the aquaporins. Because of the relatively small number of aquaporins, ultrasmall pores, FWT accounts for approximately 50% of peritoneal ultrafiltration during hypertonic dwells [30, 239, 262, 263].

Structural and functional changes in the peritoneal membrane that occur over time due to exposure to local glucose, glucose degradation products, advanced glycation end products, and mechanical and osmotic stress can all affect osmotic water transport and decrease OCG [63-66, 123, 136, 218].

Several methods have been proposed to estimate OCG [33, 239, 256, 264-266]. One of the most commonly used methods is the 2-hour double mini-PET test developed by La Milia et al., which involves two consecutive 1-hour dwells with different glucose concentrations: first with 1.5% glucose and then with 4.25% glucose

dialysis solutions [239]. OCG is calculated as the ratio between the difference in ultrafiltered volume and the difference in osmotic gradient between the two dwells:

$$OCG = \frac{V_{4.25} - V_{1.5}}{19.3T(G_{4.25} - G_{1.5})} 1.7 \cdot 1000 \quad (7)$$

However, this and many other proposed methods for assessing osmotic conductance to glucose rely on ultrafiltration measurements, which significantly limits their clinical applicability. The primary challenge lies in the variable residual volume in the peritoneal cavity, which introduces inaccuracies in UF quantification [253]. This variability can lead to imprecise OCG calculations, potentially affecting treatment decisions. Although the above equation is theoretically correct, the inclusion of two volume measurements and two glucose concentrations may considerably inflate the variances.

To overcome this limitation, alternative methods that avoids the need for direct UF measurements are being explored. One promising method involves the use of the sodium dip phenomenon to estimate UF, and consequently, OCG. This approach utilizes a single dialysate sodium measurement to accurately assess UF without volumetric measurements [256].

Normal baseline OCG values are usually between 3 to 4 μL/min/mm Hg. An OCG > 2.5 μL/min/mm Hg may be considered normal, whereas an OCG < 2 μL/min/mm Hg may indicate decreased osmotic conductance [34]. Decreased OCG can result from long-term peritoneal dialysis and may serve as a predictor of progressive peritoneal sclerosis [267].

Empagliflozin

Empagliflozin is a potent and selective inhibitor of sodium-glucose cotransporter 2 (SGLT2), a key transporter involved in glucose reabsorption in the kidney. Initially developed as a treatment for glycemic control in type 2 diabetes, empagliflozin has since demonstrated significant benefits that go far beyond its primary glucose-lowering effects [198, 268].

These multiple effects have significantly expanded the use of empagliflozin as a glucose lowering agent. There are still areas where the potential of SGLT2 inhibitors such as empagliflozin have not been fully explored. One such area is their use in patients undergoing peritoneal dialysis, and future studies could further expand the therapeutic applications of these drugs [201].

Fluorodeoxyglucose

2-deoxy-D-glucose is a glucose analog that differs from normal glucose in that the hydroxyl group (-OH) at the C-2 position is replaced by hydrogen [269].

The C1, C3, and C6 positions are critical for the entry of glucose and its analogs into cells via GLUT transporters, whereas the C2 and C3 positions are essential for entry via SGLT transporters [270]. This distinction enables differentiation between the transport routes using various glucose analogs. Because 2-DG lacks a hydroxyl group at the C2 position (**Figure 12**), it can only enter cells through GLUT transporters [270].

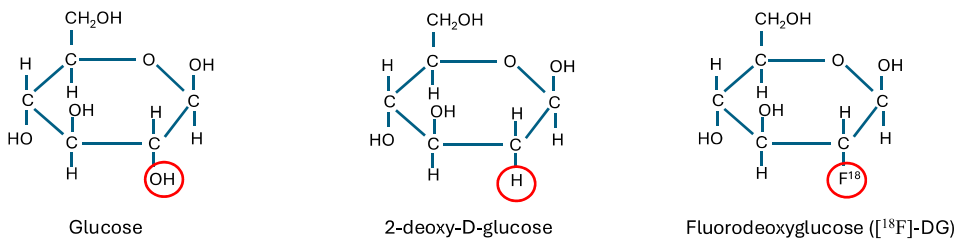

Figure 12. Structural comparison of glucose, 2-deoxy-D-glucose and fluorodeoxyglucose.

Once inside the cell, 2-deoxy-D-glucose is phosphorylated to 2-deoxy-D-glucose-6-phosphate by hexokinase. However, unlike glucose-6- phosphate, this compound cannot isomerize to fructose-6-phosphate due to the absence of the hydroxyl group, thereby blocking further glycolytic pathway. As a result, 2-deoxy-D-glucose-6-phosphate is temporarily trapped and accumulates inside the cell [271, 272] **(Figure 13)**. However, if the rate of 2-deoxy-D-glucose entry into the cell exceeds the phosphorylation rate, some 2-deoxy-D-glucose may remain unphosphorylated and be transported out of the cell [273], as shown in **Figure 13**.

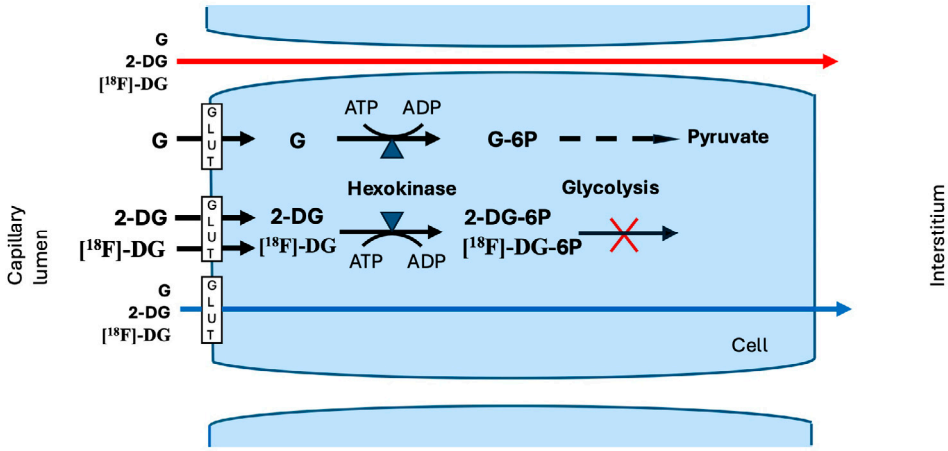

Figure 13. Schematic representation of glucose(G), 2-deoxy-D- glucose (2-DG) and [^{18}F]-DG metabolism in the cell and transportation through and between cells.

2-deoxy-D-glucose, labeled with the radioisotope fluorine-18 (fluorodeoxyglucose, abbreviated [^{18}F]-DG), is widely used to evaluate glucose metabolism in living tissues.

Phloretin and phlorizin

Phloretin and its glycoside, phlorizin, belong to the dihydrochalcone flavonoid subgroup and occur naturally in various berries and fruits, particularly apples. Phlorizin was first discovered in 1835 in the bark of Malus domestica roots (apple tree) [274-277]. Its concentration varies significantly among apple varieties and different parts of the fruit, ranging from 12 to 418 mg/kg in the peel and 4 to 20 mg/kg in the fruit pulp [277]. Following ingestion, phlorizin undergoes rapid enzymatic hydrolysis by β-glucosidase, converting it into phloretin and glucose [275]. (**Figure 14**) Phloretin demonstrates limited bioavailability (approximately 8.7%) owing to poor absorption and rapid metabolism. Its relatively short half-life of approximately 2.8 hours, indicates its rapid clearance [276, 277].

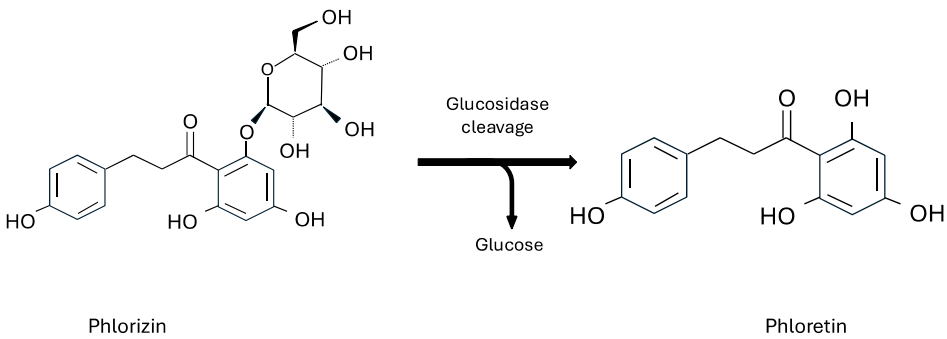

Figure 14. Structure of phlorizin and phloretin.

Phlorizin has an antidiabetic effect through the non-selective inhibition of SGLT transporters, which is limited due to its fast hydrolysis to phloretin [275]. Phloretin exerts its antidiabetic effects primarily through its interaction with GLUT channels rather than with SGLT transporters. By inhibiting GLUT2, it reduces glucose absorption in the intestine and influences the expression of GLUT4 [278]. Furthermore, phloretin decreases the levels of advanced glycation end-products (AGEs) and their receptors. Beyond its antidiabetic properties, it exhibits antioxidant, anti-inflammatory, cardioprotective, and antitumor activities [278].

Recent experimental studies have demonstrated that intraperitoneal administration of phloretin and phlorizin (which metabolizes to phloretin) effectively reduces glucose diffusion across the peritoneal membrane while enhancing ultrafiltration efficiency [196, 197, 204].

Ritonavir and Indinavir

Ritonavir and indinavir, first-generation HIV protease inhibitors, are associated with notable metabolic side effects, such as the development of insulin resistance and dyslipidemia [279]. These metabolic disturbances are thought to result from these drugs inhibitory effects on glucose transporters (GLUTs). Ritonavir inhibits both GLUT1 and GLUT4 channels almost equally, while indinavir exhibits a stronger inhibitory effect on GLUT4 than on GLUT1 channels [279].

BAY-876

GLUT1 inhibitors interfere with glycolysis by limiting glucose availability. BAY-876 is a synthetic GLUT1 antagonist with high inhibitory activity (IC_{50} of 2 nmol/L in cell-free systems) [280]. However, its poor water solubility complicates traditional routes of administration, such as oral or intravenous administration. Nevertheless, in both in vitro and in vivo studies, BAY-876 showed high efficacy in inhibiting GLUT1 expression. This inhibition resulted in reduced glycolysis and a substantial decrease in tumor growth.

Many studies have demonstrated that the inhibitory effect of BAY-876 on GLUT1 varies significantly depending on cell type or line. Studies in tumor cells have reported half-maximal inhibitory concentration (IC_{50}) values ranging from 60 to 188 nmol/L, and some cell lines were completely insensitive to BAY-876.

Aims and Hypotheses

Study I

Glucose is impermeable across cell membranes, and the entry of glucose into cells occurs via a family of hexose transporters, such as sodium and glucose co-transporters (SGLT-1 and 2), allowing secondary active glucose transport (independent of the glucose gradient), and facilitative glucose transporters (GLUTs), allowing facilitated diffusion (dependent on the glucose gradient).

Although SGLT2-inhibitors are widely prescribed for glycemic control in type 2 diabetes, prevention of kidney disease and the treatment of heart failure, their role in the absorption of glucose from peritoneal dialysis solution has not been extensively studied. This study aims to evaluate whether SGLT2 inhibitors can reduce glucose absorption during peritoneal dialysis.

This experimental study uses a rat model of peritoneal dialysis with and without empagliflozin, a potent and selective SGLT2 inhibitor, to test the hypothesis that SGLT2 inhibition can significantly reduce glucose absorption and/or glucose diffusion capacity through the peritoneal membrane.

Study II

The transport of glucose across cellular membranes is facilitated by two distinct types of glucose transporters: sodium-independent and sodium-dependent glucose transporters. Most cells express more than one type of glucose transporters.

The transport of various hexoses and molecules such as myoinositol, urate, glucosamine, and ascorbate across membranes is facilitated by GLUTs. Fourteen GLUT (GLUT1–14) have been identified in humans. Among these, GLUT1–4 have been extensively studied and are regarded as the primary facilitators of cellular glucose uptake. Recent studies have shown that non-selective GLUT inhibition, using intraperitoneal phloretin, reduces glucose absorption and ultrafiltration in an experimental model of peritoneal dialysis. GLUT1–4 have been also identified in the peritoneal membrane of both animals and humans.

This experimental PD study aims to investigate the impact of both selective and non-selective GLUT channel blockers on the transport of [^{18}F]-DG—a radiolabeled form of deoxyglucose—from the bloodstream into the dialysate. The study seeks to clarify the relative contributions of intracellular and paracellular pathways to

glucose transport during PD, and to explore strategies to reduce glucose absorption, ultimately improving PD outcomes and minimizing glucose-related complications.

This study evaluates the hypothesis that inhibiting GLUT with both selective and non-selective blockers alters the transport of [^{18}F]-DG from the bloodstream to the dialysate during PD, and that different GLUT inhibitors exert distinct effects on [^{18}F]-DG transport.

Study III

Osmotic conductance to glucose (OCG) determines ultrafiltration in patients undergoing peritoneal dialysis and can be used in clinical practice to monitor peritoneal membrane integrity in patients undergoing long-term peritoneal dialysis. The double mini-peritoneal equilibration test (dm-PET) is an increasingly utilized method for assessing OCG, but the results of this test show significant variability upon repeat measurements, which reduces its reliability.

This study aims to assess how fluctuations in residual volume affect the accuracy of the double mini-peritoneal equilibrium test (dm-PET) for measuring osmotic conductance for glucose (OCG) in patients undergoing peritoneal dialysis. In addition, it seeks to develop and validate a new, simple, and rapid single-dwell method for OCG measurement that could enhance long-term peritoneal dialysis monitoring and management.

This study tests the hypothesis that the large variability of the dm-PET OCG values are partly due to variations in residual volume and proposes a new, simple, and rapid test to measure OCG (Single-dwell OCG).

Study IV

In patients undergoing peritoneal dialysis, evaluating the characteristics of the peritoneal membrane, particularly solute and water transport, is crucial for selecting an appropriate dialysis regimen and tracking changes in membrane function over time. Although the standard 4-hour peritoneal equilibration test (PET) is widely used in clinical practice, it has notable limitations.

This study aims to investigate the reliability and clinical utility of a new, shorter 60-minute combined peritoneal equilibration test (CombiPET) as a potential alternative to conventional PET.

This study tests the hypothesis that a novel 60-minute combined peritoneal equilibrium test (CombiPET) offers reliable and clinically relevant information on peritoneal membrane properties—specifically, solute transport rate and ultrafiltration capacity—in patients undergoing peritoneal dialysis. Furthermore, it posits that CombiPET results are sufficiently reliable and reproducible to guide peritoneal dialysis treatment and monitor changes in membrane function over time.

Materials and Methods

Laboratory animals and experimental protocols

Animal models, especially rat models, are widely used to study the physiological and pathological aspects of peritoneal dialysis due to the similarity of basic physiological processes between rodents and humans during peritoneal dialysis [281-284]. A non-uremic rat model is often employed in short-term experiments to investigate the permeability of the peritoneal membrane to water and solutes. This model was applied in *Studies I* and *II*. The experimental studies included in this thesis aimed to investigate the role of glucose transporters in glucose transport across the peritoneal membrane during PD.

For the experimental PD *Studies I* and *II*, 9–10-week-old male Sprague-Dawley rats were used. The studies received approval from Lund University's Ethics Committee for Animal Research, and all animals were cared for and used in accordance with the National Institutes of Health guidelines for laboratory animals (Dnr 5.8.18-05699/2018 and Dnr 5.8.18-08386/2022).

Experimental peritoneal dialysis was performed with a 20 mL fill volume in both experimental studies. The dwell time was set to 120 minutes in *Study I* and 60 minutes in *Study II*. Solute clearance over time in the rat model is nearly double that in humans, resulting in dialysate equilibrium after approximately 120 min. At this point, the dialysate-to-plasma (D/P) ratio for creatinine is approximately 0.70 [206] and that for urea is approximately 0.80 [285], which is consistent with the results of a 240-minute human peritoneal equilibration test [123]. Therefore, a 60-minute dwell time in rats provides similar small solute removal (in terms of D/P ratio) to a 120-min dwell time in humans, whereas 120 min in rats is equivalent to 240 min in humans. This faster small solute transfer is attributed to the greater ratio of peritoneal surface area to body size in rats than in humans [283]. A higher ratio of peritoneal surface area to dialysate volume increases the mass transfer coefficient, allowing more efficient removal of substances, such as creatinine and urea, per unit time.

Study I experimental protocol

This study examined the effect of empagliflozin on glucose and water transport across the peritoneal membrane during peritoneal dialysis. Two different concentrations of peritoneal dialysis fluid were used (1.5% and 4.25%), resulting in four groups of eight animals each: two sham groups and two empagliflozin groups **(Figure 15)**.

