

**Associations of skin
autofluorescence, a marker of tissue
advanced glycation end-product
accumulation, in persons receiving
dialysis.**

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Dedication

*THIS THESIS IS DEDICATED TO THE MEMORY OF MY
BELOVED GRANDFATHER (PAPÁ SERGIO) AND
GRANDMOTHER (ABUELITA), WHO SURELY WOULD
BE SO PROUD OF ME IF THEY COULD HAVE SEEN ME
FINISH MY PhD*

Statement of originality

I certify that this thesis is the result of my own work which has been mainly undertaken during my period of registration for this degree at The University of Nottingham. I have complied with the word limit for my degree (as stated in the Quality Manual). I hereby declare that I have not submitted this material, either in whole or part, for a degree at any other institution.

Abstract

Background: Advanced glycation end-products (AGEs) are uremic toxins that are markedly increased in persons receiving dialysis due to increased production, impaired excretion and inefficient removal. Endogenous sources of AGEs include hyperglycaemia, oxidative stress, and systemic inflammation. Foods that are cooked under dry heat conditions and high temperatures, cigarette smoke and glucose degradation products formed during the manufacturing of peritoneal dialysis (PD) solutions are also important sources of AGEs. Malnutrition, a major and frequent complication in persons receiving dialysis, may also contribute to AGE formation through its association with oxidative stress and inflammation. Tissue AGE accumulation can be assessed using skin autofluorescence (SAF). It has been reported that elevated SAF and malnutrition are each associated with increased mortality in the dialysis population, but no studies have investigated these potentially related associations together. Moreover, an increase over time in SAF predicts higher mortality on haemodialysis (HD); nevertheless, factors that contribute to changes in SAF over time in dialysis populations have not been adequately investigated.

Objectives: In Study 1, we aimed to investigate in cross-sectional analysis the determinants of increased SAF in persons receiving HD and performing PD, with a particular focus on nutritional factors including markers of malnutrition and dietary AGE intake. We also investigated in a longitudinal prospective study the rate of change in SAF over 1 year, the factors associated with these changes and the prognostic value of SAF and malnutrition for predicting

mortality in persons receiving dialysis. In Study 2, we conducted a non-randomised observational proof of principle study that aimed to investigate whether improvement of nutritional status by providing individualised dietetic advice would result in a decrease in SAF in malnourished persons on dialysis.

Methods: In Study 1, we included 120 HD and 31 PD patients. SAF was measured using a validated Autofluorescence Reader at baseline, 3, 6, 9 and 12 months. Detailed assessment of dietary intake and nutritional status was undertaken at baseline, 6 and 12 months by means of three 24-hour dietary recalls, a validated food frequency questionnaire, anthropometric measurements, handgrip strength (HGS) and the 7-point scale Subjective Global Assessment (SGA). Routine biochemical variables were also measured. Survival time was defined as the number of days between the baseline assessment and the date of death, censoring due to kidney transplantation or 30 September 2018.

For Study 2, we followed-up 1 PD and 27 HD patients with malnutrition for 6 months, who received individualised nutritional advice regarding food fortification and oral nutritional supplements according to estimated nutritional requirements. SAF was measured at baseline, 3 and 6 months. Detailed assessment of dietary intake and nutritional status (same assessments as in Study 1) was undertaken at baseline and 6 months. We compared the results with a control group of malnourished dialysis patients (n=41 HD and 8 PD) taken from Study 1.

Results: In cross-sectional analysis, SAF was higher in malnourished persons receiving HD, dietary AGE intake was not positively associated with SAF, and lower serum albumin, lower protein intake, lower HGS, diabetes, smoking and dialysis vintage were independent predictors of increased SAF. In persons performing PD, cross-sectional analysis reported that higher SAF was negatively associated with dietary AGE intake, serum albumin, HGS and energy, protein and fat intake. SAF was also positively associated with dialysis vintage and total glucose exposure. In prospective analysis, the mean SAF trend observed was an increase of 0.30 ± 0.63 arbitrary units (AU) per year but this varied from a decrease of 0.43 ± 0.45 AU to an increase of 1.05 ± 0.42 AU per year in the 1st and 4th quartiles, respectively. Development/prevalence of malnutrition over 1 year, HD as first dialysis modality and current smoking were independent determinants of increasing in SAF in persons on dialysis. Multivariable Cox proportional hazards analysis identified SAF (HR 1.44 [95%CI 1.05-1.97]; $p=0.02$), malnutrition (2.35 [1.16-4.78]; $p=0.02$) and chronological age (1.60 [1.10-2.33]; $p=0.01$) as independent predictors of mortality.

In Study 2, in the intervention group all nutritional intake components, including AGEs, as well as SGA and serum albumin improved significantly, while SAF levels remained stable over 6 months. In contrast, in the control group SAF increased significantly during the observation period when there was no improvement in nutritional intake and several markers of malnutrition, though dietary AGE intake and SGA did increase.

Conclusions: Our findings suggest that malnutrition might be a more important risk factor for increased SAF than high dietary AGE intake in persons receiving dialysis, and that correction of malnutrition may be an important strategy to decrease SAF. This hypothesis was supported in part by our prospective interventional study in which improvement in nutritional status was associated with stable SAF levels despite an increase in dietary AGE intake. We also observed that higher SAF and malnutrition were independently associated with increased mortality in this population, indicating that the association between increased SAF and mortality is not attributable to malnutrition alone. Further long-term studies of dietetic interventions with larger sample sizes are needed to test this hypothesis further and evaluate the impact on long-term outcomes, including survival.

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Abbreviations list

Abbreviation	Term
ACE- I	Angiotensin-converting enzyme inhibitors
AGEs	Advanced glycation end-products
AOPPs	Advanced oxidation protein products
APD	Automated peritoneal dialysis
AU	Arbitrary units
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CAPD	Continuous ambulatory peritoneal dialysis
CANUSA	Canada-USA
CI	Confidence intervals
CKD	Chronic kidney disease
CKD-MBD	Chronic kidney disease-mineral bone disorder
CFL	Collagen-linked fluorescent
CEL	N-carboxyethyl-lysine
CML	N-carboxymethyl-lysine
CRP	C reactive protein
DEXA	Dual-energy x-ray absorptiometry
DMS	Dialysis Malnutrition Score
EPO	Erythropoietin
ESKD	End-stage kidney disease
FFQ	Food frequency questionnaire
FGF-23	Fibroblast growth factor 23
GCP	Good clinical practice

GDPs	Glucose degradation products
GFR	Glomerular filtration rate
HbA1c	Haemoglobin A1c
HD	Haemodialysis
HDF	Haemodiafiltration
HDL	High-density lipoprotein cholesterol
HGS	Handgrip strength
HOCl	Hypochlorous acid
HR	Hazard ratio
IBW	Ideal body weight
IL-6	Interleukin- 6
IQR	Interquartile range
ISRNM	International Society of Renal Nutrition and Metabolism
KDIGO	Kidney Disease Improving Global Outcomes
KDOQI	Kidney Disease Outcomes Quality Initiative
kU	Kilounits
LDL	Low-density lipoprotein cholesterol
LVH	Left ventricular hypertrophy
MAC	Mid-arm circumference
MAMC	Mid-arm muscle circumference
MCP-1	Monocyte chemoattractant protein-1
MIS	Malnutrition-Inflammation Score
MUST	Malnutrition universal screening tool
NADPH	Nicotinamide adenine dinucleotide phosphate

NKF	National Kidney Foundation
NICE	National Institute for Health and Care Excellence
nPCR	Normalized protein catabolic rate
ONS	Oral nutritional supplements
OR	Odds ratio
PD	Peritoneal dialysis
PEW	Protein-energy wasting
PIS	Participant Information Sheet
PTH	Parathyroid hormone
pmp	Per million population
PVD	Peripheral vascular disease
RA	Renal Association
RAGE	Receptor of AGEs
ROS	Reactive oxygen species
RRT	Renal replacement therapy
SAF	Skin autofluorescence
SD	Standard deviation
SGA	Subjective Global Assessment
SU	Standard Unit
TNF- α	Tumour necrosis factor- α
TSF	Triceps skinfold thickness
UK	United Kingdom
VCAM-1	Vascular adhesion molecule-1

Chapter 1. Introduction, background and literature review

1.1 Definition of chronic kidney disease

The definition of chronic kidney disease (CKD) originally proposed by the Kidney Disease Outcomes Quality Initiative (KDOQI) in 2002⁽¹⁾ and confirmed in the Kidney Disease Improving Global Outcomes (KDIGO) guidelines⁽²⁾ is the following: “abnormalities of kidney structure or function, present for more than 3 months, with implications for health”. Kidney structure abnormalities refer to the presence of kidney damage that is usually detected by histology and imaging, genetic disorders or a history of kidney transplant, while kidney function abnormalities denote a glomerular filtration rate (GFR) $<60 \text{ ml/min/1.73 m}^2$ and proteinuria (urinary protein to creatinine ratio $>200 \text{ mg/g}$) or albuminuria (urinary albumin to creatinine ratio $>30 \text{ mg/mmol}$)⁽³⁾.

According to the KDIGO guidelines⁽²⁾, GFR and albuminuria are the main kidney measures used for the classification of CKD into different stages and risk categories for CKD-related adverse outcomes, such as all-cause and cardiovascular mortality (refer to Figure 1.1.1). In 2014, the National Institute for Health and Care Excellence (NICE) guidelines for CKD in the United Kingdom (UK)⁽⁴⁾ also adopted and included the KDIGO 2013 classification of CKD using GFR and albuminuria categories.

End-stage kidney disease (ESKD) is the final stage of CKD (i.e. CKD stage G5) and occurs when the GFR is $<15 \text{ ml/min/1.73 m}^2$. At this point, there may

be clear manifestations of uremic complications such as metabolic acidosis, electrolyte disturbances (e.g. high potassium and phosphate blood levels), fluid retention, malaise, gastrointestinal symptoms (e.g. nausea, vomit, diarrhoea), poor appetite, muscle cramps and cognitive impairment which usually indicate the need for renal replacement therapy (RRT) in the form of dialysis or kidney transplant⁽²⁾.

				Persistent albuminuria categories		
				Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
GFR categories (ml/min/1.73 m ²)	Description and range					
	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	ESKD	<15			

Figure 1.1.1 The KDIGO classification system for chronic kidney disease showing prognosis by glomerular filtration rate and albuminuria categories

Abbreviations: ESKD, end-stage kidney disease; GFR, glomerular filtration rate

Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red, very high risk.

Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012

Clinical practice guideline for the evaluation and management of chronic kidney disease.

Kidney Int. 2013;3(1):1-150.

1.2 Epidemiology and risk factors for chronic kidney disease

Chronic kidney disease is a worldwide public health issue with a high global estimated prevalence of 8-16% in adult populations; consequently, this high prevalence has contributed to the progressive increase in persons with ESKD in need of RRT programmes, either peritoneal dialysis (PD), haemodialysis (HD) or kidney transplant, especially in affluent countries with universal access to healthcare⁽⁵⁾. For instance, according to the UK Renal Registry 20th Annual Report, the incidence of persons starting RRT (i.e. dialysis treatments and kidney transplant recipients) in 2016 was 118 per million population (pmp)⁽⁶⁾ compared to 96 pmp in 1998 reported in the UK Renal Registry 3rd Annual Report⁽⁷⁾, while the prevalence in 2016 of RRT was 962 pmp corresponding to 63,162 persons receiving RRT, an 83% increase from 1998 (i.e. 526 pmp). Furthermore, from 1998 to 2016, the prevalence of persons on HD and with a functioning kidney transplant increased 164% and 128%, respectively, while there was a 39% decrease in the prevalence of persons on PD^(7, 8). Figure 1.2.1 displays the RRT incidence rates in the countries of the UK from 1990-2016 and Figure 1.2.2 shows the growth in prevalence by treatment modality at the end of each year from 1997–2016.

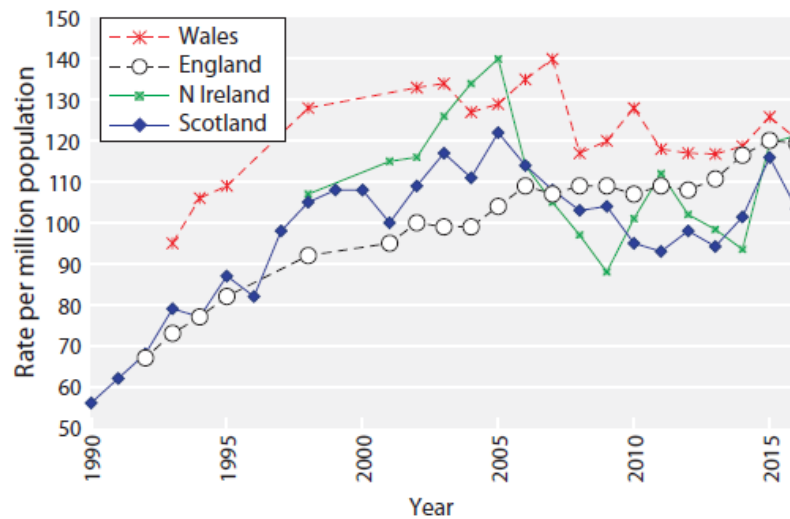


Figure 1.2.1 Renal replacement therapy incidence rates in the countries of the UK 1990-2016

Hole B, Gilg J, Casula A, Methven S, Castledine C. UK Renal Registry 20th annual report: Chapter 1 UK Renal replacement therapy adult incidence in 2016: National and centre-specific analyses. *Nephron*. 2018;139(Suppl 1):13-46.

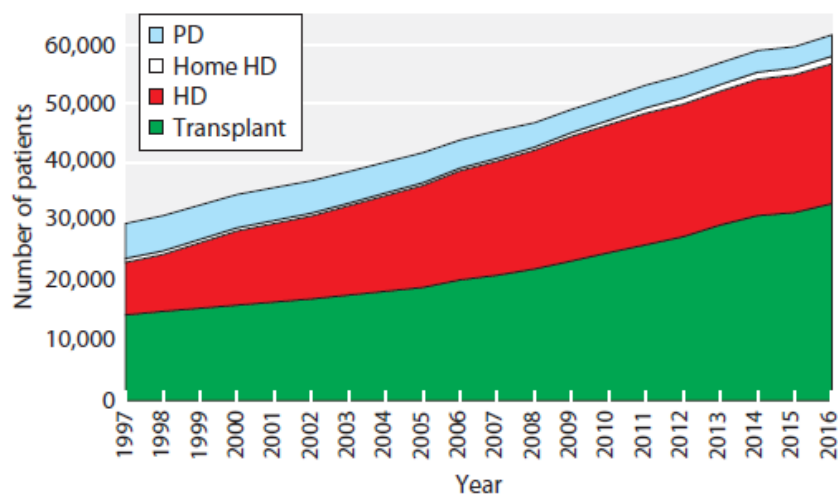


Figure 1.2.2 Growth in prevalence by treatment modality at the end of each year 1997–2016

MacNeill SJ, Ford D, Evans K, Medcalf JF. UK Renal Registry 20th Annual Report: Chapter 2 UK Renal replacement therapy adult prevalence in 2016: National and centre-specific analyses. *Nephron*. 2018;139(Suppl 1):47-74.

Besides its high prevalence, CKD is also associated with significant healthcare costs. In 2009-2010, for instance, CKD contributed £1.44 to £1.45 billion to the National Health Services costs in the UK, with ~£780 million spent on RRT⁽⁹⁾. Furthermore, outpatient HD treatment had an estimated annual cost of £32,000-£35,000 per patient in 2008⁽¹⁰⁾.

In the twentieth century, infectious diseases such as malaria, tuberculosis, influenza or acquired immunodeficiency syndrome were considered the major causes of morbidity and mortality worldwide. In contrast, CKD and other non-infectious diseases (e.g. cardiovascular disease, diabetes, chronic respiratory disease and cancer) have recently become the most common causes of premature death globally⁽¹¹⁾. For example, CKD was placed 27th in 1990 as one of the most important contributing causes for all-cause mortality worldwide, and rose to 18th in 2010⁽⁵⁾. Cardiovascular disease is closely linked to CKD and it is the most important risk factor for mortality in this population. Several studies have reported that lower estimated GFR and higher levels of albuminuria are independent risk factors for increased risk of all-cause and cardiovascular mortality⁽¹²⁾; furthermore, persons on HD and PD have two to ten times higher risk of cardiovascular mortality compared to the general population⁽¹³⁾.

According to the UK Renal Registry 20th Annual Report in 2016, diabetes continued to be the single most common cause of CKD (28.6%), followed by other aetiologies (17.2%) and uncertain aetiology (14.6%)⁽⁶⁾. Even though hypertension accounted for just 6.3% of the primary aetiologies of CKD in the

UK⁽⁶⁾, it has been considered alongside diabetes as the most common cardiovascular risk factor which accelerates the progression of CKD⁽¹¹⁾. CKD risk factors (including diabetes and hypertension) have been classified into the following categories⁽¹⁴⁾:

Table 1.2.1 Classification of risk factors for chronic kidney disease

Category	Risk factors
Susceptibility factors: Increase the possibility of kidney damage	Old age, family history of CKD, reduced renal mass, low birth weight, black population and other ethnic minorities, hypertension, diabetes, obesity and low socioeconomic level.
Initiating factors: directly initiate kidney damage	Autoimmune diseases, systemic and urinary infections, nephrolithiasis, obstruction of the lower urinary tract, nephrotoxic drugs (NSAIDs), hypertension and diabetes.
Progression factors: exacerbate kidney damage and accelerate the progression of CKD to ESKD	Persistent proteinuria, poor glycaemic and blood pressure control, smoking, dyslipidaemia, anaemia, cardiovascular disease and obesity.

Abbreviations: CKD, chronic kidney disease; ESKD, end-stage kidney disease; NSAIDs, non-steroidal anti-inflammatory drugs.

Adapted from: Martínez-Castelao A, Górriz JL, Segura-de la Morena J, Cebollada J, Escalada J, Esmatjes E, et al. Consensus document for the detection and management of chronic kidney disease. *Nefrologia*. 2014;34(2):243-262.

1.3 Description of renal replacement therapy: focus on dialysis

As stated above and according to the 2018 NICE guidelines on RRT⁽¹⁵⁾, it is suggested that persons with ESKD start a form of RRT when indicated by the impact of uremic symptoms on the day-to-day activities, biochemical disturbances, uncontrollable fluid overload, or at a GFR of around 5 to 7 ml/min/1.73 m² in the absence of any symptomatology. RRT comprises different treatments for persons with ESKD that assist or replace renal excretory function and so prolong survival. RRT includes kidney transplantation or dialysis. Kidney transplantation is a major surgical procedure that involves the transplant of a healthy kidney into a person with ESKD. Kidneys for transplantation may be obtained from a deceased or a living donor⁽¹⁶⁾. For the purpose of the thesis, this and the following sections will focus only on persons receiving HD and performing PD. We chose to study persons receiving dialysis because they represent the group with the highest reported skin autofluorescence (SAF) levels, thought to be due to increased levels of oxidative stress and severely reduced renal excretion of advanced glycation end-products (AGEs).

Dialysis refers to an artificial process of removing solutes including uremic toxins from the blood and adjusting fluid and electrolyte imbalances. There are two types of dialysis: PD and HD. Both dialysis modalities follow the physiological principles of diffusive clearance where small solutes (e.g. urea, creatinine), electrolytes (e.g. potassium, phosphate) and water will move down

their concentration gradient, from areas of high concentration (i.e. blood) to areas of low concentration (i.e. dialysate, an electrolyte solution containing the normal constituents of plasma) through a natural membrane in the abdomen (i.e. the peritoneum), in the case of PD, or an artificial extracorporeal membrane linked to a dialysis machine, in the case of HD⁽¹⁷⁾.

Besides the traditional HD technique (i.e. diffusion), there are other types of HD that use either a convection technique (i.e. haemofiltration [HF]) or a combination of diffusion and convection techniques (i.e. haemodiafiltration [HDF]) to remove excess body fluid and small to middle- and large-sized uremic toxins (e.g. β_2 -microglobulin and AGEs)⁽¹⁸⁾. Convective dialysis modalities use a pressure gradient instead of a concentration gradient that enhances the removal of fluid and waste products. In these modalities, a positive hydrostatic pressure moves water and solutes from blood to dialysate and because fluid removal is substantial, substitution fluid is administered to achieve the target fluid balance⁽¹⁷⁾.

The efficiency to remove uremic toxins not only depends on the dialysis technique used but also on the type and material of the dialysis membrane. Dialysis membranes are classified according to their water permeability into low-flux or high-flux membranes. Low-flux membranes have small pores and therefore are only able to clear small-sized molecules into the dialysate. In contrast, high-flux membranes have been developed with larger pore sizes to allow greater clearances of medium-sized molecules⁽¹⁹⁾.

Dialysis membranes can be made with either cellulose or synthetic polymers. The earliest and most used dialysis membranes consisted of natural cellulose derived from cotton; however, these were associated with increased inflammatory response⁽¹⁹⁾. This disadvantage was mitigated by the fabrication of a synthetic cellulose-derived material (i.e. cellulose acetate) and by surface-coating cellulosic membranes with polyethylene glycol, polyacrylonitrile or vitamin E⁽²⁰⁾. Nonetheless, cellulose-based membranes belong to the class of low-flux membranes, thus only allowing clearance of small-sized uremic toxins. Conversely, synthetic polymer-based membranes have larger pore size (thus better removal of middle-sizes molecules), higher hydraulic permeability and reduced induction of inflammatory mediators (i.e. improved biocompatibility). Therefore, synthetic dialysis membranes made with polymers such as polysulfone, polyethersulfone and polymethylmethacrylate, among others, have become the material of choice for high-flux HD therapies^(19, 20).

Peritoneal dialysis can be classified into continuous ambulatory PD (CAPD) or automated PD (APD). CAPD has to be performed manually by the person with ESKD or carer and it usually involves 4 exchanges of dialysis fluid daily, whereas APD involves the use of a mechanical device that delivers and drains the dialysate. CAPD has been associated with several psychosocial and physical problems (e.g. lack of motivation, technique failure and recurrent peritonitis), while APD has been reported to have some advantages such as reduced incidence of peritonitis, better acceptability, more time for work, family and social activities and lower presence of physical symptoms. Even

though APD is associated with a faster decline on residual renal function, it has been suggested as a better alternative than CAPD⁽²¹⁾.

In broad terms, PD solutions can be classified into glucose or non-glucose-containing solutions. The osmotic agent used in conventional PD solutions (i.e. glucose-based solutions) is dextrose. Glucose concentrations of conventional PD solutions are generally expressed as a percentage of either dextrose monohydrate or dextrose anhydrous. The following glucose concentrations are routinely available to use in the clinical setting: 1.36% or 1.5%, 2.27% or 2.5% and 3.86% or 4.25% for dextrose anhydrous or monohydrate, respectively⁽²²⁾. Glucose-containing solutions have more disadvantages than benefits. First of all, long-term exposure to glucose has been associated with anatomical and functional alterations of the peritoneum, such as oxidative damage of the mesothelial cells and thickening of the peritoneal membrane, which ultimately leads to ultrafiltration failure, increased incidence of peritonitis and PD discontinuation⁽²³⁾. In addition to the direct detrimental effects of high glucose exposure on the peritoneal membrane, the so-called glucose degradation products (GDPs), which are generated in PD fluid during heat sterilization and storage, promote the formation of AGEs in the peritoneum. GDPs and AGEs may cause changes in the structure and function of the peritoneal membrane, such as mesothelial damage and increased peritoneal vascularization and fibrosis, which ultimately decrease the long-term viability of the peritoneum as a dialysis membrane⁽²⁴⁾. Finally, long-term exposure to glucose-based solutions and GDPs has been implicated in the development of metabolic complications, including hyperglycaemia, hyperinsulinemia, increased body fat mass and

hypertriglyceridemia, which will eventually lead to cardiovascular complications⁽²³⁾.

Despite all of the negative effects that glucose-containing solutions have, they continue to be the indispensable dialysate for PD. Nevertheless, in order to minimise the deleterious effects of conventional PD solutions on the peritoneal membrane, it has been suggested that daily exchanges consist of a combination of conventional PD solutions with newer and more biocompatible PD solutions, including low GDP solutions, icodextrin- (i.e. glucose polymer) and aminoacid-containing solutions. Low GDP solutions have shown to improve the preservation of residual renal function and urine output, while icodextrin-based solutions have been associated with increased peritoneal ultrafiltration and reduction of uncontrolled episodes of fluid overload⁽²⁵⁾. The use of amino acid-based PD solutions has shown improvements in nutritional parameters, such as serum albumin and normalized protein catabolic rate (nPCR), and are therefore used as a supplementary treatment for persons on PD with malnutrition⁽²³⁾.

The following section will describe the complications of CKD in persons receiving dialysis, with a particular focus on cardiovascular disease, protein-energy wasting (PEW) and oxidative stress, as well as the relationship between these three major complications.

1.4 Complications of chronic kidney disease in the dialysis population

Persons receiving dialysis have lost renal endocrine and exocrine functions and as a result are at risk of developing a variety of complications which are reviewed below.

Anaemia: Relative erythropoietin (EPO) deficiency has been considered the most important aetiological factor for anaemia. Additionally, it has been suggested that disordered iron homeostasis, gastrointestinal bleeding, severe hyperparathyroidism, systemic inflammation and shortened red blood cell survival also contribute to the development of this common feature in persons on dialysis^(26, 27). Anaemia has been associated with an increase in uremic symptoms such as fatigue, tiredness, weakness, low energy to perform activities of daily living, decreased physical functioning, loss of appetite and increased time sleeping⁽²⁸⁾ and also with high rates of cardiovascular complications, hospitalizations and mortality risk⁽²⁹⁾.

CKD-Mineral bone disorder (CKD-MBD): CKD-MBD is a complex disorder in the dialysis population that develops due to reduced activation of vitamin D and an increase of phosphate levels (i.e. hyperphosphataemia). Consequently, intestinal calcium absorption is reduced and serum calcium concentration tends to drop causing increased secretion of parathyroid hormone (PTH), which leads to the common complication known as secondary hyperparathyroidism. High PTH levels increase bone resorption to maintain the

serum calcium concentration. Levels of fibroblast growth factor 23 (FGF-23), another phosphaturic hormone, also become elevated and have been recognised to play an important role in the pathogenesis of CKD-MBD⁽²⁶⁾. The abnormalities that characterise CKD-MBD interact in a complex series of mechanisms to cause arterial calcification, left ventricular hypertrophy (LVH) and increased cardiovascular mortality. Each component of CKD-MBD (i.e. high phosphate, PTH and FGF-23 levels and low calcium and vitamin D levels) has been associated with an increase in all-cause and cardiovascular mortality risk⁽³⁰⁻³²⁾.

Metabolic acidosis: This complication occurs when the kidneys are unable to cope with the daily net fixed acid production causing an imbalance between the daily consumption and production of acids and bases and their renal excretion. Metabolic acidosis has several deleterious consequences such as increased protein catabolism leading to PEW (i.e. malnutrition), potential exacerbation of secondary hyperparathyroidism which worsens CKD-MBD, decrease in functional capacity and increased risk of mortality⁽³³⁾.

Hyperkalaemia: High potassium levels are a frequent complication in the dialysis population due to impairment in the potassium excretory capacity of the kidneys. Hyperkalaemia has been associated with higher rates of cardiovascular complications and mortality⁽²⁾.

Alterations in glycaemic control: Persons receiving dialysis, irrespective of their diabetic status, experience glycaemic abnormalities due to multiple mechanisms related to the gradual decline in kidney function, the uremic milieu and the dialysis procedure itself⁽³⁴⁾. Hyperglycaemia and glucose intolerance develop as a result of insulin resistance, which might be worsened by some uremic toxins; secondary hyperparathyroidism and vitamin D deficiency may increase glucose levels because of their negative impact on insulin secretion⁽³⁵⁾. In addition, persons on PD have a higher risk of hyperglycaemia due to significant exposure to high glucose concentrations in the dialysis fluid⁽³⁴⁾. Not only high glucose levels are observed in persons on dialysis, but also hypoglycaemia. Factors contributing to low glucose levels in persons with or without diabetes undergoing dialysis include the following: decreased renal and hepatic insulin clearance or impaired renal insulin degradation resulting in increased insulin half-life, PEW and decreased food intake due to anorexia⁽³⁵⁾.

Fluid overload: This phenomenon occurs because the kidneys are no longer able to optimally control fluid balance and therefore fail to remove extra sodium and water from the body causing fluid retention. Extracellular overhydration leads to hypertension⁽³⁶⁾, LVH^(37, 38), congestive heart failure⁽³⁹⁾ and respiratory abnormalities, including pleural effusion and pulmonary oedema⁽⁴⁰⁾. Additionally, it has been reported that fluid overload is a strong predictor of increased all-cause and cardiovascular mortality^(41, 42).

Dyslipidaemia: In the general population, this cardiovascular and mortality risk factor is characterised by high levels of low-density lipoprotein (LDL) cholesterol and low levels of high-density lipoprotein (HDL) cholesterol⁽⁴³⁾. In contrast, persons on dialysis show distinctive patterns of dyslipidaemia and a “reverse epidemiology phenomenon”^(43, 44). Hypertriglyceridemia and reduced HDL cholesterol are characteristic features in this population and are related to cardiovascular disease progression and mortality⁽⁴⁵⁾; however, higher total cholesterol is paradoxically associated with lower risk of cardiovascular disease and mortality in the absence of malnutrition and inflammation. In contrast, low levels of total and LDL cholesterol have been associated with an increased risk of death in persons receiving dialysis, possibly reflecting the presence of a greater degree of malnutrition and inflammation⁽⁴⁶⁾.

Inflammation: Chronic inflammation is commonly observed in persons receiving dialysis and is considered an important cardiovascular and mortality risk factor⁽⁴⁷⁾. The underlying aetiology is only partly understood and it has been suggested that the following factors may contribute to its development: increased production and decreased clearance of pro-inflammatory cytokines, fluid overload, oxidative stress and decreased levels of antioxidants, metabolic acidosis, chronic and recurrent infections, including those related to dialysis access, altered metabolism of adipose tissue, intestinal dysbiosis, coexistence of comorbid conditions (e.g. diabetes, hypertension, coronary heart disease) and the dialysis procedure itself⁽⁴⁸⁾.

1.4.1 Cardiovascular disease: a major complication in persons receiving dialysis

Cardiovascular disease is the principal cause of morbidity and mortality in the dialysis population; for instance, it has been demonstrated that persons on dialysis are 8.8 times more likely to die of cardiovascular disease than the general population⁽⁴⁹⁾. Traditional cardiovascular risk factors which are present in both the dialysis and general population but are more prevalent in individuals with ESKD include hypertension, diabetes and dyslipidaemia.

Hypertension is a well-known contributing factor for the development of cardiovascular disease in persons receiving dialysis, particularly those undergoing HD. Fluid overload has been considered the main cause of hypertension in this population and both risk factors closely interact and lead to cardiac dilatation, LVH, deterioration of systolic and diastolic left ventricular function, myocardial infarction, heart failure, stroke and ultimately increased cardiovascular and all-cause mortality⁽⁵⁰⁾. LVH is the most important cardiac abnormality observed in the HD population; it has been independently and strongly associated with lower survival and, more importantly, worsening of LVH has been identified as the strongest predictor of sudden cardiac death in persons receiving HD after adjusting for traditional cardiovascular risk factors such as male sex, older age and presence of hypertension^(51, 52). LVH is also a strong predictor of cardiovascular events in the PD population; however, this complication is less prevalent in persons who undergo PD than HD, which might be related with the effect of preserved residual renal function (and therefore urine output) on fluid overload⁽⁵³⁾.

The presence of diabetes in persons receiving dialysis has been associated with higher risk of developing cardiovascular co-morbidities such as ischaemic heart disease and peripheral vascular disease (PVD). The pathogenesis behind the high incidence of cardiovascular disease could in part involve the formation of AGEs which are uremic toxins that will eventually cross-link with proteins in the arterial wall, increase pro-inflammatory cytokine synthesis and promote atherogenesis, arterial stiffness and endothelial dysfunction, which together significantly increase the risk of overall and cardiovascular mortality⁽⁵⁴⁾.

Dyslipidaemia is another well-known cardiovascular and mortality risk factor in the dialysis population. Some atherogenic lipid molecules (e.g. carbamylated LDL, lipoprotein [a] and apolipoprotein [a]) have shown to be independent predictors of coronary artery disease, stroke, myocardial infarction and death⁽⁵⁴⁾; nonetheless, as discussed previously, hypercholesterolemia is associated with lower cardiovascular mortality in the absence of the malnutrition-inflammation syndrome⁽⁴⁶⁾. On the other hand, one large randomised trial reported that lowering cholesterol with statin treatment in persons with CKD (including those receiving dialysis) resulted in a 17% reduction in atherosclerotic events⁽⁵⁵⁾, though two other trials found no benefit of statin therapy in persons on dialysis^(56, 57).

These traditional cardiovascular risk factors do not fully explain the high prevalence and incidence of cardiovascular disease among persons receiving dialysis. ESKD-related complications such as anaemia, CKD-MBD, malnutrition-inflammation syndrome and oxidative stress have been strongly

suggested to be non-traditional cardiovascular risk factors that contribute to the high risk of cardiovascular events observed in the ESKD population⁽⁵⁸⁾.

ESKD-associated anaemia is reported to be an independent predictor of cardiovascular morbidity and mortality⁽²⁹⁾. Besides hypertension and fluid overload, anaemia has also been implicated in the development of LVH in the dialysis population and it has been reported that EPO treatment for the correction of anaemia is associated with a regression of LVH⁽⁵⁴⁾. Furthermore, levocarnitine therapy improves EPO responsiveness index (defined as average weekly units of EPO divided by clinical dry weight and average blood haemoglobin) and decreases LVH in persons on dialysis who are resistant to EPO treatment⁽⁵⁹⁾. Nevertheless, randomised trials have observed that normalisation of haemoglobin with EPO treatment is associated with adverse effects in persons with CKD including increased risk of stroke⁽⁶⁰⁾ and all-cause mortality⁽⁶¹⁾.

