Ana Paula dos Santos Bernardo

ALTERAÇÕES METABÓLICAS EM DIÁLISE PERITONEAL: EXPRESSÃO SISTÉMICA E FUNCIONAL DA MEMBRANA PERITONEAL

METABOLIC DERANGEMENTS IN PERITONEAL DIALYSIS: SYSTEMIC IMPACT AND FUNCTIONAL EXPRESSION OF PERITONEAL MEMBRANE

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Orientadora

Professora Doutora Anabela Soares Rodrigues

Professora Auxiliar Convidada

Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto

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Metabolic derangements in peritoneal dialysis:
Systemic impact and functional expression of peritoneal membrane

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I. Bernardo A, Fonseca I, Rodrigues A, Carvalho MJ, Cabrita A. Predictors of residual renal function loss in peritoneal dialysis: is previous renal transplantation a risk factor? Adv Perit Dial. 2009;25:110-4.

II. Bernardo AP, Contesse SA, Bajo MA, Rodrigues A, Del Peso G, Ossorio M, Cabrita A, Selgas R. Peritoneal membrane phosphate transport status: a cornerstone in phosphate handling in peritoneal dialysis. Clin J Am Soc Nephrol. 2011 Mar;6(3):591-7. doi: 10.2215/CJN.06960810. Epub 2010 Nov 29.

III. Bernardo AP, Fonseca I, Rodrigues A, Carvalho MJ, Cabrita A. Overweight rather than malnutrition is widely prevalent in peritoneal dialysis patients. Adv Perit Dial. 2009;25:119-24.

IV. Bernardo AP, Fonseca I, Oliveira JC, Santos O, Carvalho MJ, Cabrita A, Rodrigues A. Adipokines in peritoneal dialysis: relevant clinical impact according to body composition. Ther Apher Dial. 2015 Apr;19(2):144-53. doi: 10.1111/1744-9987.12239. Epub 2014 Nov 3.

V. Bernardo AP, Oliveira JC, Santos O, Carvalho MJ, Cabrita A, Rodrigues A. Insulin Resistance in nondiabetic peritoneal dialysis patients: associations with body composition, peritoneal transport and peritoneal glucose absorption. Clin J Am Soc Nephrol. 2015 Oct 27. pii: CJN.03170315. [Epub ahead of print].

VI. Bernardo AP, Bajo MA, Santos O, del Peso G, Carvalho MJ, Cabrita A, Selgas R, Rodrigues A. Two-in-one protocol: simultaneous small-pore and ultrasmall-pore peritoneal transport quantification. Perit Dial Int. 2012 Sep-Oct;32(5):537-44. Epub 2012 Mar 1.

VII. Bernardo AP, Oliveira JC, Santos O, Carvalho MJ, Cabrita A, Rodrigues A. Hepatocyte growth factor signalizes peritoneal membrane failure in peritoneal dialysis. BMC Nephrol. 2014 Dec 17;15:201. doi: 10.1186/1471-2369-15-201.

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CONTENTS

ABBREVIATIONS

APD – automated peritoneal dialysis

BCM – body cell mass

BCM® - body composition monitor

BMI – body mass index

CA125 – cancer antigen-125

CAPD – continuous ambulatory peritoneal dialysis

CHP – Centro Hospitalar do Porto

CKD – chronic kidney disease

ECW – extracellular water

EMT – epithelial-to-mesenchymal transition

EPS - encapsulating peritoneal sclerosis

FWT – free water transport

HD - hemodialysis

HGF - hepatocyte growth factor

HOMA- homeostasis model assessment

ICBAS – Instituto de Ciências Biomédicas Abel Salazar

IGF-I – insulin-like growth factor I

IGFBP-1 – insulin-like growth factor binding protein 1

IL-6 – interleukin 6

LAR – leptin/adiponectin ratio

LTI – lean tissue index

nPCR – protein catabolic rate

NSS – nutrition status score

PD – peritoneal dialysis

PET – peritoneal equilibration test

Rel.FM – relative fat mass

RRF – residual renal function

SGA – subjective global assessment

UF - ultrafiltration

UFF – ultrafiltration failure

VEGF – vascular endothelial growth factor

I – Abstract

I - Abstract

- Abstract
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I. ABSTRACT

Peritoneal dialysis is a successful end stage renal disease therapy, although a dismal long-term outcome has been reported in some studies, suggesting loss of PD benefits in the long-term, with a slightly higher risk for overall mortality and cardiovascular death, compared with hemodialysis. The reasons associated with this are not fully understood, but several authors hypothesized an association with metabolic derangements, due to the potential systemic effects associated with the use of glucose (the main osmotic agent used in PD prescriptions), maximally expressed by patients who present a peritoneal membrane fast transport status. In order to improve knowledge in this field, we developed a series of cross sectional and prospective clinical researches, to investigate the systemic impact of intrinsic aspects of the peritoneal dialysis technique, such as peritoneal membrane transport status, peritoneal glucose absorption and peritoneal dialysis modality, on relevant clinical issues: residual renal function, phosphate removal, nutritional status, insulin resistance and cardiovascular events, in a population of incident and prevalent patients under peritoneal dialysis. The deleterious effects of intra-peritoneal glucose into the membrane level are well established, leading to peritoneal membrane structural alterations that will conduct to ultrafiltration failure. For that reason, this thesis focused also on the functional evaluation of the peritoneal membrane, establishing a new peritoneal equilibration test to evaluate simultaneously small solute transport and water transport pathways, and explores new peritoneal effluent biomarkers that could timely signalize peritoneal membrane dysfunction, even before clinically relevant ultrafiltration failure develops.

We started to investigate the factors conditioning residual renal function (RRF) decline in 148 consecutive incident PD patients, treated in our Unit between January 2000 and June 2007 (**chapter V.I**). Our clinical investigation has evidenced that patients on PD after renal transplantation experience similar short term RRF protection, without a shorter peritonitis-free survival, compared with patients starting PD as their first renal replacement therapy. We also concluded that diabetes was the only independent predictor of anuria, after PD start.

In a second clinical investigation about peritoneal membrane phosphate transport (**chapter V.II**), we concluded that in hyperphosphatemic and or anuric patients, the decision on the optimal PD modality should also take into account peritoneal phosphate transport characteristics, beyond the traditional adequacy parameters (urea Kt/V and creatinine peritoneal clearance). Increasing dwell times or transfer to CAPD, could be effective strategies to improve phosphate handling in patients with inadequate phosphate control on APD, especially if they are slow phosphate transporters.

We also performed a cross sectional study in order to evaluate the nutritional status of 57 PD patients in treatment in our Unit (**chapter V.III**), according to the most recent diagnostic criteria for nutritional assessment in chronic kidney disease patients. We have documented a high overweight and obesity prevalence, and a low incidence of protein-wasting. In a subsequent study, we have explored the associations between body composition, using a bioimpedance device (Body Composition Monitor), peritoneal membrane transport status, peritoneal glucose absorption and adipokines profile with cardiovascular events, in a group of 66 PD patients, prospectively followed for 47 months (**chapter V.IV.I**). Our results clearly demonstrate that body mass index (BMI) underestimates the real prevalence of obesity, compared with body composition assessment using bioimpedance analysis, in PD patients. Nevertheless, we could not found any association between peritoneal glucose absorption and small solute transport status with obesity. However, according to our clinical investigation, cardiovascular events were associated with obesity. This study enabled us, additionally, to clarify that adipokines profile depends on patient´s body composition, as leptin/adiponectin ratio was predicted by relative fat mass and lean tissue index, independently of peritoneal glucose absorption. We have also concluded that a higher leptin/adiponectin ratio (LAR) was associated with new cardiovascular events, and can represent a new atherogenic index in patients under PD, without protein wasting.

We also conducted a cross sectional study in order to assess insulin resistance prevalence and their determinants in a population non-diabetic patients, currently on treatment in our Unit (**chapter V.IV.II**). Our results show that obesity and adipokines profile play a major role in insulin resistance development in PD, independently of glucose absorption and small solute transport status. Given the already established association between insulin resistance, adipokines, and cardiovascular events (as our previous investigation demonstrates – **chapter V.IV.I**), efforts should be put in obesity prevention, correct diagnosis and management. Contemporary PD treatments minimize glucose exposition, and the lack of correlations between peritoneal glucose absorption, insulin resistance indices and body composition parameters, that we documented, puts on evidence that other presumably more powerful factors may contribute to the metabolic syndrome in PD patients. Finally, with this investigation, we also concluded that fast transporters, under updated PD prescriptions, can be adequately managed without higher risk of developing obesity or an insulin resistant state.

Finally, since the peritoneum is a determining interface between the dialysis system and the systemic milieu, we focused on functional membrane evaluation, with a full characterization of both small-solute transport and water transport pathways across the membrane (**chapter V.V.I**). We have further explored new effluent biomarkers that could timely

signalize peritoneal membrane deterioration (**chapter V.V.II**). We have concluded that quantification of free water transport (FWT) is important for detecting causes of ultrafiltration failure (UFF), beyond an increase in effective capillary surface, and that such quantification is feasible during a 4-hour, 3.86% glucose PET evaluation, with a temporary drainage of the peritoneal cavity at 60 minutes (**chapter V.V.I**). In a subsequent study (**chapter V.V.II**), 68 PD patients were evaluated using the Two-In-One protocol, and hepatocyte growth factor (HGF) in the effluent was measured, in order to explore its association with water transport pathways and ultrafiltration failure profile. Since HGF is known to ameliorate peritoneal fibrosis in an ex-vivo study, a clinical investigation like ours looks opportune. Our results demonstrate that dialysate HGF concentration is significantly higher among patients with UFF, especially if FWT is impaired, suggesting that it might be a useful marker of progressive peritoneal membrane dysfunction.

KEY WORDS: adipokines, body composition, cardiovascular disease, fast transporters, free water transport, hepatocyte growth factor, hyperphosphatemia, insulin resistance, nutrition status, obesity, overweight, peritoneal dialysis, peritoneal equilibration test, peritoneal glucose absorption, peritoneal phosphate clearance, protein wasting, renal graft failure, residual renal function, ultrafiltration failure.

I. RESUMO

A diálise peritoneal constitui uma eficaz terapêutica substitutiva da função renal, embora alguns estudos sugiram uma perda de vantagens da DP a longo prazo, com um maior risco de morte de causa cardiovascular associado a esta técnica, quando comparada com a hemodiálise. As razões associadas a estes resultados menos favoráveis não foram, até ao momento, completamente clarificadas. Contudo, vários autores colocam a hipótese de que o uso de glicose intraperitoneal (usada como agente osmótico na maioria das prescrições em diálise peritoneal) possa associar-se a resultados deletérios, a longo prazo, através dos seus potenciais efeitos sistémicos. Nesta linha conceptual, os doentes com perfil de transporte de membrana peritoneal rápido seriam os mais afetados. No sentido de amplificar o atual conhecimento científico nesta área, desenvolvemos uma série de estudos transversais e prospetivos envolvendo doentes incidentes e prevalentes na técnica, que nos permitiram investigar o impacto sistémico de aspetos intrínsecos à diálise peritoneal, tais como o tipo de transporte da membrana peritoneal, a absorção da glicose intraperitoneal e o tipo de modalidade de diálise peritoneal, em questões clínicas relevantes, nomeadamente: função renal residual, remoção peritoneal de fósforo, estado nutricional, insulinorresistência e eventos cardiovasculares. Os efeitos deletérios da glicose intraperitoneal ao nível da membrana peritoneal estão já bem documentados, originando com o tempo determinadas alterações estruturais na membrana conducentes à falência de ultrafiltração. Face ao exposto, a presente tese pretendeu estudar também a membrana peritoneal do ponto de vista funcional, estabelecendo um novo teste de equilíbrio peritoneal que permitisse avaliar, em simultâneo, o transporte de pequenos solutos e de água, explorando novos biomarcadores que permitissem sinalizar precocemente a disfunção da membrana peritoneal, antes mesmo do desenvolvimento de uma situação tardia de falência de ultrafiltração.

Começámos por investigar os fatores que condicionam a perda de função renal residual num grupo de 148 doentes consecutivos e incidentes, tratados na nossa Unidade entre janeiro de 2000 e junho de 2007 (**capítulo V.I**). A investigação clínica efetuada evidenciou que os doentes em diálise peritoneal após falência do enxerto renal apresentam, quando comparados com doentes que iniciaram diálise peritoneal como primeira técnica substitutiva da função renal, semelhante proteção da função renal residual, com tempo de sobrevida livre de peritonite similar. Concluímos ainda que a diabetes foi o único fator preditor independente de anúria após o início de diálise peritoneal.

Numa segunda investigação clínica acerca do transporte peritoneal de fósforo (**capítulo V.II**), concluímos que, nos doentes com hiperfosfatemia e ou anúria, a decisão quanto à modalidade terapêutica mais eficaz deve ter em consideração as características da

membrana quanto ao transporte de fósforo, para além dos tradicionais parâmetros de adequação em diálise (Kt/V ureia e clearance peritoneal de creatinina). Aumentar os tempos de permanência das trocas ou transferir o doente para uma modalidade de diálise peritoneal contínua de ambulatório, poderão ser estratégias eficazes no sentido de melhorar a remoção dialítica de fósforo em doentes no regime de diálise peritoneal automática que exibam um controlo do fósforo inadequado, especialmente se os mesmos apresentarem um estado de lento transporte da membrana peritoneal, no que ao fósforo diz respeito.

Efetuámos também um estudo transversal no sentido de avaliar o estado nutricional de 57 doentes em tratamento na nossa Unidade (**capítulo V.III**), de acordo com os mais recentes critérios de avaliação do estado nutricional na doença renal crónica. Documentámos uma elevada prevalência de excesso de peso e de obesidade e uma baixa incidência de desnutrição proteica nos doentes avaliados. Num estudo subsequente, explorámos as associações entre vários parâmetros de composição corporal avaliados por bioimpedância (Body Composition Monitor), estado de transporte da membrana peritoneal e perfil de adipocinas com os eventos cardiovasculares, numa população de 66 doentes em diálise peritoneal que foram prospetivamente estudados ao longo de um tempo médio de seguimento de 47 meses (**capítulo V.IV.I**). Os resultados obtidos demonstram que o índice de massa corporal subestima a real prevalência de obesidade nos doentes em diálise peritoneal, quando comparado com a avaliação da composição corporal efetuada por bioimpedância. Contudo, não encontrámos qualquer associação entre absorção de glicose intraperitoneal ou transporte peritoneal com obesidade. Todavia, de acordo com esta investigação clínica, existe uma associação entre obesidade no doente em diálise peritoneal e novos eventos cardiovasculares. Este estudo permitiu-nos ainda, adicionalmente, clarificar que o perfil de adipocinas, nos doentes em diálise peritoneal, é dependente da composição corporal, uma vez que a percentagem relativa de massa gorda e o índice de massa magra foram preditores do rácio leptina/adiponectina, independentemente da absorção intraperitoneal de glicose. Concluímos, por fim, que um rácio elevado de leptina/adiponectina se associa a novos eventos cardiovasculares, podendo representar um novo índice aterogénico na população de doentes em diálise peritoneal sem desnutrição proteica.

No sentido de avaliar a prevalência de insulinorresistência e de estabelecer os seus determinantes, efetuámos um estudo transversal que incluiu doentes não diabéticos em tratamento na nossa Unidade (**capítulo V.IV.II**). Os resultados obtidos evidenciam que a obesidade e o perfil de adipocinas desempenham um papel determinante no desenvolvimento de um estado de insulinorresistência nos doentes em diálise peritoneal, independentemente da absorção intraperitoneal de glicose e do estado de transporte da membrana peritoneal.

Dada a associação que previamente documentámos entre insulinorresistência, adipocinas, e novos eventos cardiovasculares (**capítulo V.IV.I**), esforços devem ser feitos no sentido de implementar medidas de prevenção da obesidade, mas também de otimizar o seu correto diagnóstico e sua gestão terapêutica. Os atuais regimes de prescrição terapêutica na diálise peritoneal minimizam a exposição a glicose, e, adicionalmente, a ausência de correlações encontradas entre a absorção de glicose intraperitoneal, os índices de insulinorresistência e os parâmetros de composição corporal, colocam em evidência que outros fatores, porventura mais determinantes, possam contribuir para o desenvolvimento de um síndrome metabólico nos doentes em diálise peritoneal. Por fim, a investigação clínica efetuada permitiu concluir que os doentes com perfil de rápido transporte de membrana peritoneal podem ser adequadamente tratados em diálise peritoneal, através de regimes de prescrição atuais e individualizados, sem que apresentem maior risco de desenvolver obesidade ou insulinorresistência.

Por último, sendo o peritoneu uma interface determinante entre o sistema de dialise e o meio interno, efetuámos uma avaliação funcional da membrana peritoneal, caracterizando o transporte de pequenos solutos e da água (**capítulo V.V.I**). Explorámos novos biomarcadores no efluente peritoneal suscetíveis de sinalizar precocemente uma situação de disfunção da membrana peritoneal (**capítulo V.V.II**). Concluímos que a quantificação do transporte de água livre é importante para a deteção de outras causas de falência de ultrafiltração, para além de um aumento da superfície capilar efetiva, e que essa quantificação pode ser feita durante um teste de equilíbrio peritoneal de 4 horas, com 3.86% de glicose, e que envolve uma etapa adicional de drenagem temporária da cavidade peritoneal aos 60 minutos (**capítulo V.V.I**). Num estudo posterior (**capítulo V.V.II**), 68 doentes em diálise peritoneal foram avaliados através do protocolo “Two-in-One”, tendo sido adicionalmente medido no efluente o “hepatocyte growth factor” (HGF), no sentido de explorar a sua associação com a disfunção da membrana peritoneal. Tendo sido já previamente documentado, num estudo ex-vivo, que o HGF pode condicionar uma melhoria do grau de fibrose peritoneal, uma investigação clínica como esta é oportuna. Os resultados que obtivemos evidenciam que a concentração de HGF no efluente peritoneal é significativamente maior nos doentes com falência de ultrafiltração de membrana, especialmente se o transporte de água livre estiver comprometido, o que sugere que possa ser um biomarcador útil de disfunção da membrana peritoneal.

PALAVRAS-CHAVE: absorção de glicose intraperitoneal, adipocinas, clearance peritoneal de fósforo, composição corporal, desnutrição proteica, doença cardiovascular, estado nutricional, excesso ponderal, falência de enxerto renal, falência de ultrafiltração, função renal residual, hepatocyte growth factor, hiperfosfatemia, insulinorresistência, obesidade, rápido transportador, transporte de água livre, teste de equilíbrio peritoneal.

II – Introduction

1. Peritoneal Dialysis – a successful end stage renal disease therapy

2. Clinical outcomes in PD: the role of adequacy, small-solute transport and ultrafiltration on patient and technique survival

3. Controversies on the systemic impact of peritoneal glucose absorption and membrane fast transport status

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II. INTRODUCTION

1. Peritoneal Dialysis – a successful end stage renal disease therapy

Peritoneal dialysis (PD) has been used as a successful end stage renal disease (ESRD) therapy over the last years, with significant differences in technique prevalence distribution all around the world (1). Current evidence shows that survival outcomes are now globally, at least, equivalent for both dialysis techniques (2-5), as PD results have improved during the past decade (3, 4). Cardiovascular disease is still the main cause of mortality in both hemodialysis and peritoneal dialysis patients (6). However, it seems that results are slightly different in terms of early versus long term survival between the two modalities. In fact, several studies have showed, in a consistent way, that peritoneal dialysis patients have a lower risk of death in the first years of ESRD (3, 7, 8), with the magnitude and length of time which this lower risk for death is evident depending on age, diabetes and the presence of associated comorbidities. The early survival benefit observed in peritoneal dialysis has been partially attributed to a better preservation of residual renal function by this technique compared to hemodialysis (9), that would offset the potentially deleterious systemic consequences of daily glucose loading. In fact, patients with residual renal function have lower degrees of inflammation and malnutrition, less hypertension with less cardiac hypertrophy, less anemia with less erythropoietin resistance, and a better phosphate control (10), all of which may explain the better overall and cardiovascular survival observed in the first years of peritoneal dialysis. As for long term survival comparisons between the two techniques, a recent study by Kumar and colleagues (8) as showed that, in an as-treated analysis, the 3-9 years survival was similar between the two modalities. However, others (11) have demonstrated that PD was associated with an increased risk for overall mortality and cardiovascular death, after two years of treatment. The reason for this dismal outcome after the first years of dialysis is uncertain. Although speculative, it is feared that with time, PD patients could be prone to suffer from protein wasting for one hand, and for the other, that peritoneal exposure to dextrose could lead to hyperglycemia, insulin resistance, dyslipidemia and metabolic syndrome (8, 11). In line with these hypotheses, the increased risk for cardiovascular death seen, with time on PD, could be a consequence of malnutrition-inflammation-atherosclerosis syndrome, or a consequence of metabolic syndrome and the systemic impact of peritoneal glucose exposure. In order to better understand the relation of cardiovascular risk with nutrition status in PD, contemporary studies about the impact of protein wasting and obesity in PD patient´s outcomes, with full body composition characterization, are needed.

2. Clinical outcomes in PD: the role of adequacy, small-solute transport and ultrafiltration on patient and technique survival

An association between greater clearance of small molecular weight solutes and better clinical outcomes has been reported in a prospective cohort study (12). As residual renal function was not examined separately as a risk factor for clinical outcomes, renal and peritoneal clearances were assumed as comparable, and therefore additive. However, reanalysis of the CANUSA (13) data indicates that the contribution of residual renal function is even more important than peritoneal creatinine clearance in terms of patient survival, confirming previous observations from Maiorca et al. (14) and Diaz-Buxo et al. (15). This concept was additionally reinforced by the ADEMEX study (16), which showed that increasing further small solute clearances conferred no survival benefit in PD patients as a whole, or in the subgroup of anurics, and confirming that only residual renal function was predictive of outcome in these patients. It seems that the advantage conferred by preserved residual renal function (RRF) may be related to the volume of urine excreted and to the maintenance of an euvolemic state, as for each additional 250 mL of urine excreted per day, the relative risk of death was decreased by 36% (13). Better preservation of renal endocrine function and better clearance of middle and larger molecular weight uremic toxins are also plausible reasons for better outcomes in patients maintaining RRF, as clearances of middle molecules and protein-bound solutes cannot be equalled by peritoneal dialysis (17). Residual renal function plays a central role in dialysis patients, whichever the modality, being linked with patient survival, but it has been focused more in PD. As a result, determining the factors that affect its loss has become an important issue in the care of patients under PD. Several factors have been associated with loss of RRF: larger body mass index, congestive heart failure, diabetes, hypotensive episodes, use of diuretics and peritonitis episodes (18-20). However, results are not consistent, and the investigations performed often exclude patients with a failed renal graft. There is also much debate about peritoneal membrane transport status and PD modality impacts on residual renal function (20), a clinically relevant aspect that deserves further clarification.

In terms of adequacy, studies have simply focused on urea and creatinine clearances (12, 16) and the same is true about the most recent guidelines (21). However, theoretically, targets for others solutes removal by dialysis, as phosphate, should also be included, as we know that hyperphosphatemia is common in peritoneal dialysis patients, especially when residual renal function is lost (22), and it is a well-known factor for cardiovascular morbidity and mortality in these patients (23-25). Research into the relationship between phosphate clearance, membrane transport status and PD modality is needed, because optimization of

peritoneal phosphate clearance may be a reasonable goal for adequacy in the absence of compelling evidence for a link between urea clearance and outcome on PD (16).

One of the first studies that explored the association between peritoneal membrane transport and technique and patient survival evidenced that fast transporters had a similar 2 year survival probability compared with non-fast transporters (26). However, the 2 year probability of combined patient and technique survival was significantly lower in fast transporters. The authors hypothesized that fast transporters could be prone to develop malnutrition, fluid overload and insulin resistance with a more atherogenic lipid profile, that would translate into a decreased patient and technique survival (26). More recently (27, 28), the use of automated peritoneal dialysis was able to optimize volume control in fast transporters abolishing this previously reported worse outcome (29), but doubts still remain concerning metabolic derangements. In order to explore this relevant issue, studies that specifically address the impact of fast transport status on body composition changes and insulin resistance development, with time on PD, are lacking. Future studies about the impact of fast transport status on outcomes should also focus dialysis prescription in terms of peritoneal dialysis modality and dialysis solutions used, in order to assess if potential deleterious systemic consequences of membrane fast transport could be mitigated by an adequate PD prescription.

The importance of ultrafiltration in PD patient´s outcomes was first evidenced in the EAPOS study (30). According to this prospective study, baseline predictors of poor survival in a group of 177 anuric patients under automatic peritoneal dialysis were age, nutritional status, diabetes and ultrafiltration (ultrafiltration < 750 mL/24h; P=0.047). These results stress the importance to monitor ultrafiltration profile changes, with time on PD, in order to timely adjust dialysis prescription.

In summary, in order to improve both patient and technique survival, future investigations have first to clarify how particular aspects of therapy, such as peritoneal glucose absorption, membrane transport status and PD modality, impact systemically in these patients, in terms of preservation of residual renal function, maintenance of phosphate control, and development of protein wasting, obesity and insulin resistance. At the same time, as ultrafiltration is a major determinant of patient and technique survival, studies should also focus on membrane evaluation, with a full characterization of both small-solute transport and water transport pathways across the membrane, and exploring new biomarkers that could timely signalize peritoneal membrane deterioration, even before clinically relevant ultrafiltration failure develops. Investigation about the systemic impact of inner aspects of the technique,

and improving knowledge on functional evaluation of peritoneal membrane are, nowadays, the new challenges in peritoneal dialysis field.

3. Controversies on the systemic impact of peritoneal glucose absorption and membrane fast transport status

Glucose has been used over the past 3 decades as the main osmotic agent in peritoneal dialysis, allowing for sustained ultrafiltration, and enabling good results in terms of patient and technique survival. Approximately 75% of the dialysate glucose is absorbed (31, 32) , and although there is a difference in the kinetics of glucose oxidation between intraperitoneal glucose and an equivalent oral dose, the cumulative glucose oxidation and glucose balance over a 6 hour study is similar for both routes of administration (33). Such findings raises the question about the potential role of glucose absorption in obesity and insulin resistance development in PD patients (34). In fact, several studies documented that, with time on PD, there is an increasing prevalence of obesity and overweight, but a direct association with peritoneal glucose absorption or fast transport status is not proven (35, 36).

Insulin resistance and hyperinsulinemia are already present in early stages of chronic kidney disease (CKD) (37) and peritoneal dialysis seems to improve insulin sensitivity similarly to hemodialysis, at least for a short time frame (38). However, following glucose ingestion, peritoneal dialysis patients displayed an increase in the glycemic and insulinemic responses compared to healthy subjects, suggesting an insulin resistant state (31, 39). There is a pathophysiologic link between obesity and insulin resistance, as in obese patients the adipocytes will release substantial amounts of free fatty acids and adipocytokines, like tumour necrosis factor-alfa, interleukin-6 and leptin, that will promote insulin resistance trough different pathways (40, 41). Obesity, insulin resistance, dyslipidemia and hypertension are considered a cluster of risk factors for type 2 diabetes and cardiovascular disease in the general population, and define the metabolic syndrome (42-44). As compared with hemodialysis or pre-dialysis patients, uremic patients under PD seem to have a higher risk for metabolic syndrome (45, 46) because of increased risk of metabolic disturbances such as hyperglycemia, hyperinsulinemia and weight gain. However, results are not consensual about the predictive power of metabolic syndrome as a cluster of risk factors for cardiovascular or all-cause mortality in PD patients (47-50), and this lack of evidence in PD may be explained by several reasons. First, we have to consider that some of the risk factors in metabolic syndrome, as higher cholesterol levels and higher body mass index (BMI) are associated with a better nutrition status, and therefore are most likely paradoxically associated with better

clinical outcomes (51-54). Second, even the most recently proposed diagnostic criteria for metabolic syndrome in peritoneal dialysis patients (55) have important limitations in terms of obesity evaluation. The authors proposed measuring obesity in PD patients according to BMI, instead of using waist circumference, as it is proposed by the National Cholesterol Education Program Adult Treatment Panel III (42) and the International Diabetes Federation, for the general population (43). In fact, it must be considered that waist circumference may not reliably reflect abdominal visceral fat content in PD patients, due to the presence of a catheter, lax skin condition after repeated distention of the abdomen by peritoneal dialysis fluid, and by the potential interference of residual volume inside the abdomen cavity. However, we may also argue against the use of BMI to characterize obesity, since a recent study has demonstrated that, in general population, BMI is an inaccurate obesity classification method when compared with measurement of relative body fat by dual X-ray absorptiometry (56). As CKD patients can present simultaneous changes in nutritional status, body fat content and hydration status, BMI may have even lower sensitivity and specificity in obesity characterization in patients under dialysis. This was recently documented in a study reporting that a BMI < 30 Kg/m^2 does not exclude the presence of obesity in a group of incident and prevalent patients under hemodialysis, evaluated by anthropometry (57). Similar studies in the peritoneal dialysis field are needed. The limitations in the diagnostic criteria, the lack of standardized definition and the bias imposed by the absence of body composition assessment, may explain the controversies about the systemic impacts of metabolic syndrome on PD patient outcomes. Although patients on PD are highly susceptible to the metabolic complications included in metabolic syndrome, there is no consensus about the clinical impact of these metabolic derangements when analysed both separately and as a whole (48-50). Therefore, rather than using a cluster of risk factors with unproven cut-off values, specific risk factor evaluation should be done, with metabolic risk assessment combined with parameters of inflammation and nutrition to fully assess the real systemic impact of metabolic derangements in PD.

The euglycemic hyperinsulinemic clamp is regarded as the reference method for insulin sensitivity assessment (58, 59). However, this method is expensive and not suitable to use in clinical practice. Homeostasis model assessment (HOMA-IR) is easy to perform, and was validated as a valuable alternative of measuring insulin resistance, in a static state, both in non-CKD patients (60, 61), in patients with CKD stages 3 and 4 (62, 63) and in CKD patients on dialysis (64). A single study has evidenced that insulin resistance evaluated by HOMA-IR is a predictor of cardiovascular disease in PD patients (65), however the specific role of wasting, obesity, peritoneal glucose absorption and small solute transport was not addressed. It was already established that insulin resistance in CKD patients has likely contributions from

uremic toxins, inflammation, protein-wasting, adipokines, metabolic acidosis and vitamin D deficiency that lead to acquired defects in the insulin-receptor signalling pathway (66). In order to evaluate the potential benefit of a therapeutic intervention targeting insulin resistance in PD patients, investigations should first clarify the sources of insulin resistance in this particularly population. What causes insulin resistance in PD: protein-wasting or obesity? Future investigations about insulin resistance in PD should assess the clinical relevant associations with nutritional status, and further clarify the potential role of peritoneal glucose absorption and peritoneal membrane fast transport status.

Adipokines have also been implicated in the pathogenesis of chronic inflammation and insulin resistance associated with obesity, in general population (40, 67). A recent study documented the same relationship in a group of patients under hemodialysis (64), showing further that insulin resistance indices that incorporate adipokines (like HOMA corrected by adiponectin and leptin/adiponectin ratio) were better correlates of glucose-disposal rate measured by hyperinsulinemic euglycemic clamp. Similar studies in PD field are lacking. Leptin contributes to endothelial dysfunction, atherosclerosis and insulin resistance, and adiponectin has insulin-sensitizing, anti-inflammatory and anti-atherogenic effects (40, 41). In line with this view, leptin/adiponectin ratio was been recently proposed as a new atherogenic index in type 2 diabetic patients without CKD. That concept has been supported by recent investigations that evidenced a strong correlation between pulse wave velocity and carotid intima-media thickness with leptin/adiponectin ratio (68, 69). This ratio is also markedly elevated in peritoneal dialysis patients, and one singly study demonstrates an association with mortality (70). However, this study was unable to assess the potential role of peritoneal glucose absorption and peritoneal membrane transport status on adipokines profile.

The impact of body composition on adipokines is another relevant aspect in PD that was not addressed by any clinical investigation until now. This is a major question when we are evaluating patients with CKD because both obesity and protein-wasting will alter the normal regulation of adipokines (71-73). There is experimental evidence that adiponectin may be a reflection or induce protein wasting, by stimulating energy expenditure (72). Once more, the absence of knowledge about patient´s body composition can be a bias that explains the contradictory results about adiponectin and outcomes in patients with CKD (70, 74-78), and this issue needs to be clarified in future investigations.

Nutritional assessment should be part of routine care in dialysis patients (79, 80) as protein wasting is common in end stage renal disease patients (81, 82) and is a major contributing factor to morbidity and mortality in patients under dialysis (12, 83). The first large scale cross-sectional comparison study of nutritional status between patients on peritoneal

dialysis and hemodialysis, evidenced that PD patients had significantly lower albumin levels, and a higher proportion of malnutrition by subjective global assessment (42.3% versus 30.8%) (84). Serum albumin robustly associates with death and hospitalization in ESRD patients under dialysis (85), is easily and reproducibly measured and responds to appropriate interventions (86). However, a recent investigation performed in a cohort of incident and prevalent dialysis patients (87) evidenced that serum albumin correlated poorly with several other markers of nutrition status and therefore, the authors concluded, that its value as a reliable marker of nutritional status in ESRD patients is limited. For that reason, comparative studies on nutritional status between patients under hemodialysis and peritoneal dialysis that rely on serum albumin levels should be carefully interpreted. In fact, no single marker of nutrition status has been judge to be the best to evaluate CKD patients. For that reason, a panel of experts have recently recommended the simultaneous use of biochemical criteria; evidence of low body weight, reduced body fat or weight loss; a decrease in muscle mass and low protein or energy intake, in order to diagnose protein-energy wasting in CKD patients (81). With the widespread use of bioimpedance analysis, a recent multicentre study in contemporary dialyzed patients (88) has showed, contrary to previous studies, that protein-wasting is more prevalent in hemodialysis patients, while PD patients seem to be relatively protected from catabolism, by presenting a significant higher lean tissue index than their matched pairs on hemodialysis. Given the controversy about nutritional status in PD, new studies in contemporary PD populations should be done with full nutritional status characterization, according to the most recent proposed criteria (81).

The problem of the impact of glucose absorption or membrane fast transport on nutritional status was also not adequately addressed. Churchill and colleagues (26) hypothesized that fast transporters would had greater loss of proteins in dialysate that would not be compensated by protein ingestion, as the greater glucose absorption would lead to a decreased appetite. They made that assumption based in the fact that dialysate protein and albumin loses at baseline were higher in fast transporters, and that serum albumin was significant lower, when compared with others transport categories. However, baseline subjective global assessment, lean body mass percentage and normalized protein catabolic rate (nPCR) were similar across peritoneal transport categories, and nPCR was not different, in a 18 months follow up, between fast and non-fast transporters. Another study documented the same correlation between membrane transport and albumin, but not between membrane transport and overall nutritional status in PD patients (89). New studies about nutritional status evaluation in fast transporters are needed in order to understand if those patients are more susceptible to protein-wasting or if they are a high risk group for obesity. Given the fact that

systemic inflammation is an independent predictor of survival in PD patients (90), its association with obesity and insulin resistance should also be clarified, as there is no knowledge yet about the factors that trigger systemic inflammation in these patients. This is even more crucial to investigate, as the Global Fluid Study (90) has evidenced that systemic and intraperitoneal cytokine networks are dissociated, with the second one being the most important determinant of small-solute transport status, without affecting survival.

Finally, as cardiovascular disease is the first cause of mortality in PD, future studies should focus on its relationship with nutritional status. Such a study would clarify if the concept of reversal epidemiology, explained by a high prevalence of protein-energy-wasting is applicable to PD patients, or if obesity and insulin resistance are in fact the major determinants of cardiovascular events, as in the general population.

4. Structural and functional changes of the peritoneal membrane with time on PD

Dialysate hypertonicity, acidic pH, the presence of glucose degradation products, high concentrations of lactate (91, 92) and glucose itself (93-95) contributes to structural alterations that occur in the peritoneal membrane with time on PD, in a complex process known by epithelial-to-mesenchymal transition (EMT) (96). Yanez-Mo et al. (96) demonstrated that peritoneal mesothelial cells showed a progressive loss of epithelial characteristics and acquired a fibroblast-like phenotype. According to that process, epithelial cells lose polarity, cell-cell contacts and undergo a dramatic remodelling of their cytoskeleton. A portion of these fibroblast-like cells of mesothelial origin invade the submesothelium stroma, because of their increased migratory capacity, and contribute to PD induced fibrosis of the peritoneum, in a process that involves TGF-β1 signaling. However, fibrosis is not the only structural alteration evidenced by the peritoneal membrane with time on PD. In parallel with fibrosis, the peritoneum shows an increase in angiogenesis, in a process that involves vascular endothelial growth factor (VEGF) production by the non-epithelioid, fibroblast-like cells (97).

With time on PD, all this structural alterations will translate into functional changes, with the loss of ultrafiltration capacity of the membrane being the final step (98, 99). The angiogenesis process will translate in a progressive higher small solute transport rate, with an acquired fast transport peritoneal membrane status (97). This fast transport status is responsible for a faster osmotic gradient dissipation and will promote a decrease in water transport through the ultra-small pores (100). With progression of submesothelial fibrosis, a decreased in peritoneal membrane hydraulic conductance will occur, restricting further the

water transport trough the aquaporins, and lowering the membrane osmotic conductance to glucose (101, 102).

As peritoneal biopsies are not routinely performed in clinical practice, structural alterations are hardly measured in the clinic. The above mentioned correlations between structure and function allow for clinical assessment of the peritoneal membrane characteristics, through a peritoneal equilibration test, and are fundamental to monitor, as this will guide PD prescription.

4.1. Functional evaluation of peritoneal membrane

The original peritoneal equilibration test (PET) proposed by Twardowski (103) evaluates the capacity of the peritoneal membrane to transport solutes and its ability to generate ultrafiltration. However, this test does not provide sufficient information to discriminate between causes of ultrafiltration failure. To answer that question, a modified PET, was proposed (104). This test, using 3.86% glucose solution instead of 2.27% glucose solution, not only provides better information on ultrafiltration (because the larger drained volume makes the result less subject to measurements errors, and is more sensitive for detecting clinically significant ultrafiltration failure), but also allows the sodium sieving measurement, as an indirect estimation of the magnitude of water transport through the aquaporins. However, it is important to bear in mind that an apparent reduction in sodium sieving can also be caused by an increase in peritoneal small solute diffusion, or by a reduction in the ultrafiltration coefficient of the peritoneal membrane (105). Therefore, a reliable tool to quantify both the peritoneal free water transport and small-pore solute and water transport would be of great practical importance.

Recently, two methods have been developed to measure free water transport in PD patients (106, 107), both validated by computer simulations using the three-pore model (108). The standard permeability analysis test by Smit (107) is accurate to measure both free water transport and small-solute transport, but involves several different measurements and sophisticated calculations, making this test difficult to apply in clinical practice. The mini-PET by La Milia (106) is a simple and accurate method that allows measurement of free water transport, but overestimates the dialysate-to-plasma ratio of creatinine ($D/P_{creatinine}$), since it is measured at 60 minutes, and the $D/P_{creatinine}$ at 60 minutes is not equivalent to the $D/P_{creatinine}$ measured in a 4-hour PET (109). To overcome this limitations, Cnossen et al. (110), performed a study in which they compared, in 10 prevalent PD patients, small solute transport assessed in a 4-hour, 3.86% glucose PET, with temporary complete drainage of the peritoneum after 1 hour (allowing for simultaneous quantification of free water transport), concluding that the interim step did not influence the $D/P_{creatinine}$ measured at 4 hours. This new method is simple

to use in clinical practice and overcome the limitations of the mini-PET (106) that has only one hour of duration. For that reason, this new and promising approach, should be re-evaluated, in a larger PD population, in order to assess its clinical relevance.

4.2. Effluent biomarkers as an early sign of peritoneal membrane alterations: controversies

The use of biomarkers as an early sign of peritoneal membrane alterations is currently under debate (111-113), especially because clinical factors cannot give an accurate individual prediction for peritoneal sclerosis (101). In a very recent research (102), patients with encapsulating peritoneal sclerosis (EPS) presented, compared with long-term PD controls, an early loss of both ultrafiltration capacity and sodium sieving two years before the onset of overt EPS. The loss of ultrafiltration capacity in EPS patients was disproportionate to the increase in MTAC of creatinine, and suggested an uncoupling between peritoneal fluid and solute transport. In a multivariate analysis, the loss of sodium sieving was a powerful and independent risk factor associated with encapsulating peritoneal sclerosis, outperforming other clinical variables as younger age at dialysis onset, longer PD duration, lower residual renal function, beta blocker use and peritonitis rates (102). Since a reduced free water transport is the most powerful indicator for the development of EPS, and is not simply explained by an increase in vascular capillary surface, it is important to search for a biomarker that correlates both with ultrafiltration and free water transport, and not only with membrane small solute transport status. The most recent studies about peritoneal effluent biomarkers compared cytokines in patients who developed EPS with PD controls, and present contradictory results (111-113). Besides, none of them has explored the associations between cytokines measured in the effluent and water transport pathways, namely with free water transport that could be significantly compromised several years before EPS onset (102), and for that reason, more informative, allowing a timely diagnosis of peritoneal membrane dysfunction.

Hepatocyte growth factor (HGF) is known to play a crucial role in the repairing process of tissues and preventing organ fibrosis (114-117). Human peritoneal mesothelial cells constitutively synthetize HGF, and treatment of human peritoneal mesothelial cells with HGF blocks high glucose-induced epithelial-to-mesenchymal transition (93). Recent clinical research underscores that the protective role of HGF in peritoneal fibrosis is due to inhibition of transforming growth factor β1 signaling (117). One single clinical study compared effluent HGF concentration according to small solute transport status (118). However, the authors did not explore any association between effluent HGF and ultrafiltration failure, neither with water transport pathways quantification. It is thus relevant to increase the knowledge on HGF clinical value in patients under PD, as it may possible point to new diagnostic opportunities and

therapeutic avenues. Therefore, clinical investigation under this subject is mostly important and needed.

III – Aims and Outline of the Thesis

III. AIMS AND OUTLINE OF THE THESIS

The aim of this thesis was to investigate the systemic impact of intrinsic aspects of the peritoneal dialysis technique, such as peritoneal glucose absorption, peritoneal membrane transport status, and peritoneal dialysis modality on residual renal function preservation, maintenance of phosphate control, and development of protein-energy wasting, obesity and insulin resistance in a population of incident and prevalent patients under peritoneal dialysis. At the same time, this thesis focused also on the functional evaluation of the peritoneal membrane, establishing a new peritoneal equilibration test to evaluate simultaneously small solute transport and water transport, and explores new peritoneal effluent biomarkers that could timely signalize peritoneal membrane deterioration, even before clinically relevant ultrafiltration failure develops.

In **chapter V.I**, the factors that influenced residual renal function decline in our incident peritoneal dialysis population were investigated. We aimed to test the hypothesis that the previous renal replacement therapy modality, specifically transition to PD after renal graft failure, impacts residual renal function loss.

In **chapter V.II**, the peritoneal membrane phosphate transport status was defined, in a large prevalent peritoneal dialysis population, and its associations with hyperphosphatemia, phosphate peritoneal clearance and peritoneal dialysis modality were investigated. We tested the hypothesis that phosphate serum levels are dependent on peritoneal membrane phosphate transport status, with a clinically relevant association with dialysis regimens.

In **chapter V.III**, the nutritional status of a contemporary peritoneal dialysis population, in treatment in our Unit, was investigated with the simultaneous use of different nutritional assessment tools, including biochemical criteria, body composition assessment using anthropometry, and characterization of dietary intake.

In chapter **V.IV.I** we aimed to test the hypothesis that leptin/adiponectin ratio, in patients under peritoneal dialysis, is dependent on patient´s body composition, and impacts on cardiovascular mortality. In this prospective study, the impact of adipokines profile as a predictor of cardiovascular events was investigated, taking into consideration patient´s body composition assessed by bioimpedance analysis and the potential roles of peritoneal glucose absorption and peritoneal membrane small solute transport status. The relationship between cardiovascular disease and nutrition status in PD patients was assessed. The value of body mass index to measure obesity was evaluated against full body composition characterization with bioimpedance analysis. Finally, the potential roles of peritoneal glucose absorption and fast transport status on obesity development and adipokines profile were also investigated.

In chapter **V.IV.II**, insulin resistance, using homeostasis model assessment method (HOMA-IR) and HOMA corrected by adiponectin (HOMA-AD), was evaluated in a group of non-diabetic, contemporary patients, under peritoneal dialysis. Our aim was to test the hypothesis that insulin resistance is dependent on body composition parameters, peritoneal glucose absorption and peritoneal membrane small-solute transport status. Fast transporters were particularly examined, since there are some concerns in the contemporary literature, about the metabolic systemic consequences associated with a fast membrane transport status.

In **chapter V.V.I**, functional characterization of the peritoneal membrane was performed in a multicentre study using a new peritoneal membrane equilibration test (the «Two-in-one Protocol»). Small-solute transport status, free water transport and small-pore ultrafiltration were simultaneously assessed and quantified. Ultrafiltration failure causes were analysed. The relationship between the different water transport pathways was assessed, both in situations with and without ultrafiltration failure.

In **chapter V.V.II,** we tested the hypothesis that hepatocyte growth factor (HGF), a biomarker with important roles in the repairing process of tissues and organ fibrosis prevention, signalizes peritoneal membrane derangements. Therefore, the relationship between effluent HGF and free water transport and small solute transport were investigated. Patients with fast transport status and free water compromise were particularly studied, since they are considered a high risk group in terms of peritoneal membrane dysfunction.

Chapter VI includes a general discussion. Future investigations and perspectives are also debated.

Chapter VII includes a summary of the most important conclusions.

IV – Methods

IV. METHODS

Specific methodological issues in this investigation are highlighted.

Concerning nutritional assessment, the investigations performed involved several tools, in order to achieve an accurate nutritional assessment of the studied PD patients, according to the most recent criteria proposed by the International Society of Renal Nutrition and Metabolism (81).

In the first study performed **(chapter V.III)**, nutrition status was assessed by Subjective Global Assessment (SGA) and by a nutrition status score (NSS) based on biochemical and anthropometric measurements. The SGA was performed by a single trained observer, who was blinded for the biochemical results of the patients. SGA integrates a four-item, 7-point scale, based on history of weight change, appetite, gastrointestinal symptoms and physical examination of body fat and muscles (119, 120). An SGA score of 6 – 7 indicates a well-nourished individual ("A"); 3-5 a mildly-to-moderately malnourished individual ("B"); and 1-2, a severely malnourished individual ("C"). Anthropometric measurements were made on biceps, triceps, and subscapular skinfold thickness by a single observer, immediately after peritoneal dialysate drainage, using a conventional Harpenden skinfold caliper. Each measurement was repeated three times, and the average result was registered. Mid-arm circumference (MAC) was also measured, and mid-arm muscle circumference (MAMC) was calculated using the formula (121): MAMC (mm) = MAC (MM) – 3.14 x Triceps Skinfold (mm). Body density was calculated based on the sum of skinfold thickness values, using the equations of Durnin and Womersly (122). Fat mass and lean body mass were obtained from calculated body density and body weight. The NSS used consisted of eight components: body mass index, percentage of ideal body weight (calculated according to Butheau and Metropolitan Life Insurance Company formulas), triceps and subscapular skinfold thickness, MAMC, serum albumin, total lymphocyte count and subjective physical examination. Skinfold measurements and MAMC were compared with the 50th percentile for the appropriate age and sex, and were expressed as percentages (123).

In 2009 the PD Unit adopted, as a standard, the use of whole body bioimpedance in order to evaluate patient´s body composition, using the Body Composition Monitor device (BCM®, Fresenius Medical Care, Bad Homburg, Germany). For that reason, in the subsequent investigations performed **(chapters V.IV.I and V.IV.II)**, anthropometric measurements were no longer used, and were substituted by bioimpedance analysis. The bioimpedance method applied was validated by isotope dilution methods (124), by accepted reference body composition methods (125) and by extensive clinical assessment of the hydration state (126).

Body composition assessments were done with full abdomen during the PET procedure. Lean tissue index (LTI), fat tissue index (FTI), relative fat mass (rel.FM), body cell mass (BCM) and relative over hydration (rel.OH) were measured. Only measurements achieving simultaneously high quality (>90%) and lower error percentage (<35%), showing an inverted U curve, in the device monitor, were considered valid. Due to biophysical reasons, the bioimpedance spectroscopy does not measure sequestered fluid in the trunk (127), therefore the presence or absence of PD fluid in the abdomen does not influence body composition parameters related with nutritional status (128). Women with relative fat mass above 30% and men with relative fat mass above 25% were considered obese (56). The values for LTI were compared to an age and gender-matched reference population and according to this, patients with LTI inferior to the 10^{th} percentile were considered to have protein wasting (129). Patients that simultaneously presented with relative fat mass <10% and LTI < reference range, were characterize as having protein-energy wasting (81).

Concerning biochemical assessments, albumin was measured by the same methodology in the different studies performed (bromocresol green - Cobas Integra 800 – Roche Diagnostics). Serum leptin and adiponectin, were measured using the same enzyme-linked immunosorbent assays in the different studies performed (Mediagnost, Reutlingen, Germany).

In the **chapter V.IV.II**, insulin resistance was assessed using homeostasis model assessment (HOMA-IR), homeostasis model assessment correct by adiponectin (HOMA-AD) and by leptin/adiponectin ratio, according to the following equations (64): HOMA-IR = fasting serum insulin (µU/mL) x fasting serum glucose (mg/dL)/405; HOMA-AD = fasting serum insulin (µU/mL) x fasting serum glucose (mg/dL)/adiponectin (µg/mL); leptin/adiponectin ratio = leptin (ng/mL)/adiponectin (µg/mL). In order to measure plasma insulin and plasma glucose without a significant interference from peritoneal glucose absorption, the preceding overnight dwell was standardized to 1.36% glucose solution in all patients (31). Venous blood samples were obtained after an 8-10 hours overnight fasting period, in the morning of the peritoneal equilibration test (PET). Blood samples were drawn after the last dwell drainage and before starting the PET. Insulin was measured by a sandwich assay on an electrochemiluminescence (ECLIA) immunoassay analyzer (Cobas E 170 – Roche Diagnostics GmbH). Patients with HOMA-IR ≥ 2.2 were considered to present insulin resistance, as this value corresponds to HOMA-IR percentile 50 in the larger validation study about HOMA-IR against the euglycemic, hyperinsulinemic clamp in non-diabetic patients (60).

In **chapters V.IV.I** and **V.IV.II**, peritoneal glucose absorption was calculated by subtracting the 24-h drained glucose (measured) from the total glucose influx by the dialysate.

In **chapter V.V.I** a new peritoneal equilibration test (the "Two-in-One Protocol") was tested in a large population of incident and prevalent patients under PD. This protocol allowed free water transport (FWT) quantification, beyond a simple calculation of sodium sieving. None of the patients evaluated had peritonitis during the study, or the preceding 6 weeks. During the procedure, PD solutions low in glucose degradation products were used, according to the individual patient's prescription. The volume of dialysis solution was determined by weight, without flushing the system and before filling the peritoneum. Blood and dialysate samples (each approximately 10 mL) were taken at instillation of the dialysate and after 60 and 240 minutes. At 60 minutes, an additional measurement of ultrafiltration (UF) by total drainage of the peritoneal cavity was performed. This drained volume was weighed and then immediately re-infused. Finally, after 240 minutes, the peritoneal cavity was drained and the volume obtained was weighed. During the study PET, FWT was calculated as follows: FWT (mL) = Total UF volume at 60 minutes (mL) – UF through the small pores at 60 minutes (mL). The UF through the small pores (SPUF) at 60 minutes was calculated as follows: SPUF (mL) = [NaR (mmol) × 1000] / PNa, where NaR (mmol) is sodium removal and PNa is the sodium concentration in plasma assessed by indirect ion-selective electrodes. The NaR was calculated as [Dialysate V at 60 minutes (L) × Dialysate Na at 60 minutes (mmol/L)] – [Dialysate V instilled (L) × Dialysate Na at 0 minutes (mmol/L)]. Using a simple algorithm, a correction for FWT, as described by Venturoli and Rippe (108), was also performed: $FWT_{corrected}$ = Total UF at 60 minutes + 15 – 0.92 × SPUF, where the "15" represents cumulative lymphatic absorption during 60 minutes (18 mL) minus the cumulative UF through the large pores during 60 minutes (approximately 3 mL). The FWT fraction was also evaluated. PETs with an ultrafiltered volume ≤ 400 mL/4h were considered to represent ultrafiltration failure (UFF) (130).