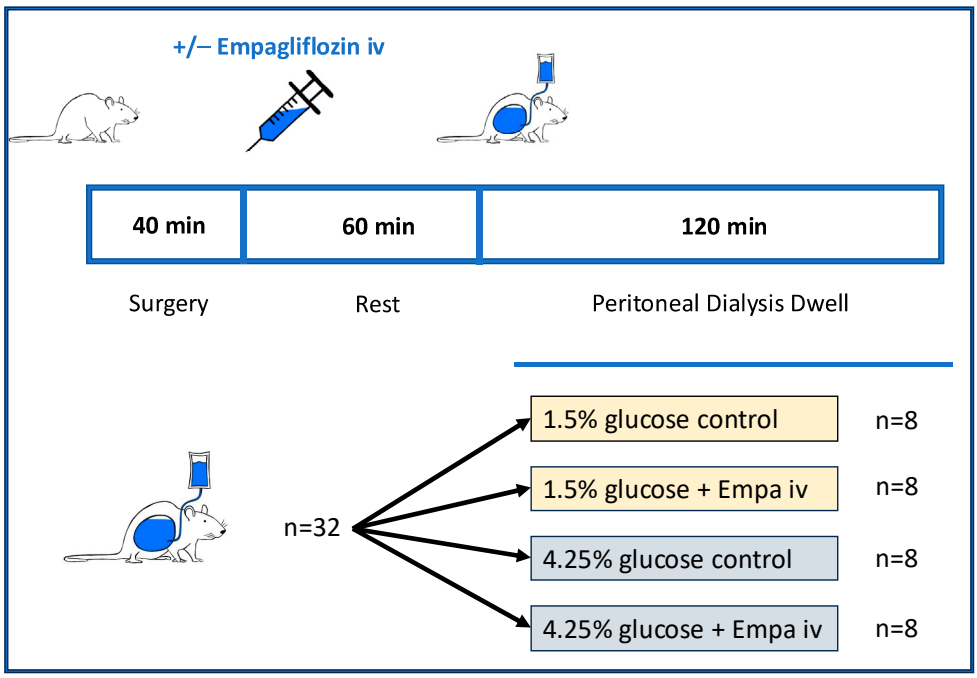

Figure 15. Schematic diagram of the experimental setup with empagliflozin. Peritoneal dialysis was performed in rats using 1.5% or 4.25% glucose solution. *iv*, intravenously; Empa, empagliflozin.

Empagliflozin, a selective SGLT2 inhibitor with an IC_{50} of 1.3 nM, was administered intravenously 0.8 µg min^{-1} kg^{-1} (Merck, Darmstadt, Germany) in the treatment groups, while the sham groups received vehicle alone. Dimethyl sulfoxide (DMSO) was used to enhance the solubility of empagliflozin. Based on first-order renal elimination, it was assumed that the steady-state plasma concentration would reach approximately 200 nM, ensuring effective inhibition of SGLT2 during the experiment. The intravenous route ensures effective blood concentrations, allowing the detection of the classic effects of SGLT2 inhibition. The low molecular weight of empagliflozin (~450 Da) enables it to diffuse easily into the peritoneal dialysate.

Peritoneal dialysis began 60 minutes after the start of the infusion, using 20 mL of the assigned PD fluid. Samples from the dialysate were collected at several time

points (0, 60, and 120 minutes of dialysis), and blood samples were drawn before and after dialysis. Urine was collected to evaluate systemic exposure to empagliflozin by measuring volume and glucose concentration.

A range of parameters reflecting glucose transport across the peritoneal membrane was assessed, including glucose diffusion capacity, glucose clearance from dialysate to plasma, glucose absorption, and D/D0 glucose in both treated and untreated animals. Sodium and osmotic water transport parameters, such as ultrafiltration rate, sodium clearance, and sodium excretion, were evaluated. Changes of 15–20% in these parameters were considered significant.

To address concerns about insufficient empagliflozin levels in the peritoneal cavity, a separate experiment was conducted in which four animals received intraperitoneal empagliflozin directly into 1.5% glucose PD solution at a concentration ten times higher than that used in the main study.

A more detailed description of the study protocol can be found in *Study I*.

Study II experimental protocol

Study II investigated how inhibiting GLUT transporters affects the diffusion of the radiolabeled glucose analog ^{18}F-deoxyglucose ([^{18}F]-DG) from plasma to dialysate. The experiment included four groups of animals: a control group and three groups treated with phloretin (50 mg/L), ritonavir (60 mg/L) or BAY-876 (25 mg/L) in 2.3% peritoneal dialysis fluid. The doses of these inhibitors were substantially higher than the respective IC_{50} values for GLUT1 and GLUT4. Dimethyl sulfoxide (DMSO) was added to improve the solubility of GLUT inhibitors in peritoneal dialysis fluid. The BAY-876 group included nine animals, but one was excluded due to pump failure, while each of the other groups had eight animals **(Figure 16)**.

On the day of the experiment, [^{18}F]-DG was obtained from the Radiopharmaceutical Unit (part of the Department of Radiation Physics) at Skåne University Hospital Lund in quantities of 200–500 MBq. A small amount of [^{18}F]-DG was then mixed with saline to create a solution for intravenous infusion at a rate of 50 µL/min. Dialysate and plasma samples were collected at regular intervals for radioactivity measurement, with all values corrected for tracer decay due to the short half-life of [^{18}F]-DG. Radioactivity samples were analyzed using a gamma counter (Wizard 1480; Wallac Oy, Turku, Finland).

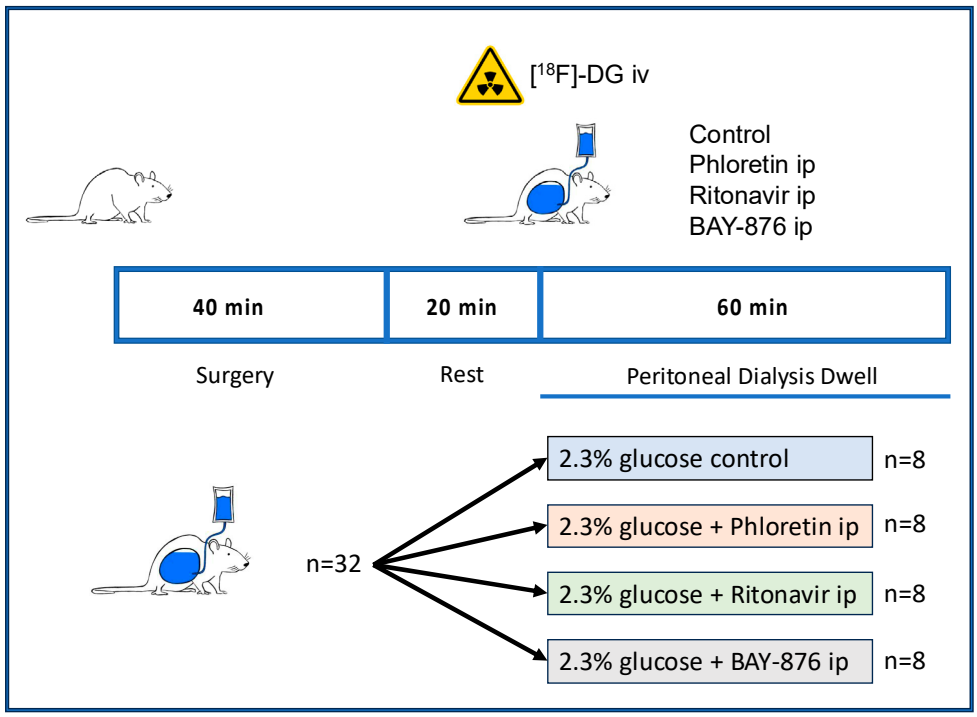

Figure 16. Schematic diagram of the experimental setup with ^{18}F-deoxyglucose. Peritoneal dialysis was performed in rats using 2.3% glucose solution with or without selective GLUT1 blocker BAY-876, non-selective GLUT blocker phloretin, and GLUT1/GLUT4 blocker ritonavir. [^{18}F]-DG, ^{18}F-deoxyglucose; *iv*, intravenously; *ip*, intraperitoneally.

Several variables were evaluated during the study, including the diffusion capacity of ^{18}F-deoxyglucose, glucose, and other small solutes, as well as ultrafiltration efficiency.

In a separate study, the effect of intraperitoneal indinavir on glucose transport across the peritoneal membrane was examined. This study included a treatment group that received indinavir (50 mg/L) in 1.5% peritoneal dialysis fluid and a control group. The indinavir dose was approximately four times the IC_{50} for GLUT4 and half the IC_{50} for GLUT1. [^{18}F]-DG was not used in this investigation **(Figure 17)**.

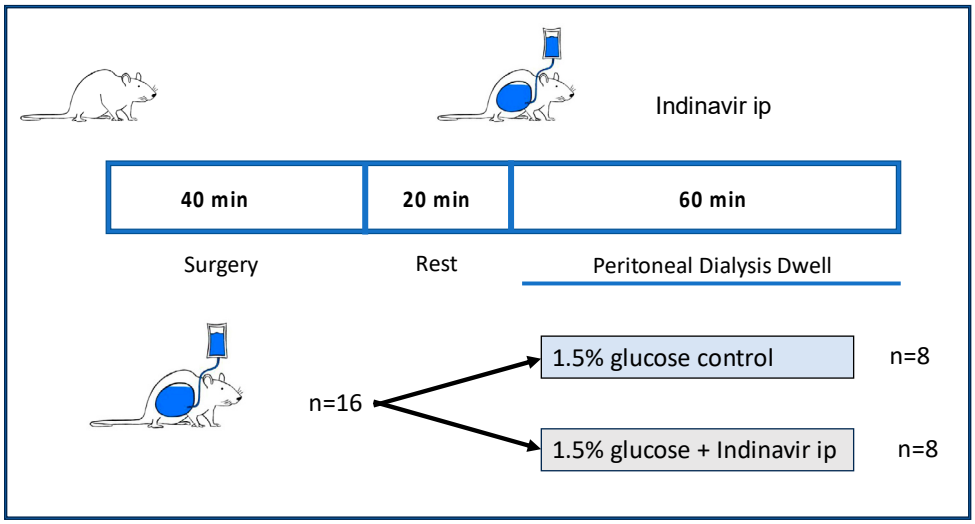

Figure 17. Schematic diagram of the experimental setup with indinavir. Peritoneal dialysis was performed in rats using 1.5% glucose solution with or without the selective GLUT4 blocker indinavir. *ip*, intraperitoneally.

GLUT1 immunostaining was performed after surgically removing the peritoneum from rats, in order to determine the localization of GLUT1 in the peritoneal membrane.

A more detailed description of the study protocol can be found in *Study II*.

The GLUT channel inhibitors used in *Study II* are summarized in **Table 6.**

Table 6. GLUT inhibitors used in the *Study II*

Inhibitor	Origin	Study	Administration	Channel	IC_{50}[μM]	Reference
Phloretin	Malus domestica	III	ip	GLUT1 GLUT2 GLUT3 GLUT4	0.33±0,04 <4 0.82±0,04 ~9.4	[168, 286]
Indinavir	Synthetic	III	ip	GLUT4	20.7±3	[279]
Ritonavir	Synthetic	III	ip	GLUT1 GLUT4	7.0±1.1 7.9±0.4	[279]
BAY-876	Synthetic	III	ip	GLUT1	~0.002	[164, 287, 288]

iv, intravenously; *ip*, intraperitoneally

Protocols for clinical studies

In *Studies III* and *IV* the protocols for both studies were carefully designed and analyzed prior to initiation of each study so as to discover lack of reproducibility, lack of standardized clinical procedures, variability due to different test operators, and inconsistency in measurement tools. Thus errors, ambiguous instructions or logistical challenges were identified and corrected before the investigation began.

To ensure consistency, all procedures were performed by a single trained medical staff member in *Studies III* and *IV*. Each phase of the test was performed in the same sequence at the same time of day. In the second study, both test days were scheduled for the same week to avoid possible changes in the peritoneal membrane. Dialysis solution infusion, drainage, and dwell times were standardized in both studies. However, the drainage time was slightly extended in the second study because some patients in the first study had large residual volumes after the drainage phase due to incomplete peritoneal emptying.

To minimize errors in the calculations of ultrafiltration, due to overfilling of peritoneal dialysis solution bags and dialysate sampling, all bags, with and without peritoneal solutions, as well as all clamps and solution packs, were weighed on an electronic scale before and after each procedure. The bags containing the remaining fluid used to collect the dialysate during the test were also carefully weighed, and any dialysate loss was accounted for in the calculations. This method ensured accurate calculation of the infused and drained solutions with minimal errors.

The blood and peritoneal fluid samples were immediately transported to the laboratory for analysis on the same day.

Study III protocol

In this study, 21 patients on peritoneal dialysis underwent modified double mini-PET (dm-PET), which included 2 consecutive 1-hour dwells with glucose solutions 1.5% and 4.25% and measurements of residual volume (RV) in the peritoneal cavity before, during, and after the procedure **(Figure 18.A)**. Nine patients underwent the test twice, resulting in 30 dm-PETs. However, two dm-PET results were excluded from the final analysis because of unmeasurable albumin levels in the dialysis solution.

Based on computer simulations using the three-pore model (TPM), a new single-dwell method to estimate osmotic conductance to glucose (OCG) was developed. The study analysed several key parameters, including water transport, sodium sieving, sodium removal, and OCG. OCG was calculated using both the double mini-PET (with and without accounting for residual volume) and the new single-dwell method.

To validate the single-dwell method, the analysis was extended to a larger cohort of 32 patients who underwent a total of 61 Uni-PET tests (which incorporate dm-PET). In this validation phase, the OCG was calculated using the single-dwell formula, without considering the RV data **(Figure 18.B)**.

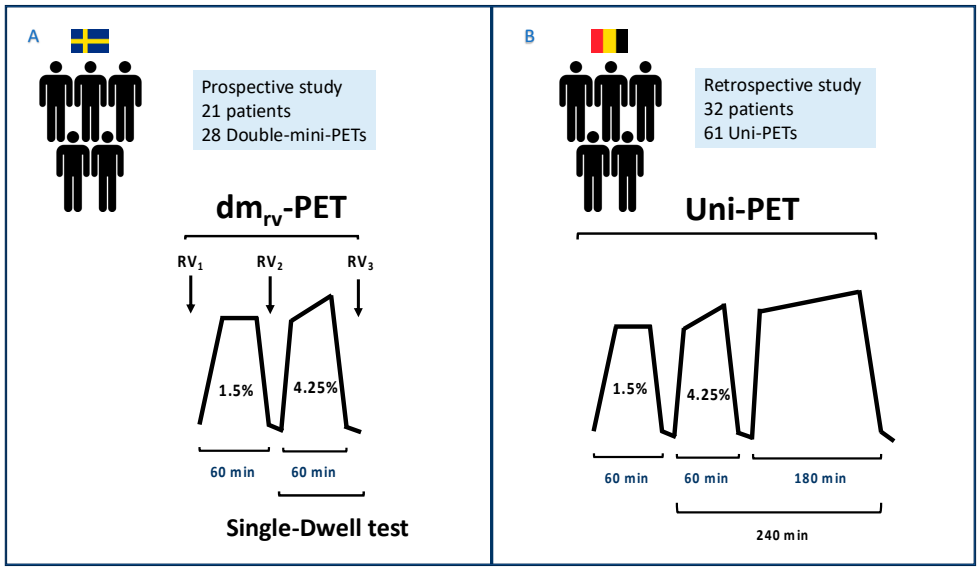

Figure 18. *Study III* design. **A.** The prospective part of the study included 21 patients who underwent 30 double mini-PETs (two tests were excluded from the analysis). **B.** Retrospective part of the study included 32 patients who underwent 61 Uni-PETs.

Study IV protocol

This study involved 34 patients on peritoneal dialysis, each undergoing three tests within the same week: a conventional Peritoneal Equilibration Test (PET) using 2.3% glucose solution, a Combined PET (CombiPET) using 4.25% glucose solution on day 1, and a repeat CombiPET on day 2 (**Figure 19.A)**. Osmotic conductance to glucose (OCG), diffusive conductance (expressed as the ratio of diffusion area to length, $A_0/\Delta x$), and albumin clearance from plasma to dialysate were measured. The study assessed CombiPET's reproducibility using intraclass correlation coefficients (ICC) and compared its agreement with conventional PET using isocratic modeling. To provide a comprehensive characterization of peritoneal membrane function in terms of both osmotic glucose conductivity and diffusion conductivity, the study incorporated results from 193 tests involving 132 patients **(Figure 19.B)**. This analysis included data from *Study III*, *Study IV*, and a Belgian cohort.

