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# ICODEXTRIN METABOLISM IN PERITONEAL DIALYSIS: CLINICAL AND EXPERIMENTAL STUDIES

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To the memory of my father
and to my family in Mexico and Sweden

# ABSTRACT

Icodextrin is a glucose polymer which is used as osmotic agent to provide sustained ultrafiltration (UF) during the long (8-16 hours) dwell in end-stage renal disease patients undergoing peritoneal dialysis (PD). The aims of this thesis were to study: the metabolism of icodextrin in the peritoneal cavity and in the circulation of PD patients and non-uremic rats, the role of α-amylase in its hydrolysis, and the rate of breakdown of a synthetic maltoheptaose (G7) *ex vivo* which we propose as a novel measure of amylase mediated oligosaccharide metabolism.

**Study I**. Gel-filtration HPLC was applied to determine high and low molecular weight icodextrin molecules in dialysate and plasma from PD patients using glucose solution or icodextrin (after the first dwell or chronically). Total hydrolysis of the samples was used to validate the results. 39 % of the infused icodextrin was absorbed from the peritoneal cavity during the long dwell. The plasma concentration of icodextrin metabolites was significantly higher and α-amylase activity significantly lower in the icodextrin groups.

**Study II**. An assay for measurement of total α-amylase activity in serum containing icodextrin degradation products was validated. The study demonstrated that the low values of plasma α-amylase activity in PD patients using icodextrin are correctly determined.

**Study III**. In 23 non-uremic rats undergoing chronic PD using either glucose- or icodextrin-based dialysis solutions, a 4-hour dwell with icodextrin was performed twice, at days 10 and 21. There was a significant decrease in amylase activity in plasma (which is much higher than in humans) and an increase in dialysate. About 60 % of the infused icodextrin was absorbed from the peritoneal cavity at the end of the dwell. No icodextrin metabolites were detected in plasma at the end of the dwell.

**Study IV**. The rate of degradation of G7 in plasma from healthy controls, and PD patients using only glucose solution or glucose in combination with icodextrin was investigated *ex vivo*. Samples were spiked with G7 and/or synthetic amylase, and incubated 4 hours at 37$^{o}$C. The G7 degradation rate was lower in plasma from icodextrin patients but it was also reduced in PD patients using glucose, in spite of the higher amylase activity, as compared with the controls. This suggests that the amylase mediated carbohydrate metabolism is reduced in PD patients. It is possible that this could contribute to reduced hyperglycemic changes, especially in patients using icodextrin.

**Study V**. When investigating also pre-dialysis (PreD) and hemodialysis (HD) patients, we found that the rate of degradation of G7 did not differ from the controls. Amylase activity was increased in the PreD, HD and GLU patients, and decreased in the ICO patients. The rate of G7 degradation per unit of amylase activity was reduced in PreD and GLU patients. The rate of G7 degradation was related to the endogenous amylase activity. These findings suggest that the amylase mediated oligosaccharide metabolism is altered in uremic patients, although this needs to be confirmed in larger studies.

**Study VI**. The relationship between the efficacy of icodextrin in changing UF, fluid status and residual urine volume versus the concentration of plasma icodextrin metabolites was investigated. There was no relationship between plasma concentrations of icodextrin metabolites and any of the other clinical parameters, including change in daily UF, urine volume, fluid or inflammatory status. Icodextrin was not associated with a greater fall in urine output despite its larger effect on the volume of extra-cellular fluid.

# LIST OF PUBLICATIONS

I. Determination of high and low molecular weight molecules of icodextrin in plasma and dialysate using gel-filtration chromatography, in peritoneal dialysis patients.
**Elvia García-López**, Björn Anderstam, Olof Heimbürger, Gianpaolo Amici, Andrzej Werynski, and Bengt Lindholm.
*Perit Dial Int. 25; 181-91, 2005*

II. Determination of α-amylase activity in serum and dialysate from patients using icodextrin based peritoneal dialysis fluid.
Björn Anderstam, **Elvia García-López**, Olof Heimbürger, and Bengt Lindholm.
*Perit Dial Int. 23; 146-150, 2003*

III. Icodextrin metabolism and alpha-amylase activity in non-uremic rats undergoing chronic peritoneal dialysis.
**Elvia García-López**, Krzysztof Pawlaczyk, Björn Anderstam, A. Rashid Qureshi, Malgorzata Kuzlan- Pawlaczyk, Olof Heimbürger, Andrzej Werynski, and Bengt Lindholm.
*Perit Dial Int. 27;415-23, 2007*

IV. Rate of synthetic oligosaccharide degradation as a novel measure of amylase activity in peritoneal dialysis patients.
**Elvia García-López**, Andrzej Weryński, Olof Heimbürger, José C. Divino Filho, Bengt Lindholm, and Björn Anderstam.
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V. Abnormal amylase mediated degradation rate of maltoheptaose (G7) in end-stage renal disease patients.
**Elvia García-López**, Björn Anderstam, Olof Heimbürger, Abdul Rashid Qureshi, Mohamed E Suliman, Ann Christin Bragfors-Helin, Peter Stenvinkel, Peter Barany, Andrzej Werynski, José C. Divino Filho, and Bengt Lindholm.
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VI. Longitudinal relationships between fluid status, inflammation, urine volume and plasma metabolites of icodextrin in peritoneal dialysis patients randomised double-blind to glucose or icodextrin for the long exchange.
Simon J. Davies, **Elvia García-López**, Graham Woodrow, Kieron Donovan, Jorg Plum, Paul Williams, Ann-Cathrine Johansson, Hans-Peter Bosselmann, Olof Heimburger, Ole Simonsen, Andrew Davenport, Bengt Lindholm, Anders Tranæus, Jose C. Divino Filho.
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## ABBREVIATIONS

| | |
|---|---|
| 1st ICO | First time use of icodextrin group |
| ANP | Atrial natriuretic peptide |
| APD | Automated peritoneal dialysis |
| BP | Blood pressure |
| CAPD | Continuous ambulatory peritoneal dialysis |
| CRP | C-reactive protein |
| Da | Daltons |
| D1 | Dwell 1 |
| D2 | Dwell 2 |
| D/P | Dialysate to plasma concentration ratio |
| ECFv | Extra-cellular fluid volume |
| EPS | Ethylidene |
| G2 | Maltose |
| G3 | Maltotriose |
| G4 | Maltotetraose |
| G5 | Maltopentaose |
| G6 | Maltohexaose |
| G7 | Maltoheptaose |
| GFR | Glomerular filtration rate |
| GLU | Glucose patient's group |
| GPDF | Glucose-based peritoneal dialysis fluid |
| HA | Hyaluronan |
| HD | Hemodialysis |
| HMW | High molecular weight |
| HPLC | High performance liquid chromatography |
| HOMA | Homeostasis model assessment |
| ICO | Icodextrin patient's group |
| IPDF | Icodextrin-based peritoneal dialysis fluid |
| LDL-C | Low density lipoprotein-cholesterol |
| LMW | Low molecular weight |
| Mn | Number average molecular weight |
| Mw | Weight average molecular weight |
| PD | Peritoneal dialysis |
| PET | Peritoneal equilibration test |
| PNP | Paranitrophenol |
| PreD | Pre-dialysis |
| RRF | Residual renal function |
| TC | Total cholesterol |
| TG | Triglycerides |
| TBWv | Total body water volume |
| UF | Ultrafiltration |

# 1 GENERAL INTRODUCTION

Peritoneal dialysis (PD) is one of the methods of renal replacement therapy. The first clinical application of PD was reported in 1923 by Ganter who treated a patient with uremia (1). After 1946, PD was successfully applied in the treatment of acute renal failure (2). About fifteen years later PD became accepted as a long term treatment for chronic uremia following the work of several investigators (3-5), and in 1976 continuous ambulatory peritoneal dialysis (CAPD) was developed as a convenient treatment modality for chronic renal failure (6). However, initially CAPD was hampered by a high peritonitis rate (7, 8). The introduction of the flexible plastic dialysate containers in 1977 was a major improvement (9).

Further improvements of the PD techniques have included the introduction of the double bag Y-shaped disconnected lines, which reduced the incidence of peritonitis (10), and the development of continuous cyclic peritoneal dialysis (CCPD) (11) and other forms of automated peritoneal dialysis (APD). These technique have lifestyle benefits and also allow the prescription of more individualized dialysis regimens (12).

The choices made in the design of conventional PD solutions were based on attempting to mimic extra cellular water (without potassium), tempered by a series of compromises to allow production, and stability under storage conditions. From the start glucose was being used as the osmotic agent, buffered with lactate alone and with a low pH, which allowed heat sterilization without causing caramelization of the glucose. The new generation of glucose-based PD solutions have a more physiological pH and contain less glucose degradation products (13).