Functional evaluation of the membrane was performed in all investigations using the Two-in-One PET. Creatinine and sodium were measured in both plasma and dialysate. Glucose was assessed in dialysate. Creatinine and glucose were measured using standard automated analyser techniques. For creatinine, the Jaffé compensated method was used. The dialysate creatinine concentration was corrected for interference by glucose according to our laboratory standards. Sodium in dialysate and plasma was measured using indirect ion-selective electrodes.

In chapter **V.V.II**, a new effluent biomarker was investigated in order to explore its associations with small solute transport and membrane water transport pathways. Effluent HGF was determined by ELISA technique according to the manufacturer's instructions (IBL – Immuno-Biological Laboratories Co. Ltd). The intra and inter-assay variations were

respectively 8,8% and 10,0%. The sensitivity was 11pg/mL. The assay was considered highly specific for the cytokine, and no significant cross-reactivity was observed.

V – Results

V.I Predictors of residual renal function loss in PD patients

Predictors of residual renal function loss in peritoneal dialysis: is previous renal transplantation a risk factor?

V.II Peritoneal membrane phosphate transport status

Peritoneal membrane phosphate transport status: a cornerstone in phosphate handling in peritoneal dialysis

V.III Evaluation of nutritional status in contemporary PD patients

Overweight rather than malnutrition is widely prevalent in peritoneal dialysis patients

V.IV The impact of peritoneal glucose absorption and fast transport status on obesity, insulin resistance and new cardiovascular events in PD patients

V.IV.I *Adipokines in Peritoneal Dialysis: relevant clinical impact according to body composition*

V.IV.II *Insulin resistance in non-diabetic peritoneal dialysis patients: associations with body composition, peritoneal transport and peritoneal glucose absorption*

V.V Functional characterization of the peritoneal membrane

V.V.I *Two-in-One Protocol: simultaneous small-pore and ultrasmall-pore peritoneal transport quantification*

V.V.II *Hepatocyte growth factor signalizes peritoneal membrane failure in peritoneal dialysis*

V – Results

V.I. Predictors of residual renal function loss in PD patients

Bernardo A, Fonseca I, Rodrigues A, Carvalho MJ, Cabrita A. Predictors of residual renal function loss in peritoneal dialysis: is previous renal transplantation a risk factor? Adv Perit Dial. 2009;25:110-4.

Short Summary

Residual renal function (RRF) plays a major role in dialysis patient´s clinical outcomes, and for that reason, preservation of residual renal function loss is an important goal in peritoneal dialysis population.

In this study we investigated the factors associated with RRF decline in a population of 148 consecutive patients treated in our Unit.

Our study showed that RRF was not significantly different between patients who started peritoneal dialysis (PD) as theirs first option, and those who started PD after renal transplantation. Transfer from hemodialysis was the only significant predictor of baseline anuria. After the start of PD, diabetes was the only independent predictor of anuria. Age, gender, PD after renal graft failure, PD modality and fast transport status were not predictors of anuria.

This investigation allowed us to conclude that diabetes was the most important determinant of the time course of RRF decline in our population, and that peritoneal dialysis after renal transplant failure can offer similar short-term RRF protection compared to that seen in PD-first patients.

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Predictors of Residual Renal Function Loss in Peritoneal Dialysis: Is Previous Renal Transplantation a Risk Factor?

Ana Bernardo,[1] Isabel Fonseca,[2] Anabela Rodrigues,[2] Maria J. Carvalho,[2] António Cabrita[2]

Preservation of residual renal function (RRF) is an important goal in peritoneal dialysis (PD). The present study explored the factors conditioning RRF decline in a PD population.

We studied 148 consecutive patients. Age, sex, diabetes, previous renal replacement therapy time and modality [hemodialysis (HD), renal transplantation (RT), or PD first], peritoneal transport, PD prescription [automated (APD) or continuous ambulatory], and peritonitis were investigated as possible determinants of RRF decline.

In 22 patients (15%), PD was started after RT. Residual renal function was not significantly different between patients who started PD as their first option and those who started after RT, either at baseline or after 1 year on PD. Baseline dialysate-to-plasma creatinine was also similar between those groups.

Transfer from HD was the single significant predictor of baseline anuria [odds ratio (OR): 6.3; $p < 0.001$*). After the start of PD, diabetes was the only predictor of anuria (OR: 2.5;* $p = 0.02$*). Age, sex, reason for PD, PD after graft failure, peritonitis, use of APD, and fast transport were not predictors of anuria. Despite slow tapering of immunosuppression, peritonitis-free survival was not shortened in patients who started PD after RT.*

Diabetes was a determinant of the time course of RRF decline in PD. Peritoneal dialysis after RT failure offered short-term RRF protection that was similar to that seen in PD-first patients.

Key words
Residual renal function, renal graft failure

From: [1]Nephrology Department, Hospital Amato Lusitano, Castelo Branco, and [2]Nephrology Department, Centro Hospitalar do Porto, Hospital Santo Antonio, Porto, Portugal.

Introduction

In recent years, greater focus has been given to residual renal function (RRF) in patients on chronic dialysis therapy because RRF plays an important role in the maintenance of fluid balance and biochemical homeostasis in end-stage renal disease patients on dialysis. Several studies have reported that RRF is better preserved in peritoneal dialysis (PD) than in hemodialysis (HD) patients. There is a consensus that RRF has a major effect on quality of life and outcome in PD patients, and therefore preservation of RRF becomes a goal of adequacy beyond the limited role of Kt/V (1). As a result, determining the factors that affect loss of RRF has become an important issue in the care of PD patients.

Several factors have been associated with loss of RRF (2–4): larger body mass index, presence of diabetes, presence of congestive heart failure, use of diuretics, hypotensive events, episodes of peritonitis, inflammation, peritoneal fast transport status, and use of automated PD (APD). However results are not consistent, and studies often exclude patients with a failed renal graft, which is becoming one of the most frequent causes of dialysis initiation (5). Most patients with a failed renal graft are initiated on HD as their next renal replacement modality, and concerns about the success of PD in this population have been expressed, given that few studies have considered outcome in patients with renal graft failure on PD. Sasal *et al.* (6) and Davies (7) reported a more rapid loss of RRF in PD patients with a failed renal allograft than in patients who had never undergone kidney transplantation. However de Jonge *et al.* (8) reported no significant difference in the decline of RRF between PD patients with renal graft failure and never-transplanted patients starting PD.

Faced with these conflicting findings about predictors of RRF loss among transplantation patients, we explored the factors conditioning RRF decline in our PD population.

Patients and methods

All consecutive incident patients commencing PD at our center between January 2000 and June 2007 were included in the study (n = 148). More than 50% of the patients were treated with PD solutions low in glucose degradation products. When needed for ultrafiltration, icodextrin was the standard prescription in the unit; use of hypertonic 3.86% glucose solution has long been abandoned and is only exceptionally prescribed.

Baseline factors such as age, sex, diabetes, total previous time on renal replacement therapy (RRT), previous modality [HD, transplantation (RT), PD as first modality], peritoneal transport category, and PD prescription [continuous ambulatory PD (CAPD) or automated PD (APD)], and peritonitis episodes were investigated as possible determinants of RRF decline. Using timed urine collections, we measured RRF as the arithmetic mean of urinary urea and creatinine clearance at baseline and at approximately 3-month intervals thereafter. The date of development of complete anuria was also recorded.

The subgroup of patients started on PD after renal graft failure was compared with PD-first patients. The RT patients all received tapering immunosuppression: immediate withdrawal of antiproliferative drugs (azathioprine, mycophenolate mofetil, sirolimus) after PD induction, with a slow reduction of calcineurinic drugs and prednisolone over several months.

Statistical analysis

All data are expressed as mean ± standard deviation or median with interquartile range (IQR). Comparisons between groups of continuous variables used the Student independent t-test or the Mann–Whitney test, as appropriate. Proportions of categorical variables were compared using the chi-square test.

The outcomes examined were anuria at PD baseline (logistic regression) and time to loss of RRF (anuria) after the start of PD (survival analysis by the Kaplan–Meier method and Cox regression for multivariate analysis). Kaplan–Meier survival curves were compared using the log-rank test. The Cox proportional hazards model was used to examine the effects of demographic, clinical, and dialysis variables on the outcome variable. The relative risks for loss of RRF (anuria) were determined by univariate and multivariate Cox regression analysis and are presented as hazard ratios (HRs) with a 95% confidence interval (CI).

A p value below 0.05 was considered to be statistically significant. Statistical analyses were performed using the statistical software package SPSS (version 15.0: SPSS, Chicago, IL, U.S.A.).

Results

Of the 148 consecutive incident patients included in the study, 69% were women (102 of 148). The mean age of the patients was 47.2 ± 16.0 years, 26 (17.6%) had diabetes, and 84 (56.8%) were on APD. Table I shows the baseline characteristics of the study patients.

Patients had been on renal replacement therapy for a mean of 3.9 ± 6.5 years (range: 0 – 34 years): 72 (48.6%) had transferred from HD (PD-after-HD group), 22 (14.9%) started PD after renal graft failure (PD-after-RT group), and the remaining 54 (36.5%) initiated dialysis with PD as the first modality (PD-first group). Of the 148 patients, 49 (33.1%) were anuric [glomerular filtration rate (GFR) < 1 mL/min] at baseline: 31 (63.3%) in the PD-after-HD group, 8 (16.3%) in the PD-after-RT group, and 10 (20.4%) in the PD-first group.

As expected, patients transferring from HD (mainly because of vascular access failure) had a higher prevalence of anuria (42.3% vs. 24.2%, p = 0.023), and this association was confirmed by multivariate

TABLE I Characteristics of the study patients

Characteristic	*[n (%)]*
Patients	148
Sex (men)	46 (31.1)
With diabetes	26 (17.6)
On APD	84 (56.8)
Reason for PD	
Choice	68 (45.9)
Access failure	80 (54.1)
Renal replacement therapy	
PD first	72 (48.6)
PD after HD	54 (36.5)
PD after RT	22 (14.9)
Baseline anuria	49 (33.1)
With peritonitis	74 (50)

APD = automated peritoneal dialysis; PD = peritoneal dialysis; HD = hemodialysis; RT = renal transplantation.

analysis. Transfer from HD was the only significant predictor of baseline anuria (OR: 6.3; $p < 0.001$) after adjustment for age, sex, decision for PD (optional or after access failure), previous renal replacement therapy, and diabetes (Table II).

Peritonitis-free survival was similar in PD-after-RT patients and PD-first patients (estimate: 391 days vs. 378 days; $p = 0.92$).

At baseline, median RRF was not significantly different between PD-first patients and PD-after-RT patients: 5.8 (IQR: 4.3 – 8.3 mL/min) versus 8.1 (IQR: 4.2 – 16.1 mL/min; Mann–Whitney *U*: $p = 0.26$). After 1 year on PD, no significant differences in median RRF emerged: 3.9 (IQR: 2.0 – 6.7 mL/min) versus 4.5 (IQR: 0.4 – 5.6 mL/min; Mann–Whitney *U*: $p = 0.74$). Baseline peritoneal transport as measured by dialysate-to-plasma (D/P) creatinine from a 4-hour peritoneal equilibration test using 3.86% glucose was also similar between the groups (0.76 ± 0.13 vs. 0.73 ± 0.11; Mann–Whitney *U*: $p = 0.42$).

Diabetes was significantly associated with an increase in the risk of RRF decline, with a mean estimate of 19.7 months to anuria in diabetic patients as compared with 37.6 months in nondiabetic patients ($p = 0.028$, Figure 1). Time to anuria was shorter in PD-after-RT patients (estimate: 23.4 months in the PD-after-RT group, 29.6 months in the PD-after-HD group, and 36 months in the PD-first group), but the PD-after-RT group was small ($n = 14$), and the differences did not reach statistical significance.

By multivariate analysis (and excluding all patients with anuria at baseline), the predictors for anuria developing after the start of PD were diabetes (OR: 2.27; $p = 0.033$) and previous time on RRT (OR: 1.005; $p = 0.028$). Age, sex, peritonitis, reason for initiating PD (access failure or choice), previous RRT modality (PD first, PD after RT), baseline D/P creatinine, and PD prescription (APD) were not significant predictors for the development of anuria (Table III).

TABLE II Predictors of anuria at baseline of peritoneal dialysis (logistic regression[a])

Predictor	*OR*	*95% CI*	p *Value*
Reason (access failure vs. optional)	1.8	0.7 to 4.1	0.18
Diabetes (yes vs. no)	0.5	0.2 to 1.5	0.21
Age (years)	1.0	0.9 to 1.0	0.81
PD after HD vs. PD first	6.3	2.6 to 9.3	<0.001
PD after RT vs. PD first	2.3	0.7 to 7.6	0.17

OR = odds ratio; CI = confidence interval; PD = peritoneal dialysis; HD = hemodialysis; RT = renal transplantation.

[a] After including age, diabetes, reason for peritoneal dialysis [PD (after access failure or optional)], and dialysis modality before PD, transfer from hemodialysis was the only factor independently associated with the presence of anuria at the beginning of PD.

Discussion

Preservation of RRF is an important goal in the management of PD patients. However, in the literature, results concerning the factors that influence the rate of decline of RRF are conflicting.

Diabetes mellitus as one of the predictors of RRF loss has been addressed by several studies, but not all (9). In a recent study that enrolled 270 incident PD patients (18% with diabetes), Liao *et al.* (2) reported that the annual rate of RRF decline in patients with diabetes was almost twice that of patients without diabetes (2.38 ± 1.38 mL/min/1.73 m^2 vs. 1.14 ± 1.27 mL/min/1.73 m^2). In the present study, we also found that diabetes was a major determinant of RRF decline, given that in the multivariate analysis, it was the only predictor of anuria.

The contribution of peritonitis episodes to RRF decline is more inconsistent. Some studies (2,10) reported that peritonitis rate was an independent risk factor for the decline of RRF in PD patients, but the link remains unclear. Along with others (11), we were not able to document any association between anuria and peritonitis rate.

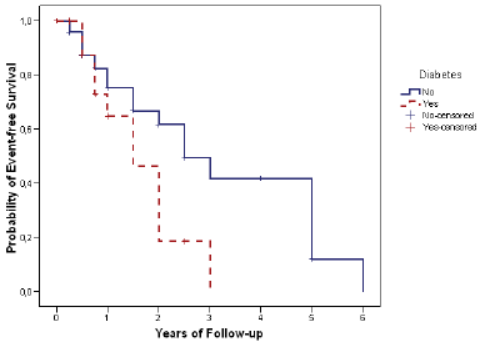

FIGURE 1 Effect of diabetes on residual renal function (Kaplan–Meier method). Time to the loss of residual renal function (anuria) was significantly shorter in patients with diabetes ($p = 0.028$).

TABLE III Predictors of anuria (loss of residual renal function) after the start of peritoneal dialysis (Cox proportional hazards analysis[a])

Predictor	*HR*	*95% CI*	p *Value*
Peritonitis episodes (1 vs. 0)	0.6	0.3 to 1.4	0.25
Peritonitis episodes (≥2 vs. 0)	0.9	0.4 to 1.9	0.76
Reason for PD (access failure vs. optional)	1.5	0.8 to 3.0	0.22
Diabetes (yes vs. no)	2.5	1.1 to 5.5	0.02
Age (years)	1.0	0.9 to 1.0	0.46
Sex (men vs. women)	1.1	0.5 to 2.3	0.75
PD after HD vs. PD first	0.9	0.4 to 2.3	0.87
PD after RT vs. PD first	2.1	0.8 to 5.4	0.12

HR = hazard ratio; CI = confidence interval; PD = peritoneal dialysis; HD = hemodialysis; RT = renal transplantation.

[a] Considering the variables sex, age, reason for peritoneal dialysis [PD (access failure/option)], type of previous renal replacement therapy, and number of peritonitis episodes in the model, diabetes was the only independent predictor of residual renal loss after PD start.

In a number of studies, fast peritoneal transport status has been associated with increased technique failure and mortality, but a recent investigation highlighted the heterogeneity of fast transport status both in cause and in outcome (12). Johnson *et al.* (11) reported that a high D/P ratio was a risk factor for rapid loss of RFF. The reason for that association is uncertain, but the authors speculate that inflammation may have caused both an increase in the peritoneal transport rate and a decline of RRF. We were not able to document this association between high peritoneal transport status and RRF loss in our group, but inflammation status presumably depends more on associated comorbidity and PD treatment strategy. Information on peritoneal transport profiles in renal graft failure patients under PD treatment is scarce, but notably, we did not find faster transport rates in our PD-after-RT group than in our PD-first patients.

Evidence has also emerged that APD, as compared with CAPD, might have a deleterious effect on RRF. It is hypothesized that the acute changes in volume and osmotic load induced at each nightly PD session could potentially accelerate deterioration of RRF. However, we—like others (2,11,13)—were not able to document any difference in decline of RRF between patients on cycler-assisted PD and patients on CAPD. Our study lends further support to the argument that APD therapy *per se* does not significantly affect the decline of RRF.

In the integrated care model of renal replacement therapy, patients with renal allograft failure returning to a second chronic PD program represent a special subgroup of end-stage renal disease patients. The reasons that very few patients initiate PD after failed allograft may be related to uncertainty about the success of PD in this patient population (5): outcome studies are few, and there are concerns about an increased risk of therapy-related infection. We previously reported similar cumulative patient and technique survival and no difference in peritonitis-free survival between our PD patients coming from transplant and our incident PD patients (14). Favorable results have also been reported by other groups (15,16). But contradictory findings have been reported for the rate of decline of RRF in this population: one study suggested that patients on PD after a failed renal transplant have a faster rate of RRF decline than do other patients on PD (7); and yet another observed similar findings in PD patients with renal graft failure as compared with matched PD patients who had never received a graft (17).

Like de Jonge *et al.* (8), we found no difference in the decline of RRF between patients starting PD after renal transplant failure and a group starting PD as their first renal replacement therapy. A determining factor might be immunosuppression management after PD induction (18). Our policy of slowly tapering immunosuppressors—immediate withdrawal of antiproliferative drugs (azathioprine, mycophenolate mofetil, sirolimus) because of the major infectious risk, but slow reduction of calcineurinic drugs and prednisolone over several months—presumably protect renal graft function. However, this issue remains open.

Conclusions

In spite of the limitations typically found in our study and others (for example, limited number of PD

patients with renal graft failure, absence of prospective investigation of the link between immunosuppression tapering protocol and outcomes), our findings suggest that, compared with PD-first patients, PD-after-RT patients experience similar short-term RRF protection without a shorter peritonitis-free survival.

Diabetes seems to be an unmodifiable baseline epidemiologic risk factor for development of anuria after PD start, but further multicenter prospective studies are needed to examine whether good glycemic control and other modifiable determinants of RRF loss can be identified, and to evaluate whether interventions targeting those determinants could slow the deterioration of RRF in patients on chronic PD. The promising role of icodextrin and alternative solutions low in glucose degradation products in reducing RRF decline must also be validated (19).

References

1 Marrón B, Remón C, Pérez–Fontán M, Quirós P, Ortíz A. Benefits of preserving residual renal function in peritoneal dialysis. Kidney Int Suppl 2008;(108):S42–51.
2 Liao CT, Shiao CC, Huang JW, *et al*. Predictors of faster decline of residual renal function in Taiwanese peritoneal dialysis patients. Perit Dial Int 2008;28(Suppl 3):S191–5.
3 Chung SH, Heimbürger O, Stenvinkel P, Quareshi AR, Lindholm B. Association between residual renal function, inflammation and patient survival in new peritoneal dialysis patients. Nephrol Dial Transplant 2003;18:590–7.
4 Rabindranath KS, Adams J, Ali TZ, *et al*. Continuous ambulatory peritoneal dialysis versus automated peritoneal dialysis for end-stage renal disease. Cochrane Database Syst Rev 2007;2:CD006515.
5 Perl J, Bargman JM, Davies SJ, Jassal SV. Clinical outcomes after failed renal transplantation—does dialysis modality matter? Semin Dial 2008;21:239–44.
6 Sasal J, Naimark D, Klassen J, Shea J, Bargman JM. Late renal transplant failure: an adverse prognostic factor at initiation of peritoneal dialysis. Perit Dial Int 2001;21:405–10.
7 Davies S. Peritoneal dialysis in the patient with a failing renal allograft. Perit Dial Int 2001;21(Suppl 3):S280–4.
8 de Jonge H, Bammens B, Lemahieu W, Maes BD, Vanrenterghem Y. Comparison of peritoneal dialysis and haemodialysis after renal transplant failure. Nephrol Dial Transplant 2006;21:1669–74.
9 Holley JL, Aslam N, Bernardini J, Fried L, Piraino B. The influence of demographic factors and modality on loss of residual renal function in incident peritoneal dialysis patients. Perit Dial Int 2001;21:302–5.
10 Shin SK, Kang SW, Seo BJ, *et al*. Risk factors influencing the decline of residual renal function in continuous ambulatory peritoneal dialysis patients. Perit Dial Int 1999;19:138–42.
11 Johnson D, Mudge D, Steutevant J, *et al*. Predictors of decline of residual renal function in new peritoneal dialysis patients. Perit Dial Int 2003;23:276–83.
12 Rodrigues AS, Martins M, Korevaar JC, *et al*. Evaluation of peritoneal transport and membrane status in peritoneal dialysis: focus on incident fast transporters. Am J Nephrol 2007;27:84–91.
13 Moist LM, Port FK, Orzol SM, *et al*. Predictors of loss of residual renal function among new dialysis patients. J Am Soc Nephrol 2000;11:556–64.
14 Oliveira N, Carvalho MJ, Fonseca I, *et al*. Peritoneal dialysis after renal transplant failure. Port J Nephrol Hypertens 2008;22:183–8.
15 Mujais S, Story K. Patient and technique survival on peritoneal dialysis in patients with failed renal allograft: a case-control study. Kidney Int Suppl 2006;(103):S133–7.
16 Badve S, Hawley C, McDonald S, *et al*. Effect of previously failed kidney transplantation on peritoneal dialysis outcomes in the Australia and New Zealand patient populations. Nephrol Dial Transplant 2006;21:776–83.
17 Schiffl H, Mucke C, Lang SM. Rapid decline of residual renal function in patients with late renal transplant failure who are re-treated with CAPD. Perit Dial Int 2003;23:398–400.
18 Messa P, Ponticelli C, Berardinelli L. Coming back to dialysis after kidney transplant failure. Nephrol Dial Transplant 2008;23:2738–42.
19 Kim SG, Kim S, Hwang YH, *et al*. Could solutions low in glucose degradation products preserve residual renal function in incident peritoneal dialysis patients? A 1-year multicenter prospective randomized controlled trial (Balnet Study). Perit Dial Int 2008;28(Suppl 3):S117–22.

Corresponding author:
Ana Bernardo, MD, Av Pedro Álvares Cabral, Castelo Branco 6000-085 Portugal.
E-mail:
anabernardo@portugalmail.pt

V – Results

V.II. Peritoneal membrane phosphate transport status

Bernardo AP, Contesse SA, Bajo MA, Rodrigues A, Del Peso G, Ossorio M, Cabrita A, Selgas R. Peritoneal membrane phosphate transport status: a cornerstone in phosphate handling in peritoneal dialysis. Clin J Am Soc Nephrol. 2011 Mar;6(3):591-7. doi: 10.2215/CJN.06960810. Epub 2010 Nov 29.

Short Summary

Phosphate control impacts dialysis outcomes, but few studies have addressed the importance of PD modality and peritoneal membrane transport status concerning dialytic phosphate clearance.

With this clinical investigation, we aimed to define peritoneal phosphate transport in peritoneal dialysis patients, and to explore its association with hyperphosphatemia, phosphate clearance and PD modality.

We evaluated 264 patients treated in Peritoneal Dialysis Unit from La Paz University Hospital.

We were able to show that residual renal function was independently, negatively, associated with hyperphosphatemia and that, in anuric patients, peritoneal membrane phosphate transport status was the only significant predictor of hyperphosphatemia. We also evidenced that phosphate and creatinine peritoneal membrane transport status characterization can differ in the same patient, and that CAPD treatment is associated with an increase in phosphate clearance of 13.6% among phosphate slow-average transporters, and 38.4% among phosphate slow transporters.

Our study contributes to our understanding of peritoneal phosphate clearance by highlighting the importance of establishing peritoneal membrane phosphate transport status. In hyperphosphatemic or anuric patients, the decision on the optimal PD modality should also take into account peritoneal phosphate transport characteristics, beyond urea Kt/V and peritoneal creatinine clearance. Increasing dwell times or transfer to CAPD could be effective strategies to improve phosphate handling in patients with inadequate phosphate control on APD.

Article

Peritoneal Membrane Phosphate Transport Status: A Cornerstone in Phosphate Handling in Peritoneal Dialysis

Ana Paula Bernardo, Sebastián Azorin Contesse,† Maria Auxiliadora Bajo,† Anabela Rodrigues,‡ Gloria Del Peso,† Marta Ossorio,† António Cabrita,‡ and Rafael Selgas†*

*Nephrology Department, Amato Lusitano Hospital, Castelo Branco, Portugal; †Nephrology Department, La Paz University Hospital, Madrid, Spain; and ‡Nephrology Department, Centro Hospitalar do Porto, Porto, Portugal

Correspondence: Dr. Ana Paula Bernardo, Av. Pedro Álvares Cabral, 6000-085 Castelo Branco, Portugal. Phone: 00351-914778868; Fax: 00351-272000257; E-mail: anabernardo@portugalmail.pt

Summary

Background and objectives Phosphate control impacts dialysis outcomes. Our aim was to define peritoneal phosphate transport in peritoneal dialysis (PD) and to explore its association with hyperphosphatemia, phosphate clearance (PPhCl), and PD modality.

Design, setting, participants, & measurements Two hundred sixty-four patients (61% on continuous ambulatory PD [CAPD]) were evaluated at month 12. PPhCl was calculated from 24-hour peritoneal effluent. Phosphate (Ph) and creatinine (Cr) dialysate/plasma (D/P) were calculated at a 4-hour 3.86% peritoneal equilibration test.

Results D/PPh correlated with D/PCr. PPhCl correlated better with D/PPh than with D/PCr. Prevalence of hyperphosphatemia (>5.5 mg/dl) was 30%. In a multiple regression analysis, only residual renal function was independently, negatively associated with hyperphosphatemia; in anuric patients, only D/PPh was an independent factor predicting hyperphosphatemia. D/PPh was 0.57 ± 0.10, and according to this, 16% of the patients were fast, 31% were fast-average, 35% were slow-average, and 17% were slow transporters. PPhCl was 37.5 ± 11.7 L/wk; it was lower in the slow transporter group (31 ± 14 L/wk). Among fast and fast-average transporters, PPhCl was comparable in both PD modalities. In comparison to automated PD, CAPD was associated with increased PPhCl among slow-average (36 ± 8 *versus* 32 ± 7 L/wk) and slow transporters (34 ± 15 *versus* 24 ± 9 L/wk).

Conclusions In hyperphosphatemic, particularly anuric, patients, optimal PD modality should consider peritoneal phosphate transport characteristics. Increasing dwell times and transfer to CAPD are effective strategies to improve phosphate handling in patients with inadequate phosphate control on automated PD.

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Introduction

Hyperphosphatemia is common among dialysis patients and is a major risk factor for cardiovascular mortality in both hemodialysis and peritoneal dialysis (PD) patients (1–7). In PD patients, phosphate control is known to deteriorate as residual renal function (RRF) declines (8,9). In fact, although the importance of RRF in maintaining serum phosphorus levels in PD patients has already been established (8), the role of peritoneal phosphate clearance in achieving adequate phosphate homeostasis has not been well studied. Dialytic phosphate clearance is the product of nonmodifiable peritoneal transport characteristics and the modifiable components of dialysis prescription. Whether creatinine may be used as a surrogate marker of phosphate transport is now under debate. Some authors consider that creatinine clearance measurements provide a good estimate of phosphorus clearance (10), whereas others showed that the peritoneal transport state defined by the creatinine equilibration pattern is poorly predictive of daily phosphate clearance (11). In fact, until now, no studies have tried to define peritoneal membrane phosphate transport status in an prevalent adult PD population. Finally, few studies have addressed the importance of PD modality concerning dialytic phosphate clearance (10,12).

Because of this, the objectives of our study were to define peritoneal membrane phosphate transport status in a large, prevalent adult PD population and to explore its association with hyperphosphatemia, phosphate peritoneal clearance, and PD modality.

Materials and Methods

This was a cross-sectional and retrospective study conducted at the La Paz University Hospital Home Dialysis Unit. From the 348 prevalent patients on PD that were treated in our unit from January 1992 until

January 2009, 264 patients were included in this cross-sectional observational study. We excluded 84 patients with <1 year of PD treatment. Each patient included was evaluated at 12 months (1 year) after starting PD. Our patients were receiving standard prescriptions of continuous ambulatory peritoneal dialysis (CAPD) or automated peritoneal dialysis (APD), according to European best practice recommendations (13). Solutions with high calcium content (3.5 mEq/L) were standardly prescribed in the unit, unless the patient had sustained hypercalcemia. Phosphate binders were prescribed, as appropriate, to maintain serum phosphorus concentration between 3.5 and 5.5 mg/dl, according to the 2003 Kidney Disease Outcomes Quality Initiative guidelines for mineral metabolism (14). Residual renal function was calculated as the average of 24-hour urinary urea and creatinine clearances. Standard parameters of dialysis adequacy were determined by measuring total (renal and peritoneal) weekly urea clearance (Kt/V) and creatinine clearance using standard methods (15). Dialysate creatinine concentration was corrected for interference by glucose according to a reference formula determined by our laboratory. Protein equivalent nitrogen appearance (nPNA) was calculated using methods described by Randerson *et al.* (16) and normalized to actual weight. Phosphate renal and peritoneal clearances were calculated as follows: peritoneal phosphate clearance (L/wk per 1.73 m^2) = (dialysate phosphate in mg/dl/plasma phosphate in mg/dl) × 24-hour effluent dialysate volume (L) × 7 (corrected for 1.73 m^2 BSA); renal phosphate clearance (L/wk per 1.73 m^2) = (urine phosphate in mg/dl/plasma phosphate in mg/dl) × 24-hour urinary volume (L) × 7 (corrected for 1.73 m^2 BSA). Additionally, we performed a 3.86%, 4-hour glucose peritoneal equilibration test (PET). Dialysate/plasma (D/P) creatinine (Cr) ratio and D/P phosphate (Ph) ratio were measured at 4 hours. The 24-hour dialysate collection was performed exactly on the day before the realization of the PET for all patients. Concerning phosphate transport status, our patients were classified as slow (D/P Ph < 0.47), slow-average (0.47 ≤ D/P Ph <0.57), fast-average (0.57 ≤ D/P Ph <0.68), or fast (D/P Ph ≥ 0.68) transporters, according to the mean ± SD of the dialysate/plasma phosphate ratio (D/P Ph). We also divided our patients by the dialysate/plasma creatinine ratio (D/P Cr) as slow (D/P Cr ≤ 0.49), slow-average (0.50 ≤ D/P Cr ≤ 0.64), fast-average (0.65 ≤ D/P Cr ≤ 0.80), and fast (D/P Cr ≥ 0.81) transporters according to the criteria defined by Twardowski *et al.* (17).

Results were expressed as frequencies and percentages for categorical variables, mean ± SD for continuous variables, and median and interquartile range for nonparametric data. Correlations between two continuous variables were expressed as Pearson's or Spearman's correlations coefficients. Differences between hyperphosphatemic (serum phosphate > 5.5 mg/dl) and normophosphatemic (serum phosphate ≤ 5.5 mg/dl) patients were evaluated using unpaired *t* test, Mann-Whitney *U* test, and χ^2 test as appropriate. In the subanalysis of anuric patients, we analyzed peritoneal phosphate clearance both as a continuous and a categorical variable, according to the mean value of peritoneal phosphate clearance (37.5 L/wk per 1.73 m^2). Multivariate analysis using conditional logistic regression and multiple linear regression models (as appropriated) were performed to analyze the determinants of hyperphosphatemia (serum phosphate being analyzed both as a continuous and categorical variable) at 1 year of treatment, first in all of the patients and then focused on anuric patients. The following variables were included in the models: PD modality as a categorical variable (CAPD *versus* APD) and nPNA, peritoneal urea Kt/V, peritoneal creatinine clearance, peritoneal phosphate clearance, D/P Ph, D/P Cr, and RRF as continuous variables. We compared clearances of small solutes (urea, creatinine, and phosphate) according to PD modality (CAPD *versus* APD) using the *t* test and Mann-Whitney *U* test, as appropriate. Peritoneal phosphate transport was categorized according to mean ± SD of D/P Ph. We compared clearances of small solutes (urea, creatinine, and phosphate) across the four categories of peritoneal membrane phosphate transport status using one-way ANOVA and Kruskal-Wallis tests, as appropriate. We finally explored the adequacy and phosphate clearances according to PD modality, controlling for peritoneal phosphate transport status. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS, version 15.0, for Windows software (SPSS, Chicago, IL).

Results

Patient baseline characteristics are listed in Table 1. The baseline evaluation (at 3 months) concerning peritoneal membrane small solute transport and residual renal function (data not shown) was not significantly different from that at 1 year. In the PET at 1 year, mean 4-hour D/P ratio was 0.70 ± 0.1 for creatinine and 0.57 ± 0.1 for phosphate. A close correlation was observed between D/P Ph and D/P Cr at 1 year ($r = 0.81$, $P < 0.0001$; Figure 1, left).

Correlation between Creatinine Transport and Phosphate Transport

Peritoneal phosphate clearance at 1 year correlated better with peritoneal creatinine clearance at 1 year than with peritoneal urea Kt/V at 1 year ($r = 0.650$, $P < 0.0001$ and $r = 0.451$, $P < 0.0001$, respectively). Peritoneal phosphate clearance at 1 year correlated better with D/P Ph at 1 year than with D/P Cr at

Table 1. Population baseline characteristics

	Total (n = 264)
Sex (male)	160 (60.6%)
Age (years)	51.4 ± 16.0
CAPD/APD	160 (60.6%)/104 (39.4%)
Diabetes mellitus	46 (17.4%)
Renal diagnosis	
Chronic glomerulonephritis	47 (17.8%)
Tubulointersticial nephropathy	47 (17.8%)
Diabetic nephropathy	37 (14.0%)
Poliquistic autossomic dominant nephropathy	29 (11.0%)
Hypertensive nephrosclerosis	29 (11.0%)
Unknown	38 (14.4%)
Others	37 (14.0%)

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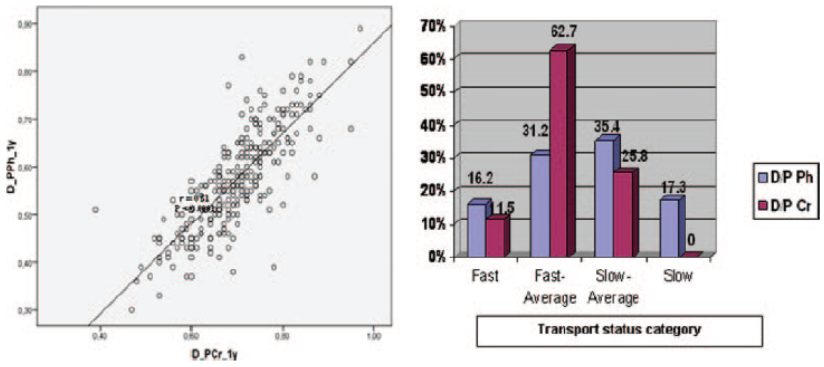

Figure 1. | (Left) Relation between D_P Ph_1y (dialysate to plasma phosphate ratio) and D_PCr_1y (dialysate to plasma creatinine ratio). (Right) Categorization of patients concerning phosphate peritoneal transport (according to mean ± SD of D/P Ph) and creatinine peritoneal transport (according to Twardowski *et al.*).

1 year ($r = 0.490$, $P < 0.0001$ and $r = 0.400$ $P < 0.0001$, respectively).

Hyperphosphatemia, RRF, and Phosphate Clearance

At 1 year of treatment, 79 patients (30%) had hyperphosphatemia (serum phosphate levels >5.5 mg/dl). Phosphate levels correlated negatively with residual renal function and renal phosphate clearance ($r = -0.290$, $P < 0.0001$ and $r = -0.225$, $P = 0.008$, respectively) but did not correlate with nPNA or with peritoneal small solute clearances. In a multiple regression analysis, only RRF was independently and negatively associated with hyperphosphatemia [Exp(B) = 0.75; 95% confidence interval, 0.64 to 0.89; $P = 0.001$]. The same results were obtained considering serum phosphate as a continuous variable (data not shown). In fact, serum phosphate levels were >5.5mg/dl in 43.6% of anuric patients ($n = 78$) *versus* 24.2% of patients with RRF ($n = 186$, $P = 0.002$). Among patients with RRF, those with hyperphosphatemia had significantly lower renal phosphate clearances than patients with serum phosphate ≤5.5mg/dl (28.2 ± 21.1 *versus* 45.5 ± 30.1 L/wk per 1.73 m^2, $P = 0.018$), whereas peritoneal phosphate clearance was similar in the two groups (34.7 ± 10.7 *versus* 34.6 ± 10.9 L/wk per 1.73 m^2; Table 2).

Anuric Patients

Anuric hyperphosphatemic patients had lower mean levels of phosphate peritoneal clearance and a statistically significant slower membrane phosphate transport rate (Table 2). Concerning adequacy parameters among anuric patients, we observed that 97.2% had a peritoneal Kt/V of 1.7 or greater and 83.3% had a peritoneal creatinine clearance of 45 L/wk per 1.73 m^2 or greater. However, only 68.8% had a peritoneal phosphate clearance of 37.5 L/wk per 1.73 m^2 or greater (equal or greater than the mean peritoneal phosphate clearance of the population in this analysis). In fact, serum phosphate levels were >5.5mg/dl in 62.5% of anuric patients with peritoneal phosphate clearance <37.5 L/wk per 1.73 m^2 ($n = 24$) *versus* 33.9% of anuric patients with a peritoneal phosphate clearance of ≥37.5 L/wk per 1.73 m^2 ($n = 54$, $P = 0.019$). In a multiple regression analysis, only D/P Ph was independently and negatively associated with hyperphosphatemia in anuric patients [Exp(B) = 0.003, 95% confidence interval, 0.001 to 0.50; $P = 0.026$]. We obtained the same results considering serum phosphate as a continuous variable (data not shown).

Modality of PD Controlling for Peritoneal Membrane Phosphate Transport

To compare phosphate clearances according to PD modality, also controlling for peritoneal membrane phosphate transport rate, we studied 260 patients (we excluded 4 patients without 1-year PET evaluation): 157 (60.4%) were treated with CAPD and 103 (39.6%) with APD. We detected a different categorization of patients concerning phosphate and creatinine peritoneal transports. Relative to peritoneal phosphate transport, 42 (16.2%) patients were classified as fast transporters (D/P Ph ≥ 0.68), 81 (31.2%) as fast-average transporters (0.57 ≤ D/P Ph <0.68), 92 (35.4%) as slow-average transporters (0.47 ≤ D/P Ph < 0.57), and 45 (17.3%) as slow transporters (D/P Ph < 0.47). Concerning peritoneal creatinine transport, 30 patients (11.5%) were classified as fast transporters (D/P Cr ≥ 0.81), 163 (62.7%) as fast-average transporters (0.65 ≤ D/P Cr ≤ 0.80), 67 (25.8%) as slow-average transporters (0.50 ≤ D/P Cr ≤ 0.64), and 0 as slow transporters (D/P Cr ≤ 0.49; Table 3; Figure 1, right).

According to PD modality, patients treated with APD had higher peritoneal urea Kt/V than patients treated with CAPD (1.71 ± 0.5 *versus* 1.45 ± 0.3, $P < 0.0001$), but there was no difference in peritoneal creatinine clearance or in peritoneal phosphate clearance between those treated with APD or CAPD (Table 3). Patients with fast-transport membranes had a higher peritoneal urea Kt/V, a higher peritoneal creatinine clearance, and a higher peritoneal phosphate clearance than the rest of categories (Table 4).

When peritoneal phosphate clearance by modality was examined for each phosphate membrane category, there was no significant difference in phosphate clearance be-

Table 2. Dialysis adequacy, phosphate clearance, and peritoneal transport status in patients with a serum phosphorous level of ≤5.5 mg/dl *versus* >5.5 mg/dl and stratified according to residual renal function at 1 year of treatment

	Serum Phosphate				All Patients $n = 264$
	Patients with RRF		Anuric Patients		
	≤5.5 mg/dl ($n = 141$)	>5.5 mg/dl ($n = 45$)	≤5.5 mg/dl ($n = 44$)	>5.5 mg/dl ($n = 34$)	
Total weekly urea Kt/V	2.7 ± 0.7[a]	2.1 ± 0.4[a]	2.1 ± 0.3	2.0 ± 0.3	2.4 ± 0.7
Peritoneal weekly urea Kt/V	1.5 ± 0.4	1.4 ± 0.3	2.1 ± 0.3	2.0 ± 0.3	1.6 ± 0.4
Renal weekly urea Kt/V	1.2 ± 0.8[a]	0.7 ± 0.5[a]			0.8 ± 0.8
Total weekly CCr (L/wk per 1.73 m^2)	93.7 ± 34.5[a]	68.0 ± 20.4[a]	52.6 ± 10.3	53.7 ± 7.8	79.0 ± 32.7
Peritoneal weekly CCr (L/wk per 1.73 m^2)	35.5 ± 9.8	34.2 ± 7.3	52.6 ± 10.3	53.7 ± 10.1	39.4 ± 11.8
Renal weekly CCr (L/wk per 1.73 m^2)	58.2 ± 37.1[a]	33.8 ± 22.2[a]			39.6 ± 38.2
Total weekly CPh (L/wk per 1.73 m^2)	80.1 ± 29.9[a]	62.9 ± 21.5[a]	46.6 ± 10.6	43.4 ± 11.3	61.6 ± 27.2
Peritoneal weekly CPh (L/wk per 1.73 m^2)	34.6 ± 10.9	34.7 ± 10.7	46.6 ± 10.6	43.4 ± 11.3	37.5 ± 11.7
Renal weekly CPh (L/wk per 1.73 m^2)	45.5 ± 30.1[a]	28.2 ± 21.1[a]			22.2 ± 29.6
RRF (ml/min per 1.73 m^2)	5.8 ± 3.7[a]	3.7 ± 2.5[a]			3.8 ± 3.8
D/P Cr	0.69 ± 0.1	0.70 ± 0.1	0.73 ± 0.1	0.71 ± 0.1	0.70 ± 0.09
D/P Ph	0.56 ± 0.1	0.58 ± 0.1	0.63 ± 0.1[b]	0.57 ± 0.1[b]	0.57 ± 0.11
nPNA (g/kg per day)	1.2 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	1.1 ± 0.2	1.2 ± 0.3

Values are expressed as mean ± SD. CCr, creatinine clearance; CPh phosphate clearance.
[a]$P < 0.05$ patients with RRF and serum phosphorous ≤5.5 mg/dl *versus* patients with RRF and serum phosphorous >5.5 mg/dl, according to independent sample *t* test.
[b]$P < 0.05$ anuric patients with serum phosphorous ≤5.5 mg/dl *versus* anuric patients with phosphorous >5.5 mg/dl, according to independent sample *t* test.

Table 3. Peritoneal membrane transport status and solute clearances according to PD modality, at 1 year of treatment

Variable	CAPD	APD	*P*
Peritoneal urea Kt/V	1.45 ± 0.3	1.71 ± 0.5	<0.0001[a]
Peritoneal creatinine clearance (L/wk per 1.73 m^2)	38.7 ± 9.1	40.0 ± 13.5	0.527
Peritoneal phosphate clearance (L/wk per 1.73 m^2)	36.5 ± 9.2	36.7 ± 12.1	0.928
D/P Cr	0.69 ± 0.1	0.71 ± 0.1	0.319
fast transporter	17/30 (56.7%)	13/30 (43.3%)	
fast-average transporter	98/163 (60.1%)	65/163 (39.9%)	
slow-average transporter	42/67 (62.7%)	25/67 (37.3%)	
slow transporter	0	0	
D/P Ph	0.61 ± 0.1	0.61 ± 0.1	0.884
fast transporter	23/42 (54.8%)	13/42 (45.2%)	
fast-average transporter	49/81 (60.5%)	32/81 (39.5%)	
slow-average transporter	52/92 (56.5%)	40/92 (43.5%)	
slow transporter	33/45 (73.3%)	12/45 (27.7%)	

Values are expressed as frequencies and percentages for categorical variables, mean ± SD for continuous variables. Patients are categorized, concerning creatinine transport status, as slow (D/P Cr ≤ 0.49), slow-average (0.50 ≤ D/P Cr ≤ 0.64), fast-average (0.65 ≤ D/P Cr ≤ 0.80), and fast (D/P Cr ≥ 0.81) transporters, according to the criteria defined by Twardowski *et al.*.
Patients are categorized, concerning phosphate transport status, as slow (D/P Ph < 0.47), slow-average (0.47 ≤ D/P Ph < 0.57), fast-average (0.57 ≤ D/P Ph < 0.68), or fast (D/P Ph ≥ 0.68) transporters, according to mean ± SD of dialysate/plasma phosphate ratio (D/P Ph).
[a]$P < 0.005$ comparisons between patients in CAPD *versus* APD using the *t* test if data were parametric and the Mann-Whitney *U* test if nonparametric.

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Table 4. Solute clearances comparison across peritoneal membrane phosphate transport status, at 1 year of treatment

Variable	Fast	Fast-Average	Slow-Average	Slow	*P*
Peritoneal urea Kt/V	1.87 ± 0.5	1.63 ± 0.5	1.58 ± 0.4	1.51 ± 0.4	0.016[a]
Peritoneal creatinine clearance (L/wk per 1.73 m^2)	49.3 ± 12.2	41.8 ± 13.9	37.1 ± 8.8	34.3 ± 12.2	0.005[a]
Peritoneal phosphate clearance (L/wk per 1.73 m^2)	47.4 ± 12.6	39.4 ± 9.9	34.0 ± 7.6	31.4 ± 14.3	<0.0001[a]

Values expressed as mean ± SD. Patients classified as slow, slow-average, fast-average. or fast transporters, according to mean ± SD of dialysate plasma phosphate ratio (D/P Ph) at a 4-hour, 3.86% glucose PET.
[a]$P < 0.005$ intergroup solute clearances comparison across the four transport groups using one-way ANOVA if data were parametric and Kruskal-Wallis test if nonparametric.

tween the modalities in the fast and fast-average transport categories (Table 5). However, in the slow-average and slow categories, treatment with CAPD was associated with significantly higher phosphate clearance than with APD (Table 5). We did not found any significant difference in RRF or in renal phosphate clearance between the four groups (slow, slow-average, fast-average, and fast transporters) that could indirectly interfere on peritoneal phosphate transport. There was no significant difference in peritoneal creatinine clearance between the two PD modalities across any of the membrane transport categories (Table 5).

Discussion

Our study highlighted that, other than RRF, peritoneal phosphate transport rate and clearance evaluations are additional parameters that can be used to optimize phosphorus control in PD patients.

Hyperphosphatemia is highly prevalent in PD patients and is a strong predictor of overall and cardiovascular mortality (1–4). Although elimination of inorganic phosphate by dialysis is a cornerstone in the management of hyperphosphatemia, the subject of phosphate handling by PD is scarcely addressed and has not been completely studied. Phosphate transport across the peritoneum is influenced by osmotic, chemical, and electrical gradients, as well as by transmembranous active phosphate transporters and thus is more complex than peritoneal urea or creatinine transport (18–20). As in Sedlacek *et al.* (10), our study showed that peritoneal phosphate clearance is more closely related with peritoneal creatinine clearance than with peritoneal urea clearance. This finding must be related to some special features of the phosphate molecule, because the molecular weight of phosphate (96 Da) lies right between those of urea (60 Da) and creatinine (130 Da), and the molecular radius of phosphate (2.8 Å) is closer to that of creatinine (3.0 Å) than urea (1.8 Å). However, concerning peritoneal membrane transport status, we showed a closer relation with peritoneal phosphate clearance and D/P Ph than with D/P Cr in a 4-hour, 3.86% glucose PET. This finding is even more important once residual renal function is lost. In that circumstance, the peritoneal membrane phosphate transport status plays the most important role in the management of hyperphos-

Table 5. Peritoneal urea, creatinine, and phosphate clearances according to peritoneal membrane phosphate transport status and PD modality, at 1 year of treatment

	CAPD	APD	*P*
Peritoneal Kt/V			
fast	1.47 ± 0.3	1.99 ± 0.4	0.026[a]
fast-average	1.74 ± 0.51	1.56 ± 0.5	0.054
slow-average	1.66 ± 0.2	1.46 ± 0.4	0.242
slow	1.58 ± 0.3	1.44 ± 0.3	0.720
Peritoneal creatinine clearance			
fast	49.6 ± 8.8	54.4 ± 12.8	0.249
fast-average	41.0 ± 14.2	42.3 ± 14.0	0.775
slow-average	41.1 ± 6.2	37.1 ± 11.5	0.968
slow	37.2 ± 5.1	31.8 ± 13.2	0.279
Peritoneal phosphate clearance			
fast	46.9 ± 12.6	48.1 ± 13.0	0.755
fast-average	39.3 ± 10.4	39.6 ± 9.3	0.865
slow-average	35.9 ± 7.8	31.6 ± 6.6	0.006[a]
slow	33.9 ± 15.2	24.5 ± 9.0	0.049[a]

Values expressed as mean ± SD. Patients classified as slow, slow-average, fast-average, or fast transporters, according to mean ± SD of dialysate plasma phosphate ratio (D/P Ph) at a 4-hour, 3.86% glucose PET.
[a]$P < 0.005$ intergroup comparisons between patients in CAPD *versus* APD using the *t* test if data were parametric and the Mann-Whitney U test if nonparametric.

phatemia by PD. Hence, when we divided patients according to the classical Twardowski categories of peritoneal membrane transport status, we found that only 67 patients (25.8%) had a slow-average transport status, and none fell into the slow transport status category. However, when divided according to mean ± SD of D/P Ph, 137 patients (52.7%) had a slow-average/slow transport status, and these patients had significantly lower values of peritoneal small solute clearances than the patients in the fast/fast-average transport category. This means that D/P Cr is not a sufficiently accurate measure to classify patients for peritoneal membrane phosphate transport status. The aqueous layer that surrounds the phosphate molecule, increasing its effective molecular weight, may be one of the reasons why creatinine apparently reaches a more rapid equilibration across the peritoneal membrane than phosphate (18,20,21).

Previous studies that made direct comparisons of phosphate clearance between CAPD and APD have not shown significant differences in peritoneal phosphate clearance (10,22) or worse peritoneal phosphate clearance on CAPD (23). These studies were limited by small sample size and no adjustment for peritoneal membrane transport category. Sedlaceak *et al.* (10) and Badve *et al.* (12) reported that phosphate clearance is increased in fast transporters compared with slow transporters in CAPD and APD when peritoneal transport status is defined according to Twardowski categories of D/P Cr in a 4-hour, 3.86% glucose PET. Our study expands on the work of these groups because we compared phosphate clearance between CAPD and APD by adjusting for peritoneal membrane phosphate transport category. In fact, when we compared modalities across each peritoneal phosphate transport category, we found that PD modality is a significant determinant of phosphate handling, in subgroups of patients, with superior peritoneal phosphate clearance associated with CAPD treatment among slow and slow-average transporters. Thus, our data suggest that peritoneal membrane phosphate transport status should also be considered when optimizing the PD prescription, especially among anuric patients. Like Wang *et al.* (8), we showed that anuric PD patients have poorer phosphate control than those with residual renal function, and in these patients, serum phosphate is an inverse function of the peritoneal phosphate clearance. Our findings that treatment with CAPD was associated with an increase in phosphate clearance of 13.6% among slow-average transporters and 38.4% among slow transporters, compared with APD, suggest that PD prescription may play an important role in phosphate handling, especially among anuric patients with slow transport categories.