CKD-MBD (i.e. hyperphosphataemia, secondary hyperparathyroidism, high FGF-23 levels, hypo- and hypercalcaemia and hypovitaminosis D) is another important non-traditional risk factor for cardiovascular morbidity and mortality. The pathogenesis of this association has been suggested to be related with the development of vascular calcification, arterial stiffness and LVH^(54, 62). Among the abnormalities that characterise CKD-MBD, increased levels of FGF-23 have been independently implicated in cardiovascular mortality⁽⁶³⁾. Some pathophysiological mechanisms that might partially explain this association are the acceleration of vascular calcification through increases in

serum phosphate and calcium levels, the development of LVH by a direct action of FGF-23 on cardiac FGF receptors, increased oxidative stress causing impairment of endothelial function (i.e. arterial stiffness) and the regulation of renal sodium absorption that may lead to the development of hypertension⁽⁶⁴⁾.

The persistent inflammatory state seen in persons receiving dialysis characterized by an increased activity of C reactive protein (CRP) and pro-inflammatory cytokines (i.e. interleukin-6 [IL-6] and tumour necrosis factor- α [TNF- α]) increases protein catabolism, muscle wasting and resting energy expenditure and decreases hepatic protein synthesis (e.g. albumin and prealbumin) and appetite, leading ultimately to the development of malnutrition, better known as PEW⁽⁶⁵⁾. PEW has been strongly associated with overall and cardiovascular mortality in the dialysis population⁽⁶⁶⁻⁶⁸⁾, but the mechanisms involved in this association are only partly understood; nonetheless, the following factors have been proposed: derangements in muscle and adipose tissue metabolism as well as impairments in the gastrointestinal, haematological and immune systems, increased production of pro-inflammatory cytokines (i.e. systemic inflammation) and most importantly the high prevalence of cardiovascular disease⁽⁶⁵⁾. It has also been reported that hypoalbuminemia (<38 g/L)⁽⁶⁹⁾, hypocholesterolaemia (<2.58 mmol/L)⁽⁴⁶⁾, low body mass index (BMI; <23 kg/m²)⁽⁷⁰⁾ and reduced dietary protein intake (<1.0 g/kg/day)⁽⁷¹⁾ (i.e. all markers of PEW) are strong predictors of mortality in the dialysis population.

Oxidative stress is another abnormality that may contribute to the development of complications in persons receiving dialysis and it has become an area of interest, along with systemic inflammation and endothelial dysfunction, because of its strong association with cardiovascular disease and mortality risk⁽⁷²⁾. Markers of oxidative stress are particularly increased in persons on dialysis and this oxidative burden is linked with systemic inflammation. The close interrelationship between oxidative stress and systemic inflammation has been implicated in cardiovascular disease because it accelerates the progression of atherosclerosis and endothelial cell injury, leading eventually to myocardial infarction, stroke and cardiovascular mortality⁽⁷³⁾.

The following section will focus on the definition of PEW, the main causes associated with its development, the most important malnutrition-related complications, as well as methods of assessment and nutritional interventions for addressing malnutrition in the dialysis population.

1.4.2 Protein-energy wasting: definition, aetiology, consequences, assessment and interventions

DEFINITION

The definition of malnutrition involves any nutritional deficiency, excess or imbalance in energy, macronutrient and micronutrient intake. The term malnutrition refers not only to undernutrition (i.e. muscle wasting and low body weight), but also to overnutrition (i.e. overweight and obesity)⁽⁷⁴⁾.

However, in persons with ESKD undergoing dialysis, the term *malnutrition* is more frequently used as a synonymous with *undernutrition*.

Over the last couple of decades, a number of terms for referring to malnutrition have been used in both the renal dietetic practice and research, including uremic malnutrition⁽⁷⁵⁾, uremic or renal cachexia⁽⁷⁶⁾, protein-energy malnutrition⁽⁷⁵⁾, malnutrition-inflammation atherosclerosis syndrome⁽⁷⁷⁾ and malnutrition-inflammation complex/cachexia syndrome⁽⁷⁸⁾. Nevertheless, in 2008 the International Society of Renal Nutrition and Metabolism (ISRNM) suggested a single term, “Protein-energy wasting”⁽⁷⁹⁾, which has improved communication and clarified thinking across renal multidisciplinary care teams. This proposed terminology has also provided a more accurate description of the well-known association between muscle and energy wasting, inadequate total dietary intake, systemic inflammation, oxidative stress, hypercatabolism and other metabolic abnormalities⁽⁷⁹⁾.

Protein energy wasting can be defined as a depletion state of body protein stores, total body fat and muscle mass⁽⁷⁹⁾. According to the ISRNM, three of the following four criteria have to be met in order to identify PEW in persons receiving dialysis: 1) biochemical criteria (i.e. low serum albumin, prealbumin or cholesterol); 2) body mass (i.e. low BMI, reduced total body fat or weight loss); 3) muscle wasting (i.e. decrease in muscle mass, reduced mid-arm muscle circumference [MAMC] or low serum creatinine normalized by body surface area); and 4) low protein or energy intake^(79, 80). Table 1.4.1 shows the full criteria for the identification of PEW in the dialysis population. For the purpose of the thesis, the term “malnutrition” will be used as a synonymous with “PEW”.

Malnutrition is one of the major and most frequent complications in persons receiving dialysis. Several studies conducted in the dialysis population have reported a wide variation in the prevalence of malnutrition, which may be attributed in part to the different nutritional markers and/or assessment methods used for the evaluation of the nutritional status⁽⁷⁸⁾. For instance, malnutrition in persons on HD has been found to be present in 24% and 34% according to a reduced MAMC (i.e. <50th percentile)⁽⁸¹⁾ and a BMI <23 kg/m²⁽⁸²⁾, respectively, while use of the original and modified versions of the Subjective Global Assessment (SGA; refer to Nutritional scoring tools section for further details) identified a prevalence of malnutrition varying from 37% to 61.5% in persons receiving dialysis^(83, 84).

Table 1.4.1 Readily utilizable criteria for the identification of protein-energy wasting in persons receiving dialysis

CRITERIA	PARAMETERS
Biochemical criteria	Serum albumin <38 g/L Serum prealbumin (transthyretin) <30 mg/dl Serum cholesterol <2.58 mmol/L
Body mass	Body mass index <23 kg/m ² Unintentional weight loss over time: 5% over 3 months or 10% over 6 months Total body fat percentage <10%
Muscle wasting	Reduction in muscle mass: 5% over 3 months or 10% over 6 months MAMC reduction >10% in relation to 50 th percentile of reference population SCr/BSA ≤380 µmol/L
Dietary intake	Unintentional low DPI <0.80 g/kg/day for at least 2 months. Unintentional low DEI <25 kcal/kg/day for at least 2 months

Abbreviations: DEI, dietary energy intake; DPI, dietary protein intake; MAMC, mid-arm muscle circumference; SCr/BSA, serum creatinine/body surface area.

Adapted from: 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 Int.* 2008;73(4):391-398.

AETIOLOGY

Malnutrition is usually associated with a decrease in total dietary intake mainly due to loss of appetite and dietary restrictions; however, malnutrition in persons with ESKD undergoing dialysis cannot be explained by insufficient nutrient intake alone⁽⁶⁵⁾. Malnutrition in persons receiving dialysis is the result of an interaction between multiple ESKD-related mechanisms, including uremic toxicity, anorexia (which leads to inadequate dietary intake), presence of co-morbid conditions and endocrine disorders, nutrient losses during dialysis, metabolic acidosis, systemic inflammation and oxidative stress^(65, 85).

Loss of kidney function plays an important role in the development of malnutrition. Uremic toxicity leads to anorexia (i.e. loss of appetite) due to an increase in circulating levels of satiety factors (e.g. cholecystokinin, glucagon and serotonin) and appetite suppressors such as leptin and insulin⁽⁸⁶⁾. In addition, the accumulation of uremic toxins contributes to the high prevalence of gastrointestinal symptoms, such as nausea, vomiting, constipation and diarrhoea⁽⁸⁷⁾.

Inadequate dietary intake is another frequent and important cause of malnutrition in persons receiving dialysis. Poor appetite has been generally considered as one of the major causes of inadequate energy, macro and micronutrient intake in the dialysis population⁽⁶⁵⁾. Additional factors associated with inadequate dietary intake include diet restrictions (i.e. fluid and sodium restrictions and low phosphate and potassium diets), older age, systemic chronic inflammation, presence of gastrointestinal symptoms and underlying

co-morbidities, poor socioeconomic status, depression, physical inability to obtain, prepare and eat food, fluid overload^(85, 88), and bloating, postprandial fullness and early satiety in the case of persons on PD⁽⁸⁹⁾.

Nutrient losses during the dialysis procedure itself also contribute to the onset and worsening of malnutrition in persons with ESKD. It has been reported that during a single HD procedure, a loss of up to 10 g of amino acids and some peptides occur⁽⁹⁰⁾. Similarly, significant protein losses into the PD dialysate (ranging from 3-9 g/day) have been observed, which can be aggravated in the presence of peritonitis (e.g. up to more than 15 g/day)⁽⁹¹⁾. Not only protein losses have been reported during dialysis, but also water-soluble vitamins such as thiamine, riboflavin, pyridoxine, cobalamin, folic acid and ascorbic acid⁽⁹²⁾, as well as trace elements including zinc, selenium and manganese⁽⁹³⁾.

The majority of the typical co-morbidities (e.g. diabetes, cardiovascular disease [heart failure and PVD], liver disease, pulmonary disease and infections) and endocrine abnormalities (e.g. insulin resistance and secondary hyperparathyroidism) observed in the dialysis population contribute to the development of malnutrition by increasing resting energy expenditure, muscle wasting, systemic inflammation and pain, as well as reducing appetite^(65, 85).

As stated previously in section 1.4 Complications of chronic kidney disease in the dialysis population, the kidney's decreased capacity to excrete acids and to synthesize bicarbonate leads to the development of metabolic acidosis⁽⁹⁴⁾.

Several mechanisms have been proposed to be involved in the association

between metabolic acidosis and malnutrition ⁽⁹⁵⁾: first, metabolic acidosis contributes significantly to increased protein catabolism and negative nitrogen balance by increasing corticosteroid levels and stimulating the activity of branched chain ketoacid dehydrogenase (an enzyme that breaks down branched-chain amino acids which are involved in muscle synthesis), caspase-3 (a proteolytic enzyme) and the ATP-dependent ubiquitin-proteasome system (a pathway for digestion of protein into peptides and amino acids); secondly, metabolic acidosis decreases muscle protein and albumin synthesis by inducing insulin resistance and reducing the synthesis of growth hormone, insulin-like growth factor and thyroid hormone; finally, metabolic acidosis has been linked with systemic inflammation by increasing the production of TNF- α (a key pro-inflammatory cytokine), which is associated with hypercatabolism, increased muscle breakdown and anti-anabolic effects⁽⁶⁵⁾.

In the last couple of decades, systemic inflammation has come to be considered one of the most important aetiological factors of malnutrition in persons receiving dialysis. Systemic inflammation, defined by increased levels of CRP, IL-6 and TNF- α , is highly prevalent in the dialysis population and is associated with higher hospitalization rates and increased cardiovascular morbidity and mortality risk⁽⁹⁶⁾. A number of mechanisms may explain the key role of inflammation in causing malnutrition, including the rise in resting energy expenditure, muscle insulin resistance and protein and lipid oxidation, as well as the reduction in the synthesis and half-life of albumin, muscle protein synthesis and appetite^(65, 96). Furthermore, pro-inflammatory cytokines such as IL-6 and TNF- α have been associated with an increase in muscle protein

catabolism by stimulating caspase-3 activity and by increasing glucocorticoid levels, which leads to an impairment of the insulin-like growth factor signalling (an anti-anabolic effect)⁽⁶⁵⁾.

Oxidative stress (i.e. imbalance between oxidant production and antioxidant defence), another common complication observed in persons receiving dialysis, has been shown to be strongly associated with higher overall and cardiovascular mortality⁽⁹⁷⁾. Moreover, oxidative stress is linked with systemic inflammation and this interaction may further contribute to the development of malnutrition by exacerbating the detrimental effects on the nutritional status, including increased protein catabolism, muscle wasting and resting energy expenditure, and decreased albumin synthesis⁽⁹⁶⁾.

Decreased functional capacity also plays an important role in the development of malnutrition. Persons receiving dialysis are more prone to lose the ability to perform activities of daily living or any physical activity due to coexisting comorbidities, such as heart failure, PVD, anaemia, rheumatological, psychiatric and neurological disorders, chronic pulmonary diseases and cancer, as well as fluid overload and older age⁽⁶⁵⁾. Consequently, low levels of physical activity worsen hypercatabolism of proteins and muscle mass loss⁽⁹⁸⁾.

Finally, an important but underappreciated risk factor for the development of malnutrition is the psychosocial component. The most frequent psychosocial problems that have a severe impact on the nutritional status of persons receiving dialysis are depression, anxiety, family issues, dietary and time

restrictions (e.g. time travelling to dialysis sessions and time to socialize), functional limitations and financial concerns⁽⁹⁹⁾.

CONSEQUENCES

Some of the factors associated with the development of malnutrition described above have also been considered as important and frequent complications of the PEW syndrome seen in the dialysis population⁽⁶⁵⁾. For instance, infections are a risk factor of malnutrition but, at the same time, presence of malnutrition increases the susceptibility to infections, which might be partially explained by the negative effects of malnutrition on the immune system^(100, 101).

Cardiovascular disease is another aetiological factor and one of the most important complications associated with malnutrition^(65, 96). It has been suggested that the high prevalence of cardiovascular disease observed in persons receiving dialysis is associated with the link between malnutrition, systemic inflammation and oxidative stress⁽¹⁰²⁾. This association will be discussed in more detail in further sections. As described above, decreased physical activity exacerbates the deleterious effects of inflammation on the nutritional status (i.e. increased protein breakdown and muscle wasting); therefore, the presence of malnutrition ultimately leads to increased muscle weakness and frailty, and vice versa^(65, 98).

On the basis of the risk factors and complications described above, it is not surprising that several prospective studies have found that malnutrition is an independent and strong predictor of all-cause and cardiovascular mortality in the dialysis population^(66, 68, 103, 104). Moreover, the presence of malnutrition has

been strongly associated with increased rates of hospitalization and poor quality of life^(67, 105), resulting in a greater use of healthcare resources and higher costs in comparison with persons who are well-nourished⁽¹⁰⁶⁾. For instance, it has been reported that the annual healthcare cost of disease-related malnutrition in the UK was £7.3 billion in 2003. Additionally, treatment of persons with malnutrition involves an annual extra cost of over £5.3 billion due to longer, more frequent and more expensive hospital stays, as well as long-term care⁽¹⁰⁷⁾.

METHODS OF ASSESSMENT

Due to the fact that malnutrition is associated with severe adverse clinical outcomes in the dialysis population, it is critical to use appropriate methods of nutritional assessment in order to identify those persons that are at nutritional risk or are already malnourished and, consequently, prevent and/or treat their malnutrition⁽⁸⁵⁾.

In general, the methods used for the evaluation of the nutritional status in persons receiving dialysis can be grouped in the following categories:

1. Body composition:

The most common techniques used for the assessment of body composition in the dialysis population are anthropometry, bioelectrical impedance analysis (BIA) and dual-energy x-ray absorptiometry (DEXA).

Anthropometry refers to the standard and systematic measurement of body dimensions (i.e. body size and shape). In broad terms, anthropometric measurements can be classified into basic measurements (e.g. weight and height), skinfold thicknesses (e.g. triceps, subscapular, abdominal) and circumferences (e.g. head, arm and waist). From these measurements, it is possible to calculate different anthropometric indexes/percentages, such as BMI, body fat percentage and MAMC⁽¹⁰⁸⁾. The main advantages of anthropometry include its simplicity and cost-effectiveness for assessing the nutritional status and for indirectly estimating total body fat and muscle mass; nevertheless, interpretation of anthropometric assessment in the dialysis population might be significantly affected by the lack of anthropometric reference standards, the high inter- and intra-observer variability and variable hydration status⁽¹⁰⁹⁾. Despite these drawbacks, it has been previously reported that a decline in mid-arm circumference (MAC) and skinfold measurements (i.e. sum of subscapular, biceps and triceps), as well as lower MAMC (a surrogate measure of muscle mass) and lower total body fat percentage are associated with higher all-cause mortality in persons receiving dialysis⁽¹¹⁰⁻¹¹³⁾.

According to the Renal Association (RA) Clinical Practice Guideline on Nutrition in CKD⁽¹¹⁴⁾ and the ISRNM criteria⁽⁷⁹⁾, BMI and percentage of unintentional dry weight loss (of $\geq 5\%$ in 3 months or $\geq 10\%$ in 6 months) have been considered as key anthropometric markers of malnutrition in persons receiving dialysis. Both anthropometric parameters share the advantages of being convenient, inexpensive and easy to calculate as weight, height and time can be automatically transferred to electronic medical records⁽¹¹⁵⁾; however,

Body mass index and percentage of dry weight loss can be confounded by the presence of fluid overload and they lack the ability to differentiate between muscle and fat mass. Furthermore, BMI does not distinguish between visceral and peripheral body fat accumulation and its interpretation can be influenced by factors such as age, gender and muscle mass⁽¹¹⁶⁾. Nonetheless, both BMI and unintentional weight loss have been strongly associated with an increased mortality risk in persons receiving dialysis^(117, 118).

Bioelectrical impedance analysis is a quick, safe, convenient and easy to perform technique that determines the resistance to the flow of an electrical current through the fat-free mass and the fat mass body compartments. In other words, BIA can directly measure and, therefore, differentiate between total body water (i.e. extra- and intracellular water), total body fat mass and lean body mass (i.e. muscle mass) according to their electrical conductivity properties⁽¹⁰⁹⁾. Another advantage of BIA over anthropometry is the estimation of the phase angle, which is a measure of nutritional health of muscle cell membranes and total cell integrity⁽¹⁰⁹⁾. Phase angle has been found to be an independent predictor of malnutrition as assessed by the SGA, muscle weakness (i.e. low handgrip strength [HGS]) and mortality in persons receiving dialysis⁽¹¹⁹⁻¹²¹⁾. One of the main limitations of using BIA is the need of expert and trained staff who can manage and interpret data appropriately⁽¹¹⁶⁾.

Additionally, fluid overload influences BIA measurement, leading to inaccurate estimations of muscle and fat mass; however, in order to improve the accuracy of the method, it has been suggested that all BIA measurements

should be performed post HD⁽¹²²⁾ or with the peritoneum drained of PD fluid⁽¹²³⁾.

Dual-energy x-ray absorptiometry scan is the most precise and reliable method that non-invasively measures the three major body compartments, namely fat mass, lean mass and bone mineral mass and density, based on the different x-ray properties of each body tissue⁽¹²⁴⁾. It is also less affected by overhydration compared with anthropometry and BIA, and its accuracy makes it a useful method for long-term monitoring of the nutritional status in persons receiving dialysis. Nevertheless, it is a costly technique in all aspects, from acquiring the DEXA scanner to maintenance of the equipment and conducting the procedure itself. It also requires a well-trained and specialised operator⁽¹²⁵⁾ and cannot be performed on the dialysis unit.

2. Biochemistry

According to the ISRNM expert panel, the main biochemical markers used for nutritional assessment in the dialysis population are serum albumin, serum prealbumin, serum cholesterol and serum creatinine⁽⁷⁹⁾.

For a very long time, serum albumin has been the most extensively studied marker of malnutrition in the dialysis population; hypoalbuminaemia and drops in serum albumin over time are strong and independent predictors of increased all-cause and cardiovascular mortality in persons receiving dialysis^(126, 127).

However, it is well-known that factors other than malnutrition may cause hypoalbuminaemia; systemic inflammation decreases hepatic albumin

synthesis⁽⁹⁶⁾ and low serum albumin levels are associated with fluid overload⁽¹²⁸⁾. Despite these drawbacks, some studies have reported improvements in serum albumin levels after following different dietetic interventions⁽¹²⁹⁾ and it has therefore been suggested to continue using serum albumin as a marker of malnutrition in persons on dialysis.

Serum prealbumin is another important nutritional marker as it significantly correlates with several other measures of the nutritional status including energy and protein intake, as well as body fat and muscle mass⁽¹³⁰⁾. Serum prealbumin levels are also decreased in the presence of systemic inflammation and it has been reported that both low prealbumin levels and a decrease of prealbumin over time are independently associated with an increased risk of death in persons on HD and PD^(131, 132).

Serum cholesterol is one of the most controversial biochemical parameters used for nutritional assessment in the dialysis population. While hypercholesterolemia is associated with increased cardiovascular mortality risk in the general population⁽¹³³⁾, it has the opposite association in persons receiving dialysis (i.e. better survival is observed with high cholesterol levels). It has been suggested that this paradoxical association could be explained by hypocholesterolaemia reflecting poor dietary intake and a greater degree of malnutrition and inflammation, which eventually leads to a higher mortality risk⁽⁴⁶⁾.

Finally, serum creatinine has been reported to be significantly associated with DEXA-measured lean body mass⁽¹³⁴⁾ and nPCR⁽¹³⁵⁾, and it has therefore been identified as a surrogate marker of skeletal muscle mass and dietary protein intake, respectively. Low serum creatinine levels have also been associated with increased risks of death in HD and PD populations^(136, 137). Nonetheless, serum creatinine is also affected by systemic inflammation⁽¹³⁵⁾ and its concentration depends on the level of residual renal function, dialysis dose and endogenous degradation⁽¹²⁵⁾. Thus, these factors should be taken into account when using serum creatinine for the assessment of the nutritional status.

3. Dietary intake

In the dialysis population, energy, macronutrient and micronutrient intake is generally assessed by means of 24-hour dietary recalls or food diaries conducted usually over 3 or 7 days. Additionally, dietary protein intake can be measured by estimating the nPCR⁽¹¹⁴⁾.

24-hour dietary recalls are a convenient and quick method of dietary intake assessment that involves a face to face or telephone interview conducted by an experienced dietitian who collects precise and comprehensive information regarding food and drink intake during a 24-hour period. In persons receiving HD, 24-h dietary recalls generally include information from a dialysis day, a non-dialysis day and a weekend day, while in persons performing PD, diet recalls obtain information from two weekdays and one weekend day. Interviewees are usually asked to estimate portion sizes in household measures and to describe all of the ingredients used for cooking, as well as the cooking

methods and type of food and drink (e.g. white or wholemeal bread, full fat or semi-skimmed milk) consumed. However, 24-hour dietary recalls rely on interviewee's memory, their cooperation and communication to provide accurate information, their estimation of portion sizes and their understanding of the questions⁽¹³⁸⁾.

Food diaries may be slightly more accurate and complete in obtaining dietary information than 24-hour dietary recalls because they can record data for a longer period of time and supplementary relevant diet information can be obtained once the food diary has been recorded and returned to the dietitian. Oral and written instructions on how to complete the 3 or 7-day food diary are usually provided by a trained dietitian. Written instructions normally include coloured pictures of portion sizes, as well as guidance on what information needs to be reported (i.e. type of food or drink, cooking methods, brand names, household measures, source of food or drink, amount of food or drink leftover). The main limitations of completing food diaries are the risk of incomplete or inaccurate description of food/drink intake and lack of compliance with the instructions⁽¹³⁸⁾.

Normalized protein catabolic rate, a measure of total body nitrogen balance, is a valid and clinically useful method of assessment of dietary protein intake. It is estimated by using equations that take into account the rate of change of blood urea nitrogen levels between two consecutive dialysis sessions in the case of persons on HD, and the 24-hour urea nitrogen content of urine and peritoneal dialysate plus the 24-hour change in total body urea nitrogen in the

case of persons performing PD. Nonetheless, there are some important limitations that have to be considered when assessing dietary protein intake using nPCR: 1) nPCR equations provide an accurate estimation of dietary protein intake only when individuals are in a steady state, which means that nPCR might overestimate or underestimate protein intake under catabolic or anabolic conditions, respectively; and 2) nPCR depends on daily protein intake and it can therefore change rapidly from day to day⁽¹²⁵⁾.

Notwithstanding the limitations of the methods of dietary intake assessment described above, it is important to highlight that both low energy intake (<25 kcal/kg/day) and low protein intake (<1.0 g/kg/day in HD and <0.73 g/kg/day in PD) estimated by either 24-hour dietary recalls, food diaries or nPCR are associated with increased overall mortality in the dialysis population^(71, 139-141)

4. Functional markers

Functional capacity, defined as the physical ability to perform activities of daily living, is markedly decreased in persons receiving dialysis and it can be both a cause and a consequence of malnutrition in this population⁽¹⁴²⁾.

Therefore, a lot of attention has been given to the use of several functional markers, such as the sit-to-stand test, gait speed and the physical component of the quality of life short-form SF-36 questionnaire, for the nutritional assessment of persons receiving dialysis. Some of these functional markers have been strongly associated with an increased risk of death and future hospitalizations in both the HD and PD populations^(143, 144); nevertheless, there is still insufficient evidence about the potential relevance for the

implementation of these physical functioning parameters in the routine clinical and dietetic practices, and they have also not yet been validated as markers of malnutrition in the dialysis population.

An alternative test is HGS, a simple, non-invasive, quick, reliable and validated method of nutritional and functional assessment in the dialysis population, which is basically a measure of muscle function and strength⁽¹⁴⁵⁾. In HD and PD populations, HGS has been shown to be significantly lower in persons with malnutrition as assessed by the SGA and its modified versions^(145, 146). In addition, it has been reported that HGS is significantly and positively associated with several nutritional markers, such as serum albumin, serum creatinine and lean body mass as assessed by DEXA, BIA and anthropometry^(146, 147). Furthermore, some studies have suggested that systemic inflammation does not affect HGS, which may be an important advantage over other inflammatory-dependent methods of nutritional assessment (i.e. biochemical variables and nPCR)⁽¹⁴⁶⁾. HGS has also been found to be a strong and an independent predictor of all-cause mortality in the dialysis population⁽¹⁴⁸⁾. More importantly, decreased muscle strength measured by HGS is more strongly associated with poor survival than low muscle mass in persons receiving dialysis⁽¹⁴⁹⁾. However, the main limitations of using HGS as a method of nutritional assessment are the lack of reference values in the dialysis population and the inconsistency regarding the techniques used for its measurement, for instance, position and time of measurement (e.g. standing or sitting position, before or after dialysis), as well as choice of arm (e.g. dominant or non-dominant, non-fistula arm)⁽¹⁴⁶⁾.

5. Nutritional scoring tools

Nutritional scoring tools use a combination of objective and subjective nutritional markers in order to minimize the limitations of the methods of nutritional assessment described above when used in isolation⁽⁷⁴⁾.

Over the last couple of decades, several nutritional scoring tools have been developed and implemented in different UK clinical care settings (i.e. primary care, home, acute or long-term care) for routine nutritional assessments. For instance, the Malnutrition Advisory Group of the British Association for Parenteral and Enteral Nutrition developed the malnutrition universal screening tool (MUST) in 2003, which uses BMI, history of weight loss and acute disease state to identify adults who are at risk of developing malnutrition or are already malnourished⁽¹⁵⁰⁾. Similar to the MUST, the Nutritional Risk Screening tool determines the risk of malnutrition by gathering information regarding BMI, unintentional weight loss and severity of disease state as a reflection of increased nutritional requirements⁽¹⁵¹⁾. The Mini-Nutritional Assessment is another nutritional scoring tool generally used for the nutritional assessment of the elderly population and it is administered in the form of a questionnaire that includes the following nutritional markers: anthropometric parameters such as BMI, history of weight loss and mid-arm and calf circumferences; lifestyle, medication, mobility and neuropsychological questions; dietary parameters (i.e. number of meals, food and fluid intake and autonomy of feeding); and a subjective assessment regarding self-perception of health and nutrition⁽¹⁵²⁾. In spite of the usefulness of the aforementioned nutritional scoring tools for

assessing the nutritional status, none of them have yet been validated in persons receiving dialysis.

In 1993, the original version of the SGA was recognized as a valid, useful and reliable nutritional scoring tool for the nutritional assessment in the dialysis population as it showed strong and significant associations with several markers of malnutrition, such as serum albumin, BIA-phase angle, MAMC, fat percentage and nPCR⁽¹⁵³⁾. The SGA consists of a comprehensive evaluation of the history of weight loss, changes in dietary intake, presence of gastrointestinal symptoms, functional capacity, disease state and its relation to nutritional requirements, and a physical examination that includes a subjective assessment of subcutaneous fat and muscle loss, and presence of oedema. According to the SGA results, the nutritional status can be classified into three categories: A=well-nourished, B=moderate malnutrition or at risk of malnutrition and C=severe malnutrition⁽¹⁵⁴⁾.

A modified version of the SGA, the 7-point scale, was proposed by the Canada-USA (CANUSA) Peritoneal Dialysis Study Group in 1996. It uses a 7-point Likert scale to rate the same core components of the original version of the SGA. The overall SGA score is rated subjectively based on the ratings of the six individual components: a rating of 6-7 indicates a well-nourished state, 3-5 represents mild to moderate malnutrition and 1-2 indicates severe malnutrition⁽¹⁵⁵⁾. The CANUSA Study was the first one to report an association between malnutrition as assessed by the 7-point scale SGA and an increased mortality and hospitalization risk. Several other studies have confirmed that the

7-point scale SGA is a strong predictor of mortality, frequent hospitalizations and poor quality of life^(66, 105, 156, 157).

Since the recognition of the 7-point scale SGA as a valid and reliable nutritional scoring tool for predicting clinical outcomes in persons receiving dialysis, a wide range of other nutritional scoring tools for the dialysis population have been emerged. For example, the renal modified SGA or better known as the Dialysis Malnutrition Score (DMS) is based on the seven original components of the SGA; however, it uses a different scoring system: each component is rated with a score from 1 (normal) to 5 (very severe impairment); therefore, the sum of all seven components ranges from 7-35. There are four overall ratings: scores of 7-10 represent persons with normal nutritional status; 11-20 denotes mild malnutrition; 21-28 moderate malnutrition; and 29-35 severe malnutrition⁽¹⁵⁸⁾. The Malnutrition-Inflammation Score (MIS) is another nutritional scoring tool that also takes into consideration the seven original components of the SGA plus BMI, serum albumin and total iron binding capacity; the 10 components are rated with a score from 0 (normal) to 3 (very severe) and the overall score can therefore range from 0 to 30; the higher the score the more severe the degree of malnutrition⁽¹⁵⁹⁾.

Although the DMS and the MIS have been shown to be associated with a number of nutritional markers, including BMI, MAMC, fat percentage, serum creatinine and serum albumin, as well as with increased mortality and hospitalizations rates and decreased quality of life^(67, 158-160), the 7-point scale SGA is still the nutritional scoring tool recommended by the National Kidney

Foundation (NKF) KDOQI Nutrition Guidelines⁽¹²⁵⁾ and the RA Clinical Practice Guideline on Nutrition in CKD⁽¹¹⁴⁾.

NUTRITIONAL INTERVENTIONS

The nutritional assessment should involve the use of multiple markers of malnutrition in order to provide a comprehensive nutritional interpretation that allows the renal dietitian to develop, implement and monitor an appropriate intervention that may lead to an improvement in nutritional status⁽¹⁶¹⁾.

Nutritional interventions should aim to meet energy and protein requirements for persons on dialysis (i.e. 30-35 kcal/kg/day and 1.0-1.2 g/kg/day, respectively)^(114, 162) in order to maintain body composition, prevent any further weight loss, especially lean body mass, or replenish already depleted protein and fat stores⁽⁸⁵⁾.

According to the RA Clinical Practice Guideline on Nutrition in CKD⁽¹¹⁴⁾, individualised nutritional counselling provided by an experienced renal dietitian remains the cornerstone component for the treatment of malnutrition in the dialysis population. Individualised nutritional counselling aims to improve dietary intake by the provision of a personalised dietetic advice based on estimated energy and protein requirements, as well as a restriction of potassium, phosphate, sodium and fluid intake, if needed⁽⁸⁵⁾. Examples of dietetic advice may include suggestions on how to decrease potassium, phosphate, sodium and fluid intake, improve blood sugar control for persons with diabetes, individualised meal plans and food fortification advice. A number of studies conducted in persons receiving HD and PD have reported

that personalised nutritional counselling not only improves several nutritional markers such as BMI, triceps and subscapular skinfold thicknesses, MAC, serum albumin, serum cholesterol and protein intake, but also prevents the worsening of malnutrition as assessed by the SGA and delays further decrease in lean body mass as assessed by BIA⁽¹⁶³⁻¹⁶⁵⁾.

The use of oral nutritional supplements (ONS) is the next suitable step for treating malnutrition if dietary intake is still below the estimated nutritional requirements for persons on dialysis and/or food intake cannot be further increased by individualised nutritional counselling alone⁽¹¹⁴⁾. Oral nutritional supplements come in a variety of presentations: liquids (juice or milkshake type), semi-solids (jelly, yogurt or smooth pudding style) and powders, which can be mixed with either water or milk, preferably full fat milk to increase energy density. The volume of ONS ranges from 125-350 ml or 30-40 ml in the case of high energy density shots. Oral nutritional supplements of 125 ml or 30 ml shots are usually the preferred choices for those persons on dialysis with fluid restrictions⁽¹⁶⁶⁾. In order to meet energy and protein requirements, ONS have been formulated to increase energy and protein intake by 7-10 kcal/kg/day and 0.3-0.4 g/kg/day, respectively⁽⁸⁵⁾.

Several randomised controlled clinical trials have reported that provision of ONS in the form of protein powders and standard, home-prepared or renal-specific formulas have a number of positive short- and long-term effects in persons receiving HD and PD⁽¹⁶⁷⁻¹⁷³⁾. For instance, the majority of the studies reported an improvement in serum albumin and nPCR^(167-169, 171-173); some

studies showed a significant increase in total energy and protein intake^(167, 168, 170, 173) and a few observed improvements in BMI^(167, 169), SGA score^(168, 170), serum prealbumin⁽¹⁶⁹⁾, body weight and skinfold measurements⁽¹⁶⁷⁾, quality of life⁽¹⁷⁰⁾ and functional capacity⁽¹⁷¹⁾.