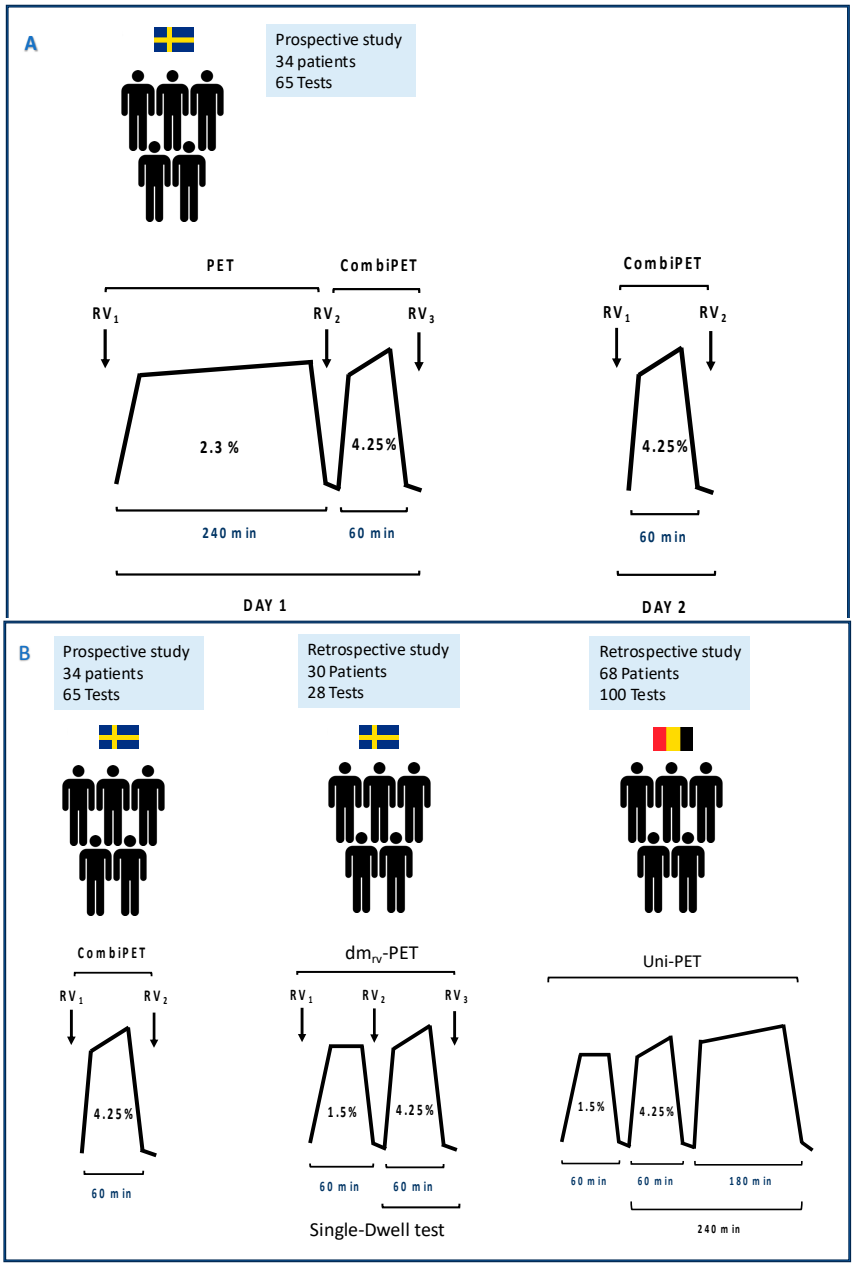

Figure 19. *Study IV* design. **A.** A total of 34 patients were enrolled in the prospective part of the study. The tests were performed over two-days. On Day 1, the patients underwent a PET test with 2.3% glucose solution, followed by a new combined peritoneal equilibrium test (CombiPET) with 4.25% glucose solution. On Day 2, CombiPET was repeated. **B.** Data from 193 tests performed on 132 patients were used to provide a comprehensive description of peritoneal membrane function. The analysis included results from *Studies III* and *IV* and the Belgian cohort.

The peritoneal fluid flow equation or Ohm´s law

In electrical circuits, Ohm's law is a crucial physics principle that defines the interrelation of voltage (*V*), current (*I*), and resistance (*R*), mathematically represented as *V=IR*. In the context of peritoneal dialysis, similar concepts can be applied to understand the dynamics of fluid and solute transport. In PD, ultrafiltration involves the movement of water across the peritoneal membrane driven by osmotic and hydrostatic pressure gradients. This is analogous to the voltage driving the current through a resistor in an electrical circuit. The peritoneal membrane acts as a barrier with specific permeability characteristics, similar to electrical resistance. Factors such as membrane thickness and pore size influence "resistance" to fluid flow.

The relationship between pressure gradients (Δ*P*), flow rate (*UF rate=UFR*), and membrane resistance (*R*) in PD can be conceptually likened to electrical circuits.

Osmotic conductance to glucose is a crucial parameter in peritoneal dialysis which reflects the membrane's ability to transport water in response to a glucose osmotic gradient. The osmotic conductance to glucose is essentially inverse to resistance, representing the ability of the membrane to facilitate water transport driven by glucose osmosis ($R = \frac{1}{OCG}$). The UFR can be expressed as:

$$UFR = OCG \cdot \Delta P \tag{8}$$

Van't Hoff's law, describes the osmotic pressure gradient acting across the peritoneal membrane due to the solute:

$$\Delta P = Pressure\ gradient = RT\Delta C \tag{9}$$

where, Δ*P* is the osmotic pressure gradient. *RT* is the product of the gas constant (*R*) and absolute temperature (*T*) in Kelvin (*RT*≈19.3 mmHg/mmol/L). The normal body thermodynamic temperature is 310 K (approximately 37°C). ΔC represent the molar solute concentration gradient.

$$\Delta P = RT\Delta C \approx 19.3 \cdot \Delta C \approx 19.3 \cdot (\bar{G} - 40) \tag{10}$$

This approximation simplifies the calculations when precise conditions are known, facilitating estimation of pressure changes owing to concentration differences.

$\bar{G}$ represents the average glucose concentration during a single dwell, which is calculated under the assumption that the dialysate glucose concentration decreases in a monoexponential manner, as described below:

$$\bar{G} = (C_0 - C_T)/ln\frac{C_0}{C_T} \quad (11)$$

where C_0 and C_T are the glucose concentrations measured immediately after instillation and before fluid drainage, respectively.

The glucose gradient is countered by an apparent net average concentration gradient of 40 mmol/L, which is primarily composed of urea, sodium, and glucose.

The UFR and OCG can now be expressed as:

$$UFR = 19.3 \cdot OCG \cdot (\bar{G} - 40)/1000 \quad (12)$$

and

$$OCG = \frac{UFR}{19.3(\bar{G}-40)} \cdot 1000 \quad (13)$$

Results

Study I

This study investigated the effects of empagliflozin, a selective SGLT2 blocker, on peritoneal water and solute transport during peritoneal dialysis in rats using two concentrations of PD solutions (1.5% and 4.25% glucose). A modified equation was developed to measure the small solute diffusion capacity, which accounts for the convective and free-water transport. The classic model for assessing diffusion capacity during peritoneal dialysis is the Henderson-Nolph model [124], which assumes diffusion of small solutes into a constant volume in the peritoneal cavity (**Figure 20**, top pane). The modified method, here called the isocratic model, allows for a variable volume and takes free-water transport and convective solute flow into consideration (**Figure 20**, bottom pane).

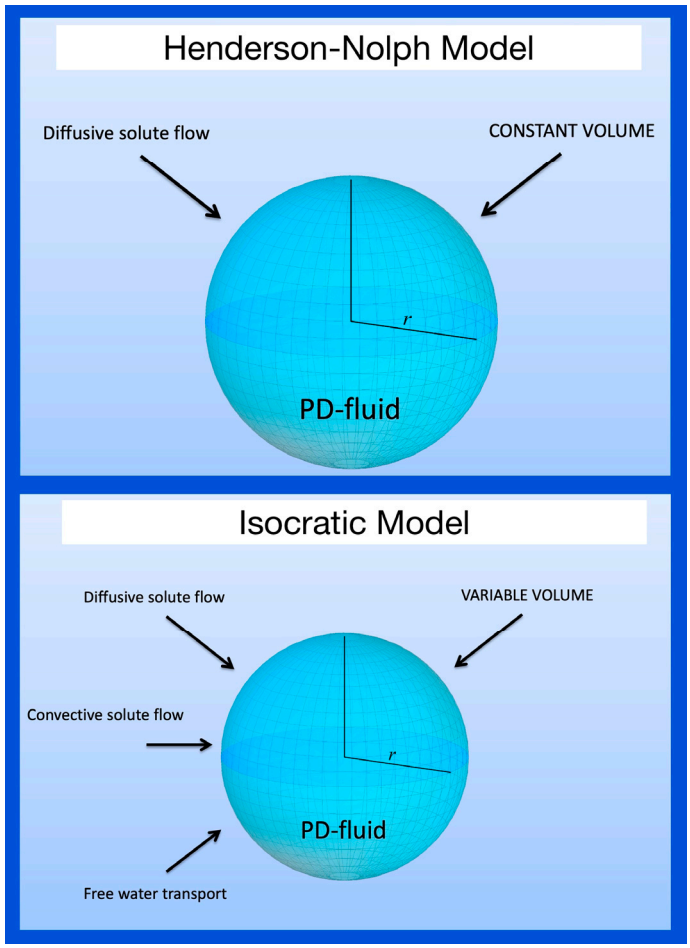

Figure 20. Classic Henderson-Nolph model and isocratic model for the diffusion capacity.

Key findings in Study I:

- The intravenous administration of empagliflozin during peritoneal dialysis had no significant effect on glucose clearance, absorption, or glucose diffusion capacity across the peritoneal membrane. No significant changes in sodium or water transport across the peritoneal barrier were observed. The main findings are summarized in the **Table 7**.
- Although empagliflozin did not affect peritoneal transport, it had significant systemic effects. When administered intravenously, empagliflozin significantly increased urinary glucose excretion and reduced plasma glucose levels after PD compared with sham groups, confirming its systemic effect **(Table 8)**.

Table 7. Glucose, sodium and osmotic water transport parameters

Group	MTAC glucose (isocratic) µL min^{-1}	Glucose clearance[a] µL min^{-1}	Glucose absorption mg	UF rate µL min^{-1}	Sodium removal per liter UF mmol L^{-1}
1.5% Glucose					
Control	178 (159 - 184)	126 (107 -133)	165 (147 - 174)	25 (20 - 29)	108 (84 - 120)
Empagliflozin	167 (149 - 180)	121 (107 -131)	157 (144 - 166)	27 (23 - 31)	112 (96 - 124)
4.25% Glucose					
Control	181 (173 - 194)	140 (133 - 150)	474 (443 - 487)	80 (73 - 84)	101 (100 - 102)
Empagliflozin	181 (172 - 182)	141 (134 - 145)	472 (444 - 487)	86 (78 - 89)	101 (96 - 108)
2 x 2 ANOVA					
p-Value (Empagliflozin)	0.35	0.67	0.72	0.17	0.73
p-Value (Glucose strength)	0.18	< 0.01	< 0.001	< 0.001	0.58
p-Value (Interaction)	0.97	0.94	0.77	0.71	0.91
95% CI for Empagliflozin treatment effect[b]	[–16 to 24]	[–15 to 15]	[–37 to 26]	[–15 to 8]	[–17 to 12]

Values are median (IQR)

[a] Dialysate-to-plasma clearance

[b]Averaged over both glucose strengths

Table 8. Empagliflozin effects on urinary output, glucose excretion and plasma glucose

Group	Urinary glucose output µg min^{-1} kg^{-1}	Urine output µL min^{-1} kg^{-1}	Plasma glucose[a] mmol L^{-1}
1.5% Glucose			
Control	0.2 (0.1 - 0.3)	25 (23 - 29)	12.2 (10.9 - 14.6)
Empagliflozin	1.5 (1.3 - 2.6)	38 (31 - 58)	11.0 (10.5 - 12.8)
4.25% Glucose			
Control	0.7 (0.2 - 2.2)	39 (28 - 56)	17.0 (16.2- 18.0)
Empagliflozin	3.3 (2.9 - 4.5)	56 (51 - 63)	14.9 (14.4- 15.2)
2 x 2 ANOVA			
p-Value (Empagliflozin)	< 0.001	< 0.05	< 0.05
p-Value (Glucose strength)	< 0.001	< 0.05	< 0.001
p-Value (Interaction)	0.25	0.94	< 0.05
95% CI for Empagliflozin treatment effect	[1 to 3]	[1 to 24]	[–4.0 to – 0.5]

Values are median (IQR)

[a] After PD

Study II

This study investigated the role of GLUT channels on glucose transport using an experimental peritoneal dialysis model with the radioactive glucose analog [^{18}F]-DG. The main findings are summarized in the **Figure 21**.

Key findings in Study II:

- Non-selective GLUT channel blockade with phloretin and ritonavir, which inhibit both GLUT1 and GLUT4 channels, significantly reduced the diffusion capacity of [^{18}F]-DG and improved the ultrafiltration rate. This suggests that GLUT channels play an important role in glucose transport during peritoneal dialysis and confirms the results of previous studies using phloretin.
- Selective GLUT1 channel blockade with BAY-876 did not result in significant changes in [^{18}F]-DG diffusion or water transport, possibly because of its poor solubility and insufficient concentration in the dialysate. Selective blockade of GLUT4 channels with indinavir did not affect water transport or glucose diffusion. Although BAY-876 did not affect [^{18}F]-DG, glucose, or water transport in this study, the overall findings suggest that at least some glucose is transported across peritoneal membrane cells during peritoneal dialysis and that GLUT1 channels are most likely responsible for this glucose transport.
- Peritoneal biopsy specimens analyzed by immunohistochemical staining with specific GLUT1 antibodies revealed diffuse GLUT1 positivity in vascular endothelial cells, whereas no GLUT1 expression was observed on the surfaces of mesothelial cells, myocytes, or fibroblasts.

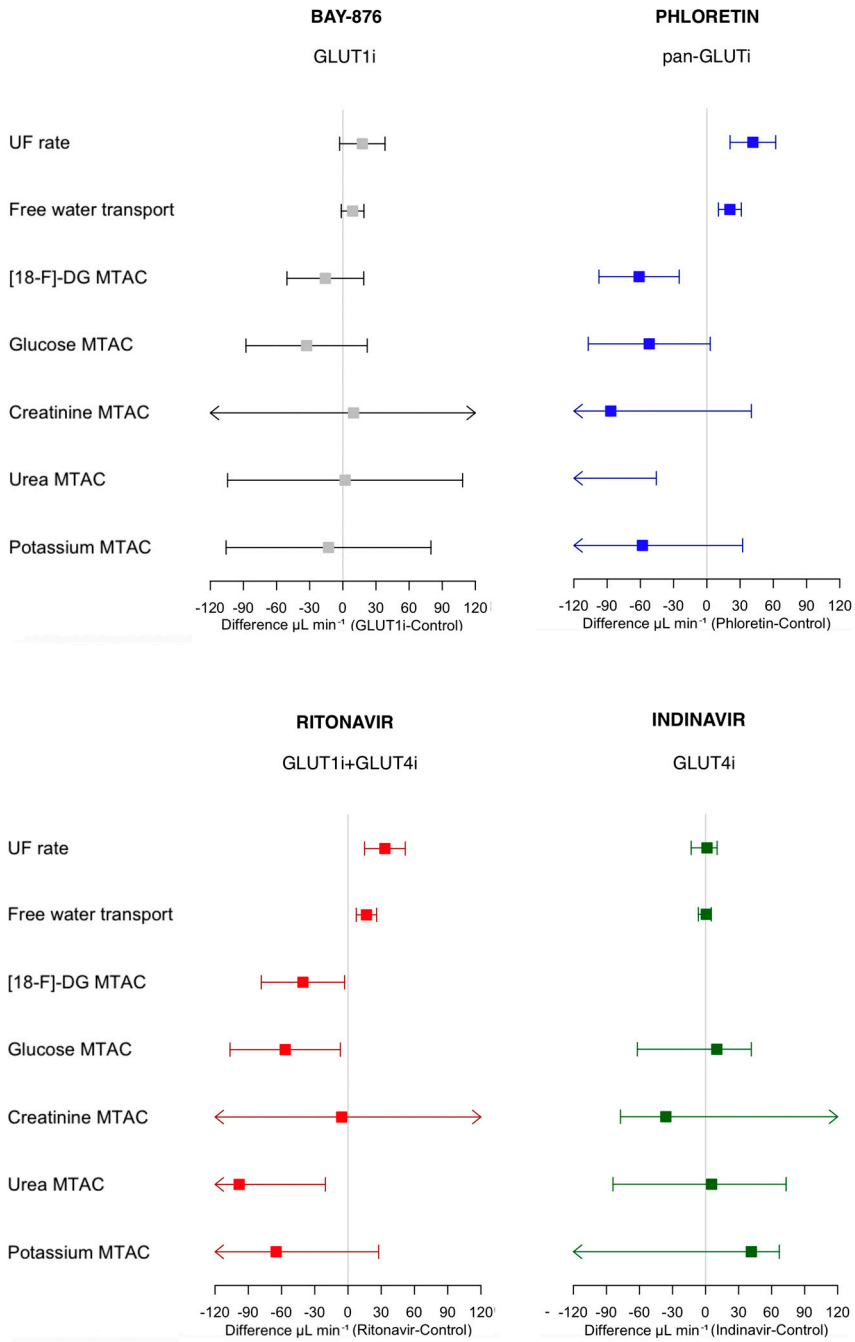

Figure 21. Treatment effects for BAY-876, phloretin, ritonavir, and indinavir on UF rate, free-water transport, isocratic diffusion capacities (MTACs) for [^{18}F]-DG (not present in indinavir experiments), glucose, creatinine, urea, and potassium.