The International Society for Peritoneal Dialysis guidelines on PD adequacy recommend a urea Kt/V target of ≥1.7 and a separate target of ≥45 L/wk per 1.73 m^2 of creatinine clearance (13). Our data suggest that focusing only on urea and creatinine for assessment of dialysis adequacy may result in inadequate clearance of phosphate. In fact, although the majority of our anuric patients accomplished those adequacy targets, 43.6% of them had hyperphosphatemia. From these, 44% had a peritoneal phosphate clearance <37.5 L/wk per 1.73 m^2, which might be amenable by adjusting PD prescription to peritoneal membrane phosphate transport rate, because 80% of them were slow/slow-average phosphate transporters, and 50% of these patients were on APD.

To our knowledge, this is the first study that defines peritoneal membrane phosphate transport status in a large, prevalent adult PD population and explores its association with hyperphosphatemia and peritoneal phosphate clearance.

One limitation is that this is a cross-sectional and observational study. Although we assessed a cross-section of the PD population who were receiving adequate dialysis on CAPD or APD, we did not attempt to assess the effect of tidal regimens, dwell times, or number of cycles on phosphate clearance. Another limitation of our study is that the phosphate binding prescription was not included. However, the lack of correlation between indirect protein intake (nPNA) with serum phosphate that we found, the universal uncertainty about phosphate binder accomplishment, and the fact that we focused our study on peritoneal phosphate elimination, may neutralize the effect of these limitations.

Conclusions

This study contributes to our understanding of peritoneal phosphate clearance by highlighting the importance of establishing peritoneal membrane phosphate transport status. In hyperphosphatemic and/or anuric patients, the decision on the optimal PD modality should also take into account peritoneal phosphate transport characteristics and not just urea Kt/V or peritoneal creatinine clearance. Increasing dwell times or transfer to CAPD could be effective strategies to improve phosphate handling in patients with inadequate phosphate control on APD.

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Disclosures

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References

1. Krediet RT, Balafa O: Cardiovascular risk in the peritoneal dialysis patient. *Nat Rev Nephrol* 6: 451–460, 2010
2. Ansell D: Serum phosphate and outcomes in PD patients. *Nephrol Dial Transplant* 22: 667–668, 2007
3. Noordzij M, Korevaar JC, Bos WJ, Boeschoten EW, Dekker FW, Bossuyt PM, Krediet RT: Mineral metabolism and cardiovascular morbidity and mortality risk: Peritoneal dialysis patients compared with haemodialysis patients. *Nephrol Dial Transplant* 21: 2513–2520, 2006
4. Melamed ML, Eutace JA, Plantiga L, Jaar BG, Fink NE, Klag MJ, Powe NR: Changes in serum calcium, phosphate, and PTH and the risk of death in incident dialysis patients: A longitudinal study. *Kidney Int* 70: 351–357, 2006
5. Kalantar-Zadeh K, Kuwae N, Regidor DL, Kovesdy CP, Kilpatrick RD, Shinaberger CS, McAllister CJ, Budoff MJ, Salusky IB, Kopple JD: Survival predictability of time-varying indicators of bone disease in maintenance hemodialysis patients. *Kidney Int* 70: 771–780, 2006
6. Young EW, Albert JM, Satayathum S, Goodkin DA, Pisoni RL,

Akiba T, Akizawa T, Kurokawa K, Bommer J, Piera L, Port FK: Predictors and consequences of altered mineral metabolism: The Dialysis Outcomes and Practice Patterns Study. *Kidney Int* 67: 1179–1187, 2005
7. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM: Mineral metabolism, mortality, and morbidity in maintenance hamodialysis. *J Am Soc Nephrol* 15: 2208–2218, 2004
8. Wang AY, Woo J, Sea MM, Law MC, Lui SF, Li PK: Hyperphosphatemia in Chinese peritoneal dialysis patients with and without residual kidney function: What are the implications? *Am J Kidney Dis* 43: 712–720, 2004
9. Pag DE, Knoll GA, Cheung V: The relationship between residual renal function, protein catabolic rate, and phosphate and magnesium levels in peritoneal dialysis patients. *Adv Perit Dial* 18: 189–191, 2002
10. Sedlacek M, Dimaano F, Uribarrri J: Relationship between phosphorus and creatinine clearance in peritoneal dialysis: Clinical implications. *Am J Kidney Dis* 36: 1020–1024, 2000
11. Schmitt CP, Borzych D, Nau B, Wuhl E, Zurowska A, Schaefer F: Dialytic phosphate removal: A modifiable measure of dialysis efficacy in automated peritoneal dialysis. *Perit Dial Int* 29: 465–471, 2009
12. Badve SV, Zimmerman DL, Knoll GA, Burns KD, McCormick BB: Peritoneal phosphate clearance is influenced by peritoneal dialysis modality, independent of peritoneal transport characteristics. *Clin J Am Soc Nephrol* 3: 1711–1717, 2008
13. Dombros N, Dratwa M, Feriani M, Gokal R, Heimburger O, Krediet R, Plum J, Rodrigues A, Selgas R, Struijk D, Verger C: EBPG Expert Group on Peritoneal Dialysis: European best practice guidelines for peritoneal dialysis. Adequacy of peritoneal dialysis. *Nephrol Dial Transplant Suppl* 9: ix24–ix27, 2005
14. National Kidney Foundation: K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis* 42[Suppl 3]: S1–S201, 2003
15. Nolph KD, Moore HL, Twardowski ZJ, Khanna R, Prowant B, Meyer M, Ponferrada L: Cross-sectional assessment of weekly urea and creatinine clearances in patients on continuous ambulatory peritoneal dialysis. *ASAIO J* 38: M139–M141, 1992
16. Randerson DH, Chapman GV, Farrell PC: Amino acid and dietary status in CAPD patients. In: *Peritoneal Dialysis*, edited by Atkins RC, Farrell PC, Thomson N, Edinburgh, Scotland, Churchill-Livingstone, 1981, pp 180–191
17. Twardowski Z, Nolph KD, Khanna R, Prowant BF, Ryan LP, Moore HL, Nielsen MP: Peritoneal equilibration test. *Perit Dial Int* 7: 138–148, 1987
18. Graff J, Fugleberg S, Brahm J, Fogh-Andersen N: The transport of phosphate between the plasma and dialysate compartments in peritoneal dialysis is influenced by an electric potential difference. *Clin Physiol* 16: 291–300, 1996
19. Rippe B, Venturoli D, Simonsen O, Arteaga J: Fluid and electrolyte transport across the peritoneal membrane during CAPD according to the three-pore model. *Perit Dial Int* 24: 10–27, 2004
20. Kuhlmann MK: Phosphate elimination in modalities of hemodialysis and peritoneal dialysis. *Blood Purif* 29: 137–144, 2010
21. Badve S, McCormick BB: Phosphate balance on peritoneal dialysis. *Perit Dial Int* 28[Suppl 2]: S26–S32, 2008
22. Evenepoel P, Bammens B, Verbeke K, Vanrenterghem Y: Superior dialytic clearance of beta 2-microglobulin and p-cresol by high-flux hemodialysis as compared to peritoneal dialysis. *Kidney Int* 70: 794–799, 2006
23. Gallar P, Ortega O, Gutierrez M, Munoz M, Hilara L, Oliet A, Rodrígues I, Giménez E, Vigil A: Influencing factors in the control of phosphorous in peritoneal dialysis. Therapeutic options. *Nefrologia* 20: 355–361, 2000

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V – Results

V.III. Evaluation of nutritional status in contemporary PD patients

Bernardo AP, Fonseca I, Rodrigues A, Carvalho MJ, Cabrita A. Overweight rather than malnutrition is widely prevalent in peritoneal dialysis patients. Adv Perit Dial. 2009;25:119-24.

Short Summary

Protein wasting is common among chronic kidney disease patients under dialysis, contributing to morbidity and mortality. Few studies evaluate the nutritional status of contemporary peritoneal dialysis (PD) patients.

In this clinical investigation, we studied 57 PD patients treated in our Unit. We assessed their nutritional status according to the most recent criteria of International Society of Renal Nutrition and Metabolism.

We concluded that overweight rather than malnutrition was widely prevalent in our population, but we could not find any association between overweight and obesity with demographic or modality related factors, neither with fast transport, nor with markers of systemic inflammation. However, we documented a potential atherogenic lipid profile in the overweight group, which can be seen as a potential risk factor for cardiovascular disease. This clinical investigation brings up de question whether overweight is a protective condition, associated with lower comorbidity and a lower metabolic rate, or whether it instead represents a clinical menace. In our subsequent clinical investigations **(chapter V.IV.I and V.IV.II)** we tried to specifically address this issue.

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Overweight Rather Than Malnutrition Is Widely Prevalent in Peritoneal Dialysis Patients

Ana P. Bernardo,[1] Isabel Fonseca,[2] Anabela Rodrigues,[2] Maria J. Carvalho,[2] António Cabrita[2]

Peritoneal dialysis (PD) patients seem to maintain a better nutrition status than do hemodialysis patients, and some develop overweight. The clinical relevance of overweight in PD is uncertain. We assessed nutrition status and evaluated the prevalence of overweight in PD patients, and we explored the association of overweight with demographic, clinical, and dialysis factors.

The study group included 57 patients (31.5% men; 12.3% with diabetes; mean age: 49.5 ± 14.9 years) on PD for 2.9 ± 2.7 years. Nutrition status was assessed by subjective global assessment (SGA), body mass index (BMI), and a nutrition status score (NSS) based on biochemical and anthropometric measurements.

By SGA, 70.2% of patients were classified as having a normal nutrition status; none had severe malnutrition. Based on the NSS, only 4 patients were identified as mildly-to-moderately malnourished. By BMI, 50.9% of the patients were overweight (BMI ≥ 25 kg/m^2). No relationship was found for BMI or NSS with dialysis time, Kt/V, residual renal function, or peritoneal transport. Similar results were obtained considering only overweight patients. Overweight patients had higher levels of serum albumin (p = 0.014), homocysteine (p = 0.003), and total–to–high-density lipoprotein cholesterol ratio (p = 0.048).

Instead of malnutrition, overweight was highly prevalent in our PD patients. Overweight was not associated with demographic- or modality-related factors, nor with fast transport or markers of systemic inflammation.

Key words
Nutrition status, overweight

From: [1]Nephrology Department, Hospital Amato Lusitano, Castelo Branco, and [2]Nephrology Department, Centro Hospitalar do Porto, Porto, Portugal.

Introduction

Malnutrition is common among patients with end-stage renal disease (ESRD), and it undoubtedly contributes to morbidity and mortality. An assessment of nutrition status should be part of routine care in dialysis patients, but may be particularly challenging in this population. Standard parameters of nutrition assessment are often invalid in ESRD, which leads to difficulty in identifying and assessing nutrition status (1).

In ESRD, the nutrition profile is quite different by dialysis modality. In fact, although malnutrition is common in hemodialysis patients, recent studies have demonstrated that some peritoneal dialysis (PD) patients maintain good nutrition status, and some develop overweight or obesity, depending on the assessment method used (1).

Many methods have been used to assess nutrition status in patients on dialysis. No single marker of nutrition has been judged the best to evaluate these patients. Several parameters should therefore be evaluated together, including assessment of body composition, history of weight loss, estimation of dietary protein intake, and some evaluation of protein stores. Subjective global assessment (SGA) is a useful instrument that gives a global score of protein–energy nutrition status. Anthropometry quantifies subcutaneous fat mass and muscle mass, providing reliable information about nutrition status. Finally, several biochemical markers (serum albumin, prealbumin, and transferrin) have been used to evaluate visceral protein stores and nutrition status. Of these biochemical markers, serum albumin has so far been the one most commonly used to assess malnutrition, and hypoalbuminemia has sometimes been used—often erroneously, because confounding non nutrition factors influence its serum levels—to diagnose malnutrition (1,2).

Body mass index (BMI) is used both as a measure of obesity and of malnutrition. Low BMI is well

known to be strongly associated with worse outcome in hemodialysis patients, because it reflects a poor nutrition status, which in turn is one of the major determinants of survival in that population (3). By contrast, patients who are obese seem to have a survival advantage (4). Most, but not all, studies show a similar survival benefit for patients on PD (4,5). Although this research places obesity in the dialysis population in a new light, it might be that for the individual patient on PD, obesity may still be considered a clinical menace because of cardiovascular morbidity and mortality. In the general population, dyslipidemia associated with obesity is one of the central features contributing to the increased cardiovascular risk in obese patients. There are no reports about the relevance of lipid profile in overweight and obese PD patients.

In the present study, we evaluated nutrition status and prevalence of overweight in PD patients. We also explored the relationship between overweight and demographic, clinical, and dialysis modality factors.

Patients and methods

All stable patients who were being treated in our PD unit and who had been on PD for a minimum of 3 months were eligible for inclusion in this cross-sectional study. We enrolled 57 PD patients.

Nutrition assessment

Nutrition status was assessed by SGA and BMI, and by a nutrition status score (NSS) based on biochemical and anthropometric measurements.

The SGA was performed by a single trained observer who was blinded for the biochemical results of the patients. In brief, SGA is based on a history of weight change, appetite, and gastrointestinal symptoms, and a physical examination of body fat and muscles. The four-item 7-point system was used (6,7). An SGA score of 6 – 7 indicates a well-nourished individual ("A"); 3 – 5, a mildly-to-moderately malnourished individual ("B"); and 1 – 2, a severely malnourished individual ("C").

Height was measured, body dry weight was determined, and BMI (kilograms per square meter) was calculated. Underweight was defined as a BMI below 18.5 kg/m^2, overweight was defined as a BMI in the range 25 – 30 kg/m^2, and obesity was defined as a BMI above 30 kg/m^2.

Anthropometric measurements were made on biceps, triceps, and subscapular skinfold thickness by a single observer, immediately after peritoneal dialysate drainage, using a conventional Harpenden skinfold caliper. Each measurement was repeated three times, and the average result was registered. Mid-arm circumference (MAC) was also measured, and mid-arm muscle circumference (MAMC) was calculated using the formula

$$\text{MAMC (mm)} = \text{MAC (mm)} - 3.14 \times \text{Triceps Skinfold (mm)}. \quad [1]$$

Body density was calculated based on the sum of skinfold thickness values, using the equations of Durnin and Womersley (8). Fat mass and lean body mass were obtained from calculated body density and body weight.

Nutrition status was also assessed by a NSS that consisted of eight components: BMI, percentage of ideal body weight (calculated according to Butheau and Metropolitan Life Insurance Company formulas), triceps and subscapular skinfold thickness, MAMC, serum albumin, total lymphocyte count, and subjective physical examination. Skinfold measurements and MAMC were compared with the 50th percentile for the appropriate age and sex and are expressed as a percentage (9). Each component of the NSS has a score from 3 (normal) to 6 (very severe). Thus, a score of 28 or lower denotes a normal nutrition status; higher scores are indicators of malnutrition (mild, moderate, or severe) as described in Table I.

Laboratory investigations

A fasting venous blood sample was taken before the morning exchange. Laboratory blood investigations included hemoglobin; serum albumin and prealbumin; transferrin; total, high-density lipoprotein (HDL), and low-density lipoprotein cholesterol; triglycerides; homocysteine; and C-reactive protein (CRP). All biochemical tests were performed in our hospital laboratory.

Dialysis adequacy, urea kinetics, and estimation of dietary protein intake

Adequacy of dialysis was calculated from a 24-hour urine and dialysate collection. Weekly Kt/V and creatinine clearance (CCr) were determined using standard methods (10). Residual glomerular filtration rate was calculated as the average of 24-hour

TABLE I Nutrition status score, based on biochemical and anthropometric measurements

Measure	*Normal nutrition*	*Mild*	*Malnutrition Moderate*	*Severe*
Body mass index (kg/m^2)	≥18.5	17.5–18.4	16.5–17.4	<16.5
Percentage of ideal body weight	≥90	80–89	60–79	<60
Triceps skinfold thickness (%)[a]	≥90	80–89	60–79	<60
Subscapular skinfold thickness (%)[a]	≥90	80–89	60–79	<60
Mid-arm muscle circumference (%)[a]	≥90	80–89	60–79	<60
Serum albumin (g/dL)	≥3.5	3.0–3.4	2.5–2.9	<2.5
Total lymphocyte count (*n*/μL)	≥1500	1200–1499	900–1199	<900
Physical exam (subjective)	Normal	Mild depletion	Moderate depletion	Severe depletion
Score	3	4	5	6
Overall score	≤28	29–32	33–35	>35

[a] Measurements of triceps and subscapular skinfold thicknesses and of MAMC were compared with percentile 50 for the same age and sex (9). The result was then expressed as a percentage.

urinary urea and CCr, as described elsewhere (11). Peritoneal membrane transport was calculated from a 4-hour peritoneal equilibration test (12).

Dietary protein intake was estimated from the protein equivalent of nitrogen appearance (PNA), using the PD Adequest software (Baxter Healthcare Corporation, Deerfield, IL, U.S.A.). This PNA was normalized for actual body weight to obtain the nPNA (grams per kilogram of body weight in 24 hours).

Statistical analysis

Data are expressed as mean ± standard deviation for continuous variables (or as otherwise stated) and as proportions for categorical variables. Normality of data was assessed using the Kolmogorov–Smirnov test. The independent Student *t*-test was used to ascertain differences between groups, and because CRP is not a normally-distributed variable, it was analyzed based on log-transformed CRP values. For categorical variables, differences were determined using the chi-square test. Correlations between variables were established using Pearson correlation coefficient or the Spearman rank test for small groups. A 0.05 level of significance was used in all statistical analyses, which were conducted using SPSS, version 15.0 (SPSS, Chicago, IL, U.S.A.).

Results

The study enrolled and evaluated 57 PD patients (20 men, 37 women; mean age: 49.5 ± 14.9 years). These patients had been on PD for 2.9 ± 2.7 years (range: 0.2 – 17.4 years), with 28 of the patients (49.1%) being treated with continuous ambulatory PD (CAPD) and 29 (50.9%) being treated with automated PD (APD) using a cycler. Of these patients, only 7 (12.3%) had diabetes.

The mean BMI in these patients was 24.9 ± 4.1 kg/m^2, with 26 (45.6%) being of normal weight, 25 (43.9%) being overweight, 4 (7%) being obese, and only 2 (3.5%) being underweight. Anthropometry results, classified according to sex and age, ranged across all percentiles. Table II summarizes the results. Analyzing the SGA, 40 patients (70.2%) scored an overall "A," indicating that they were well nourished. The remaining 17 patients (29.8%) scored an overall "B," indicating that they were mildly-to-moderately malnourished. No patient fell into the severely malnourished "C" category. According to the NSS, only 4 patients were identified as mildly-to-moderately malnourished.

No correlation was found for either the NSS or the BMI with time on dialysis, Kt/V, residual renal function, or peritoneal transport. Similar results were obtained considering only patients with a BMI of 25 kg/m^2 or more.

Considering BMI classification, and after excluding the 2 underweight patients, we divided the 55 remaining subjects into a "normal weight" group (n = 26) and an "overweight" group (patients with a BMI of 25 kg/m^2 or more, n = 29). No significant differences were found between the two groups in relation to age, PD duration, or PD modality. Table III shows the comparisons between the groups.

Overweight patients had higher levels of serum albumin (4.0 ± 0.4 g/dL vs. 3.7 ± 0.4 g/dL, p = 0.014), and were more frequently scored "A" than

TABLE II Skinfold thickness measurements

	Percentile[a]											
	<5		5–15		16–25		26–50		50–75		>75	
	(n)	*(%)*	*(n)*	*(%)*	*(n)*	*(%)*	*(n)*	*(%)*	*(n)*	*(%)*	*(n)*	*(%)*
Triceps skinfold thickness	6	10.5	7	12.3	7	12.3	19	33.3	9	15.8	9	15.8
Mid-arm muscle circumference	7	12.3	4	7.3	6	10.5	14	24.6	13	22.8	13	22.8

[a] According to Frisancho (9).

TABLE III Comparison of demographic, biochemical, and dialysis parameters between normal weight and overweight peritoneal dialysis patients

Variable	*Normal weight (n=26)*	*Overweight (n=29)*	p *Value*[a]
Automated peritoneal dialysis (%)	53.8	48.3	0.89
Subjective global assessment score "A" (%)	53.8	89.7	0.007[b]
Age (years)	49.2±14.9	51.5±14.1	0.56
Time on peritoneal dialysis (years)	2.5±1.7	3.2±3.4	0.31
D/P creatinine (by 3.86% PET)	0.76±0.13	0.75±0.18	0.76
Kt/V	2.2±0.96	2.3±0.5	0.65
Residual renal function (mL/min/1.73 m^3)	2.3±3.4	3.8±3.6	0.10
Serum albumin (g/dL)	3.7±0.4	4.0±0.4	0.014[b]
Daily nPCR (g/kg)	1.23±0.39	1.16±0.0.29	0.46
Serum homocysteine (µmol/L)	15.5±6.0	20.7±6.2	0.003[b]
C-Reactive protein (mg/L)[c]	0.23±0.78	0.33±1.72	0.352
Serum LDL cholesterol (mg/dL)	108.8±29.6	114.7±40.2	0.67
Serum HDL cholesterol (mg/dL)	52.5±15.5	45.6±14.4	0.09
Serum triglycerides (mg/dL)	140.8±71.0	192.1±109.4	0.047[b]
Serum lipoprotein(a) (mg/dL)	75.0±74.4	80.2±78.6	0.80
LDL/HDL cholesterol	2.26±0.89	2.70±1.09	0.11
Total/HDL cholesterol	3.85±1.14	4.54±1.38	0.048[b]

[a] Percentages compared by chi-square test; means compared by independent Student *t*-test.
[b] Significant.
[c] Expressed as a geometric mean.
D/P = dialysate-to-protein; nPCR = normalized protein catabolic rate; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

were normal-weight patients (89.7% vs. 53.8%, p = 0.007). Serum homocysteine values were more elevated in overweight patients (20.7 ± 6.2 µmol/L vs. 15.5 ± 6.0 µmol/L, p = 0.003), and we also found a higher total cholesterol/HDL cholesterol ratio (4.54 ± 1.38 vs. 3.85 ± 1.14, p = 0.048) and enhanced serum triglyceride levels (140.8 ± 71.0 mg/dL vs. 192.1 ± 109.4 mg/dL, p = 0.047) in that group. No significant differences were found in CRP between the normal-weight and overweight patients.

Discussion

Overweight was widely prevalent in our PD population, and the incidence of malnutrition was very small according to the subjective and objective methods used in this study.

It is widely accepted that many PD patients are malnourished. This acceptance results from the belief that low serum albumin concentrations reflect poor nutrition status. However a number of non nutrition factors—such as increased protein loss in dialysate, chronic or repeated infections, inflammation, hypervolemia, acidemia, and suboptimal clearance of uremic toxins—may contribute to low serum albumin (13,14). Hypoalbuminemia does not therefore necessarily indicate malnutrition as commonly assumed, which could eventually overestimate the prevalence of malnutrition in PD patients.

The most accepted methods of assessment of nutrition status in non renal patients are anthropometric measurements. Triceps skinfold and MAMC measurements are used to estimate fat mass and lean

body mass respectively. Measurements below the 15th percentile are thought to indicate poor nutrition status and reduced muscle mass. Most of our patients (77.2%) had a triceps skinfold higher than the 15th percentile, and a similar result was obtained in MAMC (80.7%). These anthropometric results corroborate the good nutrition status found in our patients by SGA.

Because of co-morbidity in chronic renal patients, the combination of objective and subjective parameters has been established as the best approach in nutrition assessment (1). In the present study, we used also a NSS based on biochemical and anthropometric parameters to assess nutrition status in our PD patients. This method produced a similar finding of well-nourished patients.

Obesity has become a national epidemic, with more than 65% of Americans currently above ideal body weight. A recent study in Portugal (15) describes a comparable finding: 53.6% of the population is overweight (BMI $\geq$ 25 kg/m^2). Modern lifestyles encourage overconsumption of energy and discourage expenditure of energy. Minor gaps (fewer than 100 kcal daily) in the balance of energy consumption and expenditure lead to a gradual but steady weight gain (16).

Conventionally, obesity is defined as body fat in excess of 25% in adult men and 35% in adult women. In Caucasians, these body fat percentages correspond to a BMI of 30 kg/m^2; a BMI of 25 kg/m^2 corresponds to body fat percentages of 20% in men and 30% in women. Accordingly, a BMI range of 25 – 29.9 kg/m^2 is defined for overweight, and 30 kg/m^2 or more indicates obesity. Considering these cutoffs, overweight was widely prevalent in our sample. As in the general population, and as reported in several other studies (17,18), the prevalence of obesity in PD patients has risen dramatically since the mid-1980s.

We found no association for overweight with demographic- or modality-related factors, nor with fast transport status. This slightly surprising result should turn our attention to the lifestyle of PD patients. In our population, the rate of peritoneal glucose absorption appears to play no role in determining obesity, suggesting that trends in the rate of obesity are related more to a reduction in energy expenditure than to an increase in caloric intake. However, we did not differentiate between overweight and normal-weight patients concerning the use of glucose-sparing solutions, which can be a limitation of our study. Nevertheless, overweight patients exhibited better indices of nutrition than did normal-weight patients with similar small-solute clearances. A particularly curious finding is that the protein catabolic rate (PCR), normalized to actual weight, although higher in the overweight group, did not reach a statistically significantly value. A possible explanation is that normalization to actual weight in obese patients creates inappropriately low values for nutrition indices, such as PCR, that are derived from urea nitrogen. We suggest that normalization of those indices by ideal weight would be preferable.

Several authors have reported an association between BMI and markers of inflammation and have concluded that obesity is a proinflammatory state in PD patients (19). We measured only CRP as an inflammatory marker, and we found no association between CRP and overweight. However, there are other inflammatory markers more related to obesity (because they are released by the adipose tissue) that were not measured in our study.

Finally, we documented a potential atherogenic lipid profile in the overweight group, which can be a risk factor for cardiovascular disease. We might be concerned about our overweight patients, given that they have an average total/HDL cholesterol ratio that is significantly higher than that in normal-weight patients, although the effects of dyslipidemia in uremic patients does not match the gloomy outcome of such a metabolic profile in a nonuremic population.

Conclusions

We can conclude that the nutrition assessment data obtained in this study underline the prevalence of overweight in the PD population. This profile is different from that of hemodialysis patients if one considers the high prevalence of reported malnutrition in that group. Whether overweight is a protective condition, associated with lower comorbidity and a lower metabolic rate, or whether it instead represents a clinical menace deserves further investigation.

References

1 Locatelli F, Fouque D, Heimbürger O, *et al*. Nutritional status in dialysis patients: a European consensus. Nephrol Dial Transplant 2002;17:563–72.
2 Hakim RM, Levin N. Malnutrition in hemodialysis patients. Am J Kidney Dis 1993;21:125–37.

3 Kopple JD, Zhu X, Lew N, Lowrie E. Body weight-for-height relationship predicts mortality in maintenance hemodialysis patients. Kidney Int 1999;56:1136–48.
4 Kalantar–Zadeh K, Abbott KC, Salahudeen AK, Kilpatrick RD, Horwich TB. Survival advantages of obesity in dialysis patients. Am J Clin Nutr 2005;81:543–54.
5 Ikizler TA. Nutrition and peritoneal dialysis. In: Mitch WE, Klahr S, eds. Handbook of nutrition and the kidney. 5th ed. Philadelphia: Lippincott, Williams and Wilkins; 2005: 228–44.
6 Enia G, Sicuso C, Alati G, Zoccali C. Subjective global assessment of nutrition in dialysis patients. Nephrol Dial Transplant 1993;8:1094–8.
7 Visser R, Dekker FW, Boeschoten EW, Stevens P, Krediet RT. Reliability of the 7-point subjective global assessment scale in assessing nutritional status of dialysis patients. Adv Perit Dial 1999;15:222–5.
8 Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77–97.
9 Frisancho AR. New norms of upper limb fat and muscle areas for assessment of nutritional status. Am J Clin Nutr 1981;34:2540–5.
10 Nolph KD, Moore HL, Twardowski ZJ, *et al*. Cross-sectional assessment of weekly urea and creatinine clearances in patients on continuous ambulatory peritoneal dialysis. ASAIO J 1992;38:M139–42.
11 Van Olden RW, Krediet RT, Struijk DG, Arisz L. Measurement of residual renal function in patients treated with continuous peritoneal dialysis. J Am Soc Nephrol 1996;7:745–8.
12 Twardowski ZJ. The fast peritoneal equilibration test. Semin Dial 1990;3:141–2.
13 Jones CH, Newstead CG, Will EJ, Smye SW, Davison AM. Assessment of nutritional status in CAPD patients: serum albumin is not a useful measure. Nephrol Dial Transplant 1997;12:1406–13.
14 Kaysen GA. Biological bases of hypoalbuminemia in ESRD. J Am Soc Nephrol 1998;9:2368–76.
15 Carmo I, Santos O, Camolas J, *et al*. Overweight and obesity in Portugal: national prevalence in 2003–2005. Obes Rev 2008;9:11–19.
16 Hill JO, Wyatt HR, Reed GW, Peters JC. Obesity and the environment: where do we go from here? Science 2003;299:852–5.
17 Johnson D. What is the optimal fat mass in peritoneal dialysis patients? Perit Dial Int 2007;27(Suppl 2):S250–4.
18 Li P, Kwan B, Szeto C, Ko G. Metabolic syndrome in peritoneal dialysis patients. Nephrol Dial Transplant 2008;4:206–14.
19 Stompór T, Sulowicz W, Dembinska–Kiec A, Janda K, Wójcik K, Zdzienicka A. An association between body mass index and markers of inflammation: is obesity the proinflammatory state in patients on peritoneal dialysis? Perit Dial Int 2003;23:79–83.

Corresponding author:
Ana Bernardo, MD, Av Pedro Álvares Cabral, Castelo Branco 6000-085 Portugal.
E-mail:
anabernardo@portugalmail.pt

V – Results

V.IV. The impact of peritoneal glucose absorption and fast transport status on obesity, insulin resistance and new cardiovascular events in PD patients.

V.IV.I Bernardo AP, Fonseca I, Oliveira JC, Santos O, Carvalho MJ, Cabrita A, Rodrigues A. Adipokines in peritoneal dialysis: relevant clinical impact according to body composition. Ther Apher Dial. 2015 Apr;19 (2):144-53. doi: 10.1111/1744-9987.12239. Epub 2014 Nov 3.

V.IV.II Bernardo AP, Oliveira JC, Santos O, Carvalho MJ, Cabrita A, Rodrigues A. Insulin Resistance in nondiabetic peritoneal dialysis patients: associations with body composition, peritoneal transport and peritoneal glucose absorption. Clin J Am Soc Nephrol. 2015 Oct 27. pii: CJN.03170315. [Epub ahead of print].

Short Summary (manuscript V.IV.I)

As in general population, overweight is widely prevalent in peritoneal dialysis patients. Being a risk factor for cardiovascular disease in general population, the clinical impacts of obesity and overweight are uncertain in peritoneal dialysis patients. Also, adipokines impact on clinical outcomes is not adequately addressed in peritoneal dialysis patients, as previous studies have not taken into account important specificities of body composition of this particular population.

We aimed to investigate the impact of leptin/adiponectin ratio (LAR) as a predictor of cardiovascular events in PD, taking into consideration patient´s body composition and the potential role of glucose load. We prospectively followed 66 patients for 47.0±28.2 months. New cardiovascular events were evaluated and body composition parameters were assessed by bioimpedance analysis.

With this clinical investigation, we were able to document that body mass index is an inaccurate method to classify obesity in PD, since it underestimates its prevalence, compared with body composition assessment using bioimpedance analysis. We confirmed our previous observation that obesity is widely prevalent in contemporary PD patients, being present in 73.4% of our population. Fat tissue index was higher in patients with cardiovascular events, but body composition parameters related with lean mass were similar between the two groups.

A link between obesity and new cardiovascular events was also documented, since the percentage of patients with obesity was significantly higher in patients who suffered new cardiovascular events compared with patients without new cardiovascular events (93.8% versus 66.7%, P=0.029). We also were able to document that LAR is determined by relative fat mass and lean tissue index, so that a higher leptin/adiponectin ratio is only significantly associated with cardiovascular events in peritoneal dialysis patients without protein-wasting.

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Adipokines in Peritoneal Dialysis: Relevant Clinical Impact According to Body Composition

Ana Paula Bernardo,[1,2] Isabel Fonseca,[1,2] José Carlos Oliveira,[3] Olivia Santos,[1] Maria João Carvalho,[1] António Cabrita,[1] and Anabela Rodrigues[1,2]

[1]Nephrology Department, [3]Clinical Pathology Department, Santo António Hospital, Oporto Hospital Center and [2]UMIB/ICBAS/Porto University, Oporto, Portugal

Abstract: Adipokines impact on clinical outcomes is not adequately addressed in peritoneal dialysis (PD). We investigated the impact of leptin/adiponectin ratio (L/A) as a predictor of cardiovascular events (CVE) in PD, taking into consideration patient's body composition and the potential role of glucose load. We prospectively followed 66 prevalent PD patients for 47.0 ± 28.2 months. New CVE were evaluated. Lean tissue index (LTI), relative fat mass (relFM) and relative overhydration (relOH) using multifrequency bioimpedance (BCM) were assessed; serum lipids, interleukin-6 (IL-6), leptin and adiponectin were measured. We established the determinants of L/A using multiple linear regression and the impact of L/A on CVE. Obesity was present in 47 (73.4%) patients according to relFM, and in seven (10.6%) according to body mass index (BMI). Leptin and L/A exhibited a stronger correlation with relFM (both r = 0.62, $P < 0.0001$) than with BMI (r = 0.46 and r = 0.51, respectively, both $P < 0.0001$). L/A showed a significant correlation with triglycerides (r = 0.41, $P = 0.001$) and HDL-cholesterol (r = –0.358, $P = 0.003$), better than isolated leptin or adiponectin. RelFM (RR = 0.130, 95%confidence interval [CI]:0.086–0.174, $P < 0.0001$) and LTI (RR = 0.194, 95%CI:0.037–0.351, $P = 0.016$) were independent predictors of L/A ($R^2 = 0.67$). Patients who suffered new CVE were older (59.12 ± 12.41 vs. 47.52 ± 13.84years, $P = 0.003$) and had a higher relOH (11.28 ± 7.29 vs. 6.60 ± 8.16%, $P = 0.028$). L/A was significantly higher in patients with CVE[2.29 (1.79) vs. 0.65 (1.73), $P = 0.028$] but this association was only put on evidence after excluding patients with wasting. BMI is an inaccurate method to classify obesity in PD since it underestimates its prevalence compared with body composition assessment using BCM. High adiponectin and low leptin are associated with a more favorable metabolic risk profile in PD. The L/A is determined by relFM and by LTI. A higher L/A is associated with CVE in PD patients without wasting. **Key Words:** Adipokines, Cardiovascular disease, Obesity, Peritoneal dialysis.

As in the general population (1,2), overweight is widely prevalent in peritoneal dialysis (PD) patients (3–5). Being a risk factor for cardiovascular disease (CVD) and death in general population (6), the clinical impacts of obesity and overweight are uncertain in PD patients (7–9). These conflicting results are partially explained by the use of body mass index (BMI) to characterize obesity. BMI is imprecise, since it does not distinguish lean body mass, fat mass and extracellular water. This characterization, being important in the general population, is even more crucial in chronic kidney disease (CKD) since those patients can present simultaneous changes in nutritional status, body fat content and hydration status (10) that have different impacts on outcome.

Adipose tissue is now recognized as a new endocrine organ, since biologically active molecules secreted by adipocytes, like leptin and adiponectin, are important determinants of inflammation, atherosclerosis and insulin-resistance (11,12). Although in the general population, high plasma leptin and low plasma adiponectin have been demonstrated to be risk factors for CVD (13–15), in CKD patients, similar evidence is less clear, as there are contradictory results about the impact of adiponectin and leptin on cardiovascular outcomes (16–21). These opposing results could be partially explained by differences in nutritional status and body composition of the studied populations that were not taken into consideration.

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Address correspondence and reprint requests to Dr Ana Paula Bernardo, MD, Largo Prof., Abel Salazar, 4099-001 Porto, Portugal. Email: anabernardo@portugalmail.pt

Leptin and adiponectin have opposite effects on the cardiovascular system and, in line with this view, two recent studies, in obese and type 2 diabetic patients without CKD, have established that leptin/adiponectin ratio (L/A) correlates better with pulse wave velocity and carotid intima-media thickness than plasma leptin and adiponectin alone (22,23). This preliminary observation suggests that L/A is a new atherogenic index, and that patients with a higher ratio could be prone to developing cardiovascular events. To our knowledge, only one study demonstrated an association between L/A and mortality in a group of non-diabetic PD patients (24). However, this study was unable to assess the patient's body composition and it did not address the potential role of glucose load and peritoneal membrane transport characteristics on adipokines profile. Low plasma leptin and high plasma adiponectin can both reflect a protein wasting condition (21,25), a diagnosis that cannot be ruled out with a simple BMI measurement, and which itself impacts patient outcome. In order to determine the impact of adipokines on CKD patient's clinical outcomes, studies must give detailed information about patient's body composition.

With the purpose to overcome this knowledge limitation, we performed a prospective study to investigate the impact of L/A as a predictor of cardiovascular events in PD patients, taking into consideration the nutritional status and body composition. We characterized body composition using multifrequency bioimpedance (BCM, Fresenius Medical Care, Bad Homburg, Germany) and we further explored the potential role of glucose load and peritoneal membrane small solute transport status on adipokines and obesity profile.

PATIENTS AND METHODS

Patients

This prospective observational study enrolled 66 PD patients attending the Centro Hospitalar do Porto—Peritoneal Dialysis Unit. All patients were treated with low glucose degradation product solutions. As a standard, only 1.36% and 2.27% glucose solutions were used. Hypertonic exchanges were used only by exception and for a short period of time.

All patients provided informed consent and the study had been approved by Centro Hospitalar do Porto Ethics Committee.

Study design

Patients were prospectively followed from December 2008 until death, renal transplantation, transfer to hemodialysis, or end of study (July 2013). Mean follow-up time was 47.0 ± 28.2 months. The primary endpoint was cardiovascular event. Transient ischemic attack and stroke were considered as cerebrovascular events. Coronary artery event was diagnosed when the patient had a myocardial infarction or an unstable angina episode. Peripheral artery disease was diagnosed in the presence of acute limb ischemia, major arterial thrombotic episode, or in the presence of intermittent claudication and significant arterial stenosis on angiography. Sudden death, in a patient with previously known coronary artery disease, was also considered as a cardiovascular event.

Laboratory measurements

Fasting venous blood samples were obtained at 9 am during a peritoneal equilibration test (PET). The preceding overnight dwell was standardized to 1.36% glucose solution.

Serum was allowed to clot at room temperature and then was separated from cells within 60 min and stored at –70°C until analysis for adiponectin, leptin and interleukin-6 (IL-6). Serum total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured with an autoanalyzer (COBAS Integra 800, Roche Diagnostics GmbH, Berlin, Germany).

Plasma leptin and adiponectin were measured using enzyme-linked immunosorbent assays (Mediagnost, Reutlingen, Germany). IL-6 was measured using enzyme-linked immunosorbent assay (Diasource, Louvain-La-Neuve, Belgium).

Dialysis adequacy, urea kinetics, peritoneal glucose absorption and estimation of dietary protein intake

Adequacy of dialysis was calculated from a 24-h urine and dialysate collection. Weekly Kt/V was determined using standard methods (26). Residual glomerular filtration rate was calculated as the average of 24-h urinary urea and creatinine as described elsewhere (27).

Small solute peritoneal membrane transport was calculated from a 4-h, 3.86% glucose peritoneal equilibration test, according to the two-in-one protocol (28).

Glucose drained during 24 h was measured. Peritoneal glucose absorption was calculated by subtracting the 24-h drained glucose from the total glucose influx by the dialysate.

Dietary protein intake was estimated from protein catabolic rate and normalized to actual body weight (nPCR) using the PD Adequest software (Baxter Healthcare Corporation, Deerfield, IL, USA).

Body composition assessment

We performed multifrequency whole body bioimpedance assessment using Body Composition Monitor (BCM, Fresenius Medical Care, Bad Homburg, Germany). The bioimpedance method applied was validated by isotope dilution methods (29), by accepted reference body composition methods (30,31) and by extensive clinical assessment of the hydration state (32). Only measurements achieving simultaneously high quality (> 90%) and lower error percentage (<35%) were considered valid. According to this we were able to analyze body composition parameters from 64 patients.

Due to biophysical reasons, bio-impedance spectroscopy does not measure sequestered fluid in the trunk (33), therefore presence or absence of PD fluid in the abdomen does not influence the reading of hydration status (34,35) nor influence body composition parameters related with nutritional status (36). BCM assessments were done with a full abdomen during the PET procedure. We assessed lean tissue index (LTI), fat tissue index (FTI), relative lean tissue mass (relLTM), relative fat mass (relFM), body cell mass (BCM), and relative over hydration (relOH).

Women with relative fat mass above 30% and men with relative fat mass above 25% were considered obese (37).

The values for LTI were compared to an age and gender-matched reference population and according to this, patients with LTI inferior to the 10th percentile were considered to have protein wasting (38).

Statistical analysis

Distribution of continuous variables was tested by Kolmogorov–Smirnov test. Data with normal distribution are expressed as mean ± standard deviation. Asymmetrically distributed data are reported as medians and interquartile ranges.

Pearson's and Spearman's correlation analyses were used, as appropriate, to assess a linear correlation between adipokines and leptin/adiponectin ratio with several metabolic, inflammatory and body composition parameters.

Continuous variables were compared between groups using Student's *t*-test, or Mann–Whitney test as appropriate. The χ^2 test was used for categorical variables. Comparison between overweight/obese and normal weight patients, according to BMI, was performed after excluding two underweight patients.

Multivariate analysis was done to identify independent predictors of L/A, considering the significant associated variables recognized by bivariate analysis. The L/A was natural log-transformed due to skewed distribution of L/A ratio.

Values of $P < 0.05$ were considered statistically significant. Statistical analyses were performed using the SPSS software application (version 21.0: SPSS, Chicago, IL, USA) for Windows.

RESULTS

Baseline characteristics of the study population

Table 1 lists baseline patient characteristics. Mean residual renal function was 4.51 ± 4.53 mL/min per 1.73 m^2, and 14 patients (21.2%) were anuric.

Mean $D/P_{creatinine}$ was 0.75 ± 0.10 and 13 patients (19.7%) were categorized as fast transporters (28). Mean BMI was 25.36 ± 4.44 kg/m^2, and mean relative fat mass was 31.9 ± 10.9% (28.9 ± 10.52% in men and 34.83 ± 10.67% in women).

The association of adipokines and L/A ratio with metabolic, inflammatory and body composition parameters

Leptin/adiponectin ratio showed a significant correlation with triglycerides (r = 0.41, $P = 0.001$) and HDL cholesterol (r = –0.358, $P = 0.003$), better than leptin or adiponectin isolated (Fig. 1).

Leptin and L/A exhibited a stronger correlation with FTI (r = 0.67 and r = 0.66, respectively, both

TABLE 1. *Baseline characteristics of the study patients*

Variable	Mean ± SD Median (Range)*
Gender (men/women)	34/32
Age (years)	50 ± 14
Diabetics [*n* (%)]	12 (18.2%)
Time on PD (months)	7.5 (119.8)*
APD/CAPD	35/31
Glucose load (g/day)	147 ± 77
Glucose absorbed (g/day)	54.5 ± 28.6
$D/P_{Creatinine}$	0.75 ± 0.10
RRF (mL/min per 1.73 m^2)	4.5 ± 4.5
Weekly Kt/V urea	2.4 ± 0.8
nPCR (g/kg)	1.12 ± 0.33
IL-6 (pg/mL)	24 (258)*
Leptin (ng/mL)	17 (172)*
Adiponectin (μg/mL)	17.5 ± 7.6
BMI (kg/m^2)	25.4 ± 4.4
relOH (%)	7.8 ± 8.3
LTI (kg/m^2)	13.4 ± 3.1
FTI (kg/m^2)	11.3 ± 5.2
relLTM (%)	54.4 ± 15.2
relFM (%)	31.9 ± 10.9

Values are expressed as frequencies for categorical variables, and as mean ± standard deviation or median and range for continuous variables. APD, automated peritoneal dialysis; BMI, body mass index; CAPD, continuous ambulatory peritoneal dialysis; FTI, fat tissue index; LTI, lean tissue index; nPCR, normalized protein catabolic rate; relFM, relative fat mass; relLTM, relative lean tissue mass; relOH, relative overhydration; RRF, residual renal function.

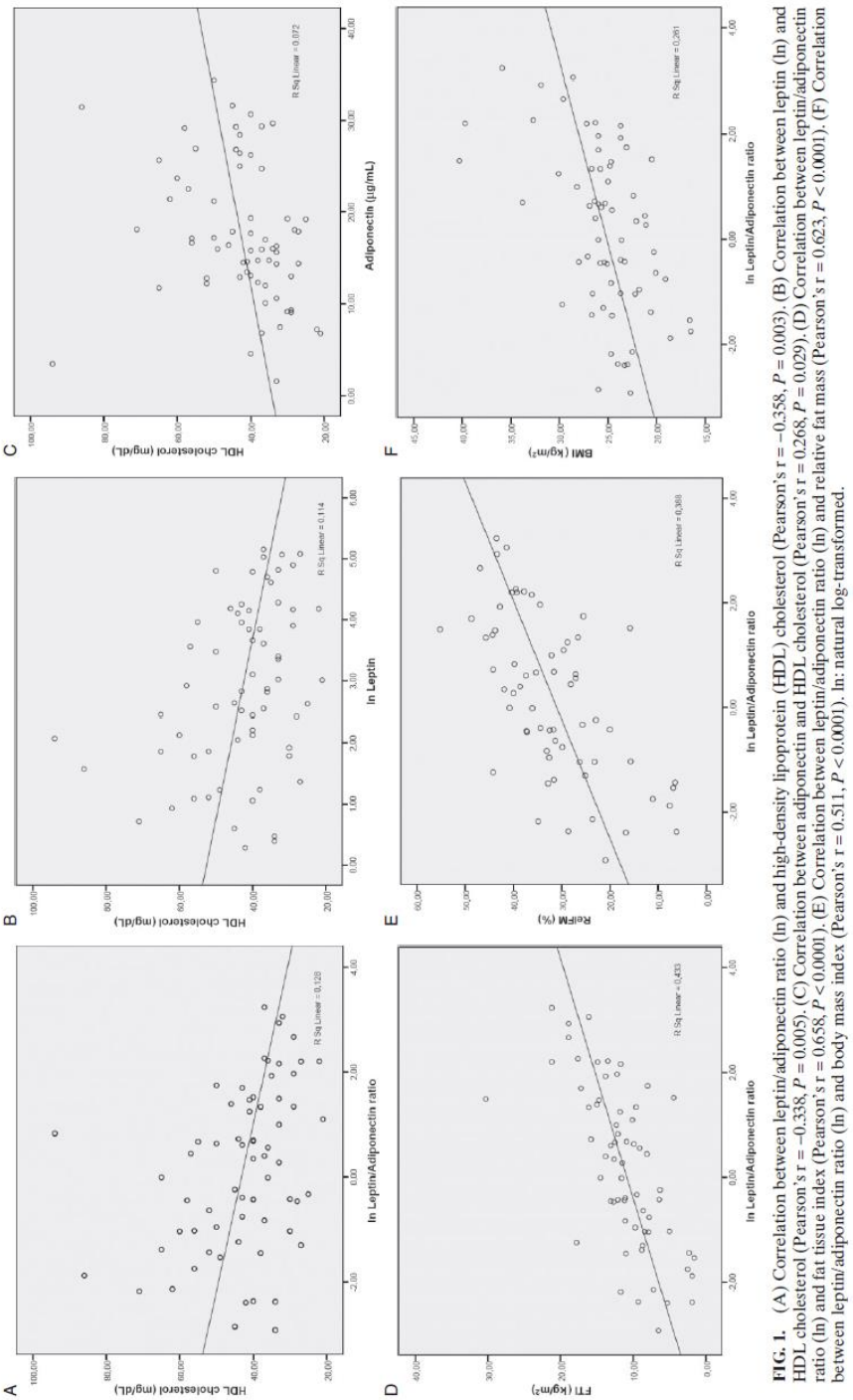

FIG. 1. (A) Correlation between leptin/adiponectin ratio (ln) and high-density lipoprotein (HDL) cholesterol (Pearson's r = −0.358, $P = 0.003$). (B) Correlation between leptin (ln) and HDL cholesterol (Pearson's r = −0.338, $P = 0.005$). (C) Correlation between adiponectin and HDL cholesterol (Pearson's r = 0.268, $P = 0.029$). (D) Correlation between leptin/adiponectin ratio (ln) and fat tissue index (Pearson's r = 0.658, $P < 0.0001$). (E) Correlation between leptin/adiponectin ratio (ln) and relative fat mass (Pearson's r = 0.623, $P < 0.0001$). (F) Correlation between leptin/adiponectin ratio (ln) and body mass index (Pearson's r = 0.511, $P < 0.0001$). ln: natural log-transformed.

TABLE 2. *Comparison between overweight/obese and normal weight patients according to body mass index (BMI) and comparison between obese and non-obese patients according to relative fat mass (relFM)*

	BMI (kg/m^2)			relFM (%)		
	>18.5 kg/m^2 <25 kg/m^2 (n = 30)	≥25 kg/m^2 (n = 34)	P	<25% (men) <30%(women) (n = 17)	≥25% (men) ≥30%(women) (n = 47)	P
Age (years)	48 ± 14	54 ± 14	0.128	43 ± 13	52 ± 14	0.029[b]
Time on PD (months)	6.8 (31.5)	8.7 (15.6)	0.856	8.2 (30.4)	6.8 (16.8)	0.820
RRF (mL/min per 1.73 m^2)	3.7 ± 3.6	5.9 ± 5.2	0.110	3.9 ± 3.7	4.8 ± 4.9	0.627
Kt/V	2.5 ± 0.6	2.3 ± 0.9	0.473	2.4 ± 0.6	2.4 ± 0.9	0.558
D/P$_{Creatinine}$	0.75 ± 0.1	0.75 ± 0.1	0.956	0.75 ± 0.1	0.75 ± 0.1	0.828
nPCR (g/kg)	1.17 ± 0.29	1.10 ± 0.4	0.521	1.26 ± 0.3	1.08 ± 0.4	0.028[b]
Glucose absorbed (g/day)	55 ± 23	52 ± 33	0.627	60 ± 22	53 ± 31	0.082
Triglycerides (mg/dL)	148 (94)	152 (141)	0.439	118 (49)	196 (120)	0.001[b]
HDL cholesterol (mg/dL)	48 ± 16	38 ± 10	0.003[a]	49 ± 14	41 ± 13	0.019[b]
Leptin (ng/mL)	12.6 (21.1)	38.0 (55.2)	0.007[a]	5.9 (10.5)	28.7 (53.9)	<0.0001[b]
Adiponectin (μg/mL)	19.6 ± 7.4	15.8 ± 7.7	0.047[a]	20.1 ± 6.1	16.3 ± 7.8	0.035[b]
L/A ratio	0.59 (1.67)	2.04 (6.97)	0.003[a]	0.35 (0.58)	1.96 (4.82)	<0.0001[b]
IL-6 (pg/mL)	20.4 (14.6)	25.0 (17.5)	0.117	16.2 (16.3)	26.1 (16.5)	0.026[b]
LTI (kg/m^2)[†]	13.2 ± 3.0	13.5 ± 3.2	0.745	16.0 ± 3.4	12.4 ± 2.5	<0.0001[b]
FTI (kg/m^2)[†]	9.0 ± 3.4	14.0 ± 5.0	<0.0001[a]	5.38 ± 2.49	13.4 ± 4.1	<0.0001[b]
BCM (kg)[†]	19.9 ± 7.2	21.0 ± 7.7	0.569	25.94 ± 8.38	18.6 ± 5.8	0.001[b]
RelOH (%)[†]	6.6 ± 8.2	9.0 ± 8.5	0.266	5.84 ± 7.61	8.5 ± 8.5	0.199
Cardiovascular events	6/30 (20%)	10/34 (29.4%)	0.564	1/17 (5.9%)	15/47 (31.9%)	0.048[b]

Values are expressed as mean ± standard deviation or median and interquartile range for continuous variables, and frequencies and percentages for categorical variables. BCM, body cell mass; BMI, body mass index; FTI, fat tissue index; LTI, lean tissue index; nPCR, protein catabolic rate; relFM, relative fat mass; relOH, relative overhydration; RRF, residual renal function. [†]Only patients with BCM measurements achieving simultaneously high quality (>90%) and lower error percentage (<35%) were considered. [a]$P < 0.05$ comparisons between overweight and normal weight patients according to body mass index using the t test if data were parametric and the Mann–Whitney U-test if nonparametric. [b] $P < 0.05$ comparisons between obese and non-obese patients according to relative fat mass (relFM) using the Mann–Whitney U-test and χ^2 test, as appropriate.