Finally, the use of enteral nutritional feeding is suggested when dietary intake is still inadequate after following dietary advice and using ONS, while the use of intradialytic parenteral nutrition or intraperitoneal amino acids may be recommended if enteral nutrition is not tolerated or is clinically inappropriate⁽¹¹⁴⁾.

In summary, malnutrition is one of the most important and prevalent complications in the dialysis population. Multiple risk factors are associated with the development of malnutrition and systemic inflammation, oxidative stress and cardiovascular disease play a major role. As a result, the presence of malnutrition is associated with a number of significant adverse consequences, including increased number of hospitalizations, higher rates of infections, poor survival and decreased quality of life. Therefore, nutritional assessment should be comprehensive enough and include a combination of different methods of nutritional evaluation that allow renal dietitians to identify those persons on dialysis at nutritional risk or already malnourished, and then plan, implement and monitor an appropriate individualised nutritional intervention that may either prevent the development of malnutrition or improve the nutritional status. Consequently, prevention of malnutrition and/or improvement in several nutritional markers after following the nutritional intervention (e.g.

personalised dietary counselling alone or use of ONS) may improve clinical outcomes (i.e. reduced healthcare costs, hospitalizations and mortality rates and increased quality of life). Therefore, further studies are critical to test the clinical and economic impact of nutritional interventions in the dialysis population.

1.4.3 Oxidative stress: a cardiovascular and malnutrition risk factor in the dialysis population

Oxidative stress can be defined as cellular, tissue and/or organ dysfunction that results from the disturbance in the balance between excessive generation and/or insufficient removal of highly oxidant compounds such as reactive oxygen species (ROS) and insufficient antioxidant defence mechanisms^(97, 174).

Free radicals, better known as ROS, are constantly formed in our bodies under normal conditions and several cells involved in tissue repair processes such as macrophages, platelets, fibroblasts and endothelial cells use free radicals at low concentrations to improve wound healing and infection control at the wound site⁽¹⁷⁵⁾. However, under pathological conditions, such as uraemia, there is an inappropriate activation of ROS production and subsequent tissue damage.

Several mechanisms of ROS production have been identified, with the mitochondrial respiratory chain and the activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system being the most powerful sources of oxidants in the body. Superoxide anion, which is immediately converted into hydrogen peroxide (i.e. a highly unstable free radical), has been considered the most important ROS generated through NADPH activation because it will act, along with hydrogen peroxide, as the precursor of more powerful oxidants that will eventually be implicated in cell membrane lipid degradation, protein aggregation and DNA damage⁽¹⁷⁶⁾.

Free radicals are inactivated by several antioxidant systems that prevent tissue damage from ROS attack. Antioxidant systems can be classified into the following:

- **Endogenous antioxidant system**

This system includes the antioxidant enzymes superoxide dismutase, which converts superoxide anion to hydrogen peroxide, and glutathione peroxidase, which removes hydrogen peroxide formed from superoxide anion⁽¹⁷⁶⁾.

- **Exogenous antioxidant system**

This system comprises fat-soluble vitamins such as A and E, which are responsible of protecting cell membranes from lipid peroxidation, as well as vitamin C, which inactivates free radicals that are generated in the blood and cytosol⁽¹⁷⁶⁾. Additionally, serum albumin has been considered one of the most important antioxidants due to its ligand binding and ROS-trapping properties. For example, serum albumin prevents the synthesis of the highly toxic hydroxyl radical by binding to free copper and iron ions and, consequently, limiting their interaction with hydrogen peroxide. Also, serum albumin has the ability to scavenge hypochlorous acid (HOCl), a strong oxidant compound, preventing further tissue damage⁽¹⁷⁷⁾.

Direct measurement of ROS is quite challenging because of their high reactivity, low blood concentrations and short half-lives; therefore, more stable molecules (e.g. proteins, lipids and nucleic acids) that interact with and are modified by ROS have been used to reflect systemic oxidative stress⁽¹⁷⁸⁾. The

most common stable oxidized molecules, better known as biomarkers of oxidative stress, are as follows:

- **Lipids:** isoprostanes (e.g. F2-isoprostane), malondialdehyde, oxidized LDL and HOCl-LDL are the most well-known biomarkers for lipid peroxidation⁽¹⁷⁸⁾.
- DNA oxidation, 8-oxodG and RNA oxidation are the most important biomarkers of nucleic acid oxidation⁽¹⁷⁴⁾.
- **Proteins:** 3-nitrotyrosine, 3-chlorotyrosine, dityrosine and protein thiols are some examples of biomarkers for protein oxidation⁽¹⁷⁴⁾. Nevertheless, the so-called advanced oxidation protein products (AOPPs), which share similar characteristics with AGEs, have been identified in persons on dialysis, and the strong association between AOPPs and AGEs contributed to the development of the term “carbonyl stress”, which is the process of formation of AGEs through an interaction between oxidation and glycation⁽¹⁷⁹⁾.

There is evidence that suggests that biomarkers of lipid peroxidation, protein oxidation and carbonyl stress are significantly increased in persons on dialysis, and that systemic inflammation contributes significantly to this pro-oxidant state mainly by activating the NADPH oxidase system^(180, 181). Besides systemic inflammation, several other uraemia- and dialysis-related factors have been implicated in the development of oxidative stress in persons receiving dialysis, for instance, increased synthesis of superoxide anion by polymorphonuclear leukocytes, bio-incompatibility of dialysis membranes,

increased NADPH oxidase activity due to endotoxemia, homocysteinemia which induces a pro-oxidant state by interacting with hydrogen peroxide, decreased clearance of oxidants, impairment in the endogenous enzyme antioxidant system (i.e. superoxide dismutase and glutathione peroxidase), decreased vitamin E content in erythrocytes and mononuclear cells and nutrient dialysis losses, especially albumin, vitamin C, zinc and selenium⁽¹⁸¹⁾.

In the dialysis population, elevated mediators of oxidative stress and pro-inflammatory cytokines play a synergistic role in the development of malnutrition and cardiovascular disease. This association will be described in the following section.

1.4.4 Systemic inflammation, oxidative stress, malnutrition and cardiovascular disease: a vicious cycle

For several years now, systemic inflammation has been recognised as a persistent and chronic complication in the dialysis population. Several reports have indicated that both persons with CKD and those undergoing HD and/or PD treatment have high levels of pro-inflammatory cytokines and CRP^(96, 182). As described in previous sections, several factors contribute to the development of systemic inflammation such as increased production and decreased clearance of pro-inflammatory cytokines, uremic milieu, oxidative stress, frequent infections and thrombotic events, presence of comorbidities, altered intestinal microbiota and the dialysis procedure itself⁽⁴⁸⁾. Chronic inflammation induces a number of mechanisms related to increased muscle proteolysis, decreased appetite and hypoalbuminemia, which ultimately lead to the development of malnutrition⁽⁶⁵⁾. It has been well-documented that elevated CRP, IL-6 and TNF- α are strong predictors of overall and cardiovascular mortality in the dialysis population^(96, 182). This association may be explained by the fact that increased levels of circulating pro-inflammatory cytokines and CRP promote LVH, endothelial dysfunction and vascular calcification, leading to the development of atherosclerosis⁽¹⁸²⁾.

Persons on dialysis are at a higher risk of accumulating several mediators of oxidative stress than the general population mainly due to increased production, decreased excretion and impaired antioxidant system^(96, 181).

Systemic inflammation causes oxidative stress and vice versa: superoxide anion increases the expression and activity of pro-inflammatory cytokines which, consequently, stimulate NADPH oxidase activity resulting in a further overproduction of ROS. Oxidative and inflammatory processes have synergistic effects on increasing protein catabolism, resting energy expenditure and loss of lean body mass, and decreasing hepatic production of pre-albumin and albumin; this interaction may therefore worsen the nutritional status of persons receiving dialysis⁽⁹⁶⁾. Oxidative stress, alongside inflammation, causes endothelial dysfunction which accelerates the progression of atherosclerosis; hence, this link may further contribute to the high all-cause and cardiovascular mortality risk observed in the dialysis population^(72, 73, 96, 97).

Malnutrition, an independent and strong predictor of all-cause and cardiovascular mortality in the dialysis population^(66, 68, 103, 104), exacerbates the deleterious atherogenic effects of systemic inflammation and oxidative stress by increasing the susceptibility to infections and cardiovascular disease risk^(101, 183). Cardiovascular disease itself worsens nutritional status by increasing inflammatory and oxidative stress biomarkers, which consequently promote further muscle wasting⁽⁹⁶⁾. It therefore seems reasonable to suggest that a vicious cycle between chronic inflammation, oxidative stress, malnutrition and cardiovascular disease may explain in part the high rates of overall and cardiovascular mortality observed in the dialysis population⁽¹⁰²⁾ (Figure 1.4.1).

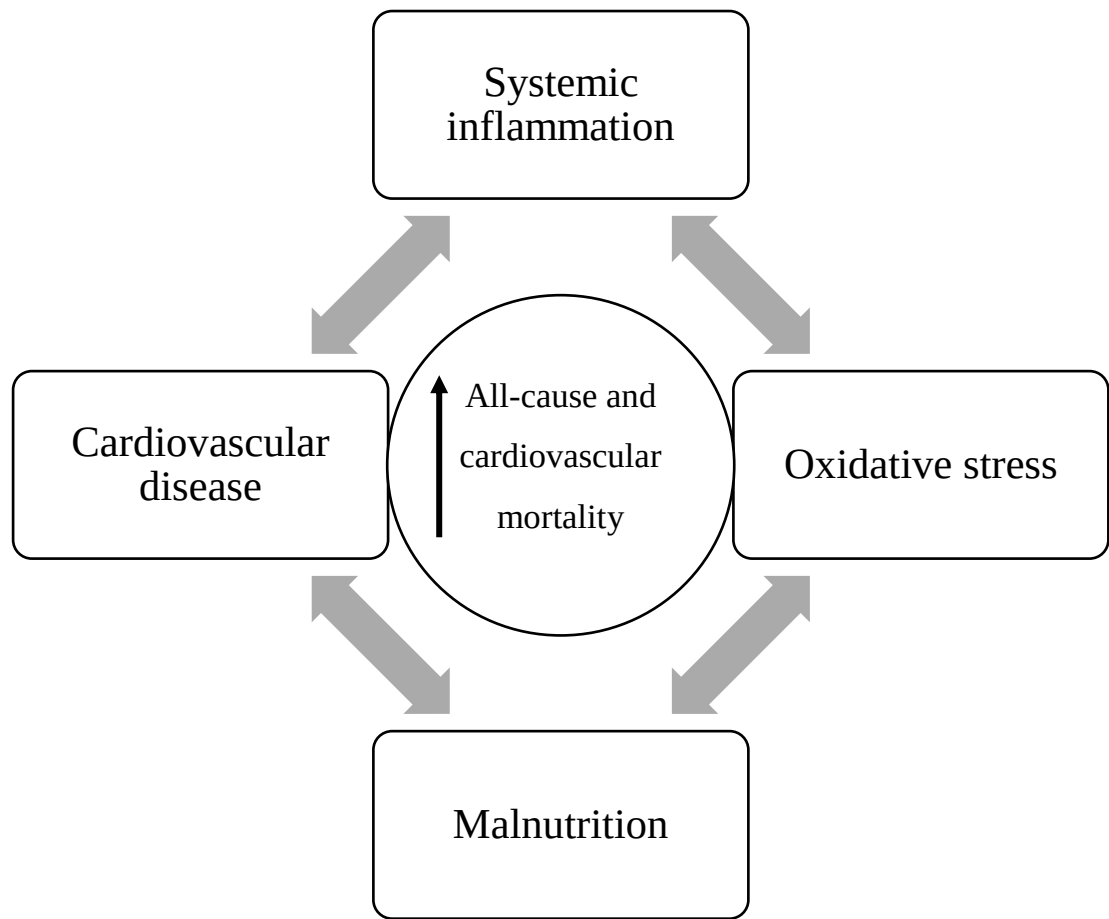


Figure 1.4.1 The vicious cycle of chronic inflammation, oxidative stress, malnutrition and cardiovascular disease and its association with overall and cardiovascular mortality in persons receiving dialysis

Adapted from: Stenvinkel P, Heimbürger O, Lindholm B, Kaysen GA, Bergström J. Are there two types of malnutrition in chronic renal failure? Evidence for relationships between malnutrition, inflammation and atherosclerosis (MIA syndrome). *Nephrol Dial Transplant*. 2000;15(7):953-960.

1.5 Advanced glycation end-products: definition, formation and measurement

Advanced glycation end-products are a diverse group of highly oxidant and heterogeneous substances that have been linked with diabetes, cardiovascular disease and other chronic conditions⁽¹⁸⁴⁾. Moreover, AGEs are uremic toxins that are markedly increased in persons on dialysis, due to increased production, impaired excretion and inefficient removal⁽¹⁸⁵⁾.

Formation of AGEs starts with a non-enzymatic and reversible reaction between reducing sugars (e.g. glucose molecules) and free amino groups of proteins, lipids and nucleic acids called the Maillard reaction. However, AGEs are also formed more rapidly during oxidative stress with the subsequent formation of reactive carbonyl compounds (i.e. carbonyl stress). At this point, AGE synthesis is irreversible and they will eventually cross-link with tissue proteins, causing a direct alteration of protein structure and function. It seems that collagen in the skin and vascular basement membranes are especially susceptible to AGE accumulation and subsequent adverse effects (e.g. increased vascular stiffness and endothelial dysfunction). AGEs also interact with specific AGE receptors that lead to the activation of systemic inflammation by increasing the release of pro-inflammatory cytokines and, consequently, exacerbate tissue damage⁽¹⁸⁵⁾.

Besides increased oxidative and carbonyl stress, several factors have been identified as contributors to the increased endogenous formation of AGEs in persons receiving dialysis, which include the following: hyperglycaemia, interaction of blood with the dialysis membrane and high glucose exposure from PD fluid⁽¹⁸⁴⁾. Furthermore, GDPs formed during manufacturing process of PD solutions (e.g. heat sterilization and storage) enhance the formation of AGEs in the peritoneum resulting in peritoneal membrane fibrosis and additional poorly understood systemic effects⁽¹⁸⁶⁾.

In addition to the endogenous formation of AGEs, these uremic toxins can also be formed exogenously, either by cigarette smoking or through food consumption⁽¹⁸⁴⁾. It has been reported that glycotoxins found in tobacco extracts and tobacco smoke are able to interact rapidly with amino groups of proteins and thus enhance the formation of AGEs⁽¹⁸⁷⁾.

Additionally, AGEs exist naturally in uncooked foods. The content of AGEs is higher in foods rich in proteins and fats. According to the classification of foods into groups by the American Diabetes Association, it has been reported that items included in the fat group such as butter, processed cream cheese, margarine and mayonnaise had the highest amount of AGEs, followed by oils and nuts. In addition, meat and meat-substitute food groups have shown high AGE levels, with bacon, full fat cheeses, red meats and poultry being the items with the highest content of AGEs. High fibre and carbohydrate foods (e.g. grains, legumes, breads and starchy vegetables) contain relatively low amounts

of AGEs. Among this group, the lowest values of AGEs have been detected in skimmed milk, fruits and vegetables⁽¹⁸⁸⁾.

In order to increase safety, convenience, flavour, colour and appearance, a wide variety of foods are exposed to processing techniques including dry-heat cooking methods at high temperatures⁽¹⁸⁹⁾. This cooking or thermal food processing promotes the formation of new AGEs, even in carbohydrate foods that show the lowest AGE content compared to other food groups. A clear example of this is the processing of commercially prepared breakfast cereals and snacks (e.g. toasted frozen waffles, Rice Krispies and biscotti), which contain 600-1,000 kilounits (kU) AGE/serving, while natural toasted bread contains only 30 kU AGE/serving⁽¹⁸⁸⁾. Cooking methods and temperature play a critical role in the formation of AGEs. Grilling, roasting, broiling, baking and frying are some of the cooking techniques that significantly increase the content of AGEs in food. For instance, a serving of chicken breast boiled for 1 hour has 1,000 kU of AGEs, while the same item broiled at 230°C for 15 minutes yields 5,250 kU of AGEs^(188, 189). Therefore, high fat and high protein diets, dry-heated processed foods and cooking techniques using dry heat and high temperatures significantly increase AGE formation.

It has been previously reported that about 10% of ingested dietary AGEs will be intestinally absorbed⁽¹⁹⁰⁾, of which only one third will be excreted by the kidneys or through faeces, while the remaining two thirds will eventually accumulate in tissue proteins⁽¹⁹¹⁾. It has also been suggested that intestinally absorbed dietary AGEs appear to be directly and significantly associated with

circulating AGE levels and several other markers of inflammation and oxidative stress in persons with diabetes and in those undergoing dialysis treatment^(191, 192).

Even though most of the AGEs have not yet been characterised, methylglyoxal derivatives, N-carboxymethyl-lysine (CML) and pentosidine are some that have been well described. Methylglyoxal is a low molecular weight carbonyl compound that reacts with free amino groups of lysine and arginine and with thiol groups of cysteine forming AGEs. CML is a non-fluorescent AGE formed by direct addition of glyoxal to lysine and it is the most prevalent AGE found in foods, while pentosidine, an arginine and a lysine residue crosslinked to a pentose, is a fluorescent AGE that forms protein-protein crosslinks⁽¹⁹³⁾.

Several analytical techniques to assess tissue AGE accumulation have been developed. The most accurate but very time consuming method for measuring AGEs is high-performance liquid chromatography. Gas or liquid chromatography/mass spectrometry, tandem mass spectrometry and liquid chromatography/tandem mass spectrometry are also accurate methods for determining AGE levels, particularly CML; however, they are time consuming and expensive. Immunoassay techniques such as the enzyme-linked immunosorbent assay using, for instance, anti-CML monoclonal antibody, are simpler to conduct, lower in cost and have been widely used for diabetes research purposes. Nonetheless, they may lack specificity, have low reproducibility, AGEs may form during sample processing due to high

temperature use, and blood and urine samples do not always reflect tissue AGE levels⁽¹⁹⁴⁾.

Taking advantage of the fluorescent properties of some AGEs, these molecules can also be determined by using techniques that measure tissue autofluorescence. One of these techniques is the well-known collagen-linked fluorescent method (CLF). The fluorescence of collagen is determined in skin biopsy material at excitation/emission of 370/440 nm (i.e. long wavelength fluorescence) providing an accurate measurement of tissue AGE accumulation; however, this is an invasive, complex and expensive procedure⁽¹⁹⁴⁾. In 2004, Meerwaldt et al.⁽¹⁹⁵⁾ developed a non-invasive, operator independent, quick (less than 5 minutes) and easy to perform technique to measure SAF that uses the fluorescent properties of some AGEs, like the extensively used CLF method. SAF is measured non-invasively in the forearm using an instrument called the Autofluorescence Reader (AGE Reader). SAF is estimated as the ratio between emission and excitation, expressed as arbitrary units (AU), by dividing the average reflected light intensity (i.e. emission) between 420 and 600 nm by the amount of excitation light between 300 and 420 nm. It has been reported in validation studies that the emission spectrum of 420-600 nm strongly correlates with skin accumulation of AGEs assessed on skin biopsy⁽¹⁹⁴⁾.

Although SAF has advantages over other techniques for measuring tissue accumulation of AGEs, it has some limitations: first, it cannot detect non-fluorescent AGEs, such as CML; secondly, it cannot determine concentrations

of individual AGEs because fluorescence provides information of group reactivity. Nevertheless, SAF measurements have been validated in persons with diabetes and in those undergoing HD treatment with specific circulating AGEs, including pentosidine and CML, and with CLF in skin biopsies^(195, 196). Another disadvantage of SAF is that skin colour and pigmentation affect SAF measurements. Due to the high absorption of the excited light in persons with very dark skin, SAF might not be reliable in this population⁽¹⁹⁷⁾; however, recent studies in non-Caucasian populations (e.g. Japanese) have shown a strong and independent association between SAF and cardiovascular morbidity and mortality⁽¹⁹⁸⁻²⁰⁰⁾. In addition, the AGE reader version 2.4.3 has been improved in order to allow measurements in darker skin; nonetheless, measurements are still not possible in persons with skin reflectivity below 6% (i.e. approximately Fitzpatrick type V-VI skin colour, the darkest skin colour type)⁽²⁰¹⁾. Finally, fluorescent chemical compounds other than fluorescent AGEs (e.g. NADPH) might also be detected by the AGE reader and therefore interfere with the measurement of skin accumulation of AGEs⁽¹⁹⁵⁾; nevertheless, several studies have repeatedly confirmed the strong association between SAF and cardiovascular morbidity as well as all-cause and cardiovascular mortality in persons on dialysis, which will be discussed in the following section.

1.6 Advanced glycation end-product accumulation and adverse outcomes in persons receiving dialysis

The relationship between increased levels of tissue AGE accumulation assessed by SAF and adverse outcomes such as cardiovascular morbidity and overall and cardiovascular mortality in the dialysis population has been investigated by several researchers in cross-sectional and prospective studies.

Several factors have been associated with higher SAF values in persons on dialysis in cross-sectional studies, for instance, presence of diabetes and cardiovascular disease, chronological age and dialysis vintage in both dialysis modalities^(198, 199, 202) and glucose exposure from PD dialysis fluid in persons performing PD^(199, 203). The type of PD solution may also be a factor that contributes to higher SAF. It has been reported that persons performing PD who use lower GDP dialysis fluid have significantly lower SAF levels than those who use standard glucose PD solutions in univariable but not in multivariable analysis; however, lower GDP dialysis fluid users had shorter dialysis vintage and the majority were non-smokers, which may also explain this finding⁽²⁰⁴⁾.

In a cross-sectional study conducted by Oleniuc et al.⁽²⁰²⁾, it was found that participants on dialysis ($n= 157$ HD and 102 PD) had higher SAF levels than those in the non-dialysis CKD group ($n=32$) and that the lowest SAF values were observed in the group with diabetes but without CKD ($n=19$), which may

reflect better renal clearance of AGEs in the non-dialysis CKD and non-CKD diabetes groups. Furthermore, SAF has been shown to be an independent predictor of aortic stiffness, enhanced pulse wave reflection (which is associated with LVH), systemic inflammation measured by CRP and endothelial dysfunction in persons receiving dialysis⁽²⁰⁵⁻²⁰⁷⁾.

It has been reported that persons on both dialysis modalities have similar SAF levels, irrespective of the diabetes status⁽²⁰³⁾. However, Jiang et al.⁽¹⁹⁹⁾ showed that SAF was significantly higher in PD ($n=613$) compared to HD participants ($n=1775$) in each age group and dialysis vintage category, regardless of the presence or absence of diabetes, which might be related to increased historical glucose exposure in PD participants that may lead to a greater accumulation of AGEs. In addition, glucose exposure dose from PD dialysis fluid and SAF levels were the strongest cardiovascular risk factors in PD participants even after adjustment by age, gender, smoking and other traditional and CKD-related risk factors, suggesting that SAF is independently associated with the development of cardiovascular disease in persons on PD⁽¹⁹⁹⁾.

The majority of the prospective observational studies investigating the association between SAF, cardiovascular morbidity and overall and cardiovascular mortality have been conducted in the HD population. One of the first studies carried out in this area was the study of Meerwaldt et al.⁽¹⁹⁶⁾ in which 109 HD participants were followed-up for 3 years. The investigators observed that SAF was independently associated with the presence of cardiovascular disease at baseline (OR 8.8 [95%CI 2.8 to 28.1]), it was an

independent and strong predictor of all-cause mortality (HR 3.9 [95%CI 1.9 to 8.1]), with cardiovascular disease being the main cause of death, and it was a better independent predictor of mortality than age, presence of diabetes, duration of CKD and serum triglyceride levels, LDL cholesterol and PTH.

Furuya et al.⁽²⁰⁸⁾ after following-up 64 HD participants for 36 months found that those who developed cardiovascular disease had significantly higher SAF values (3.88 ± 0.18 vs. 3.19 ± 0.13 AU; $p < 0.01$) and that increased SAF levels were independently associated with presence of cardiovascular disease after adjusting for confounders (OR 2.96 [95%CI 1.26–8.16]). Observational studies with longer follow-up periods (i.e. 5-6 years) but relatively small sample sizes ($n=105-128$ participants) have also reported that SAF is an independent and strong predictor of overall⁽²⁰⁹⁾ and cardiovascular mortality⁽²⁰⁰⁾. Nongnuch and Davenport⁽²¹⁰⁾ followed-up 332 HD patients for 30 months and observed that increased SAF was an independent risk factor for overall mortality (HR 2.7 [95%CI 1.40-5.22], with cardiovascular disease being the main cause of death.

Only one study has been conducted in 198 PD participants, in which after 3 years of follow-up the researchers reported that pre-existing cardiovascular disease, icodextrin use, leukocyte count, diastolic blood pressure and total cholesterol were risk factors for high SAF levels and that increased SAF predicted higher rates of mortality regardless of cardiovascular disease status⁽²¹¹⁾. Siriopol et al.⁽²¹²⁾ carried out a 30 month-observational study in 202 HD and 102 PD participants. The investigators found an association between increased SAF levels and elevated risk of all-cause mortality and sepsis-related

mortality in PD but not in HD participants. In contrast to other studies, there was no association with cardiovascular disease or cardiovascular mortality.

Another prospective study conducted in 130 non-dialysed CKD stage 5, 93 PD and 38 HD participants for 25 months found that presence of cardiovascular disease, higher Framingham cardiovascular disease risk score, haemoglobin and body fat mass index, and lower HGS were independent determinants of higher SAF. Furthermore, one standard deviation (SD) increase in SAF was independently associated with overall mortality, after adjusting for the Framingham risk score⁽²¹³⁾.

Arsov et al.⁽²¹⁴⁾ have been the first researchers that have assessed the 1 year-increase in SAF in persons on HD ($n=169$) and its association with overall and cardiovascular mortality. As shown in some of the studies described above^(196, 200, 212), diabetic HD participants had elevated SAF values at baseline as compared with non-diabetic participants; after 1 year, SAF levels increased significantly from 3.24 ± 0.87 AU to 3.39 ± 0.76 AU (mean Δ SAF: 0.16 ± 0.06 ; $p=0.03$). The 1-year increase in SAF showed a positive correlation with dialysis vintage. Multivariable Cox regression analysis showed that 1-year increase in SAF was an independent predictor of overall (HR 2.52 [95CI 1.35–4.71]) and cardiovascular mortality (HR 3.01 [95%CI 1.47–6.17]); furthermore, HD participants that had higher 1-year Δ SAF than the median value (i.e. 3.39 ± 0.76 AU) had higher overall mortality compared with the HD participants with lower 1-year Δ SAF than the median value.

On the other hand, factors that contribute to the increase in SAF over time in the dialysis population have not been adequately investigated. One prospective study has reported that dialysis vintage, lower dietary AGE intake and lower calorie intake were associated with 1-year increase in SAF in univariable analysis in a HD population ($n=156$), but that a BMI lower than or higher than 24 kg/m^2 was the sole independent predictor for this observed change⁽²¹⁵⁾. Another 1-year observational study conducted in 180 HD participants found in multivariable analysis that older age and use of insulin, lanthanum carbonate and warfarin were independent predictors of the 1-year increase in SAF, while a vegetarian diet and residual urine output $>250 \text{ ml/day}$ were significantly and independently associated with a lower or stable SAF level⁽²¹⁶⁾.

It is important to highlight that nutritional status has not been previously assessed in any of the aforementioned studies that have investigated the association between higher SAF and mortality in the dialysis population. It would seem, therefore, that further investigations regarding the association between SAF, dietary AGE intake and nutritional markers, factors associated with changes in SAF levels over a period of time, and the association of SAF and malnutrition with adverse outcomes in both dialysis modalities are needed in order to better understand the association of skin AGE accumulation with the high morbidity and mortality rates experienced by persons on dialysis.

1.7 Interventions for reducing advanced glycation end-product accumulation in the dialysis population: focus on diet

As described in previous sections, increased tissue accumulation of AGEs assessed by SAF, has been increasingly recognised as an important factor in the pathogenesis of cardiovascular morbidity and mortality in persons receiving dialysis. This close association between increased skin accumulation of AGEs, morbidity and mortality needs to be assessed by interventions designed to reduce SAF levels.

In the past several years, some conventional drugs for the treatment of persons with CKD and on dialysis have been assessed for their effectiveness in reducing AGEs. For example, the widely used antihypertensive medications angiotensin II type 1 receptor antagonists and angiotensin-converting enzyme inhibitors (ACE-I), prevent *in vitro* formation of pentosidine and CML⁽²¹⁷⁾. Azelnidipine, a calcium channel blocker and blood pressure lowering drug, decreased serum levels of AGEs in non-diabetic and hypertensive participants with CKD stages 1-2⁽²¹⁸⁾. In a study by Vlassara et al.⁽²¹⁹⁾, the non-calcium phosphate binder sevelamer carbonate was shown to be effective in reducing serum levels of CML, methylglyoxal and 8-isoprostane (i.e. oxidative stress markers) in diabetic participants with CKD stages 3-4 and to reversibly bind AGE-modified bovine serum albumin *in vitro* at intestinal, but not stomach, pH.

Other non-conventional drugs have also been tested to prevent AGE accumulation. One of these drugs is the relatively novel synthetic AGE-breaker TRC4186, which splits the preformed AGE protein cross-links in tissues. It has been reported that this AGE-breaker has the ability to delay the progression of diabetes-related complications, to preserve cardiac function and to decrease the severity of renal failure in animal models through its AGE-breaking activity; moreover, this drug has proved to be safe and well-tolerated in healthy subjects⁽²²⁰⁾. Pyridoxamine (a form of vitamin B₆), a known AGE inhibitor, has been shown to prevent the formation and skin collagen accumulation of CML and pentosidine in a hyperlipidaemic, non-diabetic and obese animal model⁽²²¹⁾. It seems that these conventional and non-conventional drugs are promising treatment options that could be used for the reduction of serum levels of AGEs and, possibly, for the prevention and decrease of skin accumulation of these uremic toxins.

Since excessive levels of AGEs are associated with many metabolic disturbances such as insulin resistance, endothelial dysfunction, oxidative stress and systemic inflammation, which are also associated with adverse outcomes⁽¹⁹¹⁾, it has been suggested that restriction of dietary AGE intake by decreasing consumption of highly processed foods and by using high water content cooking techniques that avoid very high temperatures, such as poaching, steaming, stewing and boiling, could possibly be a promising future therapeutic option for reducing circulating AGE levels and SAF⁽²²²⁾.

Animal studies have shown that low AGE diets prevent the development of atherosclerosis, diabetic nephropathy and insulin resistance⁽²²³⁻²²⁵⁾. Some randomised controlled clinical trials in human subjects have investigated the effectiveness of a restricted AGE diet on circulating AGE levels and markers of systemic inflammation, cardiovascular disease and oxidative stress.

Nine studies have been conducted in healthy populations. In a study by Semba et al.⁽²²⁶⁾, 24 healthy middle-aged to older adults were randomly allocated to a high AGE diet group ($n=12$) or to a low AGE diet group ($n=12$) and followed-up for 6 weeks. At the end of the dietary intervention, serum and urinary CML significantly decreased only in the low AGE diet group; there were no significant differences in the high AGE diet group or between groups in inflammatory markers (IL-6, CRP and TNF- α receptors I and II), vascular adhesion molecule-1 (VCAM-1), a marker of endothelial dysfunction, or serum and urinary CML.

Uribarri et al.⁽²²⁷⁾ randomly assigned 18 healthy participants to a high AGE or low AGE diet. After 4 months of follow-up, serum AGEs (i.e. CML and methylglyoxal), TNF- α and 8-isoprostane were significantly lower in the low AGE diet group compared to the high AGE diet group and serum AGEs and 8-isoprostane significantly decreased in the low AGE diet group between baseline and the end of the study. Similar results were observed in 30 healthy participants where, after 4 months, the low AGE dietary intervention showed lower values of serum CML, methylglyoxal, 8-isoprostane, TNF- α and VCAM-1 in comparison with baseline and high AGE diet group values⁽²²⁸⁾.

In a group of overweight adults over the age of 60, the participants allocated to a low AGE diet ($n=8$) significantly decreased serum AGEs, 8-isoprostane, VCAM-1 and TNF- α after 4 months of follow-up in comparison with participants in the usual diet group (high AGE content; $n=10$)⁽²²⁹⁾. Mark et al.⁽²³⁰⁾ after following-up 73 overweight women for 4 weeks found that urinary CML and methylglyoxal concentrations were significantly lower in the low AGE diet group ($n=37$) in comparison with their baseline and high AGE diet group ($n=36$) values. Macías-Cervantes et al.⁽²³¹⁾ randomly assigned 43 overweight or obese men to a low AGE diet ($n=14$), exercise + habitual diet (high in AGEs; $n=14$) or low AGE diet + exercise ($n=15$) and followed them up for 12 weeks. At the end of the study, serum CML and methylglyoxal levels were significantly decreased in the low AGE diet and low AGE diet + exercise groups compared to their baseline levels, while no significant changes were observed in serum AGEs in the exercise group + habitual diet.

Harcourt et al.⁽²³²⁾ conducted a cross-over randomised clinical trial involving 2 weeks each on a low and a high AGE-containing diet in 11 overweight and obese male adults. Intergroup comparisons showed that plasma CML was significantly decreased in the high AGE diet group, whereas urinary CML and 8-isoprostane and the inflammatory marker monocyte chemoattractant protein-1 (MCP-1) plasma concentrations were significantly increased following high AGE consumption. Another cross-over randomised trial conducted in 20 overweight adults who followed a low and a high AGE diets alternately for 2 weeks found no significant intragroup or intergroup differences in serum CML, N-carboxyethyl-lysine (CEL) and methylglyoxal; however, urinary CEL was

significantly decreased in the low AGE diet group compared to the high AGE diet group⁽²³³⁾. Baye et al.⁽²³⁴⁾ also conducted a cross-over randomised trial in which 20 overweight or obese adults followed a low AGE diet or a high AGE diet alternately for 2 weeks. Intragroup and intergroup comparisons showed no significant differences in any of the assessed inflammatory markers (i.e. CRP, IL-6, TNF- α , and MCP-1).