Glucose has served well as the prototypical osmotic agent in PD for more than four decades, because it offers many of the characteristics required of a cheap, safe, and effective osmotic agent. The disadvantages of glucose include: its rapid dissipation from the peritoneum resulting in limited ultrafiltration (UF) during a long dwell in so called high and high-average transporters; the metabolic responses to absorbed glucose such as hyperglycemia, hyperinsulinemia, hyperlipidemia and obesity; and, the local effects of glucose, glucose degradation products, and hyperosmolality on peritoneal membrane structure and function (14-16). Alternative osmotic agents with lower

molecular weight as amino acids and glycerol will be absorbed more rapidly than glucose, therefore resulting in a shorter period of positive UF (14). Several large molecular weight osmotic agents, such as starch derived glucose polymers, dextran, gelatin, albumin and polypeptides, have been used in various experimental studies (17). Based on the work carried out on dextran (18) Milner suggested a potential role for the starch-based glucose polymer, dextrin (19, 20). It is from this idea, which proposed administering a modified version of the dextrin based nutritional supplement product, Caloreen™, as the osmotic agent in PD solutions that the present product, icodextrin, has been developed. Since the capillary wall is easily permeable to water and small solutes but restricts the passage of large molecular weight solutes, the osmotic effect of a colloid substance during PD is much more prolonged than the osmotic effect of small solutes. Therefore, even with a relatively low osmolality, the colloid osmotic pressure may ensure sustained osmotic transport of water (21). The main osmotic effect of the polymers occurs over the so called small pores and free water transport (without solutes) resulting in sodium sieving through the ultra small pores (22). The presently used 7.5 % icodextrin solution is in fact hypo-osmolar to plasma, but will in general result in a sustained net UF for more than 14 hours due to the sustained colloid osmotic gradient (22). The icodextrin-based solution does not seem to affect peritoneal solute transport characteristics in the short term (23), but the large osmotic fluid flow through the small pores will result in increased clearance of sodium, as well as of low molecular proteins like β2-microglobulin and leptin (22-24).

# 2 ICODEXTRIN: REVIEW OF THE LITERATURE

## 2.1 INTRODUCTION

Icodextrin is a specific fraction of dextrin (a starch derived, water soluble, glucose polymer) isolated by fractionation of hydrolyzed cornstarch (19). The structure of icodextrin is similar to glycogen, and is based on polysaccharide polymers of D-glucopyranose linked by more than 90 % α-(1-4) and less than 10 % α-(1-6) glucosidic bonds. In contrast to glycogen, icodextrin has a lower percentage of α-(1-6) linkages and hence is less highly branched. The majority of icodextrin polymers (> 85 %) have a molecular weight between 1638 to 45,000 Daltons (Da), with only 6 % having a molecular weight less than 1638 Da. Since icodextrin is a polydispersed mixture of polymers with varying chain lengths (2 to 300 of linked glucose molecules), its molecular weight is characterized by both a number average (Mn) and a weight average (Mw) molecular weight. The Mn for icodextrin, that is the arithmetic mean of the molecular weights of the individual glucose polymer molecules, range from 5,000 to 6,500 Da and the Mw (equal to the sum of [weight of molecules times molecular weight] for all the molecular species divided by the total weight of all molecules) range from 13,000 to 19,000 Da (19, 25).

Conventional PD solutions contain glucose almost universally as osmotic agent (16). The amount of ultrafiltration (UF) achieved over a given dwell time depends largely on the strength of the concentration of glucose used (1.36 %, 2.27 % or 3.86 % anhydrous glucose), and the higher the concentration, the higher the UF volume, **Figure 1**. However, glucose has several disadvantages, due in part to its rapid absorption across the peritoneal membrane and the resulting dissipation of the osmotic gradient (16, 26-28). These include UF of short duration, especially in patients with fast peritoneal membrane transport (i.e. so called high and high-average transporters), and metabolic disturbances (16, 26-30). In addition, long-term exposure to high glucose concentration contributes to progressive peritoneal alterations that eventually may lead to UF capacity failure, a common cause of transfer to hemodialysis (HD) (26, 31-33).

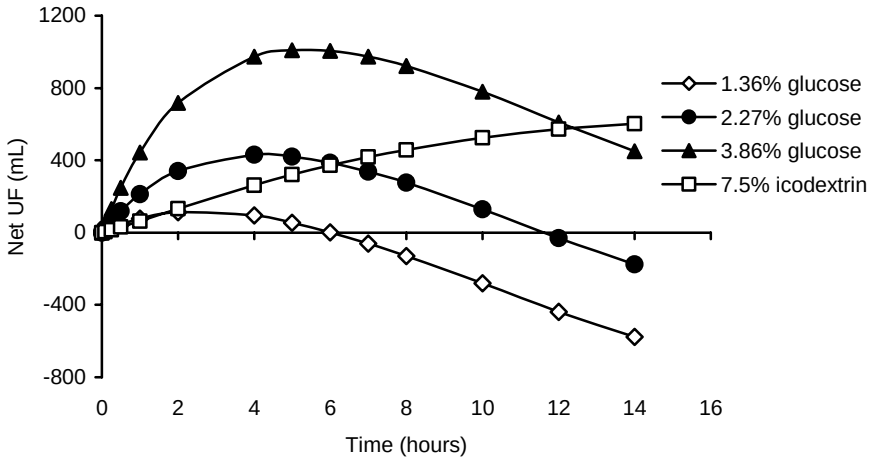


***Figure 1****. Profile of net UF in high average transport patients with the use of different PD glucose solution concentration and 7.5 % icodextrin-based solution during the time of a dwell (34).*

Whereas glucose and other small molecules are absorbed from the peritoneal cavity primarily by diffusion across the peritoneal capillary endothelium (14), the diffusion component is limited for icodextrin as icodextrin cannot easily pass through the small pores due to its high molecular weight. Icodextrin absorption occurs primarily via the relatively slow convective fluid movement out of the peritoneal cavity (14, 35). As a result the absorption of the osmotic agent is much slower than for glucose, resulting in a longer duration of the osmotic gradient. Thus, the pressure created by icodextrin will decline only slowly during the dwell and a positive net UF is therefore sustained throughout the long dwell (21, 22, 36, 37). Nevertheless, about 20 % to 40 % of the administered icodextrin is absorbed from the peritoneal cavity during the long dwell (25, 35, 37, 38).

Absorbed icodextrin is hydrolyzed in plasma by circulating α-amylase, yielding oligosaccharides (also called icodextrin metabolites), such as maltose (G2), maltotriose (G3), maltotetraose (G4), maltopentahose (G5), maltohexaose (G6), and maltoheptaose (G7). The smaller oligosacharides (i.e., G2 to G4) are the principal metabolites of icodextrin observed in blood during repeated exposure to icodextrin (37, 39-43). Maltose and the other circulating icodextrin metabolites may be further metabolized to

glucose by tissue maltases, or they are excreted into the urine or into the peritoneal dialysate (25, 35).

Although the total amount of the polydispersed mixture of icodextrin polymers can be readily quantitated in plasma and other biological samples by the hydrolysis of total carbohydrates to glucose, followed by determination of glucose by conventional methods, the characterization of the plasma kinetics, metabolism and elimination of individual icodextrin molecules has been hampered by the absence of available analytical techniques, and the complexity of the polymer mixture.

The analysis of the icodextrin metabolites in plasma from PD patients treated with icodextrin-based peritoneal dialysis fluid (IPDF), has been performed with high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) (44, 45), which is a sensitive, specific, and fast (40 min) method. However, such an instrument is not available in most laboratories. An alternative method, used for several decades, was gel-filtration high performance liquid chromatography (HPLC) and in one application (using a Jeolco-6AH automatic carbohydrate analyzer) oligosaccharides (similar to icodextrin metabolites) were determined (46). This method required, however, long analysis time (9-10 hours) and was insensitive.

Two alternative approaches were investigated in the present study. The first one, which was used at the beginning of our investigations, was HPLC with a gel-filtration media. One such column records the profile of all icodextrin molecules (Mw up to 66 kDa), but does not reliably quantify low molecular weight (LMW) icodextrin metabolites (G2-G7). However, LMW molecules smaller than about 2 kDa, i.e., maltose (G2; Mw 360 Da) up to maltoheptaose (G7; Mw 1153 Da) can be accurately measured by using another gel-filtration column (38). The second alternative which we started to use in 2004 is the use of isocratic anion exchange chromatography for the analysis of icodextrin metabolites (BioBasic AX; 150 x 4.6mm, 5µm. Thermo Electron Corporation, Woburn, MA, USA). The two techniques are comparable regarding the results obtained, but the major advantage with the new technique is the much shorter elution time, as the complete analysis of a sample takes 10 – 12 minutes compared to 90 min with the previous gel-filtration HPLC. For the analysis of the high molecular weight (HMW) fractions of icodextrin in dialysate we started to use a more modern

gel-filtration column (Shodex, Protein Series KW-802-5, Showa Denko K.K. Kanagawa, Japan) The results are comparable with the older technique, but again the major advantage is the much shorter elution time, as the running time for a dialysate sample is only 15 minutes (data not published yet).

Icodextrin and other polysaccharides are metabolized effectively by the enzyme α-amylase, (Mw 57.6 kDa). The principal sources of plasma amylase are the pancreas and the salivary glands, although this enzyme is also detectable in other organs, such as lungs, muscle, adipose tissue, and small intestine. α-Amylase is present in different isoforms, i.e. pancreatic and salivary.

Low plasma α-amylase activity has been reported in patients using icodextrin (38, 47, 48), although some authors have stated that this is due to interference of icodextrin with the analytical methods (39, 49-52), because icodextrin competes with the polysaccharide reagent. No systematic analyses of the validity of routine methods for determination of α-amylase in presence of icodextrin have been reported previously.