$P < 0.0001$) and relative fat mass (both r = 0.62, $P < 0.0001$) than with BMI (r = 0.46 and r = 0.51, respectively, both $P < 0.0001$) (Fig. 1).

Adiponectin was negatively correlated with FTI (r = –0.27, $P = 0.034$), and although not statistically significant there was a negative trend between adiponectin and BCM (r = –0.143), a body composition parameter that is mostly related with protein wasting.

We did not find any correlation between leptin/adiponectin ratio and other variables such as age, time on dialysis or with parameters of dialysis adequacy.

Adipokines, metabolic and inflammatory parameters comparison according to BMI and relative fat mass

According to BMI, two patients (3.0%) were classified as underweight (BMI ≤ 18.5 kg/m^2), 30 patients (45.5%) were normal weight (18.5 kg/m^2 < BMI < 25 kg/m^2), 27 patients (40.9%) were considered overweight (25 kg/m^2 ≤ BMI < 30 kg/m^2) and seven patients (10.6%) were obese (BMI ≥ 30 kg/m^2). Together, overweight and obesity were present in 34 (51.5%) patients.

Concerning relative fat mass (relFM), 47 patients (73.4%) were considered to be obese (24 women with relFM >30% and 23 men with relFM > 25%).

Time on PD, RRF, Kt/V, glucose absorption and D/P$_{creatinine}$ were not different, both in overweight/obese and normal weight groups (according to BMI), and obese and non-obese groups (according to relFM) (Table 2).

The difference between adiponectin, leptin and L/A was more significant when patients were compared according to relFM than according to BMI (Table 2).

Although IL-6 did not differ significantly between patients categorized according with BMI, IL-6 was significantly higher in obese than in non-obese patients characterized according to relFM [26.07 (16.50) pg/mL vs. 16.23 (16.28) pg/mL, $P = 0.026$].

We did not find any association between a fast transporter status and obesity, neither in patients with less than one year in PD, nor in long term patients.

There were no significant differences in the percentage of patients who presented a cardiovascular event, between overweight/obese and normal weight

TABLE 3. *Predictors of leptin/adiponectin ratio (ln) using multivariable linear regression*

	Regression coefficient	95% CI	*P*
RelFM (%)	0.130	0.086–0.174	<0.001
LTI (kg/m^2)	0.194	0.037–0.351	0.016
Glucose absorption (g/day)	–0.004	–0.014–0.007	0.501

ln, natural log-transformed. CI, confidence interval; $R^2 = 0.67$. LTI, lean tissue index; RelFM, relative fat mass.

patients, compared according to BMI (29.4% vs. 20.0%, $P = 0.564$). However, the percentage of obese patients, according to relFM, who presented a cardiovascular event was significantly higher compared with non-obese patients (31.9% vs. 5.9%, $P = 0.048$).

Determinants of leptin/adiponectin ratio

In a multivariate linear regression model adjusted for peritoneal glucose absorption, relFM and LTI were independent predictors of L/A (Table 3). This model explained 67% of the variability of L/A, and after including these three variables, no other contributed significantly to explain the L/A variability, namely relative over hydration, triglycerides and HDL cholesterol.

L/A ratio and clinical outcomes

During the follow-up period, 16 patients (24.2%) suffered cardiovascular events: 12 patients had one cardiovascular event and four patients presented two cardiovascular events. Cerebrovascular events were the most common (seven strokes and two transient ischemic attack) followed by coronary artery disease (three myocardial infarction, one unstable angina and two sudden deaths in patients with previously known coronary artery disease) and peripheral artery disease (one patient with mesenteric infarction, one patient with acute limb ischemia and three patients with intermittent claudication and significant arterial stenosis on angiography).

Patients who presented a cardiovascular event, during the follow-up period, were older (59.12 ± 12.41 vs. 47.52 ± 13.84 years, $P = 0.003$) and had a higher relOH percentage (11.28 ± 7.29 vs. 6.60 ± 8.16%, $P = 0.028$) measured by bioimpedance. Time on PD, RRF, Kt/V, $D/P_{creatinine}$, nPCR, intraperitoneal glucose absorbed, triglycerides, HDL cholesterol, IL-6, adiponectin, leptin and L/A were not significantly different between the two groups (Table 4).

Lean tissue index and BCM were not significantly different between patients with and without cardiovascular events, and the percentage of patients with

TABLE 4. *Comparison between patients with and without cardiovascular events (CVE)*

	All patients included ($n = 66$)			Excluding patients with protein wasting according to bioimpedance ($n = 40$)		
	CVE $n = 16$	No CVE $n = 50$	*P*	CVE $n = 9$	No CVE $n = 31$	*P*
Age (years)	59 ± 12	48 ± 14	0.003[a]	61 ± 10.84	47 ± 14	0.013[b]
Time on PD (months)	14.3 (40.9)	6.5 (15.6)	0.077	11.7 (37.3)	8.2 (20.7)	0.199
RRF (mL/min per 1.73 m^2)	4.1 ± 3.4	4.7 ± 4.9	0.989	5.0 ± 4.5	4.5 ± 3.4	0.606
Kt/V	2.4 ± 0.7	2.3 ± 0.9	0.636	2.3 ± 0.8	2.5 ± 0.9	0.740
$D/P_{Creatinine}$	0.74 ± 0.09	0.75 ± 0.10	0.831	0.75 ± 0.07	0.75 ± 0.09	0.935
nPCR (g/kg)	1.09 ± 0.43	1.14 ± 0.29	0.411	1.19 ± 0.59	1.23 ± 0.27	0.705
Glucose absorbed (g/day)	60.39 ± 30.04	52.66 ± 28.21	0.411	69.96 ± 32.52	51.84 ± 24.39	0.199
Triglycerides (mg/dL)	174 (109)	175 (130)	0.643	149 (111)	147 (163)	0.588
HDL cholesterol (mg/dL)	42 ± 17	43 ± 13	0.691	47 ± 21	44 ± 15	0.849
Leptin (ng/mL)	39.6 (47.5)	13.5 (56.7)	0.077	46.8 (20.7)	11.5 (30.5)	0.099
Adiponectin (μg/mL)	15.9 ± 7.3	18.1 ± 7.8	0.323	15.2 ± 8.3	18.5 ± 7.6	0.276
L/A ratio	2.1 (2.6)	0.7 (4.1)	0.070	2.29 (1.79)	0.65 (1.73)	0.028[b]
IL-6 (pg/mL)	24.7 (11.6)	23.8 (18.3)	0.510	22.9 (16.9)	21.6 (18.8)	0.483
LTI (kg/m^2)	12.6 ± 3.5	13.6 ± 2.9	0.251	14.8 ± 2.9	14.5 ± 2.9	0.503
FTI (kg/m^2)	12.9 ± 3.8	10.8 ± 5.5	0.054	11.9 ± 4.3	8.9 ± 3.9	0.064
BCM (kg)	18.9 ± 7.3	21.1 ± 7.3	0.245	23.3 ± 6.7	22.5 ± 7.8	0.656
RelOH (%)	11.3 ± 7.3	6.6 ± 8.2	0.028[a]	10.2 ± 5.6	5.3 ± 8.4	0.069

Values are expressed as mean ± standard deviation or median and interquartile range for continuous variables, and frequencies and percentages for categorical variables. BCM, body cell mass; BMI, body mass index; FTI, fat tissue index; LTI, lean tissue index; nPCR, protein catabolic rate; relOH, relative overhydration; RRF, residual renal function. [a]$P < 0.05$ comparison between patients with and without cardiovascular events using the Mann–Whitney *U*-test and χ^2 test, as appropriate. [b]$P < 0.05$ patients without protein wasting according to bioimpedance: comparison between patients with and without cardiovascular events using the Mann–Whitney *U*-test and χ^2 test, as appropriate.

an LTI value inferior to the reference range lower limit was similar between the two groups (43.8% vs. 35.4%, $P = 0.565$). Cox univariate analysis for cardiovascular events showed that LTI (used either as a categorical variable or as a continuous variable) was not a significant risk factor for cardiovascular events (Exp(B) = 0.974, 95% confidence interval (CI) [0.338–2.805], $P = 0.961$ and Exp(B) = 1.098, 95% CI [0.932–1.294], $P = 0.263$, respectively). Moreover, in a multivariate Cox analysis, LTI was not a predictor of cardiovascular events after adjustments for adipokines profile (Exp(B) = 1.131, 95% CI [0.385–3.321], $P = 0.822$).

Fat tissue index was slightly higher in patients with cardiovascular events (12.91 ± 3.81 vs. 10.75 ± 5.46 kg/m^2, $P = 0.054$). The percentage of obese patients according to relFM was significantly higher among patients with cardiovascular events (93.8% vs. 66.7%, $P = 0.029$).

Taking into account that malnutrition can be a confounding variable, and was not a high-risk factor for cardiovascular events in our population, we performed a second analysis excluding 24 patients who exhibited a LTI value inferior to the reference range lower limit (Table 4). Patients with cardiovascular events were significantly older (60.67 ± 10.84 vs. 47.42 ± 13.60 years, $P = 0.013$) and had significantly higher L/A than patients free of events [2.29 (1.79) vs. 0.65 (1.73), $P = 0.028$]. An association of the L/A ratio with cardiovascular events was also documented as a clinically relevant trend in Cox regression model analysis (Exp(B) = 6.819, 95% CI [0.837–55.558], $P = 0.07$). Although not statistically significant, patients with events had also higher FTI (11.94 ± 4.26 vs. 8.85 ± 3.95 kg/m^2) and higher prevalence of obesity defined according to relFM (88.9% vs. 54.8%, $P = 0.067$).

DISCUSSION

To our knowledge this is the first study to report the impact of adipokines on PD patient's outcome taking into consideration protein wasting and volume overload status by measuring lean mass and extracellular volume, with multifrequency bioimpedance. It highlights that nutritional parameters confound the clinical impact of adipokines profile. At the same time, this study brings some light into the conflicting results about obesity impact on PD patient outcomes.

According to BMI, 40.9% of our patients were overweight and 10.6% were obese. These results are in line with ours (3), and other (4,5) reports that state overweight as widely prevalent in PD patients, according to BMI classification. However, it is recognized that BMI does not differentiate lean mass, fat mass and extracellular water, and it is known that PD patients (while maintaining BMI) can present simultaneous changes in nutritional status, body fat content and hydration status (10), that differently impact on outcome. For that reason, studies performed in order to predict the impact of obesity or adipokines profile on patient outcomes have to fully assess patient's body composition.

In our study corporal composition was characterized using a multifrequency bioimpedance device that has recently been validated for use in everyday clinical practice (29–32). Although some recent studies using the BCM address the hydration status and volume overload issues in PD (39), there is scarce information about protein wasting and obesity characterized by body composition analysis techniques in adult PD patients. Using a single tool we were able to characterize both patient's body composition and hydration status. We defined obesity based on the most recent 2009 guidelines from the American Society of Bariatric Physicians (37). Using multifrequency bioimpedance to assess relative fat mass, we were able to show that obesity prevalence is even higher in PD patients than when using BMI classification, since it was present in 73.4% of our sample. A recent study has demonstrated, in the general population, that BMI is an inaccurate obesity classification method when compared with Dual X-ray absorptiometry measurement of relative body fat (40). Using a clinically applicable "bedside" validated method to characterize relative fat mass, we are able to demonstrate for the first time that BMI clearly underestimates obesity prevalence in PD patients.

Adipose tissue is increasingly recognized as an active endocrine organ and numerous adipokines have been implicated in the pathogenesis of chronic inflammation and insulin resistance associated with obesity (11,12). In CKD patients, adipokines accumulate in plasma, mainly because of residual renal function loss (41,42). However, fat mass is still an important determinant of leptin and adiponectin in CKD patients (41,42). In fact, we observed that both leptin and adiponectin were, respectively, positively and negatively correlated with FTI. Adipokines are also involved in the regulation of lipid and carbohydrate metabolism. As in other studies (17,43), our study evidenced significant correlations with lipid parameters. Beyond the more favorable metabolic risk profile associated with hyperadiponectinemia and the dismal metabolic profile associated with hyperleptinemia, these adipokines have also direct

opposite effects on cardiovascular system (11,12). In vitro and animal studies evidenced that leptin has different atherogenic properties and that adiponectin is an insulin-sensitizing, anti-inflammatory and anti-atherogenic adipokine (11,12). Given these different actions, plasma leptin levels were independently linked with myocardial infarction and stroke (13,14), and hypoadiponectinemia has been found to predict the development of type 2 diabetes (44) and coronary artery disease (15) in the general population.

Few studies performed on CKD patients have addressed the adipokines impact on outcome with conflicting results (16–21,24) and to our knowledge, only two have studied specifically PD patients (19,24). None of the studies have considered the nutritional and hydration status of the patients, which could explain the contradictory results obtained, since high adiponectin and low leptin can both reflect wasting, and overhydration can alter the normal regulation of adiponectin (45).

We found that cardiovascular events occurred more often in older and over-hydrated patients, which was expected (46). But the relevant input from our study was to document a significant increase of FTI and higher L/A in patients with cardiovascular events. However, such results were only put in evidence after excluding patients with protein wasting, since protein wasting can be a confounding variable that affects adipokines production. In fact, our study documented that both relFM and LTI were independent predictors of L/A, adjusting for peritoneal glucose absorption. Our results demonstrate that obesity, characterized by a higher relFM is associated with higher serum leptin and lower serum adiponectin and this will lead to a higher L/A. The opposite situation of protein malnutrition characterized by a reduced LTI is simultaneously associated with lower serum leptin and higher serum adiponectin and this will translate into a lower L/A. Given the clear biological evidence that leptin and adiponectin have opposite effects on the cardiovascular system, it has been postulated that a higher L/A could signal an imbalance between a pro-atherogenic and an anti-atherogenic molecule and those patients would be prone to cardiovascular events (22,23). Our patients who developed cardiovascular events had a higher L/A compared with patients free of events, but contrary to Park et al. (24) we did not obtain a statistically significant difference. The point is that both low leptin and high adiponectin can reflect protein wasting, therefore it was only after excluding from our analysis patients who exhibited an LTI value inferior to the lower reference range limit, that the significant association between L/A and CV events was confirmed. Our results suggest that L/A can be used as an atherogenic index in PD patients without wasting.

Because new evidence also suggests that elevated circulating adiponectin levels mirror a state of volume overload (45), we also analyzed the relOH in patients categorized according to adiponectin levels, but we did not find any statistically significant difference (data not shown).

We have also explored the potential role of peritoneal glucose absorption and fast transport status on obesity and adipokines profile. As in other studies (4,47) we were unable to prove an association between peritoneal glucose absorption and body fat mass increase, since peritoneal glucose absorbed did not significantly differ between non-obese and obese patients. Although appealing, we also did not find any association between a fast transporter status and obesity, neither in patients with less than one year in PD, nor in long term patients.

Although the small events number may have limited the power of the statistical analysis to identify independent risk factors for cardiovascular events, our study has several strengths. Knowing that wasting and volume overload can influence leptin and adiponectin plasma values, we were the first group to report the impact of adipokines on PD patient's outcome taking into consideration body composition parameters. We also explored the potential role of peritoneal glucose absorption and fast transport status on adipokines profile, an issue that was not addressed by any other group until now.

Although plasma leptin and adiponectin were measured one single time, these measurements have shown a high degree of reproducibility (48), suggesting that one single measurement may be sufficient for risk assessment.

CONCLUSIONS

Our study demonstrates that body mass index underestimates the prevalence of overweight/obesity compared with body composition assessment using multifrequency bioimpedance. High serum adiponectin and low leptin are associated with a more favorable metabolic risk profile in peritoneal dialysis. The leptin/adiponectin ratio is independently predicted by relative fat mass and lean tissue index, therefore, to evaluate the clinical impact of adipokines, details on body composition and the confounding protein wasting effect should be taken into account. A higher L/A is associated with cardiovascular events and can represent a new atherogenic index in PD patients without wasting.

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REFERENCES

1. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999–2008. *JAMA* 2010;303:235–41.
2. Carmo I, Santos O, Camolas J et al. Overweight and obesity in Portugal: national prevalence in 2003–2005. *Obes Rev* 2008;9:11–19.
3. Bernardo AP, Fonseca I, Rodrigues A, Carvalho MJ, Cabrita A. Overweight rather than malnutrition is widely prevalent in peritoneal dialysis patients. *Adv Perit Dial* 2009;25:119–24.
4. Vasselai P, Kamimura MA, Bazanelli AP et al. Factors associated with body-fat changes in prevalent peritoneal dialysis patients. *J Ren Nutr* 2008;18:363–9.
5. Locatelli F, Fouque D, Heimburger O et al. Nutritional status is dialysis patients: a European consensus. *Nephrol Dial Transplant* 2002;17:563–72.
6. Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW Jr. Body mass index and mortality in a prospective cohort of U.S. adults. *N Engl J Med* 1999;341:1097–105.
7. Aslam N, Bernardyni J, Fred L, Piraino B. Large body mass index does not predict short-term survival in peritoneal dialysis patients. *Perit Dial Int* 2002;22:191–6.
8. McDonald S, Collins JF, Johnson W. Obesity is associated with worse peritoneal dialysis outcomes in the Australia and New Zealand patient population. *J Am Soc Nephrol* 2003;14:2894–901.
9. Johnson DW, Herzing KA, Purdie DM et al. Is obesity a favorable prognostic factor in peritoneal dialysis patients? *Perit Dial Int* 2000;20:715–21.
10. Woodrow G, Devine Y, Cullen M, Lindley E. Application of bioelectrical impedance to clinical assessment of body composition in peritoneal dialysis. *Perit Dial Int* 2007;27:496–502.
11. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004;89:2548–56.
12. Rasouli N, Kern P. Adipocytokines and the metabolic complications of obesity. *J Clin Endocrinol Metab* 2008;93: S64–S73.
13. Soderberg S, Ahren B, Jansson JH et al. Leptin is associated with increased risk of miocardial infarction. *J Intern Med* 1999;246:409–18.
14. Soderberg S, Stegmayr B, Ahlbeck-Glader C, Slunga-Birgander L, Ahrén B, Olsson T. High leptin levels are associated with stroke. *Cerebrovasc Dis* 2003;15:63–9.
15. Frystic J, Berne C, Berlung L, Jensevik K, Flyvbjerg A, Zethelius B. Serum adiponectin is a predictor of coronary heart disease: a population-based 10-year follow-up study in elderly men. *J Clin Endocrinol Metab* 2007;92:571–6.
16. Becker B, Kronenberg F, Kielstein JT et al. Renal insulin resistance syndrome, adiponectin and cardiovascular events in patients with kidney disease: the mild and moderate kidney disease study. *J Am Soc Nephrol* 2005;16:1091–8.
17. Zoccali C, Mallamaci F, Tripepi G et al. Adiponectin, metabolic risk factors and cardiovascular events among patients with end-stage renal disease. *J Am Soc Nephrol* 2002;13:134–41.
18. Rao M, Li L, Tighiouart H, Jaber BL, Pereira BJ, Balakrishnan VS, HEMO Study Group. Plasma adiponectin and clinical outcomes among haemodialysis patients. *Nephrol Dial Transplant* 2008;23:2619–28.
19. Díez JJ, Estrada P, Bajo MA et al. High stable serum adiponectin levels are associated with a better outcome in prevalent dialysis patients. *Am J Nephrol* 2009;30:244–52.
20. Menon V, Li L, Wang X et al. Adiponectin and mortality in patients with chronic kidney disease. *J Am Soc Nephrol* 2006;17:2599–606.
21. Scholze A, Rattensperger D, Zidek W, Tepel M. Low serum leptin predicts mortality in patients with chronic kidney disease stage 5. *Obesity* 2007;15:1617–22.
22. Satoh N, Naruse M, Usui T et al. Leptin-to-adiponectin ratio as a potential atherogenic index in obese type 2 diabetic patients. *Diabetes Care* 2004;27:2488–90.
23. Kotani K, Sakane N, Saiga K, Kurozawa Y. Leptin:adiponectin ratio as an atherogenic index in patients with type 2 diabetes: relationship of the index to carotid intima-media thickness. *Diabetologia* 2005;48:2684–6.
24. Park JT, Yoo TH, Kim JK et al. Leptin/adiponectin ratio is an independent predictor of mortality in nondiabetic peritoneal dialysis patients. *Perit Dial Int* 2012; 33:67–74. doi:10.3747/pdi.2011.00066.
25. Park SH, Carrero JJ, Lindholm B, Stevinkel P. Adiponectin in chronic kidney disease has an opposite impact on protein-energy wasting and cardiovascular risk: two sides of the same coin. *Clin Nephrol* 2009;72:87–96.
26. Nolph KD, Moore HL, Twardowski ZJ et al. Cross-sectional assessment of weekly urea and creatinine clearances in patients on continuous ambulatory peritoneal dialysis. *ASAIO J* 1992;38:M139–42.
27. Van Olden RW, Krediet RT, Struijk DG, Arisz L. Measurement of residual renal function in patients treated with continuous peritoneal dialysis. *J Am Soc Nephrol* 1996;7:745–8.
28. Bernardo AP, Bajo MA, Santos O et al. Two-in-one protocol: simultaneous small-pore and ultrasmall-pore peritoneal transport quantification. *Perit Dial Int* 2012;32:537–44.
29. Moissl UM, Wabel P, Chamney PW et al. Body fluid volume determination via body composition spectroscopy in health and disease. *Physiol Meas* 2006;27:921–33.
30. Wabel P, Chamney P, Moissl U. Reproducibility of bioimpedance spectroscopy for the assessment of body composition and dry weight. *J Am Soc Nephrol* 2007;18:A255.
31. Moissl U, Wabel P, Chamney PW. Validation of bioimpedance spectroscopy; method for the assessment of fat free mass. *NDT Plus* 2008;1(Suppl 2):ii215. XLV Congress of the ERA-EDTA.
32. Chamney PW, Kramer M, Rode C, Kleinekofort W, Wizemann V. A new technique for establishing dry weight in hemodialysis patients via whole body bioimpedance. *Kidney Int* 2002;61:2250–8.
33. Cooper BA, Aslani A, Ryan M et al. Comparing different methods of assessing body composition in end-stage renal failure. *Kidney Int* 2000;58:408–16.
34. Parmentier SP, Schirutschke H, Schmitt B et al. Influence of peritoneal dialysis solution on measurements of fluid status by bioimpedance spectroscopy. *Int Urol Nephrol* 2013;45:229–32.
35. Lindley E, Devine Y, Hall L et al. A ward-based procedure for assessment of fluid status in peritoneal dialysis patients using bioimpedance spectroscopy. *Perit Dial Int* 2005;25(S3):S46–S48.
36. Arroyo D, Panizo N, Abad S et al. Intraperitoneal fluid overestimates hydration status assessment by bioimpedance spectroscopy. *Perit Dial Int*. doi:10.3747/pdi.2013.00187.
37. American society of bariatric physicians website. [Accessed 4 Feb 2012.] Available from URL: http://www.asbp.org/siterun_data/about_asbp/position_statements/doc72705232812956543 73.html
38. Wieskotten S, Heinke S, Wabel P et al. Bioimpedance-based identification of malnutrition using fuzzy logic. *Physiol Meas* 2008;29:639–54.

39. Devolder I, Verleysen A, Vijt D, Vanholer R, Van Biesen W. Body composition, hydration, and related parameters in hemodialysis versus peritoneal dialysis. *Perit Dial Int* 2010;30:208–14.
40. Shah NR, Baverman ER. Measuring adiposity in patients: the utility of body mass index (BMI), percent body fat, and leptin. *PLoS ONE* 2012;7:e33308. doi:10.1371.
41. Nordfors L, Lönnqvist F, Heimbürger O, Danielsson A, Schalling M, Stenvinkel P. Low leptin gene expression and hyperleptinemia in chronic renal failure. *Kidney Int* 1998;54:1267–75.
42. Huang JW, Yen CJ, Chiang HW, Hung KY, Tsai TJ, Wu KD. Adiponectin peritoneal dialysis patients: a comparison with hemodialysis patients and subjects with normal renal function. *Am J Kidney Dis* 2004;43:1047–55.
43. Tsao YT, Hsu YJ, Chu NF, Lai CH, Chiu JS, Lin SH. Association of plasma adiponectin and cardiovascular risk profiles in nondiabetic uremic patients on peritoneal dialysis. *J Nephrol* 2008;21:744–52.
44. Spranger J, Kroke A, Möhlig M et al. Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 2003;361:226–8.
45. Stenvinkel P. Adiponectin in chronic kidney diasease: a complex and context sensitive clinical situation. *J Ren Nutr* 2011;21:82–6.
46. Wizemann V, Wabel P, Chamney P et al. The mortality risk of overhydration in haemodialysis patients. *Nephrol Dial Transplant* 2009;24:1574–9.
47. Choi SJ, Kim NR, Lee WB et al. Changes in body fat mass in patients after starting peritoneal dialysis. *Perit Dial Int* 2011;31:67–73.
48. Pischon T, Hotamisligil GS, Rimm EB. Adiponectin: stability in plasma over 36 hours and within-person variation over 1 year. *Clin Chem* 2003;49:650–2.

V – Results

Short Summary (manuscript V.IV.II)

Insulin resistance has been associated with cardiovascular disease, the major cause of death in peritoneal dialysis patients. However, few studies have addressed the impact of fast transport status, dialysis prescription and nutritional status on insulin resistance development in peritoneal dialysis patients.

With this clinical investigation, we aimed to test whether insulin resistance is associated with obesity parameters, peritoneal transport rate and peritoneal glucose absorption.

We evaluated all the 51 non-diabetic patients currently treated in our Unit. Insulin resistance was evaluated with homeostasis model assessment method (HOMA-IR), HOMA correct by adiponectin (HOMA-AD) and with leptin/adiponectin ratio (LAR). Daily peritoneal glucose absorption was measured, and body composition parameters were assessed using bioimpedance analysis.

We found significant correlations between the several insulin resistance indices and body composition parameters related with fat mass in obese patients, but not in non-obese group. We didn´t found any association between insulin resistance indices and small-solute transport status, neither with peritoneal glucose absorption. An insulin resistant state was documented in 18 patients (35.3%), who presented a significant higher prevalence of obesity (84.6% versus 47.8%, P=0.04), but similar prevalence of protein-wasting (38.5% versus 13%, P=0.11). There were no significant differences in peritoneal glucose absorption, body composition parameters and insulin resistance indices between fast transporters and patients who present others peritoneal small-solute transport categories. Fat tissue index and leptin/adiponectin ratio were independent predictors of HOMA-IR, in a multivariate analysis adjusted for glucose absorption and small-solute transport ($r=0.82$, $P<0.001$).

In this clinical investigation, we were able to clarify that insulin resistance in non-diabetic peritoneal dialysis patients is associated with obesity and leptin/adiponectin ratio, independently of glucose absorption and small-solute transport status. Fast transporters, under updated therapy regimens, have no higher risk of obesity or insulin resistance.

In practice, the two studies presented in **chapter V.IV**, put in evidence that the concept of reversal epidemiology that was found in several hemodialysis populations may not be applied to PD patients. As in general population, in patients under peritoneal dialysis, obesity is related

with insulin resistance, and patients with insulin resistance, namely with higher leptin/adiponectin ratio, are more prone to suffer from cardiovascular events.

Article

Insulin Resistance in Nondiabetic Peritoneal Dialysis Patients: Associations with Body Composition, Peritoneal Transport, and Peritoneal Glucose Absorption

Ana Paula Bernardo,† Jose C. Oliveira,‡ Olivia Santos,* Maria J. Carvalho,* Antonio Cabrita,* and Anabela Rodrigues*†

*Department of Nephrology and ‡Department of Clinical Pathology, Santo António General Hospital (Hospital Center of Porto, EPE), Porto, Portugal; and †Unit for Multidisciplinary Research in Biomedicine, Abel Salazar Biomedical Sciences Institute, University of Porto, Porto, Portugal

Correspondence: Dr. Ana Paula Bernardo, Largo Prof. Abel Salazar Biomedical Sciences Institute, 4099-001 Porto, Portugal. Email: anabernardo@portugalmail.pt

Abstract

Background and objectives Insulin resistance has been associated with cardiovascular disease in peritoneal dialysis patients. Few studies have addressed the impact of fast transport status or dialysis prescription on insulin resistance. The aim of this study was to test whether insulin resistance is associated with obesity parameters, peritoneal transport rate, and glucose absorption.

Design, setting, participants, & measurements Insulin resistance was evaluated with homeostasis model assessment method (HOMA-IR), additionally corrected by adiponectin (HOMA-AD). Enrolled patients were prevalent nondiabetics attending at Santo António Hospital Peritoneal Dialysis Unit, who were free of hospitalization or infectious events in the previous 3 months (51 patients aged 50.4±15.9 years, 59% women). Leptin, adiponectin, insulin-like growth factor-binding protein 1 (IGFBP-1), and daily glucose absorption were also measured. Lean tissue index, fat tissue index (FTI), and relative fat mass (rel.FM) were assessed using multifrequency bioimpedance. Patients were categorized according to dialysis to plasma creatinine ratio at 4 hours, 3.86% peritoneal equilibration test, and obesity parameters.

Results Obesity was present in 49% of patients according to rel.FM. HOMA-IR correlated better with FTI than with body mass index. Significant correlations were found in obese, but not in nonobese patients, between HOMA-IR and leptin, leptin/adiponectin ratio (LAR), and IGFBP-1. HOMA-IR correlated with HOMA-AD, but did not correlate with glucose absorption or transport rate. There were no significant differences in insulin resistance indices, glucose absorption, and body composition parameters between fast and nonfast transporters. A total of 18 patients (35.3%) who had insulin resistance presented with higher LAR and rel.FM (7.3 [12.3, interquartile range] versus 0.7 [1.4, interquartile range], $P<0.001$, and 39.4±10.1% versus 27.2±11.5%, $P=0.002$, respectively), lower IGFBP-1 (8.2±7.2 versus 21.0±16.3 ng/ml, $P=0.002$), but similar glucose absorption and small-solute transport compared with patients without insulin resistance. FTI and LAR were independent correlates of HOMA-IR in multivariate analysis adjusted for glucose absorption and small-solute transport ($r=0.82$, $P<0.001$).

Conclusions Insulin resistance in nondiabetic peritoneal dialysis patients is associated with obesity and LAR independent of glucose absorption and small-solute transport status. Fast transport status was not associated with higher likelihood of obesity or insulin resistance.

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Introduction

Insulin resistance is already present in early stages of CKD (1), with likely contributions from uremic toxins, inflammation, malnutrition, metabolic acidosis, and vitamin D deficiency leading to acquired defects in the insulin receptor signaling pathway (2). After induction, peritoneal dialysis (PD) has been shown to initially improve insulin resistance in uremic patients, similar to hemodialysis (HD) (3). However, it is feared that with time the cumulative exposure to glucose solutions used in PD might lead to systemic hyperglycemia, obesity, and aggravate insulin resistance, which could contribute to increased cardiovascular risk (4). The incidence of overweight and obese patients is widely prevalent in contemporary PD populations (5,6), although there is much controversy about the major role of peritoneal glucose absorption on body fat accumulation over time (7,8). A single study has evidenced that insulin resistance evaluated by homeostasis model assessment (HOMA-IR) is a predictor of cardiovascular disease in PD patients (9); however, the specific role of peritoneal glucose absorption, small-solute transport, or obesity were not addressed.

Hung *et al.* (10) have shown that leptin/adiponectin ratio (LAR) and homeostasis model assessment corrected for adiponectin (HOMA-AD) were the better correlates of glucose-disposal rate measured by hyperinsulinemic euglycemic glucose clamp in chronic HD patients. LAR has been recently proposed as a new atherogenic index, since it has been associated with cardiovascular events in PD (6). However, no study has explored the association between these newly adipokine-based insulin resistance indices and the HOMA-IR in PD patients.

Therefore, we aimed to test whether insulin resistance is associated with obesity parameters, peritoneal transport rate, and glucose absorption, by using three insulin resistance indices (HOMA-IR, HOMA-AD, and LAR) and bioimpedance analysis in stable PD patients. We further evaluated insulin-like growth factor-binding protein 1 (IGFBP-1) as a marker of hepatic insulin sensitivity, and explored its association with obesity. Additionally, we evaluated the determinants of insulin resistance measured by HOMA-IR in a group of patients undergoing PD for >1 year.

Materials and Methods

Patients

This cross-sectional study enrolled all the 51 prevalent nondiabetic PD patients currently attending Santo António Hospital Peritoneal Dialysis Unit, who were free of hospitalization or infectious events in the previous 3 months. All patients were treated with low-glucose degradation product solutions; 3.86% glucose exchanges were not routinely used. Icodextrin was used in 21 patients (41.2%). None of the patients were currently taking corticosteroids. All patients provided informed consent and the study was approved by the Centro Hospitalar do Porto Ethics Committee [approval #061/12(039-DEFI/059-CES)].

Laboratory Measurements

Venous blood samples were obtained after an 8–10-hour overnight fasting period, on the morning of the peritoneal equilibration test (PET). The preceding overnight dwell was standardized to 1.36% glucose solution. Blood samples were drawn after the last dwell drainage and before starting the PET. Serum was allowed to clot at room temperature for no longer than 60 minutes and was then separated from cells by centrifugation and divided in two aliquots, one for immediate assay, the other for storage at –70°C until analysis for adiponectin, leptin, and IGFBP-1.

Creatinine was measured by the compensated Jaffe method. The dialysate creatinine concentration was corrected for interference by glucose according to our laboratory standards. Serum albumin was measured by bromocresol green dye (Cobas Integra 800, Roche Diagnostics GmbH). Insulin was measured by a sandwich assay on an electrochemiluminescence immunoassay analyzer (Cobas E 170, Roche Diagnostics GmbH). Glucose and C-reactive protein was measured using the Roche methods in a Roche autoanalyzer (COBAS Integra 800, Roche Diagnostics GmbH). Serum leptin, adiponectin, and IGFBP-1, were measured using ELISA (Mediagnost, Reutlingen, Germany).

Derived Insulin Resistance Indices

Insulin resistance was assessed using HOMA-IR, HOMA-AD, and LAR, according to the following equations (10):

$$\text{HOMA-IR} = \text{fasting serum insulin}(\mu\text{U/ml}) \times \text{fasting serum glucose}(\text{mg/dl})/405$$

$$\text{HOMA-AD} = \text{fasting serum insulin}(\mu\text{U/ml}) \times \text{fasting serum glucose}(\text{mg/dl})/\text{adiponectin}(\mu\text{g/ml})$$

$$\text{LAR} = \text{leptin}(\text{ng/ml})/\text{adiponectin}(\mu\text{g/ml}).$$

Patients with HOMA-IR ≥2.2 were considered to have insulin resistance, as this value corresponds to the HOMA-IR 50th percentile in the larger validation study about HOMA-IR against the euglycemic, hyperinsulinemic clamp in nondiabetic patients (11).

Dialysis Adequacy, Urea Kinetics, Peritoneal Glucose Absorption, and Estimation of Dietary Protein Intake

Adequacy of dialysis was calculated from 24-hour urine and dialysate collection. Weekly Kt/V was determined using standard methods (12). Residual renal function was calculated as the average of 24-hour urinary urea and creatinine, as described elsewhere (13). Small-solute peritoneal membrane transport was calculated from a 4-hour, 3.86% glucose PET according to the two-in-one protocol (14). Glucose drained over 24 hours was measured. Peritoneal glucose absorption was calculated by subtracting the 24-hour drained glucose from the total glucose influx by the dialysate. Dietary protein intake was estimated from protein catabolic rate normalized to actual body weight (nPCR) using the PD Adequest software (Baxter Healthcare Corporation, Deerfield, IL).

Body Composition Assessment

We performed multifrequency whole body bioimpedance assessment using the Body Composition Monitor (Fresenius Medical Care, Bad Homburg, Germany). The bioimpedance method applied was validated by isotope dilution methods (15), by accepted reference body composition methods (16,17), and by extensive clinical assessment of the hydration state (18). Body composition assessments were done with full abdomen during the PET procedure, taking into account the fact that the patient was weighed after draining out peritoneal effluent and thereafter 2 liters of peritoneal solution were instilled.

We measured lean tissue index (LTI), fat tissue index (FTI), relative fat mass (rel.FM), body cell mass (BCM), and relative overhydration (rel.OH). Only measurements simultaneously achieving high quality (>90%) and low error percentage (<35%), showing an inverted U curve in the device monitor, were validated.

Due to biophysic reasons, the bioimpedance spectroscopy does not measure sequestered fluid in the trunk (19), and therefore the presence or absence of PD fluid in the abdomen does not influence body composition parameters related to nutritional status (20). Women with rel.FM >30% and men with rel.FM >25% were considered obese (21).

The values for LTI were compared with an age- and gender-matched reference population and, according to this, patients with LTI <10th percentile were considered to

have protein wasting (22). Patients that simultaneously presented with rel.FM<10% and LTI <reference range were characterized as having protein-energy wasting (23).

Statistical Analyses

The data are presented as means±SD or as median with the interquartile range. Pearson and Spearman correlation analyses were used, as appropriate, to assess a linear correlation between insulin resistance indices and several metabolic, inflammatory, and body composition parameters, peritoneal glucose absorption, and small-solute transport status.

Continuous variables were compared between groups using *t*-test or Mann–Whitney *U* test, as appropriate. The chi-squared test was used for categorical variables. Comparison between obese and nonobese patients was performed according to body mass index (BMI) and according to rel.FM by bioimpedance analysis. We also performed comparisons concerning fast transport status and insulin resistance state.

Independent correlates of HOMA-IR were assessed by multivariate analysis, considering the significant associated variables recognized by bivariate analysis, and adjusted for peritoneal glucose absorption, small-solute transport, and time on PD. LAR, C-reactive protein, and leptin were natural log-transformed due to the skewed distribution. Values of $P<0.05$ were considered statistically significant. Statistical analyses were performed using SPSS software application (version 21; SPSS, Chicago, Illinois).

Results

General Features of the Study Population

Table 1 lists features of the study population. Mean dialysate to plasma ratio $(D/P)_{creatinine}$ was 0.8±0.1; 13.7% were fast transporters. Mean fasting glucose and insulin were within normal ranges. Insulin resistance was detected in 18 patients (35.3%) according to HOMA-IR. Using BMI, 20 patients (39%) were considered overweight or obese. According to rel.FM, 25 patients (49%) were obese. The nutritional spectrum included nine obese patients with sarcopenia (LTI <reference range) and two with protein-energy wasting (rel.FM<10% and LTI <reference range).

The prevalence of obesity (according to rel.FM) was significantly higher in patients on PD for >1 year (61%), than in patients on PD for <1 year (20%) (odds ratio [OR] =6.29; 95% CI, 1.50 to 26.31; P=0.01).

Table 1. Characteristics of the study patients

Variable	Mean±SD or Median (Interquartile Range)
Age, years	50.4±15.9
Time on PD, months	36.3±33.6
Sex (female), %	58.8
APD/CAPD	22/29
HOMA-IR	2.1±1.4
Fasting plasma insulin, μU/ml	9.6±5.5
Fasting plasma glucose, mg/dl	84.9±11.8
HOMA-AD	42.0±33.6
IGFBP-1, ng/ml	16.0±14.6
Adiponectin, mcg/ml	24.3±7.9
Leptin (ng/ml)	29.2 (64.2)
LAR	1.0 (3.3)
Albumin, g/dl	3.9±0.4
Prealbumin, mg/dl	34.2±7.6
nPCR, g/kg per day	1.1±0.3
C-reactive protein (mg/L)	3.1 (5.7)
$D/P_{creatinine}$	0.8±0.1
Weekly Kt/V urea	2.2±0.5
RRF, ml/min per 1.73 m^2	3.1±2.9
BMI, kg/m^2	24.0±4.1
FTI, kg/m^2	9.8±5.4
rel.FM, %	28.8±12.3
LTI, kg/m^2	13.9±3.7
rel.OH, %	6.4±9.1
Glucose load, g/day	146.5±89.2
Glucose absorbed, g/day	60.1±29.5
Causes of renal failure	**Number of patients**
Chronic glomerulonephritis (%)	28 (55)
Tubulointerstitial nephritis (%)	5 (9.8)
ADPRD (%)	4 (7.8)
CAKUT (%)	3 (5.9)
Chronic pyelonephritis (%)	3 (5.9)
CKD of unknown etiology (%)	8 (15.7)

Values are expressed as frequencies for categorical variables and as mean±SD or median (interquartile range) for continuous variables. PD, peritoneal dialysis; APD, automated peritoneal dialysis; CAPD, continuous ambulatory peritoneal dialysis; HOMA-IR, homeostasis model assessment insulin resistance index; HOMA-AD, homeostasis model assessment corrected for adiponectin; IGFBP-1, insulin-like growth factor binding protein 1; LAR, leptin/adiponectin ratio; nPCR, normalized protein catabolic rate; D/P, dialysate to plasma ratio; RRF, residual renal function; BMI, body mass index; FTI, fat tissue index; rel.FM, relative fat mass; LTI, lean tissue index; rel.OH, relative overhydration; ADPRD, autosomal dominant polycystic renal disease; CAKUT, congenital anomalies of the kidney and urinary tract.

Correlations between Insulin Resistance Indices, Small-Solute Transport, Glucose Absorption, and Body Composition Parameters

HOMA-IR correlated better with FTI than with BMI both in the general population and in the obese group (Table 2). HOMA-IR correlated strongly with HOMA-AD. Notably, significant correlations were found between HOMA-IR and leptin, and LAR and IGFBP-1 in the obese group, but not in the nonobese group (Table 2). Instead of the above positive correlation in the obese group, LAR<1 was documented in the two patients with protein-energy wasting, with high HOMA-IR.

There were no correlations between insulin resistance indices and renal function, Kt/V urea, peritoneal transport, or glucose absorption (Table 2). A significant correlation of HOMA-IR with lean mass was not found.

Metabolic and Inflammatory Parameters according to BMI and rel.FM

According to BMI, there were no differences in fasting insulin, HOMA-IR, or HOMA-AD between normal weight and obese patients. However, according to rel.FM, HOMA-IR,

Table 2. Correlations between HOMA-IR and other insulin resistance indices (HOMA-AD and LAR), small-solute transport ($D/P_{creatinine}$), glucose absorbed, and body composition parameters

	All Patients N=51		Obese Patients According to rel.FM n=25		Nonobese Patients According to rel.FM n=26	
	r	*P* Value	r	*P* Value	r	*P* Value
Age	−0.001	0.10	−0.26	0.22	0.07	0.75
Time on PD	−0.15	0.30	−0.23	0.26	−0.34	0.19
HOMA-AD	0.73	<0.001[a]	0.77	<0.001[a]	0.77	<0.001[a]
IGFBP-1	−0.43	0.01[a]	−0.56	0.01[a]	−0.28	0.25
Leptin	0.48	<0.001[a]	0.65	<0.001[a]	0.15	0.47
Adiponectin	−0.05	0.77	0.04	0.87	−0.03	0.90
LAR	0.55	<0.001[a]	0.74	<0.001[a]	−0.09	0.72
$D/P_{creatinine}$	0.06	0.68	0.16	0.50	0.24	0.26
Glucose absorbed	−0.14	0.33	−0.08	0.72	−0.22	0.29
BMI	0.44	0.001[a]	0.43	0.03[a]	0.16	0.44
FTI	0.46	0.001[a]	0.54	0.01[a]	−0.19	0.36
LTI	−0.14	0.34	−0.06	0.78	0.32	0.11

PD, peritoneal dialysis; HOMA-AD, homeostasis model assessment corrected for adiponectin; IGFBP-1, insulin-like growth factor binding protein 1; LAR, leptin/adiponectin ratio; D/P, dialysate to plasma ratio; BMI, body mass index; FTI, fat tissue index; rel.FM, relative fat mass; LTI, lean tissue index.
[a]$P<0.05$.

HOMA-AD, and fasting insulin were significantly higher in obese patients and the difference in LAR was more pronounced (Table 3). Similarly, C-reactive protein did not differ significantly between patients categorized according to BMI, but was significantly higher in obese patients characterized by rel.FM (3.6 [7.4] versus 1.3 [4.4], $P=0.03$).

Time on PD, residual renal function, Kt/V, peritoneal glucose absorption, and $D/P_{creatinine}$ did not differ significantly between groups, according to BMI and rel.FM.

Fast Transport Status Associations with Insulin Resistance, Glucose Absorption, and Body Composition Parameters

Fast transporters were more frequently prescribed automated PD (57.1% versus 40.9%, $P=0.62$) and icodextrin (71.4% versus 36.4%, $P=0.05$). There were no significant differences between fast transporters and nonfast transporters concerning fasting glucose (88.0±9.1 versus 85.0±12.0 mg/dl, $P=0.47$), fasting insulin (10.5±5.1 versus 7.8±5.7 μU/ml, $P=0.63$), HOMA-IR (2.3±1.3 versus 2.1±1.4, $P=0.55$), HOMA-AD (34.7±26.9 versus 45.4±36.2, $P=0.37$), LAR (3.1 [5.8] versus 3.8 [5.5], $P=0.37$), and IGFBP-1 (24.5±27.0 versus 12.3±8.0, $P=0.55$). PD regimens also allowed similar daily peritoneal glucose absorption. There were no differences concerning body composition parameters related both with fat mass, lean mass, and relative overhydration between fast transporters and the other small-solute transport categories. The prevalence of both wasting (28.6% versus 15.4%, $P=0.59$) and obesity (28.6% versus 48.7%, $P=0.43$) were not significantly different between the two groups.

Insulin Resistance in Prevalent Patients

Patients with >1 year on PD, classified as insulin resistant (HOMA≥2.2), presented significantly higher values of HOMA-AD and LAR (70.7±40.4 versus 23.5±11.4, and 7.3 [12.3] versus 0.7 [1.4] respectively, both $P<0.001$], and significantly lower values of IGFBP-1 (8.2±7.2 ng/ml versus 21.0±16.3 ng/ml, $P=0.002$). Body composition parameters related to fat mass were significantly higher in insulin resistant patients, while parameters related to lean mass were similar between the groups (Table 4).

The prevalence of obesity according to rel.FM was significantly higher in the insulin-resistant group than in patients with HOMA<2.2 (84.6% versus 47.8%, $P=0.04$). The prevalence of protein wasting according to bioimpedance was similar between the two groups (Table 4). Mean daily peritoneal glucose absorption was not significantly different between the two groups (Table 4).

Determinants of HOMA-IR in PD

In a multivariate linear regression model adjusted for peritoneal glucose absorption, small-solute transport, C-reactive protein, and time on PD, only FTI and LAR were independently correlated with HOMA-IR ($r=0.82$, $P<0.001$). HOMA-IR was 0.16 units higher per kg/m² of FTI (95% CI, 0.05 to 0.26, $P=0.01$) and 0.10 units higher per unit of LAR (95% CI, 0.01 to 0.2, $P=0.03$).

Discussion

To the best of our knowledge this is the first study that explores the relationships between HOMA-IR and new adipokines-based insulin resistance indices with peritoneal glucose absorption, small-solute transport, and body composition assessed by bioimpedance in PD patients. It also sheds light on the factors that predict insulin resistance in PD patients, showing the role of obesity and adipokines in this population. PD is associated with an increased risk of cardiovascular mortality after the first year (24). Though speculative, it is feared that glucose absorption could explain this outcome by promoting body fat gain and insulin resistance (2,4). Only two studies have specifically addressed the relationship of HOMA-IR with outcomes in PD (9,25).

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Table 3. Comparison between overweight/obese and normal weight patients according to BMI and comparison between obese and nonobese patients according to rel.FM

	Normal Weight N=31 18.5≤BMI<25 kg/m^2	Overweight/Obese n=20 BMI≥25 kg/m^2	*P* Value	Nonobese n=26 rel.FM<25% (Men), <30% (Women)	Obese n=25 rel.FM≥25% (Men), ≥30% (Women)	*P* Value
Time on PD, months	30.1±31.4	48.1±34.9	0.07	28.3±30.8	46.2±34.7	0.06
Age, years	45.4±114.8	59.0±14.4	0.002	44.9±14.1	56.7±15.8	0.01
Glucose, mg/dl	83.2±12.2	88.5±11.0	0.12	86.0±12.2	84.8±11.8	0.73
Insulin, μU/ml	8.6±4.6	11.6±6.3	0.06	8.0±3.6	11.6±6.5	0.02
HOMA-IR	1.8±1.1	2.6±1.6	0.06	1.7±0.9	2.5±1.6	0.04
HOMA-AD	32.9±24.3	51.6±40.9	0.09	27.5±19.2	52.4±39.0	0.01
LAR	0.7 (2.0)	1.9 (8.9)	0.01	0.4 (0.9)	2.8 (8.0)	<0.001
IGFBP-1, ng/ml	18.7±16.6	12.0±11.3	0.15	18.1±17.7	13.7±11.8	0.37
nPCR, g/kg per day	1.1±0.2	1.0±0.3	0.05	1.2±0.2	1.0±0.1	0.01
Albumin, g/dl	3.9±0.4	3.9±0.4	0.97	3.9±0.4	4.0±0.4	0.87
C-reactive protein, mg/L	3.2 (4.4)	2.8 (10.6)	0.27	1.3 (4.4)	3.6 (7.4)	0.03
Adiponectin, mcg/ml	26.2±7.3	23.2±7.2	0.21	26.9±7.0	23.2±7.3	0.11
Leptin (ng/ml)	14.7 (42.7)	49.8 (169.9)	0.01	12.8 (24.7)	53.4 (162.6)	<0.001
Weekly Kt/V urea	2.2±0.5	2.1±0.5	0.39	2.2±0.5	2.2±0.5	0.80
RRF, ml/min per 1.73 m^2	3.2±2.5	2.8±3.3	0.57	3.8±3.4	2.3±1.8	0.05
$D/P_{creatinine}$	0.8±0.1	0.8±0.1	0.49	0.8±0.1	0.7±0.1	0.02
Glucose load, g/day	128.3±74.6	168.7±100.4	0.14	134.5±74.8	154.6±98.6	0.43
Glucose absorbed, g/day	55.5±26.6	63.6±30.6	0.33	59.9±25.2	57.8±31.4	0.80
BMI, kg/m^2	22.1±1.9	28.1±3.2	<0.001	22.9±2.5	26.0±4.4	0.004
Fat mass, kg	14.2±3.6	27.1±10.2	<0.001	12.1±4.7	27.0±8.7	<0.001
FTI, kg/m^2	7.4±3.4	13.9±5.3	<0.001	6.0±2.3	14.0±4.3	<0.001
rel.FM, %	24.8±10.9	36.0±11.1	0.001	19.2±7.4	39.1±6.7	<0.001
LTI, kg/m^2	14.2±3.6	13.6±4.0	0.63	16.6±3.2	11.4±2.0	<0.001
BCM, kg	21.7±8.4	21.0±9.9	0.79	27.3±8.6	15.7±4.7	<0.001
rel.OH, %	7.3±9.6	4.80±8.06	0.34	5.1±9.2	7.5±8.8	0.35

Values are expressed as mean±SD or median (interquartile range) as appropriate. BMI, body mass index; rel.FM, relative fat mass; PD, peritoneal dialysis; HOMA-IR, homeostasis model assessment insulin resistance index; HOMA-AD, homeostasis model assessment corrected for adiponectin; LAR, leptin/adiponectin ratio; IGFBP-1, insulin-like growth factor binding protein 1; nPCR, normalized protein catabolic rate; RRF, residual renal function; D/P, dialysate to plasma ratio; FTI, fat tissue index; LTI, lean tissue index; BMC, body cell mass; rel.OH, relative overhydration.