Study III

A novel single-dwell method (Single-dwell OCG) to estimate osmotic conductance to glucose (OCG) was developed and validated based on computer simulations derived from the 3-pore model (TPM) for membrane permeability. The single-dwell OCG method was also validated in an independent Belgian cohort consisting of 32 PD patients (which underwent a total of 61 Uni-PETs) **(Figure 22)**.

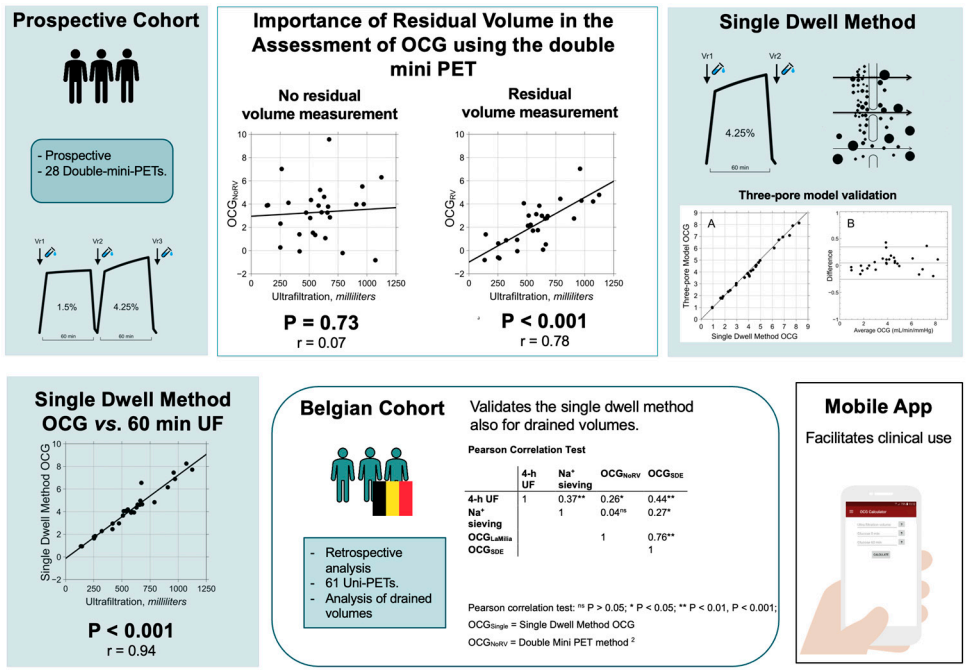

Figure 22. The prospective part of the study included 21 patients who underwent 30 double mini-PETs (two tests were excluded from the analysis). Conventional double mini-PET OCGs did not correlate with UF (P = 0.73) unless residual volumes were taken into account (P < 0.001). Using the three-pore model, the study investigated whether OCG could be accurately determined based on a single 4.25% dwell (the latter part of the double mini-PET). Lastly, in a separate Belgian cohort of 61 Uni-PETs, the single dwell method OCG was correlated with the double mini-PET OCG (Pearson's r = 0.76). To facilitate the use of the single-dwell method, a convenient mobile application was developed. (Adapted from poster at the 2019 EuroPET conference in Ljubljana, Slovenia)

Key findings in Study III:

- Single-dwell OCG had a better correlation with actual UF compared to other methods: the conventional double mini-PET and the double mini-PET taking residual volumes into account (dmRV method).
- These findings were replicated in the retrospective Belgian validation cohort, where OCG calculated using the single-dwell method was closely

correlated with parameters of osmotic water transport, even when RVs were not considered, that is, with only drained volumes.

- The single-dwell and dmRV methods were able to identify all patients with low OCG **(Table 9)**.

Table 9. Evaluation of the diagnostic precision of different methods for measuring osmotic conductance to glucose

Index Methods	**Reference Method (OCGtpm)**	
dm-PET, no residual volume	**OCGtpm > 2**	**OCGtpm < 2**
OCGdmp > 2	16	4
OCGdmp < 2	7	1
dm-PET, including residual volumes		
OCGdmpRV > 2	16	0
OCGdmpRV < 2	7	5
Single-dwell equation, including residual volumes		
OCGsd > 2	23	0
OCGsd < 2	0	5

OCG, osmotic conductance to glucose; dm-PET, double mini-peritoneal equilibration test; dmp, double mini-peritoneal equilibration test; dmpRV, double mini-peritoneal equilibration test taking residual volumes into account; sd, single-dwell method; tpm, the 3-pore model.

Nine patients underwent double mini-PET procedure twice. One double mini-PET was excluded from analysis due to missing residual volume data. Eight patients are deemed to be an insufficient number for a reliable repeatability analysis. Nevertheless, visual inspection could yield some information regarding repeatability. In **Figure 23**, OCG values obtained on the first occasion are shown with those obtained on the second occasion.

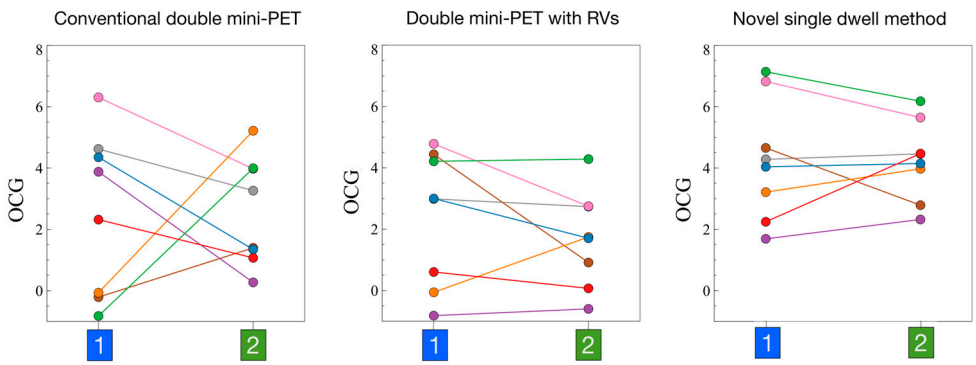

Figure 23. Repeated OCG tests in eight patients. OCG (y-axis) is expressed in µL/min/mmHg.

From visual inspection it appears that the conventional double mini-PET has rather poor repeatability. The underlying reason for this was hypothesized to, at least in part, be due to large variances in conventional OCGs. This conclusion was supported by a Monte Carlo error propagation analysis (**Figure 24**), which showed a much lower variance for the single-dwell OCG method than for the double mini-PET methods.

Monte Carlo Stochastic Error Propagation Analysis

a

INPUT VARIANCE

Parameter	CV %
Dialysate albumin conc.	3.9
Dialysate glucose conc.	1.1
Dwell time	0.5
Drained volume	5.0

b

OUTPUT VARIANCE

Parameter	CV %
OCG_{dmp}	45.4
OCG_{dmpRV}	53.5
OCG_{sd}	21.2 ***

OCG, osmotic conductance to glucose; OCG_{dmp}, OCG calculated from the double-mini peritoneal equilibration test (PET) [4]; OCG_{dmpRV}, OCG calculated from the double-mini PET taking residual volumes into account; OCG_{sd}, OCG calculated using the Single Dwell Method; *** P < 0.001 compared to OCG_{dmpRV}.

c

Contribution of Inputs to the Output Variance

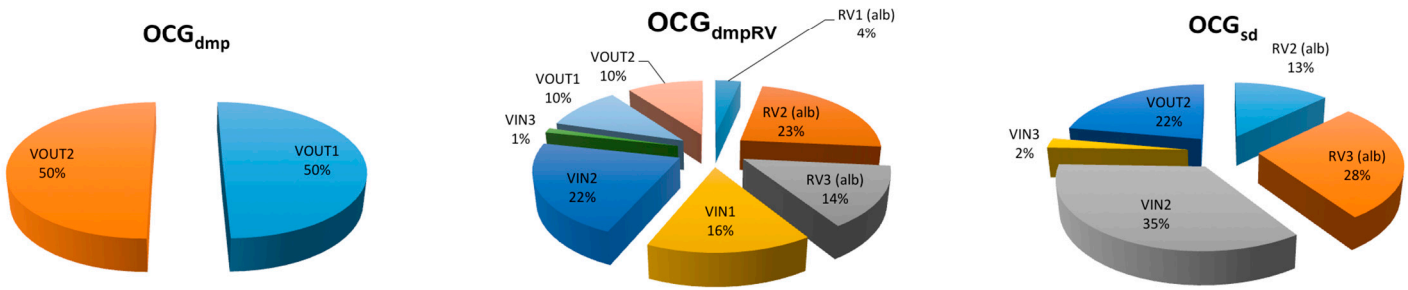

Figure 24. Error propagation and sensitivity analysis. RV, residual volume; VIN1, volume in for 1.5% dwell, VIN2; volume in for the 4.25% dwell; VOUT1, drained volume for 1.5% dwell; VOUT2, drained volume for the 4.25% dwell; RV1, residual volume before the 1.5% dwell; RV2, residual volume before the 4.25% dwell; RV3, residual volume after the 4.25% dwell. The Sobol method was used for variance-based sensitivity analyses using the sensi package for R.

Study IV

This study investigated the efficacy and reliability of a novel 60-minute combined peritoneal equilibration test (CombiPET) as an alternative to the standard 4-hour PET in patients undergoing peritoneal dialysis.

Key findings in Study IV:

- CombiPET showed excellent reproducibility for both osmotic conductance to glucose (OCG) and diffusive conductance ($A_0/\Delta x$), confirming its clinical robustness and ability to provide accurate measurements of the capacities for water and small solute transfer in patients treated with PD (**Figure 25**).
- Applying an isocratic model to predict solute concentrations yielded strong agreement between the modelled and measured solute transport values (**Figure 26**). These findings demonstrate that despite its significantly shorter duration, CombiPET can provide solute transport information comparable to that of conventional PET.

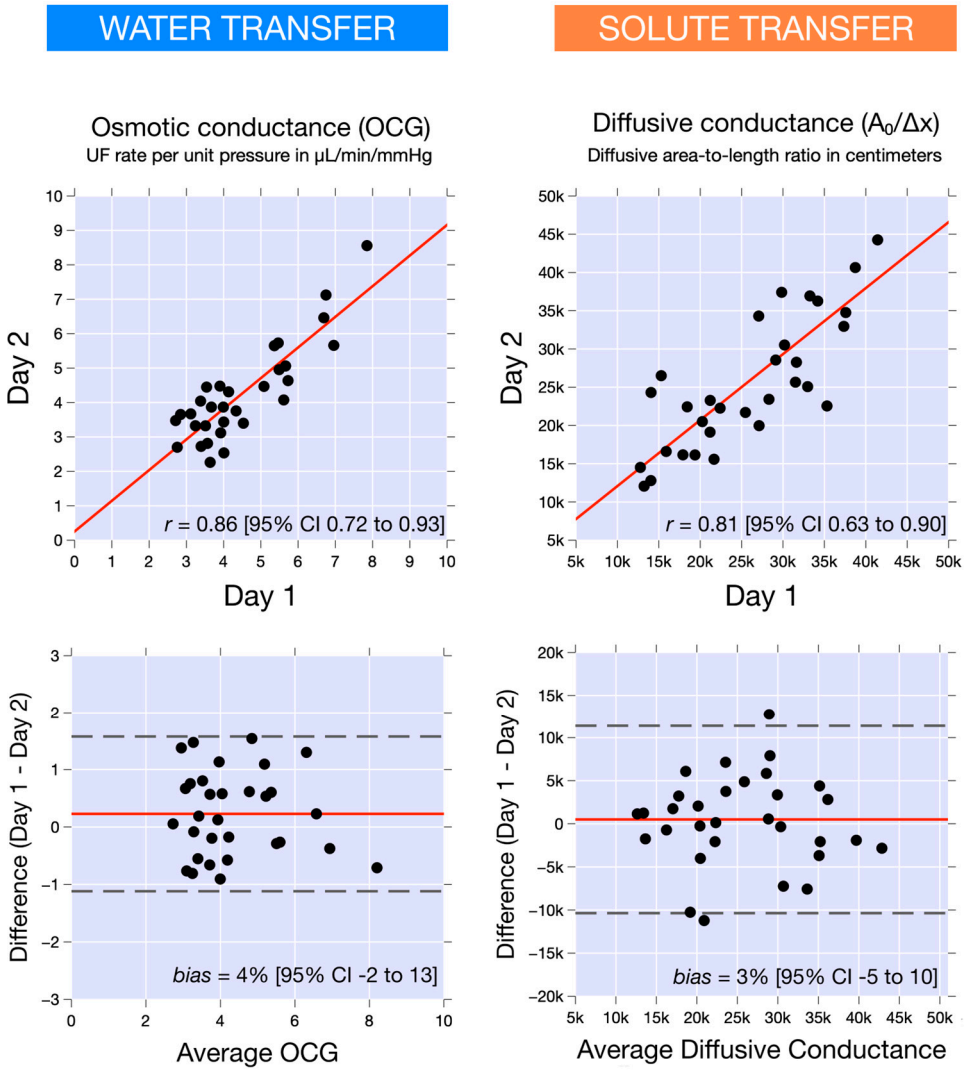

Figure 25. CombiPET reliability results. The figure presents Bland-Altman plots comparing the results obtained from Day 1 and Day 2 tests for water transfer (osmotic conductance to glucose, left pane) and solute transfer (small solute diffusive conductance, right pane).

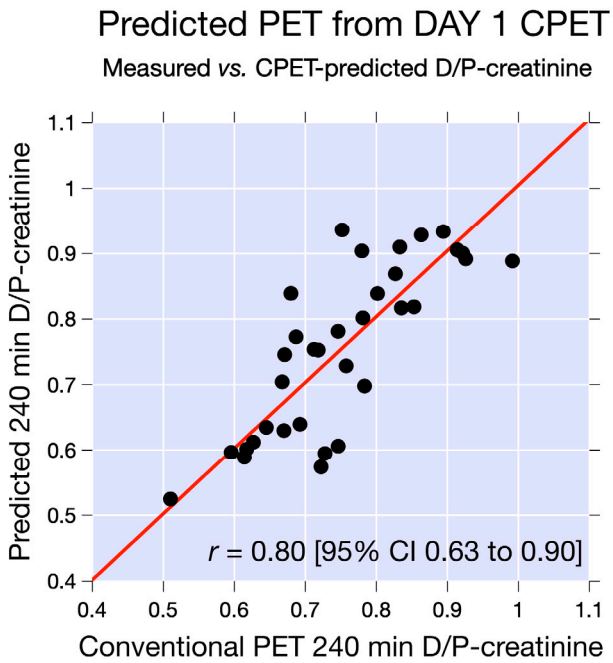

Figure 26. Correlation between the modelled (predicted PET) and measured D/P creatinine (conventional 240 min PET).

Discussion

Glucose and its journey across the peritoneal membrane

After initiating peritoneal dialysis treatment, fewer than 50% of all patients adhere to this method over a two-year period [198, 289, 290]. This decline is attributed to the elevated mortality rates observed among patients on dialysis as well as transitions to hemodialysis or kidney transplantation. Factors contributing to the discontinuation of PD and transition to HD include infectious and mechanical complications, alterations in living circumstances or health status, insufficient home support, diminished residual kidney function, inadequate removal of uremic toxins, and suboptimal fluid management [289].

Considering the numerous benefits of PD compared with HD, it is essential to develop innovative approaches to extend the duration of PD treatment which would contribute to greater use of this dialysis method.

One of the main factors limiting PD is the deleterious effects of the dialysis solution on the peritoneal membrane. High glucose concentrations can have negative effects on the peritoneal membrane as well as adverse systemic effects. Moreover, the high glucose permeability of the peritoneal membrane leads to loss of the glucose gradient and reabsorption of the dialysis solution, causing many patients to struggle with overhydration.

Despite these drawbacks, glucose remains a relatively safe and readily available osmotic agent in peritoneal solutions, making its replacement difficult. For example, using icodextrin more than once a day can cause maltose accumulation, as maltase, the enzyme needed to break down maltose into glucose, is found only in the intestinal epithelium and renal tubules. Alternative osmotic agents, such as glycerol, xylitol, or L-carnitine, face similar issues due to their low molecular weight and potential systemic side effects.