## 2.2 PHARMACODYNAMIC PROPERTIES OF ICODEXTRIN

Icodextrin has a Mw of 16,800 Da and Mn of 5,300 Da (19). As icodextrin molecules range from 2 to 300 glucose units in length and only a small proportion is under 10 units, this limits uptake into the circulation by diffusion and therefore systemic absorption is mainly by the lymphatics. The links between the individual glucose moieties are predominantly α-(1-4) glucosidic bonds which are highly susceptible to degradation by enzymes, such as amylases, whereas the proportion of less susceptible α-(1-6) linkages is < 10 % (19).

### 2.2.1 Mechanism of action

With a calculated osmolality of 282 - 286 mOsmol/L, the 7.5 % icodextrin solution is iso-osmolar with respect to normal plasma and produces transcapillar UF by a mechanism resembling “colloid” osmosis (21-23). The phenomenon of osmotic water transport between iso-osmolar solutions is possible because the peritoneal membrane is permeable to small and large molecular weight solutes; hence, the flow of water occurs in the direction of the relative excess of relatively impermeable and large molecules

(i.e. icodextrin), rather than down the solute concentration (osmolality) gradient. This is explained by the heteroporous structure of the peritoneal membrane with a high osmotic pressure from icodextrin over the small pore system as icodextrin cannot pass through the small pores (the reflection coefficient for the largest icodextrin fractions is close to 1, glucose is about 0.03) whereas the water flow through the aquaporins is very small due to the very low difference in the osmolarity and the small total area of aquaporins. As the UF with icodextrin solution will occur almost entirely through the small pores, no sieving of solutes will be observed with this solution (53).

### 2.2.2 Effects on ultrafiltration (UF)

In controlled clinical trials in patients undergoing CAPD (37, 40, 41, 54) or APD (39, 55-58), intraperitoneal administration of 7.5 % icodextrin for the long (8 to 16 hours) exchange produced UF volumes which exceeded those with 1.36 %, 2.27 % and 3.86 % glucose solutions. More recently the use of a combination of glucose and icodextrin has been demonstrated to further improve the UF (43, 59-61) during a long dwell (up to 16 hours).

### 2.2.3 Effect on fluid balance and cardiovascular parameters

As a consequence of the increase in UF, icodextrin improved fluid balance in PD patients when compared with 1.36 % or 2.27 % glucose in a single centre non-randomized study (58) and subsequently in two multicenter randomized trials (62, 63).

In the larger multicenter study (62), 50 PD patients with < 750 mL daily urine output (all high or high-average transporters) underwent double-blind therapy using icodextrin or 2.27 % glucose for the long-dwell exchange. After six months of treatment, the icodextrin recipients had lost weight whereas there was a tendency for weight gain in the glucose recipients. The improvement in fluid status with icodextrin was apparent within the first month of treatment and sustained throughout the remainder of the study. Blood pressure (BP) was well controlled in this study; no clear relationship between changing body composition and 24-hour ambulatory BP was observed (62).

In the smaller study (63), 19 PD patients receiving icodextrin for 4 months experienced significant reductions in body weight (2 %) and extracellular water (ECW) (10 %),

compared with nonsignificant increases of 0.5 % in body weight and 10 % in ECW, in 13 patients receiving 1.36 % glucose. Interestingly, there was an unrelated, but significant 5% reduction in left ventricular hypertrophy (LVH) in the icodextrin group, whereas there were no changes of BP in the icodextrin or glucose groups. Another report (58) showed that icodextrin used for the long dwell effectively improved the BP control in 6 out of 14 patients in a single center, non-randomized study, compared with 2.27 % glucose. In another prospective crossover study, eight CAPD patients were investigated on two different study days. CAPD was carried out with 1.36 % and 3.86 % glucose solutions on one study day, and with 1.36 % glucose and icodextrin 7.5 % on the other day. BP was significantly higher during 3.86% glucose dwells as compared with 1.36% glucose or icodextrin dwells (64).

### 2.2.4 Metabolic effects

On account of its slower rate of absorption from the peritoneal cavity and/or the fact that its eventual metabolism into glucose occurs within tissues and not in the blood (see below), the metabolic effects of icodextrin are generally less pronounced than those of standard glucose solutions (65).

Plasma glucose and insulin levels were not significantly different following a single 12-hour dwell with icodextrin in a pharmacokinetic study in 13 patients (25), an observation which supports the claim that, in contrast to glucose-based dialysis fluid (GPDF), acute administration of icodextrin does not lead to hyperglycemia or hyperinsulinemia (65). A more recent study (66) reported that non-diabetic CAPD patients using IPDF (n = 17) had significantly lower fasting serum insulin, 10.15 ± 6.87 U/mL as compared with the patients using only GPDF (n = 27), 20.59 ± 17.86 U/mL. The homeostasis model assessment (HOMA) index was also significantly lower in the icodextrin group 2.3 ± 1.7 than in the glucose group 4.8 ± 4.1. A multicenter study which included 49 diabetic patients showed that overall there were no significant differences in the HbA1C or plasma glucose levels; however, in 27 patients who started with HbA1C ≥ 6.5 %, the mean values decreased significantly during icodextrin treatment, reaching the lowest value at 6 month (67). In a multicenter, open-label, randomized clinical trial in 59 high and high average diabetic patients on PD in Mexico, 30 patients were randomized to icodextrin and 29 to glucose PD solution, and the patients were followed 6 months. UF was significantly higher, ECFv was reduced,

and BP and the dose of antihypertensives decreased significantly in the icodextrin group (68).

The carbohydrate (glucose) load following administration of icodextrin once daily for the long dwell in CAPD or APD patients has been reported to be lower than that with 2.27 % glucose [by 8 %, n=17 (55)] and 3.86 % glucose [by 55 %, n=65 (37, 56)], as well as when using a combination with glucose-, amino acid-, and icodextrin-based dialysis solution (43, 69).

The glucose load is thought to lead or contribute to dyslipidemia in PD patients, although a direct association has been difficult to demonstrate (65). Some (65, 67, 70, 71), but not all (66, 72) studies have reported a relatively favorable effect of icodextrin on the plasma lipid profile compared with glucose in terms of significant reduction of total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C), and triglycerides (TG) levels. For example, in a 12-week randomized crossover study in 21 non-diabetic patients TC and LDL-C levels decreased significantly from baseline (70). Similarly, TC and LDL-C were significantly reduced in a secondary analysis of 88 patients (65) who participated in a 6-month non-blind, randomized trial (n=209) (37). TG levels were also reduced from baseline in a 6-month study in 12 non-diabetic patients (71) and in a 8-week study including both diabetic and non-diabetic patients. More recently, Barbazono et al (67) reported significantly decreased levels of TCL, DL-C and TG in a 12-month study in 51 diabetic patients.

### 2.2.5 Effects on peritoneal membrane characteristics

In PD, the local biocompatibility of an agent refers to its ability to permit long term dialysis without any clinical significant changes in the functional characteristics of the peritoneum. There has been a debate over the years as to how biocompatible icodextrin is for the peritoneal membrane. In its favor, it is iso-osmotic, low in glucose degradation products and perhaps, most important of all, glucose free. Preclinical studies suggest that icodextrin may be more biocompatible as osmotic agent than glucose (73). *In vitro* (74-76) and rat (77) studies have shown significantly reduced formation of early and advanced glycation end-products (implicated in the functional deterioration of the peritoneal membrane in long term PD patients (78)) with icodextrin compared with standard glucose solution. However, this effect has not been confirmed

clinically (79-81), in part because of the short duration of the studies and probably because of the "carry-over" effects of glucose used for the other exchanges (73).

Icodextrin may have less deleterious effects than hyperosmolar glucose solutions on peritoneal membrane function according to the findings of a prospective, multicenter study in 177 anuric APD patients (82). At baseline, 43 (24 %) patients used only 1.36 % glucose, whereas 134 patients used at least one exchange using glucose 2.27 % or 3.86 %, and 82 (46 %) patients were using icodextrin 7.5 % for the long dwell as a part of their regimen. Patients that did not use icodextrin but used higher glucose concentration had the most marked changes in longitudinal membrane function.

Peritoneal membrane transport characteristics were essentially unaffected by icodextrin and glucose in two randomized multicenter clinical trials in which a total of 194 and 13 stable CAPD patients were followed for 1 and 2 years respectively (40, 83, 84). Mesothelial markers and peritoneal defense characteristics (white cell counts) were also unchanged after two years of treatment with icodextrin or glucose (84). More recently, it has been reported that the use of a daily exchange of glucose-free dialysis solution leads to a better preservation of mesothelial cell mass (85).

### 2.2.6 The effect of icodextrin on serum amylase

The usual reason for measuring amylase levels is to confirm or exclude the diagnosis of pancreatitis. The serum amylase levels reflect a balance between the rate of entry into the circulation of salivary and pancreatic amylase from all sources and the rate of amylase removal from the circulation (86). Amylase is a relatively stable enzyme with a molecular weight of 57.6 kDa (87) that hydrolyzes internal $\alpha$-(1-4) glucosidic bonds resulting in the production of maltose and other oligosaccharide (25, 38-41, 48, 88). Amylase is essential for the digestion of dietary starch, in the intestinal tract and it is also responsible for the rapid breakdown of starch reaching the circulation.