Table 4. Differences in laboratory parameters, small-solute transport, glucose absorption, and body composition parameters according to insulin resistance in patients on PD for >1 year

	HOMA-IR<2.2 *n*=23	HOMA-IR≥2.2 *n*=13	*P* Value
Age, years	53.7±16.7	51.9±15.8	0.63
Glucose, mg/dl	83.6±10.9	91.9±10.0	0.03
Insulin, μU/ml	6.6±2.1	15.8±5.9	<0.001
HOMA-IR	1.4±0.4	3.6±2.3	<0.001
HOMA-AD	23.5±11.1	70.7±40.4	<0.001
Leptin/adiponectin ratio	0.7 (1.4)	7.3 (12.3)	<0.001
IGFBP-1, ng/ml	21.0±16.3	8.2±7.2	0.002
nPCR, g/kg per day	1.1±0.3	0.9±0.2	0.17
Albumin, g/dl	3.9±0.4	4.0±0.5	0.39
C-reactive protein (mg/L)	2.7 (5.6)	3.5 (5.1)	0.75
Adiponectin, mcg/ml	25.9±7.7	22.8±6.9	0.30
Leptin (ng/ml)	14.7 (37.2)	140.7 (228.1)	<0.001
Weekly Kt/V urea	2.2±0.5	2.0±0.5	0.11
RRF, ml/min per 1.73 m^2	2.5±3.3	2.4±1.8	0.72
$D/P_{creatinine}$	0.8±0.1	0.8±0.1	1.00
Glucose load, g/day	168.2±87.5	162.0±95.4	0.75
Glucose absorbed, g/day	62.0±25.9	63.4±35.2	0.85
BMI, kg/m^2	24.3±3.0	26.6±5.3	0.28
Fat mass, kg	18.0±8.3	28.3±12.0	0.01
FTI, kg/m^2	9.1±4.3	14.7±5.9	0.004
rel.FM, %	27.2±11.5	39.4±10.1	0.002
LTM, kg	40.2±14.6	31.5±10.6	0.12
Obesity according to rel.FM (%)	11/23 (47.8)	11/13 (84.6)	0.04
Protein wasting according to LTI (%)	3/23 (13)	5/23 (38.5)	0.11

Values are expressed as mean ±SD or median (interquartile range) as appropriate.PD, peritoneal dialysis; HOMA-IR, homeostasis model assessment insulin resistance index; HOMA-AD, homeostasis model assessment corrected for adiponectin; IGFBP-1, insulin-like growth factor binding protein 1; nPCR, normalized protein catabolic rate; RRF, residual renal function; D/P, dialysis to plasma ratio; FTI, fat tissue index; rel.FM, relative fat mass; LTM, lean tissue mass; LTI, lean tissue index.

These studies presented contradictory results and did not address the role of glucose absorption and fast transport status on insulin resistance, a major limitation. In our study, peritoneal glucose absorption was not correlated with any insulin resistance indices, nor with fat mass, and did not differ in insulin-resistant patients versus the lower HOMA-IR group. Our results also reproduce our previous observation that BMI underestimates obesity prevalence in PD (6), and that obesity increases in patients on PD for >1 year (61%). This supports other studies reporting increased fat mass with time on PD (8,26–28). However, none of the body fat mass composition parameters showed correlation with glucose absorption or fast transport status. In line with our finding, others documented a lack of association between peritoneal glucose exposure and body fat gain in patients on PD (7,29).

We also demonstrate that significant differences in HOMA-IR, HOMA-AD, and LAR were only evident when patients were compared according to rel.FM and not according BMI. Again, these results underline the impact of obesity on insulin resistance development in PD patients, as HOMA-IR, HOMA-AD, and LAR were significantly higher in obese patients compared to the results in nonobese patients, when categorized according to rel.FM.

A clinically relevant conclusion is that the relationship between insulin resistance and the adipokines ratio (LAR) is regulated by body composition parameters. We have previously reported that rel.FM and LTI predicted LAR independently of peritoneal glucose absorption (6). The results of the present study corroborate this, underlining that insulin resistance is aggravated in both extremes of the nutritional spectrum: obese patients exhibited higher LAR values, strongly positively correlated with HOMA-IR, but the two patients with protein-energy wasting also evidenced insulin resistance while LAR was <1. We measured IGFBP-1, which has recently been validated as a surrogate marker of visceral fat, and is strongly inversely related to liver fat content measured by the gold standard method, proton magnetic resonance spectroscopy (30). We report a significant negative correlation between HOMA-IR and IGFBP-1 in our population, especially in obese patients (r=–0.56, P=0.01), in line with IGFBP-1 being a marker of hepatic insulin sensitivity.

Concerning fast transport status, it is feared that higher glucose absorption could induce metabolic alterations and cardiovascular events (2). In a previous study of PD patients followed up on for 47 months, no difference was found concerning small-solute transport rate between patients with and without cardiovascular events (6). In the present study, we also did not find any significant difference concerning LAR, IGFPB-1, glucose absorption, or body composition parameters between fast transporters and other categories of small-solute transport. Only two studies have assessed the correlations between peritoneal transport and insulin resistance measured by HOMA-IR (31,32), concluding that $D/P_{creatinine}$ was not significantly associated

with HOMA-IR. Our results not only support such evidence, but expand on the knowledge regarding fast transport status systemic consequences, highlighting the fact that fast transporters under updated therapy regimens have no higher likelihood of obesity or overhydration compared with nonfast transporters. The use of new solutions, icodextrin, and short dwell times could contribute to minimizing glucose exposure and systemic effects.

Also relevant was to document that patients categorized as insulin resistant presented significantly higher adipokine-based insulin resistance indices, lower IGFBP-1, and significantly higher values of body composition parameters related with fat mass, in spite of similar BMI scores.

Leptin and adiponectin play important and opposite roles in the regulation of cardiovascular and metabolic homeostasis. We recently reported that LAR can be used as a new atherogenic index, as it is associated with cardiovascular events in PD patients without protein wasting (6). According to our present investigation this association between LAR and cardiovascular events might be explained through development of insulin resistance. In fact, given the direct actions of adiponectin on insulin sensitivity both in skeletal muscle and liver (33,34), a higher LAR can been seen not only as a biomarker of insulin resistance, but also as a predictor of insulin resistance. Our study also highlights the fact that obesity is associated with insulin resistance in PD patients, independently of peritoneal glucose absorption and fast transport status.

Our study is limited by its cross-sectional design and sample size, and for that reason we aim for a longitudinal extension of this investigation. Validation of insulin resistance indices against the euglycemic hyperinsulinemic clamp in the PD field would be valuable, since it is regarded as the reference method for insulin sensitivity assessment. However, this method is expensive and not suitable for use in clinical practice. HOMA-IR, however, is easy to perform and validated as a valuable alternative of insulin sensitivity both in non-CKD patients (11,35), in CKD patients stages III and IV (36,37), and in HD patients (10).

There are also strengths to this study. The few studies that have calculated HOMA-IR in PD patients lack any standardization of PD prescription concerning the last dwell period, before collecting blood to analyze insulin and glucose (9,25). We standardized these procedures not only in a fasting state, but also after the overnight dwell with 1.36% glucose solution, before the PET. Such a protocol minimizes any potential effect of peritoneal glucose absorption, while allowing for dialysis maintenance, as a previous study by Heaton *et al.* showed that there are no differences in fasting plasma glucose and insulin between undialyzed PD patients (with no PD solution in the abdomen) and patients on 1.36% glucose solution, after the first 60 minutes of a 1.36% glucose dwell (38). Assuming this, other investigators focused on metabolic issues, performing measurements after the 1.36% glucose solution overnight dwell (39,40).

Insulin assay can vary considerably, especially if antibodies that crossreact with insulin or split-proinsulin products are used (41): we used an insulin-specific assay, thereby minimizing the interference exerted on the HOMA-IR score. The systemic impact of peritoneal glucose absorption is scarcely addressed, and the few studies about insulin resistance in PD do not take into account the impact of protein-energy wasting and obesity in its development. In the present investigation, we have not only measured peritoneal glucose absorption, but also assessed body composition by bioimpedance method. This may suffer from methodologic errors, but is validated as a clinical tool to evaluate body composition in ESRD patients (19,20,42,43).

To summarize, our results evidence that obesity and adipocytokines profile are associated with insulin resistance in PD, independently of glucose absorption and small-solute transport status. Patients with insulin resistance, namely with higher LAR, are more prone to suffer from cardiovascular events (6). In order to possibly break such a chain of events, efforts should be put into obesity prevention, as well as correct diagnosis and treatment. One of the most feared systemic consequences of glucose absorption in PD is obesity and/or development of insulin resistance. However, fast transport status under contemporary PD regimens was not associated with obesity or insulin resistance. Current PD treatments minimize glucose exposition, and the lack of correlations between peritoneal glucose absorption and insulin resistance indices and body composition parameters puts forward evidence that other factors may contribute to a metabolic syndrome development in PD patients, perhaps in a more powerful way. Beyond genetics, future efforts should be made towards determining resting energy expenditure (44) and the role of energy intake and physical exercise.

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Disclosures

None.

References

1. Sechi LA, Catena C, Zingaro L, Melis A, De Marchi S: Abnormalities of glucose metabolism in patients with early renal failure. *Diabetes* 51: 1226–1232, 2002
2. Fortes PC, de Moraes TP, Mendes JG, Stinghen AE, Ribeiro SC, Pecoits-Filho R: Insulin resistance and glucose homeostasis in peritoneal dialysis. *Perit Dial Int* 29[Suppl 2]: S145–S148, 2009
3. Kobayashi S, Maejima S, Ikeda T, Nagase M: Impact of dialysis therapy on insulin resistance in end-stage renal disease: comparison of haemodialysis and continuous ambulatory peritoneal dialysis. *Nephrol Dial Transplant* 15: 65–70, 2000
4. Kim YL, Cho JH, Choi JY, Kim CD, Park SH: Systemic and local impact of glucose and glucose degradation products in peritoneal dialysis solution. *J Ren Nutr* 23: 218–222, 2013
5. Bernardo AP, Fonseca I, Rodrigues A, Carvalho MJ, Cabrita A: Overweight rather than malnutrition is widely prevalent in peritoneal dialysis patients. *Adv Perit Dial* 25: 119–124, 2009
6. Bernardo AP, Fonseca I, Oliveira JC, Santos O, Carvalho MJ, Cabrita A, Rodrigues A: Adipokines in peritoneal dialysis: relevant clinical impact according to body composition. *Ther Apher Dial* 19: 144–153, 2015
7. Vasselai P, Kamimura MA, Bazanelli AP, Pupim LB, Avesani CM, da Mota Ribeiro FS, Manfredi SR, Draibe SA, Cuppari L: Factors associated with body-fat changes in prevalent peritoneal dialysis patients. *J Ren Nutr* 18: 363–369, 2008

8. Fernström A, Hylander B, Moritz A, Jacobsson H, Rössner S: Increase of intra-abdominal fat in patients treated with continuous ambulatory peritoneal dialysis. *Perit Dial Int* 18: 166–171, 1998
9. Li Y, Zhang L, Gu Y, Hao C, Zhu T: Insulin resistance as a predictor of cardiovascular disease in patients on peritoneal dialysis. *Perit Dial Int* 33: 411–418, 2013
10. Hung AM, Sundell MB, Egbert P, Siew ED, Shintani A, Ellis CD, Bian A, Ikizler TA: A comparison of novel and commonly-used indices of insulin sensitivity in African American chronic hemodialysis patients. *Clin J Am Soc Nephrol* 6: 767–774, 2011
11. Bonora E, Targher G, Alberiche M, Bonadonna R, Saggiani F, Zenere MB, Monauni T, Muggeo M: Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 23: 57–63, 2000
12. Nolph KD, Moore HL, Twardowski ZJ, Khanna R, Prowant B, Meyer M, Ponferrada L: Cross-sectional assessment of weekly urea and creatinine clearances in patients on continuous ambulatory peritoneal dialysis. *ASAIO J* 38: M139–M142, 1992
13. van Olden RW, Krediet RT, Struijk DG, Arisz L: Measurement of residual renal function in patients treated with continuous ambulatory peritoneal dialysis. *J Am Soc Nephrol* 7: 745–750, 1996
14. Bernardo AP, Bajo MA, Santos O, del Peso G, Carvalho MJ, Cabrita A, Selgas R, Rodrigues A: Two-in-one protocol: simultaneous small-pore and ultrasmall-pore peritoneal transport quantification. *Perit Dial Int* 32: 537–544, 2012
15. Moissl UM, Wabel P, Chamney PW, Bosaeus I, Levin NW, Bosy-Westphal A, Korth O, Müller MJ, Ellegård L, Malmros V, Kaitwatcharachai C, Kuhlmann MK, Zhu F, Fuller NJ: Body fluid volume determination via body composition spectroscopy in health and disease. *Physiol Meas* 27: 921–933, 2006
16. Wabel P, Chamney P, Moissl U: Reproducibility of bioimpedance spectroscopy for the assessment of body composition and dry weight. *J Am Soc Nephrol* 18: A255, 2007
17. Moissl U, Wabel P, Chamney PW: Validation of bioimpedance spectroscopy method for the assessment of fat free mass. NDT Plus 1(Suppl2):ii215.XLV Congress of the ERA-EDTA, 2008
18. Chamney PW, Kramer M, Rode C, Kleinekofort W, Wizemann V: A new technique for establishing dry weight in hemodialysis patients via whole body bioimpedance. *Kidney Int* 61: 2250–2258, 2002
19. Cooper BA, Aslani A, Ryan M, Zhu FY, Ibels LS, Allen BJ, Pollock CA: Comparing different methods of assessing body composition in end-stage renal failure. *Kidney Int* 58: 408–416, 2000
20. Arroyo D, Panizo N, Abad S, Vega A, Rincón A, de José AP, López-Gómez JM. Intraperitoneal fluid overestimates hydration status assessment by bioimpedance spectroscopy. *Perit Dial Int* 35: 85–89, 2015
21. American Society of Bariatric Physicians: Overweight and obesity evaluation and management. Available at: http://www.asbp.org/siterun_data/about_asbp/position_statements/doc7270523281295654373.html. Accessed February, 14, 2012
22. Wieskotten S, Heinke S, Wabel P, Moissl U, Becker J, Pirlich M, Keymling M, Isermann R: Bioimpedance-based identification of malnutrition using fuzzy logic. *Physiol Meas* 29: 639–654, 2008
23. Fouque D, Kalantar-Zadeh K, Kopple J, Cano N, Chauveau P, Cuppari L, Franch H, Guarnieri G, Ikizler TA, Kaysen G, Lindholm B, Massy Z, Mitch W, Pineda E, Stenvinkel P, Treviño-Becerra A, Wanner C: A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. *Kidney Int* 73: 391–398, 2008
24. Johnson DW, Dent H, Hawley CM, McDonald SP, Rosman JB, Brown FG, Bannister K, Wiggins KJ: Association of dialysis modality and cardiovascular mortality in incident dialysis patients. *Clin J Am Soc Nephrol* 4: 1620–1628, 2009
25. Sanchez-Villanueva R, Estrada P, del Peso G, Grande C, Diez JJ, Iglesias P, Gonzalez E, Aguilar-Rodriguez A, Selgas R, Bajo MA; Grupo de Estudios Peritoneales de Madrid de REDINREN (Red Renal de Investigación de la RETICS 06/0016, del Instituto de Salud Carlos III); del IRSIN (Instituto Reina Sofía de Investigación Nefrológica): Repeated analysis of estimated insulin resistance using the HOMAIR index in nondiabetic patients on peritoneal dialysis and its relationship with cardiovascular disease and mortality. *Nefrologia* 33: 85–92, 2013
26. Stenvinkel P, Lindholm B, Lönnqvist F, Katzarski K, Heimbürger O: Increases in serum leptin levels during peritoneal dialysis are associated with inflammation and a decrease in lean body mass. *J Am Soc Nephrol* 11: 1303–1309, 2000
27. Søreide R, Dracup B, Svarstad E, Iversen BM: Increased total body fat during PD treatment. *Adv Perit Dial* 8: 173–176, 1992
28. Cho KH, Do JY, Park JW, Yoon KW: Effect of icodextrin dialysis solution on body weight and fat accumulation over time in CAPD patients. *Nephrol Dial Transplant* 25: 593–599, 2010
29. Fan S, Davenport A: Does increased glucose exposure lead to increased body fat and reduced lean body mass in anuric peritoneal dialysis patients? *Eur J Clin Nutr* 68: 1253–1254, 2014
30. Kotronen A, Lewitt M, Hall K, Brismar K, Yki-Järvinen H: Insulin-like growth factor binding protein 1 as a novel specific marker of hepatic insulin sensitivity. *J Clin Endocrinol Metab* 93: 4867–4872, 2008
31. Chen HY, Kao TW, Huang JW, Chu TS, Wu KD: Correlation of metabolic syndrome with residual renal function, solute transport rate and peritoneal solute clearance in chronic peritoneal dialysis patients. *Blood Purif* 26: 138–144, 2008
32. da Silva DR, Figueiredo AE, Antonello IC, Poli de Figueiredo CE, d'Avila DO: Solutes transport characteristics in peritoneal dialysis: variations in glucose and insulin serum levels. *Ren Fail* 30: 175–179, 2008
33. Rasouli N, Kern PA: Adipocytokines and the metabolic complications of obesity. *J Clin Endocrinol Metab* 93[Suppl 1]: S64–S73, 2008
34. Kershaw EE, Flier JS: Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 89: 2548–2556, 2004
35. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412–419, 1985
36. Shoji T, Emoto M, Nishizawa Y: HOMA index to assess insulin resistance in renal failure patients. *Nephron* 89: 348–349, 2001
37. Jia T, Huang X, Qureshi AR, Xu H, Ärnlöv J, Lindholm B, Cederholm T, Stenvinkel P, Risérus U, Carrero JJ: Validation of insulin sensitivity surrogate indices and prediction of clinical outcomes in individuals with and without impaired renal function. *Kidney Int* 86: 383–391, 2014
38. Heaton A, Johnston DG, Burrin JM, Orskov H, Ward MK, Alberti KGMM, Kerr DNS: Carbohydrate and lipid metabolism during continuous ambulatory peritoneal dialysis (CAPD): the effect of a single dialysis cycle. *Clin Sci (Lond)* 65: 539–545, 1983
39. Li PK, Kwan BC, Szeto CC, Ko GT: Metabolic syndrome in peritoneal dialysis patients. *NDT Plus* 1: 206–214, 2008
40. Szeto CC, Chow KM, Kwan BC, Chung KY, Leung CB, Li PK: New-onset hyperglycemia in nondiabetic chinese patients started on peritoneal dialysis. *Am J Kidney Dis* 49: 524–532, 2007
41. Robbins DC, Andersen L, Bowsher R, Chance R, Dinesen B, Frank B, Gingerich R, Goldstein D, Widemeyer HM, Haffner S, Hales CN, Jarrett L, Polonsky K, Porte D, Skyler J, Webb G, Kallagher K: Report of the American Diabetes Association's task force on standardization of the insulin assay. *Diabetes* 45: 242–256, 1996
42. Parmentier SP, Schirutschke H, Schmitt B, Schewe J, Herbrig K, Pistrosch F, Passauer J: Influence of peritoneal dialysis solution on measurements of fluid status by bioimpedance spectroscopy. *Int Urol Nephrol* 45: 229–232, 2013
43. Lindley E, Devine Y, Hall L, Cullen M, Cuthbert S, Woodrow G, Lopot F: Award-based procedure for assessment of fluid status in peritoneal dialysis patients using bioimpedance spectroscopy. *Perit Dial Int* 25[Suppl 3]: S46–S48, 2005
44. Wang X, Axelsson J, Nordfors L, Qureshi AR, Avesani C, Barany P, Schalling M, Heimbürger O, Lindholm B, Stenvinkel P: Changes in fat mass after initiation of maintenance dialysis is influenced by the uncoupling protein 2 exon 8 insertion/deletion polymorphism. *Nephrol Dial Transplant* 22: 196–202, 2007

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V – Results

V.V. Functional characterization of peritoneal membrane

V.V.I Bernardo AP, Bajo MA, Santos O, del Peso G, Carvalho MJ, Cabrita A, Selgas R, Rodrigues A. Two-in-one protocol: simultaneous small-pore and ultrasmall-pore peritoneal transport quantification. Perit Dial Int. 2012 Sep-Oct;32(5):537-44. Epub 2012 Mar 1.

V.V.II Bernardo AP, Oliveira JC, Santos O, Carvalho MJ, Cabrita A, Rodrigues A. Hepatocyte growth factor signalizes peritoneal membrane failure in peritoneal dialysis. BMC Nephrol. 2014 Dec 17;15:201. doi: 10.1186/1471-2369-15-201.

Short Summary (manuscript V.V.I)

Glucose, the main osmotic agent used in peritoneal dialysis (PD), contributes to peritoneal membrane structural changes, with time on the technique. This structural alterations will translate into functional changes, with the loss of ultrafiltration capacity of the membrane being the final step. Reduced free water transport (FWT) contributes to ultrafiltration failure and should be seen as a sign of more severe functional deterioration of the peritoneal membrane. However, the modified peritoneal equilibration test (PET) does not allow to quantify FWT trough the aquaporins.

The aim of our clinical investigation was to quantify simultaneously, in a large PD population, small solute transport, FWT and small-pore ultrafiltration using a single PET procedure. We evaluated 70 patients attending at Peritoneal Dialysis Units from Hospital de Santo António – Porto, and Hospital Universitário La Paz – Madrid. Our investigation shows that total ultrafiltration correlated better with effectively measured FWT than with any other indirect measurement of sodium sieving. FWT and total ultrafiltration, both decline with time on PD. Eleven patients were diagnosed with ultrafiltration failure, presenting significant higher small-solute transport parameters and lower FWT. The water transport trough small-pores correlates positively with FWT in patients without ultrafiltration failure, but negatively in patients with ultrafiltration failure.

In this clinical investigation we tested a new PET (the “Two-in-One Protocol”), in a large PD population, showing that measurement of FWT and small-pore ultrafiltration quantification is feasible in a single PET procedure. This study also contributes to clarify additional causes of ultrafiltration failure, beyond increased effective capillary surface. The proposed Two-in-One Protocol is a simple method for timely detection of membrane failure, capable of document the most common peritoneal membrane dysfunctions, and can be used in routine annual PD patient management, so that therapy can be properly adjusted.

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TWO-IN-ONE PROTOCOL: SIMULTANEOUS SMALL-PORE AND ULTRASMALL-PORE PERITONEAL TRANSPORT QUANTIFICATION

Ana Paula Bernardo,[1] M. Auxiliadora Bajo,[2] Olivia Santos,[1] Gloria del Peso,[2] Maria João Carvalho,[1] António Cabrita,[1] Rafael Selgas,[2]* and Anabela Rodrigues[1]*

Nephrology Department,[1] Centro Hospitalar do Porto, Hospital de Santo António, and ICBAS/UP, Porto, Portugal; and Nephrology Department,[2] La Paz University Hospital, Madrid, Spain

♦ ***Background:*** **Reduced free water transport (FWT) through ultrasmall pores contributes to net ultrafiltration failure (UFF) and should be seen as a sign of more severe functional deterioration of the peritoneal membrane. The modified peritoneal equilibration test (PET), measuring the dip in dialysate Na concentration, estimates only FWT. Our aim was to simultaneously quantify small-solute transport, FWT, and small-pore ultrafiltration (SPUF) during a single PET procedure.**

♦ ***Methods:*** **We performed a 4-hour, 3.86% glucose PET, with additional measurement of ultrafiltration (UF) at 60 minutes, in 70 peritoneal dialysis patients (mean age: 50 ± 16 years; 61% women; PD vintage: 26 ± 23 months). We calculated the dialysate-to-plasma ratios (D/P) of creatinine and Na at 0 and 60 minutes, and the Na dip ($Dip_{D/PNa60'}$), the delta dialysate Na 0–60 (ΔDNa_{0-60}), FWT, and SPUF.**

♦ ***Results:*** **Sodium sieving (as measured by ΔDNa_{0-60}) correlated strongly with the corrected $Dip_{D/PNa60'}$ ($r = 0.85$, $p < 0.0001$) and the corrected FWT ($r = 0.41$, $p = 0.005$). Total UF showed better correlation with FWT than with indirect measurements of Na sieving ($r = 0.46$, $p < 0.0001$ for FWT; $r = 0.360$, $p < 0.0001$ for $Dip_{D/PNa60'}$). Corrected FWT fraction was 0.45 ± 0.16. A negative correlation was found between time on PD and both total UF and FWT ($r = -0.253$, $p = 0.035$ and $r = -0.272$, $p = 0.023$ respectively). The 11 patients (15.7%) diagnosed with UFF had lower FWT (89 mL vs 164 mL, $p < 0.05$) and higher D/P creatinine (0.75 vs 0.70, $p < 0.05$) than did the group with normal UF. The SPUF correlated positively with FWT in the normal UF group, but negatively in UFF patients ($r = -0.709$, $p = 0.015$). Among UFF patients on PD for a longer period, 44.4% had a FWT percentage below 45%.**

♦ ***Conclusions:*** **Measurement of FWT and SPUF is feasible by simultaneous quantification during a modified 3.86% glucose PET, and FWT is a decisive parameter for detecting causes of UFF in addition to increased effective capillary surface.**

* These authors contributed equally to the paper.

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KEY WORDS: Peritoneal equilibration test; aquaporins; peritoneal transport; ultrafiltration failure.

Ultrafiltration failure (UFF) is an important cause of treatment drop-out in long-term peritoneal dialysis (PD). Heimbürger *et al.* (1) reported a cumulative risk for the development of permanent loss of net ultrafiltration (UF) of 3% after 1 year and 31% after 6 years of PD treatment. At that time, UF loss was associated mostly with a large effective peritoneal surface area (1–3) or, eventually, with a high lymphatic absorption rate (1).

Later, once aquaporins in the capillary peritoneal wall were demonstrated, some authors showed that impaired water transport through the ultrasmall pores could contribute to net UFF in PD (4,5). That finding was based on an observed reduction of sodium sieving during a 3.86% glucose peritoneal equilibration test ("modified PET"). In the first hour of a modified PET, a strong osmotic gradient over the aquaporins induces free water transport (FWT) from the capillaries to the dialysate, resulting in a decrease (by dilution) of the dialysate sodium concentration. This dip in dialysate sodium is hence an indirect method for estimating the magnitude of water transport through the ultrasmall pores. However, it is important to bear in mind that an apparent reduction in sodium sieving can also be caused by increases in peritoneal small-solute diffusion, or by a reduction in the UF coefficient (LpS) of the peritoneal membrane (6,7). Therefore, a reliable tool to quantify both peritoneal FWT and small-pore solute and fluid transport would be of great practical importance.

Correspondence to: A.P. Bernardo, Largo Prof. Abel Salazar, Porto 4099-001 Portugal.
anabernardo@portugalmail.pt
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Recently, two methods—one by La Milia *et al.* ("mini-PET") (8), and the other by Smit *et al.* (9)—have been developed to assess aquaporin-mediated water transport in PD patients. These methods have both been validated by computer simulations using the three-pore model of peritoneal transport (10). Compared with the method from Smit *et al.*, the mini-PET is simple because it does not involve sophisticated calculations or measurement of fluid absorption by calculation of the clearance of a macromolecular marker. Although extremely accurate in measuring FWT, the mini-PET does not reproduce the standardized small-solute transport categorization, because its dialysate-to-plasma ratio of creatinine (D/P Cr) measured at 60 minutes of the dwell reveals a relatively higher rate of small-solute transport than is found in a 4-hour dwell (11). Besides, it is burdensome in clinical practice to have each patient undergo different types of PET for an evaluation of membrane status. To overcome this limitation, Cnossen *et al.* (12) performed a study in which they compared, in 10 prevalent PD patients, small-solute transport assessed in a 4-hour, 3.86% glucose PET, with temporary drainage after 1 hour (allowing for quantification of FWT), concluding that the interim step did not influence the D/P Cr result. Because no further report has yet re-evaluated the clinical relevance of their approach, we aimed to extend our present investigation into diagnosing UFF in a larger population by simultaneously evaluating small-solute transport and more accurately assessing ultrasmall- and small-pore UF using a single PET procedure.

METHODS

PATIENTS AND PROCEDURES

This cross-sectional study enrolled 70 patients (29 men, 41 women; mean age: 50 ± 16 years) attending PD units at Hospital Geral de Santo António – Centro Hospitalar do Porto, Portugal, and Hospital Universitario La Paz, Spain, between January 2008 and August 2010. Mean time on PD was 26 months (range: 1 – 121 months). Of the 70 patients, 43 (61%) were on automated PD. None of the patients had peritonitis during the study or the preceding 4 weeks. The test was performed as a routine annual evaluation of membrane status without elective selection.

A 4-hour 3.86% glucose modified PET with temporary drainage at 60 minutes (13) was performed in all patients. During the procedure, we used PD solutions low in glucose degradation products [either Balance (Fresenius Medical Care, Bad Homburg, Germany) or Physioneal (Baxter Healthcare Corporation, Deerfield, IL, USA) according to the individual patient's prescription]. The volume of dialysis solution was determined by weight, without flushing the system and before filling the peritoneum. Blood and dialysate samples (each approximately 10 mL) were taken at instillation of the dialysate and after 60 and 240 minutes. At 60 minutes, we performed an additional measurement of UF by total drainage of the peritoneal cavity. This drained volume was weighed and then immediately reinfused. Finally, after 240 minutes, the peritoneal cavity was drained and the drained volume was weighed.

A PET with an ultrafiltrate volume of 400 mL or less at 4 hours was considered to represent UFF. By that definition, UFF was detected in 11 patients (15.7%), who were analyzed separately and compared with the stable group.

MEASUREMENTS

Creatinine and sodium were measured in both plasma and dialysate. Glucose was assessed in dialysate. Creatinine and glucose were measured using standard automated analyzer techniques. For creatinine, the Jaffé compensated method was used. The dialysate creatinine concentration was corrected for interference by glucose according to laboratory standards. Sodium in dialysate and plasma was measured using indirect ion-selective electrodes.

CALCULATIONS

We calculated the dialysate-to-plasma sodium (D/P_{Na}) at the beginning ($D/P_{Na0'}$) and at 1 hour ($D/P_{Na60'}$) of the study PET. The $Dip\_D/P_{Na60'}$ is the difference between the $D/P_{Na0'}$ and the $D/P_{Na60'}$. The mass transfer area coefficient for creatinine (MTAC Cr), calculated by the simplified Garred model (14,15), was used to estimate the dialysate sodium concentration attributable to diffusion (16,17). The resulting value was then subtracted from the sodium concentration measured in the dialysate at 60 minutes. Thus, we were able to calculate the $D/P_{Na60'}$ and the respective $Dip\_D/P_{Na60'}$, with and without correction for sodium diffusion, according to previous methods used to assess unit reference values (11). As a more simple clinical tool, the difference in dialysate sodium ($\Delta D_{Na0-60'}$) was also measured by subtracting the dialysate sodium at 60 minutes ($D_{Na60'}$) from the initial dialysate sodium ($D_{Na0'}$) (13).

During the study PET, FWT was calculated as follows:

FWT (mL) = Total UF volume at 60 minutes (mL) – UF through the small pores at 60 minutes (mL).

The UF through the small pores (SPUF) at 60 minutes was calculated as follows:

SPUF (mL) = [NaR (mmol) × 1000] / PNa,

where NaR (mmol) is sodium removal and PNa is the sodium concentration in plasma assessed by indirect ion-selective electrodes. The NaR was calculated as

[Dialysate V at 60 minutes (L) × Dialysate Na at 60 minutes (mmol/L)] – [Dialysate V instilled (L) × Dialysate Na at 0 minutes (mmol/L)].

Using a simple algorithm, we also performed a correction for FWT as described by Venturoli and Rippe (10):

$FWT_{corrected}$ = Total UF at 60 minutes + 15 – 0.92 × SPUF,

where the "15" represents cumulative lymphatic absorption during 60 minutes (18 mL) minus the cumulative UF through the large pores during 60 minutes (approximately 3 mL). The FWT fraction was also evaluated.

In addition, patients were characterized by peritoneal transport status as described by Twardowski *et al.* (18).

STATISTICAL ANALYSIS

Data with normal distribution are expressed as mean ± 1 standard deviation, with 95% confidence intervals. Asymmetrically distributed data are reported as medians and interquartile ranges.

Pearson and Spearman correlation analyses were used, as appropriate, to investigate possible relations between the parameters of peritoneal transport.

Comparisons of the parameters of peritoneal transport between patients on PD for less than 2 years and for 2 or more years were performed using the Student t-test or Mann–Whitney U-test (according to the variables involved).

We also used the unpaired Student t-test or Mann-Whitney U-test to compare patients with and without UFF according to the variables involved.

Values of p less than 0.05 were considered statistically significant. Statistical analyses were performed using the SPSS software application (version 15.0: SPSS, Chicago, IL, USA) for Windows.

RESULTS

SOLUTE AND FLUID TRANSPORT PARAMETERS

Table 1 summarizes the peritoneal transport characteristics from the PET performed in the 70 study patients. Notably, FWT accounted for 35.8% of the UF at 60 minutes, and once corrected (for sodium diffusion, cumulative UF volume through the large pores, and cumulative lymphatic absorption at 60 minutes), its contribution increased to a mean of 45.3%. With respect to small-solute transport characteristics, 2 patients (3%) were categorized as slow transporters (D/P Cr ≤ 0.49), 19 (27%) as slow-average (0.50 ≤ D/P Cr ≤ 0.64), 42 (60%) as fast-average (0.65 ≤ D/P Cr ≤ 0.80), and 7 (10%) as fast transporters (D/P Cr ≥ 0.81).

CORRELATIONS BETWEEN TOTAL UF, SODIUM SIEVING, AND FWT QUANTIFICATION

Total UF at 4 hours showed a better correlation with quantified FWT than with any indirect measurement of sodium sieving ($r = 0.46$, $p < 0.0001$ for FWT; $r = 0.48$, $p < 0.0001$ for $FWT_{corrected}$; $r = -0,29$, $p < 0.0001$ for $D/P_{Na60'}$; $r = -0.45$, $p < 0.0001$ for $D/P_{Na60'corrected}$; $r = 0.36$, $p < 0.0001$ for $Dip\_D/P_{Na60'}$; $r = 0.44$, $p < 0.0001$ for $Dip\_D/P_{Na60'corrected}$; and $r = 0.32$, $p = 0.005$ for $\Delta D_{Na0-60'}$).

TABLE 1
Peritoneal Transport Characteristics in 70 Stable Patients Assessed During a 4-Hour, 3.86% Glucose Peritoneal Equilibration Test with Temporary Drainage at 60 Minutes

Variable	Value	
	Mean±SD	95% CI
Total UF at 4 h (mL)	665.73 ± 235.39	609.60 to 721.85
UF at 1 h (mL)	425.25 ± 21.74	381.87 to 468.63
SPUF (mL)	274.03 ± 133.32	240.28 to 304.45
FWT (mL)	152.14 ± 88.51	131.52 to 174.25
$FWT_{corrected}$ (mL)	189.03 ± 92.09	167.39 to 211.89
%FWT	35.79 ± 17.59	31.8 to 40.24
$\%FWT_{corrected}$ (mL)	45.34 ± 16.58	41.39 to 49.29
MTAC Cr (mL/min)	9.15 ± 4.11	8.13 to 10.11
D/P Cr	0.70 ± 0.10	0.68 to 0.73
D_{240}/D_0 glucose	0.35 ± 0.15	0.32 to 0.39
$D/P_{Na60'}$	0.89 ± 0.04	0.88 to 0.91
$D/P_{Na60'corrected}$	0.84 ± 0.06	0.82 to 0.86
$Dip\_D/P_{Na60'}$	0.04 ± 0.02	0.03 to 0.04
$Dip\_D/P_{Na60'corrected}$	0.10 ± 0.05	0.09 to 0.11

SD = standard deviation; CI = confidence interval; UF = ultrafiltration; SPUF = UF through the small pores at 60 minutes; FWT = free water transport at 60 minutes; $FWT_{corrected}$ = FWT with an algorithm correction according to Venturoli *et al.* (10); MTAC = mass transfer-area coefficient; Cr = creatinine; D/P = dialysate-to-plasma ratio; D_{240}/D_0 = end (240 min)–to–initial dialysate ratio; $D/P_{Na60'}$ = D/P sodium at 60 minutes; $D/P_{Na60'corrected}$ = D/P sodium at 60 minutes corrected for diffusion; $Dip\_D/P_{Na60'}$ = difference between initial and 60-minute D/P sodium; $Dip\_D/P_{Na60'corrected}$ = difference between initial and 60-minute D/P Na, corrected for diffusion.

We found a significant correlation between sodium sieving measurements and FWT quantification, especially when FWT was corrected for diffusion [Figure 1(A,B)]. Additionally, a simple tool such as $\Delta D_{Na0-60'}$ correlated strongly with the corrected dip [$r = 0.85$, $p < 0.0001$, Figure 1(C)] and with FWT ($r = 0.41$, $p < 0.0001$), which is relevant for routine bedside evaluation.

SHORT- COMPARED WITH LONG-TERM PD TREATMENT

Of the study patients, 41 (59%) had been on PD for fewer than 2 years (short-term PD), and 29 patients (41%) had been on PD for 2 or more years (long-term PD). Although none of the comparisons were statistically significant, the long-term group had lower FWT and total UF values (data not shown). However, we observed a statistically significant trend to achieve less total UF and less FWT over time on PD [Figure 2(A,B)].

UFF AND WATER TRANSPORT PATHWAYS

Among the 70 study patients, 11 (15.7%) had UFF (total UF ≤ 400 mL). Although the difference was not statistically significant, patients with normal UF had been on PD treatment for a shorter period of time (Table 2). Compared with stable patients, patients with UFF had a significantly lower mean FWT, a higher D/P Cr, and a higher MTAC Cr (Table 2). Among 9 patients with UFF who had been on PD for more than 18 months (82%), 2 (22.2%) were fast transporters, and 4 (44.4%) had a FWT percentage corrected for sodium diffusion of less than 45% (the mean for the entire population). Notably, as Figure 3 shows, SPUF correlated positively with FWT in the non-UFF group ($r = 0.257$, $p = 0.032$), but more strongly and negatively in the UFF group ($r = -0.709$, $p = 0.015$).

DISCUSSION

To our knowledge, the present study is the first to report simultaneous small-pore and ultrasmall-pore peritoneal transport quantification in a large PD population through the use of a 4-hour 3.86% glucose PET with temporary drainage at 60 minutes. The UF quantification at 60 minutes allows for FWT to be measured as first described by La Milia and colleagues (8). Our study expands the work of the La Milia group, because the 4-hour PET with the added procedure allows for both FWT and small-pore UF to be measured at the same time that peritoneal solute transport is being quantified in a standardized way, without the overestimation that might occur when transport categorization is based on a D/P Cr obtained after a 60-minute dwell (11).

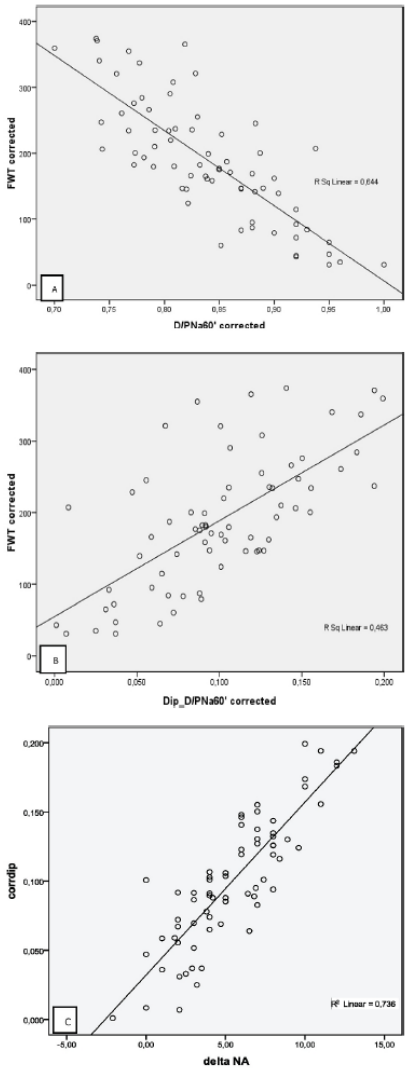

Figure 1 — Correlations between (A) the dialysate-to-plasma (D/P) sodium at 60 minutes corrected for diffusion ($D/P_{Na60'corrected}$) and free water transport with an algorithm correction ($FWT_{corrected}$) according to Venturoli and Rippe (10) (Pearson $r = -0.80$, $p < 0.0001$); (B) the difference ("delta") of the initial D/P sodium ($D/P_{Na0'}$) and the 60-minute D/P sodium corrected for diffusion ($Dip\_D/P_{Na60'corrected}$) and $FWT_{corrected}$ according to Venturoli and Rippe (10) (Pearson $r = 0.68$, $p < 0.0001$); and (C) the corrected dip and delta sodium (Pearson $r = 0.85$, $p < 0.0001$).

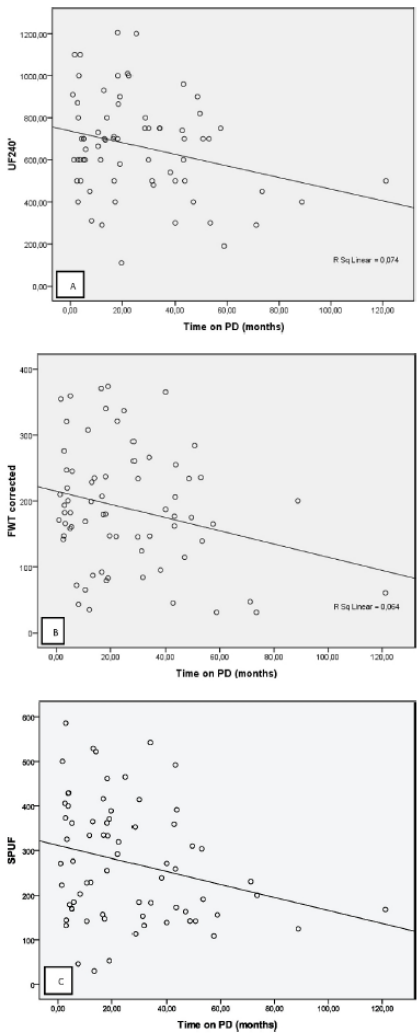

Figure 2 — Simultaneous small-pore and ultrasmall-pore peritoneal transport evaluation and its profile with time on peritoneal dialysis (PD). Correlations between (A) total ultrafiltration ($UF_{240'}$) and time on PD (Pearson $r = -0.272$, $p = 0.023$); (B) free water transport with an algorithm correction ($FWT_{corrected}$) according to Venturoli and Rippe (10) and time on PD (Pearson $r = -0.253$, $p = 0.035$); and (C) small-pore ultrafiltration (SPUF) with time on PD (Pearson $r = -0.243$ $p = 0.003$).

Even when no correction was made for sodium diffusion, the results obtained for FWT (mean FWT at 60 minutes: 152 mL) were quite similar to those obtained with a more accurate and sophisticated method that used a volume marker [mean FWT at 60 minutes: 135 mL according to Smit *et al.* (9); median FWT at 60 minutes: 154 mL according to Parikova *et al.* (19)]. We also emphasize that the results obtained regarding the contribution of FWT to 60-minute UF are in line with those reported by La Milia—mean $FWT_{corrected}$ being 45% in our study, and mean FWT percentage being 46% in the report by La Milia *et al.* (8). Also, skilled studies from Waniewski *et al.*, whatever the adjustments and methodology used, found a FWT fraction of 0.40 ± 0.12, which is equivalent to the fraction that we documented (20,21).

The fact that FWT correlates better with total UF than with any indirect measure of sodium sieving is evidence that reduced water flow through aquaporins is somehow not the only factor connected with reduced sodium sieving (7). As expected, the algorithm correction for sodium diffusion that we used allowed us to correct some underestimation of FWT because of the presence of sodium diffusion from the circulation (10). To correct the calculations for diffusive sodium transport, the MTAC Cr estimated using the simplified Garred formula [following the methodology previously published by the Krediet group (16)], was also used by Asghar and Davies (22) to investigate peritoneal fluid pathways. Although the simplified Garred model may be less accurate than the Waniewski model, the good correlation between these two models allows for the use of the former model in routine clinical evaluation (11). However, we suggest that peritoneal transport quantification can rely on a two-in-one protocol of 4-hour PET that simultaneously provides information on D/P creatinine (at 4 hours) and 60-minute FWT quantification, avoiding correction formulas and indirect estimates of sodium sieving that have been found to be less discriminative.

We also found a slight, but consistent, tendency for FWT to decline with time on PD. Such impairment might occur as a consequence of interstitial membrane changes, lowering the capacity of glucose to exert its osmotic effect on the ultrasmall pores. To explain the apparently "paradoxical" observation that, although FWT was lower in patients with UFF than in those without UFF, the FWT percentage simultaneously failed to reach statistical significance when those groups were compared, it must be emphasized that the decrease in aquaporin function indeed cannot be cited as the single reason for the FWT decrease. Because of dissipation of the glucose osmotic gradient, FWT depends on and is inversely correlated with increased MTAC Cr—the more common cause of UFF. Our results accord with those of Parikova *et al.* (23): that is, by analyzing fluid transport pathways and their determinants in patients with UFF, documented early-stage UFF is

associated with a decline in FWT dependent on increased effective capillary surface area without significant decrease of FWT contribution. Later, aquaporin dysfunction and additional interstitial changes combine to cause a significant decline in the FWT fraction with loss of glucose osmotic conductance. Interestingly, we found that

TABLE 2
Simultaneous Small-Pore and Ultrasmall-Pore Peritoneal Transport Evaluation for Peritoneal Dialysis (PD) Patients With and Without Ultrafiltration (UF) Failure

	Ultrafiltration failure			
	Patients without		Patients with	
	Mean±SD	95% CL	Mean±SD	95% CL
Patients (*n*)	59		11	
Time on PD (months)	23.43±21.65	17.79, 29.08	38.15±28.25	19.17, 57.14
Total UF at 4 h (mL)	732.40±188.78[a]	683.19, 781.59	308.18±93.68[a]	245.25, 371.12
UF at 1 h (mL)	453.25±179.89[a]	406.37, 500.13	280.91±83.27[a]	224.97, 336.85
SPUF (mL)	289.43±136.50[b]	253.86, 325.00	191.44±75.39[b]	140.79, 242.08
FWT (mL)	163.83±87.03[b]	141.14, 186.51	89.47±70.69[b]	41.98, 136.96
$FWT_{corrected}$ (mL)	201.96±90.50[c]	178.37, 225.54	119.73±68.85[c]	73.47, 165.98
%FWT	36.96±16.29	32.71, 41.20	29.53±23.33	13.86, 45.20
$\%FWT_{corrected}$ (mL)	46.17±15.79	42.06, 50.29	40.88±20.59	27.04, 54.71
MTAC Cr (mL/min)	8.74±4.07[b]	7.68, 9.80	11.38±3.70[b]	8.90, 13.87
D/P Cr	0.70±0.10[b]	0.67, 0.72	0.75±0.07[b]	0.70, 0.80
D_{240}/D_0 glucose	0.37±0.16	0.33, 0.41	0.27±0.03	0.25, 0.30
$D/P_{Na60'}$	0.90±0.04[b]	0.89, 0.90	0.93±0.04[b]	0.90, 0.95
$D/P_{Na60'corrected}$	0.83±0.06[b]	0.81, 0.85	0.89±0.07[b]	0.85, 0.94
$Dip\_D/P_{Na60'}$	0.04±0.02	0.03, 0.05	0.03±0.02	0.02, 0.05
$Dip\_D/P_{Na60'corrected}$	0.11±0.04[b]	0.10, 0.12	0.06±0.05[b]	0.03, 0.09

SD = standard deviation; CL = confidence limits; SPUF = UF through the small pores at 60 minutes; FWT = free water transport at 60 minutes; $FWT_{corrected}$ = FWT with an algorithm correction according to Venturoli and Rippe (10); MTAC = mass transfer-area coefficient; Cr = creatinine; D/P = dialysate-to-plasma ratio; D_{240}/D_0 = end (240 min)–to–initial dialysate ratio; $D/P_{Na60'}$ = D/P sodium at 60 minutes; $D/P_{Na60'corrected}$ = D/P sodium at 60 minutes corrected for diffusion; $Dip\_D/P_{Na60'}$ = difference between initial and 60-minute D/P sodium; $Dip\_D/P_{Na60'corrected}$ = difference between initial and 60-minute D/P Na, corrected for diffusion.
[a] t-Test $p < 0.0001$, comparing patients with and without ultrafiltration failure.
[b] t-Test $p < 0.05$, comparing patients with and without ultrafiltration failure.
[c] t-Test $p < 0.005$, comparing patients with and without ultrafiltration failure.

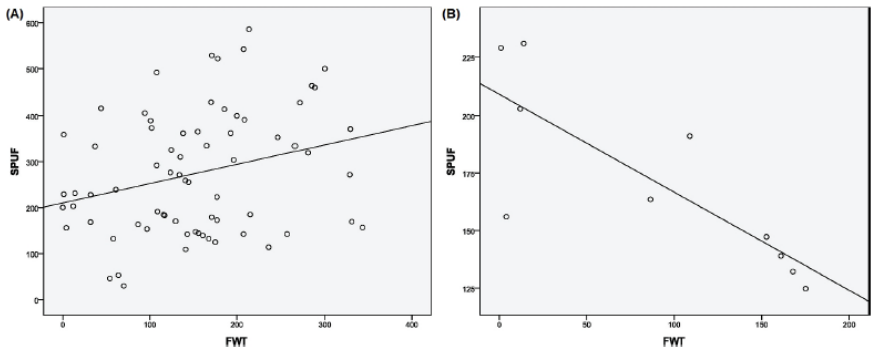

Figure 3 — Small-pore and ultrasmall-pore peritoneal water transport pathways. (A) Free water transport (FWT) and small-pore water transport both contribute to net ultrafiltration in patients without ultrafiltration failure ($r = 0.257$, $p = 0.032$). (B) Disproportionate decline in FWT in patients with ultrafiltration failure ($r = -0.709$, $p = 0.015$).

small-pore and aquaporin water transport both positively correlate in non-UFF patients; however, in acquired UFF, a disproportionate profile was documented, suggesting that ultrasmall-pore transport indeed depends on other than the effective capillary surface. Interstitial fibrosis may explain this "uncoupling" between changes in small-pore transport and in FWT that we observed in patients with UFF (24).

Hence, our procedure allows for an exploration of various time-dependent changes of the ultrasmall- and small-pore water transport pathways. It also enables the identification of causes of UFF in our population, with important repercussions for PD prescription. Among the 11 patients with UFF, 5 had a $FWT_{corrected}$ contribution to UF at 60 minutes that was less than 45%, and only 2 of those 5 were categorized as fast transporters. That finding suggests again a higher compromise of FWT, disproportionate to higher small-solute transport. This understanding is of particular importance from a clinical viewpoint, because these patients will benefit most from icodextrin instead of hypertonic glucose prescriptions. Extension of the two-in-one protocol (5-hour "uni-PET") or performance of a double mini-PET (25) to measure glucose osmotic conductance as a second evaluation step could aid in the diagnosis of UFF. However, we skipped such tests because we already adopt icodextrin prescription whenever a patient presents increased effective capillary surface or reduced FWT, abolishing the use of hypertonic 3.86% solutions in regular prescriptions.