In all likelihood the continuous endothelial layer of capillaries and venules is the main limiting factor for the transport of glucose and other small solutes during peritoneal dialysis, whereas the mesothelium and interstitial layer do not have a significant limiting role. According to the three-pore theory, glucose transfer primarily occurs through the interendothelial spaces of blood vessels known as small pores. However, glucose channels are present in all human cells because glucose cannot pass through cell membranes via simple diffusion. The peritoneal

membrane cells also contain these channels, including SGLT1, SGLT2, GLUT1, GLUT2, GLUT3, and GLUT4. These channels have been extensively studied and characterized in both human and small rodent mesothelial cell cultures, as well as in peritoneal membrane biopsies. Most of the information regarding the expression of these channels pertains to mesothelial cells. Moreover, the expression of SGLT and GLUT channels in the submesothelial layer adjacent to the blood vessel wall has been investigated. However, very little is known about their presence in the endothelium of the small peritoneal vessels or interstitial cells.

Study I aimed to investigate the effect of the selective SGLT2 inhibitor empagliflozin on glucose transport during peritoneal dialysis in an experimental rat model. Empagliflozin was administered intravenously in the main study, and a small group of animals received the drug intraperitoneally. Systemic effects, such as polyuria and glucosuria, were noted in animals that received empagliflozin, confirming the blocking effect on the SGLT channels in the renal tubules. However, the hypothesis that empagliflozin might reduce glucose transport across the peritoneal membrane was not confirmed, and no significant effect was observed on either glucose transport or water transport.

It is important to acknowledge the limitations of this study, such as a small sample size and the use of animals with normal kidney function, which may not accurately reflect the physiological conditions of PD. This study may also have been underpowered to detect smaller effects. The acute nature of the study could be perceived as a limitation, but it can also be regarded as a strength, since the purpose of the study was to elucidate whether a significant amount of glucose is transferred via SGLT2-channels or not– rather than to study chronic effects.

Although the findings of this study were negative, it does not provide irrefutable evidence that SGLT2 inhibitors do not affect the peritoneal membrane via alternative mechanisms should they be administered over an extended period. These inhibitors might exert beneficial effects on peritoneal function and longevity owing to their anti-inflammatory and antifibrotic properties, as well as their capacity to mitigate oxidative stress. Several studies describing the effects of SGLT2 inhibitors have suggested that this class of drugs may have additional vascular benefits by modulating vascular endothelial cell activation and reducing endothelial dysfunction. Experimental studies in rodents have shown less thickening of the peritoneal membrane in animals treated with SGLT2 inhibitors during PD than in control animals. The inhibition of SGLT2 is linked to the prevention or reduction of reactive oxygen species generation. This process typically results in DNA damage, which contributes to age-related changes and the transformation of mesothelial cells to mesenchymal cells in peritoneal tissues. Additionally, SGLT2 inhibition may interfere with molecular pathways involving HIF-1α, TGF-β, and p-Smad3 proteins.

Currently, with several SGLT2 inhibitors available for oral use, there is a growing interest in potential benefits in the context of peritoneal dialysis. Multiple clinical

studies have been initiated to investigate the safety of this drug class and their positive effects on residual kidney function or membrane transport properties. To date, only a few studies have been published [202].

The role of GLUT channels in glucose transport and their potential impact on peritoneal membrane damage during long-term dialysis is less well understood than that of SGLT channels. Mesothelial cells express GLUT1, GLUT2, GLUT3, and GLUT4 channels, and their expression levels can be altered by peritoneal dialysis solutions. Although there is virtually no information on the presence of GLUT channels in the small vessels of the peritoneal membrane, these channels are well known in other regions of the vascular endothelium and have been extensively studied in the blood-brain barrier (BBB), where glucose transport via paracellular pathways is practically nonexistent. It is also known that peripheral endothelial cells express GLUT1, GLUT3, and GLUT4, but do not express the low-affinity glucose transporter GLUT2.

Several GLUT channel inhibitors are currently known, including natural compounds such as phlorizin and phloretin found in apple root bark and fruit peels, as well as synthetic inhibitors such as BAY-876. The GLUT-inhibiting properties of these substances are of particular interest in oncology because of the Warburg effect observed in malignant tumors [167, 291]. Scientists are also investigating the relationship between GLUT channels and neurodegenerative diseases such as Alzheimer's disease [169]. However, to date, no GLUT blocker has been approved for clinical use.

Previous experimental peritoneal dialysis studies using phlorizin, an SGLT1 and SGLT2 channel inhibitor, believed to be rapidly hydrolyzed to phloretin, and phloretin, a non-selective GLUT channel inhibitor, have shown a significant reduction in glucose diffusion across the peritoneal membrane and increased ultrafiltration. These results suggest that glucose transport during peritoneal dialysis occurs not only through paracellular spaces (small pores), but also into or across peritoneal membrane cells.

Study II further investigated the role of GLUT channels in peritoneal dialysis using the radiolabeled glucose analog [^{18}F]-DG (fluorodeoxyglucose), which enters cells via GLUT channels. [^{18}F]-DG is particularly valuable for investigating glucose metabolism and is extensively used in clinical diagnostics, especially in positron emission tomography (PET) scanning. Some [^{18}F]-DG becomes trapped inside the cell because after rapid phosphorylation to [^{18}F]-DG-6-phosphate, it cannot proceed further in the glycolytic pathway due to the lack of a hydroxyl group. However, evidence suggests that not all [^{18}F]-DG entering the cell is phosphorylated, if its rate of entry exceeds the rate of phosphorylation. Approximately 30–60% of [^{18}F]-DG remains unphosphorylated, allowing it to be transported not only into the cell but also out of the cell.

We investigated the effects of four different GLUT channel blockers, phloretin, ritonavir, indinavir, and BAY-876, in an experimental peritoneal dialysis model on the transport of glucose and [^{18}F]-DG across the peritoneal membrane from blood to peritoneal fluid. The aim of this study was to test the hypothesis that glucose transport during peritoneal dialysis not only occurs via paracellular pathways but also via intracellular or transcellular pathways.

The results showed that phloretin (a non-selective GLUT channel inhibitor) and ritonavir (a GLUT1 and GLUT4 inhibitor) significantly reduced the mass transfer area coefficient (MTAC) of [^{18}F]-DG, while BAY-876 (a selective GLUT1 inhibitor) and indinavir (a selective GLUT4 inhibitor) had no significant effect on [^{18}F]-DG or glucose transport. Although the lack of an effect of BAY-876 and indinavir complicates the interpretation of these results, the overall findings suggest that at least some glucose is transported across peritoneal membrane cells during peritoneal dialysis and that GLUT1 channels are most likely responsible for this transport. Creatinine MTAC remained unchanged after GLUT channel blockade, which supports the hypothesis that the decrease in [^{18}F]-DG MTAC was due to the inhibition of transcellular [^{18}F]-DG transport rather than paracellular transport (**Figure 27**).

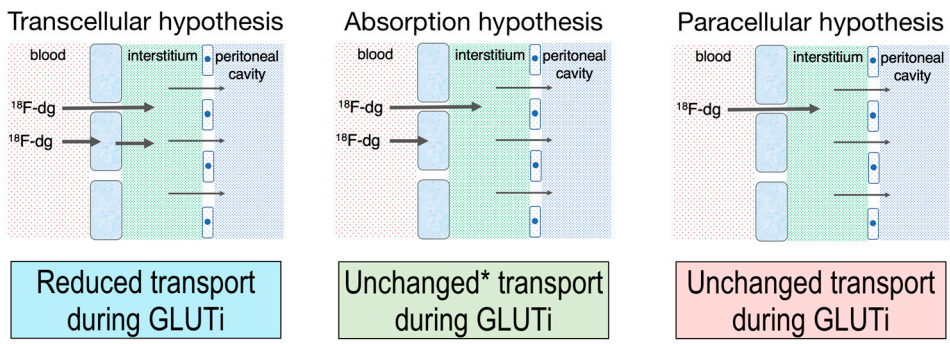

Figure 27. Three hypotheses on how [^{18}F]-DG transport occurs across the peritoneal membrane. If transport occurs transcellularly (left pane), then the effect of GLUT-inhibition would be a reduction in [^{18}F]-DG MTACs. If [^{18}F]-DG is mainly absorbed and trapped in cells (middle pane), then [^{18}F]-DG MTACs would be unchanged during GLUT-inhibition (* or possibly increased due a higher [^{18}F]-DG concentration locally in the microcirculation). Lastly, if [^{18}F]-DG is transferred via the paracellular route, then GLUT-inhibition would have no effect.

Peripheral capillary endothelial cells are known to express GLUT1 channels; however, other research groups investigating glucose transport across the peritoneal membrane have focused on the peritoneal mesothelium. In *Study II*, we demonstrated the presence of GLUT1 channels in small peritoneal vessels in the rat using immunohistochemistry. Interestingly, GLUT1 was not detected in mesothelial

cells or other cells of the peritoneal interstitial layer, reinforcing the concept that the vascular endothelium is the main barrier.

Phloretin has been repeatedly found to be effective in reducing the diffusion of glucose and [^{18}F]-DG across the peritoneal membrane during experimental peritoneal dialysis. Thus, phloretin might be a potential agent for improving the efficiency of peritoneal dialysis by reducing both local and systemic adverse effects of glucose as well as by alleviating fluid balance problems, thereby improving patient outcomes. However, there are some issues that need to be solved before its application in clinical practice, such as safety studies and addressing its poor water solubility and low intestinal absorption. Given the importance of reducing the side effects of glucose, in view of the potential therapeutic properties of phloretin, there is ongoing work to develop synthetic or semi-synthetic phloretin derivatives or formulations that improve its solubility and bioavailability. This would have important implications not only in peritoneal dialysis but also in oncology.

In conclusion, *Studies I* and *II* provide new insights into peritoneal transport during dialysis and challenge the basis of the three-pore model, opening possibilities for future improvements in the methodology of peritoneal dialysis. The finding that glucose appears to pass directly through the peritoneal cells, as evidenced in *Study II*, represents a major departure from traditional views on solute transfer across the peritoneal membrane. Not only glucose, but also urea is most likely transferred in a transcellular fashion [196], possibly via urea transporter B (UT-B) [292]. It is conceivable that other solutes also might pass through alternative pathways. For example, creatinine is known to be transported across cell membranes via organic cation transporters (OCTs), with OCT2 being well characterized for its relevance in creatinine tubular secretion (responsible for 10–40% of renal creatinine excretion) [293]. How much of the creatinine transfer during PD occurs via OCTs remains an open question. Large variances exist in creatinine clearance between patients and a high clearance is associated with worse outcomes for patients treated with glucose-based fluids only. Organic anion transporters (OATs) are present in vascular endothelial cells and facilitate the transport of a wide range of organic anions across cell membranes. These include endogenous metabolites (e.g., uric acid), drugs (e.g., furosemide and penicillin), toxins, and many other exogenous compounds [294]. Given the vascular nature of the peritoneal membrane, it is plausible that OATs are present in the endothelial cells of the peritoneal capillaries and contribute to solute transport during PD treatment. Thus, there is little doubt that the list of molecules capable of crossing the peritoneal barrier via pathways other than the interendothelial small pores will expand rapidly in the coming years.

Novel Functional Tests in Peritoneal Dialysis Treatment

Peritoneal dialysis is a kidney replacement therapy that utilizes the peritoneal membrane as a natural filter to remove excess fluid and uremic toxins from the blood stream. The effectiveness of peritoneal dialysis varies significantly among patients owing to differences in dialysis regimens, residual kidney function, and the unique properties of each person's peritoneal membrane. Unlike hemodialysis filters which have known characteristics, the "peritoneal filter" is unique to each person, and its solute and water transport capabilities become apparent first after treatment is initiated. Moreover, these properties can change over time due to the effects of the dialysis solutions, mechanical forces, and inflammatory processes.

To optimize treatment outcomes, nephrologists must tailor dialysis prescriptions according to each patient's specific membrane properties and personal goals. This personalized approach requires the continuous monitoring of membrane functions to allow timely adjustments to treatment strategy.

Various methods for assessing peritoneal membrane function have been proposed, but they are often questioned in clinical practice due to their complexity, time and personnel requirements, and the belief that membrane characteristics can be understood by evaluating daily dialysis results. Some propose a greater focus on patients' quality of life and individual needs rather than basing dialysis regimens on test results. The relatively short duration of PD treatment for many patients and limited knowledge of the benefits of regular testing further reduces enthusiasm for routine assessments of membrane function.

Although routine testing is not widely recommended, peritoneal membrane function assessment can help clarify the causes of patient deterioration and be helpful in making difficult decisions, such as changing dialysis regimen or transitioning to hemodialysis. In particular, reliable and reproducible peritoneal membrane tests are important in PD research, especially when developing new dialysis solutions or investigating the effects of various drugs on the peritoneum (e.g., SGLT2 channel blockers or GLUT blockers).

Considering the various arguments for and against, the need of a functional membrane test, which is simple and reliable and capable of characterizing the multiple functions of the peritoneal membrane in a single assessment is evident. Several tests have been developed to evaluate the characteristics of the peritoneal membrane. The most common is the 4-hour Peritoneal Equilibration Test, which is typically performed using 2.3% glucose solutions, during which solute permeability is measured. A variant of this test, utilizing a 4.25% glucose solution, also allows the assessment of sodium sieving and ultrafiltration. Although shorter-duration PETs have been proposed, the traditional 4-hour PET continues to be the standard. The 2-hour double mini-PET, which evaluates osmotic water conductance and sodium sieving, can be used for assessing impaired water transport.

Study III examined the influence of residual volume on double mini-PET results and introduced a simpler, one-hour single-exchange test using a 4.25% glucose solution. This new test provided more accurate estimates of osmotic conductance to glucose (OCG) than the double mini-PET and was validated in an independent cohort. However, the need to determine the residual volume and reliance on albumin as an endogenous marker introduces complexities that may not be feasible in all clinics. In summary, the results from *Study III* showed that residual volumes should be considered when using the double mini-PET method.

Study IV focused on overcoming the inherent limitations that result from performing volumetric assessments of ultrafiltration during PD. This was accomplished by combining solute diffusion and water transport assessments in a single test. The proposed CombiPET test demonstrated excellent reproducibility of OCG and diffusive conductance ($A0/\Delta x$), highlighting its robustness and reliability in clinical practice. Strong correlations between the simulated and measured solute concentrations confirmed that CombiPET provides information comparable to standard PET in a significantly shorter time.

This test introduced an innovative method for calculating ultrafiltration rates based on changes in dialysate sodium concentration, making traditional ultrafiltration calculation methods redundant, thereby significantly increasing the reliability of the test. This test showed that an integrated assessment of both osmotic and diffusive conductance is necessary to avoid misinterpretation. Although sodium sieving effectively identifies patients with intact water permeability, it can be misleading in cases of low sodium sieving. Moreover, with CombiPET various fill volumes and glucose concentrations can be used during the test without compromising its reliability.

This study has some limitations. Small sample sizes limit the generalizability of the findings, and certain computational simplifications and modeling methods may not reflect all clinical scenarios, potentially introducing bias. Prospective studies are needed to confirm whether improvements in peritoneal membrane testing result in better patient outcomes and long-term benefits.

In conclusion, the development of more efficient and comprehensive peritoneal membrane assessment tests such as CombiPET represents a significant advancement in PD management. These new methods offer a possibility for personalized and effective treatment strategies, potentially improving patient outcomes and quality of life. However, further research is needed to fully validate its clinical utility and long-term benefits in diverse patient populations.

Conclusions

Study I

Using the selective SGLT2 channel blocker empagliflozin in an experimental rat peritoneal dialysis model did not confirm the hypothesis that SGLT2 inhibitors reduce glucose transport across the peritoneal membrane or enhance ultrafiltration. However, these findings do not entirely exclude the possibility of subtle effects on glucose transport or long-term improvements in membrane structure unrelated to glucose transport.

Study II

Investigations into the role of GLUT channels during experimental peritoneal dialysis in a rat model, involving both selective and non-selective GLUT blockers, verified their contribution to glucose transport and ultrafiltration. Non-selective GLUT channel blockers, such as phloretin and ritonavir, decreased the transport of the glucose analog [^{18}F]-DG across the peritoneal membrane and increased ultrafiltration. These findings indicate that GLUT channels are at least partly responsible for the loss of glucose gradient observed during PD. Although the exact GLUT channel subtype remains unclear, GLUT1 is the most likely candidate. A deeper understanding of these glucose transport mechanisms may have significant clinical implications, potentially mitigating the harmful effects of glucose exposure and prolonging the duration of effective peritoneal dialysis treatment.

Study III

The residual peritoneal volume has a significant impact on the accuracy of osmotic conductance to glucose measurements when using the double mini peritoneal equilibration test (dm-PET). The single-dwell OCG method may offer a more reliable tool for monitoring peritoneal membrane integrity in patients with long-term PD.

Study IV

The CombiPET test permits rapid, reliable, and reproducible measurements of both osmotic conductivity for glucose and diffusion permeability, making it an excellent alternative to several existing tests of peritoneal membrane function. By providing a simple and dependable method for monitoring membrane performance, CombiPET facilitates more informed clinical decision-making and timely detection of functional changes. This, in turn, can improve patient outcomes and save time for both patients and health care providers.