In persons with pre-diabetes, diabetes and the metabolic syndrome, six randomised clinical trials have investigated the effect of diet on circulating AGEs and markers of inflammation, cardiovascular disease and oxidative stress. In a 24-week randomised controlled clinical trial, 57 pre-diabetic adults were allocated to a low AGE diet ($n=29$) or to a standard diet but with high AGE content ($n=28$). At the end of the follow-up period, the low AGE diet group showed a significant reduction in CRP and carotid intima-media thickness in comparison with their baseline values. No significant changes were observed in the standard diet group⁽²³⁵⁾. Vlassara et al.⁽²³⁶⁾ conducted a 1-year randomised clinical trial in obese persons with the metabolic syndrome who were allocated to a regular/high AGE diet ($n=49$) or to a low AGE diet ($n=51$). Serum, intracellular and urinary CML and methylglyoxal, as well as 8-isoprostane, TNF- α , VCAM-1 and the receptor of AGEs (RAGE) were significantly decreased in the low AGE diet group, while the high AGE diet group showed significant increases in serum, intracellular and urinary AGEs, 8-isoprostane and RAGE.

Cai et al.⁽²³⁷⁾ allocated type 1 and 2 diabetic participants to a low AGE diet group ($n=11$) or to a high AGE diet group ($n=13$) and found that after 6 weeks of follow-up the high AGE diet group showed significantly higher values of oxidized-LDL and serum CML compared to baseline and low AGE diet group. In a study by Vlassara et al.⁽²³⁸⁾, 24 diabetic participants (11 in a 2-week crossover and 13 in a 6-week study) were randomly assigned to either a high AGE or low AGE diet. Intra and intergroup comparisons in the 2-week crossover trial showed that serum AGEs and VCAM-1 significantly increased in the high AGE diet group and significantly decreased in the low AGE diet group. Results of the 6-week study reported a significant reduction of TNF- α , CRP, VCAM-1 and serum AGEs in the low AGE diet group ($n=6$) compared to baseline and high AGE diet group ($n=7$) levels.

Uribarri et al.⁽²²⁷⁾ randomly assigned 18 diabetic participants to a high AGE ($n=12$) or low AGE diet ($n=6$). Similar results were observed as in the study with healthy participants⁽²²⁷⁾ (see above). Luévano-Contreras et al.⁽²³⁹⁾ conducted a 6-week randomised prospective study in 26 type 2 diabetic subjects equally allocated to a high AGE or low AGE diet. Significant reductions were observed in levels of TNF- α and malondialdehyde (a marker of lipid peroxidation) in the low AGE diet group in comparison with their baseline and high AGE diet group values.

Dietary AGE restriction studies in the CKD and PD populations are scarce. Only four studies (one in CKD stage 3, one in non-diabetic persons with CKD stages 3 and 4, and two in persons performing PD) have been published.

Vlassara et al.⁽²²⁸⁾ followed-up for 4 weeks nine CKD stage 3 participants who were randomly assigned to a low AGE or to their usual diet (high in AGEs). The low dietary AGE intervention group showed a significant decrease in serum CML, methylglyoxal, 8-isoprostane, TNF- α and VCAM-1 in comparison with baseline and high AGE diet group values. In the study conducted in non-diabetic persons with CKD stages 3 and 4⁽²⁴⁰⁾, participants were randomly allocated to a low AGE diet ($n=13$) or a usual/high AGE diet ($n=13$). After 12 weeks of follow-up, no significant differences were observed in both groups regarding serum total AGEs, pentosidine, TNF- α , IL-6 and CRP, as well as urinary 8-isoprostane; however, both groups did show a significant reduction in malondialdehyde levels compared to their baseline.

In the study conducted by Peppas et al.⁽²⁴¹⁾, it was observed that after 1 month of follow-up PD participants randomised to the low AGE diet ($n=9$) had significant reductions in CML and methylglyoxal levels, while the high AGE diet group ($n=9$) showed a significant increase in both serum AGEs. At the end of the study, TNF- α and VCAM-1 were significantly lower in PD participants following the low AGE diet compared to the high AGE diet group. Similarly, Yacoub et al.⁽²⁴²⁾ found that a 1-month dietary AGE restriction significantly reduces CML and methylglyoxal levels in persons performing PD ($n=10$), while these same serum AGEs significantly increased in the PD group following a high AGE diet ($n=10$). Of note, none of these studies assessed the impact of dietary AGE restriction on SAF levels.

In summary, published evidence suggests that following a low AGE diet is an effective therapy for reducing pro-inflammatory biomarkers, particularly TNF- α , biomarkers of oxidative stress (e.g. 8-isoprostane) and endothelial dysfunction (i.e. VCAM-1) and circulating AGEs such as CML and methylglyoxal in healthy, diabetic, CKD and PD participants. Nevertheless, the majority of the randomised clinical trials described above included very small sample sizes, had relatively short follow-ups (2-16 weeks), were considered of low quality and did not control for confounding factors such as medication use, supplement intake, smoking status and nutritional status. Therefore, these limitations need to be considered before drawing conclusions regarding the effectiveness of dietary AGE restrictions on oxidative stress, inflammation, cardiovascular risk markers and serum AGE levels, particularly in persons on dialysis.

Although considerable research has been devoted to the effects of low AGE diets on circulating AGE levels and biomarkers of oxidative stress, inflammation and endothelial dysfunction in healthy and diabetic populations, rather less attention has been paid to the effectiveness of dietary AGE reductions on SAF levels in persons on dialysis. In fact, there has been only one cross-sectional study conducted in 332 HD participants that has reported a significant difference in SAF levels between vegetarian and non-vegetarian participants (2.71 ± 0.6 vs. 3.31 ± 0.97 ; $p=0.002$)⁽²⁴³⁾. Additionally, a 1-year observational study conducted in 180 HD participants has shown that following a vegetarian diet is independently associated with lower SAF levels over time⁽²¹⁶⁾. Assuming that a low AGE diet may have similar effects as a

vegetarian diet, further prospective and randomised trials need to be conducted in order to determine whether dietary restriction of AGEs could reduce tissue AGE accumulation assessed by SAF and, consequently, decrease cardiovascular morbidity and mortality risk in the dialysis population. Even though low dietary AGE interventions seem to be a promising therapeutic approach for reducing circulating AGE and SAF levels, it is important to consider that following a restrictive diet may put persons on dialysis at risk of developing malnutrition or worsen the nutritional status of those who are already malnourished.

It is therefore the purpose of the thesis to investigate the determinants of increased SAF, the factors associated with change in SAF levels over time and the association between SAF and survival in persons receiving HD and PD (Study 1), as well as the impact of a dietetic intervention to correct malnutrition on SAF (Study 2).

The results of this thesis will provide a framework to increase the understanding of the interaction between SAF, dietary AGE intake, malnutrition and adverse outcomes in persons on dialysis and may identify ways in which these could be improved. Published observational studies suggest that reduction of SAF levels will in turn be associated with a reduction in the high morbidity and mortality rates associated with chronic dialysis and, consequently, quality of life and healthcare costs. Testing these hypotheses is beyond the scope of the studies presented.

1.8 Aims and objectives

Primary objectives:

Study 1

- To investigate changes in SAF levels over time in persons on PD and HD.
- To investigate SAF levels as a determinant of survival in persons receiving dialysis.

Study 2

- To investigate whether improvement of nutritional status by providing individualised dietetic advice results in a decrease in SAF in a dialysis population.

Secondary objectives:

Study 1

- To investigate the association of increased SAF levels with morbidity, dietary AGE intake and nutritional markers, including dietary intake, biochemistry, HGS, anthropometry and SGA at baseline.
- To determine the most important factors associated with change in SAF over time.

Study 2

- To investigate the effect of individualised dietetic advice on dietary AGE intake and nutritional markers, including dietary intake, biochemistry, HGS, anthropometry and SGA.

Chapter 2. Materials and methods

2.1 Study design and configuration

Study 1 was a longitudinal prospective study in which persons receiving dialysis were followed-up for 1 year.

Study 2 was an observational non-randomized proof of principle study in which malnourished persons on dialysis received intensive individualised dietetic advice and were followed-up for 6 months. We considered it unethical to randomise malnourished persons on dialysis to no intervention and therefore compared the results with a historical control group of persons on dialysis with malnutrition taken from Study 1, who were seen by their usual dietitian once a month and received standard nutritional counselling.

Because of the nature of the intervention (i.e. dietetic), blinding of participants and investigators was not possible. Nevertheless, in order to reduce the risk of bias, nutritional status evaluation by SGA was conducted by an experienced dietitian who did not participate in other methods of nutritional assessment and did not conduct SAF measurements.

Data from Studies 1 and 2 were collected at the Renal Unit of the Department of Nephrology at the Royal Derby Hospital (University Hospitals of Derby and Burton NHS Foundation Trust).

2.2 Selection and withdrawal of participants

2.2.1 Recruitment

Potential participants on HD and PD were recruited from a single centre (i.e. Royal Derby Hospital). Initial details of Studies 1 and 2 and their respective participant information sheets (PIS; Refer to Appendix 2.3) were provided by either dialysis nurses, a research nurse or the investigator. Potential participants had the opportunity to read through the PIS and think about taking part in the studies for at least 24 hours. They also had the opportunity to ask any questions regarding the studies before signing the consent form.

2.2.2 Informed consent

The process for obtaining participant informed consent was in accordance with Good Clinical Practice (GCP) guidelines. Detailed information was provided on both studies to ensure that participants understood the purpose and potential risks involved. Studies 1 and 2 did not include participants who were unable to represent their own interests or were particularly susceptible to coercion.

All participants gave written informed consent. Informed Consent Forms were signed and dated by the participants before they underwent any procedures, assessments or interventions related to Studies 1 or 2. One copy of the consent form was kept by the participant, one was kept by the investigator, and a third was retained in the participants' hospital records.

It was explained to the potential participants that the decision regarding participation in both studies was entirely voluntary. It was also emphasized to the participants that consent regarding study participation could be withdrawn at any time without penalty or affecting the quality of their future medical care.

2.2.3 Eligibility criteria

Inclusion criteria

HD cohort:

- Age: >18 years (no upper age limit).
- At least three dialysis sessions per week for 3-4 hours.
- Dialysis with high-flux polysulphone, polyarylethersulfone and polyvinylpyrrolidone dialyzers.
- Able to give informed consent.

PD cohort:

- Age: >18 years (no upper age limit).
- Dialysis with lactate/bicarbonate-buffered solutions with different glucose concentrations as prescribed for routine clinical care.
- Able to give informed consent.

Exclusion criteria

- Does not wish to participate.
- Pregnancy or breastfeeding or intending pregnancy.
- Expected survival less than one year (for Study 1).
- Persons with dark skin colour.

All patients who met the inclusion criteria were invited to take part in both studies. Patients on HD were invited when they attended their HD sessions, while those on PD were invited when they have clinic appointments. A total of 151 participants were recruited from September 2016 to August 2017 for Study 1 (Chapters 3-6) and 32 participants were recruited from January to June 2018 for Study 2 (Chapter 7) (Figure 2.2.1).

2.2.4 Participant withdrawal

Participants were withdrawn from the study if they withdrew their consent at any point, received a renal transplant or declined to follow the dietetic intervention (Study 2). Participants were made aware that their withdrawal would not affect their future care. Participants were also made aware (via the information sheet and consent form) that should they withdraw the data collected to date would not be erased and, with their consent, would still be used in the final analysis.

2.3 Data collection

For both studies, relevant participant characteristics were extracted from electronic medical records and from a self-report questionnaire at baseline and included the following:

- Demographic characteristics: chronological age, sex and ethnicity.
- Educational level. Participants were classified into 2 categories: 1) No educational qualifications and 2) Educational qualifications, which included participants with GCSE, A levels, NVQ levels, first degree and/or higher degree.

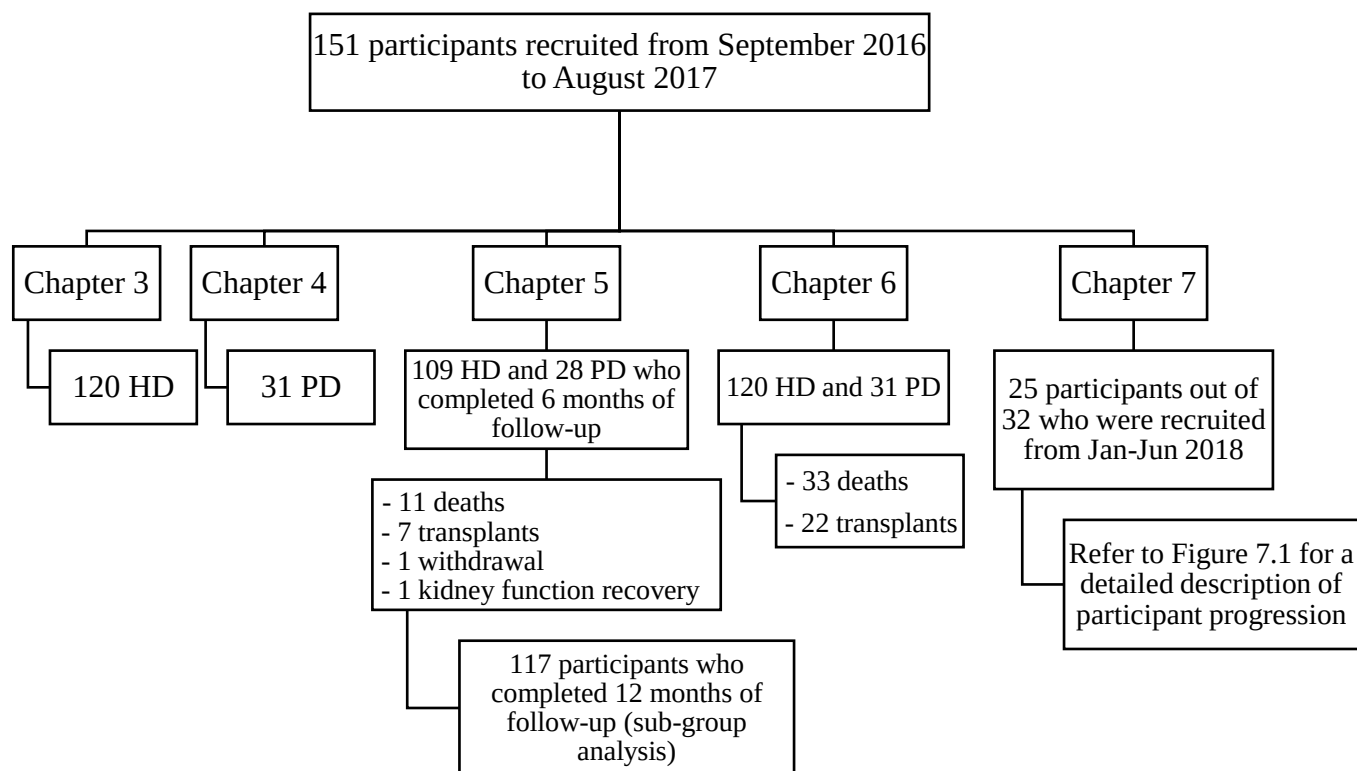


Figure 2.2.1 Flowchart of participant allocation to each Results Chapter of the thesis.

- Occupation status, defined as employed or unemployed (which included retired participants).
- Duration of nephrology care, defined as time since first contact with the nephrology department.
- Dialysis vintage, defined as time since first dialysis treatment.
- Dialysis adequacy: Kt/V was estimated weekly in PD and per session in HD.
- Co-morbidities: current co-morbidities at the time of recruitment included diabetes, hypertension, obesity and cancer. Diabetes and cancer were defined by clinical diagnosis. Participants were considered to be hypertensive according to the K/DOQI clinical practice guidelines on hypertension (i.e. pre-dialysis systolic blood pressure ≥ 140 mmHg and pre-dialysis diastolic blood pressure ≥ 90 mmHg)⁽²⁴⁴⁾, or if currently

on anti-hypertensive drugs. Obesity was defined according to the World Health Organization (WHO) criteria⁽²⁴⁵⁾ as having a BMI ≥ 30 kg/m².

- History of cardiovascular disease, defined as presence of coronary heart disease and/or PVD, as well as previous myocardial infarction, heart failure, atrial fibrillation and/or stroke.
- Smoking status. Participants were classified as being current, former or non-smokers.
- Glucose exposure: in the PD population, information regarding PD solutions was extracted and included lactate/bicarbonate-buffered 1.36% and 3.86% glucose (Physioneal or Dianeal; Baxter®), 7.5% icodextrin (Extraneal; Baxter®) and 1.1% amino acid-containing solutions (Nutrineal; Baxter®). Solutions with 2.27% glucose were not used in the Derby renal unit. The volume of 1.36% and 3.86% glucose-containing solutions used per week at the time of recruitment was obtained and the history of glucose exposure was then estimated by multiplying the dialysis vintage by the weekly glucose load as previously described⁽²⁰³⁾.
- Medications: current medication at the time of recruitment was obtained and included ACE-I, angiotensin receptor blockers, alpha blockers, beta blockers, calcium channel blockers, aldosterone antagonists, aspirin, statins, vitamin B complex, iron, folic acid, calcium-based phosphate binders, sevelamer carbonate, lanthanum carbonate, calcitriol, EPO, cinacalcet, 1-alfacalcidol and proton pump inhibitors. Insulin and oral hypoglycaemic agents were also recorded in the case of participants with diabetes.

- Routine blood/laboratory tests were analysed in a local NHS laboratory at the Royal Derby Hospital, which is serviced and managed according to national standards and governance frameworks. Routine pre-dialysis blood tests were recorded at baseline, 6, and 12 months and included haemoglobin, haemoglobin A1c (only in persons with diabetes), urea, creatinine, potassium, phosphate, corrected calcium, sodium, albumin, total protein, total cholesterol, triglycerides, intact PTH and CRP.

2.4 Study assessments

STUDY 1

Skin autofluorescence measurement

Tissue AGE accumulation was assessed by measuring SAF at baseline, 3, 6, 9 and 12 months. SAF was measured using a validated Autofluorescence Reader Standard Unit (SU) version 2.4.3 (AGE Reader SU, DiagnOptics Technologies BV, Aarhusweg 4-9, Groningen, The Netherlands) (Figure 2.4.1). The AGE Reader SU has CE approval (reference number: 2085393CE01) to be used as a medical device and thus has passed strict safety standards. The amount of ultraviolet light exposure is actually far below the safety limits.



Figure 2.4.1 The Autofluorescence Reader Standard Unit

The method for measuring SAF has been previously described by Meerwaldt et al.⁽¹⁹⁶⁾ Briefly, the AGE Reader SU shines ultraviolet light on a skin area of approximately 1 cm² (i.e. illumination window), protected from surrounding light, with an excitation light range of 300-420 nm (maximum intensity at 370 nm). Emitted light from the skin in the range of 300-600 nm is measured with a spectrometer (AVS-USB2000, Avantes Inc., Eerbeek, The Netherlands) using a 200-µm glass fibre (Figure 2.4.2). SAF was calculated in AU by dividing the average emitted light per nm (i.e. fluorescent light coming from the skin) in the range between 420 and 600 nm by the average excitation light intensity per nm in the range between 300 and 420 nm (i.e. $\text{SAF} = \text{emitted light [420–600 nm]} / \text{excitation light [300–420 nm]}$).

For each participant, three measurements were performed on the volar surface of the lower arm at approximately 10 cm below the elbow, ensuring that the area had normal skin without visible vessels, scars, tattoos or other abnormalities. The mean value of the three SAF measurements was used for statistical analysis. For HD participants, only the non-fistula arm was used and readings were undertaken within the first hour of HD treatment.

Skin colour and pigmentation may affect SAF measurements. Due to the high absorption of the excited light in persons with very dark skin (i.e. skin reflectivity below 6%)⁽²⁴⁶⁾, SAF might not be reliable in this population and persons with dark skin (i.e. Fitzpatrick skin colour type V-VI) were therefore excluded. If the skin reflectivity is <6%, the AGE Reader indicates that the

signal is too low to obtain a valid reading. It has been previously reported that SAF readings have a coefficient of variation of 7-8%⁽²⁴⁷⁾.

The AGE Reader SU was operated from the investigator's laptop using the proprietary AGE Reader SU software. After successful completion of the measurement, within a few seconds the "SAF measurement report" was shown on the laptop screen and results were automatically stored in dedicated folders on the laptop (Figure 2.4.3).

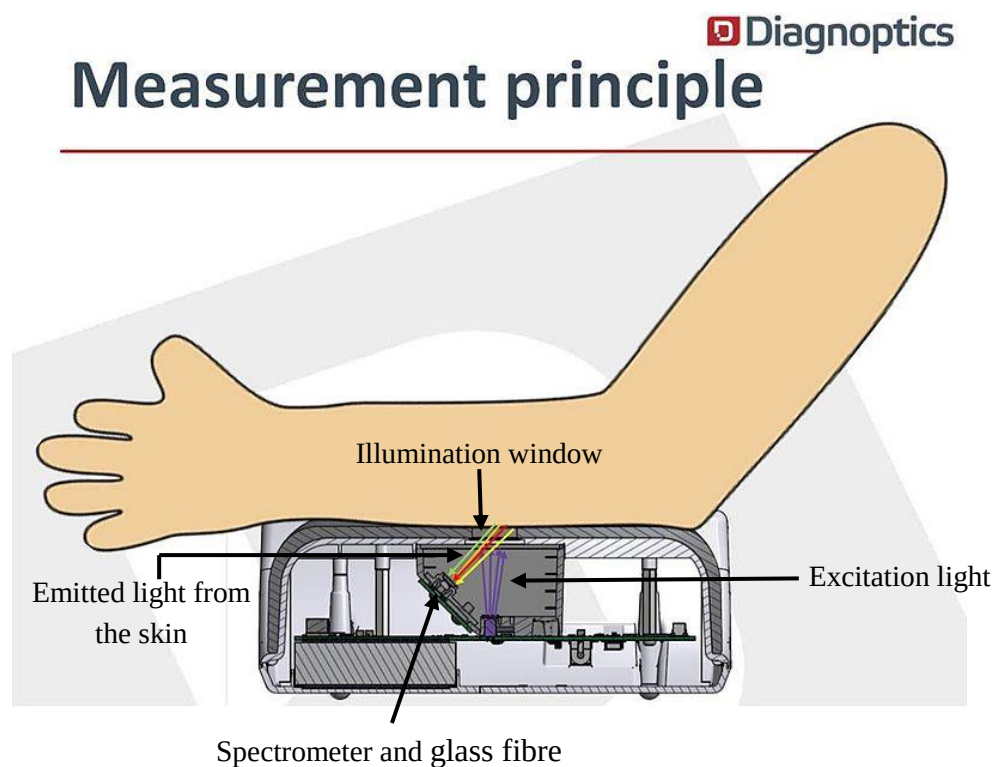


Figure 2.4.2 Schematic view of the skin autofluorescence measurement principle

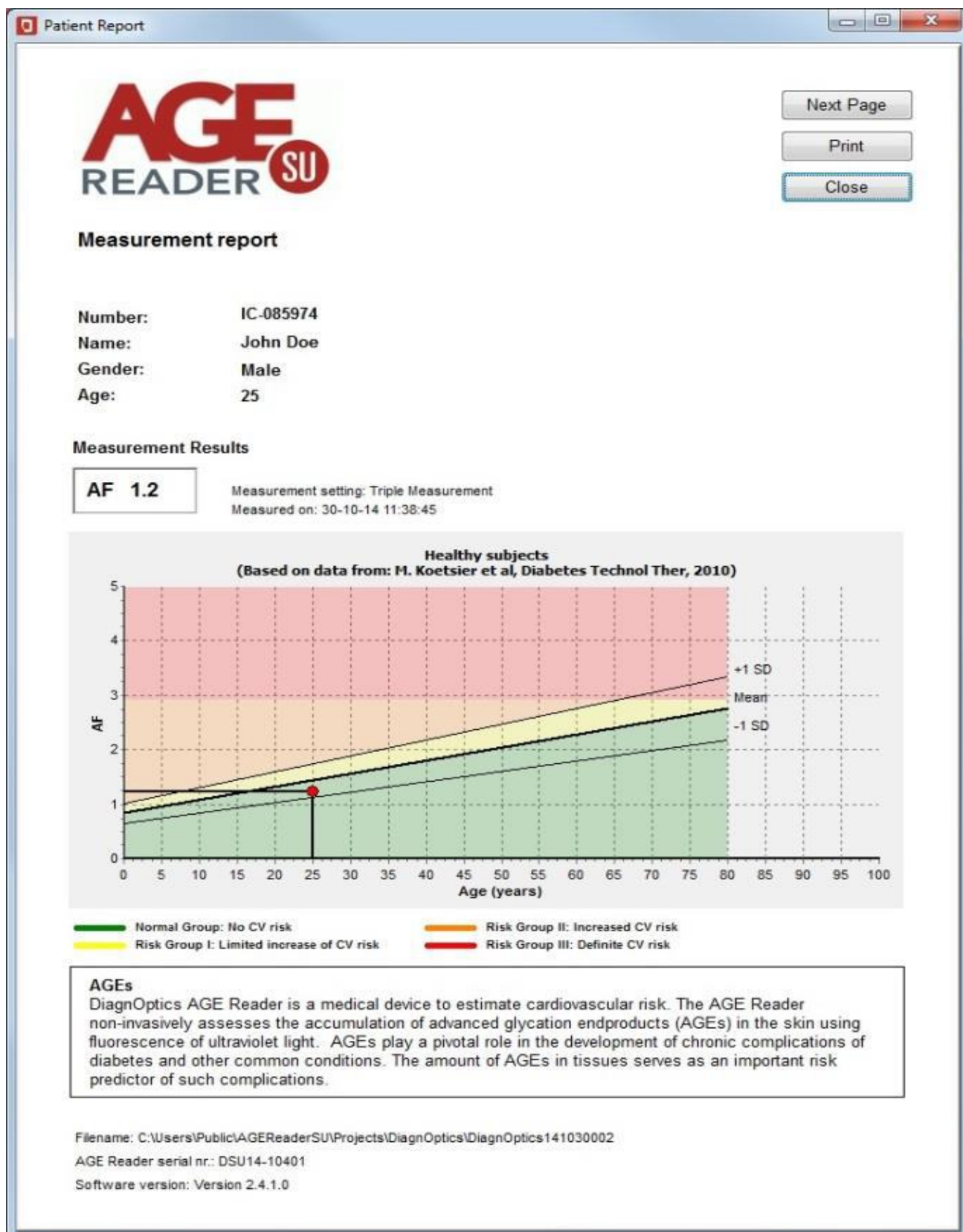


Figure 2.4.3 Skin autofluorescence measurement report example

Nutritional assessments

Nutritional assessments were performed at baseline, 6 and 12 months and included the following:

- 24-hour dietary recalls

Dietary intake was assessed by means of three 24-hour dietary recalls, including one dialysis day, one non-dialysis day and one weekend day for HD participants, and two weekdays and one weekend day for PD participants.

Participants were asked to remember all foods and drinks they had the previous day. Information regarding type (e.g. white or brown bread, butter or margarine, full fat or semi-skimmed milk), portion size (e.g. level, rounded or heaped teaspoon/tablespoon, cup or mug [of known volume], thin, medium or thick slice of bread/cheese) and source of food or drink (e.g. homemade, restaurant, takeaway) with brand names and cooking method (e.g. boiled, fried, grilled, steamed, oven-baked) was recorded as thoroughly as possible. Any food or drink not consumed and the amount was also recorded.

Average energy, protein and fat intake were calculated by using the software Dietplan 7 (Forestfield Software Limited, West Sussex, UK). Average daily energy and protein intake were then calculated in kilocalories and grams, respectively, per ideal body weight (IBW).

- Food frequency questionnaire

Dietary AGE intake (reported in kU/day) was estimated with a food frequency questionnaire (FFQ) previously validated in persons with diabetes⁽²⁴⁸⁾. The FFQ consists of 90 food items grouped in 7 categories: breakfast foods/cereals,

biscuits/pastries/snacks, fats, meats (including deli meat or lunchmeat, beef, pork, chicken, turkey, and fish or seafood), cheese, eggs and combination foods. American names of several food items were changed to British names in order to facilitate the understanding between the participant and the investigator (e.g. cookies were changed to biscuits, cinnamon sweet roll to cinnamon swirl, potato chips to potato crisps, etc.) (See Appendix 2.1).

The FFQ has 11 options of frequencies with assigned frequency codes: 0 for never, 0.01 for 1 to 6 times per year, 0.028 for 7 to 11 times per year, 0.033 for once a month, 0.08 for 2 to 3 times per month, 0.14 for once a week, 0.29 for twice a week, 0.5 for 3 to 4 times per week, 0.79 for 5 to 6 times per week, 1 for once a day, and 2 for 2 or more per day. The AGE content per food was calculated by multiplying the frequency code by the amount of food ingested in grams and then by the amount of AGEs in 100 g of food (data extracted from Goldberg's AGE food content tables⁽¹⁸⁸⁾). For example, a participant eats 40 g of Weetabix 2-3/month; therefore, the AGE content calculation was as follows: $0.08 \text{ (frequency code)} \times 0.4 \text{ (amount of food)} \times 1403 \text{ (AGE content in 100 g of Weetabix)} = 44.9 \text{ kU}$. Total dietary AGE intake was then calculated by summing the AGE content from each foodstuff.

Goldberg's AGE food content tables⁽¹⁸⁸⁾ have data on approximately 540 foods; thus, some food items and cooking methods were not found in the database and the AGE content from the closest food analogue and similar cooking technique available in the database was therefore used. For example,

there was no AGE content for oven cooked salmon in the database, thus we used broiled salmon instead.

- Anthropometry

Anthropometric measurements including weight, height, MAC and triceps skinfold thickness (TSF) were measured according to international standard methods as previously described⁽¹⁰⁸⁾. BMI was reported in kg/m² and MAMC was calculated by using the following standard equation: $MAMC (cm^2) = MAC - (3.14 * TSF)$, where MAC and TSF were measured in cm.

- Handgrip strength

Handgrip strength was measured with the Takei 5401 handgrip digital dynamometer (Takei Scientific Instruments Co., Ltd., Tokyo, Japan). For the HD participants, HGS measurement was undertaken within the first hour of HD treatment and the non-fistula arm was used. For the PD participants, HGS was measured during PD clinic visits and the dominant arm was used if the participant did not have a fistula. Ideally a standing position with feet a hip width apart was used for conducting the HGS measurement; when this was not possible, the participant was sitting upright with the arm extended straight down by the side of the bed/chair. The dynamometer's grip range was adjusted until the second joint of the participant's forefinger was bent through 90 degrees. The participant gripped the dynamometer, exerted full force without letting their arm touch their body and then relaxed.

- Subjective global assessment

The 7-point scale SGA was used to assess the nutritional status. SGA is a comprehensive and validated nutritional assessment tool⁽¹⁰⁵⁾, widely used in both the clinical and research fields and is recommended by the NKF KDOQI Nutrition Guidelines⁽¹²⁵⁾ as well as the RA Clinical Practice Guideline on Nutrition in CKD⁽¹¹⁴⁾. It uses a 7-point Likert scale to rate the following components: history of weight loss, dietary intake, gastrointestinal symptoms, functional capacity, disease state/co-morbidities and physical examination, which includes a subjective assessment of subcutaneous fat and muscle mass loss, and presence of oedema. The overall SGA score is rated subjectively based on the ratings of the six individual components. There are three overall SGA ratings: well-nourished (ratings of 6 or 7 in most categories), mild-moderate malnutrition (ratings of 3 to 5 in at least three categories) and severe malnutrition (ratings of 1 or 2 in at least three categories) (See Appendix 2.2). For statistical analysis, participants were classified as being well-nourished (SGA score of 6 or 7) or malnourished (SGA scores from 1 to 5).

STUDY 2

SAF was measured using the AGE Reader SU at baseline, 3 and 6 months according to the method described above. Detailed assessment of dietary intake and nutritional status was undertaken at baseline and 6 months, including energy, protein, fat and dietary AGE intake, HGS, anthropometric measurements and SGA using the same materials and methods as described above. Routine biochemical variables were also measured at baseline and 6 months.

Participants received individualized nutritional advice and support formulated and delivered by experienced dietitians consisting of food fortification recommendations and oral nutritional supplementation aiming to achieve estimated nutritional requirements (i.e. energy [30-35 kcal/kg/day] and protein intake [1.1-1.2 g/kg/day])⁽¹¹⁴⁾. Advice was individualized according to patient needs and food preferences. Participants were then followed up for 6 months.

Food fortification involved enhancing the energy and protein content of meals and snacks without increasing the portion sizes of foods. Examples of food fortification advice included the following: use full fat dairy products, add cream cheese to bread/crackers/mashed potato, add extra butter or margarine to foods, use salad cream/mayonnaise/salad dressing on sandwiches and salads, fry foods in oil/butter, add sugar or honey to cereals, porridge and drinks, and add custard, cream, jam, lemon curd or syrup to cakes, pies and fresh or tinned fruit.

Oral nutritional supplements included Fortisip Compact (2.4 kcal/ml), Fortisip (1.5 kcal/ml) and Fortijuice (1.5 kcal/ml) (Nutricia Advanced Medical Nutrition®, Wiltshire, United Kingdom), as well as Renapro® shot (Stanningly Pharma, BioCity, Pennyfoot Street, Nottingham, United Kingdom) and Fresubin® 5kcal Shot (Fresenius Kabi Ireland, Balbriggan, Dublin, Ireland). Table 2.4.1 shows detailed macro- and micronutrient composition of the aforementioned oral nutrition supplements.

Table 2.4.1 Average macronutrient and micronutrient composition of oral nutritional supplements per 100 ml.

	Fortisip compact	Fortisip	Fortijuice	Fresubin 5kcal	Renapro shot
Energy (kcal)	240	150	150	500	137
Protein (g)	9.6	6.0	4.0	0.0	33
Fat (g)	9.3	5.8	0.0	53.8	<0.1
Carbohydrate (g)	29.7	18.4	33.5	4.0	2.0
Fibre (g)	<0.5	0.3	0.0	0.4	0.0
Sodium (mg)	96	90	9.7	---	190
Potassium (mg)	236	159	9.3	---	18
Phosphorus (mg)	174	78	12	---	1.5
Calcium (mg)	174	91	30	---	27
Vitamin A (µg)	240	123	188	---	---
Vitamin E (mg)	3.0	1.9	2.3	14	---
Vitamin D (µg)	1.8	1.1	1.3	---	---
Vitamin C (mg)	24	15	19	---	---
Folic acid (µg)	64	40	50	---	---
Vitamin B ₁₂ (µg)	0.70	0.32	0.39	---	---

Participants received precise oral and written instructions on how and when to take the supplements and how to follow the food fortification advice provided.