One of the limitations of our study is the inability to quantify the lymphatic absorption rate, which is not feasible day-to-day in the clinic, and whose methodology is still under debate (26). It is a known simplification of the three-pore model to assume that absorption of fluid from the peritoneal cavity is constant, at a rate of 0.3 mL per minute. In fact, peritoneal fluid absorption varies between patients and might even be a cause of UFF, but peritoneal absorption remains a difficult problem for investigators, and no clinical procedure is able to exclude peritoneal absorption except by using a macromolecular tracer such as dextran (19) or radioiodinated human serum albumin (27,28). However, high lymphatic absorption is recognized to be mostly a cause of inherent UFF and, more rarely, a cause of acquired UFF (23,29). The fact that UFF presented mostly in our patients with longer time on PD may counteract the effect of this limitation.

CONCLUSIONS

Quantification of FWT is important for detecting causes of UFF beyond increased effective capillary surface. Such quantification is feasible during a routine 4-hour 3.86% PET evaluation with temporary drainage at 60 minutes. This two-in-one protocol provides reliable and useful clinical information concerning both small-solute transport and the various water transport pathways. Furthermore, complicated calculations needed to correct for sodium diffusion are avoided, allowing the test to be easily applied in daily clinical practice. The proposed two-in-one protocol, documenting the most common peritoneal membrane dysfunctions and giving prognostic information, is a simple method for timely detection of membrane failure. We therefore advocate using this PET evaluation in routine annual PD patient management so that therapy can be properly adjusted.

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DISCLOSURES

The authors have no financial conflicts of interest to declare.

REFERENCES

1. Heimbürger O, Waniewski J, Werynski A, Tranæus A, Lindholm B. Peritoneal transport in CAPD patients with permanent loss of ultrafiltration capacity. *Kidney Int* 1990; 38:495–506.
2. Krediet RT, Imholz AL, Struijk DG, Koomen GC, Arisz L. Ultrafiltration failure in continuous ambulatory peritoneal dialysis. *Perit Dial Int* 1993; 13(Suppl 2):S59–66.
3. Selgas R, Bajo MA, Castro MJ, del Peso G, Aguilera A, Fernández–Perpén A, *et al.* Risk factors responsible for ultrafiltration failure in early stages of peritoneal dialysis. *Perit Dial Int* 2000; 20:631–6.
4. Monquil MC, Imholz AL, Struijk DG, Krediet RT. Does impaired transcellular water transport contribute to net ultrafiltration failure during CAPD? *Perit Dial Int* 1995; 15:42–8.
5. Ho-dac-Pannekeet MM, Atasever B, Struijk DG, Krediet RT. Analysis of ultrafiltration failure in peritoneal dialysis patients by means of standard peritoneal permeability analysis. *Perit Dial Int* 1997; 17:144–50.
6. Krediet RT, Lindholm B, Rippe B. Pathophysiology of peritoneal membrane failure. *Perit Dial Int* 2000; 20(Suppl 4):S22–42.
7. Rippe B, de Arteaga J, Venturoli D. Aquaporins are unlikely to be affected in marked ultrafiltration failure: results from a computer simulation. *Perit Dial Int* 2001; 21(Suppl 3):S30–4.

8. La Milia V, Di Filippo S, Crepaldi M, Del Vecchio L, Dell'Oro C, Andrulli S, *et al.* Mini-peritoneal equilibration test: a simple and fast method to assess free water and small solute transport across the peritoneal membrane. *Kidney Int* 2005; 68:840–6.
9. Smit W, Struijk DG, Ho-Dac-Pannekeet MM, Krediet RT. Quantification of free water transport in peritoneal dialysis. *Kidney Int* 2004; 66:849–54.
10. Venturoli D, Rippe B. Validation by computer simulation of two indirect methods for quantification of free water transport in peritoneal dialysis. *Perit Dial Int* 2005; 25:77–84.
11. Rodrigues AS, Silva S, Bravo F, Oliveira JC, Fonseca I, Cabrita A, *et al.* Peritoneal membrane evaluation in routine clinical practice. *Blood Purif* 2007; 25:497–504.
12. Cnossen TT, Smit W, Konings CJ, Kooman JP, Leunissen KM, Krediet RT. Quantification of free water transport during the peritoneal equilibration test. *Perit Dial Int* 2009; 29:523–7.
13. van Biesen W, Heimbürger O, Krediet R, Rippe B, La Milia V, Covic A, *et al.* Evaluation of peritoneal membrane characteristics: a clinical advice for prescription management by the ERBP working group. *Nephrol Dial Transplant* 2010; 25:2052–62.
14. Garred LJ, Canud B, Farell PC. A simple kinetic model for assessing peritoneal mass transfer in chronic ambulatory peritoneal dialysis. *ASAIO J* 1983; 6:131–7.
15. Teixidó J, Cofan F, Borrás M, Bonet J, Bonal J, Galimany R, *et al.* Mass transfer coefficient: comparison between methods. *Perit Dial Int* 1993; 13(Suppl 2):S47–9.
16. Zweers MM, Imholz AL, Struijk DG, Krediet RT. Correction of sodium sieving for diffusion from the circulation. *Adv Perit Dial* 1999; 15:65–72.
17. Westra WM, Smit W, Zweers MM, Struijk DG, Krediet RT. Diffusion correction of sodium sieving applicable in a peritoneal equilibration test. *Adv Perit Dial* 2003; 19:6–9.
18. Twardowski ZJ, Nolph KD, Khanna R, Prowant BF, Ryan LP, Moore HL, *et al.* Peritoneal equilibration test. *Perit Dial Bull* 1987; 7:138–47.
19. Parikova A, Smit W, Struijk DG, Zweers MM, Krediet RT. The contribution of free water transport and small pore transport to the total fluid removal in peritoneal dialysis. *Kidney Int* 2005; 68:1849–56.
20. Waniewski J, Debowska M, Lindholm B. Water and solute transport through different types of pores in peritoneal membrane in CAPD patients with ultrafiltration failure. *Perit Dial Int* 2009; 29:664–9.
21. Waniewski J, Debowska M, Lindholm B. Ultrafiltration and absorption in evaluating aquaporin function from peritoneal transport of sodium. *Perit Dial Int* 2007; 27:687–90.
22. Asghar RB, Davies SJ. Pathways of fluid transport and reabsorption across the peritoneal membrane. *Kidney Int* 2008; 73:1048–53.
23. Parikova A, Smit W, Struijk DG, Krediet RT. Analysis of fluid transport pathways and their determinants in peritoneal dialysis patients with ultrafiltration failure. *Kidney Int* 2006; 70:1988–94.
24. Rippe B, Venturoli D. Simulations of osmotic ultrafiltration failure in CAPD using a serial three-pore membrane/fiber matrix model. *Am J Physiol Renal Physiol* 2007; 292:F1035–43.
25. La Milia V, Limardo M, Virga G, Crepaldi M, Locatelli F. Simultaneous measurement of peritoneal glucose and free water osmotic conductances. *Kidney Int* 2007; 72:643–50.
26. Krediet RT. The effective lymphatic absorption rate is an accurate and useful concept in the physiology of peritoneal dialysis. *Perit Dial Int* 2004; 24:309–13.
27. Waniewski J, Debowska M, Lindholm B. How accurate is the description of transport kinetics in peritoneal dialysis according to different versions of the three-pore model? *Perit Dial Int* 2008; 28:53–60.
28. Waniewski J, Sobiecka D, Debowska M, Heimbürger O, Weryński A, Lindholm B. Fluid and solute transport in CAPD patients before and after permanent loss of ultrafiltration capacity. *Int J Artif Organs* 2005; 28:976–86.
29. Smit W, Parikova A, Struijk DG, Krediet RT. The difference in causes of early and late ultrafiltration failure in peritoneal dialysis. *Perit Dial Int* 2005; 25(Suppl 3):S41–5.

V – Results

Short Summary (manuscript V.V.II)

Ultrafiltration failure is still a challenging complication of peritoneal dialysis, and its prevalence increases with time on PD. In long-term patients, ultrafiltration failure is more severe and often associates with free water transport (FWT) compromise. Since it is known that increased submesothelial fibrosis is an early and progressive lesion ultimately associated with ultrafiltration failure and free water transport compromise, the search for an effluent marker related to membrane fibrosis process, and exhibiting a good correlation with ultrafiltration and FWT, would be clinically important. Hepatocyte growth factor (HGF) counteracts peritoneal fibrosis, in animal models and in-vitro studies, but no study has ever explored effluent HGF in peritoneal dialysis patients with ultrafiltration failure.

Our cross sectional study enrolled 68 patients treated in our Unit. All the patients performed a peritoneal equilibration test according to our “Two-in-One Protocol”. HGF in the effluent was also measured.

A strong negative correlation was documented between HGF and both FWT and total ultrafiltration volume. Patients with ultrafiltration failure had higher dialysate HGF, specially the patient´s sub-group who presented FWT compromise.

With this clinical investigation we were able to document that HGF concentration is significantly higher among patients with ultrafiltration failure, especially if FWT is impaired, being a potential sign of peritoneal membrane deterioration.

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Hepatocyte growth factor signalizes peritoneal membrane failure in peritoneal dialysis

Ana Paula Bernardo[1,3,4*], José C Oliveira[2], Olívia Santos[1], Maria J Carvalho[1], António Cabrita[1] and Anabela Rodrigues[1,3]

Abstract

Background: Hepatocyte growth factor (HGF) counteracts peritoneal fibrosis in animal models and in-vitro studies, but no study explored effluent HGF in peritoneal dialysis (PD) patients with ultrafiltration failure (UFF). Our aim was to assess the relationship between effluent HGF with UF profile, free water transport (FWT) and small-solute transport.

Methods: We performed 4-hour, 3.86% PET with additional UF measurement at 60 minutes in 68 PD patients. $MTAC_{creatinine}$, FWT, small-pore ultrafiltration, and effluent HGF were quantified.

Results: Effluent HGF negatively correlated with UF ($r = -0.80$, $p = 0.009$) and FWT ($r = -0.69$, $p = 0.04$). Patients with UFF had higher dialysate HGF (103 pg/mL vs 77 pg/mL, $p = 0.018$) and, although not statistically significant, those with FWT compromise had also higher dialysate HGF compared with subgroup of UFF without FWT compromise (104 pg/mL vs 88 pg/mL, $p = 0.08$). FWT ≤ 45% without clinical UFF was documented in some patients who also had increased effluent HGF.

Conclusions: Dialysate HGF concentration is significantly higher among patients with UFF, specially, if FWT is impaired, being a sign of peritoneal membrane deterioration.

Keywords: Hepatocyte growth factor, Peritoneal membrane, Ultrafiltration failure, Water transport

Background

Ultrafiltration failure (UFF) is still a challenging complication of peritoneal dialysis and its prevalence increases with time on PD [1-3]. In long-term patients, UFF is more severe and often associated with free water transport (FWT) compromise [4-6]. The two-in-one peritoneal equilibration test allows simultaneous quantification of FWT and small pore ultrafiltration, being a simple method for timely detection of membrane failure, as we previously reported [7]. Since it is known that increased submesothelial fibrosis is an early and progressive lesion ultimately associated with UFF [8], the search for an effluent marker related to membrane fibrosis process and exhibiting a good correlation with ultrafiltration and FWT would be clinically important. Such a marker could timely detect peritoneal membrane failure. Besides it should be desirable that such marker could signalize peritoneal membrane deterioration even before clinically relevant UFF.

Hepatocyte growth factor (HGF) is known to play a crucial role in the repairing process of tissues and preventing organ fibrosis [9-12]. Yu et al. demonstrated, for the first time, that human peritoneal mesothelial cells constitutively synthesized HGF [13], and that treatment of human peritoneal mesothelial cells with HGF blocks high glucose-induced epithelial-to-mesenchymal transition (EMT). More recently, Ueno T, et al. showed that HGF secreted by mesenchymal stem cells was implicated in the inhibition of the transforming growth factor β1 signaling and ameliorated peritoneal fibrosis in an **ex-vivo** study [12]. It is thus relevant to increase the knowledge on HGF clinical value, in patients under PD, as it may possibly point to new diagnostic opportunities and therapeutic avenues. Therefore clinical investigation under this subject is mostly important and needed.

Impaired FWT is assumed to indicate a more severe functional and structural membrane lesion due to aquaporin disfunction or interstitial changes [6,14-16],

* Correspondence: anabernardo@portugalmail.pt
[1]Nephrology Department, St. António Hospital - Oporto Hospital Center, Oporto, Portugal
[3]UMIB/ICBAS/UP, Oporto, Portugal
Full list of author information is available at the end of the article

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but there are no clinical studies exploring the associations between effluent HGF, ultrafiltration failure, FWT and small-solute transport. For that reason, we performed a clinical investigation in order to assess, in a prevalent PD population, the relation between dialysate HGF and the ultrafiltration profile, FWT quantification, and small-solute transport.

Methods

Patients and procedures

This cross sectional study enrolled 68 patients of our Unit. All the patients performed a 4-hour, 3.86% glucose modified peritoneal equilibration test (PET) with total temporary drainage at 60 minutes ["Two-in-one" protocol, as published before [7]]. This protocol allows free water transport quantification, beyond a simple calculation of sodium sieving [7]. None of the patients had peritonitis during the study or the preceding 6 weeks. During the procedure, we used PD solutions low in glucose degradation products, according to the individual patient's prescription. The volume of dialysis solution was determined by weight, without flushing the system and before filling the peritoneum. Blood and dialysate samples (each approximately 10 mL) were taken at instillation of the dialysate and after 60 and 240 minutes. At 60 minutes, we performed an additional measurement of UF by total drainage of the peritoneal cavity. This drained volume was weighed and then immediately reinfused. Finally, after 240 minutes, the peritoneal cavity was drained and the volume obtained was weighed.

PETs with an ultrafiltered volume ≤ 400 mL/4 h were considered to represent ultrafiltration failure (UFF).

All patients provided written informed consent for participation, and the study was approved by the Ethics Committee of St. António Hospital – Oporto Hospital Center.

Measurements

Creatinine and sodium were measured both in plasma and dialysate. For creatinine, the Jaffé compensated method was used. The dialysate creatinine concentration was corrected for interference by glucose according to our laboratory standards. Sodium was measured using indirect ion-selective electrodes. Effluent samples taken at 4 hour were immediately stored at −70°C, until they were used to measure HGF, VEGF and CA125. Effluent CA125 was determined with an electrochemiluminescence method on an automated analyzer (COBAS e-411, Roche Diagnostics GmbH). Effluent HGF and VEGF levels were determined by ELISA technique according to the manufacturer's instructions (IBL – Immuno-Biological Laboratories Co. Ltd). The intra and inter-assay variations were 8,8%% and 10,0%, respectively for HGF and 5,9%, and 9,4% for VEGF. The sensitivity was 11 pg/mL for HGF and 1 pg/mL for VEGF. Both the assays are considered highly specific for the cytokines, and no significant cross-reactivity was observed.

Calculations

Patients were characterized by peritoneal transport status as described by Twardowski et al. [17]. The $MTAC_{creatinine}$ was calculated by the simplified Garred model [18].

FWT and UF through the small pores (SPUF) at 60 minutes were calculated as we previously described [7]. Using a simple algorithm, we also performed a correction for FWT as described by Venturoli and Rippe [19].

Statistical analysis

Except for time on PD, HGF, HGF/CA125 and VEGF/CA125, all variables had normal distribution. Results are expressed as mean ± SD or as median and interquartile range.

Pearson correlation analysis was used in order to explore possible relations between HGF (with logarithmic transformation) and ultrafiltration profile, FWT and small-solute transport.

For comparison of small solute transport, water transport pathways and effluent markers between patients with and without ultrafiltration failure, Mann–Whitney U test was used.

In order to study our patients ultrafiltration failure profile we made a comparison of small solute transport, water transport pathways and effluent markers between patients with FWT ≤ 45% and $D/P_{Creatinine} \geq 0.81$ and patients without FWT compromise and non-fast transport category, using Mann–Whitney U test.

Unpaired Student t-Test or Mann Whitney U-test to compare patients with FWT ≤ 45% and FWT > 45%, as appropriate, according to the variables involved.

Results

Solute, fluid transport parameters and concentration of cytokines in dialysate

Table 1 summarizes the peritoneal transport characteristics evaluated with a combined ("two-in-one") PET performed in 68 study patients [35 men; mean age: 50 ± 14 years; 14 patients were diabetic; 16 were anuric; 36 were on APD; PD vintage 18.7 ± 23.5 months (range 1 – 121 months)]. According to small solute transport characteristics, 1 (1.5%) patient was classified as slow transporter ($D/P_{Creatinine} \leq$ 0,49), 10 (14.7%) as slow-average ($0,50 \leq D/P_{Creatinine} \leq$ 0.64), 41 (60.3%) as fast-average ($0,65 \leq D/P_{Creatinine} \leq 0.80$), and 16 (23.5%) as fast transporters ($D/P_{Creatinine} \geq 0,81$). Concerning water transport pathways, FWT accounted for 37.15% of the UF at 60 minutes, and once corrected (for sodium diffusion, cumulative UF volume through the large pores, and cumulative lymphatic absorption at 60 minutes), its contribution increased to a mean value of 45.46%.

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Table 1 Peritoneal transport characteristics and effluent cytokines in 68 stable patients assessed during a 4-hour, 3.86% glucose peritoneal equilibration test with temporary drainage at 60 minutes

Variable	Mean ± SD Median (IQR 25%-75%)
Total UF at 4 h (mL)	669,12 ± 226,76
SPUF (mL)	323,36 ± 129,34
FWT (mL)	183,26 ± 63,02
$FWT_{corrected}$(mL)	224,12 ± 66,51
%FWT	37,15 ± 11,79
$\%FWT_{corrected}$	45,46 ± 11,11
D/P $_{Creatinine}$	0,76 ± 0,12
MTAC $_{Creatinine}$ (mL/min)	11,32 ± 7,14
Effluent HGF (pg/mL)	77,17 (68,83 – 94,31)
Effluent VEGF (pg/mL)	13,96 ± 4,92
CA125 (U/mL)	19,57 ± 11,31

SD = standard deviation; UF = ultrafiltration; SPUF = UF through the small pores at 60 minutes; FWT = free water transport at 60 minutes; $FWT_{corrected}$ = FWT with an algorithm correction according to Venturoli and Rippe [16]; MTAC = mass transfer area coefficient; D/P = dialysate-to-plasma ratio; HGF = hepatocyte growth factor; VEGF = vascular endothelial growth factor; CA 125 = cancer antigen 125.

HGF correlations with ultrafiltration, FWT and small-solute transport

HGF measured in the effluent significantly correlated with total ultrafiltration at a 4 h, 3.86% glucose PET ($r = -0.358$, $p = 0.003$), with FWT ($r = -0.407$, $p = 0.001$) and $MTAC_{creatinine}$ ($r = 0.355$, $p = 0.003$). These correlations were even stronger when we focused the analysis in patients with ultrafiltration failure (Figures 1A,B,C). In those patients, HGF exhibited a strong negative correlation with total ultrafiltration and FWT ($r = -0.802$, $p = 0.009$ and $r = -0.690$, $p = 0.04$, respectively) and a strong positive correlation with $MTAC_{creatinine}$ ($r = 0.747$, $p = 0.021$). No correlation was found between dialysate HGF concentration and small pore water transport (neither in global population, nor in the UFF group).

Patients with ultrafiltration failure compared with stable patients

Among the 68 study patients, 9 (13.2%) had UFF (total UF ≤ 400 mL/4 h). Although not statistically significant, patients with UFF had been on PD for a longer time, had higher $D/P_{creatinine}$ and $MTAC_{creatinine}$(Table 2). HGF concentration was significant higher in patients with UFF (median 103.0 pg/mL IQR [79.8–110.8]) compared with stable patients (median 77.1 pg/mL IQR [68.1–92.6], $p = 0.018$). Although not statistically significant, patients with UFF had also a higher ratio HGF/CA125 (Table 2).

Ultrafiltration failure profile

From the 9 patients with UFF, 3 had a more severe profile characterized by FWT compromise (FWT ≤45%) and increased ($D/P_{creatinine} \geq 0{,}81$). Those patients had significant lower ultrafiltration volume at a 4 h PET (166.7 ± 57.4 mL vs 375.0 ± 41.8 mL, $p = 0.024$), lower FWT quantification (128.67 ± 26.50 mL vs 183.5 ± 12.14 mL, $p = 0.024$) and higher $MTAC_{creatinine}$ (25.40 ± 2.63 mL/min vs 9.62 ± 2.45 mL/min, $p = 0.024$) (Figure 2A,B,C). Although not statistically significant, patients with the more severe UFF profile had also higher values of HGF measured in the effluent (104.3 pg/mL vs 88.94 pg/mL, $p = 0.085$) (Figure 2D).

Tree other patients showed a less severe UFF profile, with increased effective capillary surface but preserved FWT (>45%). And still 3 incident patients (3 – 6 months on PD), were average transporters with preserved FWT, in whom higher lymphatic absorption was by exclusion presumed.

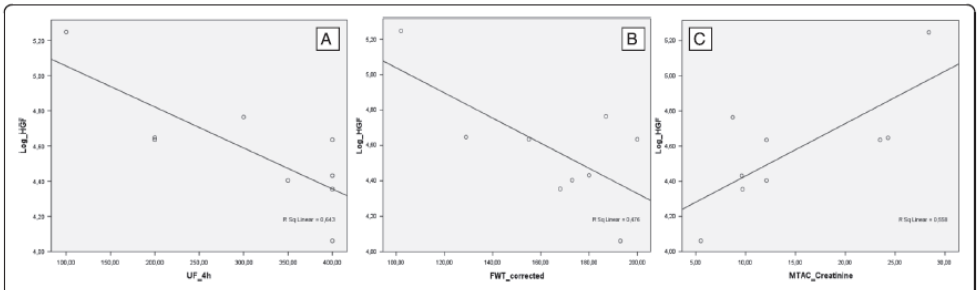

Figure 1 HGF correlations with ultrafiltration, FWT and small-solute transport. (A) Correlation between effluent hepatocyte growth factor (HGF) and total ultrafiltration at a 4 h, 3.86% glucose PET (UF240), in patients with ultrafiltration failure (Pearson $r = -0.802$, $p = 0.009$); **(B)** correlation between effluent hepatocyte growth factor (HGF) and FWT corrected according to Venturoli and Rippe [16], in patients with ultrafiltration failure (Pearson $r = -0.690$, $p = 0.04$); **(C)** correlation between effluent hepatocyte growth factor (HGF) and creatinine mass transfer area coefficient ($MTAC_{creatinine}$), in patients with ultrafiltration failure (Pearson $r = 0.747$, $p = 0.021$).

Table 2 Comparison of small solute transport, water transport pathways and effluent markers between patients with and without ultrafiltration failure

	Patients with UFF Mean ± SD Median (IQR 25-75%)	Patients without UFF Mean ± SD Median (IQR 25-75%)
Patients (n)	9	59
Time on PD (months)	6.0 (4.0 – 35.5)	7.0 (4.0 – 27.0)
Total UF at 4 hour (mL)	305.56 ± 113.04[a]	724.58 ± 184.38[a]
SPUF (mL)	192.89 ± 59.41[a]	343.27 ± 125.68[a]
FWT (mL)	134.89 ± 35.07[b]	190.64 ± 63.25[b]
$FWT_{corrected}$(mL)	165.22 ± 31.93[c]	233.10 ± 65.95[c]
%FWT	41.87 ± 13.07	36.43 ± 11. 53
$\%FWT_{corrected}$	51.16 ± 12.29	44.59 ± 10.77
$D/P_{Creatinine}$	0.81 ± 0.12	0.75 ± 0.11
$MTAC_{Creatinine}$ (mL/min)	14.88 ± 8.23	10.78 ± 6.87
Effluent HGF (pg/mL)	103.01 (79.83 – 110.78)[d]	77.07 (68.05 – 92.58)[d]
Effluent VEGF (pg/mL)	15.10 ± 5.68	13.79 ± 4.82
CA125 (U/mL)	24.63 ± 19.63	18.89 ± 9.75
HGF/CA125 (pg/U)	6.22 (2.28 – 8.27)	4.64 (3.30 – 6.36)
VEGF/CA125 (pg/U)	0.70 (0.53 – 1.39)	0.76 (0.54 – 1.08)

SD = standard deviation; UF = ultrafiltration; SPUF = UF through the small pores at 60 minutes; FWT = free water transport at 60 minutes; $FWT_{corrected}$ = FWT with an algorithm correction according to Venturoli and Rippe [16]; MTAC = mass transfer area coefficient; D/P = dialysate-to-plasma ratio; HGF = hepatocyte growth factor; VEGF = vascular endothelial growth factor; CA 125 = cancer antigen 125.
[a]Mann–Whitney U test p <0.0001, comparing patients with and without ultrafiltration failure.
[b]Mann–Whitney U test p = 0.006, comparing patients with and without ultrafiltration failure.
[c]Mann–Whitney U test p = 0.002, comparing patients with and without ultrafiltration failure.
[d]Mann–Whitney U test p = 0.018, comparing patients with and without ultrafiltration failure.

FWT profile in patients without UFF

From the 59 studied patients without UFF, 33 had a FWT ≤45%. Those patients had a mean UF volume at 4 h PET equivalent to patients with FWT > 45% (Table 3). Although not statistically different, patients with FWT ≤ 45% had higher effluent HGF, when compared with patients with FWT > 45%. In spite of higher effluent HGF concentration, the HGF/CA125 ratio was significantly lower in patients without UFF and FWT ≤45% compared with patients without UFF and FWT >45% (3.65 IQR [2.96-5.60] vs 5.19 IQR 4.02-8.37], p = 0.014).

Discussion

To our knowledge, the present study is the first to report a clinical significant relation between HGF measured in the effluent and ultrafiltration profile, FWT quantification and small-solute transport in peritoneal dialysis patients. Since HGF is involved in the process of peritoneal submesothelial fibrosis [12,13], this study also suggest some structural-functional correlations.

Although impaired FWT is frequently associated to aquaporin dysfunction, several studies provided indirect evidence that FWT can also be impaired in situations of decreased peritoneal water permeability due to interstitial changes, especially in long term patients [6]. Simulations of osmotic ultrafiltration failure in CAPD using a serial three-pore membrane/fiber matrix model [14] documented the uncoupling of small solute transport from LpS in computer simulations of UFF, whenever changes both in vasculature and in interstitium are taken into account, supporting further the role of the peritoneal membrane interstitium in fluid transport. Moreover, Devuyst and Rippe stated in a recent review [16] that reduced LpS in long-term PD has been attributed to reductions in AQP1-meditated water transport, but it might also be the result of a combination of increased vascularization and fibrotic scar tissue in the peritoneum.

When we analyze the reasons for UFF in our study we distinguish 3 groups of patients. Three had a more severe profile characterized by FWT compromise (FWT ≤45%) on top of increased effective capillary surface, also with higher values of effluent HGF. Three patients presented an increased effective capillary surface but FWT was not impaired. Another 3 incident patients were average transporters with normal FWT. A high effective lymphatic absorption rate could be the reason for UFF in those incident patients since high lymphatic absorption is recognized to be mostly a cause of inherent UFF [5]. This variability in UFF patterns that we found was also documented by Waniewsky et al. [15] that recognized that UFF due to high peritoneal absorption could be associated with normal or decrease fractional contribution by transcellular pores to hydraulic conductivity. It is noteworthy that mean HGF in those 3 patients (with a functional UFF) was significantly lower compared with the 3 patients that had impaired FWT [77.84 (67.9 – 79.8) vs 104.3 (103.7 – 147.16), p = 0.05].

In contrast with previous studies [4,6,20], $MTAC_{creatinine}$ was higher but not statistically different between patients with and without UFF. This is due to the presence of many incident fast transporters (mean time on PD 6 ± 4 months) in the non UFF group.

Report and interpretation of causes of UFF as well as absolute and fractional FWT may vary according to the PD vintage. Our results are in accordance with Parikova et al., [6] that documented an early stage UFF associated with decrease of absolute FWT dependent on increased effective capillary surface without significant decrease of FWT contribution, while later the loss of osmotic conductance to glucose lead to a significant decrease of FWT fraction. In fact, all 9 patients with UFF had a significant decrease in absolute FWT compared with patients without UFF, but only 3 had a reduction in FWT contribution to 60 minutes ultrafiltration.

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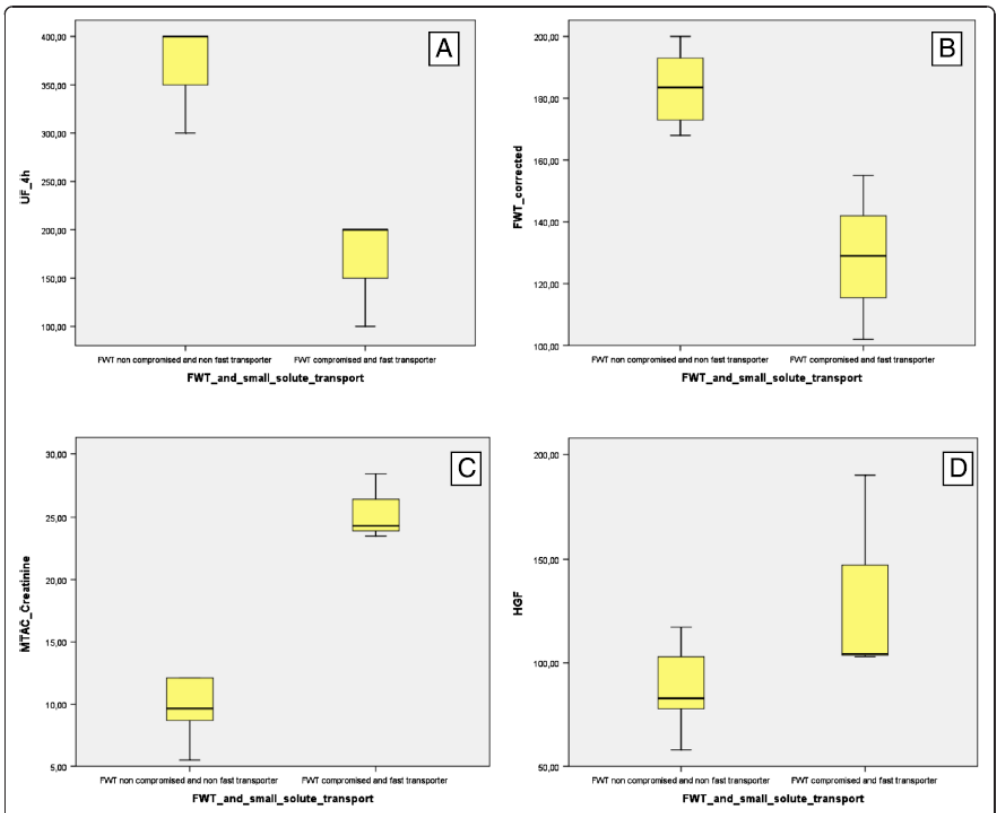

Figure 2 Ultrafiltration failure profile. Comparison within patients with UFF, between 6 patients with non compromised FWT and non fast transporters versus 3 patients with FWT ≤ 45% and fast transporters: (A) Total ultrafiltration at a 4 h, 3.86% glucose PET (375.0 ± 41.83 mL vs 166.67 ± 57.74 mL, p = 0.024). **(B)** FWT quantification at 60 minutes in a 4 h, 3.86% glucose PET (183.5 ± 12.14 mL vs 128.67 ± 26.50 mL, p = 0.024) **(C)** $MTAC_{creatinine}$ (9.62 ± 2.45 mL/min vs 25.40 ± 2.63 mL/min, p = 0.024). **(D)** Effluent Hepatocyte Growth Factor (88.94 pg/mL vs 104.3 pg/mL, p = 0.085). Box plot representation: 75% percentile, 25% percentile, median, and maximum and minimum values.

Moreover, our study adds further clinical evidence to the recent report from Nakamura S. et al. [21]. Those authors studied focal HGF expression in peritoneum biopsies of a small number of peritoneal dialysis patients, with and without UFF. Although they did not measure dialysate HGF concentration, they demonstrated an increased expression of HGF in peritoneal tissues of CAPD patients with low ultrafiltration capacity compared with those with a normal ultrafiltration profile. Given the already mentioned protective effects of HGF on peritoneal fibrosis [11-13], we hypothesized that the increased peritoneal HGF expression demonstrated by Nakamura S. et al. [21], and the higher dialysate HGF concentration that we found in our patients with UFF, can be seen as a reactive mechanism to peritoneal membrane lesion. This is also supported by the fact that patients with more severe forms of UFF (with FWT compromise besides an increase in small-solute transport), presented higher dialysate HGF concentration.

The effluent HGF concentration on patients under PD was addressed, until now, by one single clinical study [22]. However, Mizuiri S et al. [22], only compared dialysate HGF concentration according to small solute transport status, and found that fast transporters had higher effluent HGF concentration compared with others small solute transport categories, which we also reproduce (data not shown). Unfortunately such study gave no information about UFF or water transport pathways quantification.

As others [23] we also found significantly higher dialysate VEGF concentration in fast transporters compared

Table 3 FWT profile in patients without UFF: comparison of small solute transport, water transport pathways and effluent markers between patients with FWT ≤ 45% and FWT > 45%

	Patients without ultrafiltration failure	
	Patients with FWT ≤ 45% Mean ± SD Median (IQR 25-75%)	Patients with FWT > 45% Mean ± SD Median (IQR 25-75%)
Patients (n)	33	26
Time on PD (months)	6.0 (4.0 – 19.0)	8.5 (5.0 – 30.25)
Total UF at 4 hour (mL)	739.39 ± 210.56	705.77 ± 146.51
SPUF (mL)	412.9 ± 108.54[a]	254.81 ± 83.74[a]
FWT (mL)	173.42 ± 60.43[b]	212.50 ± 60.98[b]
$FWT_{corrected}$(mL)	221.51 ± 66.11	247.81 ± 63.97
%FWT	29.10 ± 6.50[a]	45.74 ± 9. 66[a]
$\%FWT_{corrected}$	37.56 ± 5.62[a]	53.52 ± 8.96[a]
$D/P_{Creatinine}$	0.76 ± 0.08	0.73 ± 0.14
$MTAC_{Creatinine}$ (mL/min)	10.45 ± 5.14	11.20 ± 8.69
Effluent HGF (pg/mL)	77.17 (68.42 – 93.89)	75.89 (66.47 – 86.51)
Effluent VEGF (pg/mL)	14.19 ± 4.96	13.28 ± 4.68
CA125 (U/mL)	22.28 ± 10.90[c]	14.56 ± 5.82[c]
HGF/CA125 (pg/U)	3.65 (2.96 – 5.60) [d]	5.19 (4.02 – 8.37) [d]
VEGF/CA125 (pg/U)	0.68 (0.51 – 0.90)	0.89 (0.67 – 1.29)

Variables presented as mean ± SD or median (interquartile range) accordingly. SD = standard deviation; UF = ultrafiltration; SPUF = UF through the small pores at 60 minutes; FWT = free water transport at 60 minutes; $FWT_{corrected}$ = FWT with an algorithm correction according to Venturoli and Rippe [16]; MTAC = mass transfer area coefficient; D/P = dialysate-to-plasma ratio; HGF = hepatocyte growth factor; VEGF = vascular endothelial growth factor; CA 125 = cancer antigen 125.
a) T-test p <0.0001, comparing patients with FWT ≤ 45% and FWT > 45%.
b) T-test p = 0.017, comparing patients with FWT ≤ 45% and FWT > 45%.
c) T-test p = 0.001, comparing patients with FWT ≤ 45% and FWT > 45%.
d) Mann–Whitney U test p = 0.014, comparing patients with FWT ≤ 45% and FWT > 45%.

with non-fast transporters (data not shown). Although it remains uncertain if this increase in VEGF production corresponds to an increased production of intraperitoneal vasoactive substances, as might occur in incident fast transporters, or if it is the result of epithelial-to-mesenchymal transition, dialysate VEGF was not discriminative of UFF. On the contrary, effluent HGF was informative not only about UFF but also highlighted more severe UFF profile, with FWT compromise.

We are aware that the mass of mesothelial cells could affect the levels of intraperitoneal growth factors in PD patients. Although there are some controversies about the use of CA125 as an index of mesothelial cells mass or their functional properties [24-26], we also documented a higher HGF/CA125 ratio in UFF patients compared with stable patients, indicating a reactive increased HGF production beyond that we would expect for the mesothelial cell mass.

Since there are no clinical studies that had examined plasma and effluent HGF, and we found a correlation with small-solute transport, we might question whether effluent HGF concentration could depend on plasma HGF levels. We think that this is not plausible for various reasons. First, the peritoneal permeability is expected to be poor, since HGF is a heterodimeric molecule composed of a 69 KDa alpha subunit and a 34 KDa beta subunit. Second and mostly important, human peritoneal cells constitutively synthesized HGF [13]. For these reasons, we believe that the HGF protein detected in the effluent is locally produced.

The use of effluent biomarkers as an early sign of peritoneal membrane alterations is currently under debate [27-29] specially because clinical factors cannot give an accurate individual prediction for EPS [30].

In a very recent report [27], MCP-1, IL-6 and CCL15 were found at higher levels in the dialysate of patients who subsequently developed EPS. However, by logistic regression analysis, these cytokines did not improve prediction of future EPS above known clinical factors, as PD vintage and peritoneal small solute transport. On the contrary, Sampimon et al. [28] concluded that dialysate appearance rate of CA125 and IL-6 combined was potentially useful for an early diagnosis of EPS. None of these studies explored the associations between the cytokines measured in the dialysate and ultrafiltration or water transport pathways in a peritoneal equilibration test. These would be of great importance as we know that in the 2 years that precede an EPS diagnosis, a proportion of patients with EPS present an uncoupling between the membrane ultrafiltration capacity and the peritoneal membrane small solute transport [30]. This fact gives even more strength to the necessity of finding a biomarker that correlates both with ultrafiltration and with water transport pathways, and not only to the membrane small solute transport status. HGF can be easily measured, without specific preparation of the dialysate sample, by commercially available highly specific assay (IBL-Immuno-Biological Laboratories Co.Ltd), with acceptable inter-assay variability (10%); it is constitutively synthesized by human peritoneal mesothelial cells, blocks high glucose-induced epithelial-to-mesenchymal transition (EMT) and was implicated in the inhibition of the transforming growth factor β1 signaling, ameliorating peritoneal fibrosis in an ex-vivo study [13]. According to our present investigation, dialysate HGF concentration increases as ultrafiltration decreases in a 4-hour, 3,86% glucose PET. The fact that dialysate HGF concentration is even higher among patients with FWT compromise and fast transport status increases the likelihood of effluent HGF concentration being related with peritoneal deterioration, as a reactive repairing mechanism, and not with a

functional characteristic, as for example an higher effective lymphatic absorption rate, hardly measurable in clinic.

In this study we reported a mean $FWT_{corrected}$ fraction of 45%, which is in line with our previous studies [7], and with the FWT fraction reported by La Milia [31]. A FWT ≤ 45% is clinical relevant in patients with UFF, since it may signalize aquaporin dysfunction or interstitial fibrosis with glucose osmotic conductance compromise, with important repercussions for PD prescription. However, there is no knowledge yet about the clinical value of FWT fraction on patients without UFF. We think that this is another relevant aspect of our investigation: although we did find that patients without UFF but with FWT fraction ≤45% had higher dialysate HGF concentration compared with patients with preserved FWT fraction, the first group had a significant lower mean value of HGF/CA125 ratio compared with the second one. This trend is completely different from that we observed in patients with UFF, in whom we found a higher HGF/CA125 ratio. We hypothesized that this may represent an intermediate level of peritoneal dysfunction, where the patient may already have interstitial changes that lead to a reactive increase in HGF production, while not severe enough to present as UFF. At this point, those patients may still have a preserved mesothelial cell mass which explains the lower HGF/CA125 ratio that we found in this group. Aging, uremia, diabetes are indeed often associated with membrane changes already at PD start possibly justifying in some patients selective FWT compromise in absence of clinically relevant UFF [32-35].

Our study is limited by its cross sectional design and small number of patients with UFF. A longitudinal study is being conducted in order to document the dynamic profile of HGF production and its relationship with peritoneal membrane water transport changes. More studies are needed to increase the knowledge on HGF clinical value, in patients under PD, as it may possibly point to new diagnostic opportunities and therapeutic avenues in the ultrafiltration failure field.

Conclusions

Since HGF ameliorated peritoneal fibrosis in an **ex-vivo** study [12], a clinical study as ours looks opportune. Our results demonstrated, for the first time, that dialysate HGF concentration is significantly higher among patients with ultrafiltration failure, specially if free water transport is impaired, being an useful marker of progressive peritoneal deterioration.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

APB and AR designed the study, analyzed and interpreted the data. JCO, OS, MJC and AC gave scientific contributions in their field of expertise. All authors revised the manuscript and contributed to its improvement. All authors read and approved the final manuscript.

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Author details

[1]Nephrology Department, St. António Hospital - Oporto Hospital Center, Oporto, Portugal. [2]Clinical Pathology, St. António Hospital - Oporto Hospital Center, Oporto, Portugal. [3]UMIB/ICBAS/UP, Oporto, Portugal. [4]Largo Prof. Abel Salazar, 4099-001 Porto, Portugal.

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References

1. Heimbürger O, Waniewski J, Werynski A, Tranaeus A, Lindholm B: **Peritoneal transport in CAPD patients with permanent loss of ultrafiltration capacity.** *Kidney Int* 1990, **38**:495–506.
2. Krediet RT, Imholz AL, Struijk DG, Koomen GC, Arisz L: **Ultrafiltration failure in continuous ambulatory peritoneal dialysis.** *Perit Dial Int* 1993, **13**(Suppl 2):S59–S66.
3. Krediet RT, Lindholm B, Rippe B: **Pathophysiology of peritoneal membrane failure.** *Perit Dial Int* 2000, **20**(Suppl4):S22–S42.
4. Smit W, Schouten N, van den Berg N, Langedijk MJ, Struijk DG: **Krediet RT; Netherlands Ultrafiltration Failure Study Group. Analysis of the prevalence and causes of ultrafiltration failure during long-term peritoneal dialysis: a cross-sectional study.** *Perit Dial Int* 2004, **24**:562–570.
5. Smit W, Parikova A, Struijk DG, Krediet RT: **The difference in causes of early and late ultrafiltration failure in peritoneal dialysis.** *Perit Dial Int* 2005, **25**(S3):S41–S45.
6. Parikova A, Smit W, Struijk DG, Krediet RT: **Analysis of fluid transport pathways and their determinants in peritoneal dialysis patients with ultrafiltration failure.** *Kidney Int* 2006, **70**:1988–1994.
7. Bernardo AP, Bajo MA, Santos O, Del Peso G, Carvalho MJ, Cabrita A, Selgas R, Rodrigues A: **Two-in-one protocol: simultaneous small-pore and ultrasmall-pore peritoneal transport quantification.** *Perit Dial Int* 2012, **32**(5):537–544.
8. Del Peso G, Jiménez-Heffernan JA, Bajo MA, Aroeira LS, Aguilera A, Fernández-Perpén A, Cirugeda A, Castro MJ, de Gracia R, Sánchez-Villanueva R, Sánchez-Tomero JA, López-Cabrera M, Selgas R: **Epithelial-to-mesenchymal transition of mesothelial cells is an early event during peritoneal dialysis and is associated with high peritoneal transport.** *Kidney Int Suppl.* 2008, (108):S26–33.
9. Shukla MN, Rose JL, Ray R, Lathrop KL, Ray A, Ray P: **Hepatocyte growth factor inhibits epithelial to myofibroblast transition in lung cells via Smad7.** *Am J Respir Cell Mol Biol* 2009, **40**:643–653.
10. Yang J, Dai C, Liu Y: **A novel mechanism by which hepatocyte growth factor bloks tubular epithelial to mesenchymal transition.** *J Am Soc Nephrol* 2005, **16**:68–78.
11. Nakamura S, Niwa T: **Pyridoxal phosphate and hepatocyte growth factor prevent dialysate-induced peritoneal damage.** *J Am Soc Nephrol* 2005, **16**:144–150.
12. Ueno T, Nakashima A, Doi S, Kawamoto T, Honda K, Yokoyama Y, Doi T, Higashi Y, Yorioka N, Kato Y, Kohno N, Masaki T: **Mesenchymal stem cells ameliorate experimental peritoneal fibrosis by suppressing inflammation and inhibiting TGF-β1 signaling.** *Kidney Int* 2013, **84**:297–307.
13. Yu MA, Shin KS, Kim JH, Kim YI, Chung SS, Park SH, Kim YL, Kang DH: **HGF and BMP-7 ameliorate high glucose-induced epithelial-to-mesenchymal transition of peritoneal mesothelium.** *J Am Soc Nephrol* 2009, **20**:567–581.
14. Rippe B, Venturoli D: **Simulations of osmotic ultrafiltration failure in CAPD using a serial three-pore membrane/fiber matrix model.** *Am J Physiol Renal Physiol* 2007, **292**:F1035–F1043.
15. Waniewski J, Debowska M, Lindholm B: **Water and solute transport through different types of pores in peritoneal membrane in CAPD patients with ultrafiltration failure.** *Perit Dial Int* 2009, **29**:664–669.
16. Devuyst O, Rippe B: **Water transport across the peritoneal membrane.** *Kidney Int* 2014, **85**(4):750–758.
17. Twardowski ZJ, Nolph KO, Khanna R, Prowant BF, Ryan LP, Moore HL, Nielsen MP: **Peritoneal equilibration test.** *Perit Dial Bull* 1987, **7**:138–147.
18. Garred LJ, Canaud B, Farrell PC: **A simple kinetic model for assessing peritoneal mass transfer in chronic ambulatory peritoneal dialysis.** *ASAIO J* 1983, **6**:131–137.

19. Venturoli D, Rippe B: **Validation by computer simulation of two indirect methods for quantification of freee water transport in peritoneal dialysis.** *Perit Dial Int* 2005, **25**:77–84.
20. Waniewski J: **Peritoneal fluid transport: mechanisms, pathways, methods of assessment.** *Arch Med Res* 2013, **44**:576–583.
21. Nakamura S, Tachikawa T, Tobita K, Miyazaki S, Sakai S, Morita T, Hirasawa Y, Weigle B, Pischetsrieder M, Niwa T: **Role of advanced glycation end products and growth factors in peritoneal dysfunction in CAPD patients.** *Am J Kidney Dis* 2003, **41**(Suppl 1):S61–S67.
22. Mizuiri S, Hemmi H, Arita M, Tai R, Hattori Y, Muto A, Suzuki Y, Ohashi Y, Sakai K, Aikawa A: **Effluent markers related to epithelial mesenchymal transition with adjusted values for effluent cancer antigen 125 in peritoneal dialysis patients.** *Int J Nephrol* 2011, **2011**:261040.
23. Pecoits-Filho R, Araújo MR, Lindholm B, Stenvinkel P, Abensur H, Romão JE Jr, Marcondes M, De Oliveira AH, Noronha IL: **Plasma and dialysate Il-6 and VEGF concentrations are associated with high peritoneal solute transport rate.** *Nephrol Dial Transplant* 2002, **17**:1480–1486.
24. Rodrigues AS, Martins M, Korevaar JC, Silva S, Oliveira JC, Cabrita A: **Castro e Melo J, Krediet RT. Evaluation of peritoneal transport and membrane status in peritoneal dialysis: focus on incident fast transporters.** *Am J Nephrol* 2007, **27**:84–91.
25. Krediet R: **Dialysate cancer antigen 125 concentration as marker of peritoneal membrane status in patients treated with chronic peritoneal dialysis.** *Perit Dial Int* 2001, **21**:560–567.
26. Breborowicz A, Breborowicz M, Pyda M, Połubinska A, Oreopoulos D: **Limitations of CA125 as an index of peritoneal mesothelial cell mass.** *Nephron Clin Pract* 2005, **100**:c46–c51.
27. Goodlad C, Tam FWK, Ahmad S, Bhangal G, North BV, Brown EA: **Dialysate cytokine levels do not predict encapsulating peritoneal sclerosis.** *Perit Dial Int* 2014, **34**(6):594–604.
28. Sampimon DE, Korte MR, Barreto DL, Vlijm A, Waart R, Struijk DG, Krediet RT: **Early diagnostic markers for encapsulating peritoneal sclerosis: a case–control study.** *Perit Dial Int* 2010, **30**:163–169.
29. Barreto DL, Coester AM, Struijk DG, Krediet RT: **Can effluent matrix metalloproteinase 2 and plasminogen activator inhibitor 1 be used as biomarkers of peritoneal membrane alterations in peritoneal dialysis patients?** *Perit Dial Int* 2013, **33**(5):529–537.
30. Lambie ML, John B, Mushahar L, Huckvale C, Davies S: **The peritoneal osmotic conductance is low well before the diagnosis of encapsulating peritoneal sclerosis is made.** *Kidney Int* 2010, **78**:611–618.
31. La Milia V, Di Filippo S, Crepaldi M, Del Vecchio L, Dell'Oro C, Andrulli S, Locatelli F: **Mini-peritoneal equilibration test: a simple and fast method to assess free water and small solute transport across the peritoneal membrane.** *Kindey Int* 2005, **68**:840–846.
32. Williams JD, Craig KJ, Topley N, Von Ruhland C, Fallon M, Newman GR, Mackenzie RK: **Williams GT; Peritoneal Biopsy Study Group. Morphologic changes in the peritoneal membrane of patients with renal disease.** *J Am Soc Nephrol* 2002, **13**:470–479.
33. Honda K, Hamada C, Nakayama M, Miyazaki M, Sherif AM, Harada T: **Hirano H; Peritoneal Biopsy Study Group of the Japanese Society for Peritoneal Dialysis. Impact of uremia, diabetes, and peritoneal dialysis itself on the pathogenesis of peritoneal sclerosis: a quantitative study of peritoneal membrane morphology.** *Clin J Am Soc Nephrol* 2008, **3**:720–728.
34. Oliveira L, Rodrigues A: **Previous renal replacement therapy time at start of peritoneal dialysis independently impact on peritoneal membrane ultrafiltration failure.** *Int J Nephrol* 2011, **2011**:685457. doi:10.4061/2011/685457. Epub 2011 Sep 29.
35. Shimaoka T, Hamada C, Kaneko K, Io H, Sekiguchi Y, Aruga S, Inuma J, Inami Y, Hotta Y, Horikoshi S, Kumasaka T, Tomino Y: **Quantitative evaluation and assessment of peritoneal morphologic changes in peritoneal dialysis patients.** *Nephrol Dial Transplant* 2010, **25**(10):3379–3385. doi:10.1093/ndt/gfq194. Epub 2010 Apr 22.

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VI – General Discussion

- Predictors of residual renal function loss and phosphate handling in peritoneal dialysis.
- Nutrition and body composition in contemporary peritoneal dialysis patients: clinical relevant associations.
- Nutritional status and adipokines in peritoneal dialysis patients: relationship with cardiovascular events.
- Insulin resistance in non-diabetic peritoneal dialysis patients: associations with body composition but not with peritoneal glucose absorption or peritoneal membrane fast transport status.
- Peritoneal membrane functional evaluation: free water transport quantification and the potential value of effluent hepatocyte growth factor to timely characterize peritoneal membrane dysfunction.
- Future investigations.

VI. GENERAL DISCUSSION

The number of end stage renal disease patients treated by peritoneal dialysis (PD) has increased over the last years, all around the world (1). The clinical outcomes in patients under PD improved in the last decade (2, 4), and the most recent studies comparing survival in peritoneal dialysis and hemodialysis showed similar outcomes in the first years of end stage renal disease therapy (2-5). In spite of being a successful end stage renal disease therapy, there are inner aspects of the technique that require additional investigation, in order to improve further patient´s clinical outcomes, such as metabolic derangements and the systemic impact of the functional characteristics of the peritoneal membrane.