Populärvetenskaplig sammanfattning på Svenska

Kronisk njursjukdom påverkar cirka 10 procent av världens befolkning och utgör en stor utmaning för både individens hälsa och hälso- och sjukvårdssystemen. På grund av längre livslängd och att allt fler människor som lever med riskfaktorer som fetma, fysisk inaktivitet, diabetes, högt blodtryck och hjärt-kärlsjukdomar, ökar risken också för kronisk njursvikt.

Njursvikt kan delas in i stadier: från njursvikt med intakt njurfunktion (stadium I) till terminal njursvikt (stadium V). När njurfunktionen är kraftigt nedsatt och endast en liten del av dess kapacitet återstår, kan patienter uppleva symtom som minskad aptit, illamående, trötthet, muskelsvaghet, klåda, svullnader och högt blodtryck. Blodprover visar ofta störningar i kalcium-, fosfor-, kalium- och syra-basbalansen, samt förhöjda nivåer av urea och kreatinin. När njurfunktionen når en kritiskt låg nivå blir dialys eller njurtransplantation nödvändig för överlevnad. Dialys är en behandlingsmetod som kan ersätta eller komplettera njurarnas funktion och finns i två former: peritonealdialys och hemodialys.

I detta arbete fokuseras på peritonealdialys.

Introduktion

Peritonealdialys är den vanligaste formen av hemdialys och är i många fall billigare för samhället än hemodialys. Dessutom ökar den patientens självständighet och uppmuntrar till aktivt deltagande i egenvård. Metoden använder bukhinnan som ett naturligt filter för att hjälpa kroppen att eliminera slaggprodukter och överflödig vätska, vilket åtminstone delvis kompenserar för nedsatt njurfunktion. Förenklat kan bukhinnan ses som en sil med många porer i olika storlekar, genom vilka vatten och små molekyler som kreatinin, urea och glukos kan passera. Däremot kan större molekyler som blodproteiner och blodkroppar i princip inte passera genom detta filter.

Dialysvätskor med särskild sammansättning—som förutom vatten innehåller olika typer av salter och buffertar, för att reglera syra-basbalansen, samt osmotiska ämnen som glukos, aminosyror eller icodextrin—infunderas regelbundet i bukhålan och dräneras ut genom en speciell silikonkateter. Katetern placeras genom ett mindre

kirurgiskt ingrepp och är lämplig för långtidsbehandling. Procedurerna att genomföra peritonealdialys är enkla, och de flesta patienter lär sig snabbt att utföra dem hemma; vid behov kan utbildad personal bistå.

De viktigaste processerna under dialys är *diffusion* (när lösta ämnen rör sig från högre till lägre koncentration), *osmos* (när det osmotiska ämnet i dialysvätskan skapar en koncentrationsgradient som får vattnet att röra sig från blodet genom bukhinnan och in i bukhålan) samt *konvektion* (där lösta ämnen transporteras tillsammans med vattenflödet).

Trots alla sina fördelar har peritonealdialys vissa nackdelar, bland annat den begränsade kapaciteten hos bukhinnan för både vätskeavlägsnande och eliminering av uremiska toxiner.

För att säkerställa vätskeavlägsnande under peritonealdialys tillsätts ett osmotiskt verkande medel, oftast glukos, till dialysvätskorna. På grund av sin lilla storlek passerar glukos lätt från bukhålan in i blodet genom bukhinnan, och om en olämplig behandlingsregim används kan detta leda till vätskeretention i kroppen. Den snabba absorptionen av glukos från dialysvätskor bidrar också till ett onödigt högt kaloriintag, vilket kan leda till insulinresistens eller till och med diabetes, samt störningar i lipidmetabolismen, ökad fettvävnad och viktökning. Den höga glukoskoncentrationen i dialysvätskorna har också en direkt negativ inverkan på bukhinnans funktion och struktur. Trots de kända negativa lokala och systemiska effekterna av glukos är glukosbaserade dialysvätskor de mest använda i klinisk praxis.

Glukoskanaler, vattenkanaler (akvaporiner) och intercellulära förbindelser är extremt viktiga strukturer i alla biologiska barriärer. De är nära relaterade till cellernas förmåga att reglera transporten av vatten och näringsämnen samt att anpassa sig till olika miljöförhållanden, vilket gör dem särskilt viktiga i samband med dialys.

Glukoskanaler tillhör en uråldrig och allmänt förekommande familj av proteiner som finns i alla levande organismer, från bakterier till människor. De är nödvändiga för energiförsörjningen, eftersom glukos är den primära energikällan för många organismer. Under evolutionen har olika typer av glukoskanaler utvecklats, anpassade för specifika funktioner och vävnader. Glukoskanaler delas in i två huvudgrupper baserat på deras mekanism för att transportera glukos över cellmembraner. Den första gruppen är de natriumberoende SGLT-kanalerna, även kallade sodium-glucose linked transporters eller sodium-glucose cotransporters. Dessa använder natriumgradienten som drivkraft för att transportera glukos mot dess koncentrationsgradient och kräver indirekt energi i form av ATP. SGLT-kanalerna inkluderar SGLT1, som främst finns i tarmepitelet, och SGLT2, som dominerar i njurtubuli och transporterar glukos tillsammans med natrium.

Den andra gruppen är de natriumoberoende GLUT-kanalerna, även kallade facilitative glucose transporters. Dessa transporterar glukos genom underlättad diffusion utan att direkt använda energi och uttrycks i de flesta celltyper i kroppen. GLUT-familjen är ännu mer varierad än SGLT- familjen, med minst 14 kända typer, var och en med specifika funktioner och vävnadsfördelning. Exempelvis är GLUT4 viktig för insulinreglerad glukostransport i muskler och fettvävnad, medan GLUT2 är involverad i glukossensoriska mekanismer i levern och bukspottkörteln. GLUT1 är allmänt förekommande och fungerar som primär glukosleverantör till hjärnan. Mångfalden och specialiseringen av dessa kanaler är avgörande för anpassning till förändrade näringsförhållanden och energibehov. Minst fyra typer av GLUT-kanaler (GLUT1, GLUT2, GLUT3 och GLUT4) samt båda typerna av SGLT-kanaler (SGLT1 och SGLT2) finns även i bukhinnan.

Akvaporiner (AQP) är specialiserade vattenkanaler som möjliggör snabb och selektiv transport av vatten genom cellmembranen. I människokroppen har 13 olika AQP-typer identifierats (AQP0–AQP12), var och en med unika funktioner och vävnadsfördelning. I bukhinnan anses akvaporin-1 spela en nyckelroll i vattentransporten över membranet.

Intercellulära förbindelser är nödvändiga för celladhesion och kommunikation. Genom dessa strukturer bildas olika vävnader och organ, och effektiva barriärer skapas mellan kroppens inre och yttre miljö. Sannolikt sker huvuddelen av utbytet mellan dialysvätska och blod under peritonealdialys sker genom dessa intercellulära förbindelser. Under de senaste åren har dock intresset ökat för glukostransportmekanismer genom själva cellmembranen och deras potentiella roll under peritonealdialys.

Syftet med detta arbete är att bidra till en bättre förståelse av mekanismerna för glukostransport över bukhinnan, samt att utveckla ett lättillgängligt test för att bedöma bukhinnans funktion. Detta skulle möjliggöra inte bara valet av den mest lämpliga behandlingsregimen, utan också tidig upptäckt av oönskade förändringar i bukhinnan under dialysbehandlingen.

Studierna I och *II* fokuserar på glukostransporten över bukhinnan, medan *III* och *IV* ägnas åt utveckling och validering av ett funktionellt test.

Glukos och dess resa genom bukhinnan

Studier I och II

I denna del presenteras två experimentella studier på råttor av peritonealdialys. Syftet är att klargöra glukoskanalernas roll i glukostransporten över bukhinnan och att ifrågasätta det hittills dominerande antagandet att betydande glukostransport under peritonealdialys endast sker genom intercellulära utrymmen. I *Studie I* och *II* testas hypotesen att glukosupptaget från dialysvätskorna kan minskas genom att

blockera glukoskanalerna. Detta skulle möjliggöra att en hög glukoskoncentration i bukhålan bibehölls under längre tid, minska de oönskade effekterna av glukos på bukhinnan och hela organismen, samt underlätta avlägsnandet av överflödig vätska från kroppen.

I *Studie I* undersöktes blockering av SGLT2-kanaler med hjälp av empagliflozin, ett läkemedel som ofta används för att behandla diabetes och även förskrivs för att bromsa progressionen av hjärtsvikt och kronisk njursvikt. Under experimentet injicerades empagliflozin direkt till djurens blod under pågående peritonealdialys. Studien visade ingen signifikant effekt på glukostransporten över membranet och förbättrade inte heller ultrafiltrationen eller vätskeavlägsnandet.

I *Studie II* användes också en djurmodell för peritonealdialys, men här studerades transporten av glukos och en radioaktiv glukosanalog fluorodeoxyglucose ([^{18}F-DG]) över bukhinnan genom att blockera GLUT-kanaler med olika hämmare, såsom phloretin, ritonavir, indinavir och BAY-876, som skiljer sig åt i sin förmåga att blockera olika GLUT-kanaler. Under studien minskade [^{18}F]-DG-diffusionskapaciteten signifikant, och ultrafiltrationen ökade vid användning av phloretin och ritonavir, medan de andra två hämmarna inte visade någon effekt. Även om BAY-876, en GLUT1-blockerare, och indinavir, en GLUT4-blockerare, inte hade någon effekt på glukos- eller [^{18}F]-DG transporten, tyder de övergripande fynden på att åtminstone en del glukos transporteras över peritonealceller under peritonealdialys och att GLUT1-kanaler mest sannolikt är ansvariga för denna glukostransport.

I studie II kunde GLUT1-kanaler påvisas i bukhinnans små kärl från råtta med hjälp av immunhistokemi.

Resultaten från dessa två studier ger ny kunskap om transportprocesserna genom bukhinnan och öppnar möjligheter att i framtiden förbättra vården av patienter som behandlas med peritonealdialys. Genom att optimera glukostransporten och minska den skadliga effekten av höga glukosnivåer kan man förlänga den tid patienter kan behandlas med peritonealdialys och minska negativa effekter av glukos. Därför är det nödvändigt att fortsätta forskningen och genomföra kliniska prövningar för att omsätta dessa resultat i praktiken och göra dem tillgängliga som behandlingsalternativ vid kronisk njursjukdom.

Nya funktionstester vid peritonealdialysbehandling

Studier III och IV

Eftersom varje patients bukhinna är unik är det viktigt att känna till dess transportegenskaper för både vatten och lösta ämnen för att kunna välja rätt dialysbehandling. Det finns flera tester för att utvärdera bukhinnans egenskaper, men de används sällan i klinisk praxis på grund av tidskrävande procedurer och

bristande reproducerbarhet och tillförlitlighet. Syftet med dessa studier var att utveckla ett snabbt och tillförlitligt kliniskt test för att bestämma bukhinnans egenskaper, vilket skulle underlätta användningen i kliniken och valet av lämplig behandling.

Vattentransport över bukhinnan blir särskilt viktig för patienter som genomgår peritonealdialys när urinproduktionen minskar, för att upprätthålla vätskebalansen och undvika övervätskning. En stor andel patienter i peritonealdialys lider av kroniskt vätskeöverskott, vilket avsevärt ökar risken för komplikationer som högt blodtryck, hjärtförstoring och hjärtsvikt. Vätskeöverskott bidrar till sämre patientutfall och kan leda till att peritonealdialys behandlingen misslyckas. Under peritonealdialysen beror mängden av vatten som avlägsnas genom bukhinnan på membranets egenskaper, dialysvätskans sammansättning, tiden som dialysvätskan är kvar i bukhålan och återabsorptionshastigheten av vätska från bukhålan.

Treportsmodellen är en matematisk modell som möjliggör beräkningar och förutsägelser av transport av vatten och lösta ämnen över bukhinnan under peritonealdialys. Enligt treporsteorin, när glukosbaserade dialysvätskor används, transporteras vatten genom bukhinnans mikrovaskulära endotel på två sätt: via paracellulära vägar (små porer) och transcellulära vägar (akvaporiner eller ultrasmå porer). Vatten som transporteras genom paracellulära vägar bär med sig lösta små ämnen, medan fri vattentransport sker genom akvaporiner utan medföljande ämnen. Effektiviteten av det glukosinducerade osmotiska flödet genom små porer är relativt låg, medan den genom akvaporiner har 100%-ig effektivitet.

Den vanligaste orsaken till otillräcklig vattenborttagning under peritonealdialys är en hög glukostransporthastighet över bukhinnan, kombinerat med olämpligt val av dialysvätska och/eller behandlingsregim. Med tiden kan vattentransporten genom bukhinnan försämras på grund av skador på membranet orsakade av dialysvätskorna, mekanisk och osmotisk stress samt återkommande peritoniter.

Glukosens förmåga att framkalla ultrafiltration eller vattenborttagning genom bukhinnan kallas glukosens osmotiska konduktans (OCG). Denna parameter återspeglar glukosens effektivitet som en osmotisk agent.

Flera kliniska och matematiska metoder har föreslagits för att utvärdera OCG. En av de mest använda metoderna är det två timmar långa dubbla mini-PET-testet, som involverar två på varandra följande påsbyten med en timmes dialystid i bukhålan med olika glukoskoncentrationer. OCG beräknas som förhållandet mellan skillnaden i ultrafiltrerad volym och skillnaden i osmotisk gradient mellan de två dialyserna. Minskad OCG kan vara resultatet av långvarig peritonealdialys och kan indikera progressiva förändringar i bukhinnan.

I *Studie III* undersöktes påverkan av restvolymen, dvs kvarvarande vätska, i bukhålan på bestämningen av bukhinnans vattengenomsläpplighet som svar på glukos inducerat osmotiskt tryck med hjälp av det så kallade dubbla mini-PET testet.

Detta test jämfördes med ett nytt, kortare en timmes test. Studien involverade patienter i peritonealdialys som genomgick både det dubbla minitestet och det nya korta testet. Resultaten visade att bestämning av restvolymen är mycket viktig för att kunna noggrant beräkna den ultrafiltrerade vätskemängden och erhålla exakta testresultat som beskriver membranens vattengenomsläpplighet. Testens reproducerbarhet har dock inte undersökts i denna studie.

Syftet med *Studie IV* var främst att undersöka om den nya metoden för OCG-bestämning kunde reproducera resultat vid upprepade mätningar. Det nya testet, som föreslogs för att mäta OCG i Studie III, visade sig ha svagheter vid upprepade mätningar. I *Studie IV* infördes i stället en metod för att uppskatta vattenborttagning med hjälp av natrium, vilket gav utmärkta resultat även vid upprepade mätningar.

Under peritonealdialys rör sig lösta ämnen som urea, kreatinin, glukos och elektrolyter mellan blodet och dialysvätskan genom bukhinnan. I klinisk praxis är peritoneal equilibrerings test (PET) den mest använda metoden för att utvärdera transporthastigheten av lösta ämnen över bukhinnan. På grund av sin enkelhet används PET allmänt i många kliniker och fungerar ofta som referensmetod. Standard-PET är ett fyra timmar långt test.

I *Studie IV* jämfördes också det korta testet (så kallad CombiPET) med det klassiska peritoneal equilibrerings testet, som vanligtvis används för att bestämma membranens permeabilitet för små lösta ämnen. Det visade sig att det korta, CombiPET, testet kunde ersätta det långa fyra timmars testet.

Genom att ändra beräkningsmetoden kunde det nya en timmes testet ersätta både det två timmar långa dubbla mini-PET testet och det fyra timmar långa PET-testet. Det korta testet kunde också reproducera resultaten vid upprepade utföranden, vilket framhäver dess tillförlitlighet och stabilitet.

Ett sådant test möjliggör långsiktig övervakning av bukhinnan hos patienter som genomgår peritonealdialys. Det kan avslöja både funktionella förändringar och tidiga tecken på progressiva strukturella förändringar i bukhinnan. Genom att upptäcka dessa förändringar tidigt kan behandlingsmetoden anpassas för att förebygga allvarliga komplikationer, som inkapsulerande peritoneal skleros, en allvarlig komplikation som innebär ärrbildningar i bukhinnan. Testet hjälper även doktorn att välja optimal behandling baserat på patientens individuella membranegenskaper. Dessutom effektiviserar det arbetet för vårdpersonalen genom att spara tid vid behandlingsplanering och uppföljning.

Popular summary in English

Introduction

Chronic kidney disease affects approximately 10 percent of the world's population and poses a significant challenge to both an individual's health and healthcare systems. Due to longer life expectancy and an increasing number of people living with risk factors, such as obesity, physical inactivity, diabetes, high blood pressure, and cardiovascular diseases, the risk of chronic kidney failure is also increasing.