Participants were closely monitored by the dietitian at least once a week to encourage adherence to nutritional advice, and 24-hour dietary recalls were also conducted to ensure compliance with the advice provided. Each participant was also reviewed quarterly with the clinical lead renal dietitian in order to modify the dietetic advice, if required.

2.5 Statistical analysis

Data management and statistical analyses were performed using statistical software SPSS version 24.0 (IBM Corporation, Chicago, IL). Data are presented as mean $\pm$ SD, median (interquartile range [IQR]), percentages, odds

ratio (OR [95% CI, confidence intervals]) or hazard ratios (HR [95% CI]), as appropriate.

To compare baseline versus final evaluations, Wilcoxon test was used in the case of continuous variables and McNemar test in the case of categorical variables. Intergroup comparisons were performed using Mann Whitney U test for continuous variables and Chi-squared test or Fisher's exact test for categorical variables. Spearman's correlation coefficient was used to determine the significance and strength of associations between continuous variables. Multivariable linear regression analysis was used to identify independent determinants of AGE accumulation as assessed by SAF. Variables included were all significantly associated with SAF in univariable analysis.

For Study 1, the rate of change of SAF between baseline, 3, 6, 9 and 12 months (i.e. SAF trend) was calculated by fitting a regression line using the "SLOPE" function in Microsoft Excel 2013, where the y-axis represented SAF values and the x-axis represented time points⁽²⁴⁹⁾. Participants were then classified into quartiles according to their SAF trend values. Comparisons of continuous variables across all quartiles were performed using Kruskal-Wallis test. Multivariable logistic regression analysis was performed to identify the determinants associated with changes in SAF over 1 year. Nagelkerke R² for the model and Hosmer and Lemeshow test p value were reported.

Kaplan-Meier survival curves were computed according to baseline SAF above or below the median value and nutritional status at baseline. Cox proportional hazards models were used to investigate the prognostic value of AGE accumulation as assessed by SAF and presence of malnutrition for predicting mortality. Since SAF and malnutrition have both been independently associated with increased mortality in the dialysis population, we created a product term (i.e. two-way interaction term) and included it in the regression model in order to test if there was an interaction between SAF and malnutrition. For all statistical analyses, a p-value less than 0.05 was considered to have statistical significance.

Sample size calculation was performed by using the software nQuery Advanced v8.0 (Statistical Solutions Limited, Boston, Massachusetts). The primary outcome for sample size determination in Study 1 was survival in relation to baseline SAF above or below the median value in persons on dialysis. Power calculation showed that with a sample size of 150 participants split in 2 groups (SAF above the median [$n=75$] and SAF below the median [$n=75$]), the analysis would have more than 80% power to detect a HR of 2.7, assuming that the total number of events (i.e. deaths) observed is 34 and the average probability of the event over the course of the study is 0.1 and 0.35 in group 1 (SAF below the median) and group 2 (SAF above the median), respectively.

Since Study 2 was a proof of principle study, we considered it reasonable to include 40 dialysis participants (either HD or PD).

2.6 Ethical and regulatory aspects

Ethical approval for both studies was granted by the local Research Ethics Committee (East Midlands – Nottingham 1. REC reference: 16/EM/0243) and the respective NHS Research & Development department (i.e. Derby Teaching Hospitals NHS Foundation Trust; DHRD/2016/056). Studies 1 and 2 were conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996, the principles of GCP, and the Department of Health Research Governance Framework for Health and Social Care, 2005. Studies 1 and 2 were registered on www.clinicaltrials.gov (NCT02878317).

We submitted an amendment to the protocol for Health Research Authority approval. Analysis of baseline data from Study 1 identified strong associations between higher SAF and malnutrition, suggesting that correction of malnutrition may be a more important dietetic intervention to decrease SAF levels than a low AGE diet. We further reasoned that placing persons with malnutrition on a restrictive diet may worsen their malnutrition and we have therefore adapted our original research plan to include an observational study to assess the impact of correcting malnutrition on SAF by providing intensive individualised nutritional advice rather than a randomised trial of dietary AGE restriction.

Refer to Appendix 2.3 Ethical approval documentation for consultation of ethical approval letters, final version of the protocol and PIS for Studies 1 and 2.

Chapter 3. The association of nutritional factors and skin autofluorescence in persons receiving haemodialysis

The work in this chapter has been published in the Journal of Renal Nutrition:

Viramontes Hörner D, Selby NM, Taal MW. The association of nutritional factors and skin autofluorescence in persons receiving hemodialysis. J Ren Nutr. 2019; 29(2):149-155.

The work in this chapter has also been presented as a poster at the following conferences:

- *“Skin autofluorescence and dietary advanced glycation end-products as risk factors in dialysis patients”. World Congress of Nephrology, Mexico City, 2017 and British Renal Society Conference, Nottingham, 2017.*
- *“Impact of nutritional status on skin autofluorescence in haemodialysis patients”. European Renal Association-European Dialysis and Transplantation Association Conference, Madrid, 2017.*

ABSTRACT

Background: AGEs are uremic toxins that result from hyperglycaemia, oxidative stress, and systemic inflammation. AGEs are also formed in food during cooking. On the other hand, malnutrition may contribute to AGE formation through its association with oxidative stress and inflammation. AGE accumulation can be measured by SAF and elevated SAF is independently

associated with higher mortality on HD. We aimed to investigate associations between SAF, dietary AGE intake, and markers of malnutrition in persons receiving HD.

Methods: This was a single centre cross-sectional study that included 120 participants on HD dialyzing at least 3 times per week for 3-4 hours. SAF was measured using an Autofluorescence Reader. Dietary AGE, energy, protein and fat intake, HGS, anthropometric measurements and biochemistry were also assessed. SGA was performed to evaluate the nutritional status.

Results: SAF was higher in malnourished participants and correlated negatively with serum albumin and cholesterol, HGS and energy, protein and fat intake and positively with CRP and chronological age; SAF did not correlate with dietary AGE intake. Multivariable linear regression analysis showed that diabetes, smoking, serum albumin, HGS, protein intake, and dialysis vintage were independent predictors of increased SAF.

Conclusions: Markers of malnutrition were more important determinants of increased SAF than high dietary AGE intake in this HD population. Nutritional interventions aiming to reduce SAF by correcting malnutrition should therefore be investigated. The observed association between higher SAF and malnutrition may in part explain the previously reported association between higher SAF and mortality on HD.

INTRODUCTION

AGEs are uremic toxins that are markedly increased in persons on dialysis⁽¹⁸⁵⁾ and may contribute to the pathogenesis of accelerated cardiovascular disease in this population. Accumulation of AGEs in the skin can be assessed by measuring SAF and previous studies have found that SAF is elevated in

persons receiving HD^(202, 203). Moreover, higher SAF is independently associated with all-cause and cardiovascular mortality in the dialysis population^(196, 213, 214), but the mechanism underlying this association is not completely understood. AGEs form cross-links with tissue proteins, particularly collagen in the skin and vascular basement membranes, causing increased arterial stiffness and endothelial dysfunction which may contribute to the pathogenesis of cardiovascular disease⁽¹⁸⁵⁾. These changes could be predicted to increase LVH which may result in heart failure⁽²⁰⁷⁾. Additionally, AGEs interact with specific AGE receptors that lead to the activation of systemic inflammation, exacerbating tissue damage⁽¹⁸⁵⁾. This may contribute to the pathogenesis of atherosclerosis^(72, 73, 96, 97).

AGEs are formed by the non-enzymatic reaction of reducing sugars with free amino groups of proteins, lipids and nucleic acids⁽¹⁸⁴⁾. AGEs are also formed more rapidly during oxidative stress with the subsequent formation of reactive carbonyl compounds (i.e. carbonyl stress). Hyperglycaemia due to diabetes (a common cause of ESKD) and interaction of blood with the HD membrane have also been identified as contributors to the increased endogenous formation of AGEs in persons on dialysis⁽¹⁸⁴⁾. Furthermore, AGEs are normally removed from the plasma by renal excretion and those with ESKD are therefore at further risk of AGE accumulation due to decreased excretion^(250, 251).

Additionally, AGEs may enter the body from exogenous sources including cigarette smoke or food⁽¹⁸⁵⁾. AGEs are found naturally in animal-derived and high fat uncooked foods. Importantly, the AGE content of food is increased

considerably by cooking methods that require high temperatures such as frying, roasting and grilling. Furthermore, processing of food accelerates the formation of new AGEs⁽¹⁸⁹⁾. It has been reported that ~10% of the ingested AGEs are absorbed⁽²⁵²⁾ and studies in healthy, obese and diabetic participants have reported that higher dietary AGE intake is associated with increased serum AGE levels^(229, 232, 238). It is unknown whether dietary AGE intake contributes significantly to skin accumulation of AGEs in persons on dialysis.

Malnutrition is one of the most important and frequent complications in persons with ESKD undergoing dialysis⁽⁷⁸⁾ and may result from decreased dietary nutrient intake due to loss of appetite, presence of comorbidities, metabolic derangements and other factors associated with uremic toxicity⁽⁶⁵⁾. Additionally, inflammation and oxidative stress (other common conditions in the dialysis population) may further contribute to the development of malnutrition, creating a vicious cycle⁽⁹⁶⁾.

Whilst several factors associated with increased SAF have been described in the dialysis population, there are no studies that have examined the effect of diet. Several factors that lead to malnutrition may theoretically also increase SAF but conversely, increased intake of certain foods with high AGE content could also potentially contribute. Therefore, we aimed to investigate the most important determinants associated with increased SAF levels in cross-sectional analysis of baseline data in persons receiving HD, with a particular focus on nutritional factors including dietary AGE intake and markers of malnutrition.

MATERIALS AND METHODS

Please refer to Chapter 2 for a detailed description of the materials and methods used in this cross-sectional analysis.

Study population

All patients who met inclusion criteria were invited to take part in this study. This cross-sectional analysis included 120 participants on HD dialysing at least 3 times per week for 3-4 hours with high-flux polysulphone, polyarylethersulfone and polyvinylpyrrolidone dialyzers. Relevant participant characteristics were extracted from electronic medical records and from a self-report questionnaire including demographic characteristics, educational level, occupation status, duration of nephrology care, dialysis vintage, dialysis adequacy, current co-morbidities, history of cardiovascular disease and smoking status. Routine bloods/laboratory tests were also recorded.

Assessments

SAF was measured by using a validated AGE Reader as previously described⁽¹⁹⁶⁾. Average energy, protein and fat intake were assessed by means of three 24-hour dietary recalls. Energy and protein intake were reported in kcal/kg of IBW/day and g/kg of IBW/day, respectively. Fat intake was reported in g/day. A FFQ previously validated in persons with diabetes⁽²⁴⁸⁾ was used to assess dietary AGE intake (reported in kU/day). Anthropometric measurements and HGS were assessed according to standard methods. The 7-point scale SGA was performed to evaluate the nutritional status.

Statistical analyses

Power calculation showed that with 123 participants (Group 1 [malnourished]: $n=47$; Group 2 [well-nourished]: $n=76$) the analysis would have 80% power to detect a significant difference in SAF of 0.5 AU between two groups. This calculation included an inflation of 15% to allow for use of non-parametric tests. Data are presented as median (IQR) or percentages, as appropriate. Intergroup comparisons were performed using Mann Whitney U test for continuous variables and Fisher's exact test for categorical variables. Spearman's correlation coefficient was used to determine the significance and strength of associations between continuous variables. Multivariable linear regression analysis was used to identify independent determinants of AGE accumulation as assessed by SAF. Variables included were all significantly associated with SAF in univariable analysis.

RESULTS

Study population characteristics

Baseline characteristics of participants receiving HD are shown in Table 3.1. Median age, dialysis vintage and SAF were 65 (IQR 54 to 75) years, 31 (11 to 71) months and 3.3 (2.8 to 3.8) AU, respectively. The majority of the participants were male and of white ethnicity. Prevalence of diabetes, malnutrition and coronary heart disease was 40%, 38% and 42%, respectively. Median dietary AGE intake was high at 12384 (8665 to 16984) kU/day, while energy and protein intake were low compared to the recommendations for persons receiving dialysis^(114, 125).

Table 3.1 Demographic characteristics including clinical, biochemical and nutritional data

<i>Variable</i>	<i>Haemodialysis (n=120)</i>
Age (years)	65 (IQR 54 to 75)
Male [n (%)]	76 (63)
White ethnicity [n (%)]	106 (88)
Educational qualifications [n (%)]	62 (52)
Unemployed [n (%)]	93 (77)
Current smoking [n (%)]	17 (14)
Diabetes [n (%)]	49 (41)
Malnutrition [n (%)]	46 (38)
Coronary heart disease [n (%)]	51 (42)
Peripheral vascular disease [n (%)]	10 (8)
Duration of nephrology care (months)	98.0 (51.2 to 157.2)
Dialysis vintage (months)	31.0 (11.0 to 71.0)
Dialysis adequacy (<i>Kt/V</i>)	1.2 (1.0 to 1.4)
Haemoglobin (g/L)	119.0 (109.0 to 123.7)
Serum albumin (g/L)	33.0 (31.0 to 35.0)
C reactive protein (mg/L)	9.0 (4.0 to 19.0)
Total cholesterol (mmol/L)	3.8 (3.1 to 4.5)
Serum creatinine (μ mol/L)	618 (495 to 746)
Serum phosphate (mmol/L)	1.48 (1.22 to 1.78)
Serum corrected calcium (mmol/L)	2.43 (2.35 to 2.52)
Serum potassium (mmol/L)	4.7 (4.3 to 5.3)
Skin autofluorescence (AU)	3.3 (2.8 to 3.8)
Dietary AGE intake (kU/day)	12384 (8665 to 16984)*
Energy intake (kcal/kg/day)	20.2 (16.5 to 23.8)
Protein intake (g/kg/day)	0.8 (0.6 to 1.0)
Fat intake (g/day)	53.6 (37.3 to 71.5)
Dry weight (kg)	75.2 (64.1 to 92.1)
Body mass index (kg/m ²)	26.2 (23.6 to 31.1)
Handgrip strength (kg)	20.0 (12.4 to 29.0)
Mid-arm muscle circumference (cm ²)	25.5 (23.0 to 28.1)
Triceps skinfold thickness (mm)	15.6 (11.8 to 22.0)

AGE, advanced glycation end product; AU, arbitrary units; IQR, interquartile range; kU, kilounits.

*Dietary AGE intake kU/kg/day: 160.5 (110.1 to 230.3)

Table 3.2 Associations of dietary advanced glycation end-product intake in persons receiving haemodialysis

	Haemodialysis (n=120)	
Factor	Dietary AGE intake (kU/day)	p Value
Gender		
Female	9765 (8050 to 14095)	0.002
Male	14088 (9719 to 19463)	
Malnutrition		
Yes	11058 (8162 to 15496)	0.049
No	13426 (9519 to 18760)	
Educational qualifications		
Yes	14138 (9814 to 19838)	0.003
No	10934 (8198 to 15022)	
Employed		
Yes	13240 (8594 to 19213)	0.40
No	11784 (8739 to 16404)	
History of smoking		
Yes	13693 (9443 to 18758)	0.02
No	11342 (7546 to 14591)	
	Spearman's Rho	p Value
Dialysis vintage (months)	-0.061	0.51
Haemoglobin (g/L)	0.194	0.03
Serum albumin (g/L)	0.126	0.17
C reactive protein (mg/L)	-0.053	0.57
Total cholesterol (mmol/L)	0.104	0.26
Serum creatinine (µmol/L)	0.303	0.001
Serum phosphate (mmol/L)	0.260	0.004
Energy intake (kcal/kg/day)	0.276	0.002
Protein intake (g/kg/day)	0.320	<0.0001
Fat intake (g/day)	0.340	<0.0001
Body mass index (kg/m ²)	-0.010	0.91
Handgrip strength (kg)	0.268	0.004
MAMC (cm ²)	0.129	0.16

AGE, Advanced glycation end-product; kU, kilounits; MAMC, Mid-arm muscle circumference.

Table 3.3 Determinants of skin autofluorescence in univariate analysis in persons receiving haemodialysis

Factor	Haemodialysis (n=120)	
	Skin autofluorescence (AU)	p Value
<i>Malnutrition</i>		
Yes	3.7 (2.8 to 4.1)	0.04
No	3.2 (2.7 to 3.7)	
<i>Diabetes</i>		
Yes	3.6 (3.1 to 4.3)	0.001
No	3.2 (2.7 to 3.7)	
<i>Peripheral Vascular Disease</i>		
Yes	4.0 (3.5 to 5.2)	0.006
No	3.2 (2.7 to 3.8)	
<i>Gender</i>		
Female	3.4 (2.8 to 3.9)	0.90
Male	3.2 (2.7 to 3.8)	
<i>Smoking</i>		
Current	3.7 (2.8 to 3.9)	0.82
Former	3.4 (2.9 to 3.9)	
<i>History of smoking</i>		
Yes	3.4 (2.9 to 3.9)	0.021
No	3.0 (2.6 to 3.7)	
<i>Employed</i>		
Yes	3.4 (2.8 to 3.9)	0.07
No	3.0 (2.5 to 3.3)	
	Spearman's Rho	p Value
Age (years)	0.237	0.009
Dialysis vintage (months)	0.236	0.01
C reactive protein (mg/L)	0.273	0.003
Haemoglobin (g/L)	-0.096	0.29
Urea (mmol/L)	-0.203	0.03
Serum creatinine (µmol/L)	-0.094	0.31
Serum albumin (g/L)	-0.285	0.002
Total cholesterol (mmol/L)	-0.280	0.002
Serum calcium (mmol/L)	-0.191	0.04
Serum phosphate (mmol/L)	-0.069	0.45
Dietary AGE intake (kU/day)	-0.161	0.08
Energy intake (kcal/kg/day)	-0.349	<0.0001
Protein intake (g/kg/day)	-0.339	<0.0001
Fat intake (g/day)	-0.255	0.005
SGA score	-0.236	0.009
Body mass index (kg/m ²)	0.106	0.25
Handgrip strength (kg)	-0.338	<0.0001
MAMC (cm ²)	-0.084	0.36

AGE, Advanced glycation end-product; AU, arbitrary units; kU, kilounits; MAMC, Mid-arm muscle circumference; SGA, Subjective Global Assessment.

Table 3.4 Multivariable linear regression analysis to identify independent determinants of skin autofluorescence in persons receiving haemodialysis

<i>Factor</i>	<i>Unstandardized coefficient (B)</i>	<i>Standardized coefficient (β)</i>	<i>p Value</i>
Age (years)	0.001	0.014	0.87
Dialysis vintage (months)	0.003	0.233	0.003
Diabetes (no=0, yes=1)	0.379	0.210	0.009
History of smoking (no=0, yes=1)	0.480	0.261	0.001
Serum albumin (g/L)	-0.041	-0.203	0.016
Handgrip strength (kg)	-0.019	-0.241	0.003
Protein intake (g/kg/day)	-0.534	-0.168	0.038

The seven variables shown were used as independent variables. Adjusted R^2 for the model was 0.366

Factors associated with dietary AGE intake

Table 3.2 summarizes associations with dietary AGE intake in HD participants. Malnourished participants had significantly lower dietary AGE intake than those who were well-nourished. Dietary AGE intake correlated significantly and positively with other measures of dietary intake including energy, protein and fat intake, as well as HGS, haemoglobin, serum creatinine and serum phosphate. Participants with educational qualifications and a history of smoking (i.e. former and current smokers), as well as males had higher dietary AGE intake than participants with no educational qualifications, non-smokers and females, respectively.

Repeat analysis after adjustment of dietary AGE intake for body weight (kU/kg/day) showed a weak negative correlation with SAF ($r=-0.197$; $p=0.03$) and a negative correlation with MAMC ($r=-0.294$; $p=0.001$). Positive correlations with energy, protein and fat intake as well as serum phosphate and haemoglobin were confirmed. The associations with malnutrition, sex, smoking status, serum creatinine and HGS were no longer significant.

Determinants of skin autofluorescence

Table 3.3 shows the determinants of SAF in univariable analyses.

Malnourished participants had significantly higher SAF levels than those who were well-nourished. Subjective Global Assessment score (i.e. degree of malnutrition) was inversely associated with SAF. Additionally, there were significant and negative correlations between SAF and several markers of malnutrition including serum albumin, cholesterol and urea, as well as HGS and energy, protein and fat intake. Furthermore, SAF correlated significantly and positively with CRP, chronological age and dialysis vintage. Persons with diabetes, PVD and a history of smoking had also higher SAF levels than those without diabetes or PVD and non-smokers, respectively. Of note, SAF levels were not significantly correlated with dietary AGE intake in this HD population. Multivariable linear regression identified diabetes, dialysis vintage, history of smoking (current or previous), lower serum albumin, lower HGS and lower protein intake as independent determinants of increased SAF levels, but chronological age was not. The adjusted R^2 for the model was 0.366 (Table 3.4).

DISCUSSION

We have found that elevated SAF was not positively associated with dietary AGE intake in persons on HD but was associated with several markers of malnutrition, suggesting that malnutrition is a more important determinant of increased SAF than AGE ingestion in this population. We have also confirmed previously reported associations between elevated SAF and diabetes, dialysis vintage, smoking and markers of inflammation.

Dietary AGE intake was high in our study population at 12384 kU/day compared to the suggested intake of less than 8000 kU/day⁽¹⁸⁸⁾. Other studies conducted in persons receiving HD have also found a dietary AGE intake >12000 kU/day⁽¹⁹²⁾. In this cross-sectional analysis, the lack of association between higher SAF and dietary AGE intake in persons on HD was an unexpected finding. This is most likely explained by the positive association between dietary AGE intake and the intake of all dietary components including total energy, protein and fat (high fat and high protein animal-derived foods have a high AGE content)⁽¹⁸⁹⁾. Serum creatinine and serum phosphate were also positively correlated with AGE intake; this might be explained by the fact that serum creatinine is a marker of protein intake⁽¹³⁵⁾ and high protein foods are also high in phosphate⁽²⁵³⁾. Thus, persons with lower dietary AGE intake also had lower total nutritional intake and were more likely to be malnourished (Table 3.2). Furthermore, our observation that higher SAF correlated significantly and negatively with several markers of malnutrition (i.e. serum albumin, HGS, SGA score, and total energy, protein and fat intake) and that SAF was independently and negatively associated with serum albumin, protein

intake and HGS in multivariable analysis implies that malnutrition may be a more important determinant of elevated SAF in persons receiving HD than dietary AGE intake. Our interpretation is that any impact of dietary AGE intake on SAF was outweighed in this population by the greater impact of malnutrition.

The association between increased SAF and malnutrition may be explained by several mechanisms. The persistent inflammatory state seen in persons with ESKD characterized by increased levels of CRP and pro-inflammatory cytokines (e.g. IL-6 and TNF- α), worsens the nutritional status by increasing protein catabolism, muscle wasting and resting energy expenditure, as well as decreasing hepatic protein synthesis (e.g. albumin and pre-albumin)⁽⁸⁵⁾.

Furthermore, oxidative stress, another common abnormality in persons on dialysis, is closely linked with systemic inflammation and this interrelationship has been implicated in the development of malnutrition⁽⁹⁶⁾. It has therefore been suggested that a vicious circle of malnutrition, inflammation and oxidative stress accelerates the progression of atherosclerosis and endothelial dysfunction which accounts for at least some of the increase in cardiovascular mortality risk observed in the dialysis population^(78, 96). In fact, several studies have reported that malnutrition, assessed by the original and modified versions of the SGA, is a strong and independent predictor of mortality in persons receiving dialysis^(66, 68, 103, 104). As discussed above, inflammation and oxidative stress may also accelerate AGE formation. It is therefore possible that the previously reported association between increased SAF and higher all-cause as well as cardiovascular mortality in persons on HD may be explained in part by

the association between increased SAF and malnutrition that we have observed. Of note, previous studies that have reported an association between higher SAF and mortality in the dialysis population did not assess nutritional status^(196, 213, 214).

We observed that diabetes, chronological age and dialysis vintage were important determinants of increased SAF. Additionally, higher SAF was found to be more evident in HD participants with a previous history of PVD. Several cross-sectional studies conducted in the dialysis population have also found that presence of diabetes and cardiovascular disease, older age and longer dialysis vintage are associated with higher SAF^(198, 199, 202). It is well-known that cigarette smoking is an exogenous source of AGE formation⁽¹⁸⁴⁾ and in this cross-sectional analysis, smoking status was found to be an independent determinant of increased SAF. Similar associations with cigarette smoking have been reported in previous studies conducted in healthy participants^(254, 255).

The data presented should be interpreted in the light of some limitations. This study was cross-sectional and observational in design and we are therefore unable to confirm a causal relationship between higher SAF and the observed associations. Further longitudinal and interventional studies are therefore required to investigate the relationship between SAF and malnutrition.

Nevertheless, this is the first study that has investigated the association of nutritional factors and tissue AGE accumulation measured by SAF in the HD population. The sample size was relatively small and the study was conducted at a single centre. However, we were able to identify robust associations

between SAF and nutritional factors that should now be confirmed in larger studies. For technical reasons (discussed in the methods), SAF could not be assessed in persons with darker skin colour, who were excluded. Our data may therefore not be applicable to populations with dark skin colour.

CONCLUSION

We have found in this cross-sectional analysis that factors associated with malnutrition were the most important determinants of higher SAF in persons on HD, but that higher dietary intake of AGEs was not. The observed association between higher SAF and malnutrition may in part explain the previously reported association between higher SAF and mortality on HD. Our findings might have potentially important clinical implications because they suggest that correction of malnutrition may result in a reduction in SAF levels and improved survival. This hypothesis requires testing in further prospective interventional studies.

Chapter 4. Impact of nutritional factors on skin autofluorescence in persons performing peritoneal dialysis

The work in this chapter is under peer review by Clinical Kidney Journal.

ABSTRACT

Background: AGEs are markers of inflammatory and oxidative stress. Food and GDPs formed during the manufacturing of PD solutions are also important sources of AGEs. Malnutrition, oxidative stress and inflammation are closely linked, which may contribute to AGE formation. SAF, a measure of tissue AGE accumulation, is a predictor of mortality on PD. We investigated the determinants of increased SAF in persons performing PD, focusing on dietary AGE intake and markers of malnutrition.

Methods: SAF was measured in 31 PD patients using an Autofluorescence Reader. Detailed assessment of dietary intake and nutritional status was undertaken and included dietary AGE, energy, protein and fat intake, HGS, anthropometry, biochemistry and SGA.

Results: Malnourished participants tended to have higher SAF values than those well-nourished, but the difference was not statistically significant. Higher SAF was negatively associated with serum albumin, HGS and energy, protein and fat intake. Unexpectedly, SAF correlated negatively with dietary AGE intake. SAF was positively associated with dialysis vintage and total glucose exposure, and was higher in diabetic participants and current smokers.

Conclusions: SAF was associated with several markers of malnutrition and was inversely associated with dietary AGE intake. This suggests that malnutrition and glucose exposure might be more important determinants of increased SAF than high dietary AGE intake in persons on PD. Correction of malnutrition and reduction of glucose exposure may be important strategies to lower SAF. These hypotheses now require testing in prospective interventional studies.

INTRODUCTION

AGEs are a heterogeneous group of uremic toxins that accumulate in persons receiving dialysis⁽¹⁸⁵⁾. Tissue AGE accumulation can be measured non-invasively by SAF and it has been reported that persons performing PD have increased SAF levels^(202, 203). Furthermore, elevated SAF has been shown to be a predictor of cardiovascular and sepsis-related mortality in the PD population^(211, 212), but the mechanism underlying this association is not completely understood.

AGEs result from the non-enzymatic reaction of glucose molecules with free amino groups of proteins, lipids and nucleic acids (i.e. the so-called Maillard reaction) resulting in alteration in their function. AGEs may also form through other endogenous pathways, including hyperglycaemia due to diabetes (a common cause of ESKD) and oxidative stress⁽¹⁸⁴⁾. Moreover, AGEs are normally excreted by the kidneys and those with ESKD are therefore at further risk of AGE accumulation due to decreased renal clearance^(250, 251). Persons undergoing renal replacement by PD, especially those treated with glucose-

containing PD solutions, are exposed to high glucose concentrations and GDPs, which are generated in PD fluid during heat sterilization and storage, promoting the formation of AGEs in the peritoneum. GDPs and AGEs may cause anatomical and functional changes of the peritoneal membrane, such as mesothelial damage, increase peritoneal vascularization and fibrosis, which ultimately decrease the long-term viability of the peritoneum as a dialysis membrane⁽²⁴⁾. Additionally, accumulation of AGEs in the mesothelial layer, vascular walls and connective tissues of the peritoneal membrane increases with PD dialysis vintage⁽²⁵⁶⁾.

AGEs are not only generated endogenously. Cigarette smoking and food are also important sources of exogenous AGEs⁽¹⁸⁵⁾. Uncooked foods high in fat and protein content have the highest AGE levels. More importantly, foods cooked under dry and high heat conditions (e.g. frying, grilling, roasting and baking) increase their AGE content considerably. Furthermore, food processing techniques promote the formation of new AGEs⁽¹⁸⁹⁾. It has been reported that ~10% of dietary AGEs are absorbed, which significantly contributes to the total body AGE pool⁽²⁵²⁾. Studies conducted in healthy, obese and diabetic persons as well as those on PD have reported an increase in circulating AGE levels after following a high AGE diet^(229, 232, 238, 257). However, it is unknown whether dietary AGE intake contributes significantly to skin accumulation of AGEs in persons on PD.

Malnutrition is one of the most common and severe complications in persons with ESKD undergoing dialysis⁽⁷⁸⁾. Factors specifically related to the

development of malnutrition in persons performing PD may include the following: 1) inadequate dietary macro and micronutrient intake due to poor appetite and presence of gastrointestinal symptoms such as bloating, postprandial fullness and early satiety; 2) significant protein losses into the dialysate (ranging from 3-20 g/day), which is aggravated in the presence of peritonitis; and 3) presence of hypercatabolic comorbidities, infections (i.e. peritonitis), metabolic acidosis and inadequate dialysis^(89, 91). Additionally, systemic inflammation and oxidative stress (other common conditions in the dialysis population) may further contribute to the development of malnutrition, creating a vicious cycle⁽⁹⁶⁾.

In the PD population, several factors including dialysis vintage, chronological age and history of glucose exposure have been associated with increased SAF levels^(202, 203) but the impact of diet on SAF has not previously been studied. Increased dietary AGE intake may increase SAF but conversely, factors involved in the development of malnutrition may theoretically also increase SAF. Therefore, we aimed to investigate the determinants of increased SAF in persons performing PD with a particular focus on nutritional factors including dietary AGE intake and markers of malnutrition. We hypothesised that higher dietary AGE intake and malnutrition would be associated with higher SAF.

MATERIALS AND METHODS

Please refer to Chapter 2 for a detailed description of the materials and methods used in this cross-sectional study.

Study population

This cross-sectional analysis included 31 participants on PD who were dialyzed using lactate/bicarbonate-buffered 1.36% and 3.86% glucose, 7.5% icodextrin and/or 1.1% aminoacid-containing solutions. Information regarding age, sex and ethnicity, as well as duration of nephrology care, dialysis vintage, dialysis adequacy, current co-morbidities and history of cardiovascular disease was extracted from electronic medical records and recorded in a case report questionnaire. This questionnaire was also used to collect data regarding educational qualifications, socio-economic status and history of smoking. Results from routine clinical blood tests were also recorded. The volume of 1.36% and 3.86% glucose-containing solutions used per week at the time of recruitment was obtained and the history of glucose exposure was then estimated by multiplying the dialysis vintage by the weekly glucose load as previously described⁽²⁰³⁾.

Assessments

SAF was measured with a validated AGE Reader by following the method previously described by Meerwaldt et al.⁽¹⁹⁶⁾. Three 24-hour dietary recalls were used to evaluate energy, protein and fat intake. For comparison with nutritional guidelines, average daily energy and protein intake were calculated in kilocalories and grams, respectively, and expressed per kilogram of IBW. For correlations, all categories of dietary intake were expressed as absolute values. Dietary AGE intake was estimated with a FFQ previously validated in persons with diabetes⁽²⁴⁸⁾ and reported in kU/day. Anthropometric measurements and HGS were assessed according to standard methods. The 7-point scale SGA was performed to evaluate the nutritional status.

Statistical analyses

Continuous variables are presented as median (IQR) and categorical variables as percentages. Comparisons of continuous variables between two independent groups were performed using Mann Whitney U test. Non-parametric Spearman's rank correlation coefficient was used to assess the strength and direction of the monotonic relationship between continuous variables.

RESULTS

Study population characteristics

Baseline participant characteristics including demographics, clinical, biochemical and nutritional variables are presented in Table 4.1. Median age and dialysis vintage of participants performing PD were 67 (IQR 56 to 75) years and 18 (8 to 44) months, respectively. Sixty eight percent of participants were male and 87% were of white ethnicity. Diabetes, malnutrition and coronary heart disease were present in 42%, 32% and 29% of the population, respectively. Sixty eight percent of participants were treated with CAPD and 32% were treated with APD. Participants performing PD had elevated SAF levels (3.2 [2.9 to 3.7] AU) and high dietary intake of AGEs (15685 [10596 to 19977] kU/day). Participants also evidenced low protein and energy consumption in comparison with estimated nutritional requirements^(114, 125). All of the participants used 1.36% glucose-containing solutions, 35.5% used amino acid-based solutions, 87% used icodextrin and none were regularly exposed to 3.86% glucose-containing solutions.