Patients with ESRD display a mild to moderate increase of serum amylase levels (89-96). The main reason for this increase has been attributed to a reduced glomerular filtration rate or coexistent pancreatic disease (91). The exceptions are PD patients using icodextrin where amylase levels have been reported to be low (38, 47-52), although most investigators have stated that this is due to interference of icodextrin

with the analytical methods (49-52) because icodextrin could compete with the polysaccharide in the reagent.

## 2.3 PHARMACOKINETIC PROPERTIES ICODEXTRIN

Several studies have reported steady state plasma concentration of icodextrin metabolites in adults (37-41, 43, 97, 98) and pediatric (97) PD patients using icodextrin chronically for the long dwell. However, much of the knowledge about the pharmacokinetic profile of the polymer is based on the results of a study (25) in 13 patients undergoing PD conducted specifically to determine the absorption, plasma kinetics and elimination of icodextrin and its metabolites following a single long dwell exchange. In this study (25) patients underwent a 12-hour exchange with 2 L of dialysis solution containing 7.5% icodextrin followed by resumption of their standard (glucose-based) PD prescription. Total icodextrin concentration in plasma, urine and dialysate were assayed using amyloglucosidase to hydrolyze all glucose polymers to glucose (99). A median of 40.1 % of the administered dose was absorbed during the 12-hour dwell, with a rate constant for disappearance from peritoneal cavity of 5.0 g/h. Plasma levels of icodextrin rose during the dwell and declined after the drainage. Peak plasma concentration (median $C_{peak}$ = 2.23 mg/mL) was reached at the end of the dwell (median $T_{max}$ = 12.7 hours) and returned to baseline within 3 to 7 days. Icodextrin had a plasma half-life of 14.7 hours with a median clearance of 1.09 l/ hours (25).

### 2.3.1 Absorption and metabolism

The absorption of icodextrin from the peritoneal cavity can be approximated using a zero-order (linear) kinetics, consistent with a constant rate of fluid (with icodextrin) absorption via convective peritoneal lymphatic pathways (25). Thus, the amount of icodextrin absorbed depends on the dwell time and correspond to approximately 20 % to 40 % of the administered dose during 8- to 16-hour dwell (25, 35, 37, 38).

In plasma, icodextrin is hydrolyzed by α-amylases into smaller glucose polymers with lower degree of polymerization than the original polymer, G2, G3, G4, G5, etc (25, 37, 38, 40, 41). These oligosaccharides may in turn be further metabolized to glucose by the *tissue* maltases (there is no circulating maltase).

It is recommended to use only one exchange of icodextrin per day to avoid the plasma accumulation of maltose and other icodextrin metabolites (16, 100). The steady-state concentration of icodextrin metabolites in plasma (≈ 5 mg/mL) is reached within 7 -10 days, and remains relatively stable during the daily use of 7.5 % icodextrin-based dialysis solution for the long dwell in adults (37-40, 98), and in children (97). In a recent study (43) where two icodextrin exchanges were used in 12 out of 17 patients included in a crossover study for 10 days, the total concentration of circulating icodextrin metabolites was on average 3.0 mg/ml (maximum 5.62 mg/mL), a value which does not differ substantially from values obtained following the use of only one icodextrin exchange per day.

### 2.3.2 Elimination

The fate of icodextrin administered to the peritoneal cavity will depend on three situations: (**A**) the metabolic stability of the polymer in the peritoneal fluid; **(B**) the degree of absorption into de systemic circulation; (**C**) renal, dialytic, and metabolic elimination from the systemic circulation (35). Urinary excretion of icodextrin metabolites is directly related to residual renal function. Moberly et al (25) reported that approximately 7 % of the administered icodextrin is removed in the dialysate during the following three exchanges with glucose solutions after a single long icodextrin dwell. The recovery of icodextrin metabolites (G2 to G4) in the dialysate of the subsequent glucose exchanges, contributes to their elimination from blood, and shows that these metabolites diffuse from the plasma into the peritoneal cavity (25).

## 2.4 THERAPEUTIC EFFICACY

The use of 7.5 % icodextrin solution administered once-daily for the long dwell exchange has been compared with that of standard glucose solution in several studies in stable adult patients undergoing CAPD (37, 40, 54) or APD (29, 39, 55, 57, 101). More recently the use of a combination of glucose and icodextrin has been reported (43, 59-61). These trials include the evaluation of the short-term UF efficacy of icodextrin and the assessment of the long-term effects of the polymer on patient survival and quality of life. Overall, these studies confirm the role of icodextrin as an effective osmotic agent for the long dwell.

### 2.4.1 Ultrafiltration efficacy

Adequate fluid management is a major goal of PD and is necessary for achievement of good clinical outcome (102). Suboptimal fluid management is still a significant problem in subsets of PD population, particularly in patients who have high-average or high peritoneal solute transport patterns (102-105). Inadequate fluid removal is a common finding when GPDF are used for the long dwell, particularly in high-average/high transporters (34, 104). Because of its small size, glucose is rapidly transported across the peritoneal membrane, leading to progressive dissipation of the osmotic gradient, and a corresponding reduction in net UF (34).

### 2.4.2 Impact of peritonitis on UF

Despite the technological advantages, peritonitis still accounts for significant morbidity in PD patients and remains a common cause of transfer to HD (106, 107). Net UF with icodextrin, in contrast to glucose-based dialysis solution, was preserved during episodes of peritonitis in clinical trials (37, 108, 109).

### 2.4.3 Effect on patient survival

Patient survival is difficult to measure in studies of PD, because at some stage (prior to death) patients may transfer to HD (e.g. because of peritonitis or UF failure) or go on to receive a kidney transplant. Nonetheless, available data suggest, but do not prove, that the once-daily use of icodextrin during the long dwell does not adversely affect patient survival (37, 39, 40, 110).

### 2.4.4 Effect on quality of life

A one-year randomized, multicenter, double-blind study evaluated the quality of life in both control (glucose-based solution) and icodextrin groups, using the validated Kidney Disease Quality of Life (KDQOL) questionnaire, which combines the generic Short-Form (SF-36) questionnaire with a list of 35 renal disease-specific symptoms/problems and a single overall health rating. Overall, quality of life declined in both treatment groups, although patients receiving icodextrin showed a better maintenance of quality of life (40).

## 2.5 TOLERABILITY

With the exception of skin reactions, icodextrin is generally as well tolerated as conventional glucose-based solutions in clinical trials (111). New skin reactions occurred in approximately twice as many patients treated with icodextrin as those receiving glucose (10 % vs 5 %), and was related to the use of polymer in about half of the cases (5.5 % vs 1.7 %) (Baxter Healthcare, 2001). Rash typically appeared early (≤ 3 weeks), was mild or moderate in the majority of the patients (95 %), and resolved with, or in some cases without, discontinuation of icodextrin. Sterile peritonitis has been reported in patients using icodextrin, due to the presence of peptidoglycans in the dialysis fluid (112)

## 2.6 CONCLUSIONS

PD represents a complementary technique to HD and kidney transplantation in the integrated management of end-stage renal disease (113-115). A major disadvantage of PD is the long-term technique failure, due to the failure of the peritoneum to function effectively as a dialyzing membrane, either temporarily or permanently (115). UF failure has been estimated to occur in as many as 5 % of PD patients after one year of treatment, 30 % after 3 years, and 50 % after four years (116). It is attributed to the bioincompatibility of conventional PD solutions, which are characterized by high glucose concentrations, high osmolality, low pH, and use of lactate as a buffer (27, 117). In particular, glucose, through the generation of toxic glucose degradation products and advanced glycation end products, is strongly implicated in the progressive peritoneal pathophysiology that leads eventually to UF failure (78, 114). In addition, glucose has several other well recognized deficiencies as osmotic agent (27). Primarily, its rapid absorption from the peritoneal cavity leads to reduced UF and aggravation of long-term metabolic complications, such as hyperinsulineamia, hyperlipidemia and obesity.

Icodextrin may improve the UF as compared with the glucose-based dialysis solutions, and its use is associated with a reduction of the carbohydrate absorption compared with the use of hypertonic glucose solutions during the long dwell, which may help to reduce the risk of metabolic complications such as hyperlipidemia, hyperinsulinemia,

appetite suppression and obesity (118). The use of icodextrin in place of hypertonic glucose-based dialysis solution (i.e. 2.27 % and 3.86 %) reduces the peritoneal glucose exposure, which may mitigate the detrimental effects of glucose on the peritoneal membrane structure and function (73). This may extend the time that patients are able to use their PD modality of choice (62).

In conclusion, 7.5 % icodextrin solution offers an alternative to conventional glucose-based solutions for the once-daily long-dwell exchanges in PD. It is effective, generally well tolerated and is especially useful in situations of reduced or inadequate UF in patients using glucose-based PD solutions, especially in high and high-average transporters, and during episodes of peritonitis.