Kidney failure is often divided into stages, ranging from the mildest (Stage I) to the most severe (Stage V). When kidney function is severely reduced and only a small portion of its capacity remains, patients may experience symptoms, such as decreased appetite, nausea, fatigue, muscle weakness, itching, swelling, and high blood pressure. Blood tests often show disturbances in calcium, phosphorus, potassium, and acid-base balance as well as elevated levels of urea and creatinine. When kidney function reaches a critically low level dialysis or kidney transplantation is necessary for survival. Dialysis is a treatment method that can replace or supplement kidney function, it exists in two forms: peritoneal dialysis and hemodialysis.

This thesis focuses on peritoneal dialysis.

Glucose and its journey through the peritoneum

Peritoneal dialysis is the most common form of home dialysis and is usually less expensive than hemodialysis. It also increases patients' independence and encourages active participation in self-care. This dialysis method uses the peritoneum as a natural filter to help eliminate waste products and excess fluid, which at least partially compensates for reduced kidney function. The peritoneum can be seen as a sieve with many pores of various sizes through which water and small molecules such as creatinine, urea, and glucose can pass. Larger molecules, such as blood proteins and cells, cannot pass through this filter.

Dialysis fluids with a special composition, which, in addition to water, contain various types of salts, buffers to regulate the acid-base balance, and osmotic agents such as glucose, amino acids, or icodextrin, are regularly infused into the abdominal cavity and drained through a special silicone catheter. The catheter is placed via a

minor surgical procedure and is suitable for long-term treatment. The dialysis procedures are simple, most patients quickly learn to perform them at home, and trained staff can assist if necessary.

The main processes during dialysis are *diffusion* (when dissolved substances move from a higher to a lower concentration), *osmosis* (when the osmotic agent in the dialysis fluid creates a concentration gradient that causes water to move from the blood through the peritoneum and into the abdominal cavity), and *convection* (when dissolved substances are transported along with the water flow).

Despite its advantages, peritoneal dialysis has certain drawbacks, such as the limited capacity of the peritoneum for both fluid removal and elimination of uremic toxins.

To ensure fluid removal during peritoneal dialysis, an osmotic agent, usually glucose, is added to the dialysis fluids. Owing to its small size, glucose easily passes from the abdominal cavity into the blood through the peritoneum, and if an inappropriate treatment regimen is used, this can lead to fluid retention in the body. The rapid absorption of glucose from dialysis fluids also contributes to unnecessarily high calorie intake, which can lead to insulin resistance or even diabetes, as well as disturbances in lipid metabolism, increased adipose tissue, and weight gain. High glucose concentration in dialysis fluids also has a direct negative impact on the function and structure of the peritoneum. Glucose-based dialysis fluids are most commonly used in clinical practice, despite the known negative local and systemic effects of glucose.

Glucose channels, water channels (aquaporins), and intercellular junctions are important structures in all biological barriers. They are closely related to the ability of cells to regulate the transport of water and nutrients and adapt to different environmental conditions, making them particularly important in the context of dialysis.

Glucose channels are an ancient and ubiquitous family of proteins found in all living organisms from bacteria to humans. They are essential for energy supply because glucose is the primary energy source for many organisms. Throughout evolution, different types of glucose channels have been developed and adapted to specific functions and tissues. Glucose channels are divided into two main groups based on their mechanisms of glucose transport across cell membranes. The first group consists of sodium-dependent SGLT channels, also known as sodium-glucose-linked transporters or sodium-glucose cotransporters. These channels use the sodium gradient as a driving force to transport glucose against its concentration gradient and indirectly require energy in the form of ATP. SGLT channels include SGLT1, which is primarily found in the intestinal epithelium, and SGLT2, which dominates in the renal tubules and transports glucose together with sodium. The second group comprises sodium-independent GLUT channels, also known as facilitative glucose transporters. These channels transport glucose through facilitated diffusion without directly using energy, and are expressed in most cell

types in the body. The GLUT family is even more varied than the SGLT family, with at least 14 known types, each with a specific function and tissue distribution. For example, GLUT4 is important for insulin-regulated glucose transport in muscles and adipose tissue, whereas GLUT2 is involved in glucose-sensing mechanisms in the liver and pancreas. GLUT1 is widely present and serves as the primary glucose supplier to the brain. The diversity and specialization of these channels are crucial for adaptation to changing nutritional conditions and energy needs. At least four types of GLUT channels (GLUT1, GLUT2, GLUT3, and GLUT4) and both SGLT channels (SGLT1 and SGLT2) are present in the peritoneum.

Aquaporins (AQP) are specialized water channels that enable rapid and selective transport of water across cell membranes. Thirteen different AQP types have been identified in the human body (AQP0–AQP12), each with unique functions and tissue distribution. In the peritoneum, aquaporin-1 is considered to play a key role in water transport across the membrane.

Intercellular junctions are essential for cell adhesion and communication. Through these structures, different tissues and organs are formed, and effective barriers are created between the internal and external environments of the body. It is assumed that the greater part of the exchange between the dialysis fluid and blood during peritoneal dialysis occurs through these intercellular junctions. However, in recent years, interest has increased in glucose transport mechanisms through cell membranes and their potential role during peritoneal dialysis.

The purpose of this thesis was to contribute to a better understanding of the mechanisms of glucose transport across the peritoneum and develop an easily accessible test to assess peritoneal function. This would enable not only the selection of the most appropriate treatment regimen but also early detection of undesirable changes in the peritoneum during dialysis treatment.

Studies I and *II* focused on glucose transport across the peritoneum, while *studies III* and *IV* were devoted to the development and validation of a functional test.

Studies I and II

In this section, two experimental studies of peritoneal dialysis are presented. This study aimed to clarify the role of glucose channels in glucose transport across the peritoneum and to challenge the dominant assumption that significant glucose transport during peritoneal dialysis only occurs through intercellular spaces. In *Studies I* and *II*, the hypothesis was tested whether glucose uptake from dialysis fluids could be reduced by blocking the glucose channels. This would enable longer maintenance of high glucose concentration in the abdominal cavity, reduce the undesirable effects of glucose on the peritoneum and the entire organism, and facilitate the removal of excess fluid from the body.

In *Study I*, blocking of SGLT2 channels using empagliflozin was investigated. Empagliflozin is a drug often used to treat diabetes and slow the progression of heart failure and chronic kidney failure. During the experiment, empagliflozin was administered directly into the blood of animals during peritoneal dialysis. Unfortunately, this study showed no significant effect on glucose transport across the peritoneal membrane and did not improve ultrafiltration or fluid removal.

In *Study II*, an animal model of peritoneal dialysis was also used, in which the transport of glucose and a radioactive glucose analog, fluorodeoxyglucose ([^{18}F]-DG), across the peritoneum was studied by blocking GLUT channels with various inhibitors, such as phloretin, ritonavir, indinavir, and BAY-876, which differ in their abilities to block different GLUT channels. During the study, [^{18}F]-DG diffusion capacity decreased significantly, and ultrafiltration increased with the use of phloretin and ritonavir, whereas the other two inhibitors showed no effect.

Although indinavir, a GLUT4 blocker, and BAY-876, a GLUT1 blocker, had no effect on glucose or [^{18}F]-DG transport, the overall findings suggest that at least some glucose is transported across peritoneal membrane cells during peritoneal dialysis and that GLUT1 channels are most likely responsible for this glucose transport. In *Study II*, GLUT1 channels were detected in the small peritoneal vessels of rats by immunohistochemistry.

The results of these two studies provide new knowledge about the processes occurring through the peritoneum and open possibilities for future improvements in the care of patients treated with peritoneal dialysis. By optimizing glucose transport and reducing the harmful effects of high glucose levels, we can extend the time that patients can be treated with peritoneal dialysis and reduce the negative effects of glucose. Therefore, it is necessary to continue research and conduct clinical trials to translate these results into clinical practice and make them available as treatment options for chronic kidney disease.

Novel functional tests in peritoneal dialysis treatment

Because each patient's peritoneum is unique, it is important to know its transport properties for both water and solutes to select the appropriate dialysis treatment. There are several tests to evaluate peritoneal properties, but they are seldom used in clinical practice because of their time-consuming procedures and lack of reproducibility. The purpose of these studies was to develop a quick and reliable clinical test to determine peritoneal properties, which would facilitate its clinical use and the choice of appropriate treatment.

Water transport across the peritoneum becomes particularly important for patients undergoing peritoneal dialysis when urine production decreases so as to maintain fluid balance. A large proportion of patients with PD suffer from chronic fluid

overload, which significantly increases the risk of complications such as hypertension, cardiac enlargement, and heart failure. Fluid overload contributes to poor patient outcomes and can lead to PD treatment failure. During PD, the amount of water removed through the peritoneum depends on the properties of the membrane, composition of the dialysis fluid, dwell time in the abdominal cavity, and rate of fluid reabsorption from the abdominal cavity.

The three-pore model is a mathematical model that enables calculations and predictions of the transport of water and solutes across the peritoneal membrane during peritoneal dialysis. According to the three-pore theory, when glucose-based dialysis fluids are used, water is transported through the peritoneal microvascular endothelium in two ways: via paracellular pathways (small pores) and transcellular pathways (aquaporins or ultrasmall pores). Water transported through paracellular pathways carries small dissolved substances, whereas free-water transport occurs through aquaporins without accompanying substances. The efficiency of glucose-induced osmotic flow through small pores is relatively low, whereas that through aquaporins is 100%.

The most common cause of insufficient water removal during peritoneal dialysis is a high glucose transport rate across the peritoneum combined with an inappropriate choice of dialysis fluid and/or treatment regimen. Over time, water transport through the peritoneum can deteriorate owing to damage to the membrane caused by dialysis fluids, mechanical and osmotic stress, and recurrent peritonitis.

The ability of glucose to induce ultrafiltration or water removal through the peritoneum is called osmotic conductance to glucose (OCG). This parameter reflects the efficacy of glucose as an osmotic agent.

Several clinical and mathematical methods have been proposed to evaluate OCG. One of the most commonly used methods is the two-hour double mini-PET test, which involves two consecutive one-hour dwells with different glucose concentrations. OCG is calculated as the ratio between the difference in ultrafiltered volume and the difference in the osmotic gradient between the two dwells. Decreased OCG can result from long-term peritoneal dialysis and may indicate progressive changes in the peritoneum.

Studies III and IV

In *Study III*, the impact of the residual volume in the abdominal cavity on peritoneal water permeability in response to glucose-induced osmotic pressure (OCG) was investigated using a double mini-test. This test was compared with a new, shorter, one-hour test. The study involved patients on peritoneal dialysis who underwent both the double mini-PET test and the new short test. The results showed that determining the residual volume is very important for accurately calculating the amount of ultrafiltered fluid and obtaining precise test results that describe

membrane water permeability. However, the reproducibility of this test was not examined in the present study.

The main aim of *Study IV* was to investigate whether the new method for OCG determination could reproduce the same results during repeated measurements. The new test, which was used to measure OCG in the first study, also had weaknesses in repeated measurements.

In *Study IV*, a method for estimating water removal using sodium was introduced, which yielded excellent results, even in repeated measurements.

During peritoneal dialysis, solutes, such as urea, creatinine, glucose, and electrolytes, move between the blood and dialysis fluid through the peritoneum. In clinical practice, the peritoneal equilibration test (PET) is the most commonly used method for evaluating the transport rate of solutes across the peritoneum. Owing to its simplicity, PET is widely used in many clinics and often serves as the reference method. The standard PET is a four-hour test.

In *Study IV*, the short test (called CombiPET) was also compared with the classic peritoneal equilibration test (PET), which is usually used to determine membrane permeability to small solutes. It was found that the short CombiPET test could replace the long four-hour test.

By modifying the calculation method, the new one-hour test could replace both the two-hour double mini-PET test and four-hour PET test. The short test reproduced results in repeated measurements, highlighting its reliability and stability.

Such test enables long-term monitoring of the peritoneum in patients undergoing peritoneal dialysis. It can reveal both functional changes and early signs of progressive structural alterations in the peritoneal membrane. By detecting these changes early, the treatment method can be adjusted to prevent serious complications such as encapsulating peritoneal sclerosis, a complication with scar tissue in the peritoneum. This test also enables the doctor to select the optimal treatment based on the patient's individual membrane characteristics. Furthermore, it streamlines work for healthcare professionals by saving time in treatment planning and follow-up.

Santrauka lietuvių kalba

Apie 10 procentų pasaulio gyventojų kenčia nuo mažesnio ar didesnio laipsnio lėtinio inkstų nepakankamumo, kas ženkliai įtakoja tiek asmens sveikatą, tiek sveikatos priežiūros sistemas. Dėl ilgėjančios gyvenimo trukmės ir nuolat augant asmenų grupei, susiduriančiai su tokiais rizikos veiksniais kaip nutukimas, fizinis neaktyvumas, diabetas, aukštas kraujospūdis ir širdies bei kraujagyslių ligos, auga ir lėtinio inkstų nepakankamumo rizika.

Inkstų nepakankamumas gali būti skirstomas į stadijas: nuo inkstų nepakankamumo su nepažeista inkstų funkcija (I-a stadija) iki galutinės inkstų nepakankamumo stadijos (V-a stadija). Kai inkstų funkcija yra smarkiai sumažėjusi, pacientams gali pasireikšti tokie simptomai kaip sumažėjęs apetitas, pykinimas, padidėjęs nuovargis, raumenų silpnumas, niežulys, tinimai ir aukštas kraujospūdis. Kraujo tyrimai dažnai rodo kalcio, fosforo, kalio ir rūgščių-šarmų pusiausvyros sutrikimus, taip pat padidėjusį šlapalo ir kreatinino kiekį. Kai inkstų funkcija pasiekia kritiškai žemą lygį, dializė arba inkstų transplantacija tampa gyvybiškai būtina. Dializė yra gydymo metodas, kuris gali pakeisti arba papildyti inkstų funkciją ir yra dviejų formų: peritoninė dializė ir hemodializė.

Šiame darbe dėmesys skiriamas peritoninei dializei.

Įvadas

Peritoninė dializė yra dažniausia namų dializės forma. Ji daugeliu atvejų yra pigesnė nei hemodializė, be to, ji didina paciento savarankiškumą ir skatina aktyvų įsitraukimą į gydymo procesą. Peritoninės dializės metu pilvaplėvė funkcionuoja kaip natūralus filtras, padedantis organizmui pašalinti medžiagų apykaitos produktus ir perteklinį skystį, tokiu būdu, bent iš dalies, kompensuojama sumažėjusi inkstų funkcija. Supaprastintai, pilvaplėvę galima pavaizduoti kaip kiaurasamtį su daug įvairaus dydžio skylučių ar dar kitaip porų, per kurias prasiskverbia vanduo ir ištirpusios mažos molekulės, tokios kaip kreatininas, šlapalas ar gliukozė. Tačiau didesnės molekulės, tokios kaip kraujo baltymai ir kraujo ląstelės, praktiškai negali prasiskverbti pro šį filtrą.

Specialios sudėties dializės tirpalai, kurie sudaryti iš vandenyje ištirpintų įvairių rūšių druskų bei buferio, skirto reguliuoti rūgščių-šarmų pusiausvyrą, ir osmosinių medžiagų, tokių kaip gliukozė, aminorūgštys ar ikodestrinas, reguliariai įleidžiami

į pilvo ertmę ir išleidžiami per specialų silikoninį kateterį. Kateteris įstatomas nedidelės chirurginės intervencijos metu ir yra tinkamas ilgalaikiam gydymui. Peritoninės dializės atlikimo procedūros yra nesudėtingos, ir dauguma pacientų greitai išmoksta jas atlikti namuose, o prireikus, procedūras gali atlikti ir apmokytas personalas.

Svarbiausi procesai, vykstantys peritoninės dializės metu, yra *difuzija* (kai ištirpusios medžiagos juda iš didesnės koncentracijos į mažesnę), *osmozė* (kai osmosinė medžiaga, esanti dializės tirpale, sukuria koncentracijos gradientą, dėl kurio vanduo iš kraujo per pilvaplėvę gali patekti į pilvo ertmę) ir *konvekcija* (kai ištirpusios medžiagos pernešamos kartu su vandens srautu).

Nepaisant visų privalumų, peritoninė dializė turi ir tam tikrų trūkumų, tarp kurių galima paminėti ribotą pilvaplėvės pajėgumą šalinant tiek perteklinius skysčius, tiek ureminius toksinus.

Siekiant užtikrinti perteklinių skysčių iš organizmo pašalinimą, į peritoninės dializės tirpalus pridedama osmosinio agento, dažniausiai gliukozės. Dėl savo mažo molekulės dydžio gliukozė lengvai prasiskverbia iš peritoninio tirpalo pilvaplėvės ertmėje į kraują, kas, pasirinkus netinkamą gydymo režimą, gali sukelti skysčių susilaikymą organizme. Greitas gliukozės įsisavinimas iš dializės tirpalų taip pat prisideda ir prie didelio nepageidaujamo kalorijų įsisavinimo, kas gali sumažinti jautrumą insulinui ar net sukelti diabetą, lipidų apykaitos sutrikimus bei riebalinio audinio ir kūno svorio padidėjimą. Didelė gliukozės koncentracija dializės tirpaluose taip pat neigiamai veikia pilvaplėvės funkciją ir struktūrą. Nepaisant šio žinomo neigiamo vietinio ir sisteminio gliukozės poveikio, gliukozės pagrindu pagaminti dializės tirpalai yra tarp dažniausiai naudojamų.