Table 4.1 Demographic characteristics including clinical, biochemical and nutritional data

<i>Variable</i>	<i>Peritoneal dialysis (n=31)</i>
Age (years)	67 (IQR 56 to 75)
Male [n (%)]	21 (68)
White ethnicity [n (%)]	27 (87)
Educational qualifications [n (%)]	23 (74)
Unemployed [n (%)]	21 (68)
Current smoking [n (%)]	6 (19)
Diabetes [n (%)]	13 (42)
Malnutrition [n (%)]	10 (32)
Coronary heart disease [n (%)]	9 (29)
Peripheral vascular disease [n (%)]	1 (3)
Duration nephrology care (months)	54.0 (29.0 to 77.0)
Dialysis vintage (months)	18.0 (8.0 to 44.0)
Dialysis adequacy (<i>Kt/V</i>)	2.1 (1.8 to 2.8)
Total glucose exposure (g)	12376 (3808 to 41888)
Haemoglobin (g/L)	115.0 (110.0 to 122.0)
Serum albumin (g/L)	29.0 (26.0 to 32.0)
C reactive protein (mg/L)	6.0 (2.0 to 12.0)
Total cholesterol (mmol/L)	4.4 (3.7 to 5.7)
Serum creatinine (μ mol/L)	623 (506 to 842)
Serum phosphate (mmol/L)	1.47 (1.27 to 1.73)
Serum corrected calcium (mmol/L)	2.44 (2.37 to 2.55)
Serum potassium (mmol/L)	3.8 (3.4 to 4.5)
Skin autofluorescence (AU)	3.2 (2.9 to 3.7)
Dietary AGE intake (kU/day)	15685 (10596 to 19977)
Energy intake (kcal/kg/day)	17.1 (14.1 to 23.8)
Protein intake (g/kg/day)	0.8 (0.6 to 1.1)
Fat intake (g/day)	45.1 (37.1 to 66.3)
Dry weight (kg)	77.0 (68.0 to 90.4)
Body mass index (kg/m ²)	27.6 (23.2 to 30.0)
Handgrip strength (kg)	26.4 (18.3 to 39.1)
Mid-arm muscle circumference (cm ²)	25.7 (22.0 to 28.0)
Triceps skinfold thickness (mm)	16.6 (12.8 to 24.6)

AGE, advanced glycation end product; AU, arbitrary units; IQR, interquartile range; kU,

kilounits.

Factors associated with dietary AGE intake

Dietary AGE intake was not significantly associated with any other measures of nutritional intake including total energy, protein or fat intake. However, dietary AGE was positively correlated with serum phosphate, which is dependent in part on dietary intake (Table 4.2). There was no statistically significant difference in AGE intake between well-nourished and malnourished participants assessed by SGA but AGE intake did correlate positively with HGS, a composite marker of muscle function and nutritional status.

Table 4.2 Associations of dietary advanced glycation end-product intake in persons performing peritoneal dialysis

	Peritoneal dialysis (n=31)	
Factor	Dietary AGE intake (kU/day)	p Value
Gender		
Female	12754 (9939 to 16349)	0.096
Male	16243 (12732 to 21097)	
Malnutrition		
Yes	13400 (9012 to 20024)	0.35
No	15753 (12652 to 20213)	
	Spearman's Rho	p Value
Serum albumin (g/L)	-0.002	0.99
Serum phosphate (mmol/L)	0.365	0.04
Energy intake (kcal/day)	0.278	0.13
Protein intake (g/day)	0.312	0.09
Fat intake (g/day)	0.175	0.34
Body mass index (kg/m ²)	0.037	0.84
Handgrip strength (kg)	0.498	0.004
Mid-arm muscle circumference (cm ²)	-0.001	0.99

AGE, advanced glycation end product; kU, kilounits.

Determinants of skin autofluorescence

Table 4.3 shows the factors associated with SAF in univariable analyses. There were significant and positive correlations between SAF, dialysis vintage and total glucose exposure from PD solutions. Malnourished participants tended to have higher SAF values than those well-nourished, but the difference was not statistically significant (3.6 [3.1 to 3.9] vs. 3.1 [2.7 to 3.4]; $p=0.065$). A significant and negative correlation was observed between SAF and other markers of nutritional status including serum albumin and HGS. Dietary AGE and all other measures of nutritional intake (i.e. energy, protein and fat intake) were negatively correlated with SAF. Females, current smokers, participants who were unemployed and/or retired and diabetic participants had higher SAF levels than non-diabetics, males, employed participants and former or non-smokers.

DISCUSSION

We observed high levels of SAF in persons performing PD. Dialysis vintage and total glucose exposure were significant determinants of SAF. Contrary to our expectation, dietary AGE intake was negatively associated with SAF. Participants with malnutrition as assessed by SGA tended to have higher SAF than those well-nourished and higher SAF was significantly associated with several other markers of malnutrition, including lower serum albumin, lower HGS as well as lower energy, protein and fat intake. Additionally, we have confirmed previously reported associations between increased SAF and diabetes as well as smoking. These results suggest that as regards nutritional factors, malnutrition may have a greater impact on SAF than high dietary AGE intake in this population.

Table 4.3 Determinants of skin autofluorescence in univariable analysis in persons performing peritoneal dialysis

	Peritoneal dialysis (n=31)	
Factor	Skin autofluorescence (AU)	p Value
Malnutrition		
Yes	3.6 (3.1 to 3.9)	0.065
No	3.1 (2.7 to 3.4)	
Diabetes		
Yes	3.5 (3.0 to 4.0)	0.03
No	3.1 (2.7 to 3.2)	
Peripheral Vascular Disease		
Yes	3.0	0.65
No	3.2 (2.7 to 3.8)	
Gender		
Female	3.6 (3.1 to 4.0)	0.04
Male	3.1 (2.8 to 3.4)	
Smoking		
Current	3.8 (3.3 to 4.6)	0.03
Former	3.1 (2.6 to 3.2)	
History of smoking		
Yes	3.1 (2.8 to 3.8)	0.80
No	3.3 (3.0 to 3.5)	
Employed		
Yes	2.8 (2.4 to 3.0)	<0.0001
No	3.5 (3.1 to 3.8)	
	Spearman's Rho	p Value
Age (years)	0.339	0.06
Dialysis vintage (months)	0.515	0.003
Total glucose exposure (g)	0.493	0.005
C reactive protein (mg/L)	0.064	0.73
Serum albumin (g/L)	-0.540	0.002
Total cholesterol (mmol/L)	0.186	0.32
Dietary AGE intake (kU/day)	-0.449	0.01
Energy intake (kcal/day)	-0.468	0.008
Protein intake (g/day)	-0.460	0.009
Fat intake (g/day)	-0.367	0.04
SGA score	-0.233	0.21
Body mass index (kg/m ²)	-0.042	0.82
Handgrip strength (kg)	-0.527	0.002
Mid-arm muscle circumference (cm ²)	-0.154	0.41

AGE, advanced glycation end product; AU, arbitrary units; kU, kilounits; SGA, Subjective Global Assessment.

The SAF levels (3.2 [2.9 to 3.7] AU) observed in our study population were substantially higher than previously reported values in other populations, including healthy Caucasian volunteers, aged 60-70 years (2.5 ± 0.57 AU)⁽²⁵⁴⁾, persons with the metabolic syndrome (1.9 ± 0.43 AU; mean age: 45 years)⁽²⁵⁸⁾ and diabetic participants without CKD (2.8 ± 0.33 AU; mean age: 59 years)⁽²⁰²⁾, suggesting that ESKD and/or the dialysis procedure itself may contribute to increased skin accumulation of AGEs. Previous studies conducted in PD populations have reported similar SAF values (3.58 ± 0.75 AU⁽²⁰³⁾; 3.48 ± 0.7 AU⁽²⁰²⁾; 3.5 ± 0.7 AU⁽²¹²⁾). It has also been reported that persons on HD and PD have similar SAF levels^(202, 203, 212); however, Jiang et al.⁽¹⁹⁹⁾ showed that SAF was significantly higher in PD compared to HD participants in each age group and dialysis vintage category, regardless of the presence or absence of diabetes, which might be related to glucose exposure from the dialysis fluid during PD.

Malnourished PD participants evidenced higher (though not statistically significant) SAF levels than those well-nourished. In addition, higher SAF correlated significantly with other markers of malnutrition including lower serum albumin, lower HGS and lower energy, protein and fat intake. We have previously reported an association between higher SAF and markers of malnutrition in persons receiving HD⁽²⁵⁹⁾. These associations may be explained in part by the close interrelationship between malnutrition, inflammation and oxidative stress. Chronic systemic inflammation and oxidative stress have been implicated in the development of malnutrition due to their detrimental effects on nutritional status, including increased protein catabolism, muscle wasting

and resting energy expenditure, and decreased hepatic protein synthesis (e.g. albumin and pre-albumin)⁽⁹⁶⁾. Inflammation and oxidative stress are associated with endothelial dysfunction, which accelerates the progression of atherosclerosis. AGEs may contribute to the pathophysiology of cardiovascular disease observed in the ESKD population. AGEs cross-link with proteins of the extracellular matrix (e.g. collagen and elastin), altering their structural and functional properties, which translates into increased arterial stiffness and endothelial dysfunction⁽²⁰⁷⁾. In addition, AGEs worsen vascular tissue damage by binding to specific AGE receptors that induce oxidative stress and systemic inflammation⁽¹⁸⁵⁾. Together, these factors may contribute to the high cardiovascular mortality rate observed in malnourished dialysis populations^(78, 96). Furthermore, a number of prospective observational studies have shown that malnutrition strongly and independently predicts mortality in persons performing PD^(66, 104, 260). We therefore propose that the observed associations in our study population between several markers of malnutrition and elevated SAF levels might partially explain the previously reported prognostic value of increased SAF for predicting mortality in the PD population^(211, 212). It is important to highlight that nutritional status has not been previously assessed in any study that has investigated the association between higher SAF and mortality in the PD population^(202, 212, 213).

The recommended daily dietary intake of AGEs has been proposed to be less than 8000 kU⁽¹⁸⁸⁾. In our study population, we observed a high dietary AGE intake (15685 kU/day) in comparison with this recommendation. Other studies have also reported that persons performing PD have a daily dietary AGE intake

above the suggested value^(242, 257). Nevertheless, we found a negative correlation between SAF and dietary AGE intake, which was unexpected. Since high fat and high protein animal-derived foods also have a high AGE content⁽¹⁸⁹⁾, we propose that in our study lower AGE intake was a marker of poor nutritional intake, which may lead to malnutrition and high SAF. The observed negative correlation between energy, protein and fat intake and SAF, as well as a positive association between serum phosphate and dietary AGE intake (high protein foods are also high in phosphate)⁽²⁵³⁾, support this hypothesis. Additionally, HGS showed a significant and positive correlation with dietary AGE intake, which may be explained by the positive impact of nutritional intake on muscle strength^(65, 145).

In this study, dialysis vintage and history of glucose exposure from dialysis fluid (1.36% glucose-containing solutions) were positively and significantly correlated with SAF levels. High peritoneal and blood glucose concentrations likely predispose to AGE formation⁽²⁴⁾, as observed in persons with diabetes⁽²⁶¹⁾. Additionally, it has been suggested that GDPs formed during the manufacturing of PD solutions contribute to the formation of AGEs⁽²⁶²⁾. Other studies have similarly reported that elevated SAF was strongly associated with increased historical glucose exposure^(203, 211), even when persons on PD are using low-GDP containing solutions⁽²⁰³⁾. Thus, it seems likely that reduction of exposure to glucose from dialysis fluid should form a part of any strategy to reduce SAF in PD populations.

Similar to other studies conducted in dialysis populations, we observed that diabetes was associated with higher SAF^(199, 202, 203). Consistent with evidence that cigarette smoking is an exogenous source of AGE formation⁽¹⁸⁴⁾ and previous studies conducted in participants without kidney disease,^(254, 255) we observed that current smokers had higher SAF levels than non-smokers.

This study has some important limitations. First, this was a cross-sectional analysis and we were therefore unable to determine causality between higher SAF and the observed associations. In addition, the small sample size resulted in relatively low statistical power to detect significant associations and precluded multivariable analysis. Further prospective and interventional investigations with sufficient sample size are therefore needed to confirm the association between SAF and malnutrition. Nonetheless, to our knowledge, this is the first observational study that has investigated the association of dietary AGE intake, markers of malnutrition and SAF in a PD population. Secondly, SAF is affected by skin colour and pigmentation, and measurements are not reliable in persons with darker skin colour (who were excluded from our study) due to the high absorption of the excitation light. Therefore, the results observed in this cross-sectional study may not be applicable to populations with dark skin colour. Finally, we assessed glucose exposure in persons on PD only at the time of recruitment and did not take into account changes in the PD prescription that may have occurred prior to recruitment.

CONCLUSION

The present cross-sectional analysis has found that SAF was associated with several markers of malnutrition and inversely associated with dietary AGE intake, suggesting that malnutrition might be a more important risk factor for increased SAF than high dietary AGE intake in persons on PD. Our findings have significant practical and clinical applications because they suggest that improvement of the nutritional status may result in a decrease in SAF levels, whereas restriction of dietary AGE intake is unlikely to be effective and risks exacerbating malnutrition. Reduction of glucose exposure from dialysis fluid may also be important to lower SAF. Reducing SAF may in turn result in improved survival. Future prospective interventional studies are needed to test these hypotheses.

Chapter 5. Factors associated with change in skin autofluorescence in persons receiving dialysis: a prospective study

The work in this chapter is under peer review by the Journal of Nutrition.

The work in this chapter has also been presented as a poster at the following conferences:

- *“Factors associated with temporal changes in skin autofluorescence in dialysis patients”. UK Kidney Week, Harrogate, 2018 and International Congress on Renal Nutrition and Metabolism, Genoa, Italy, 2018.*
- *“Factors associated with change in skin autofluorescence in dialysis patients: a prospective study”. National Kidney Foundation Spring Clinical Meeting, Boston, US, 2019 and UK Kidney Week, Brighton, 2019.*

ABSTRACT

Background: An increase over time in SAF, a measure of tissue accumulation of AGEs, predicts higher mortality on HD. However, evidence is lacking regarding factors that contribute to changes in SAF over time in dialysis populations. We aimed to investigate the rate of change in SAF over 1 year and the factors associated with these changes in persons receiving dialysis.

Methods: This was a single-centre prospective observational study. SAF was measured in 109 HD and 28 PD patients using an Autofluorescence Reader at baseline, 3, 6, 9 and 12 months. Rate of change in SAF was calculated by fitting a regression line using the “SLOPE” function in Microsoft Excel.

Participants were classified into quartiles according to their trend values.

Dietary AGE intake and nutritional assessments were performed at baseline, 6 and 12 months.

Results: The mean SAF trend observed was an increase of 0.30 ± 0.63 AU per year but this varied from a decrease of 0.43 ± 0.45 AU to an increase of 1.05 ± 0.42 AU per year in the 1st and 4th quartiles, respectively. Increasing SAF was more common in participants who developed malnutrition during the observation period, whilst those who became well-nourished were more likely to have stable SAF (80% vs. 42%; $p=0.02$). Development/prevalence of malnutrition over 1 year, HD as first dialysis modality and current smoking were independent predictors of increasing SAF.

Conclusions: SAF increases over time in most persons on dialysis.

Independent determinants of increasing in SAF were development/prevalence of malnutrition, HD as first dialysis modality and current smoking. Strategies to reduce/prevent the rise in SAF, including prevention and/or correction of malnutrition, should be investigated in prospective studies.

INTRODUCTION

AGEs are a heterogeneous group of molecules that result from the non-enzymatic glycation of proteins, lipids and nucleic acids⁽¹⁸⁴⁾. Moreover, AGEs are uremic toxins that are markedly increased in persons with CKD, particularly on dialysis, due to increased production, impaired excretion and inefficient removal^(250, 251). AGEs are rapidly formed either endogenously during hyperglycaemia and oxidative stress or through exogenous sources such as cigarette smoke and food⁽¹⁸⁴⁾. AGEs will eventually cross-link with tissue

proteins and interact with specific AGE receptors resulting in activation of systemic inflammation and, consequently, exacerbation of tissue damage⁽¹⁸⁵⁾. Tissue AGE accumulation can be assessed non-invasively using SAF. Several cross-sectional studies have reported that persons on dialysis have increased SAF and that factors such as chronological age, diabetes, dialysis vintage and glucose exposure from PD fluid are associated with higher SAF in this population^(202, 203). We have also shown that smoking and markers of malnutrition, such as serum albumin, HGS and protein intake, are independent determinants of increased SAF in persons receiving HD⁽²⁵⁹⁾.

Previous observational studies have shown that higher baseline SAF is strongly associated with increased mortality in the dialysis population^(196, 211-213). Additionally, Arsov et al.⁽²¹⁴⁾ reported that an increase in SAF over time predicts higher mortality in HD; however, factors that contribute to the increase in SAF over time in the dialysis population have not been adequately investigated. One prospective study has reported that dialysis vintage, lower dietary AGE intake and lower calorie intake were associated with 1-year increase in SAF in univariable analysis in a HD population, but that a BMI lower than or higher than 24 kg/m² was the sole independent predictor for this observed change⁽²¹⁵⁾. Another 1-year observational study in HD patients found that older age and use of insulin, lanthanum carbonate and warfarin were independent predictors of the 1-year increase in SAF in multivariable analysis, while a vegetarian diet and residual urine output >250 ml/day were independently associated with a stable SAF⁽²¹⁶⁾. It would seem, therefore, that further investigations regarding factors that contribute to changes in SAF over

time in the dialysis population are needed. Thus, we aimed to investigate the rate of change in SAF over 1 year and the factors associated with these changes with a particular focus on nutritional aspects.

MATERIALS AND METHODS

Please refer to Chapter 2 for a detailed description of the materials and methods used in this prospective study.

Study population

One hundred and nine HD and twenty eight PD patients were enrolled in this single centre prospective observational study. Participants on HD were dialyzed at least three times per week for 3-4 h with high-flux polysulphone, polyarylethersulfone and polyvinylpyrrolidone dialyzers, while PD participants dialyzed using lactate/bicarbonate-buffered 1.36% and 3.86% glucose, 7.5% icodextrin and/or 1.1% aminoacid-containing solutions. Baseline participant characteristics including chronological age, sex, ethnicity, duration of nephrology care, dialysis vintage, dialysis adequacy, current co-morbidities and history of cardiovascular disease were extracted from electronic medical records. Information regarding educational achievement, occupation and smoking status, and routine blood tests were also recorded.

Assessments

SAF was assessed with a validated AGE Reader. The method has been previously described in more detail elsewhere⁽¹⁹⁶⁾. SAF was measured at baseline, 3, 6, 9 and 12 months. The rate of change of SAF between these five

time points (i.e. SAF trend) was then calculated by fitting a regression line using the “SLOPE” function in Microsoft Excel 2013, where the y-axis represented SAF values and the x-axis represented time points⁽²⁴⁹⁾. Participants were then classified into quartiles according to their SAF trend values. For further analysis, participants were classified as having stable SAF (Q1 and Q2) or increasing SAF (Q3 and Q4).

Detailed nutritional assessments were undertaken at baseline, 6 and 12 months and included: assessment of calorie, protein and fat intake by means of three 24-h dietary recalls; dietary AGE intake estimated with a FFQ previously validated in persons with diabetes⁽²⁴⁸⁾; anthropometric measurements, HGS, and the 7-point scale SGA to evaluate the nutritional status.

Statistical analyses

Data are presented as mean $\pm$ SD, median (IQR), percentages or OR (95% CI), as appropriate. For intragroup comparisons, Wilcoxon test was used in the case of continuous variables. Intergroup comparisons were performed using Mann-Whitney U test for continuous variables and χ^2 test or Fisher’s exact test for categorical variables. Comparisons of continuous variables across all quartiles were performed using a Kruskal-Wallis test. Multivariable logistic regression analysis was performed to identify the determinants associated with changes in SAF over 1 year (increasing versus stable). Independent variables used in the analysis were selected on the basis of significant associations in univariable analyses or biological plausibility (chronological age). Nagelkerke R^2 for the model and Hosmer and Lemeshow test p value were reported.

RESULTS

Study population characteristics

Baseline participant characteristics are shown in Table 5.1. Mean age of the whole cohort was 65 ± 14 years. Sixty six percent of participants were male, 91% were of white ethnicity and 56% commenced dialysis on HD. Current smoking and malnutrition were present in 15% and 36% of the population, respectively. Participants evidenced high dietary AGE intake (14768 ± 7659 kU/day), while energy and protein intake were found to be low in comparison with estimated nutritional requirements^(114, 125). During follow-up, 11 participants died, 7 were transplanted, 1 withdrew their consent and 1 recovered kidney function.

Factors associated with change in skin autofluorescence

Study population characteristics by SAF slope quartiles are shown in Table 5.1. The mean SAF trend observed was an increase of 0.3 ± 0.6 AU/year. Mean SAF trends by quartiles (Q) were -0.43 ± 0.45 AU/y (Q1), 0.14 ± 0.11 AU/y (Q2), 0.47 ± 0.10 AU/y (Q3) and 1.05 ± 0.42 AU/y (Q4). All participants in Q1 had a negative SAF slope. Baseline serum albumin, urea and MAMC were significantly different across SAF trend quartiles. Participants who evidenced an increase in SAF over 12 months (Q3 and Q4) were more likely to have had HD as their first modality and to be current smokers than those who had stable SAF (Q1 and Q2). Subjective Global Assessment score (i.e. degree of malnutrition) was not significantly different between stable SAF (Q1 and Q2) and increasing SAF (Q3 and Q4) groups. No associations were observed with multiple other risk factors.

Table 5.1 Baseline characteristics including clinical, biochemical and nutritional data by skin autofluorescence slope quartiles

Variable	Overall (n=137)	Q1 (n=35)	Q2 (n=34)	Q3 (n=34)	Q4 (n=34)	p Value
Skin autofluorescence slope (AU)	0.30 ± 0.63	-0.43 ± 0.45	0.14 ± 0.11	0.47 ± 0.10	1.05 ± 0.42	<0.0001
Age (years)	65 ± 14	65 ± 15	62 ± 11	65 ± 11	66 ± 18	0.4
Male [n (%)]	90 (66)	25 (71)	22 (65)	22 (65)	21 (62)	0.9
White ethnicity [n (%)]	124 (91)	31 (89)	29 (85)	30 (88)	34 (100)	0.2
Haemodialysis [n (%)]	109 (80)	29 (83)	23 (67)	26 (76)	31 (89)	0.1
<i>First dialysis modality</i>						
Haemodialysis [n (%)]	77 (56)	15 (43)	14 (41)	24 (71)	24 (71)	0.01
Peritoneal dialysis [n (%)]	60 (44)	20 (57)	20 (59)	10 (29)	10 (29)	
Qualifications [n (%)]	79 (58)	17 (49)	23 (67)	22 (65)	17 (50)	0.3
Unemployed [n (%)]	104 (76)	29 (83)	19 (56)	26 (76)	30 (88)	0.01
Current smoking [n (%)]	20 (15)	1 (3)	6 (18)	4 (12)	9 (26)	0.04
Diabetes [n (%)]	57 (42)	18 (51)	14 (41)	13 (38)	12 (35)	0.6
Malnutrition [n (%)]	49 (36)	15 (43)	14 (41)	5 (15)	15 (44)	0.03
Coronary heart disease [n (%)]	54 (39)	16 (46)	13 (38)	11 (32)	14 (41)	0.7
Peripheral vascular disease [n (%)]	9 (7)	4 (11)	4 (12)	1 (3)	0 (0)	0.1
Duration nephrology care (months)	80 (43 to 146)	91 (51 to 137)	59 (33 to 111)	93 (33 to 163)	96 (61 to 143)	0.4
Dialysis vintage (months)	27 (10 to 68)	44 (18 to 71)	29 (9 to 48)	16 (5 to 32)	36 (10 to 72)	0.06
Haemoglobin (g/L)	117 ± 13	118 ± 14	116 ± 11	116 ± 13	118 ± 13	0.4
Serum albumin (g/L)	31.6 ± 4.3	31.4 ± 3.9	32.0 ± 4.9	33.1 ± 3.4	30.1 ± 4.6	0.02

<i>Variable</i>	<i>Overall (n=137)</i>	<i>Q1 (n=35)</i>	<i>Q2 (n=34)</i>	<i>Q3 (n=34)</i>	<i>Q4 (n=34)</i>	<i>p Value</i>
C reactive protein (mg/L)	8.0 (4.0 to 17.0)	10.0 (5.0 to 23.0)	6.0 (3.0 to 13.0)	6.0 (3.0 to 12.0)	9.0 (3.0 to 31.0)	0.09
Total cholesterol (mmol/L)	4.1 ± 1.2	3.9 ± 1.0	4.3 ± 1.2	4.2 ± 1.4	3.9 ± 1.1	0.3
Urea (mmol/L)	17.5 ± 5.2	16.5 ± 5.5	18.6 ± 5.7	19.0 ± 4.8	15.7 ± 4.5	0.01
Serum creatinine (μmol/L)	642 ± 207	652 ± 179	654 ± 252	657 ± 218	603 ± 174	0.7
Serum phosphate (mmol/L)	1.57 ± 0.51	1.54 ± 0.39	1.63 ± 0.61	1.58 ± 0.55	1.53 ± 0.49	1.0
Serum corrected calcium (mmol/L)	2.43 ± 0.13	2.45 ± 0.12	2.43 ± 0.15	2.39 ± 0.11	2.47 ± 0.14	0.06
Serum potassium (mmol/L)	4.6 ± 0.7	4.7 ± 0.8	4.6 ± 0.9	4.5 ± 0.6	4.7 ± 0.7	0.4
Dietary AGE intake (kU/day)	14768 ± 7659	15330 ± 8127	16140 ± 8717	14573 ± 8439	13011 ± 6351	0.5
Energy intake (kcal/kg/day)	21.1 ± 7.6	21.1 ± 8.2	21.4 ± 8.4	20.1 ± 6.4	21.8 ± 7.5	1.0
Protein intake (g/kg/day)	0.89 ± 0.28	0.88 ± 0.31	0.89 ± 0.28	0.91 ± 0.27	0.89 ± 0.29	0.8
Fat intake (g/day)	59.1 ± 30.0	55.9 ± 30.1	59.2 ± 31.5	56.3 ± 25.0	65.0 ± 33.3	0.6
Dry weight (kg)	79.3 ± 20.2	74.6 ± 15.7	79.4 ± 17.8	84.1 ± 20.2	79.1 ± 20.7	0.3
Body mass index (kg/m ²)	27.7 ± 6.2	27.1 ± 5.3	27.1 ± 5.5	28.9 ± 5.7	27.7 ± 8.2	0.4
Handgrip strength (kg)	23.0 ± 11.0	22.1 ± 10.5	24.4 ± 12.0	24.6 ± 10.5	20.8 ± 11.0	0.3
Mid-arm muscle circumference (cm ²)	25.6 ± 3.7	24.7 ± 3.3	25.9 ± 3.7	26.6 ± 2.9	25.1 ± 4.6	0.04
Triceps skinfold thickness (mm)	17.2 ± 7.3	16.7 ± 6.0	16.6 ± 7.2	18.8 ± 7.6	16.6 ± 8.4	0.6

AGE, advanced glycation end-products; AU, arbitrary units; kU, kilounits

Table 5.2 Change in dietary intake and Subjective Global Assessment score by stable (Q1 and Q2) and increasing (Q3 and Q4) skin autofluorescence status

Variable	Baseline		Month 6		Month 12	
	Stable SAF (n=59)	Increasing SAF (n=58)	Stable SAF (n=59)	Increasing SAF (n=58)	Stable SAF (n=59)	Increasing SAF (n=58)
Dietary AGE intake (kU/day)	15310 ± 8265	14195 ± 8012	16522 ± 8475	14941 ± 7759	15760 ± 9030	16593 ± 10904†
Energy intake (kcal/kg/day)	20.4 ± 8.1	21.6 ± 7.1	20.9 ± 7.6	22.1 ± 6.9	22.3 ± 8.9	22.8 ± 10.5
Protein intake (g/kg/day)	0.84 ± 0.27	0.91 ± 0.28	0.86 ± 0.28	0.87 ± 0.27	0.88 ± 0.31	0.88 ± 0.39
Fat intake (g/day)	55.2 ± 30.6	63.2 ± 30.2	57.3 ± 27.9	61.2 ± 22.6	59.9 ± 32.3	64.9 ± 40.4
SGA score	5.8 ± 1.5	5.9 ± 1.6	6.3 ± 1.5*	6.2 ± 1.6	6.0 ± 1.6	5.9 ± 2.0

AGE, advanced glycation end-products; kU, kilounits; SAF, skin autofluorescence, SGA, Subjective Global Assessment

Values expressed as mean ± SD

*p=0.006

†p=0.01

Changes in dietary intake and SGA score over 1 year in stable SAF and increasing SAF groups are shown in Table 5.2. A significant increase in dietary AGE intake from baseline to 12 months was observed in the increasing SAF group (14195 ± 8012 vs. 16593 ± 10904 ; $p=0.01$) but not in the stable SAF group. At 6 months, SGA score improved significantly in the stable SAF group compared to baseline (6.3 ± 1.5 vs. 5.8 ± 1.5 ; $p=0.006$), but no significant change was observed in the increasing SAF group (6.2 ± 1.6 vs. 5.9 ± 1.6 ; $p=0.08$). Energy, protein and fat intake remained unchanged over 1 year in both groups. The significant improvement in SGA score in the stable SAF group might be explained by an improvement in other SGA categories rather than dietary intake, such as in gastrointestinal symptoms, functional capacity and weight gain.

Change in skin autofluorescence by nutritional status

A sub-group comparison analysis between Q1 and Q2 (stable SAF) versus Q3 and Q4 (increasing SAF) by nutritional status was conducted in 117 participants who completed 12 months of follow-up (Figure 5.1). These participants were classified according to their nutritional status over 1 year into the following groups: well-nourished throughout 1 year, malnourished throughout 1 year, well-nourished at baseline but malnourished at either 6 or 12 months, and malnourished at baseline but well-nourished at either 6 or 12 months. Participants who remained well-nourished throughout 12 months evidenced equal proportions with stable or increasing SAF, whereas those who remained malnourished and those who became well-nourished evidenced a greater proportion with stable SAF than increasing SAF. In contrast, 80% of

participants who were well-nourished initially but developed malnutrition either at 6 or 12 months evidenced increasing SAF.

Determinants of change in skin autofluorescence

Multivariable logistic regression analysis with increasing SAF versus stable SAF as the dependent variable identified HD as first dialysis modality, current smoking and the prevalence or development of malnutrition over 1 year as independent predictors of increasing SAF, but chronological age and baseline serum albumin, urea and MAMC were not (Table 5.3).

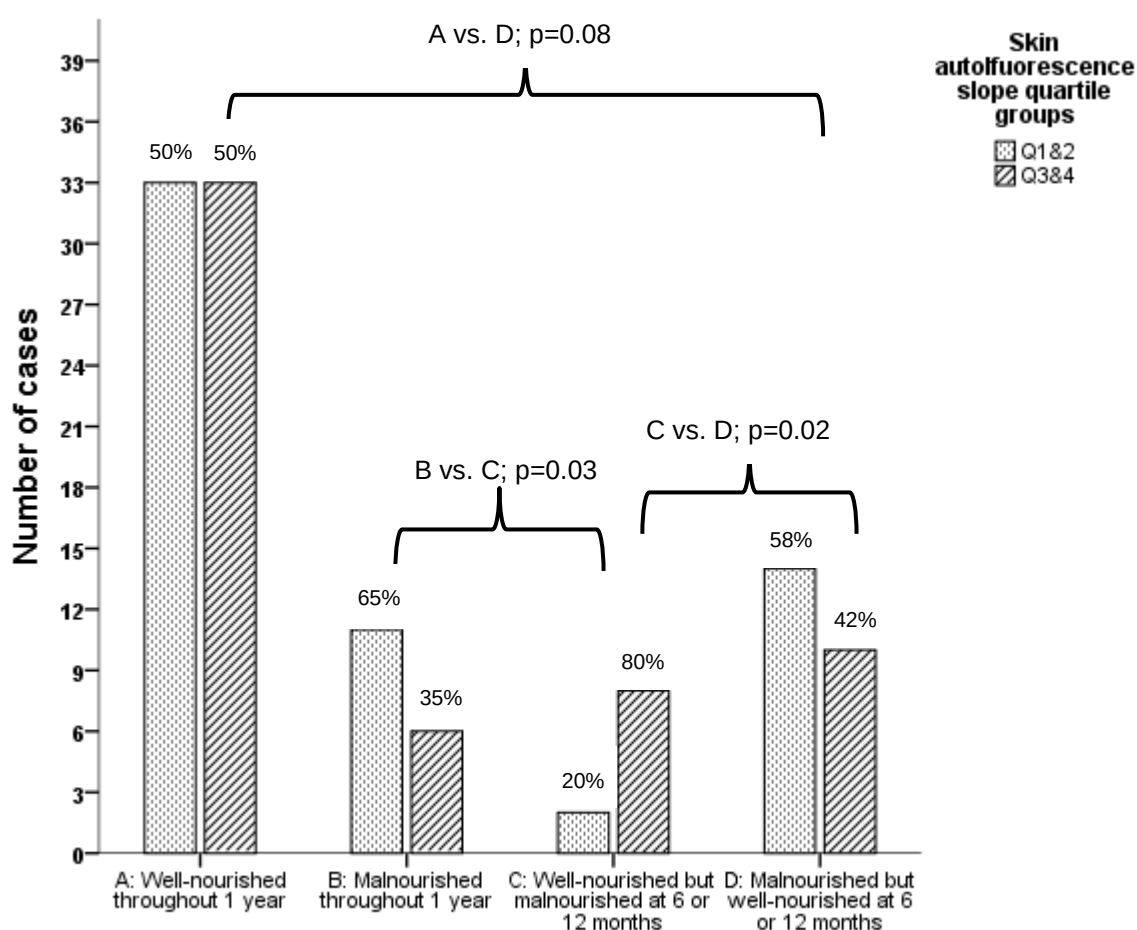


Figure 5.1 Prevalence of stable (Q1 and Q2) versus increasing (Q3 and Q4) skin autofluorescence levels over one year by change in nutritional status over one year

Table 5.3 Multivariable logistic regression analysis to identify independent determinants of change in skin autofluorescence (Increase over 12 months [Q3 & Q4] versus stable [Q1 & Q2])

<i>Factor</i>	<i>Odds ratio</i>	<i>95% CI</i>	<i>p Value</i>
Age (years)	0.99	0.97 to 1.03	0.9
First dialysis modality (Haemodialysis vs. Peritoneal dialysis)	5.17	1.99 to 13.41	0.001
Current smoking status (Current smokers vs. current non-smokers)	4.04	1.05 to 15.55	0.04
Nutritional status (Malnourished throughout the year or became malnourished vs. well-nourished throughout the year or became well-nourished)	3.07	1.10 to 8.61	0.03
Baseline serum albumin (g/L)	0.94	0.83 to 1.10	0.2
Baseline urea (mmol/L)	0.99	0.92 to 1.08	0.9
Baseline mid-arm muscle circumference (cm ²)	1.01	0.90 to 1.13	0.8

The seven variables shown were used as independent variables. Nagelkerke R^2 for the model was 0.206. Hosmer and Lemeshow test p value was 0.668

DISCUSSION

In this prospective study, we found that SAF increases over time in most persons receiving dialysis. We also identified that prevalence or development of malnutrition over 1 year, current smoking and HD as first dialysis modality were independent predictors of increasing in SAF. Interestingly, dietary AGE intake was not an independent determinant of the 1-year increase in SAF in this dialysis population. These observations confirm the findings of a previous cross-sectional study which reported that several markers of malnutrition were more important determinants of higher SAF than dietary intake of AGEs in persons receiving HD⁽²⁵⁹⁾.