# 3 AIMS OF THIS THESIS

This study was undertaken to analyze and characterize the metabolism of icodextrin in the peritoneal cavity and in the circulation of PD patients and non-uremic rats, and furthermore to analyze the breakdown of supplemented G7 into plasma samples in *ex vivo* experiments. The specific aims of the six separate studies were:

**I.** To apply HPLC with a gel-filtration media to determine HMW and LMW icodextrin molecules in dialysate and plasma from PD patients using icodextrin and to investigate the relationship between the icodextrin metabolites and plasma α-amylase activity and residual renal function.

**II.** To validate and use an assay for measurement of total α-amylase activity in serum containing icodextrin degradation products.

**III.** To study the metabolism of icodextrin and α-amylase activity following daily exposure to dialysis solutions containing either glucose or icodextrin as osmotic agent in non-uremic rats.

**IV.** To investigate *ex vivo* the rate of degradation of G7 in plasma from healthy controls, and PD patients using glucose-based or icodextrin-based dialysis solutions.

**V.** To investigate *ex vivo* the rate of degradation of G7 in plasma from pre-dialysis (PreD), hemodialysis (HD), and PD patients and to relate the G7 degradation rate to amylase activity and residual renal function**.**

**VI.** To investigate the relationship between the plasma concentration of icodextrin metabolites and the efficacy of icodextrin in changing ultrafiltration, fluid status and the relative preservation of residual urine volume.

# 4 MATERIAL AND METHODS

## 4.1 SUBJECTS AND EXPERIMENTAL DESIGN

**Study I**. PD patients were divided into three groups: patients using only GPDF (GLU group; n = 23), PD patients investigated before and after the first exchange of IPDF (1st ICO group; n = 24), and patients using IPDF chronically (IPDF group; n = 9). Venous blood samples were taken in the morning after an overnight fast and in addition, in the 1st ICO group, blood samples were taken a few hours before the first instillation of IPDF. Spent dialysate was collected from the patients in GLU (n=6) and 1st ICO (n = 13) groups. All samples were frozen and stored at -20°C to -70°C pending analyses. Total hydrolysis of plasma and dialysate to glucose, was performed with the enzyme amyloglucosidase according with the method described by Wang et al (99).

**Study II**. Plasma samples were collected from 27 PD patients using icodextrin for the long dwell; 19 PD patients using glucose for all dwells; and 12 healthy volunteers. *Experiment 1*: Samples were supplemented with commercial IPDF and incubated at 37°C. *Experiment 2:* Samples (basal and icodextrin spiked) were subjected to four different reagent concentration. *Experiment 3:* Plasma samples from PD patients were spiked with standard α-amylase. Amylase activity was measured after each one of the experiments.

**Study III**. Male Wistar rats (n = 23) with implanted peritoneal catheter were infused twice daily for 3 weeks with 20 ml icodextrin 7.5% based peritoneal dialysis fluid (ICO group, n = 12), or glucose 3.86% based peritoneal dialysis fluid (GLU group, n = 11). A 4 hour dwell study using 30 ml icodextrin was performed on day 10 (D1) and day 21 (D2) in both groups. Radiolabeled serum albumin (RISA) was used as a macromolecular volume marker. Dialysate samples were collected at 3, 15, 30, 60, 90, 120, and 240 minutes. Blood samples were drawn before the start and at the end of the dwell.

**Study IV**. Plasma samples from: healthy volunteers (Control group; n = 11), PD patients treated with glucose solution (GLU group; n = 11) and PD patients using icodextrin (ICO group; n = 19), were spiked with G7 and with G7 and in additional

studies also with synthetic porcine amylase pending incubation at 37°C for 4 hours. Samples were taken at 3 and 30 minutes and at 1, 2 and 4 hours, and analysed by HPLC to follow the degradation of supplemented G7. Total amylase and pancreatic amylase activity, as well as pancreatic amylase concentration were determined.

**Study V**. There were 15 healthy controls, 13 PreD, 13 HD, 19 GLU and 21 ICO patients. The methodology was essentially the same than in **Study IV**, with the difference that in this study we did not measure pancreatic amylase activity or concentration.

**Study VI**. Fifthy patients on CAPD or APD from centers in Germany, Sweden, and the United Kingdom were included in a prospective, multicenter, controlled, randomized, double-blind design study. After the baseline period, patients were randomized either to icodextrin 7.5 % or glucose 2.27 % for the long dwell, further study assessment visits were at 1, 3, and 6 months. Plasma samples were analysed for C-reactive protein (CRP), tumour necrosis factor (TNF-α), HMW- and LMW-hyaluronan (HA), atrial natiuretic peptide (ANP), amylase activity and icodextrin metabolites; extra-cellular fluid volume (ECFv), total body water volume (TBWv) and 24 hours urine volume collection were determined.

## 4.2 DEFINITIONS

We defined high molecular weight (HMW) molecules of icodextrin as those with a MW higher than that of the maltoheptaose (G7, Mw 1153 Da) and low molecular weight (LMW) icodextrin metabolites as G2 to G7. The latter term is also used in reference to the natural occurring circulating oligosaccharides in PD patients.

## 4.3 PREPARATION OF THE SAMPLES

For the analysis of HMW the dialysate samples were deproteinized with 4 % (final concentration) 5-sulphosalicylic acid and centrifuged for 20 minutes at 2650 g, pending injection of 250 μL into the HPLC system (Waters Corporation, Milford, MA, USA) **(Studies I and III)**.

For the quantification of LMW oligosaccharide (G2-G7) dialysate or plasma were deproteinized using Ultrafree®-MC Centrifugal filter units (Millipore Bedford, MA, USA), with a molecular weight cut off of 30,000 and were centrifuged at 5,000 g for 60 minutes, pending injection of 100 μL into the HPLC system **(Studies I to III)**. In **studies IV to VI**, plasma proteins were precipitated using 50 % acetonitrile (ACN; Merck, Darmstadt, Germany) and centrifuged for 20 minutes at 2650 g. The supernatants were then transferred to Ultrafree®-MC Centrifugal filter units, with a molecular weight cut off of 30,000, and centrifuged for 30 minutes at 5000 g, pending injection of 10 μL into the HPLC system

## 4.4 HPLC MEASUREMENTS

Gel-filtration HPLC was used for the separation of HMW icodextrin fractions and LMW icodextrin metabolites. The analytical column used for analysis of HMWs was a HiPrep® 16/60 Sephacryl S-200 High Resolution; for LMW metabolites we used a Superdex Peptide HR 10/30 analytical column (Amersham Pharmacia Biotech, Uppsala, Sweden), in **studies I to III**. As cheaper, fast, and more compact HPLC columns are now available, in 2004 we switched to a weak anion exchange column, BioBasic AX (150 x 4.6mm, 5μm. Thermo Electron Corporation, Woburn, MA, USA), for the analysis of LMW icodextrin metabolites to improve the accuracy and speed of the determinations, **studies IV to VI**

## 4.5 AMYLASE MEASUREMENTS

Plasma and dialysate α-amylase activity was determined with p-nitrophenol-maltoheptaoside as substrate by a fully automated routine method from Instrumentation Laboratory (IL Test ™) developed for a Monarch 1000 centrifugal analyzer (IL Inc. Lexington, MA; USA), **Study I-III**, and from 2004 with a Konelab 20XT routine biochemical analyzer (Thermo Clinical Labsystems Oy), **Studies IV and V.** The pancreas amylase activity assay (Diasys Diagnostic Systems GmbH, Holzheim, Germany) was specific for this enzyme. Pancreas amylase concentration was analysed with an ELISA assay (cat no K6410; from Immundiagnostik AG, Bensheim, Germany). Pancreatic amylase activity was also analyzed at the Department of Laboratory Medicine, Karolinska University Hospital, Huddinge, **Study IV**.

## 4.6 OTHER LABORATORY AND CLINICAL ANALYSIS

Plasma albumin (bromcresol purple), CRP, creatinine, urea, uric acid, calcium, and glucose concentrations were determined by using routine methods at the Department of Laboratory Medicine, Karolinska University Hospital, Huddinge **(Study V)**.

**Study VI**. TNF-α was determined by immunometric assay, (Immulite® Automatic Analyzer. Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) and both HMW and LMW-HA by high sensitivity, proteoglycan dependent time resolved immunoassay (119). ANP was extracted from plasma using Sep-pak-C18 columns and quantified with a $^{125}$I solid phase RIA (Laboserv GmbH, Giessen, Germany) (120). The extra-cellular fluid volume (ECFv) and total body water volume (TBWv) was determined using multiple frequency bioelectrical impedance analysis performed with the Hydra 4200 analyser (Xitron Technologies, San Diego, CA).

## 4.7 STATISTICAL ANALYSIS

The continuous variables were expressed as mean ± SD or unless otherwise noted. Statistical significance was set at the level of $P < 0.05$. Differences between the two groups were analyzed with the Wilcoxon-rank sums test and differences between more than two groups Kruskal-Wallis analysis of variance (ANOVA), followed by *Post hoc* Dunn´s test for non-parametric comparisons. A $\chi^2$ test was used for categorical variables. We used the non-parametric Spearman rank test (Rho) for association between continuous and ordinal variables.

In **Study I** we used Bland-Altman test (121) to confirm the agreement between the methods (HPLC and total hydrolysis) of measuring the icodextrin concentration in biological samples**.** In **Study III** we used General Linear Models (GLM) procedure to identify significant interactions between groups, dwells, and time respectively**.** In **Study V** we used Multivariate Mixed Model for adjustment for group of patients, sex, and GFR in the relation between amylase activity and G7 degradation rate. In **Study VI** we used a Mixed Model for repeated measurements to investigate the association between changing in fluid status, urine volume in glucose and icodextrin therapy.