We developed a series of cross sectional and prospective clinical researches, in order to investigate the systemic impact of intrinsic aspects of the peritoneal dialysis technique, such as peritoneal membrane transport status, peritoneal glucose absorption and peritoneal dialysis modality, on residual renal function, phosphate control, body composition and insulin resistance development in a population of incident and prevalent patients under peritoneal dialysis. At the same time, since the filter is a determining interface between the external technique and the internal milieu, this thesis focused also the functional evaluation of the peritoneal membrane, by establishing a new peritoneal equilibration test capable of evaluate simultaneously small-solute transport and water transport pathways, enabling us to diagnose the most common causes of ultrafiltration failure in our patients. We also explored new peritoneal effluent biomarkers that could timely signalize peritoneal membrane deterioration, even before clinically relevant ultrafiltration failure develops.

Predictors of residual renal function loss and phosphate handling in peritoneal dialysis

Residual renal function (RRF) plays a major role in dialysis patients, whichever the modality, but has been much more focused in PD with impact in patient´s survival (13-15). For that reason, determining the factors that affect its loss has become an important issue in the care of PD patients. Larger body mass index, diabetes, peritonitis episodes, peritoneal membrane fast transport status and the use of automated peritoneal dialysis (APD) are some of the factors that have been previously associated with residual renal function loss in PD (18-20). However, results are not consensual and studies frequently exclude patients with a failed renal graft, which has becoming one of the most frequent causes of dialysis initiation (131). We have evaluated 148 patients consecutively admitted and treated in our Unit, and we have explored the factors conditioning RRF decline in our population (**chapter V.I**). Our results

reproduce a previous report from Liao et al. (18), since diabetes was the major determinant of RRF decline in the studied population. Importantly, we could not find any association between RRF loss and peritonitis rates, peritoneal membrane fast transport status, neither with APD use in our population, replicating the findings in previous studies (18, 132, 133). Contradictory results have been reported concerning RRF decline in patients that initiate peritoneal dialysis after a failed renal transplant. Sasal et al. (134) and Davies et al. (135) reported a more rapid loss of RRF in these patients, compared with patients that have never undergone kidney transplantation. We, like de Jonge et al. (136), found no difference in the decline of RRF between patients starting PD after renal transplant failure and patients starting PD as their first renal replacement therapy. Although the small sample size of the PD after renal transplant group could have precluded a statistically significant difference, we hypothesized that our policy of slowly tapering the immunosuppressors (immediate withdrawal of the anti-proliferative drugs, but slow reduction of calcineurin inhibitors and prednisolone over several months), presumably protects renal graft function.

The robust relationship seen between RRF and survival on PD may be also partially explained by the improved control of serum phosphate levels evidenced in patients with preserved RRF (22), since a strong correlation between residual renal function and serum phosphate concentration has been reported (137). Our study on phosphate handling in PD patients (**chapter V.II**), reproduced the results from Wang study (22), as hyperphosphatemia was present in 43.6% of our anuric patients versus 24.2% in patients with RRF (P=0.002), and RRF was the only independent predictor of hyperphosphatemia in the 264 studied patients.

Hyperphosphatemia is a common clinical finding in patients with CKD under dialysis, and has emerged as an important factor for cardiovascular mortality in these patients (23-25, 138-141). Mechanistically, the link between hyperphosphatemia and mortality has been attributed to the disseminated vascular calcifications (142) and to several hemodynamic disturbances including ventricular hypertrophy, increased blood pressure, increased cardiac work and high arterial tensile stress (143). These findings raise the issue of whether dialytic phosphate removal might provide a more relevant direct measure of dialysis efficacy and adequacy than urea and creatinine clearances, especially when residual renal function is lost. In fact, when we focused on anuric patients, peritoneal membrane creatinine transport status was not a significant predictor of hyperphosphatemia, but peritoneal membrane phosphate transport status (D/P phosphate) was. This is another relevant clinical input of our investigation: although peritoneal phosphate clearance was more closely related with peritoneal creatinine clearance than with urea clearance, D/P creatinine was not a sufficiently accurate measure to classify patients for peritoneal membrane phosphate transport status, as

the characterization of patients concerning phosphate and creatinine transport was quite different (47.4% patients were fast/fast average transporters according to phosphate transport, but 74.2% were fast/fast average transporters according to creatinine transport). The fact that phosphate is mainly an intra-cellular ion (with a slow intra-extra cellular solute transfer rate); 15% to 20% of phosphate is protein bound and 5% complexes with other ions; its transport depends on osmotic, chemical and electrical gradients and also trans-membranous active phosphate transporters; and the aqueous layer that surrounds the phosphate molecule, may be some of the reasons why phosphate apparently reaches a slowly equilibration across the peritoneal membrane than creatinine (144-146).

Few studies have compared peritoneal phosphate clearance according to peritoneal dialysis modality, and the results were not consensual (147-149). These previous studies were limited by small sample size and no adjustment for peritoneal membrane transport category. Sedlacek et al. (147) and Badve et al. (148), reported that phosphate clearance is higher in fast transporters compared with slow transporters in CAPD and APD, when peritoneal transport status is defined according to Twardowski categories of D/P creatinine (103) in a 4-hour, 3.86% glucose PET. Our study **(chapter V.II)** expands on the work of these groups, because we compared phosphate clearance between CAPD and APD, by adjusting for peritoneal membrane phosphate transport category. Our data suggests that peritoneal membrane phosphate transport status should be considered in order to optimize PD prescription, as CAPD was associated with a significant increase in phosphate clearance of 13.6% among slow-average transporters, and 38.4% among slow transporters, compared with APD.

Our clinical investigation also suggest that focusing only on urea and creatinine for assessment of dialysis adequacy may not result in an adequate clearance of phosphate. In fact, although more than 97% of our anuric patients had a peritoneal Kt/V of 1.7 or greater, and 83.3% had a peritoneal creatinine clearance equal or superior to 45L/wk per 1.73m^2 [complying with the International Society for Peritoneal Dialysis guidelines on PD adequacy (21)], 43.6% of them had hyperphosphatemia, which might be amenable by adjusting PD prescription to peritoneal membrane phosphate transport rate, since 80% of them were slow/slow-average phosphate transporters, 50% of whom were on APD.

Smaller studies later published (150, 151), reproduced our conclusions.

Our clinical investigation did not assessed particularities of dialysis prescription, as the effects of tidal regimens, dwell times or number of cycles. Phosphate binding prescription was also not included, however the lack of correlation between indirect protein intake (nPNA) with

serum phosphate levels, the universal uncertainty about phosphate binder accomplishment, and the fact that we focused on peritoneal phosphate elimination minimizes this limitation.

Nutrition and body composition in contemporary peritoneal dialysis patients: clinical relevant associations

Protein-energy wasting is common among end stage renal disease patients (81, 82), and is an important predictor of mortality in this population (12, 83). The first studies that compared nutritional status in patients under different dialysis modalities concluded that protein-energy wasting was more prevalent in PD patients, based mostly on albumin serum levels and BMI (84). Although a predictor of all-cause mortality in patients under dialysis, hypoalbuminemia itself does not necessarily indicate protein wasting, as several other non-nutritional factors may contribute to lower serum albumin levels in chronic kidney disease patients (152-155). It is known that PD patients can lose 6 to 8 grams/day of protein, primarily albumin, through the peritoneal membrane. However, there is insufficient evidence that these peritoneal protein loses contribute to protein-energy wasting, and the evidence is inconsistent about their association with cardiovascular events or all-cause mortality (156). In line with this view, an observational cohort study of dialysis patients, undertaken to determine the survival predictability of serum albumin level in peritoneal dialysis and hemodialysis patients, concluded that serum albumin predicts all-cause, cardiovascular and infection-related mortality, both in PD and HD patients, but the threshold at which the risk of death increases varies by dialysis modality, being 0.2 to 0.3 g/L lower in PD patients, than for patients undergoing maintenance hemodialysis (85).

According to the most recent diagnostic criteria for protein-energy wasting in CKD patients (81), we performed a cross sectional study in order to evaluate nutritional status and the prevalence of overweight/obesity in a group of 57 PD patients in treatment in our Unit (**chapter V.III**). To evaluate our patient´s nutritional status, we performed subjective global assessment (SGA), anthropometric measurements, and we applied a nutrition status score based on serum chemistry (albumin and total lymphocyte count), body mass (BMI, percentage of ideal body weight, triceps and subscapular skinfold thickness and subjective physical examination) and muscle mass (mid-arm muscle circumference and subjective physical examination). By SGA, 70.2% of our patients were classified has having a normal nutritional status, and none had severe protein-energy wasting. According to the nutrition status score (NSS) applied, only 7% of the patients were mildly-to-moderately malnourished. The results obtained with SGA and NSS were corroborated by anthropometric measurements (compared

to an age and gender matched population), since 77.2% of our patients had a triceps skinfold thickness higher than the percentile 15th, and a similar result was obtained for mid-arm muscle circumference (80.7%). Our results were somehow latter corroborated by a recent published study that evaluated the body composition of a large number of patients under dialysis (88). That study evidenced that patients under PD maintained a better nutritional status in terms of lean body mass than their matched paired HD patients, who presented significant lower values of body cell mass and lean tissue index, measured by bioimpedance analysis (88). Contrary with the first studies on nutritional status assessment in patients under peritoneal dialysis (84), our clinical investigation has evidenced that PD patients seem to be relatively protect from catabolism as malnutrition is not, nowadays, a common situation in patient under PD (**chapter V.III**).

According to BMI, 43.9% of the patients evaluated (**chapter V.III**) were overweight (BMI ≥ 25Kg/m^2) and 7% were obese (BMI ≥ 30Kg/m^2). We found no association between overweight/obesity and demographic or modality related factors, neither with fast transport status. We also found no differences in C-reactive protein, between patients classified as overweight/obese and normal-weight patients, although we found significant higher serum homocysteine, higher triglycerides and higher total cholesterol/HDL cholesterol ratio in overweight/obese patients. As we, other authors (35, 36) documented a high prevalence of obesity in PD patients, which reinforce the results that we obtained in this clinical investigation. Low BMI is recognized to be strongly associated with worse outcome in HD patients, because it reflects a poor nutrition status, in a relation known by reverse epidemiology. In PD field, there are contradictory results about the impact of obesity in patient´s outcomes (54, 157, 158). The potential atherogenic profile that we documented in the overweight/obese group allows us to hypothesize that, as in general population, obesity may be a potential risk factor for cardiovascular disease in PD patients. This hypothesis led us to perform a second investigation, in order to understand the association between nutritional status and cardiovascular disease in patients under PD (**chapter V.IV.I**).

In a second clinical investigation, performed in a different PD cohort (**chapter V.IV.I**), we reproduce our previous observation that overweight is widely prevalent in PD patients, since 40.9% of the studied patients were overweight and 10.6% were obese. Being a simple and accessible tool to characterize obesity, it is recognize that BMI does not differentiate lean mass, fat mass and extracellular water. As it is known that PD patients (while maintaining BMI) can present simultaneous changes is nutritional status, body fat content and hydration status (159), that differently impact on outcome, we wanted to improve our knowledge on this field. For that reason, we performed additionally a full body composition assessment of our patients,

using multifrequency bioimpedance (BCM, Fresenius Medical Care, Bad Homburg, Germany). According to the percentage of relative fat mass assessed by bioimpedance analysis, women were classified as obese it they presented a relative fat mass above 30%, and men were classified as obese if their relative fat mass was superior to 25% (56). The results obtained have clearly demonstrated that our real obesity prevalence was even higher than when we used BMI classification, as 73.4% of our patients had a relative fat mass above the previously mentioned cut-offs. Using a clinically applicable "bedside" validated method to characterize relative fat mass, we were able to demonstrate that BMI clearly underestimates obesity prevalence in PD patients. Our results corroborate a clinical investigation that documented that a BMI < 30 Kg/m^2 does not exclude the presence of obesity, in a group of incident and prevalent patients under hemodialysis, evaluated by anthropometry (57). In a third clinical investigation performed to assess insulin resistance, in a group of 51 non-diabetic patients currently receiving treatment in our Unit (**chapter V.IV.II**), we obtained similar results concerning obesity evaluation, since 33% of the studied patients were considered overweight and 6% were obese according to the BMI, but according to bioimpedance analysis, the prevalence of obesity was much higher, being 49%, according to the previously mentioned cut-offs of relative fat mass.

It is important to emphasize that, the bioimpedance method applied (Body Composition Monitor – Fresenius Medical care, Bad Homburg Germany), was validated by isotope dilution methods (124), by accepted reference body composition methods (127) and by extensive clinical assessment of the hydration state (126). We performed bioimpedance analysis in the morning of the PET, and we only considered valid measurements that simultaneously presented high quality (>90%) and lower error percentage (<35%), showing, in the device monitor, an inverted U curve. Currently, there is some debate about the patient´s conditions in time of measurement of body composition by bioimpedance, namely whether the presence of intraperitoneal effluent could interfere with the results obtained. Based on a critical review of the most recent literature, we believe that the presence or absence of PD fluid in the abdomen does not influence the readings of hydration status or nutrition parameters. The reason for that assumption is based on the fact that due to bio-physical reasons, bioimpedance spectroscopy does not measure sequestered fluid in the trunk (127, 160). The issue about differences in hydration status due to the presence of PD fluid in the abdomen was specifically addressed by two recent studies (161, 162). Both studies evidenced that the difference in fluid overload and extracellular water/total body water ratio measured was negligible, neither statistically nor clinical significant, when the measurements were performed first in patients with 2 liters of glucose solution in the peritoneum, and then after the solution was drained. It is also important to emphasize that no study using the body composition monitor device has ever showed any

difference in lean body mass, in body cell mass or fat mass readings, according to the presence or absence of peritoneal dialysis solution in the abdomen. In fact, in a recent clinical investigation, Arroyo D. et al. (128), clearly demonstrated that intracellular water, lean tissue mass, lean tissue index, fat tissue mass, fat tissue index, body cell mass and adipose tissue mass remained unaffected by abdominal content.

Glucose remains the most common osmotic agent used in PD solutions because of its low price, proved efficiency and easy metabolism. However, there is the fear that with time on PD, peritoneal glucose absorption would lead to obesity, dyslipidemia and insulin resistance aggravation (34). In line with this view, fast transporters could be even more problematic, since they present a more rapidly absorption of the glucose present in the dialysis solutions (163). We have carefully explored the possible associations between daily peritoneal glucose absorption and small solute transport status with obesity, in two different clinical investigations (**chapters V.IV.I and V.IV.II**). The results obtained were similar and clearly demonstrated the lack of association between peritoneal glucose absorption and obesity, and are corroborated by two other contemporaneous clinical investigations (36, 164). We also did not found any association between fast transport status and obesity, in none of our clinical researches (**chapters V.III, V.IV.I and V.IV.II**), meaning that fast transporters can be adequately managed in PD without becoming obese, by using an individualized prescription that matches the patient´s peritoneal membrane characteristics.

Nutritional status and adipokines in peritoneal dialysis patients: relationship with cardiovascular events

Cardiovascular disease is the leading cause of death in peritoneal dialysis patients (165, 166), and its association with nutritional status has not been sufficiently investigated and clarified. Protein-energy wasting, inflammation and atherosclerosis are closely linked in chronic kidney disease patients, in an association known by malnutrition-inflammation-atherosclerosis (MIA) syndrome (82). Patients with MIA syndrome are more prone to suffer from cardiovascular events than patients with a normal nutrition status (83). For another hand, the associations between obesity and cardiovascular events are well characterized in general population, but remain largely unexplored in CKD patients under PD. With the aim of clarifying the associations between cardiovascular events and body composition and adipokines profile in PD, we conducted a prospective study including 66 prevalent patients of our Unit, followed for 47.0±28.2 months (**chapter V.IV.I**).

Our study (**chapter V.IV.I**) brings some light into the conflicting results about the impact of nutritional status in PD patient´s outcomes, concerning new cardiovascular events. As we mentioned before, we registered an elevated prevalence of obesity in the studied population (73.4%), in line with our **(chapter V.III)**, and others (36) previous researches, and a relatively low prevalence of protein-wasting (36%). Patients who presented a cardiovascular event, during the follow-up period, were older and had a higher relative over-hydration percentage, compared with patients that were free of events, which was expected (167). Concerning body composition assessment, there were no differences in lean tissue index and body cell mass between patients with and without cardiovascular events, and the percentage of patients with protein-wasting classified by bioimpedance analysis was similar between the two groups (43.8% vs 35.4%, P=0.565). Contrary to the results obtained concerning lean body mass, fat tissue index was slightly higher among patients with cardiovascular events, and the percentage of obese patients according to relative fat mass was also significantly higher in this group (93.8% versus 66.7%, P=0.029).

Knowing, from our previous studies **(chapter V.III)**, that obesity is widely prevalent in contemporaneous PD populations, we hypothesized that adipokines could be useful markers to predict cardiovascular events in these patients. Adipose tissue is now considered an active endocrine organ, and numerous adipokines have been implicated in the pathogenesis of chronic inflammation and insulin resistance associated with obesity. In vitro and animal studies evidenced that leptin has different atherogenic properties, and that adiponectin is an insulin-sensitizing, anti-inflammatory and anti-atherogenic adipokine (40, 41). Few studies performed on CKD patients have addressed the adipokines impact on outcome, with conflicting results (74-77, 168) and, until know, only two have specifically studied PD patients (70, 78). However, none of the mentioned studies considered the nutritional and hydration status of the patients, which could explain the contradictory results obtained, since besides the well-known association between adipokines and fat mass (40), high adiponectin and low leptin can both reflect wasting, and overhydration can alter the normal regulation of adiponectin (72, 73).

One of the most important results of our clinical investigation was to document that patients who presented cardiovascular events were mostly obese and had also a significant higher leptin/adiponectin ratio. However, contrary to Park et al. (70), we were only able to document such result on adipokines ratio, once we exclude from the analysis patients with protein-wasting. Our study also puts on evidence the fact that protein-wasting is a confounding variable that affects adipokines production, since we were able to demonstrate that both relative fat mass and lean tissue index were independent predictors of leptin/adiponectin ratio, after adjusting for peritoneal glucose absorption. Our results demonstrate that obesity

associates with higher leptin levels and lower adiponectin levels that will translate into a higher leptin/adiponectin ratio. The opposite situation of protein-wasting is characterized by a lower leptin value and a higher adiponectin value that will translate into a lower leptin/adiponectin ratio. Given the clear biologic evidence that leptin and adiponectin have opposite effects on cardiovascular system, we may hypothesized that in PD patients without protein-wasting, a higher leptin/adiponectin ratio is a predictor of cardiovascular events, being an atherogenic index in this population.

We have also explored the potential role of peritoneal glucose absorption and peritoneal membrane small solute transport status on obesity (has mentioned before), and on cardiovascular events. Although appealing, once more, we could not find any association between peritoneal glucose absorption and fast transport status with obesity, which reproduces our previous observation **(chapter V.III)**. Concerning cardiovascular events, we were also not able to document any association with peritoneal glucose absorption or with membrane transport characteristics. Concerning peritoneal glucose exposure, a recently published study concludes that high glucose concentrations in peritoneal dialysate are associated with all-cause and cardiovascular mortality, in a group of patients under continuous ambulatory peritoneal dialysis (169). However, the authors could not assess if the association found with cardiovascular mortality is explained by the potential deleterious systemic effects of glucose itself, or by the clinical situation underlining the necessity to use more hypertonic glucose. In fact, the authors hypothesized that the association found is explained by the fact that peritoneal glucose potentially leads to metabolic syndrome development that will translate into a higher cardiovascular mortality. However, the nutritional status of the population studied was not evaluated, body composition was not assessed, effective peritoneal glucose absorption was not measured, and they gave no information about the prevalence of metabolic syndrome in their population. They have performed an analysis on the independent risk factors associated with the use of higher dialysate glucose concentrations. They found that older patients, with lower residual renal function and with a higher small solute transport rate were more likely to require a high glucose load in terms of PD prescription. It is known that older patients (170), with RRF loss (171) and fast transporters under CAPD (172) can be prone to overhydration, which is a well-known condition that predicts higher cardiovascular mortality in patients under dialysis (167). In line with this view, the association found between high glucose in the dialysate and cardiovascular mortality could be explained through overhydration, instead of being a direct consequence of the potential deleterious systemic effects of glucose, that were not effectively measured in this study.

There is uncertainty about the clinical outcomes of fast transporters in peritoneal dialysis. Although the last meta-analysis published has demonstrated that a higher peritoneal membrane solute transport rate is associated with a higher mortality (29), other recent studies concluded that the use of continuous cycling peritoneal dialysis seems to offset some of this negative effect on mortality (27, 28, 173, 174). In line with this view, we were not able to find any association between fast transport status and higher cardiovascular events. Our policy to treat fast transporters mostly using APD, with short dwells and using icodextrin for the long dwell, may explain the similar clinical outcomes of our patients, independently of their peritoneal membrane small solute transport characteristics.

Insulin resistance in non-diabetic peritoneal dialysis patients: associations with body composition but not with peritoneal glucose absorption or peritoneal membrane fast transport status

With time on PD, obesity and insulin resistance aggravation are two feared systemic consequences of peritoneal glucose absorption, especially in patients who present a peritoneal membrane fast transport status. The associations of insulin resistance with clinical outcomes in PD were recently investigated (65, 175), but these two studies presented contradictory results. Besides, none of these studies has clarified the impact of body composition, nutritional status or other factors related with PD prescription, on insulin resistance development, which is a meaningful clinical question, since without these relevant information doctors cannot develop a successful strategy in order to ameliorate insulin resistance in their patients. Another relevant clinical issue, not sufficiently clarified, is the impact of peritoneal membrane fast transport status and glucose absorption on insulin resistance.

The results of our clinical investigation (**chapter V.IV.II**), evidenced that insulin resistance is prevalent even in non-diabetic patients on peritoneal dialysis. It also brings some light into the factors responsible for insulin resistance in patients under peritoneal dialysis, enhancing the role of obesity and adipokines in this population, as fat tissue index and leptin/adiponectin ratio were significant predictors of insulin resistance, measured by homeostasis model assessment method (HOMA-IR), independently of peritoneal glucose absorption and peritoneal small solute transport status.

For the first time in PD field, we documented a significant positive association between HOMA-IR and two other adipokines-based insulin resistance indices (HOMA corrected by adiponectin and leptin/adiponectin ratio), reproducing the results obtained by Hung et al. (64), in patients under chronic hemodialysis.

The associations between obesity and insulin resistance in our population were documented in several ways. First, insulin resistance indices were strongly correlated with body composition parameters related with fat mass (as fat tissue index and relative fat mass), but not with parameters related with lean mass (as lean tissue index and body cell mass). Second, when patients were categorized as obese and non-obese according to relative fat mass measured by bioimpedance, HOMA-IR, HOMA corrected by adiponectin (HOMA-AD) and leptin/adiponectin ratio were significantly higher in obese group. The fact that these differences were not noticeable when patients were compared concerning BMI, gives an indirect evidence that BMI is not an accurate tool to classify obesity in PD patients, which reinforces the findings of our previous clinical investigation (**chapter V.IV.I)**. Third, when we compared patients according to insulin resistance state (patients with HOMA-IR equal or superior to 2.2 and inferior to 2.2), we found that insulin resistant patients had significant higher values of relative fat mass and fat tissue index, while maintaining similar values of BMI and lean tissue index. Obesity, according to relative fat mass, was also more prevalent in insulin resistant patients than in patients with HOMA-IR < 2.2 (84.6% versus 47.8%, P=0.039), while the prevalence of wasting was similar between the two groups (38.5% versus 13%, P=0.107). Lastly, fat tissue index was an independent predictor of HOMA-IR in the studied patients.

We might be questioned about the fact that we have measured total body fat mass instead of visceral fat mass, a traditional insulin resistance predictor in general population (176, 177). However, the role of body fat distribution and the metabolic complications of obesity in general population has been recently a matter of debate and controversy (178), since there are also studies that documented a more important role of upper body subcutaneous fat mass on insulin resistance development (179). This may explain the results obtained in a previous clinical investigation about insulin resistance predictors in hemodialysis patients (64). According to Hung et al. study (64), truncal fat mass and total fat mass, assessed by DEXA, were both independent predictors of insulin resistance in a population of 10 patients under hemodialysis. We were not able to perform DEXA or abdominal CT scan on our patients, due to financial constraints. We alternatively measured serum IGFBP-1, a recently validated surrogate marker of visceral fat, strongly inversely related with liver fat content measured by the gold standard method - proton magnetic resonance spectroscopy (180). Our results evidence that IGFBP-1 has a strong and negative correlation with HOMA-IR, HOMA-AD and leptin/adiponectin ratio, and that serum IGFBP-1 is significantly lower in insulin resistant patients (with HOMA-IR equal or superior to 2.2).

Another clinical relevant result, that deserves to be discussed, is the relationship between insulin resistance and the adipokines ratio (LAR) that depends on body composition

parameters. We have previously reported that relative fat mass and lean tissue index predicted LAR independently of peritoneal glucose absorption **(chapter V.IV.I)**. The results of the present study (**chapter V.IV.II**) corroborate this evidence, underlining further that insulin resistance is aggravated in both the extremes of the nutritional spectrum, while LAR present a diverse link with HOMA-IR according to such status : 1) protein-energy wasting patients evidenced a LAR inferior to 1, and in these patients the correlation between LAR and HOMA-IR was negative, meaning that as the severity of protein-wasting increases, LAR decreases and HOMA-IR increases; 2) in the opposite situation, obese patients exhibited higher LAR values, and LAR presented a strong positive correlation with HOMA-IR. Leptin and adiponectin play important and opposite roles in the regulation of cardiovascular and metabolic homeostasis. In a previous clinical investigation **(chapter V.IV.I)** we reported that LAR could be used as a new atherogenic index, as it was associated with cardiovascular events in PD patients without protein-wasting. According to the results now obtained, we may hypothesize that the association between LAR and cardiovascular events, that we have previously documented, can be explained through insulin resistance development. In fact, given the direct actions of adiponectin on insulin sensitivity, both in skeletal muscle and liver (40, 41), a higher leptin/adiponectin ratio can been seen not only as a biomarker of insulin resistance but also as a predictor of insulin resistance, as our most recent results demonstrate (**chapter V.IV.II**).

In this clinical investigation (**chapter V.IV.II**), peritoneal glucose absorption showed no association with obesity profile, which reproduces our previous results **(chapter V.IV.I)** and others author´s observations (36, 164). We are aware that the glycemic and insulinemic responses differ after a similar oral glucose dose and intra-peritoneal glucose administrations. The cephalic phase of insulin release, and specially the enteric release of cholecystokinin, gastric inhibitory polypeptide and glucagon-like peptide 1 (GLP-1), which are bypassed during the peritoneal administration of glucose, explain the more physiological insulinemic response that is seen after oral glucose administration, compared with the intra-peritoneal route. This incretin effect partially explains the greater rate of glucose disappearance from plasma, and the greater rate of cellular utilization of glucose that is observed in the first 180 minutes after an equivalent oral glucose load compared with the intra-peritoneal administration. However, although peritoneal glucose administration is not as physiological as the oral route, we still were not able to document any association between peritoneal glucose absorption and insulin resistance in our patients. As we already mentioned, one of the most feared systemic consequences of glucose absorption in PD is precisely obesity and insulin resistance development. The fact that we didn´t found any association between peritoneal glucose absorption and insulin resistance indices, doesn´t mean that glucose is not an important variable in the complex situation of obesity and insulin resistance development in PD. More

than that, our results reinforce that with contemporary therapies, that avoid excessive glucose exposition, absorbed glucose is not associated with insulin resistance, but adiposity is. Therefore, our findings put on evidence that other variables such as dietary intake, energy expenditure, and genetics (that partially determines resting energy expenditure) might be more powerful determinants of obesity, deserving focused investigation.

Given the fact that fast transporters have been previously associated with a gloomy outcome (26, 29), we specifically studied this group concerning cardiovascular events and insulin resistance (**chapters V.IV.I and V.IV.II**), testing whether fast transporters are prone to obesity and insulin resistance development (34). The results that we obtained with two different clinical investigations cannot support such a chain of events (**chapters V.IV.I and V.IV.II**). First, none of our studies documented any association between peritoneal small solute transport status and obesity profile (**chapters V.III, V.IV.I and V.IV.II**). Second, we could not find any association between peritoneal small solute transport status and cardiovascular events, in a group of 57 PD patients followed for 47 months (**chapter V.IV.I**). Third, in a study about insulin resistance prevalence in a group on 51 non-diabetic PD patients, we also did not find any significant difference concerning leptin/adiponectin ratio, IGFBP-1, HOMA-IR, HOMA-AD, peritoneal glucose absorption, neither in body composition parameters related with fat mass, between fast transporters and the other small solute transport categories (**chapter V.IV.II**). To our knowledge, only two studies evaluated the associations between peritoneal small solute transport and insulin resistance measured by HOMA-IR (181, 182), and both evidenced a lack of correlation between D/P creatinine and HOMA-IR. Our results not only support such evidence, but further expands the knowledge about fast transport status systemic consequences, highlighting that fast transporters under updated therapy regimens have no higher risk for obesity and insulin resistance development.

Systemic inflammation was recently documented to be an independent predictor of survival in PD (90), but the factors that may trigger inflammation were not sufficiently clarified. As others (183), we found an association between inflammation and obesity. However, we were just able to document such association, when patients were characterized according to relative fat mass by bioimpedance analysis, but not when patients were classified according to BMI. Indeed our studies evidenced that IL-6 **(chapter V.IV.I)** and C-reactive protein **(chapter V.IV.II)** were significantly higher in obese patients, classified according to relative fat mass percentage. Concerning insulin resistance evaluation **(chapter V.IV.II)**, we also found that patients with HOMA-IR superior or equal to 2.2 presented higher C-reactive protein levels, although the difference was not statistically significant. The small number of patients evaluated could preclude us from obtaining a significant result. Besides, there are other inflammatory cytokines known to be more related with insulin resistant states, such as TNF-alfa, IL-1 and

IL-6 (40), that were not measured. As Lambie M. et al. (90), we also did not found any association between systemic inflammation and small solute transport status. However, contrary to that study, we could not find any association between fatal and non-fatal cardiovascular events and a fast transport status (**chapter V.IV.I**). We hypothesized, once more, that our PD prescription policy can explain our patient´s better outcome, since contrary to the Global Fluid Study, the majority of our fast transporters are prescribed with APD and icodextrin. In fact, our results are supported by others recent clinical investigations that evidence that a peritoneal membrane fast transport status is no longer associated with worse outcome, in cohorts using APD and icodextrin (27, 173, 174). The axis of pathophysiology between fast transport and reported worse outcomes is probably much more related with volume control and hypervolemia driven systemic inflammation, than merely related with glucose absorption. On the other hand, glucose and its glucose degradation products are promoters of membrane functional loss (34). Therefore, even if there is no evidence of a strong link between peritoneal glucose exposition and insulin resistance or obesity development, still glucose sparing regimens are recommendable to minimize membrane loss, hypervolemia risk and associated mortality.

Finally, some formal methodological issues deserves further discussion, namely the HOMA-IR cut-off chosen to classify patients for insulin resistance, the importance of insulin analytical determination, and the potential effects of peritoneal glucose concentration on serum insulin and glucose measurements.

Concerning the HOMA-IR cut off, patients with HOMA-IR ≥ 2.2 were considered to present insulin resistance, as this value corresponds to HOMA-IR percentile 50 in the larger validation study about HOMA-IR against the euglycemic, hyperinsulinemic clamp in non-diabetic patients (60).

It is also important to stress that insulin assays can vary considerably, especially if antibodies cross-reacting with insulin or split-proinsulin products are used (184).We have minimize any potential interference exerted on HOMA-IR score, by using an insulin-specific assay.

We calculated insulin resistance (HOMA-IR) by measuring fasting plasma glucose and fasting plasma insulin after an overnight fast (8 to 10 hours fast), but with continuation of PD therapy in a standardized way, with 1.36% glucose in the last dwell. We considered that this standardization concerning the last dwell adds accuracy to our methodology and is a strength, since the majority of studies that calculate HOMA-IR in PD patients lack in standardization of PD prescription, with maintenance of the usual prescription (65, 175, 181). We have carefully chosen to performed the last dwell with the lowest concentration of glucose in the dialysate

(1.36% glucose), based on the measured effects on plasma glucose and plasma insulin of a single 6-hour dialysis cycle with 1.36% glucose solution, that was previously investigated, in a group of non-diabetic patients on continuous ambulatory peritoneal dialysis (31). The investigation performed by Heaton A. et al. (31), clearly showed that plasma insulin was not significant different, in any moment, along the 360 minutes study, between patients without glucose in the abdomen compared with patients with 2 liters of 1.36% glucose solution. Concerning plasma glucose, the difference between undialysed patients and patients with 1.36% glucose solution in the abdomen, was only significant at 45 minutes, and the results were not different between 60 and 360 minutes. The results expressed by Heaton A. et al. (31), enabled us to perform a protocol to investigate the presence of insulin resistance in our PD population, measuring plasma glucose and insulin in a fasting state, minimizing any interference from peritoneal glucose. In patients under CAPD, the last dwell was performed at 23 h and the blood was collected at 9 am after the drainage of the peritoneal cavity and before the PET starts. In patients under APD, we also changed the prescription and told our patients to use 1.36% glucose has their last exchange. In patients under APD, the blood was also collected after drainage of the peritoneal cavity, before the PET starts, and we have ensured that the previous dwell was performed with 1.36% glucose solution. As the previously cited investigation (31) showed that there are no differences in plasma glucose and insulin between undialysed PD patients and patients with 1.36% glucose solution, after the first 60 minutes of a dwell, we think that our protocol minimizes any potential effect of peritoneal glucose absorption, while it allows the patients to maintain dialysis.

Peritoneal membrane functional evaluation: free water transport quantification and the potential value of effluent hepatocyte growth factor to timely characterize peritoneal membrane dysfunction

Over time, peritoneal dialysis results in structural alterations of the peritoneal membrane (96), hardly assessed in clinical practice. These structural alterations will promote membrane functional changes that are worth to be evaluated through peritoneal equilibrations tests, as they have therapeutic implications in terms of dialysis prescription (185). Although there are several peritoneal equilibration tests available for routine clinical practice, the type of information provided by each of them is different, and for that reason, each clinician should elect the PET that provides the most useful information in terms of peritoneal membrane functional characterization (185). We report (**chapter V.V.I**), simultaneous small-pore and ultrasmall-pore peritoneal transport quantification, through the use of a 4-hour 3.86% glucose PET with temporary drainage at 60 minutes, expanding a previous report from Cnossen et al.

(110). Our clinical investigation also expands the work of La Milia group (106), because our Two-in-One Protocol allows peritoneal ultrafiltration measurement at 1 hour [as the mini-PET (106)], while it maintains a total duration of 4 hours [as a standardized, or modified PET (104)]. This protocol enabled us to simultaneously quantify ultrafiltration through ultrasmall pores and, at the same time, characterize the membrane according to small solute transport, as in a standardized PET, without occurring the overestimation that might happen when small-solute transport categorization is based on a $D/P_{creatinine}$ obtained after a 1 hour dwell (109).

The results that we have obtained, in terms of FWT and FWT percentage, were in line with those obtained with a standard permeability test (107), a more sophisticated method hardly used in daily clinical practice. Also, skilled studies from Waniewski J. et al. (186, 187), found a FWT fraction of 0.40±0.12, which is equivalent to the FWT percentage that we obtained.

The fact that FWT correlated better with total ultrafiltration than with any indirect measure of sodium sieving was already expected, given the fact that a reduced water flow through the aquaporins is not the only plausible cause that determines a sodium sieving reduction (188).

We have performed a correction for sodium diffusion using the mass transfer area coefficient for creatinine ($MTAC_{Creatinine}$), calculated by the simplified Garred model (189). The same methodology was also followed by other groups that have investigated peritoneal fluid pathways (190, 191) and exhibits good correlation with the Waniewski model (109). We also have performed a FWT correction for lymphatic absorption and for the ultrafiltration through the large pores, during the first 60 minutes of the dwell, according to an algorithm validated by computer simulation model (108).

We documented a slight, but consistent, tendency for total ultrafiltration and FWT decline with time on PD. Patients with ultrafiltration failure evidenced a FWT significantly lower and a $D/P_{creatinine}$ significantly higher, when compared with patients without ultrafiltration failure. However, FWT fraction was similar between the two groups, which is line with Parikova et al. (100), that has demonstrated that in the first years of PD treatment, an increase of vascular peritoneal surface area will lead to a rapid disappearance of the osmotic gradient and thereby promote some reduction in FWT, but not in its contribution to total ultrafiltration.

Another relevant aspect of our clinical investigation, that deserves to be discussed, is the opposite correlation that we found between small-pore and aquaporin water transport in patients with and without ultrafiltration failure. In fact, we have documented a positive correlation between small-pore and free water transport in patients without ultrafiltration failure

(r=0.257, p=0.032), in line with the fact that, in a non-pathological setting, FWT depends on osmotic gradient and effective capillary surface (188). However, in patients with acquired ultrafiltration failure, a negative and more powerful correlation was documented between small-pore and free water transport pathways (r=-0.709, p=0.015). In order to explain this opposite profile found in ultrafiltration failure situation, we have hypothesized that some of our patients with most severe forms of ultrafiltration failure could present an interstitial fibrosis process that would explain this “uncoupling” between changes in small-pore transport and in FWT. He have formulated this hypothesis based on a previous investigation about simulations of osmotic ultrafiltration failure in CAPD, using a serial three-pore membrane/fiber matrix computational model (192). According to that study, an uncoupling of small solute transport from ultrafiltration coefficient (LpS) was documented in computer simulations of ultrafiltration failure, whenever changes in both vasculature and in interstitium were taken into account. In fact, when the authors investigated the impact of increasing the effective vascular surface area (S) concomitantly with altering the fiber composition and by adding (collagen) fibers to the interstitium, they evidenced a reduction of ultrafiltration capacity of the total capillary-interstitial barrier, while only little affecting small solute transport and glucose reflection coefficient (sigma). Our results, on functional membrane properties in ultrafiltration failure setting, were posteriorly validated and corroborated by a recent clinical research, that has analysed peritoneal membrane samples of patients with encapsulating peritoneal sclerosis (102). This study confirms that structural changes in the peritoneum interstitium account for an excessive reduction in osmotic conductance without affecting small solute transport, as we have previously hypothesized that would occur in our patients with the most severe ultrafiltration failure forms that presented, in a functional peritoneal membrane evaluation, a FWT compromise beyond of what would be explained by an increase in capillary surface.

In a clinical point of view, our study has enabled us to identify other important causes of ultrafiltration failure, beyond the increase in effective capillary surface, with repercussion in the therapeutic managing of these patients. In fact, among the 11 patients with ultrafiltration failure, five had a FWT fraction contribution to ultrafiltration at 60 minutes inferior to 45%, but only two of them were fast transporters. That finding suggests again a higher compromise of FWT, disproportionate to small-solute transport. As a therapeutic strategy, those patients with FWT severe compromise will benefit most from icodextrin instead of hypertonic glucose prescriptions. Thus, one of the most important clinical implications of using the Two-in-One protocol, to our daily clinical practice, is that it allowed us to understand and monitor longitudinally the functional characteristics of our patient´s peritoneal membrane, improving our capacity to prescribe a dialysis program that matches each patient membrane

characteristics and timely detect signs of alarm relative to the risk of encapsulating peritoneal sclerosis.

Several clinical studies, and others based on computational models, have already demonstrated that impaired free water transport indicates a more severe functional and structural lesion of the peritoneal membrane, due to aquaporin dysfunction or interstitial changes (100, 105, 193-195). In fact, a recent clinical research has documented that patients with encapsulating peritoneal sclerosis (EPS) presented early loss of ultrafiltration capacity and sodium sieving 2 years before the onset of EPS, compared with long-term PD controls (102). In a multivariate analysis, sodium sieving was the only independent risk factor associated with EPS, outperforming other clinical variables, commonly associated with a higher EPS risk, such as age at dialysis onset, PD duration, residual renal function, beta-blocker use and peritonitis rate. Furthermore, according to the same study, submesothelial thickness presented strong negative correlations with net ultrafiltration and with sodium sieving, but no correlation was found with small-solute transport evaluated with $D/P_{creatinine}$ at a 4 hours PET. These recently proven functional-structural peritoneal membrane correlations, associated with the fact that the performance of serial peritoneal biopsies to detect morphology modifications is not feasible in PD patients, makes desirable the search for a biomarker that correlates both with ultrafiltration and with free water transport, and not only to the membrane small solute transport status. The use of effluent biomarkers as an early sign of peritoneal membrane alterations is currently under debate (111-113), and studies provided different results. In a recent report (111), monocyte chemotactic protein-1 (MCP-1), interleukin-6 (IL-6) and leukotactin (CCL15), were found at higher levels in the peritoneal effluent of patients who subsequently developed EPS, although without improving prediction of future EPS risk above known clinical factors, such as PD vintage and peritoneal small solute transport status. On the contrary, Sampimon et al. (112) evidenced that dialysate appearance rate of IL-6 and CA125 combined was potentially useful for an early diagnosis of EPS. However, none of these studies has explored the associations between the measured cytokines in the effluent and water transport pathways, especially with free water transport, given the important functional-structural correlations that were already mentioned.

To our knowledge our group was the first to report a clinical significant association between hepatocyte growth factor (HGF) measured in the effluent, FWT quantification and ultrafiltration profile (**chapter V.V.II**). Given the fact that HGF is constitutively synthesized by human peritoneal mesothelial cells and it is known to block high glucose induced epithelial-to-mesenchymal transition, ameliorating peritoneal fibrosis (93), we hypothesized that the increased effluent HGF that we found in patients with ultrafiltration failure, especially in patients

with the most UFF severe forms (with FWT fraction compromise), could be seen as a reactive repairing mechanism, being an early sign of peritoneal membrane deterioration. In fact, our study adds further clinical evidence to a previous report that studied focal HGF expression in peritoneum biopsies of PD patients with and without ultrafiltration failure (196). According to Nakamura et al. (196), there was an increased expression of HGF in peritoneal tissues of CAPD patients with low ultrafiltration capacity compared with the group with a normal ultrafiltration profile. Once more, these results give strength to our hypothesis that the higher HGF concentration that we found in patients with FWT compromise, on top of an increased small-solute transport, can been seen as a reactive mechanism to peritoneal membrane lesion. The peritoneal effluent HGF concentration was measured, until now, by a single clinical study (118). However, Mizuiri S. et al. (118) have only compared effluent HGF concentration according to small-solute transport, and gave no information about ultrafiltration failure or water transport pathways profile of the population evaluated. Since there are no clinical studies that had examined plasma and effluent HGF, and we have also found a correlation with small-solute transport, we might question whether effluent HGF concentration could depend on plasma HGF levels. We think that this is not plausible for various reasons. First, the peritoneal permeability is expected to be poor, since HGF is a heterodimeric molecule composed of a 69 KDa alpha subunit and a 34 KDa beta subunit. Second, we couldn´t find any correlation between effluent HGF concentration and small-pore water transport, meaning that HGF is not significantly diffusible. Third, Yu et al. (93) demonstrated that human peritoneal cells constitutively synthesized HGF, and Nakamura et al. (196) showed that HGF can be expressed at the peritoneal membrane level, especially in PD patients with ultrafiltration failure. For these reasons, we believe that the HGF protein detected in the effluent is locally produced.

All patients included in this clinical investigation (**chapter V.V.II**) performed a peritoneal membrane transport functional test using the Two-in-One Protocol **(chapter V.V.I),** whose specifications and advantages were already discussed. The evaluation performed enabled us to classify nine patients as having ultrafiltration failure. Three had a more severe profile characterized by FWT compromise (FWT ≤45%) and increased effective capillary surface ($D/P_{creatinine}$≥0,81). Those patients had significant lower ultrafiltration volume at a 4-hour PET (166,7±57,7 mL vs 375,0±41,8mL, p=0,024), lower FWT quantification (128,7±26,5 mL vs 183,5±12,1 mL, p=0,024) and higher $MTAC_{creatinine}$ (25,4 ± 2,6 mL/min vs 9,6±2,5 mL/min,p=0.024). Patients with the more severe UFF profile had also higher values of HGF measured in the effluent (104,3 pg/mL vs 88,9 pg/mL, p=0.085). Another three patients, although not fast transporters, presented an increased effective capillary surface when compared with their previous $MTAC_{creatinine}$ result (on a previous PET).The rapid disappearance

of the osmotic gradient contributed for UFF in those patients, while FWT was not impaired. The last three patients were incident, with few months on PD (3 – 6 months). According to small solute transport they were average transporters with no FWT impairment. A high effective lymphatic absorption rate could be the reason for UFF in those incident patients, since high lymphatic absorption is recognized to be mostly a cause of inherent UFF (193). It is noteworthy that mean HGF in those three patients (with a functional UFF) was significantly lower compared with the 3 patients that had impaired FWT [77.84 (67.9 – 79.8) pg/mL vs 104.3 (103.7 – 147.16) pg/mL, p=0.05].

In this clinical investigation we reported a FWT fraction of 45% which is in line with ours **(chapter V.V.I),** and others (106) previous observations. A FWT fraction inferior to 45% is clinical relevant in patients with ultrafiltration failure, as it may signalize an aquaporin dysfunction or a process of interstitial fibrosis, that will promote a decrease in the membrane osmotic conductance to glucose (100). However, there is actually no knowledge about the clinical meaning of a FWT reduction in patients without ultrafiltration failure. This is another relevant aspect of our investigation: a FWT fraction ≤45% without clinical ultrafiltration failure was documented in some patients, who also had increased dialysate HGF concentration compared with patients with preserved FWT fraction. We can hypothesized that this may represent an intermediate level of peritoneal dysfunction, where the patient may already have some interstitial changes that lead to a reactive increase in HGF production, while not severe enough to functionally present an ultrafiltration failure. Aging, uremia and diabetes are often associated with membrane changes already at PD start, possibly justifying, in some patients, selective FWT compromise in absence of a clinically relevant ultrafiltration failure (197-199).

One of the limitations of our studies on peritoneal membrane functional evaluation is the inability of our Two-in-One protocol to quantify lymphatic absorption rate, which is not feasible in daily clinical practice, and whose methodology is still under debate (200). We are also aware that assuming a constant rate of 0.3 mL/minute for peritoneal lymphatic absorption is a simplification of the three-pore model, as it may differ between patients. Furthermore, we were able to identify a higher lymphatic absorption, as a plausible cause of ultrafiltration failure, in some of our patients, by exclusion. In fact, peritoneal absorption remains a difficult problem to investigators as no clinical procedure, feasible in daily clinical practice, is able to quantify lymphatic absorption, except by using macromolecular tracer such as dextran (201) or radioiodinated human serum albumin (202, 203).

Our studies were also limited by their cross sectional design and small number of patients with ultrafiltration failure. A longitudinal study is being conducted in order to better understand the clinical meaning of a decreased FWT in patients without ultrafiltration failure,

and also to document the dynamic profile of HGF production and its relationship with peritoneal membrane water transport changes.

Future investigations

Preservation of residual renal function is patients under peritoneal dialysis: the role of new PD solutions and beyond

In the clinical investigation performed **(chapter V.I)**, we concluded that diabetes was the only independent predictor of anuria, after PD start. Although more than 50% of the population under analyse was treated with PD solutions low in glucose degradation products, we were not able to determine the effect of these new solutions concerning residual renal function loss. This subject has been recently matter of investigation. In fact, according to a recently published meta-analysis about the effect of neutral-pH, low-glucose degradation products peritoneal dialysis solutions, on residual renal function, these more biocompatible solutions resulted in better preservation of RRF that persisted beyond 12 months (204). However, there is still room to investigate whether there is a link between uraemia per se and peritoneal structural and functional changes, and if kidney function protection may also be independently associated with peritoneal membrane preservation. Additionally, the differential qualitative impact of kidney filtration rate versus diuresis in outcomes and systemic inflammation certainly merits further investigation.

Obesity determinants in peritoneal dialysis patients: the role of dietary intake, genetics and energy expenditure

We observed, in a reproducible manner, in two different clinical investigations **(chapters V.IV.I and V.IV.II)**, a lack of association between peritoneal glucose absorption and obesity development, in patients under peritoneal dialysis. We also concluded that peritoneal glucose absorption is not associated with insulin resistance development, in our patients, but adiposity is.

Our studies were limited by their cross-sectional design, and for that reason we aim for a longitudinal extension of these investigations. Nevertheless, our findings put on evidence that other variables such as dietary intake, energy expenditure, and genetics (that partially determines resting energy expenditure) might be more powerful determinants of obesity, deserving focused investigation.

The gut releases several peptides upon feeding, which affect hypothalamic pathways involved in the regulation of satiety and food intake. Cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) signal to the brain and provide satiety signals. This gut-brain axis regulation of glucose metabolism deserves focused investigation in peritoneal dialysis field, as the intra-peritoneal route bypasses gut hormones and their action on food intake regulation by the hypothalamus.

As we, Vasselai et al. (36) also found no association between fat mass gain in PD patients and peritoneal glucose absorption. In fact, they found that patients who presented fat mass gain, after one year under PD, presented a higher ratio of caloric intake/energy expenditure, when compared with patients that lost fat mass, while the daily peritoneal glucose absorption was similar between the 2 groups. More studies focused on the relation between obesity and energy expenditure in PD field are needed.

Heritability studies, in the general population, suggest that as much as 70% of the variability in human body weight may be accounted for by genetic factors (205, 206). In peritoneal dialysis field, studies on obesity genetics are scarce. Nordfors et al. (207), documented that patients with the deletion/deletion uncoupling protein 2 genotype presented more fat tissue gain during one year in PD, than those patients with the insertion/deletion uncoupling protein 2 genotype . More studies under these subject are needed, in order to improve our knowledge on obesity pathogenesis in patients under PD, and to enhance our ability to treat these patients successfully.

Potential therapeutic options to reduce insulin resistance in patients under peritoneal dialysis

Few studies specifically addressed the potential role of icodextrin-based solutions in reducing insulin resistance in patients under peritoneal dialysis (208-212). Although the majority of these studies report a lower HOMA-IR value when patients are prescribed icodextrin for the long dwell, those results have to be cautiously interpreted. The low number of patients included (12 to 34 patients), the short follow-up time (3 to 9 months), but especially some methodological constraints related to the non-fasting state of the patients when glucose and insulin measurements are taken, preclude a definitive conclusion. In order to clarify the potential role of icodextrin in insulin resistance, in patients under PD, more studies are needed that strictly comply with the principles of a true fasting state when blood samples are collected, with a dialysis prescription protocol that minimizes any potential interference of peritoneal glucose solutions.

Synthetic activators of $PPAR_{\gamma}$, called thiazolidinediones, act as effective insulin sensitizers, thereby reducing plasma insulin levels and, theoretically, the complications associated with hyperinsulinemia. In patients under peritoneal dialysis, a single study has investigated the potential effect of oral pioglitazone on insulin resistance and adipokines balance (213). According to Li et al. (213), insulin resistance measured with HOMA-IR has significantly declined, and adipokines balance has improved, after a 12 week period of treatment with pioglitazone. However, additional prospective studies are warranted to confirm these findings, as this study has enrolled only 36 patients, and 28% of them were diabetics (a sub-group where the beneficial effects of thiazolidinediones were already expected).

Since peritoneal glucose administration bypasses the gut and the incretin effect of enhancing glucose-dependent insulin secretion, glucagon-like peptide-1 based therapies could enhance glucose metabolism in patients under PD. The particular effect of linagliptin, a dipeptidyl peptidase 4 inhibitor that is primarily eliminated via enterohepatic system, is thus a theoretical potential candidate to ameliorate insulin resistance in patients under PD.

Investigations on free water transport profile with time on PD and the dynamic profile of HGF production

Our studies on peritoneal membrane function evaluation were limited by their cross sectional design and small number of patients with ultrafiltration failure (**chapters V.V.I** and **V.V.II**). Therefore, a longitudinal study is being conducted in order to better understand the clinical meaning of a decreased FWT in patients without ultrafiltration failure, and also to document the dynamic profile of HGF production and its relationship with peritoneal membrane water transport changes.

Investigations on pharmacological agonists of the aquaporins

The identification of the ultra-small pores offers the opportunity to modulate water transport in PD. Studies in rat and mouse models (214, 215), and in patients under PD (216), have shown that an aquaporin-1 modulation can be achieved without modifying the osmotic gradient. Whether increasing the expression of aquaporin-1 in the membrane, or gating the already existing channels, could be used to treat patients with ultrafiltration failure, remains to be investigated.