Gliukozės kanalai, vandens kanalai, arba dar kitaip akvaporinai, taip pat ir tarpląstelinės jungtys yra itin svarbios visų biologinių barjerų struktūros. Jos glaudžiai susijusios su ląstelių gebėjimu reguliuoti vandens ir maistinių medžiagų pernešimą bei prisitaikymą prie įvairių aplinkos sąlygų. Šios struktūros taip pat vaidina itin svarbų vaidmenį peritoninės dializės metu.

Gliukozės kanalai yra plačiai paplitusi baltymų šeima, randama visuose gyvuose organizmuose, nuo bakterijų iki žmonių. Jie būtini energijos tiekimui, nes gliukozė yra pagrindinis daugeliui organizmų energijos šaltinis. Evoliucijos metu išsivystė įvairių tipų gliukozės kanalai, pritaikyti specifinėms funkcijoms ir įvairiems audiniams. Gliukozės kanalai skirstomi į dvi pagrindines grupes, remiantis mechanizmais, kurių dėka jie transportuoja gliukozę per ląstelių membranas. Pirmoji gliukozės kanalų grupė yra nuo natrio priklausomi SGLT kanalai, dar vadinami natrio-gliukozės kotransporteriais. Jie naudoja natrio gradientą kaip varomąją jėgą gliukozės transportavimui prieš jos koncentracijos gradientą ir netiesiogiai reikalauja energijos ATP forma. SGLT kanalai apima SGLT1, kurie daugiausiai randami žarnyno epitelyje, ir SGLT2 kanalus, kurie dominuoja inkstų

kanalėliuose. Antrąją gliukozės kanalų grupę sudaro nuo natrio nepriklausomi GLUT kanalai, dar vadinami palengvintaisiais gliukozės transporteriais. Šie kanalai transportuoja gliukozę palengvintos difuzijos būdu, tiesiogiai nenaudodami energijos, ir gali būti randami visuose organizmo ląstelių tipuose. GLUT šeima yra dar įvairesnė nei SGLT šeima. Yra žinoma mažiausiai 14 GLUT tipų, kurių kiekvienas pasižymi specifinėmis funkcijomis ir skirtingu pasiskirstymu įvairiuose audiniuose. Pavyzdžiui, GLUT4 yra svarbus insulino reguliuojamam gliukozės pernešimui raumenyse ir riebaliniame audinyje, o tuo tarpu GLUT2 dalyvauja gliukozės jutimo mechanizmuose kepenyse ir kasoje. GLUT1 yra plačiai paplitęs ir veikia kaip pagrindinis gliukozės tiekėjas smegenims. Šių kanalų įvairovė ir specializacija yra labai svarbi prisitaikant prie besikeičiančių mitybos sąlygų ir energijos poreikių. Yra žinoma, kad mažiausiai keturi GLUT kanalų tipai (GLUT1-GLUT4) bei abiejų rūšių SGLT kanalai (SGLT1 ir SGLT2) egzistuoja ir pilvaplėvėje.

Akvaporinai (AQP) yra specializuoti vandens kanalai, leidžiantys greitą ir selektyvų vandens pernešimą per ląstelių membranas. Žmogaus organizme yra identifikuota 13 skirtingų AQP tipų (AQP0–AQP12), iš kurių kiekvienas turi unikalią funkciją ir specifinį pasiskirstymą audiniuose. Manoma, kad akvaporinas-1 atlieka pagrindinį vaidmenį vandens pernešime per pilvaplėvės membraną.

Tarpląstelinės jungtys yra būtinos ląstelių sulipimui ir komunikacijai. Šių struktūrų dėka formuojasi įvairūs audiniai ir organai, o taip pat tarp kūno vidinės ir išorinės aplinkos sukuriami efektyvūs barjerai. Didžioji dalis mainų tarp dializės tirpalo ir kraujo peritoninės dializės metu vyksta per šias tarpląstelines jungtis. Tačiau pastaraisiais metais išaugo susidomėjimas ir gliukozės pernešimo mechanizmais per pačias ląstelių membranas bei jų galimu vaidmeniu peritoninės dializės metu.

Šiuo darbu siekiama prisidėti prie geresnio gliukozės pernešimo per pilvaplėvės membraną mechanizmų supratimo, taip pat sukurti nesudėtingą ir lengvai prieinamą testą pilvaplėvės funkcijai įvertinti, kurio dėka galima būtų ne tik pasirinkti tinkamiausią peritoninės dializės gydymo režimą, bet ir laiku aptikti nepageidaujamus pilvaplėvės pokyčius, atsirandančius gydymo eigoje.

I-oje ir *II-oje studijos*e buvo siekiama prisidėti prie gliukozės pernešimo per pilvaplėvę mechanizmo išaiškinimo, tuo tarpu *III-ioji* ir *IV-oji* studijos buvo skirtos peritoninės membranos funkcijos vertinimo testo kūrimui ir jo patikimumo patvirtinimui.

Gliukozė ir jos kelionė per pilvaplėvę

I-a ir II-a studijos

Šioje dalyje pristatomos dvi eksperimentinės peritoninės dializės studijos, kurių tikslas ištirti gliukozės kanalų vaidmenį gliukozės transporte per pilvaplėvę ir galbūt

užginčyti iki šiol vyraujantį įsitikinimą, kad reikšmingas gliukozės transportas peritoninės dializės metu vyksta tik per tarpląstelinius tarpus. *I-oje* ir *II-oje* studijose buvo tiriama, ar galima sumažinti gliukozės įsisavinimą iš dializės tirpalų, blokuojant gliukozės kanalus, esančius ląstelių membranose. Teigiami šių tyrimų rezultatai leistų ilgiau išlaikyti aukštą gliukozės koncentraciją pilvo ertmėje, taip pat sumažinti nepageidaujamą gliukozės poveikį pilvaplėvei ir visam organizmui, kas savo ruožtu palengvintų perteklinio skysčio pašalinimą dializės metu.

I-oje studijoje buvo tiriama SGLT2 kanalų funkcija, blokuojant juos selektyviu SGLT2 kanalų blokatoriumi empagliflozinu, kuris naudojamas diabeto gydymui, taip pat širdies nepakankamumo ir lėtinio inkstų nepakankamumo progresavimo sulėtinimui. Deja, tyrimas neparodė jokio reikšmingo poveikio gliukozės pernešimui per peritoninę membraną ir taip pat nepagerino skysčio pašalinimo.

II-oje studijoje taip pat buvo naudojamas gyvūnų peritoninės dializės modelis, tačiau čia buvo tiriamas gliukozės ir radioaktyvaus gliukozės analogo ([^{18}F]-DG) pernešimas per pilvaplėvę, blokuojant GLUT kanalus įvairiais blokatoriais, tokiais kaip floretinas, ritonaviras, indinaviras ir BAY-876, kurie skiriasi tarpusavyje GLUT kanalus blokuojančiomis savybėmis. Tyrimo metu, naudojant floretiną ir ritonavirą, [^{18}F]-DG difuzija ženkliai sumažėjo, ko pasėkoje išaugo ir ultrafiltracija. Tuo tarpu indinaviras ir BAY-876 jokio reikšmingo poveikio radioaktyvaus gliukozės analogo ir gliukozės pernešimui neturėjo. Nors GLUT4 blokatorius indinaviras ir GLUT1 blokatorius BAY-876 neturėjo įtakos gliukozės ir [^{18}F]-DG pernešimui, iš bendrų rezultatų galima daryti išvadą, kad peritoninės dializės metu bent dalis gliukozės pernešama per peritoninės membranos ląsteles ir kad už šį gliukozės pernešimą greičiausiai atsakingi GLUT1 kanalai. *II-oje studijoje* GLUT1 kanalai buvo identifikuoti mažose žiurkės pilvaplėvės kraujagyslėse, naudojant imunohistocheminį metodą.

Šių dviejų tyrimų rezultatai suteikia naujų žinių apie procesus, vykstančius pilvaplėvėje, ir atveria galimybes ateityje pagerinti peritonine dialize gydomų pacientų priežiūrą. Sumažinus gliukozės pernešimą per peritoninę membraną ir jos žalingą poveikį pačiai peritoninei membranai, galima būtų prailginti gydymo peritoninėmis dializėmis laikotarpį. Norint tai pasiekti, būtina ir toliau tęsti tiek eksperimentinius, tiek klinikinius tyrimus, kurių rezultatus vėliau būtų galiam pritaikyti ir klinikinėje praktikoje.

Nauji funkciniai testai peritoninės dializės gydyme

III-a ir IV-a studijos

Kadangi kiekvieno paciento pilvaplėvė yra unikali, yra svarbu žinoti jos gebėjimą pernešti tiek vandenį, tiek ištirpusias medžiagas, kad būtų galima parinkti individualų peritoninės dializės gydymo režimą. Yra eilė testų, skirtų pilvaplėvės

savybių įvertinimui, tačiau jie yra gana retai naudojami klinikinėje praktikoje dėl didelių laiko sąnaudų, sudėtingų procedūrų ir menko testų patikimumo.

Šių dviejų studijų tikslas buvo sukurti greitą ir patikimą testą pilvaplėvės savybėms įvertinti, kas palengvintų testų naudojimą klinikinėje praktikoje ir leistų pasirinkti optimalų gydymo rėžimą.

Sumažėjus šlapimo išskyrimui, pacientams, gydomiems peritonine dialize, vandens pašalinimas per pilvaplėvę tampa ypač svarbus, kad būtu išvengta perteklinio skysčio susikaupimo. Didelė dalis pacientų, gydomų peritonine dialize, kenčia nuo lėtinio skysčių pertekliaus, kuris žymiai padidina komplikacijų, tokių kaip aukštas kraujospūdis, širdies padidėjimas ir širdies nepakankamumas, riziką. Skysčių perteklius prisideda prie blogesnių gydymo rezultatų ir gali lemti peritoninės dializės kaip gydymo metodo nesėkmę. Per pilvaplėvę peritoninės dializės metu pašalinto vandens kiekis priklauso nuo membranos savybių, dializės tirpalo sudėties, jo išlaikymo pilvo ertmėje laiko ir taip pat dializės tirpalo reabsorbcijos iš pilvo ertmės greičio.

Trijų porų modelis yra matematinis modelis, leidžiantis atlikti vandens bei tirpių medžiagų pernešimo per pilvaplėvės membraną peritoninės dializės metu skaičiavimus ir prognozes. Pagal trijų porų teoriją, kai naudojami gliukozės pagrindu pagaminti dializės tirpalai, vanduo yra transportuojamas per peritoninės membranos mikrokraujagyslių endotelį dviem būdais: per tarpląstelinius kelius (mažas poras) ir per transląstelinius kelius (akvaporinus arba itin mažas poras). Vanduo, transportuojamas per tarpląstelinius kelius, kartu neša ir mažas ištirpusias medžiagas, o laisvo vandens transportas vyksta per akvaporinus be lydinčių medžiagų. Gliukozės sukelto osmosinio srauto efektyvumas per mažas poras yra santykinai žemas, tuo tarpu per akvaporinus yra šimtaprocentinis.

Dažniausia nepakankamo vandens pašalinimo priežastis peritoninės dializės metu yra didelis gliukozės pernešimo per pilvaplėvę greitis kartu su netinkamu dializės tirpalo ir (arba) gydymo režimo pasirinkimu. Laikui bėgant, vandens pernešimas per pilvaplėvę gali taip pat pablogėti dėl membranos pažeidimų, kuriuos sukelia dializės tirpalai, pasikartojantys peritonitai bei mechaninis ir osmosinis stresas.

Gliukozės gebėjimas sukelti ultrafiltraciją per pilvaplėvę vadinamas osmosiniu laidumu gliukozei (OCG). Šis parametras atspindi gliukozės kaip osmosinio agento efektyvumą.

Siekiant įvertinti OCG, yra pasiūlyta keletas klinikinių ir matematinių metodų. Vienas dažniausiai naudojamų metodų yra dviejų valandų trukmės dvigubas mini-PET testas, kuris apima du vienos valandos dializės keitimus su skirtingų gliukozės koncentracijų peritoniniais tirpalais. OCG apskaičiuojamas kaip ultrafiltruoto tūrio ir osmosinio gradiento skirtumo tarp dviejų keitimų santykis. Sumažėjęs OCG gali būti stebimas, kaip ilgalaikės peritoninės dializės rezultatas, ir gali atspindėti progresuojančius pokyčius pilvaplėvėje.

Trečioje studijoje buvo tiriama pilvo ertmėje esančio liekamojo tūrio įtaka gliukozės sukeltam osmosiniam vandens laidumui per pilvaplėvę (OCG), atliekant vadinamąjį dvigubą mini-PET testą. Šis testas buvo lyginamas su nauju, trumpesniu vienos valandos testu. Tyrime dalyvavo pacientai, gydomi peritonine dialize. Tyrimo metu pacientams buvo atliktas tiek dvigubas mini-PET testas, tiek naujasis trumpas testas. Rezultatai parodė, kad liekamojo tūrio nustatymas yra labai svarbus, norint tiksliai apskaičiuoti ultrafiltruoto skysčio kiekį ir gauti tikslesnius testų rezultatus, apibūdinančius peritoninės membranos vandens laidumą. Testo gebėjimas atkartoti rezultatus šioje studijoje buvo netiriamas.

IV-os studijos tikslas buvo patikrinti, ar naujas OCG nustatymo metodas galėtų atkartoti rezultatus, atliekant pakartotinius matavimus. Buvo nustatyta, kad net šis naujas testas turėjo trūkumų ir nepilnai patenkino lūkesčius, atliekant pakartotinus matavimus. *IV-os studijos* metu ultrafiltracijos apskaičiavimui buvo pasirinkta natriu paremta skaičiavimų metodika, kuri leido gauti puikius rezultatus net ir atliekant pakartotinus matavimus.

Peritoninės dializės metu ištirpusios medžiagos, tokios kaip karbamidas, kreatininas, gliukozė ir elektrolitai, juda tarp kraujo ir dializės tirpalo per pilvaplėvę. Klinikinėje praktikoje peritoninis ekvilibracijos testas (PET) yra dažniausiai naudojamas metodas ištirpusių medžiagų transporto greičiui per pilvaplėvę įvertinti. PET yra plačiai taikomas daugelyje klinikų ir dažnai naudojamas kaip referencinis metodas. Tačiau standartinio PET atlikimas užtrunka net keturias valandas.

Ketvirtoje studijoje buvo lyginamas trumpasis testas (pavadintas CombiPET) su klasikiniu PET. Paaiškėjo, kad trumpasis CombiPET yra lygiavertis ilgąjam keturių valandų PET.

Tokiu būdu, pakeitus skaičiavimo metodiką, naujasis vienos valandos testas galėjo pakeisti tiek dviejų valandų trukmės dvigubą mini-PET, tiek keturių valandų trukmės PET. Trumpasis testas taip pat galėjo puikiai atkartoti rezultatus pakartotiniuose matavimuose, kas pabrėžia testo patikimumą ir stabilumą.

Toks testas leidžia sekti funkcinius pilvaplėvės pokyčius, taip pat laiku aptikti progresuojančius struktūrinius peritoninės membranos pokyčius pacientams, gydomiems peritonine dialize. Tai leidžia, reikalui esant, laiku pakeisti gydymo metodą, siekiant išvengti rimtų komplikacijų, tokių kaip, pavyzdžiui, inkapsuliuojanti peritoninė sklerozė. Be to, šis testas palengvina optimalaus gydymo pasirinkimą, atsižvelgiant į individualias membranos savybes, ir taupo personalo laiką.

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Clinical, Experimental and Theoretical Studies of Solute and Water Removal in Peritoneal Dialysis

IT TOOK SEVERAL centuries of research and experimentation before scientists successfully harnessed the unique properties of the peritoneal membrane for kidney replacement therapy. Delving into the processes of peritoneal dialysis, one is fascinated not only by the observations and discoveries of numerous scientists but also by the complexity and mystery of the human body itself. Although the human body was not originally designed for peritoneal dialysis, it is astonishing how the peritoneum can remove fluids and toxins through the "three-pore sieve". Furthermore, despite continuous exposure—24 hours a day, sometimes for years—to non-physiological stimuli such as peritoneal dialysis solutions, it can maintain its cellular structure and function, efficiently removing fluids and toxins and thereby partially replacing kidney function.

Despite significant progress over time in understanding the mechanisms of peritoneal dialysis, much remains unexplored and enigmatic. The aims of this thesis are firstly to investigate the still unexplained processes occurring during peritoneal dialysis, which could eventually contribute to increasing the efficiency of this treatment and, most importantly, reducing the negative effects of peritoneal dialysis solutions on both the peritoneal membrane and the human body as a whole and secondly to facilitate the selection of individualized dialysis regimens in clinical practice and allow timely detection of adverse structural changes in the peritoneal membrane.

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