All statistical analyses were performed with SAS statistical software (Version 9.1; SAS Institute, Inc., Cary, NC, USA).

# 5 RESULTS AND DISCUSSION

## 5.1 HIGH MOLECULAR WEIGHT FRACTIONS (I, III)

Despite more than 10 years of clinical experience of icodextrin and a considerable wealth of research, study of its pharmacokinetics has been hampered by the complexities of the analysis required (25). In **Study I and III,** we were able to demonstrate in a dynamic situation, how the relative concentration of the various fractions of icodextrin change after a long dwell in PD patients (**Study I**) and during a 4-hour dwell in non-uremic rats undergoing chronic PD (**Study III**). In **Study I**, we found that in the non-used fresh 7.5 % icodextrin solution, the molecules between 1.08 and 43.4 kDa represent about 92 % of the total icodextrin, whereas only about 4 % of the molecules were larger than 43.5 kDa and only 3 % were smaller than 1.08 kDa. The absorption of icodextrin from the peritoneal cavity was on average 39 % for a dwell time from 10 to 12 hour. There was a relative increase in the "medium large" HMW fractions 9.89 to 21.5 kDa (from 32 % to 40 %) and also an increase of 4.4-9.89 kDa fractions (from 22 % to 24 %) respectively. The results obtained by gel-filtration HPLC yielded values of total icodextrin in dialysate and plasma that were in agreement with results on the total amount of glucose obtained by the total enzymatic hydrolysis of the carbohydrates present in the dialysate and plasma samples.

Although it is to difficult compare studies in humans and animal experiments, especially in this case when the differences in amylase activity levels are huge (42, 49, 122), it is of interest to analyze the different pattern of icodextrin degradation in the dialysate during a 4-hour dwell in humans (data not previously reported) and in a 4-hour dwell in rats (**Study III**), **Figure 2**. At the end of the 4-hour dwell in humans, an average of 61 % of the infused icodextrin remained in the peritoneal cavity, whereas in rats only an average of 40 % of the infused icodextrin remained in the peritoneal cavity after the 4-hour dwell. In the case of the 4-hour dwell in humans we did not correct the results by the UF volume, which should have modified the percentage of initial icodextrin remaining at the end of the dwell.

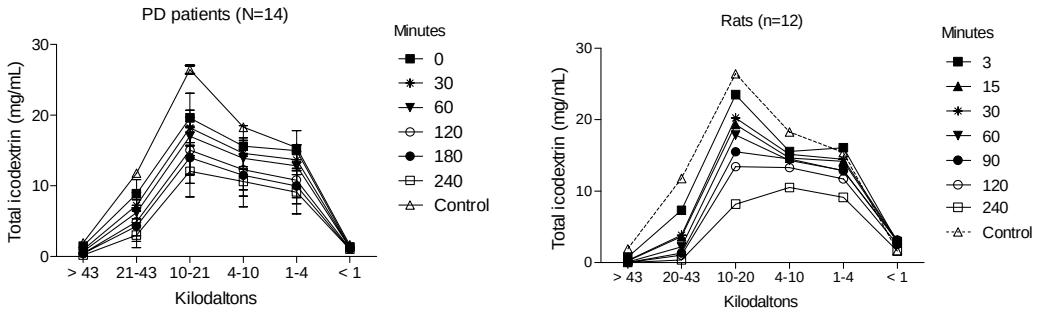


***Figure 2.*** *The HMW pattern of icodextrin fractions during a 4 hours dwell in 14 PD patients (left graph) and in 12 non-uremic rats (graph to the right).*

Since 1988 (48), there has not been any published detailed study (except ours) on the molecular distribution of the HMW fractions of icodextrin. We previously improved and validated a gel-filtration HPLC method to obtain a more accurate analysis of HMW fractions of icodextrin in the dialysate, in a shorter time; however, the results are still expressed as relative concentrations (38, 42). Furthermore, the complete analysis of a sample still took 4 hours. Therefore, we are now using a new gel filtration column (Shodex, Protein Series KW-802-5, Showa Denko K.K. Kanagawa, Japan) providing comparable results, and with this new column the time for complete analysis of a sample takes now only 15 minutes (data not shown)

## 5.2 LMW ICODEXTRIN METBOLITES (STUDIES I – VI)

Maltose (G2) was detected in the plasma of all uremic patients in a concentration ≤ 0.5 mg/mL (**Studies I, II, IV-VI**). In patients using icodextrin other circulating oligosaccharides were also present, not only G2, but also G3 and G4 and, although in a much lower concentration, larger molecules were also detected in these patients, this has been reported in many studies (25, 35, 37, 39-41, 98). In **Study III**, no icodextrin metabolites were found in plasma at the end of the dwell in non-uremic rats.

Previous to our studies there were three methods reported for the analysis of the icodextrin metabolites: (**A**) old gel-filtration HPLC method (46, 48), (**B**) high

performance anion-exchange HPLC (44), and (**C**) total hydrolysis (48, 99). With the first method the analysis of a sample took 6 hours. The second method is more sensitive, specific, and faster; however, it is not accessible in most laboratories. The third method, total hydrolysis (99) is very reliable, when it is accurately performed, but it only provides information about the total amount of carbohydrate which is broken down to glucose, and gives no details of the different icodextrin molecules. In **Studies I – III** we used gel-filtration HPLC; however, a run still took 90 minutes to complete. In **Studies IV – VI**, we changed to a weak anion exchange column (see section 4.4) and now we were able to obtain a better separation and to shorten the time of the analysis to 10 to 12 minutes, **Figure 3**. Note that the standards are available up to G7, but larger molecules could be visible if present.

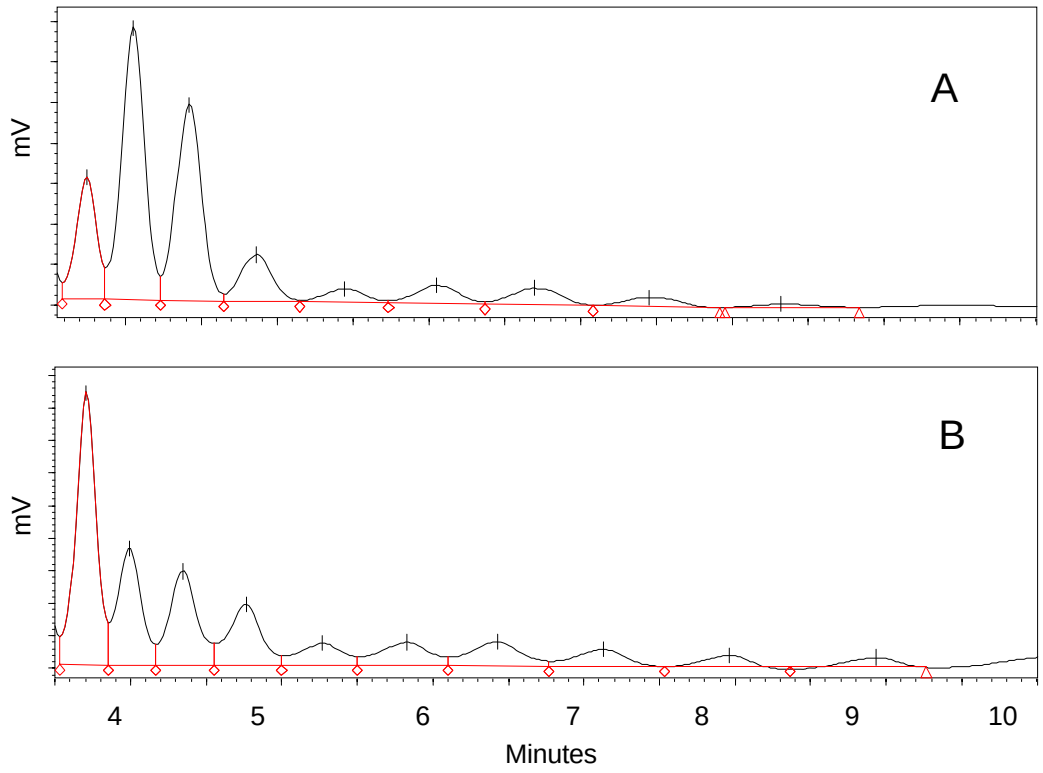


***Figure 3.*** *Chromatograms of a plasma sample (A) from a patient using icodextrin solution and a dialysate effluent sample (B). The first peak to the left correspond to glucose, followed by maltose (G2), G3, etc.*

For the study of the rate of degradation of oligosaccharides in uremic patients with different levels of amylase activity, maltoheptaose (G7) was chosen as it is one or the

largest icodextrin molecules that could be present in the circulation of patients using icodextrin-based dialysis solution. In **Studies IV and V**, together with the analysis of the degradation of G7, we also analyzed the formation from G7 of other molecules, i.e. G2, G3, G4, G5 and G6. The rate of G7 degradation is described in detail in **Studies IV** and **V**. The appearances of G2 – G4 are shown in **Figure 4**; whereas the changes in G5 and G6 were less marked (these results are not shown).