VII – Conclusions

- Predictors of residual renal function loss in PD patients
- Peritoneal membrane phosphate transport status
- Evaluation of nutritional status in contemporary PD patients
- The impact of peritoneal glucose absorption and fast transport status on obesity, insulin resistance and new cardiovascular events in PD patients
- Functional characterization of peritoneal membrane

VII. CONCLUSIONS

Predictors of residual renal function loss in PD patients

Our first clinical investigation **(chapter V.I)** has evidenced that patients on PD after renal transplantation experience similar short term residual renal function protection, without a shorter peritonitis-free survival, compared with patients starting PD, as their first renal replacement therapy. We also concluded that diabetes was the only independent predictor of anuria after PD start.

Peritoneal membrane phosphate transport status

Our second clinical investigation (**chapter V.II**) contributed to increasing our understanding on peritoneal phosphate clearance, by highlighting the importance of establishing peritoneal membrane phosphate transport status. We concluded that in hyperphosphatemic and or anuric patients, the decision on the optimal PD modality should also take into account peritoneal phosphate transport characteristics, beyond the traditional adequacy parameters (urea Kt/V and creatinine peritoneal clearance). Increasing dwell times or transfer to CAPD could be effective strategies to improve phosphate handling in patients with inadequate phosphate control on APD, especially if they are slow phosphate transporters.

Evaluation of nutritional status in contemporary PD patients

According to the most recent diagnostic criteria for nutritional assessment in chronic kidney disease patients, we have documented a high overweight and obesity prevalence, and a low incidence of protein-wasting in patients under peritoneal dialysis, using several biochemical and anthropometric parameters **(chapter V.III)**. These results were reproduced in a subsequent study, **(chapter V.IV.I)**, and we further demonstrated that body mass index (BMI) underestimates the real prevalence of obesity, compared with body composition assessment using bioimpedance analysis **(chapter V.IV.I).**

The impact of peritoneal glucose absorption and fast transport status on obesity, insulin resistance and new cardiovascular events in PD patients

According to our clinical investigation **(chapter V.IV.I)**, cardiovascular events were associated with obesity. Furthermore, we could not find any association between obesity and peritoneal glucose absorption, neither with fast transport status. This study enabled us, additionally, to clarify that adipokines profile depends on patient´s body composition, as leptin/adiponectin ratio was predicted by relative fat mass and lean tissue index, independently of peritoneal glucose absorption. In line with these results, obese patients presented higher leptin and lower adiponectin level that translated into a higher leptin/adiponectin ratio; patients with protein-wasting presented a lower leptin and a higher adiponectin that translated into a lower leptin/adiponectin ratio. Our results can therefore explain the previously reported contradictory results about adiponectin and clinical outcomes, in patients with chronic kidney disease. We have also concluded that a higher leptin/adiponectin ratio was associated with cardiovascular events, and can represent a new atherogenic index in patients under PD, without protein wasting.

In a subsequent clinical investigation about insulin resistance in non-diabetic PD patients **(chapter V.IV.II)**, we concluded that obesity and adipokines profile play a major role in insulin resistance development in PD, independently of glucose absorption and small solute transport status. In practice, this study puts on evidence that the concept of reversal epidemiology that was found in several hemodialysis populations may not be applied to PD patients. Given the already established association between insulin resistance, namely between higher LAR, and cardiovascular events (as our previous investigation demonstrates – **chapter V.IV.I**), efforts should be put in obesity prevention, correct diagnosis and treatment. One of the most feared systemic consequences of glucose absorption in PD is precisely obesity and insulin resistance development. However, contemporary PD treatments minimize such exposition, and the lack of correlations between peritoneal glucose absorption, insulin resistance indices and body composition parameters that we found, puts on evidence that other factors (such as genetics, energy expenditure and energy intake) may contribute to a metabolic syndrome development in PD patients, perhaps in a more powerful way. Finally, with this investigation, we also concluded that fast transporters can be adequately managed in PD, without becoming obese or developing an insulin resistant state. Using new solutions, with low glucose degradation products, icodextrin and short dwells could contribute to minimize glucose exposure, as an updated therapy that could successfully treat fast transporters in peritoneal dialysis.

Functional characterization of peritoneal membrane

Our clinical investigation on functional evaluation of the peritoneal membrane (**chapter V.V.I**), allowed us to conclude that quantification of free water transport (FWT) is important for detecting causes of ultrafiltration failure (UFF), beyond an increase in effective capillary surface, and that such quantification is feasible during a 4-hour, 3.86% glucose PET evaluation, with a temporary drainage of the peritoneal cavity at 60 minutes, in a Two-in-One PET Protocol.

In a subsequent clinical investigation (**chapter V.V.II**), hepatocyte growth factor (HGF) was explored as a potential new effluent biomarker that could timely signalize peritoneal membrane dysfunction. We concluded that dialysate HGF concentration is significantly higher among patients with UFF, especially if FWT is impaired, suggesting that it might be a useful marker of progressive peritoneal membrane dysfunction.

VIII - References

VIII. REFERENCES

1. Jain AK, Blake P, Cordy P, Garg AX. Global trends in rates of peritoneal dialysis. Journal of the American Society of Nephrology : JASN. 2012;23(3):533-44.
2. Remon Rodriguez C, Quiros Ganga PL. Current evidence shows that survival outcomes are equivalent for dialysis techniques. Nefrologia : publicacion oficial de la Sociedad Espanola Nefrologia. 2011;31(5):520-7.
3. Mehrotra R, Chiu YW, Kalantar-Zadeh K, Bargman J, Vonesh E. Similar outcomes with hemodialysis and peritoneal dialysis in patients with end-stage renal disease. Archives of internal medicine. 2011;171(2):110-8.
4. Kramer A, Stel V, Zoccali C, Heaf J, Ansell D, Gronhagen-Riska C, et al. An update on renal replacement therapy in Europe: ERA-EDTA Registry data from 1997 to 2006. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2009;24(12):3557-66.
5. Weinhandl ED, Foley RN, Gilbertson DT, Arneson TJ, Snyder JJ, Collins AJ. Propensity-matched mortality comparison of incident hemodialysis and peritoneal dialysis patients. Journal of the American Society of Nephrology : JASN. 2010;21(3):499-506.
6. Bloembergen WE, Port FK, Mauger EA, Wolfe RA. A comparison of cause of death between patients treated with hemodialysis and peritoneal dialysis. Journal of the American Society of Nephrology : JASN. 1995;6(2):184-91.
7. Locatelli F, Marcelli D, Conte F, D'Amico M, Del Vecchio L, Limido A, et al. Survival and development of cardiovascular disease by modality of treatment in patients with end-stage renal disease. Journal of the American Society of Nephrology : JASN. 2001;12(11):2411-7.
8. Kumar VA, Sidell MA, Jones JP, Vonesh EF. Survival of propensity matched incident peritoneal and hemodialysis patients in a United States health care system. Kidney international. 2014;86(5):1016-22.
9. Lang SM, Bergner A, Topfer M, Schiffl H. Preservation of residual renal function in dialysis patients: effects of dialysis-technique-related factors. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2001;21(1):52-7.
10. Wang AY. The John F. Maher Award Recipient Lecture 2006. The "heart" of peritoneal dialysis: residual renal function. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2007;27(2):116-24.
11. Johnson DW, Dent H, Hawley CM, McDonald SP, Rosman JB, Brown FG, et al. Association of dialysis modality and cardiovascular mortality in incident dialysis patients. Clinical journal of the American Society of Nephrology : CJASN. 2009;4(10):1620-8.

12. Adequacy of dialysis and nutrition in continuous peritoneal dialysis: association with clinical outcomes. Canada-USA (CANUSA) Peritoneal Dialysis Study Group. Journal of the American Society of Nephrology : JASN. 1996;7(2):198-207.

13. Bargman JM, Thorpe KE, Churchill DN. Relative contribution of residual renal function and peritoneal clearance to adequacy of dialysis: a reanalysis of the CANUSA study. Journal of the American Society of Nephrology : JASN. 2001;12(10):2158-62.

14. Maiorca R, Brunori G, Zubani R, Cancarini GC, Manili L, Camerini C, et al. Predictive value of dialysis adequacy and nutritional indices for mortality and morbidity in CAPD and HD patients. A longitudinal study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1995;10(12):2295-305.

15. Diaz-Buxo JA, Lowrie EG, Lew NL, Zhang SM, Zhu X, Lazarus JM. Associates of mortality among peritoneal dialysis patients with special reference to peritoneal transport rates and solute clearance. American journal of kidney diseases : the official journal of the National Kidney Foundation. 1999;33(3):523-34.

16. Paniagua R, Amato D, Vonesh E, Correa-Rotter R, Ramos A, Moran J, et al. Effects of increased peritoneal clearances on mortality rates in peritoneal dialysis: ADEMEX, a prospective, randomized, controlled trial. Journal of the American Society of Nephrology : JASN. 2002;13(5):1307-20.

17. Bammens B, Evenepoel P, Verbeke K, Vanrenterghem Y. Time profiles of peritoneal and renal clearances of different uremic solutes in incident peritoneal dialysis patients. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2005;46(3):512-9.

18. Liao CT, Shiao CC, Huang JW, Hung KY, Chuang HF, Chen YM, et al. Predictors of faster decline of residual renal function in Taiwanese peritoneal dialysis patients. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2008;28 Suppl 3:S191-5.

19. Chung SH, Heimburger O, Stenvinkel P, Qureshi AR, Lindholm B. Association between residual renal function, inflammation and patient survival in new peritoneal dialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2003;18(3):590-7.

20. Rabindranath KS, Adams J, Ali TZ, MacLeod AM, Vale L, Cody J, et al. Continuous ambulatory peritoneal dialysis versus automated peritoneal dialysis for end-stage renal disease. The Cochrane database of systematic reviews. 2007(2):Cd006515.

21. Dombros N, Dratwa M, Feriani M, Gokal R, Heimburger O, Krediet R, et al. European best practice guidelines for peritoneal dialysis. 7 Adequacy of peritoneal dialysis. Nephrology,

dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2005;20 Suppl 9:ix24-ix7.

22. Wang AY, Woo J, Sea MM, Law MC, Lui SF, Li PK. Hyperphosphatemia in Chinese peritoneal dialysis patients with and without residual kidney function: what are the implications? American journal of kidney diseases : the official journal of the National Kidney Foundation. 2004;43(4):712-20.

23. Ansell D. Serum phosphate and outcomes in PD patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2007;22(2):667-8.

24. Noordzij M, Korevaar JC, Bos WJ, Boeschoten EW, Dekker FW, Bossuyt PM, et al. Mineral metabolism and cardiovascular morbidity and mortality risk: peritoneal dialysis patients compared with haemodialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2006;21(9):2513-20.

25. Melamed ML, Eustace JA, Plantinga L, Jaar BG, Fink NE, Coresh J, et al. Changes in serum calcium, phosphate, and PTH and the risk of death in incident dialysis patients: a longitudinal study. Kidney international. 2006;70(2):351-7.

26. Churchill DN, Thorpe KE, Nolph KD, Keshaviah PR, Oreopoulos DG, Page D. Increased peritoneal membrane transport is associated with decreased patient and technique survival for continuous peritoneal dialysis patients. The Canada-USA (CANUSA) Peritoneal Dialysis Study Group. Journal of the American Society of Nephrology : JASN. 1998;9(7):1285-92.

27. Yang X, Fang W, Bargman JM, Oreopoulos DG. High peritoneal permeability is not associated with higher mortality or technique failure in patients on automated peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2008;28(1):82-92.

28. Rumpsfeld M, McDonald SP, Johnson DW. Higher peritoneal transport status is associated with higher mortality and technique failure in the Australian and New Zealand peritoneal dialysis patient populations. Journal of the American Society of Nephrology : JASN. 2006;17(1):271-8.

29. Brimble KS, Walker M, Margetts PJ, Kundhal KK, Rabbat CG. Meta-analysis: peritoneal membrane transport, mortality, and technique failure in peritoneal dialysis. Journal of the American Society of Nephrology : JASN. 2006;17(9):2591-8.

30. Brown EA, Davies SJ, Rutherford P, Meeus F, Borras M, Riegel W, et al. Survival of functionally anuric patients on automated peritoneal dialysis: the European APD Outcome Study. Journal of the American Society of Nephrology : JASN. 2003;14(11):2948-57.

31. Heaton A, Johnston DG, Burrin JM, Orskov H, Ward MK, Alberti KG, et al. Carbohydrate and lipid metabolism during continuous ambulatory peritoneal dialysis (CAPD): the effect of a single dialysis cycle. Clinical science (London, England : 1979). 1983;65(5):539-45.

32. Grodstein GP, Blumenkrantz MJ, Kopple JD, Moran JK, Coburn JW. Glucose absorption during continuous ambulatory peritoneal dialysis. Kidney international. 1981;19(4):564-7.

33. Delarue J, Maingourd C, Lamisse F, Garrigue MA, Bagros P, Couet C. Glucose oxidation after a peritoneal and an oral glucose load in dialyzed patients. Kidney international. 1994;45(4):1147-52.

34. Kim YL, Cho JH, Choi JY, Kim CD, Park SH. Systemic and local impact of glucose and glucose degradation products in peritoneal dialysis solution. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation. 2013;23(3):218-22.

35. Fernstrom A, Hylander B, Moritz A, Jacobsson H, Rossner S. Increase of intra-abdominal fat in patients treated with continuous ambulatory peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 1998;18(2):166-71.

36. Vasselai P, Kamimura MA, Bazanelli AP, Pupim LB, Avesani CM, da Mota Ribeiro FS, et al. Factors associated with body-fat changes in prevalent peritoneal dialysis patients. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation. 2008;18(4):363-9.

37. Fliser D, Pacini G, Engelleiter R, Kautzky-Willer A, Prager R, Franek E, et al. Insulin resistance and hyperinsulinemia are already present in patients with incipient renal disease. Kidney international. 1998;53(5):1343-7.

38. Kobayashi S, Maejima S, Ikeda T, Nagase M. Impact of dialysis therapy on insulin resistance in end-stage renal disease: comparison of haemodialysis and continuous ambulatory peritoneal dialysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2000;15(1):65-70.

39. Delarue J, Maingourd C, Couet C, Vidal S, Bagros P, Lamisse F. Effects of oral glucose on intermediary metabolism in continuous ambulatory peritoneal dialysis patients versus healthy subjects. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 1998;18(5):505-11.

40. Rasouli N, Kern PA. Adipocytokines and the metabolic complications of obesity. The Journal of clinical endocrinology and metabolism. 2008;93(11 Suppl 1):S64-73.

41. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. The Journal of clinical endocrinology and metabolism. 2004;89(6):2548-56.
42. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama. 2001;285(19):2486-97.
43. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. Lancet. 2005;366(9491):1059-62.
44. Ford ES. Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence. Diabetes care. 2005;28(7):1769-78.
45. Johnson DW, Armstrong K, Campbell SB, Mudge DW, Hawley CM, Coombes JS, et al. Metabolic syndrome in severe chronic kidney disease: Prevalence, predictors, prognostic significance and effects of risk factor modification. Nephrology (Carlton, Vic). 2007;12(4):391-8.
46. Young DO, Lund RJ, Haynatzki G, Dunlay RW. Prevalence of the metabolic syndrome in an incident dialysis population. Hemodialysis international International Symposium on Home Hemodialysis. 2007;11(1):86-95.
47. Park SH, Lindholm B. Definition of metabolic syndrome in peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2009;29 Suppl 2:S137-44.
48. Park JT, Chang TI, Kim DK, Lee JE, Choi HY, Kim HW, et al. Metabolic syndrome predicts mortality in non-diabetic patients on continuous ambulatory peritoneal dialysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2010;25(2):599-604.
49. Liao CT, Kao TW, Chou YH, Wu MS, Chen YM, Chuang HF, et al. Associations of metabolic syndrome and its components with cardiovascular outcomes among non-diabetic patients undergoing maintenance peritoneal dialysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2011;26(12):4047-54.
50. Szeto CC, Kwan BC, Chow KM, Leung CB, Cheng MS, Law MC, et al. Metabolic syndrome in peritoneal dialysis patients: choice of diagnostic criteria and prognostic implications. Clinical journal of the American Society of Nephrology : CJASN. 2014;9(4):779-87.
51. Fleischmann E, Teal N, Dudley J, May W, Bower JD, Salahudeen AK. Influence of excess weight on mortality and hospital stay in 1346 hemodialysis patients. Kidney international. 1999;55(4):1560-7.

52. Leavey SF, McCullough K, Hecking E, Goodkin D, Port FK, Young EW. Body mass index and mortality in 'healthier' as compared with 'sicker' haemodialysis patients: results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2001;16(12):2386-94.

53. Port FK, Ashby VB, Dhingra RK, Roys EC, Wolfe RA. Dialysis dose and body mass index are strongly associated with survival in hemodialysis patients. Journal of the American Society of Nephrology : JASN. 2002;13(4):1061-6.

54. Johnson DW, Herzig KA, Purdie DM, Chang W, Brown AM, Rigby RJ, et al. Is obesity a favorable prognostic factor in peritoneal dialysis patients? Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2000;20(6):715-21.

55. Li PK, Kwan BC, Szeto CC, Ko GT. Metabolic syndrome in peritoneal dialysis patients. NDT plus. 2008;1(4):206-14.

56. Shah NR, Braverman ER. Measuring adiposity in patients: the utility of body mass index (BMI), percent body fat, and leptin. PloS one. 2012;7(4):e33308.

57. Gracia-Iguacel C, Qureshi AR, Avesani CM, Heimburger O, Huang X, Lindholm B, et al. Subclinical versus overt obesity in dialysis patients: more than meets the eye. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2013;28 Suppl 4:iv175-81.

58. Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association. Diabetes care. 1998;21(2):310-4.

59. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. The American journal of physiology. 1979;237(3):E214-23.

60. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes care. 2000;23(1):57-63.

61. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

62. Jia T, Huang X, Qureshi AR, Xu H, Arnlov J, Lindholm B, et al. Validation of insulin sensitivity surrogate indices and prediction of clinical outcomes in individuals with and without impaired renal function. Kidney international. 2014;86(2):383-91.

63. Shoji T, Emoto M, Nishizawa Y. HOMA index to assess insulin resistance in renal failure patients. Nephron. 2001;89(3):348-9.

64. Hung AM, Sundell MB, Egbert P, Siew ED, Shintani A, Ellis CD, et al. A comparison of novel and commonly-used indices of insulin sensitivity in African American chronic hemodialysis patients. Clinical journal of the American Society of Nephrology : CJASN. 2011;6(4):767-74.

65. Li Y, Zhang L, Gu Y, Hao C, Zhu T. Insulin resistance as a predictor of cardiovascular disease in patients on peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2013;33(4):411-8.

66. Fortes PC, de Moraes TP, Mendes JG, Stinghen AE, Ribeiro SC, Pecoits-Filho R. Insulin resistance and glucose homeostasis in peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2009;29 Suppl 2:S145-8.

67. Fernandez-Real JM, Lopez-Bermejo A, Casamitjana R, Ricart W. Novel interactions of adiponectin with the endocrine system and inflammatory parameters. The Journal of clinical endocrinology and metabolism. 2003;88(6):2714-8.

68. Satoh N, Naruse M, Usui T, Tagami T, Suganami T, Yamada K, et al. Leptin-to-adiponectin ratio as a potential atherogenic index in obese type 2 diabetic patients. Diabetes care. 2004;27(10):2488-90.

69. Kotani K, Sakane N, Saiga K, Kurozawa Y. Leptin : adiponectin ratio as an atherosclerotic index in patients with type 2 diabetes : relationship of the index to carotid intima-media thickness. Diabetologia. 2005;48(12):2684-6.

70. Park JT, Yoo TH, Kim JK, Oh HJ, Kim SJ, Yoo DE, et al. Leptin/adiponectin ratio is an independent predictor of mortality in nondiabetic peritoneal dialysis patients. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2013;33(1):67-74.

71. Adamczak M, Chudek J, Wiecek A. Adiponectin in patients with chronic kidney disease. Seminars in dialysis. 2009;22(4):391-5.

72. Park SH, Carrero JJ, Lindholm B, Stenvinkel P. Adiponectin in chronic kidney disease has an opposite impact on protein-energy wasting and cardiovascular risk: two sides of the same coin. Clinical nephrology. 2009;72(2):87-96.

73. Stenvinkel P. Adiponectin in chronic kidney disease: a complex and context sensitive clinical situation. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation. 2011;21(1):82-6.

74. Becker B, Kronenberg F, Kielstein JT, Haller H, Morath C, Ritz E, et al. Renal insulin resistance syndrome, adiponectin and cardiovascular events in patients with kidney disease: the mild and moderate kidney disease study. Journal of the American Society of Nephrology : JASN. 2005;16(4):1091-8.

75. Menon V, Li L, Wang X, Greene T, Balakrishnan V, Madero M, et al. Adiponectin and mortality in patients with chronic kidney disease. Journal of the American Society of Nephrology : JASN. 2006;17(9):2599-606.

76. Zoccali C, Mallamaci F, Tripepi G, Benedetto FA, Cutrupi S, Parlongo S, et al. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. Journal of the American Society of Nephrology : JASN. 2002;13(1):134-41.

77. Rao M, Li L, Tighiouart H, Jaber BL, Pereira BJ, Balakrishnan VS. Plasma adiponectin levels and clinical outcomes among haemodialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2008;23(8):2619-28.

78. Diez JJ, Estrada P, Bajo MA, Fernandez-Reyes MJ, Grande C, del Peso G, et al. High stable serum adiponectin levels are associated with a better outcome in prevalent dialysis patients. American journal of nephrology. 2009;30(3):244-52.

79. Dombros N, Dratwa M, Feriani M, Gokal R, Heimburger O, Krediet R, et al. European best practice guidelines for peritoneal dialysis. 8 Nutrition in peritoneal dialysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2005;20 Suppl 9:ix28-ix33.

80. Clinical practice guidelines for nutrition in chronic renal failure. K/DOQI, National Kidney Foundation. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2000;35(6 Suppl 2):S1-140.

81. Fouque D, Kalantar-Zadeh K, Kopple J, Cano N, Chauveau P, Cuppari L, et al. A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. Kidney international. 2008;73(4):391-8.

82. Mak RH, Ikizler AT, Kovesdy CP, Raj DS, Stenvinkel P, Kalantar-Zadeh K. Wasting in chronic kidney disease. Journal of cachexia, sarcopenia and muscle. 2011;2(1):9-25.

83. de Mutsert R, Grootendorst DC, Axelsson J, Boeschoten EW, Krediet RT, Dekker FW. Excess mortality due to interaction between protein-energy wasting, inflammation and cardiovascular disease in chronic dialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2008;23(9):2957-64.

84. Cianciaruso B, Brunori G, Kopple JD, Traverso G, Panarello G, Enia G, et al. Cross-sectional comparison of malnutrition in continuous ambulatory peritoneal dialysis and hemodialysis patients. American journal of kidney diseases : the official journal of the National Kidney Foundation. 1995;26(3):475-86.

85. Mehrotra R, Duong U, Jiwakanon S, Kovesdy CP, Moran J, Kopple JD, et al. Serum albumin as a predictor of mortality in peritoneal dialysis: comparisons with hemodialysis.

American journal of kidney diseases : the official journal of the National Kidney Foundation. 2011;58(3):418-28.

86. Pupim LB, Cuppari L, Ikizler TA. Nutrition and metabolism in kidney disease. Seminars in nephrology. 2006;26(2):134-57.

87. Gama-Axelsson T, Heimburger O, Stenvinkel P, Barany P, Lindholm B, Qureshi AR. Serum albumin as predictor of nutritional status in patients with ESRD. Clinical journal of the American Society of Nephrology : CJASN. 2012;7(9):1446-53.

88. van Biesen W, Claes K, Covic A, Fan S, Lichodziejewska-Niemierko M, Schoder V, et al. A multicentric, international matched pair analysis of body composition in peritoneal dialysis versus haemodialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2013;28(10):2620-8.

89. Cueto-Manzano AM, Espinosa A, Hernandez A, Correa-Rotter R. Peritoneal transport kinetics correlate with serum albumin but not with the overall nutritional status in CAPD patients. American journal of kidney diseases : the official journal of the National Kidney Foundation. 1997;30(2):229-36.

90. Lambie M, Chess J, Donovan KL, Kim YL, Do JY, Lee HB, et al. Independent effects of systemic and peritoneal inflammation on peritoneal dialysis survival. Journal of the American Society of Nephrology : JASN. 2013;24(12):2071-80.

91. Holmes CJ, Faict D. Peritoneal dialysis solution biocompatibility: definitions and evaluation strategies. Kidney international Supplement. 2003(88):S50-6.

92. Topley N. Membrane longevity in peritoneal dialysis: impact of infection and bio-incompatible solutions. Advances in renal replacement therapy. 1998;5(3):179-84.

93. Yu MA, Shin KS, Kim JH, Kim YI, Chung SS, Park SH, et al. HGF and BMP-7 ameliorate high glucose-induced epithelial-to-mesenchymal transition of peritoneal mesothelium. Journal of the American Society of Nephrology : JASN. 2009;20(3):567-81.

94. Davies SJ, Phillips L, Naish PF, Russell GI. Peritoneal glucose exposure and changes in membrane solute transport with time on peritoneal dialysis. Journal of the American Society of Nephrology : JASN. 2001;12(5):1046-51.

95. Honda K, Nitta K, Horita S, Yumura W, Nihei H, Nagai R, et al. Accumulation of advanced glycation end products in the peritoneal vasculature of continuous ambulatory peritoneal dialysis patients with low ultra-filtration. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1999;14(6):1541-9.

96. Yanez-Mo M, Lara-Pezzi E, Selgas R, Ramirez-Huesca M, Dominguez-Jimenez C, Jimenez-Heffernan JA, et al. Peritoneal dialysis and epithelial-to-mesenchymal transition of mesothelial cells. The New England journal of medicine. 2003;348(5):403-13.

97. Aroeira LS, Aguilera A, Selgas R, Ramirez-Huesca M, Perez-Lozano ML, Cirugeda A, et al. Mesenchymal conversion of mesothelial cells as a mechanism responsible for high solute transport rate in peritoneal dialysis: role of vascular endothelial growth factor. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2005;46(5):938-48.

98. Davies SJ, Bryan J, Phillips L, Russell GI. Longitudinal changes in peritoneal kinetics: the effects of peritoneal dialysis and peritonitis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1996;11(3):498-506.

99. Davies SJ, Phillips L, Griffiths AM, Russell LH, Naish PF, Russell GI. What really happens to people on long-term peritoneal dialysis? Kidney international. 1998;54(6):2207-17.

100. Parikova A, Smit W, Struijk DG, Krediet RT. Analysis of fluid transport pathways and their determinants in peritoneal dialysis patients with ultrafiltration failure. Kidney international. 2006;70(11):1988-94.

101. Lambie ML, John B, Mushahar L, Huckvale C, Davies SJ. The peritoneal osmotic conductance is low well before the diagnosis of encapsulating peritoneal sclerosis is made. Kidney international. 2010;78(6):611-8.

102. Morelle J, Sow A, Hautem N, Bouzin C, Crott R, Devuyst O, et al. Interstitial Fibrosis Restricts Osmotic Water Transport in Encapsulating Peritoneal Sclerosis. Journal of the American Society of Nephrology : JASN. 2015.

103. Twardowski ZJ, Nolph KD, Khanna R, Prowant BF, Ryan LP, Moore HL, et al. PERITONEAL EQUILIBRATION TEST. Peritoneal Dialysis Bulletin. 1987;7(3):138-47.

104. La Milia V, Pozzoni P, Virga G, Crepaldi M, Del Vecchio L, Andrulli S, et al. Peritoneal transport assessment by peritoneal equilibration test with 3.86% glucose: a long-term prospective evaluation. Kidney international. 2006;69(5):927-33.

105. Rippe B, de Arteaga J, Venturoli D. Aquaporins are unlikely to be affected in marked ultrafiltration failure: results from a computer simulation. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2001;21 Suppl 3:S30-4.

106. La Milia V, Di Filippo S, Crepaldi M, Del Vecchio L, Dell'Oro C, Andrulli S, et al. Mini-peritoneal equilibration test: A simple and fast method to assess free water and small solute transport across the peritoneal membrane. Kidney international. 2005;68(2):840-6.

107. Smit W, Struijk DG, Ho-Dac-Pannekeet MM, Krediet RT. Quantification of free water transport in peritoneal dialysis. Kidney international. 2004;66(2):849-54.

108. Venturoli D, Rippe B. Validation by computer simulation of two indirect methods for quantification of free water transport in peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2005;25(1):77-84.

109. Rodrigues AS, Silva S, Bravo F, Oliveira JC, Fonseca I, Cabrita A, et al. Peritoneal membrane evaluation in routine clinical practice. Blood purification. 2007;25(5-6):497-504.

110. Cnossen TT, Smit W, Konings CJ, Kooman JP, Leunissen KM, Krediet RT. Quantification of free water transport during the peritoneal equilibration test. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2009;29(5):523-7.

111. Goodlad C, Tam FW, Ahmad S, Bhangal G, North BV, Brown EA. Dialysate cytokine levels do not predict encapsulating peritoneal sclerosis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2014;34(6):594-604.

112. Sampimon DE, Korte MR, Barreto DL, Vlijm A, de Waart R, Struijk DG, et al. Early diagnostic markers for encapsulating peritoneal sclerosis: a case-control study. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2010;30(2):163-9.

113. Barreto DL, Coester AM, Struijk DG, Krediet RT. Can effluent matrix metalloproteinase 2 and plasminogen activator inhibitor 1 be used as biomarkers of peritoneal membrane alterations in peritoneal dialysis patients? Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2013;33(5):529-37.

114. Yang J, Dai C, Liu Y. A novel mechanism by which hepatocyte growth factor blocks tubular epithelial to mesenchymal transition. Journal of the American Society of Nephrology : JASN. 2005;16(1):68-78.

115. Shukla MN, Rose JL, Ray R, Lathrop KL, Ray A, Ray P. Hepatocyte growth factor inhibits epithelial to myofibroblast transition in lung cells via Smad7. American journal of respiratory cell and molecular biology. 2009;40(6):643-53.

116. Nakamura S, Niwa T. Pyridoxal phosphate and hepatocyte growth factor prevent dialysate-induced peritoneal damage. Journal of the American Society of Nephrology : JASN. 2005;16(1):144-50.

117. Ueno T, Nakashima A, Doi S, Kawamoto T, Honda K, Yokoyama Y, et al. Mesenchymal stem cells ameliorate experimental peritoneal fibrosis by suppressing inflammation and inhibiting TGF-beta1 signaling. Kidney international. 2013;84(2):297-307.

118. Mizuiri S, Hemmi H, Arita M, Tai R, Hattori Y, Muto A, et al. Effluent markers related to epithelial mesenchymal transition with adjusted values for effluent cancer antigen 125 in peritoneal dialysis patients. International journal of nephrology. 2011;2011:261040.

119. Enia G, Sicuso C, Alati G, Zoccali C. Subjective global assessment of nutrition in dialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1993;8(10):1094-8.

120. Visser R, Dekker FW, Boeschoten EW, Stevens P, Krediet RT. Reliability of the 7-point subjective global assessment scale in assessing nutritional status of dialysis patients. Advances in peritoneal dialysis Conference on Peritoneal Dialysis. 1999;15:222-5.

121. Locatelli F, Fouque D, Heimburger O, Drueke TB, Cannata-Andia JB, Horl WH, et al. Nutritional status in dialysis patients: a European consensus. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2002;17(4):563-72.

122. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. The British journal of nutrition. 1974;32(1):77-97.

123. Frisancho AR. New norms of upper limb fat and muscle areas for assessment of nutritional status. The American journal of clinical nutrition. 1981;34(11):2540-5.

124. Moissl UM, Wabel P, Chamney PW, Bosaeus I, Levin NW, Bosy-Westphal A, et al. Body fluid volume determination via body composition spectroscopy in health and disease. Physiological measurement. 2006;27(9):921-33.

125. Wabel P, Chamney P, Moissl U, Jirka T. Importance of whole-body bioimpedance spectroscopy for the management of fluid balance. Blood purification. 2009;27(1):75-80.

126. Chamney PW, Kramer M, Rode C, Kleinekofort W, Wizemann V. A new technique for establishing dry weight in hemodialysis patients via whole body bioimpedance. Kidney international. 2002;61(6):2250-8.

127. Cooper BA, Aslani A, Ryan M, Zhu FY, Ibels LS, Allen BJ, et al. Comparing different methods of assessing body composition in end-stage renal failure. Kidney international. 2000;58(1):408-16.

128. Arroyo D, Panizo N, Abad S, Vega A, Rincon A, de Jose AP, et al. Intraperitoneal Fluid Overestimates Hydration Status Assessment by Bioimpedance Spectroscopy. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2015;35(1):85-9.

129. Wieskotten S, Heinke S, Wabel P, Moissl U, Becker J, Pirlich M, et al. Bioimpedance-based identification of malnutrition using fuzzy logic. Physiological measurement. 2008;29(5):639-54.

130. Mujais S, Nolph K, Gokal R, Blake P, Burkart J, Coles G, et al. Evaluation and management of ultrafiltration problems in peritoneal dialysis. International Society for Peritoneal Dialysis Ad Hoc Committee on Ultrafiltration Management in Peritoneal Dialysis.

Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2000;20 Suppl 4:S5-21.

131. Perl J, Bargman JM, Davies SJ, Jassal SV. Clinical outcomes after failed renal transplantation-does dialysis modality matter? Seminars in dialysis. 2008;21(3):239-44.

132. Johnson DW, Mudge DW, Sturtevant JM, Hawley CM, Campbell SB, Isbel NM, et al. Predictors of decline of residual renal function in new peritoneal dialysis patients. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2003;23(3):276-83.

133. Moist LM, Port FK, Orzol SM, Young EW, Ostbye T, Wolfe RA, et al. Predictors of loss of residual renal function among new dialysis patients. Journal of the American Society of Nephrology : JASN. 2000;11(3):556-64.

134. Sasal J, Naimark D, Klassen J, Shea J, Bargman JM. Late renal transplant failure: an adverse prognostic factor at initiation of peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2001;21(4):405-10.

135. Davies SJ. Peritoneal dialysis in the patient with a failing renal allograft. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2001;21 Suppl 3:S280-4.

136. de Jonge H, Bammens B, Lemahieu W, Maes BD, Vanrenterghem Y. Comparison of peritoneal dialysis and haemodialysis after renal transplant failure. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2006;21(6):1669-74.

137. Page DE, Knoll GA, Cheung V. The relationship between residual renal function, protein catabolic rate, and phosphate and magnesium levels in peritoneal dialysis patients. Advances in peritoneal dialysis Conference on Peritoneal Dialysis. 2002;18:189-91.

138. Krediet RT, Balafa O. Cardiovascular risk in the peritoneal dialysis patient. Nature reviews Nephrology. 2010;6(8):451-60.

139. Kalantar-Zadeh K, Kuwae N, Regidor DL, Kovesdy CP, Kilpatrick RD, Shinaberger CS, et al. Survival predictability of time-varying indicators of bone disease in maintenance hemodialysis patients. Kidney international. 2006;70(4):771-80.

140. Young EW, Albert JM, Satayathum S, Goodkin DA, Pisoni RL, Akiba T, et al. Predictors and consequences of altered mineral metabolism: the Dialysis Outcomes and Practice Patterns Study. Kidney international. 2005;67(3):1179-87.

141. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. Journal of the American Society of Nephrology : JASN. 2004;15(8):2208-18.

142. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, et al. Phosphate regulation of vascular smooth muscle cell calcification. Circulation research. 2000;87(7):E10-7.

143. Marchais SJ, Metivier F, Guerin AP, London GM. Association of hyperphosphataemia with haemodynamic disturbances in end-stage renal disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1999;14(9):2178-83.

144. Graff J, Fugleberg S, Brahm J, Fogh-Andersen N. The transport of phosphate between the plasma and dialysate compartments in peritoneal dialysis is influenced by an electric potential difference. Clinical physiology (Oxford, England). 1996;16(3):291-300.

145. Rippe B, Venturoli D, Simonsen O, de Arteaga J. Fluid and electrolyte transport across the peritoneal membrane during CAPD according to the three-pore model. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2004;24(1):10-27.

146. Kuhlmann MK. Phosphate elimination in modalities of hemodialysis and peritoneal dialysis. Blood purification. 2010;29(2):137-44.

147. Sedlacek M, Dimaano F, Uribarri J. Relationship between phosphorus and creatinine clearance in peritoneal dialysis: clinical implications. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2000;36(5):1020-4.

148. Badve SV, Zimmerman DL, Knoll GA, Burns KD, McCormick BB. Peritoneal phosphate clearance is influenced by peritoneal dialysis modality, independent of peritoneal transport characteristics. Clinical journal of the American Society of Nephrology : CJASN. 2008;3(6):1711-7.

149. Gallar P, Ortega O, Gutierrez M, Munoz M, Hilara L, Oliet A, et al. [Influencing factors in the control of phosphorus in peritoneal dialysis. Therapeutic options]. Nefrologia : publicacion oficial de la Sociedad Espanola Nefrologia. 2000;20(4):355-61.

150. Lopez-Guerra EA, Rodriguez-Garcia VH, Rodriguez-Castellanos FE. Determination of peritoneal phosphate transport as a tool for controlling serum phosphorus. Nefrologia : publicacion oficial de la Sociedad Espanola Nefrologia. 2014;34(5):584-90.

151. Botelho C, Rodrigues A, Oliveira JC, Cabrita A. Peritoneal phosphate removal varies by peritoneal dialysis regimen: an underestimated parameter of phosphate control. Journal of nephrology. 2013;26(1):183-90.

152. Ikizler TA. The use and misuse of serum albumin as a nutritional marker in kidney disease. Clinical journal of the American Society of Nephrology : CJASN. 2012;7(9):1375-7.

153. Jeejeebhoy KN. Nutritional assessment. Gastroenterology clinics of North America. 1998;27(2):347-69.

154. Kaysen GA, Rathore V, Shearer GC, Depner TA. Mechanisms of hypoalbuminemia in hemodialysis patients. Kidney international. 1995;48(2):510-6.

155. Moshage HJ, Janssen JA, Franssen JH, Hafkenscheid JC, Yap SH. Study of the molecular mechanism of decreased liver synthesis of albumin in inflammation. The Journal of clinical investigation. 1987;79(6):1635-41.

156. Balafa O, Halbesma N, Struijk DG, Dekker FW, Krediet RT. Peritoneal albumin and protein losses do not predict outcome in peritoneal dialysis patients. Clinical journal of the American Society of Nephrology : CJASN. 2011;6(3):561-6.

157. Aslam N, Bernardini J, Fried L, Piraino B. Large body mass index does not predict short-term survival in peritoneal dialysis patients. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2002;22(2):191-6.

158. McDonald SP, Collins JF, Johnson DW. Obesity is associated with worse peritoneal dialysis outcomes in the Australia and New Zealand patient populations. Journal of the American Society of Nephrology : JASN. 2003;14(11):2894-901.

159. Woodrow G, Devine Y, Cullen M, Lindley E. Application of bioelectrical impedance to clinical assessment of body composition in peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2007;27(5):496-502.

160. Than N, Woodrow G, Oldroyd B, Gonzalez C, Turney JH, Brownjohn AM. Effect of peritoneal fluid on whole body and segmental multiple frequency bioelectrical impedance in patients on peritoneal dialysis. European journal of clinical nutrition. 2000;54(5):450-1.

161. Parmentier SP, Schirutschke H, Schmitt B, Schewe J, Herbrig K, Pistrosch F, et al. Influence of peritoneal dialysis solution on measurements of fluid status by bioimpedance spectroscopy. International urology and nephrology. 2013;45(1):229-32.

162. Lindley E, Devine Y, Hall L, Cullen M, Cuthbert S, Woodrow G, et al. A ward-based procedure for assessment of fluid status in peritoneal dialysis patients using bioimpedance spectroscopy. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2005;25 Suppl 3:S46-8.

163. Gokal R, Moberly J, Lindholm B, Mujais S. Metabolic and laboratory effects of icodextrin. Kidney international Supplement. 2002(81):S62-71.

164. Fan S, Davenport A. Does increased glucose exposure lead to increased body fat and reduced lean body mass in anuric peritoneal dialysis patients? European journal of clinical nutrition. 2014;68(11):1253-4.

165. Weiner DE, Sarnak MJ. A decade after the KDOQI CKD guidelines: impact on the cardiovascular disease-CKD paradigm. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2012;60(5):710-2.

166. Foley RN. Clinical epidemiology of cardiac disease in dialysis patients: left ventricular hypertrophy, ischemic heart disease, and cardiac failure. Seminars in dialysis. 2003;16(2):111-7.

167. Wizemann V, Wabel P, Chamney P, Zaluska W, Moissl U, Rode C, et al. The mortality risk of overhydration in haemodialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2009;24(5):1574-9.

168. Scholze A, Rattensperger D, Zidek W, Tepel M. Low serum leptin predicts mortality in patients with chronic kidney disease stage 5. Obesity (Silver Spring, Md). 2007;15(6):1617-22.

169. Wen Y, Guo Q, Yang X, Wu X, Feng S, Tan J, et al. High glucose concentrations in peritoneal dialysate are associated with all-cause and cardiovascular disease mortality in continuous ambulatory peritoneal dialysis patients. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2015;35(1):70-7.

170. Van Biesen W, Williams JD, Covic AC, Fan S, Claes K, Lichodziejewska-Niemierko M, et al. Fluid status in peritoneal dialysis patients: the European Body Composition Monitoring (EuroBCM) study cohort. PloS one. 2011;6(2):e17148.

171. Wu HY, Hung KY, Hu FC, Chen YM, Chu TS, Huang JW, et al. Risk factors for high dialysate glucose use in PD patients--a retrospective 5-year cohort study. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2010;30(4):448-55.

172. Konings CJ, Kooman JP, Schonck M, Struijk DG, Gladziwa U, Hoorntje SJ, et al. Fluid status in CAPD patients is related to peritoneal transport and residual renal function: evidence from a longitudinal study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2003;18(4):797-803.

173. Lee CC, Chen KH, Tian YC, Weng CM, Yang CW, Hung CC. Initial high peritoneal transport status is not a predictor of mortality in peritoneal dialysis patients. Renal failure. 2010;32(7):788-95.

174. Johnson DW, Hawley CM, McDonald SP, Brown FG, Rosman JB, Wiggins KJ, et al. Superior survival of high transporters treated with automated versus continuous ambulatory peritoneal dialysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2010;25(6):1973-9.

175. Sanchez-Villanueva R, Estrada P, del Peso G, Grande C, Diez JJ, Iglesias P, et al. Repeated analysis of estimated insulin resistance using the HOMAIR index in nondiabetic patients on peritoneal dialysis and its relationship with cardiovascular disease and mortality. Nefrologia : publicacion oficial de la Sociedad Espanola Nefrologia. 2013;33(1):85-92.

176. Cefalu WT, Wang ZQ, Werbel S, Bell-Farrow A, Crouse JR, 3rd, Hinson WH, et al. Contribution of visceral fat mass to the insulin resistance of aging. Metabolism: clinical and experimental. 1995;44(7):954-9.

177. Seidell JC, Bjorntorp P, Sjostrom L, Kvist H, Sannerstedt R. Visceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels. Metabolism: clinical and experimental. 1990;39(9):897-901.

178. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. The Journal of clinical endocrinology and metabolism. 2008;93(11 Suppl 1):S57-63.

179. Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM. Relationships of generalized and regional adiposity to insulin sensitivity in men. The Journal of clinical investigation. 1995;96(1):88-98.

180. Kotronen A, Lewitt M, Hall K, Brismar K, Yki-Jarvinen H. Insulin-like growth factor binding protein 1 as a novel specific marker of hepatic insulin sensitivity. The Journal of clinical endocrinology and metabolism. 2008;93(12):4867-72.

181. Chen HY, Kao TW, Huang JW, Chu TS, Wu KD. Correlation of metabolic syndrome with residual renal function, solute transport rate and peritoneal solute clearance in chronic peritoneal dialysis patients. Blood purification. 2008;26(2):138-44.

182. da Silva DR, Figueiredo AE, Antonello IC, Poli de Figueiredo CE, d'Avila DO. Solutes transport characteristics in peritoneal dialysis: variations in glucose and insulin serum levels. Renal failure. 2008;30(2):175-9.

183. Stompor T, Sulowicz W, Dembinska-Kiec A, Janda K, Wojcik K, Zdzienicka A. An association between body mass index and markers of inflammation: is obesity the proinflammatory state in patients on peritoneal dialysis? Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2003;23(1):79-83.

184. Robbins DC, Andersen L, Bowsher R, Chance R, Dinesen B, Frank B, et al. Report of the American Diabetes Association's Task Force on standardization of the insulin assay. Diabetes. 1996;45(2):242-56.

185. van Biesen W, Heimburger O, Krediet R, Rippe B, La Milia V, Covic A, et al. Evaluation of peritoneal membrane characteristics: clinical advice for prescription management by the ERBP working group. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2010;25(7):2052-62.

186. Waniewski J, Debowska M, Lindholm B. Ultrafiltration and absorption in evaluating aquaporin function from peritoneal transport of sodium. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2007;27(6):687-90.

187. Waniewski J, Debowska M, Lindholm B. Water and solute transport through different types of pores in peritoneal membrane in CAPD patients with ultrafiltration failure. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2009;29(6):664-9.

188. Krediet RT, Lindholm B, Rippe B. Pathophysiology of peritoneal membrane failure. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2000;20 Suppl 4:S22-42.

189. Teixido J, Cofan F, Borras M, Bonet J, Bonal J, Galimany R, et al. Mass transfer coefficient: comparison between methods. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 1993;13 Suppl 2:S47-9.

190. Zweers MM, Imholz AL, Struijk DG, Krediet RT. Correction of sodium sieving for diffusion from the circulation. Advances in peritoneal dialysis Conference on Peritoneal Dialysis. 1999;15:65-72.

191. Asghar RB, Davies SJ. Pathways of fluid transport and reabsorption across the peritoneal membrane. Kidney international. 2008;73(9):1048-53.

192. Rippe B, Venturoli D. Simulations of osmotic ultrafiltration failure in CAPD using a serial three-pore membrane/fiber matrix model. American journal of physiology Renal physiology. 2007;292(3):F1035-43.

193. Smit W, Parikova A, Struijk DG, Krediet RT. The difference in causes of early and late ultrafiltration failure in peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2005;25 Suppl 3:S41-5.

194. Devuyst O, Rippe B. Water transport across the peritoneal membrane. Kidney international. 2014;85(4):750-8.

195. Morelle J, Devuyst O. Water and solute transport across the peritoneal membrane. Current opinion in nephrology and hypertension. 2015;24(5):434-43.

196. Nakamura S, Tachikawa T, Tobita K, Miyazaki S, Sakai S, Morita T, et al. Role of advanced glycation end products and growth factors in peritoneal dysfunction in CAPD patients. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2003;41(3 Suppl 1):S61-7.

197. Williams JD, Craig KJ, Topley N, Von Ruhland C, Fallon M, Newman GR, et al. Morphologic changes in the peritoneal membrane of patients with renal disease. Journal of the American Society of Nephrology : JASN. 2002;13(2):470-9.

198. Honda K, Hamada C, Nakayama M, Miyazaki M, Sherif AM, Harada T, et al. Impact of uremia, diabetes, and peritoneal dialysis itself on the pathogenesis of peritoneal sclerosis: a quantitative study of peritoneal membrane morphology. Clinical journal of the American Society of Nephrology : CJASN. 2008;3(3):720-8.

199. Shimaoka T, Hamada C, Kaneko K, Io H, Sekiguchi Y, Aruga S, et al. Quantitative evaluation and assessment of peritoneal morphologic changes in peritoneal dialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2010;25(10):3379-85.

200. Krediet RT. The effective lymphatic absorption rate is an accurate and useful concept in the physiology of peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2004;24(4):309-13; discussion 16-7.

201. Parikova A, Smit W, Struijk DG, Zweers MM, Krediet RT. The contribution of free water transport and small pore transport to the total fluid removal in peritoneal dialysis. Kidney international. 2005;68(4):1849-56.

202. Waniewski J, Debowska M, Lindholm B. How accurate is the description of transport kinetics in peritoneal dialysis according to different versions of the three-pore model? Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2008;28(1):53-60.

203. Waniewski J, Sobiecka D, Debowska M, Heimburger O, Werynski A, Lindholm B. Fluid and solute transport in CAPD patients before and after permanent loss of ultrafiltration capacity. The International journal of artificial organs. 2005;28(10):976-86.

204. Yohanna S, Alkatheeri AM, Brimble SK, McCormick B, Iansavitchous A, Blake PG, et al. Effect of Neutral-pH, Low-Glucose Degradation Product Peritoneal Dialysis Solutions on Residual Renal Function, Urine Volume, and Ultrafiltration: A Systematic Review and Meta-Analysis. Clinical journal of the American Society of Nephrology : CJASN. 2015.

205. Stunkard AJ, Harris JR, Pedersen NL, McClearn GE. The body-mass index of twins who have been reared apart. The New England journal of medicine. 1990;322(21):1483-7.

206. Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC, Hayakawa K. The heritability of body mass index among an international sample of monozygotic twins reared apart. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 1996;20(6):501-6.

207. Nordfors L, Heimburger O, Lonnqvist F, Lindholm B, Helmrich J, Schalling M, et al. Fat tissue accumulation during peritoneal dialysis is associated with a polymorphism in uncoupling protein 2. Kidney international. 2000;57(4):1713-9.

208. Amici G, Orrasch M, Da Rin G, Bocci C. Hyperinsulinism reduction associated with icodextrin treatment in continuous ambulatory peritoneal dialysis patients. Advances in peritoneal dialysis Conference on Peritoneal Dialysis. 2001;17:80-3.

209. Furuya R, Odamaki M, Kumagai H, Hishida A. Beneficial effects of icodextrin on plasma level of adipocytokines in peritoneal dialysis patients. Nephrology, dialysis, transplantation :

official publication of the European Dialysis and Transplant Association - European Renal Association. 2006;21(2):494-8.

210. Canbakan M, Sahin GM. Icodextrine and insulin resistance in continuous ambulatory peritoneal dialysis patients. Renal failure. 2007;29(3):289-93.

211. Takeguchi F, Nakayama M, Nakao T. Effects of icodextrin on insulin resistance and adipocytokine profiles in patients on peritoneal dialysis. Therapeutic apheresis and dialysis : official peer-reviewed journal of the International Society for Apheresis, the Japanese Society for Apheresis, the Japanese Society for Dialysis Therapy. 2008;12(3):243-9.

212. de Moraes TP, Andreoli MC, Canziani ME, da Silva DR, Caramori JC, Ponce D, et al. Icodextrin reduces insulin resistance in non-diabetic patients undergoing automated peritoneal dialysis: results of a randomized controlled trial (STARCH). Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2015.

213. Li Y, Xie QH, You HZ, Tian J, Hao CM, Lin SY, et al. Twelve weeks of pioglitazone therapy significantly attenuates dysmetabolism and reduces inflammation in continuous ambulatory peritoneal dialysis patients--a randomized crossover trial. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2012;32(5):507-15.

214. Stoenoiu MS, Ni J, Verkaeren C, Debaix H, Jonas JC, Lameire N, et al. Corticosteroids induce expression of aquaporin-1 and increase transcellular water transport in rat peritoneum. Journal of the American Society of Nephrology : JASN. 2003;14(3):555-65.

215. Yool AJ, Morelle J, Cnops Y, Verbavatz JM, Campbell EM, Beckett EA, et al. AqF026 is a pharmacologic agonist of the water channel aquaporin-1. Journal of the American Society of Nephrology : JASN. 2013;24(7):1045-52.

216. de Arteaga J, Ledesma F, Garay G, Chiurchiu C, de la Fuente J, Douthat W, et al. High-dose steroid treatment increases free water transport in peritoneal dialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2011;26(12):4142-5.

IX – Acknowledgments

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