The mean SAF trend observed in our dialysis population was an increase of 0.3 ± 0.6 AU/year. Some researchers have found a lower 1-year increase in SAF (0.15 ± 0.09 AU⁽²¹⁵⁾ and 0.16 ± 0.06 AU⁽²¹⁴⁾), while others have reported an increase of 0.48 ± 0.16 AU⁽²¹⁶⁾. However, these studies measured SAF only twice at 12 months apart, and are therefore subject to the statistical phenomenon known as “regression to the mean”, whereas we have used multiple SAF measurements to derive a more robust trend over time.

In this study, several markers of malnutrition including serum albumin, urea and MAMC were significantly different across SAF trend quartiles and the majority of the participants who developed malnutrition evidenced increasing SAF, while those who became well-nourished were more likely to have stable SAF. Moreover, prevalence or development of malnutrition over 1 year was an independent determinant of increasing in SAF in multivariable analysis. These

results confirm the previous observations of a cross-sectional study conducted in a HD population, in which presence of malnutrition, lower serum albumin and lower urea were significantly associated with higher SAF levels in univariable analysis, and lower protein intake, lower HGS and lower serum albumin (all markers of malnutrition) were independent predictors of higher SAF⁽²⁵⁹⁾. We propose that the association between malnutrition and higher SAF might be explained in part by a vicious cycle that develops between systemic inflammation, oxidative stress and malnutrition observed in the dialysis population. Systemic inflammation causes oxidative stress and vice versa; oxidative and inflammatory processes have synergistic effects on increasing protein catabolism and muscle mass loss, and decreasing appetite as well as hepatic albumin synthesis, which ultimately leads to the development of malnutrition^(65, 96). Thus, factors that contribute to the development of malnutrition are likely to increase AGE formation and malnutrition in turn exacerbates these factors.

We have also observed in this study that current smoking was a significant and independent predictor of increasing in SAF, confirming observations in previous cross-sectional studies conducted in healthy, diabetic and HD patients that have reported that smoking is independently associated with higher SAF levels^(255, 259, 263). Several mechanisms may explain these associations: first, cigarette smoking is a well-known source of exogenous AGEs⁽¹⁸⁴⁾; second, glycotoxins found in tobacco extracts and tobacco smoke are able to interact and cross-link rapidly with amino groups of proteins and thus enhance the endogenous formation of AGEs⁽¹⁸⁷⁾; finally, long-term exposure to cigarette

smoke has been linked with increased oxidative stress, systemic inflammation and endothelial dysfunction⁽²⁶⁴⁾.

Another independent determinant of the 1-year increase in SAF observed in this study was HD as first dialysis modality. It is well-known that the dialysis procedure itself (HD or PD) exacerbates oxidative stress and systemic inflammation⁽⁹⁷⁾; however, several studies have shown that persons receiving HD have higher levels of biomarkers of oxidative stress, including markers of glycooxidation, protein oxidation, lipid peroxidation and DNA oxidative damage, as well as higher levels of pro-inflammatory markers such as CRP, IL-6 and TNF- α , in comparison with persons performing PD. Moreover, dialysis losses of antioxidants are higher in HD than in PD, and endogenous antioxidant activity is significantly suppressed in HD compared to PD⁽²⁶⁵⁾. In addition, residual renal function declines faster in persons with CKD starting dialysis on HD vs. PD⁽²⁶⁶⁾, and it has been previously reported that the better preservation of residual renal function in PD is independently associated with decreased levels of oxidative stress⁽²⁶⁷⁾.

Arsov et al.⁽²¹⁵⁾ reported in univariable analysis that lower dietary AGE intake and lower energy intake were associated with a 1-year increase in SAF in a HD population, but that a BMI lower than or higher than 24 kg/m² was the sole independent predictor of this observed change; however, chronological age and smoking status, known predictors of SAF, were not included in the multivariable model. Moreover, although BMI is considered a key anthropometric measure of nutritional assessment in persons receiving

dialysis^(114, 125), it is a poor marker of malnutrition when used in isolation because it can be confounded by overhydration, it does not differentiate between muscle and fat mass and its interpretation can be influenced by age, gender and muscle mass⁽¹¹⁶⁾. Nongnuch et al.⁽²¹⁶⁾ reported that lower serum albumin is related to the 1-year increase in SAF in persons receiving HD and that following a vegetarian diet (which is low in dietary AGEs) is independently associated with a stable SAF over 1 year; however, smoking status was also not included in the multivariable analysis, and similar to the study by Arsov et al.⁽²¹⁵⁾, SAF was only measured at two different time points (baseline and 12 months), meaning that both studies are subject to the problem of regression to the mean, which may lead to inaccurate conclusions about the determinants of the change in SAF.

Limitations of this study include its observational design which allowed us to report only associations that do not necessarily imply causal relationships between risk factors and the rate of change in SAF over 1 year. Nevertheless, the associations from our longitudinal study provide more robust evidence than previous cross-sectional analyses. Additionally, not all dialysis participants had SAF measured at 12 months due to death, transplantation or loss to follow-up, which may have led to some selection bias. However, only 20 of 137 participants did not have SAF assessed at 12 months and the most common reasons were death (11 of 20) and transplantation (7 of 20). Given that SAF is a risk factor for death in the dialysis population, it is likely that those who died would have had higher SAF. Conversely, those who were transplanted would likely have had lower SAF due to better general health and less comorbid

conditions. It therefore seems likely that these two groups would have balanced each other making significant selection bias unlikely. This was a single centre study and results may therefore not be directly applicable to patients from other centres. The results require confirmation in larger multicentre studies. Finally, persons with dark skin colour were excluded from our study and the findings may therefore not be applicable to populations with darker skin colours.

CONCLUSION

In conclusion, we found in this prospective study that SAF increases over time in most persons receiving dialysis. Prevalence or development of malnutrition over 1 year, current smoking and HD as first dialysis modality were independent determinants of increasing SAF. The relative lack of association with other risk factors implies that SAF is a unique risk marker in the dialysis population. Strategies to reduce or prevent the rise in SAF, including prevention or treatment of malnutrition, should be investigated in prospective studies.

Chapter 6. Skin autofluorescence and malnutrition as predictors of mortality in persons receiving dialysis

The work in this chapter has been presented as a poster at the National Kidney Foundation Spring Clinical Meeting, Boston, US, 2019 and as an oral presentation at UK Kidney Week, Brighton, 2019.

ABSTRACT

Background: SAF, a measure of accumulation of AGEs, and malnutrition are each associated with higher mortality in dialysis populations, but no studies have investigated these potentially related associations together. We assessed SAF and malnutrition as risk factors for mortality in persons receiving dialysis.

Methods: This was a single centre prospective observational study that included 120 HD and 31 PD patients. SAF was measured using an AGE Reader. Dietary intake, HGS, anthropometry, biochemistry and SGA were also evaluated. Time to event was days from baseline to death, kidney transplantation or 30/9/2018.

Results: Median observation time was 576 days during which 33 (21.9%) patients died. Those who died had higher baseline SAF levels (3.8 ± 1.0 vs. 3.3 ± 0.8 AU; $p=0.001$) and were more likely to be malnourished (58% vs. 31%; $p=0.006$). Malnourished persons who died had higher SAF values than those who died but were well-nourished (4.2 ± 1.1 vs. 3.3 ± 0.7 AU; $p=0.007$). Survival was significantly better in participants with baseline SAF below the

median and in those well-nourished than those with baseline SAF above the median and in those malnourished, respectively. Multivariable analysis identified SAF (HR 1.44 [95%CI 1.05-1.97]; p=0.02), malnutrition (2.35 [1.16-4.78]; p=0.02) and chronological age (1.60 [1.10-2.33]; p=0.01) as independent predictors of mortality.

Conclusions: Though malnutrition and higher SAF are potentially inter-related, they were independently associated with increased mortality in this population. Interventions to improve outcomes by reducing SAF through dietary AGE restriction should therefore be careful to avoid exacerbating malnutrition and require testing in prospective studies.

INTRODUCTION

Dialysis is a life-prolonging therapy for persons with ESKD but is associated with a high mortality rate due in large part to increased cardiovascular disease^(13, 49). Complications such as malnutrition, systemic inflammation and oxidative stress have been proposed as non-traditional cardiovascular risk factors that contribute to the high risk of cardiovascular death observed in the ESKD population⁽⁵⁸⁾ but the manner in which these factors interact and their relative importance remain to be elucidated.

AGEs are uremic toxins that accumulate in persons receiving dialysis due to a combination of reduced renal clearance and increased formation^(250, 251).

Endogenous sources of AGEs include oxidative and carbonyl stress, as well as hyperglycaemia⁽²⁵⁰⁾. AGEs can also be formed exogenously, either by cigarette smoking or through the ingestion of foods that are cooked under dry heat

conditions and high temperatures, such as in grilling, roasting, frying and baking⁽¹⁸⁹⁾. Taking advantage of the fluorescent properties of some AGEs, tissue AGE accumulation can be assessed non-invasively by SAF, which has proven to be an independent predictor of all-cause and cardiovascular mortality in the dialysis population^(196, 211, 213); however, the mechanisms underlying this association are not completely understood.

AGEs are markers of oxidative stress, which is linked with systemic inflammation. Oxidative and inflammatory processes act synergistically in promoting protein catabolism, muscle wasting and reduced hepatic albumin synthesis, which together lead to the development of malnutrition in the setting of ESKD⁽⁹⁶⁾. Malnutrition, an independent predictor of mortality in the dialysis population^(66, 68, 103, 104), exacerbates the deleterious effects of systemic inflammation and oxidative stress by increasing the susceptibility to infections and cardiovascular disease risk^(101, 183). It therefore seems reasonable to suggest that a vicious cycle between inflammation, oxidative stress and malnutrition may contribute to the poor survival rates observed in persons on dialysis⁽¹⁰²⁾. Malnutrition and SAF have each been reported in separate studies to independently predict worse survival on dialysis but, to our knowledge, no studies have investigated the association of SAF and malnutrition with mortality together. Moreover, in a cross-sectional analysis, we have previously reported an independent association between higher SAF and several markers of malnutrition in persons on HD⁽²⁵⁹⁾. We therefore aimed to investigate the prognostic significance of SAF and malnutrition when evaluated together in

persons receiving dialysis. We hypothesised that higher SAF and malnutrition would interact and that this interaction would increase the risk of mortality.

MATERIALS AND METHODS

Please refer to Chapter 2 for a detailed description of the materials and methods used in this prospective observational study.

Study population

This was a single centre prospective observational study. Persons receiving HD and performing PD who were ≥ 18 years of age and had an expected survival more than 1 year were eligible. We enrolled 120 HD and 31 PD patients from September 2016 to August 2017. Participants on HD were dialysed 3-4 times per week for 3-4 hours using high-flux polysulphone, polyarylethersulfone or polyvinylpyrrolidone dialyzers. Persons performing PD dialysed using lactate/bicarbonate-buffered 1.36% and 3.86% glucose, 7.5% icodextrin and/or 1.1% aminoacid-containing solutions. The following exclusion criteria were used: pregnancy or intending pregnancy, breastfeeding, and having dark skin colour. Electronic medical records were used to collect the following participant characteristics: age, sex, ethnicity, dialysis vintage, dialysis adequacy, blood results, current co-morbidities and history of cardiovascular disease. Information regarding educational level, occupation and smoking status was obtained from direct interview.

Outcomes

Dates and causes of death were obtained from electronic medical records.

Causes of death were classified into the following groups⁽²⁶⁸⁾: cardiovascular,

infection, malignancy, treatment withdrawal and other cause of death. The classification was performed independently by two consultant nephrologists and any disagreements were resolved by discussion. Survival time was defined as the number of days between the baseline assessment and the date of death, censoring due to kidney transplantation or 30 September 2018.

Assessments

SAF was measured by using a validated AGE Reader as previously described⁽¹⁹⁶⁾. Average energy, protein and fat intake were assessed by means of three 24-hour dietary recalls. Energy and protein intake were reported in kcal/kg of IBW/day and g/kg of IBW/day, respectively. Fat intake was reported in g/day. A FFQ previously validated in persons with diabetes⁽²⁴⁸⁾ was used to assess dietary AGE intake (reported in kU/day). Anthropometric measurements and HGS were assessed according to standard methods. The 7-point scale SGA was performed to evaluate the nutritional status.

Statistical analyses

Data are expressed as mean $\pm$ SD, median (IQR), percentages or hazard ratios (HR [95% CI]), as appropriate. Missing data were omitted (CRP, n=7; HGS, n=6; MAC, MAMC and TSF, n=1). Comparisons of continuous variables between two independent groups were performed using Student *t* test or Mann Whitney U test, while intergroup comparisons for categorical variables were conducted with χ^2 test or Fisher's exact test. Kaplan-Meier survival curves were computed according to baseline SAF levels above or below the median value and nutritional status at baseline.

Cox proportional hazards models were used to investigate the prognostic value of SAF and malnutrition for predicting mortality. Due to the limited number of events (deaths) only the three variables of greatest interest that were associated with mortality in univariable analyses were included in the model. A product term (i.e. two-way interaction term) was included in the regression model to test for an interaction between SAF and malnutrition. For all statistical analyses, a p-value less than 0.05 was considered to have statistical significance. Sample size determination with mortality as the primary outcome, showed that with a sample size of 150 participants split in 2 groups (group 1: SAF below the median [$n=75$]; group 2 SAF above the median [$n=75$]), the analysis would have more than 80% power to detect a HR of 2.7, assuming that the total number of events (i.e. deaths) observed was 34 and the average probability of the event was 0.1 in group 1 and 0.35 in group 2.

RESULTS

Study population characteristics

Baseline characteristics are shown in Table 6.1. Mean age of the whole cohort was 64 ± 14 years. Sixty four percent of the population were male and 88% were of white ethnicity. Diabetes, heart disease and obesity were present in 41%, 40% and 36% of the population, respectively. Mean SAF was high at 3.4 ± 0.9 AU compared to the reference value of 2.5 ± 0.6 AU for the age group of 60-70 years⁽²⁵⁴⁾. Dietary AGE intake was also high in comparison to the recommended intake of less than 8000 kU/day⁽¹⁸⁸⁾. Participants had low calorie and protein intake compared to the estimated nutritional requirements for persons receiving dialysis⁽¹¹⁴⁾.

Table 6.1 Baseline participant characteristics by nutritional status classification

<i>Variable</i>	<i>Overall (n=151)</i>	<i>Malnourished (n=56)</i>	<i>Well-nourished (n=95)</i>	<i>p Value^b</i>
Age (years)	64 ± 14	62 ± 16	65 ± 13	0.5
Female [n (%)]	54 (36)	30 (54)	24 (25)	<0.0001
White ethnicity [n (%)]	133 (88)	47 (84)	86 (91)	0.2
Educational qualifications [n (%)]	85 (56)	28 (50)	57 (60)	0.2
Unemployed [n (%)]	114 (76)	48 (86)	66 (70)	0.03
Current smoking [n (%)]	23 (15)	16 (29)	7 (7)	<0.0001
Diabetes [n (%)]	62 (41)	21 (38)	41 (43)	0.5
Coronary heart disease [n (%)]	60 (40)	29 (52)	31 (33)	0.02
Peripheral vascular disease [n (%)]	11 (7)	5 (9)	6 (6)	0.6
Obesity [n (%)]	55 (36)	11 (20)	44 (46)	0.001
Dialysis vintage (months)	29 (IQR 10 to 69)	31 (11 to 61)	26 (10 to 67)	0.4
Dialysis adequacy (<i>Kt/V</i>) ^a	1.45 ± 0.59	1.54 ± 0.62	1.40 ± 0.56	0.1
Serum albumin (g/L)	31.5 ± 4.6	30.1 ± 5.4	32.4 ± 3.8	0.02
C reactive protein (mg/L)	8 (4 to 17)	9 (3 to 23)	8 (4 to 14)	0.6
Total cholesterol (mmol/L)	4.1 ± 1.2	4.2 ± 1.3	4.1 ± 1.1	0.9

<i>Variable</i>	<i>Overall (n=151)</i>	<i>Malnourished (n=56)</i>	<i>Well-nourished (n=95)</i>	<i>p Value^b</i>
Serum creatinine (μmol/L)	646 ± 214	607 ± 211	669 ± 214	0.06
Serum phosphate (mmol/L)	1.56 ± 0.51	1.64 ± 0.56	1.51 ± 0.47	0.2
Serum potassium (mmol/L)	4.6 ± 0.7	4.5 ± 0.7	4.7 ± 0.7	0.2
Skin autofluorescence (AU)	3.4 ± 0.9	3.6 ± 1.0	3.2 ± 0.7	0.009
Dietary AGE intake (kU/day)	14545 ± 781	12763 ± 6580	15595 ± 8315	0.02
Energy intake (kcal/kg/day)	20.9 ± 7.6	18.6 ± 7.6	22.2 ± 7.3	0.001
Protein intake (g/kg/day)	0.88 ± 0.29	0.79 ± 0.32	0.94 ± 0.25	<0.0001
Fat intake (g/day)	57.9 ± 29.7	47.9 ± 24.2	63.8 ± 31.2	<0.0001
Dry weight (kg)	79.3 ± 20.8	69.7 ± 17.8	85.0 ± 20.4	<0.0001
Body mass index (kg/m ²)	27.7 ± 6.3	25.1 ± 5.1	29.2 ± 6.4	<0.0001
Handgrip strength (kg)	23.0 ± 11.5	19.1 ± 10.9	25.1 ± 11.3	0.001
Mid-arm muscle circumference (cm ²)	25.6 ± 3.7	23.9 ± 3.6	26.6 ± 3.5	<0.0001
Triceps skinfold thickness (mm)	17.2 ± 7.2	15.1 ± 6.3	18.4 ± 7.5	0.02

AGE, advanced glycation end-product; AU, arbitrary units; IQR, interquartile range; kU, kilounits.

^aKt/V is weekly in peritoneal dialysis and per session in haemodialysis; ^bMalnourished vs. Well-nourished

Malnutrition was present in 37% of the population. SAF was significantly higher in malnourished participants compared to those who were well-nourished. Serum albumin, dietary AGE, energy, protein and fat intake, HGS and all anthropometric variables were significantly lower in malnourished versus well-nourished participants. Females, current smokers and those who were unemployed were more likely to be malnourished than males, non-smokers and employed participants, respectively. Coronary heart disease was more evident in malnourished participants (Table 6.1).

Follow-up results

Median observation time was 576 days (IQR 395 to 684 days) during which 33 (21.9%) participants died and 22 (14.6%) received a kidney transplant. The most common cause of death was infection (33.3%) followed by cardiovascular (30.3%), treatment withdrawal (15.1%), other cause of death (12.1%) and cancer (9.2%).

Table 6.2 shows baseline participant characteristics according to survival status. Participants who died had significantly higher baseline SAF and CRP levels compared to those who did not die. Non-survivors were more likely to be malnourished in comparison to survivors. Non-survivors also had significantly lower serum albumin, HGS and energy, protein and fat intake compared to survivors. Older age, lack of educational qualifications and unemployment were more evident among non-survivors than in survivors.

Table 6.2 Comparison of baseline participant characteristics between survivors and non-survivors

<i>Variable</i>	<i>Non-survivors (n=33)</i>	<i>Survivors (n=118)</i>	<i>p Value^a</i>
Age (years)	69 ± 12	62 ± 14	0.004
Female [n (%)]	13 (39)	41 (35)	0.6
Educational qualifications [n (%)]	20 (61)	46 (39)	0.03
Unemployed [n (%)]	30 (91)	84 (71)	0.02
Current smoking [n (%)]	6 (18)	17 (14)	0.6
Diabetes [n (%)]	15 (46)	47 (40)	0.6
Coronary heart disease [n (%)]	17 (52)	43 (36)	0.1
Malnutrition [n (%)]	19 (58)	37 (31)	0.006
Dialysis vintage (months)	43 (IQR 20 to 71)	25 (8 to 68)	0.1
Serum albumin (g/L)	29.5 ± 6.1	32.1 ± 3.9	0.02
C reactive protein (mg/L)	11 (5 to 29)	7 (3 to 14)	0.04
Total cholesterol (mmol/L)	4.0 ± 1.2	4.1 ± 1.2	0.6
Serum creatinine (µmol/L)	657 ± 206	643 ± 217	0.8
Skin autofluorescence (AU)	3.8 ± 1.0	3.3 ± 0.8	0.002
Dietary AGE intake (kU/day)	13381 ± 7032	14870 ± 8019	0.4
Energy intake (kcal/kg/day)	18.0 ± 6.2	21.7 ± 7.8	0.01
Protein intake (g/kg/day)	0.75 ± 0.25	0.92 ± 0.29	0.003
Fat intake (g/day)	48.6 ± 24.7	60.5 ± 30.6	0.02
Body mass index (kg/m ²)	28.3 ± 6.5	27.5 ± 6.2	0.6
Handgrip strength (kg)	18.6 ± 10.7	24.1 ± 11.5	0.008
MAMC (cm ²)	25.7 ± 4.5	25.6 ± 3.5	0.7
Triceps skinfold thickness (mm)	18.5 ± 6.7	16.9 ± 7.4	0.2

AGE, advanced glycation end-product; AU, arbitrary units; IQR, interquartile range; kU, kilounits; MAMC, mid-arm muscle circumference.

^aNon-survivors vs. survivors.

Participants who died and were malnourished ($n=19$) had significantly higher SAF levels and lower serum albumin, serum creatinine, dietary AGE, energy, protein and fat intake, BMI, HGS, MAMC and TSF in comparison to those who died but were well-nourished ($n=14$). These data are summarised in Table 6.3. Kaplan-Meier analysis showed that survival was significantly better in those participants with a baseline SAF level below the median value of 3.3 AU and in those who were well-nourished at baseline in comparison to those with baseline SAF level above the median value and in those malnourished at baseline, respectively (Figure 6.1 and Figure 6.1). Seventy six percent ($n=25$) of participants who died had SAF levels above the median value. Causes of death were not significantly different between those participants with baseline SAF level below the median value and those who were well-nourished at baseline compared to those with baseline SAF level above the median value and those malnourished at baseline, respectively (Table 6.4).

Univariable analysis identified malnutrition, no educational qualifications, higher SAF, chronological age and lower serum albumin, HGS and energy, protein and fat intake as significant determinants of higher mortality.

Multivariable Cox proportional hazards analysis identified SAF (HR 1.44 [95%CI 1.05 to 1.97] per SD; $p=0.02$), malnutrition (2.35 [1.16 to 4.78]; $p=0.02$) and chronological age (1.60 [1.10 to 2.33] per SD; $p=0.01$) as independent predictors of mortality (Table 6.5). Inclusion of a product term in the model showed no interaction between SAF and malnutrition.

Table 6.3 Comparison of non-survivor characteristics according to nutritional status at baseline

Variable	Malnourished (n=19)	Well-nourished (n=14)	p Value ^a
Age (years)	71 ± 10	68 ± 15	0.6
Female [n (%)]	10 (53)	3 (21)	0.07
Educational qualifications [n (%)]	14 (74)	6 (43)	0.2
Unemployed [n (%)]	18 (95)	12 (86)	0.6
Current smoking [n (%)]	4 (21)	2 (14)	1.0
Diabetes [n (%)]	9 (47)	6 (43)	1.0
Coronary heart disease [n (%)]	11 (58)	6 (43)	0.5
Dialysis vintage (months)	37 (IQR 22 to 72)	44 (14 to 68)	0.8
Serum albumin (g/L)	27.3 ± 6.8	32.4 ± 3.4	0.03
C reactive protein (mg/L)	17 (4 to 43)	11 (6 to 16)	0.6
Total cholesterol (mmol/L)	4.0 ± 1.4	4.0 ± 0.9	0.8
Serum creatinine (µmol/L)	588 ± 178	751 ± 209	0.02
Skin autofluorescence (AU)	4.2 ± 1.1	3.3 ± 0.7	0.02
Dietary AGE intake (kU/day)	10921 ± 5470	16719 ± 7711	0.01
Energy intake (kcal/kg/day)	15.9 ± 4.4	20.8 ± 7.3	0.03
Protein intake (g/kg/day)	0.66 ± 0.21	0.88 ± 0.26	0.03
Fat intake (g/day)	40.0 ± 14.8	60.2 ± 30.8	0.04
Body mass index (kg/m ²)	26.1 ± 4.7	31.3 ± 7.5	0.03
Handgrip strength (kg)	13 (10 to 18)	21 (15 to 27)	0.02
Mid-arm muscle circumference (cm ²)	24.2 ± 3.7	27.7 ± 4.7	0.03
Triceps skinfold thickness (mm)	16.0 ± 5.8	21.6 ± 6.7	0.03

AGE, advanced glycation end-product; AU, arbitrary units; IQR, interquartile range; kU, kilounits.

^aMalnourished vs. well-nourished.

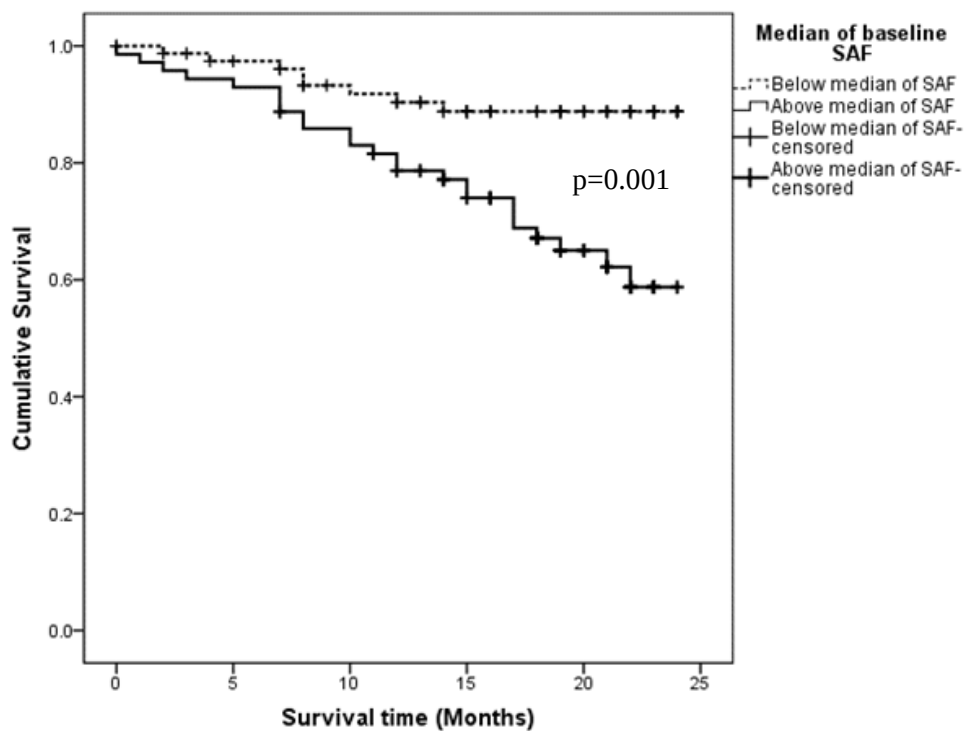


Figure 6.1 Kaplan-Meier plot of survival according to baseline skin autofluorescence above or below the median value

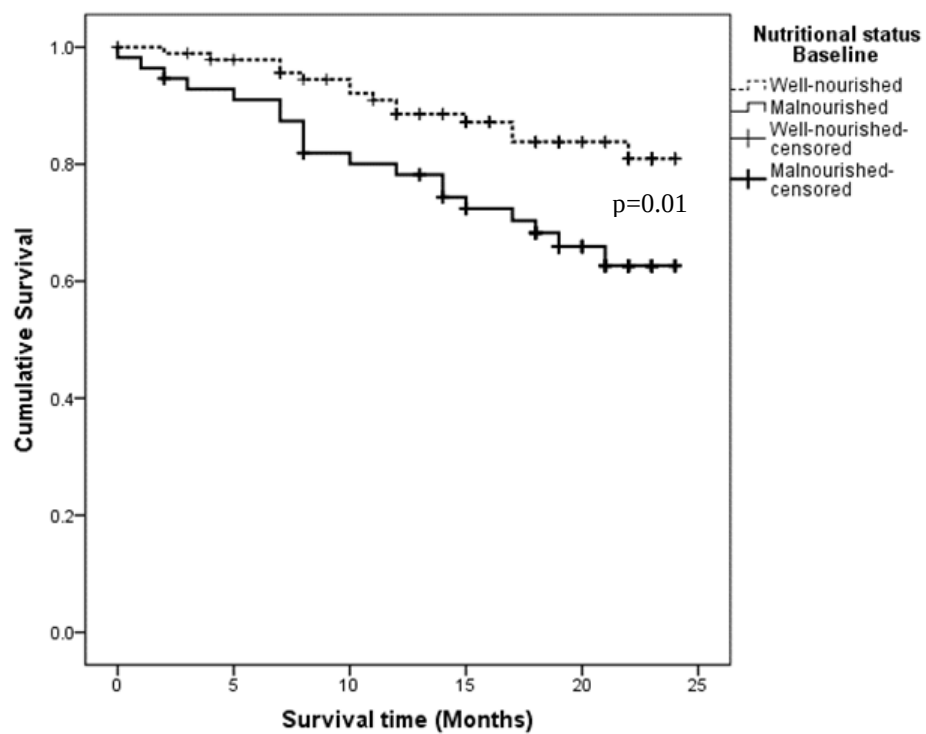


Figure 6.1 Kaplan-Meier plot of survival according to nutritional status at baseline

Table 6.4 Causes of death according to baseline skin autofluorescence below or above the median value and nutrition status at baseline.

<i>Cause of death</i>	<i>All (n=33)</i>	<i>SAF below median (n=8)</i>	<i>SAF above median (n=25)*</i>	<i>Well-nourished (n=14)</i>	<i>Malnourished (n=19)†</i>
Infection [n (%)]	11 (33.3)	3 (37.5)	8 (32.0)	5 (35.7)	6 (31.6)
Cardiovascular [n (%)]	10 (30.3)	2 (25.0)	8 (32.0)	3 (21.4)	7 (36.8)
Treatment withdrawal [n (%)]	5 (15.1)	1 (12.5)	4 (16.0)	2 (14.3)	3 (15.8)
Cancer [n (%)]	3 (9.2)	0 (0.0)	3 (12.0)	2 (14.3)	1 (5.3)
Other [n (%)]	4 (12.1)	2 (25.0)	2 (8.0)	2 (14.3)	2 (10.5)

SAF, skin autofluorescence

*SAF below median vs SAF above median p value = 0.626

†Well-nourished vs malnourished p value = 0.826

Table 6.5 Cox proportional hazards analysis showing predictors of overall mortality in persons receiving dialysis

<i>Predictor</i>	<i>Univariable analysis</i>		<i>Multivariable analysis</i>	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Age (years)	1.53 (1.04 to 2.24)	0.03	1.60 (1.10 to 2.33)	0.01
Female	1.18 (0.59 to 2.37)	0.7		
No educational qualifications	2.09 (1.04 to 4.12)	0.04		
Current smoking	1.15 (0.48 to 2.79)	0.8		
Diabetes	1.12 (0.57 to 2.23)	0.7		
Coronary heart disease	1.59 (0.80 to 3.15)	0.2		
Malnutrition	2.30 (1.15 to 4.59)	0.02	2.35 (1.16 to 4.78)	0.02
Dialysis vintage (months)	1.00 (0.99 to 1.00)	0.7		
Serum albumin (g/L)	0.89 (0.84 to 0.96)	0.001		
C reactive protein (mg/L)	1.01 (1.00 to 1.03)	0.06		
Total cholesterol (mmol/L)	0.92 (0.67 to 1.25)	0.6		
Serum creatinine (μ mol/L)	1.00 (0.99 to 1.00)	0.6		
Skin autofluorescence (AU)	1.55 (1.16 to 2.07)	0.003	1.44 (1.05 to 1.97)	0.02
Dietary AGE intake (kU/day)	1.00 (1.00 to 1.00)	0.4		
Energy intake (kcal/kg/day)	0.93 (0.88 to 0.99)	0.02		
Protein intake (g/kg/day)	0.16 (0.04 to 0.61)	0.008		
Fat intake (g/day)	0.98 (0.97 to 0.99)	0.03		
Body mass index (kg/m^2)	1.02 (0.97 to 1.07)	0.5		
Handgrip strength (kg)	0.96 (0.92 to 0.99)	0.04		
Mid-arm muscle circumference (cm^2)	1.01 (0.92 to 1.11)	0.8		
Triceps skinfold thickness (mm)	1.03 (0.98 to 1.07)	0.2		

AGE, advanced glycation end-product; AU, arbitrary units; IQR, interquartile range; kU, kilounits.