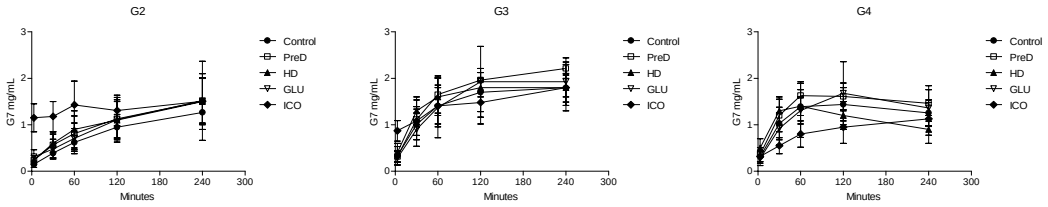


***Figure 4****. Following the spike of G7 in plasma samples, the changes of G2 –G7 were followed. The appearance of G2 and G3 seems to continue at 4 hours, while for G4 it reaches a plateau at 2 hours and then seems to start to decline.*

In **Study I**, GFR correlated negatively with total icodextrin metabolites (Rho = -0.24, p <0.05),

## 5.3 AMYLASE (STUDIES I – VI)

In our studies, total amylase activity was significantly lower in PD patients using icodextrin solution (**Studies I, II, IV- VI)** and as expected it was significantly higher in PD patients using glucose-based dialysis solution for all dwells (**Studies I, II, IV- VI**) as well as in pre-dialysis and hemodialysis patients (**Study V**). Moreover, pancreatic amylase *activity* and pancreatic amylase *concentration* were also low in patients using icodextrin (**Study IV**). Total amylase activity correlated with pancreatic amylase activity (Rho = 0.96; $p < 0.0001$) and with pancreatic amylase concentration (Rho = 0.93; $p < 0.0001$), **Study IV**.

We were able to demonstrate that there was no interference by the oligosaccharides present in plasma of PD patients using icodextrin on the routine method for analysis of

amylase by manipulating the samples in three different experiments, see section 4.1 (**Study II**).

Plasma amylase activity also decreased significantly after the 4 hour dwell with icodextrin in rats and increased during the dwell in the dialysate (**Study III**).

In an additional study (not reported elsewhere) we followed the changes of amylase activity in a PD patient having a first dwell with icodextrin for two hours. Plasma samples were taken before the infusion of icodextrin into the peritoneal cavity, and at 30, 60, 120 minutes after the infusion, and at the following day. Interestingly, the amylase activity started to decrease progressively already after 60 minutes, and this progressive decrease suggest that there is no interference. If such interference would be present, we should have expected to find a sudden and not a progressive decrease. Further studies are needed to confirm this observation.

Theoretically, pancreatic amylase can reach the bloodstream; (**A**) via the pancreatic acinar tissue, ductules or duct into the blood perfusing the pancreas; (**B**) from pancreas into the lymph; and (**C**) from the intestinal lumen into the blood perfusing the mucosa (86), whereas salivary amylase is supplied from the salivary glands and they may reach the blood via various pathways. The half life of amylase is reported to be relatively short [1 – 2 hours in the baboon (86, 123)]. The major route of entry for amylase into the circulation in healthy persons appears to be via a direct "leak" from the pancreatic acini or ducts into the blood (124). However, it is possible that in PD patients using icodextrin, more amylase will be needed first in the lymphatics due to the presence of icodextrin molecules (earlier than in blood) that require to be hydrolyzed. We believe that this could be the reason or one of the reasons for the low levels of amylase in plasma in these patients. Other possibilities could be: (**A**) the presence of an "inhibitor" in uremic plasma which alters the function of an active site of the amylase enzyme either directly or indirectly and inhibits the chemical reactions that are involved in the determination of amylase activity and concentration; or (**B**) the enhanced clearance of circulating amylase either by its elimination due to the forming of enzyme-polymer complexes or enhanced metabolism; however, we do not have any evidence for any of these mechanisms.

Despite the substantial fall in circulating amylase in patients using icodextrin, the metabolism of icodextrin did not seem to be markedly affected, and this paradoxical finding prompted us to study the rate of G7 degradation in more detail.

## 5.4 RATE OF G7 DEGRADATION (STUDIES IV, V)

The aim of **Study IV** was to see if the rate of degradation of G7 differed between the GLU and ICO patients compared with the controls. As expected, due to the low amylase levels, the degradation rate of G7 was indeed lower in the ICO group. Unexpectedly, the rate of degradation of G7 was lower also in the GLU group in spite of the higher amylase activity (**Study IV**).

These results motivated us to extend our investigation to other groups of patients, i.e. PreD and HD patients, to confirm or reject the hypothesis of an abnormal amylase dependent oligosaccharide metabolism (i.e. G7 degradation) in "all" patients with chronic kidney disease. Whereas the PreD and HD patients were found to hydrolyze the G7 at the same speed as plasma from healthy subjects with normal amylase activity, the rate was slower than could have been expected considering to the increased levels of amylase activity in these patients. The ratio *G7 degradation rate/amylase activity* (a measure of amylase efficiency) was decreased in all patients, except in the ICO patients where it tended to be increased (**Study V**).

When combining all groups the rate of G7 degradation correlated with the endogenous amylase activity (Rho = 0.74, $p < 0.0001$). Both amylase activity (Rho = 0.65, $p < 0.0001$) and G7 degradation rate (Rho = 0.62, $p < 0.0001$) correlated positively with GFR.

To validate the G7 experiments as well as to get another "view" of the amylase activity assay, we performed a series of experiments to measure the appearance of glucose (instead of the disappearance of G7 from the sample). We used the same amylase activity assay, but instead of measuring the appearance of PNP, we measured glucose, see **Figure 5.**

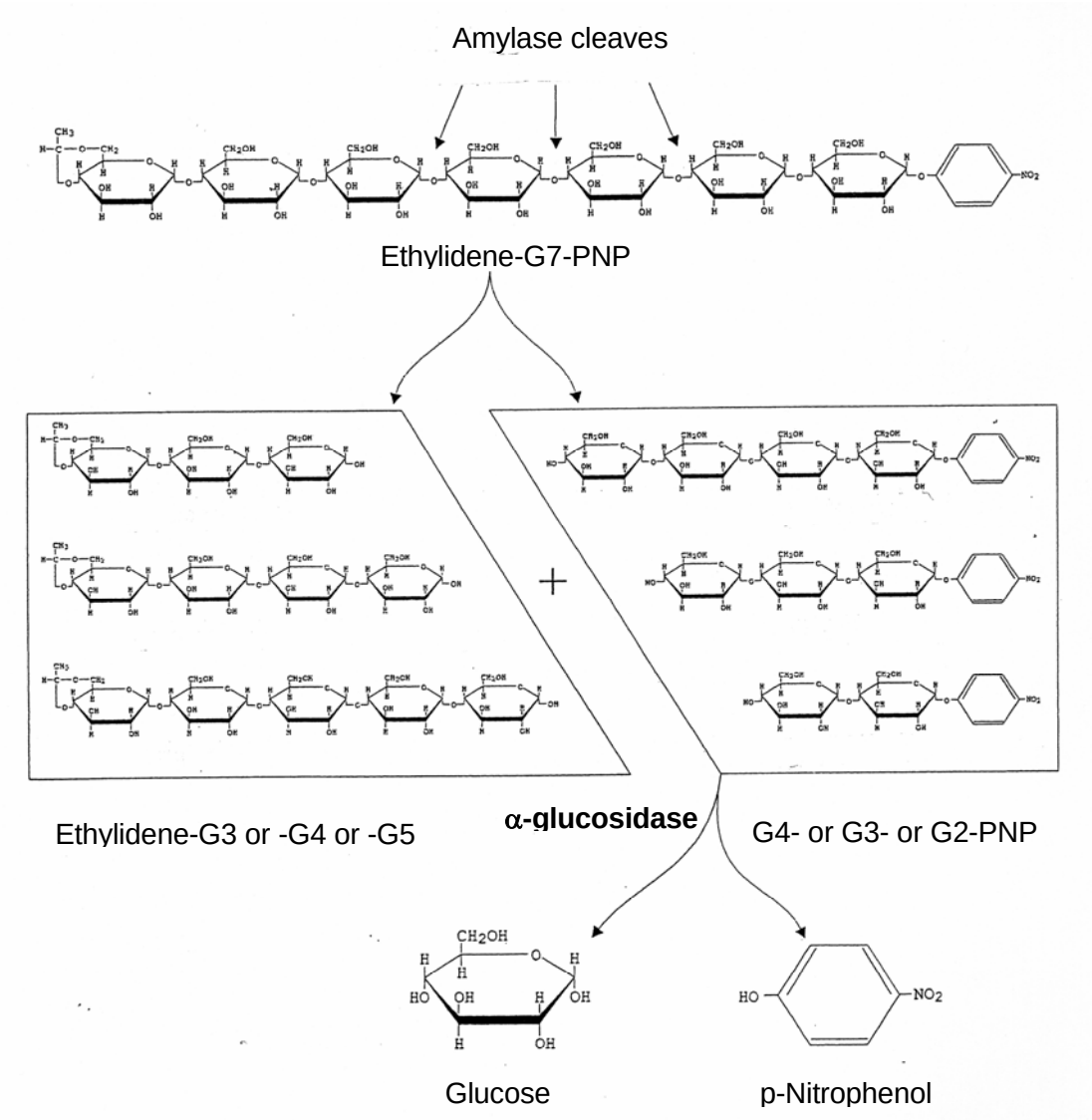


***Figure 5****. Principle of the analysis of amylase activity using EPS-G7-PNP. In normal analysis of α-amylase activity, the substrate of the reagent PNP-G7 is hydrolyzed by the α-amylase in the sample; α-amylase splits the inside glucosidic bonds of the substrate. The last glucose unit is blocked by the ethylidene group, which prevents the helper enzyme included in the reagent, α-glucosidase, from breaking down the substrate in absence of α-amylase. The complete breakdown of the PNP-G7 to glucose and PNP is accomplished by α-glucosidase. The activity of α-amylase is determined by the rate of increase in absorbance at 405 nm as PNP is produced.*

Plasma samples from healthy controls, PreD patients, HD patients, GLU patients, and ICO patients were spiked with either G7 or ethylidene G7 p-nitrophenol (EPS-G7-PNP, Roche Diagnostics, GmbH, Manheim, Germany) and in both cases α-glucosidase

(Sigma-Aldrich. St Louis, MO, USA), and samples were then incubated at 37°C for 60 minutes. Glucose was measured at 0, 2, 15, 30, and 60 min.

EPS-G7-PNP is the substrate in the commercial routine amylase assay we used. Ethylidene is a blocking substance that prevents α-glucosidase from breaking down the substrate in absence of α-amylase. α-Amylase cleaves the substrate producing smaller fragments that can be hydrolyzed by α-glucosidase.

The total amount of glucose measured was the expected; however, different glucose appearance curves were obtained when using pure G7 and EPS-G7 (**Figure 6**). When G7/α-glucosidase was spiked into the plasma there was a rapid appearance of glucose. This is because there is no blocking of the G7 molecule, in which case α-glucosidase starts to act immediately. In the case of using the EPS-G7/α-glucosidase reaction, the appearance of glucose was slower due to the effect of the EPS in all groups but not in the ICO group, because in the first steep part of the curve, the α-glucosidase in the assay first breaks down the icodextrin metabolites present already in the plasma of these patients. Therefore, a second more flat part of the curve is seen, reflecting a slow formation rate of glucose. This was another experiment showing a consequence of the low α-amylase activity in patients using icodextrin.

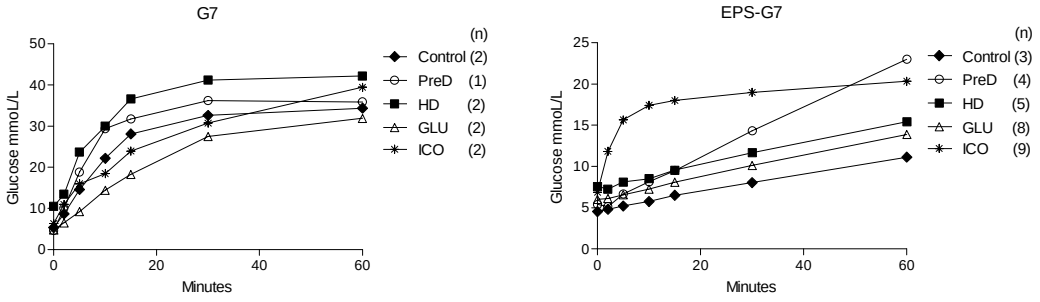


***Figure 6.*** *Appearance of glucose after the spiking with G7/α-glucosidase (left figure) and EPS-G7/α-glucosidase (right figure).*

## 5.5 FLUID STATUS, INFLAMMATION, AND ICODEXTRIN METABOLITES (STUDY VI)

Icodextrin has been shown to alter fluid status by reducing the ECFv (58, 62, 63). In our study, a significant correlation between the fall in ECFv and RRF was observed in both randomized groups (P=0.001). Icodextrin patients had non-significantly higher ANP levels at baseline, whereas by 3 (P=0.026) and 6 months (P=0.016) this differed between groups due to divergence. There was a correlation between increasing ANP and reduced ECF at 3 months, R=-0.46, P=0.007, in patients randomized to icodextrin but not to glucose. There were no relationships between fluid status and any inflammatory markers at any point of the study, with the exception of albumin at baseline, R=-0.39, P=0.007. Amylase activity at –1 month and baseline were highly correlated; r=0.89, P<0.0001. Within patient concentrations of icodextrin metabolites were highly correlated; on multivariate analysis, the only predictor of between patient variability on multivariate analysis was body weight. There was no relationship between plasma concentrations of icodextrin metabolites and any of the other clinical parameters, including change in daily ultrafiltration, urine volume, fluid or inflammatory status.

# 6 SUMMARY AND CONCLUSIONS

Despite more than 10 years of clinical experience of icodextrin and a considerable wealth of research on various aspects of icodextrin (23, 37, 40, 41, 43, 56, 59, 62, 63, 98, 101, 125, 126), the studies of its pharmacokinetics have been hampered by the complexities of the analysis required (25). With these studies we have presented an improved methodology to analyze HMW and LMW icodextrin fractions in biological samples that can be used to study the complex pattern of changes of total icodextrin in dialysate and icodextrin metabolites in plasma and dialysate. In addition, as a spin-off of these studies, we propose a novel method (using G7) to analyze *ex vivo* the rate of oligosaccharides breakdown in uremic patients, a method which also could be used in other patient groups.

**Study I**. Accurate analysis of HMW icodextrin fractions and LMW icodextrin metabolites in plasma and dialysate in PD patients can be achieved by gel-filtration HPLC with two different columns. This method can be used to study the complex pattern of changes of icodextrin and its metabolites in plasma and dialysate. This analytical approach is useful for further studies to better understand the kinetics of icodextrin and its metabolites in relation to clinical parameters such as peritoneal transport rate and ultrafiltration.

**Study II**. Low levels of α-amylase are seen in patients using IPDF; however, the clinical significance of a low α-amylase activity in patients using IPDF is unknown.

**Study III**. There are both similarities and differences in icodextrin metabolism in rats compared with PD patients. The α-amylase activity is the main determinant of the rate of icodextrin metabolism, and as α-amylase activity in rats is much higher than in humans, this results in a more rapid metabolism of icodextrin in rats compared with humans. Based on these findings, we conclude that animal models using rats are not ideal for the study of icodextrin metabolism.

**Study IV**. An *ex vivo* model to study the relationship between amylase activity and the actual rate of carbohydrate (represented by G7) breakdown was developed and showed that PD patients using glucose and icodextrin degrade G7 at a slower speed than

controls. This suggests that the amylase mediated oligosaccharide metabolism is reduced in PD patients. The clinical significance, if any, is unclear: however, it is possible that this could contribute to reduced hyperglycemic changes, especially in patients using icodextrin.

**Study V**. The rate of degradation of the saccharide molecule maltoheptaose (G7) is strongly related to the plasma amylase activity and to the residual glomerular filtration rate. The mode of therapy seems to influence this rate as it was reduced *only* in the patients undergoing PD. These results suggest that the carbohydrate load in PD patients may reduce the rate of metabolism of synthetic carbohydrates (glucose and icodextrin), a finding of potential significance for the metabolism of carbohydrates especially in uremic patients with carbohydrate intolerance as well as in diabetic patients. On the other hand, the apparent efficiency of amylase expressed as the ratio between G7 degradation rate and plasma amylase activity, was reduced in PreD patients, patients undergoing HD and in the patients undergoing PD with glucose based solution. In contrast, this ratio tended to be increased in PD patients using icodextrin solution although this finding is somewhat uncertain due to the high variability of both G7 degradation rate and amylase activity levels in these patients. The clinical significance, if any, of these finding is unclear but we speculate that these alterations might modify the effect of insulin resistance and carbohydrate intolerance in patients with chronic kidney disease.

**Study VI.** This study supports previous observational data that changes in fluid status are associated with changes in urine volume. Icodextrin was, however, not associated with a greater fall in urine output despite its larger effect on ECFv. Changes in fluid status cannot be explained by or do not influence systemic inflammation. Neither can they be explained by individual variability in plasma concentrations of icodextrin that are in turn inversely proportional to the volume of distribution.

## 6.1 FUTURE STUDIES AND COLLABORATIONS

Based on the findings of this thesis, future studies will focus us on:

- Analyses to confirm or reject our hypothesis that the amylase mediated hydrolysis of oligosaccharides is altered in uremic patients.

- Analyses of the levels of icodextrin and amylase activity in lymph in experimental studies, to further understand the kinetics of icodextrin and α-amylase.
- Clinical investigations of icodextrin metabolism in different cohorts of clinically stable patients as well as in patients with different complications such as loss of UF capacity, peritonitis, diabetes and congestive heart failure.
- Theoretical and experimental investigations of the kinetics of amylase mediated oligosaccharide metabolism.
- Analyses of the half life of amylase during different conditions *ex vivo*.
- Extending our collaborations with other centers. So far we have started collaboration with centers in the United Kingdom, Spain, Italy, Turkey, Netherlands, France, Poland, Mexico, and United States of America.

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