DISCUSSION

In this prospective observational study, we found that higher SAF and malnutrition were significant and independent predictors of increased mortality in persons receiving dialysis. We also identified that several markers of malnutrition such as serum albumin, HGS and energy, protein and fat intake were important determinants of higher mortality, and that SAF was significantly increased among those dialysis patients who died and were malnourished.

The association between SAF and increased mortality in dialysis populations has been previously investigated by several researchers. In one of the first prospective studies carried out in this area⁽¹⁹⁶⁾, higher SAF was independently associated with a 4-fold increased mortality risk over 3 years, with cardiovascular disease being the main cause of death. We have observed a 44% higher risk of all-cause mortality for each SD increase in SAF. Similarly, Mukai et al.⁽²¹³⁾ reported that each one SD increase in SAF was independently associated with a 56% higher risk of death. Several other observational studies have also reported that increased SAF independently predicts higher rates of all-cause and cardiovascular mortality in persons receiving dialysis^(200, 209-212). Multiple mechanisms may explain the association between increased SAF and mortality. AGEs cross-link proteins of the extracellular matrix (e.g. collagen and elastin), altering their structural and functional properties, which translates into increased arterial stiffness and endothelial dysfunction⁽²⁰⁷⁾ both of which contribute to the pathogenesis of cardiovascular disease. In addition, AGEs exacerbate vascular tissue damage by binding to specific AGE receptors that

induce oxidative stress and systemic inflammation⁽¹⁸⁵⁾. Systemic inflammation in turn promotes endothelial dysfunction and vascular calcification⁽¹⁸²⁾. Oxidative stress, alongside inflammation, also causes endothelial dysfunction which leads to the development and progression of atherosclerosis^(72, 73, 96, 97). Additionally, the association may be explained, at least in part, by an association between increased SAF and malnutrition⁽²⁵⁹⁾, a well described risk factor for reduced survival on dialysis, but none of the aforementioned prospective studies included an assessment of nutritional status. We have now filled this knowledge gap by showing that SAF and malnutrition are independent predictors of higher mortality on dialysis.

Malnutrition is one of the major and most prevalent complications in persons receiving dialysis. We found in our study that malnutrition, as assessed by the 7-point scale SGA, was a significant and independent determinant of increased mortality. Several prospective studies have also reported that malnutrition, evaluated by the original and modified versions of the SGA, is independently and strongly associated with overall and cardiovascular mortality in the dialysis population^(66, 68, 103, 104). This association might be partially explained by the interaction between multiple risk factors related with malnutrition that are also associated with increased mortality, including inadequate dietary intake^(71, 139-141), presence of cardiovascular disease⁽⁴⁹⁾, metabolic acidosis⁽³³⁾, and most importantly, systemic inflammation⁽⁴⁷⁾ and oxidative stress⁽⁷²⁾.

We have previously reported in a cross-sectional analysis that SAF was significantly higher among malnourished persons on HD and that markers of malnutrition such as lower serum albumin, lower protein intake and lower HGS

were independent determinants of increased SAF⁽²⁵⁹⁾. The current study extends these observations by showing that higher SAF and malnutrition were independent risk factors for higher mortality, though no interaction was found on statistical testing. Additionally, SAF was significantly higher in non-survivors who were malnourished compared to those who died but were well-nourished. Furthermore, lower serum albumin, HGS and energy, protein and fat intake were significant determinants of higher mortality in univariable analyses. The association between higher SAF and malnutrition may be explained by overlapping mechanisms that likely contribute to both. AGEs are rapidly formed during oxidative stress. Oxidative stress in turn promotes systemic inflammation and vice versa. Chronic inflammation and oxidative stress together induce a number of mechanisms including increased muscle proteolysis, decreased appetite and hypoalbuminemia, which ultimately lead to the development of malnutrition^(65, 96). Malnutrition may also contribute to AGE formation through its interaction with oxidative and inflammatory processes. Thus, a vicious cycle likely exists between chronic inflammation, oxidative stress and malnutrition to explain the association between SAF, malnutrition and increased mortality.

There are some limitations that need to be considered when interpreting our findings. First, we included subjects from a single centre and the sample size was relatively small, which prevented us from including more variables in the multivariable Cox proportional hazards model. Second, the follow-up period was relatively short and a longer observation period may have revealed more evidence of interaction between higher SAF, malnutrition and mortality. Third,

we limited our analysis to consideration of baseline variables whereas a larger study would have allowed inclusion of change in SAF and change in nutritional status as time varying factors. Nevertheless, unlike previous cohorts, this study has prospectively assessed the interaction between SAF and malnutrition for predicting mortality. The observational design of this study did not allow us to assess causality; however, our results suggest that the risk of death is considerably higher among those subjects with increased SAF and malnutrition. Finally, SAF measurements cannot be performed in subjects with Fitzpatrick skin types 5-6 (i.e. dark skin colour) who were excluded from this study; therefore, our findings may be only applicable to persons with Fitzpatrick skin types 1-4.

CONCLUSION

In conclusion, although higher SAF and malnutrition are potentially inter-related, we have found that they were independently associated with increased mortality in this dialysis population. These findings potentially provide a dual approach to improve clinical outcomes by focusing in the prevention and/or correction of malnutrition and in addressing other factors associated with increased SAF. Our observations have also strengthened the role of SAF as an independent risk factor in the dialysis population and suggest that interventions to reduce SAF may result in improved survival, though this should now be tested in prospective trials. An additional practical implication is that interventions aimed at improving outcomes by reducing SAF through dietary AGE restriction should be careful to avoid exacerbating malnutrition.

Chapter 7. Impact of dietetic intervention on skin autofluorescence and nutritional status in persons receiving dialysis: a proof of principle study

The work in this chapter has been presented as a poster at UK Kidney Week, Brighton, 2019.

ABSTRACT

Background: AGEs are uremic toxins that result from hyperglycaemia, oxidative stress, smoking and food consumption. We have previously reported that markers of malnutrition are more important determinants of increased SAF, a measure of AGE accumulation, than high dietary AGE intake in a dialysis population, suggesting that correcting malnutrition may decrease SAF. We investigated this hypothesis in a prospective proof of principle study.

Methods: We studied 1 PD and 27 HD patients with malnutrition who received individualised nutritional advice over 6 months. SAF was measured using an AGE Reader at baseline, 3 and 6 months. Detailed assessment of dietary intake and nutritional status was undertaken at baseline and 6 months. We compared the results with a control group of malnourished dialysis patients ($n=41$ HD and 8 PD) taken from Study 1.

Results: The intervention group showed a significant increase in all nutritional intake components, including AGEs, as well as SGA and serum albumin, while SAF levels remained stable over 6 months. In contrast, in the control group SAF increased significantly during the observation period when there was no

improvement in nutritional intake and several markers of malnutrition, though dietary AGE intake and SGA did increase.

Conclusion: Individualised dietetic advice was associated with improvement in dietary intake, markers of malnutrition and stable SAF levels, despite an increase in dietary AGE intake, suggesting that dietetic interventions may be effective in preventing the rise in SAF observed in malnourished dialysis populations over time. Further long-term nutritional interventions with larger sample sizes are needed to test this hypothesis and evaluate the impact on survival.

INTRODUCTION

AGEs are uremic toxins that accumulate in persons on dialysis due to reduced renal clearance and increased production^(250, 251). Key endogenous pathways leading to the formation of AGEs include hyperglycaemia due to diabetes, increased oxidative and carbonyl stress, and systemic inflammation. AGEs are also formed exogenously, either by cigarette smoking or through food consumption⁽¹⁸⁴⁾. High fat and high protein diets, dry-heated processed foods and cooking techniques using dry heat and high temperatures (e.g. grilling, roasting, broiling, baking and frying) significantly increase AGE formation^(188, 189). On the other hand, malnutrition may also be associated with AGE formation by provoking systemic inflammation and oxidative stress⁽²⁵⁹⁾. AGEs cross-link with tissue proteins; it seems that collagen in the skin and elastin in vascular basement membranes are especially susceptible to AGE accumulation⁽¹⁸⁵⁾. Due to the fluorescent nature of some AGEs, tissue AGE accumulation can be assessed using a non-invasive, operator independent and

easy to perform technique called SAF, which has been shown to be an independent predictor of mortality in the dialysis population^(196, 211, 212).

Several randomised controlled trials conducted in healthy overweight and/or obese volunteers⁽²³⁰⁻²³²⁾, persons with diabetes and the metabolic syndrome⁽²³⁶⁻²³⁸⁾, and in those with CKD⁽²⁴¹⁾ and performing PD⁽²⁴²⁾ have reported that restriction of dietary AGE intake is associated with a reduction in circulating AGE levels, suggesting that a low AGE diet may be also associated with a decrease in SAF. Nevertheless, as presented in Chapter 3, we have previously reported in a cross-sectional analysis conducted in persons on HD that presence of malnutrition was associated with higher SAF levels, that lower serum albumin, lower HGS and lower dietary protein intake (all markers of malnutrition) were independent determinants of increased SAF, and that high dietary AGE intake was not associated with higher SAF⁽²⁵⁹⁾. Additionally, in persons performing PD, higher SAF was associated with lower serum albumin and HGS as well as lower dietary energy, protein, fat and AGE intake (Chapter 4). Moreover, in a prospective study we observed that the development or prevalence of malnutrition was an independent determinant of an increasing trend in SAF over 12 months (Chapter 5). Together these findings suggest that in persons receiving dialysis, correction of malnutrition may be a better strategy to decrease SAF levels than dietary AGE restriction. We therefore aimed to investigate whether improvement of nutritional status by providing intensive individualised dietetic advice would result in a decrease in SAF in persons receiving dialysis.

MATERIALS AND METHODS

Please refer to Chapter 2 for a detailed description of the materials and methods used in this study.

Study population

This observational non-randomized proof of principle study initially included 30 HD and 2 PD patients with malnutrition (i.e. SGA score of ≤ 5) who were ≥ 18 years old and were able to give written informed consent. Participants on HD were dialysing 3-4 times per week for 3-4 hours with high-flux polysulphone, polyarylethersulfone and polyvinylpyrrolidone dialyzers, while PD participants were dialyzed using lactate/bicarbonate-buffered 1.36% glucose, 7.5% icodextrin and/or 1.1% aminoacid-containing solutions. Baseline participant characteristics including demographics, clinical and biochemical data were collected from electronic medical records and from a self-report questionnaire.

Intervention

Participants received individualized nutritional advice and support formulated and delivered by experienced dietitians consisting of food fortification recommendations and oral nutritional supplementation aiming to achieve estimated nutritional requirements (i.e. energy [30-35 kcal/kg/day] and protein intake [1.1-1.2 g/kg/day])⁽¹¹⁴⁾. Advice was individualized according to patient needs and food preferences. Participants were then followed up for 6 months.

Food fortification involved enhancing the energy and protein content of meals and snacks without increasing the portion sizes of foods. Oral nutritional supplements included Fortisip Compact (2.4 kcal/ml), Fortisip (1.5 kcal/ml), Fortijuice (1.5 kcal/ml), Renapro® shot and Fresubin® 5kcal Shot. Participants received precise oral and written instructions on how and when to take the supplements and how to follow the food fortification advice provided. Participants were closely monitored by the dietitian at least once a week to encourage adherence to nutritional advice, and 24-hour dietary recalls were also conducted to ensure compliance with the advice provided. Each participant was also reviewed quarterly with the clinical lead renal dietitian in order to modify the dietetic advice, if required.

Assessments

SAF was measured using a validated AGE Reader at baseline, 3 and 6 months according to the method previously described by Meerwaldt et al.⁽¹⁹⁶⁾. Detailed assessment of dietary intake and nutritional status was undertaken at baseline and 6 months, including energy, protein, fat and dietary AGE intake, HGS, anthropometric measurements and SGA. Because of the nature of the intervention (i.e. dietetic), blinding of participants and investigators was not possible. Nevertheless, in order to reduce the risk of bias, nutritional status evaluation by SGA was conducted by an experienced dietitian who did not participate in other methods of nutritional assessment and did not conduct SAF measurements. Routine biochemical variables were also measured at baseline and 6 months.

Controls

We considered it unethical to randomise persons with malnutrition to no intervention and therefore compared the results with a historical control group of malnourished persons on dialysis (n=41 HD and 8 PD) taken from Study 1, who were assessed at the same time points and using the same methodology.

Statistical analyses

Data are presented as mean $\pm$ SD, median (IQR) or percentages, as appropriate. For intragroup comparisons, Wilcoxon test was used in the case of continuous variables. Intergroup comparisons were performed using Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables.

RESULTS

Participants

A total of 188 dialysis patients were assessed for eligibility from January to June 2018. Of these, 142 did not meet inclusion criteria, 13 declined to participate and one died after agreeing to take part in the study. A total of 32 dialysis patients were therefore enrolled. Four patients did not complete the study protocol (3 deaths and 1 consent withdrawal). Twenty eight patients completed 6 months of intervention and were therefore included in the final analysis. Historical controls were selected from a previous cohort (i.e. Study 1) of 151 dialysis patients. Of these, 56 were classified as being malnourished (i.e. SGA score ≤ 5) at baseline. Forty nine patients completed 6 months of follow-up (i.e. 5 deaths and 2 transplants) and were included in the final analysis (Figure 7.1).

Baseline participant characteristics

Demographic, clinical, biochemical and nutritional characteristics of the intervention and historical control groups are shown in Table 7.1. Participants in the intervention group had significantly longer dialysis vintage, higher energy and fat intake and lower BMI in comparison to the historical control group at baseline, though dietary energy intake was below estimated nutritional requirements in both groups. There were no other significant differences between the groups at baseline.

Follow-up

Table 7.1 shows changes in SAF, biochemical variables and nutritional markers from baseline to 6 months. In the intervention group, we observed a significant increase in intake of all dietary components, including AGEs (13823 [10840 to 20441] kU/day vs 19074 [13372 to 27340] kU/day; $p < 0.0001$), as well as in SGA score and serum albumin. SAF levels did not change significantly over 6 months in the intervention group (3.8 ± 0.7 AU vs. 3.7 ± 0.7 AU; $p = 0.3$) (Figure 7.2). In contrast, in the historical control group there was no increase in nutritional intake, which remained below estimated nutritional requirements, except for dietary AGE intake, which did increase (11940 [8787 to 15833] kU/day vs 14697 [9427 to 19071] kU/day; $p = 0.03$). SGA score increased in the historical control group but other markers of nutrition did not change. SAF increased significantly in the control group (3.5 ± 0.9 AU vs. 3.8 ± 1.2 AU; $p = 0.03$) (Figure 7.2).

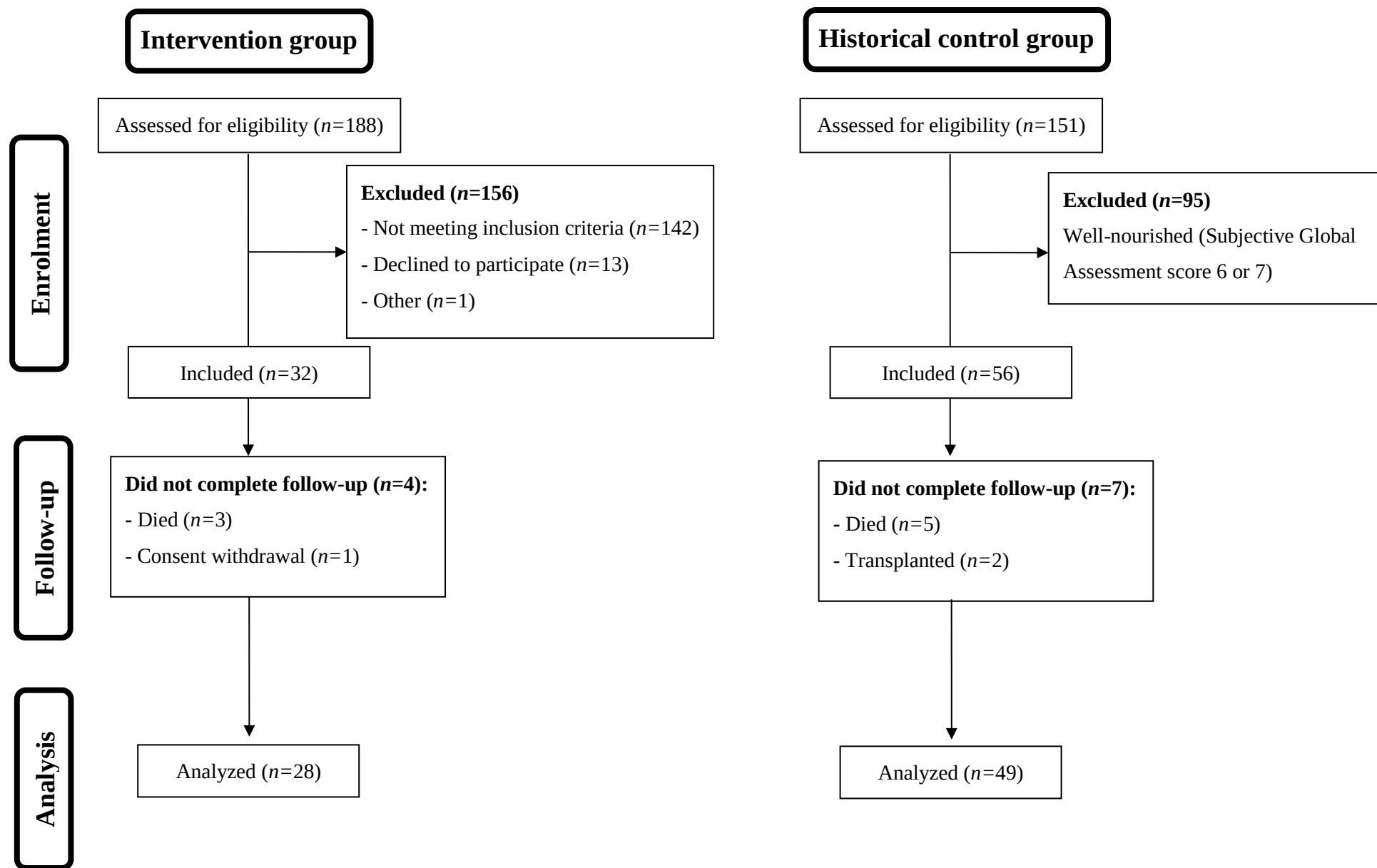


Figure 7.1 The Consolidated Standards of Reporting Trials (CONSORT) flowchart of participant progression through the study

Table 7.1 Changes in skin autofluorescence, biochemical data and nutritional markers from baseline to 6 months in intervention and historical control groups

Variable	Intervention group (n=28)			Historical control group (n=49)		
	Baseline	Month 6	p Value	Baseline	Month 6	p Value
Age (years)	65 (IQR 56 to 74)	---	---	63 (53 to 75)	---	---
Male [n (%)]	14 (50)	---	---	23 (47)	---	---
White ethnicity [n (%)]	24 (86)	---	---	43 (88)	---	---
Educational qualifications [n (%)]	19 (68)	---	---	27 (55)	---	---
Unemployed [n (%)]	26 (93)	---	---	41 (84)	---	---
Current smoking [n (%)]	5 (18)	---	---	14 (29)	---	---
Diabetes [n (%)]	13 (46)	---	---	18 (37)	---	---
Coronary heart disease [n (%)]	12 (43)	---	---	25 (51)	---	---
Dialysis vintage (months)	69.0 (35.0 to 147.0)†	---	---	29.0 (9.5 to 66.0)	---	---
Serum albumin (g/L)	30.0 (26.0 to 33.0)	31.5 (30.0 to 34.0)	0.01	32.0 (27.0 to 34.0)	31.0 (27.0 to 34.5)	0.9
C reactive protein (mg/L)	8.5 (2.3 to 30.0)	6.0 (2.0 to 21.8)	0.7	8.0 (3.0 to 22.5)	8.5 (3.0 to 18.8)	0.7
Total cholesterol (mmol/L)	3.7 (3.2 to 4.7)	3.8 (3.1 to 4.6)	0.5	4.0 (3.1 to 4.9)	4.1 (3.4 to 4.9)	1.0
Serum creatinine (µmol/L)	508 (403.5 to 623)	587(453 to 667)	0.09	571 (443.5 to 717)	559 (467.5 to 729.5)	0.5
Serum phosphate (mmol/L)	1.44 (1.19 to 1.82)	1.41 (1.10 to 1.90)	0.9	1.61 (1.25 to 1.85)	1.48 (1.29 to 1.97)	0.4
Serum potassium (mmol/L)	4.9 (4.2 to 5.5)	4.8 (4.3 to 5.2)	0.3	4.5 (3.9 to 5.2)	4.5 (4.1 to 5.1)	0.6
Skin autofluorescence (AU)	3.8 ± 0.7	3.7 ± 0.7	0.3	3.5 ± 0.9	3.8 ± 1.2	0.03
Dietary AGE intake (kU/day)	13823 (10840 to 20441)	19074 (13372 to 27340)	<0.0001	11940 (8787 to 15833)	14697 (9427 to 19071)	0.03
Energy intake (kcal/kg/day)	20.9 (16.6 to 27.3)†	30.1 (24.9 to 35.9)	<0.0001	17.9 (13.5 to 21.7)	18.9 (15.1 to 26.9)	0.053
Protein intake (g/kg/day)	0.7 (0.6 to 1.1)	1.1 (0.8 to 1.2)	<0.0001	0.8 (0.6 to 1.0)	0.8 (0.6 to 1.0)	0.9
Fat intake (g/day)	52.2 (41.1 to 69.8)†	83.5 (67.3 to 98.7)	<0.0001	44.8 (33.1 to 59.2)	49.2 (34.3 to 67.6)	0.09
Dry weight (kg)	60.9 (54.9 to 71.8)	61.4 (56.2 to 70.5)	0.8	66.4 (56.7 to 79.5)	66.8 (55.9 to 79.4)	0.4
Body mass index (kg/m ²)	22.1 (19.8 to 25.1)†	23.0 (20.4 to 24.8)	0.8	25.4 (20.5 to 28.1)	25.0 (20.6 to 29.1)	0.4
MAMC (cm ²)	23.1 (22.3 to 24.5)	23.6 (20.9 to 25.8)	0.3	24.0 (20.8 to 26.6)	24.1 (22.0 to 26.8)	0.2
Triceps skinfold thickness (mm)	12.9 (8.3 to 15.9)	13.0 (9.0 to 17.0)	0.1	14.6 (10.5 to 18.9)	15.6 (10.6 to 20.0)	0.4
Handgrip strength (kg)	15.2 (10.4 to 22.3)	16.7 (10.6 to 24.7)	0.8	17.3 (12.2 to 25.6)	18.3 (12.4 to 26.3)	0.053
SGA score	4.0 (3.25 to 5.0)	5.0 (5.0 to 6.0)	<0.0001	5.0 (3.0 to 5.0)	5.0 (3.0 to 7.0)	<0.0001

AGE, advanced glycation end-products; AU, arbitrary units; IQR, interquartile range; kU, kilounits; MAMC, mid-arm muscle circumference; SGA, subjective global assessment.

† p<0.05 Intervention vs. control at baseline. P-values in columns are for comparison of baseline and 6 month data within each group.

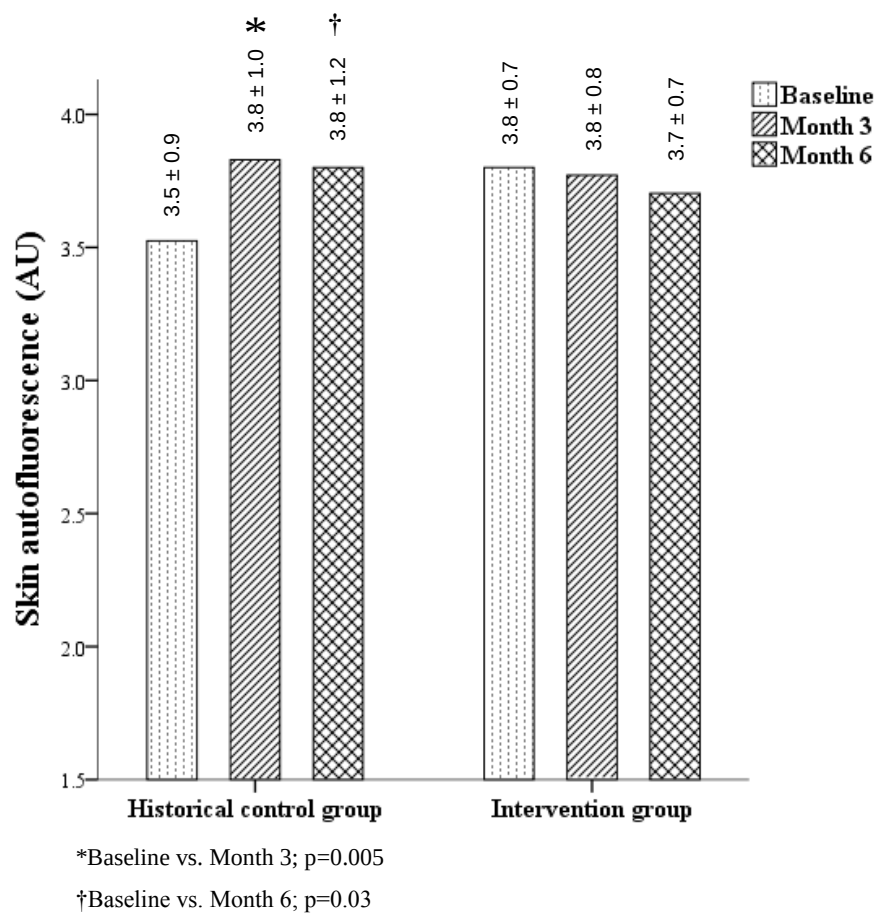


Figure 7.2 Changes in skin autofluorescence throughout the follow-up period

Table 7.2 shows individual change in SGA score in the intervention group from baseline to 6 months, as well as a detailed description of the dietetic intervention provided to each individual participant. Sixty eight percent ($n=19$) of the population improved their SGA score. Of these, 8 became well-nourished. Only one participant showed a decrease in the SGA score.

Table 7.2 Individual change in Subjective Global Assessment score from baseline to 6 months in the intervention group.

<i>Participant ID number</i>	<i>Dietetic intervention</i>	<i>SGA score baseline</i>	<i>SGA score Month 6</i>
1	Food fortification advice + 1 fortisip compact OD for 6 months	5	6
2	Food fortification advice + 1 fortisip compact BD for 6 months	3	4
3	Food fortification advice + 1 fortisip compact OD for 6 months	4	5
4	Food fortification advice for 6 months	4	5
5	Food fortification advice + 1 fortisip compact BD for 6 months	3	5
6	Food fortification advice + 1 fortisip compact OD for 2 months (stopped due to diarrhoea), then food fortification advice only for 4 months	4	4
7	Food fortification advice for 6 months	5	5
8	Food fortification advice for 6 months	3	5
9	Food fortification advice for 6 months	5	6
10	Food fortification advice for 6 months	5	5
11	Food fortification advice only for 1 month, then food fortification advice + 1 fortisip 1.5 kcal/ml for 5 months	5	5
12	Food fortification advice + 1 fortisip compact OD for 3 months, then BD for 3 months	5	5
13	Food fortification advice for 6 months	4	6
14	Food fortification advice for 6 months	5	6
15	Food fortification advice for 6 months	5	5
16	Food fortification advice + 1 fortisip compact BD for 3 months, then OD for 3 months	3	4
17	Food fortification advice for 6 months	3	5
18	Food fortification advice + 1 fortisip compact BD for 3 months, then OD for 2 months, then food fortification advice only for 1 month	4	5
19	Food fortification advice for 6 months	5	6
20	Food fortification advice for 6 months	5	5
21	Food fortification advice + 1 fortisip compact OD for 6 months	5	5
22	Food fortification advice + 1 fortisip compact OD for 1 month, then fortification advice only for 5 months	5	6
23	Food fortification advice + 1 fortisip compact OD for 5 months, then fortification advice only for 1 month	4	6
24	Fortification advice only for 2 months, then 1 fortijuice 1.5 kcal/ml OD for 4 months	5	6
25	Food fortification advice + 1 fortisip compact OD for 5 months, then food fortification advice only for 1 month	3	4
26	Food fortification advice + 1 fortisip compact OD for 3 months and BD for 3 months	4	5
27	Food fortification advice only for 3 months, then food fortification + Renapro shot OD for 1 month, then 3 shots of Fresubin 5kcal/ml OD for 2 months	4	3
28	Food fortification advice + 1 fortisip compact BD for 3 months, then OD for 1 month, then food fortification advice only for 2 months	3	4

BD, twice a day; OD, once a day; SGA, Subjective Global Assessment

DISCUSSION

In this proof of principle study, we found that intensive individualized dietetic advice and support in malnourished persons on dialysis was associated with improvement in dietary intake and markers of malnutrition as well as stable SAF levels over 6 months, despite an increase in dietary intake of AGEs. In contrast, failure to improve dietary intake in a historical control group was associated with an increase in SAF over the same time period.

Malnutrition is a highly prevalent complication in the dialysis population that is very difficult to reverse due to the interaction between several etiological factors such as uremic toxicity, poor appetite associated with dialysis routine and symptom burden of dialysis resulting in inadequate dietary intake, presence of co-morbidities, nutrient losses during dialysis, metabolic acidosis, systemic inflammation and oxidative stress^(65, 85). Nevertheless, in our study, we have observed that an intensive and individualised dietetic intervention was associated with achievement of recommended dietary energy and protein intake^(114, 125) and with an improvement in serum albumin, SGA score and fat intake. A number of interventional studies conducted in persons receiving dialysis support our findings that with personalised nutritional counselling alone⁽¹⁶³⁻¹⁶⁵⁾ and/or dietetic advice plus specific oral nutritional supplements⁽¹⁶⁷⁻¹⁷³⁾ it is possible to achieve improvements in nutritional markers including serum albumin, prealbumin and total cholesterol, energy and protein intake, BMI, TSF and MAC. These findings highlight the importance of individualised nutritional advice as the cornerstone of the treatment of malnutrition in the dialysis population⁽¹¹⁴⁾.

In the setting of improved dietary intake and nutritional status, we observed that SAF levels remained stable over 6 months in the intervention group. Conversely, in the historical control group all components of nutritional intake and other markers of malnutrition did not improve, and this was associated with a significant increase in SAF levels. The SGA score did improve in the historical control group; however, SGA may be influenced by factors that are not directly attributable to nutrition such as infections and other comorbid conditions. These observations confirm the previous findings of our prospective observational study (Study 1), in which prevalence or development of malnutrition over 1 year was an independent determinant of increasing in SAF (Chapter 5). We propose that factors that contribute to the development of malnutrition, such as systemic inflammation and oxidative stress, are likely to increase tissue AGE accumulation and malnutrition in turn exacerbates these factors, creating a vicious cycle^(65, 96).

A small number of randomised controlled clinical trials in the CKD and PD populations^(228, 241, 242), with small sample sizes and short follow-up periods, have shown that dietary AGE restriction was significantly associated with a decrease in serum CML and methylglyoxal levels (circulating AGEs). On the other hand, following a high AGE diet resulted in a significant increase in these same serum AGEs, suggesting that dietary interventions that increase AGE intake may therefore increase SAF. However, we have previously reported in a cross-sectional analysis that SAF was significantly higher among malnourished persons on HD and that markers of malnutrition such as lower serum albumin, lower protein intake and lower HGS were more important determinants of

increased SAF than high dietary AGE intake, which was not positively associated with SAF (Chapter 3)⁽²⁵⁹⁾. Furthermore, in a PD population, higher dietary AGE intake was associated with better nutritional intake and lower SAF (see Chapter 4). The present intervention study extends these observations by showing that SAF levels stabilised over 6 months in association with improvements in nutritional status in malnourished persons on dialysis, even though dietary AGE intake increased significantly, suggesting that correction of malnutrition is a more important intervention to prevent an increase in SAF than restriction of dietary AGE intake in a dialysis population. The latter may risk exacerbating malnutrition and should probably be avoided in those who are malnourished.

This study has some important limitations that need to be highlighted. First, this was a single centre proof of principle study. Second, the recruitment target was not achieved (i.e. 40 participants) and the follow-up period was relatively short, which resulted in low statistical power to detect changes in some of the variables measured. We did not observe a reduction in SAF in the intervention group but this may have been due to the relatively small number of participants and short duration of follow-up. Nevertheless, we have established the feasibility of intensive individualised dietary intervention to improve nutritional status and stabilise SAF, which continued to increase in the historical control group. Further multicentre clinical trials with larger sample sizes and longer follow-up, testing specific nutritional interventions are therefore needed to test more robustly whether correction of malnutrition may result in a decrease in SAF and improved outcomes. The intervention and

historical control groups were matched for most variables at baseline, though the intervention group evidenced longer dialysis vintage as well as higher dietary energy and fat intake but lower BMI. Dietary energy intake was below estimated nutritional requirements in both groups and both were selected for evidence of malnutrition, so we do not consider these differences to have impacted our findings. Ideally, this intervention should be tested in a prospective randomised controlled trial but we had concerns that not providing adequate nutritional support to persons with malnutrition would be unethical. Finally, the results observed in this study may not be applicable to populations with dark skin colour (who were excluded from our study) because SAF measurements cannot be performed in persons with darker skin colour due to the high absorption of the excitation light.

CONCLUSION

With intensive individualised dietetic advice we observed improvement in dietary intake and markers of nutritional status that was associated with stable SAF levels over 6 months, despite an increase in dietary AGE intake. In contrast, failure to improve dietary intake in a historical control group was associated with an increase in SAF. This suggests that individualised nutritional advice may be effective in preventing the rise in SAF observed in malnourished persons on dialysis over time and that the benefits of improving nutritional intake are probably outweighed by any adverse effects of increased dietary AGE intake. Studies of nutritional interventions with larger sample sizes and longer follow-up are needed to test this hypothesis and evaluate the impact on long-term outcomes, including survival.

Chapter 8. Conclusions

The work in this thesis has focused on the determinants of increased SAF, changes in SAF levels over time and the factors associated with these changes, SAF and malnutrition as predictors of mortality, and the impact of intensive individualised dietetic advice on nutritional status and SAF levels in persons receiving HD and performing PD.

SAF, a marker of tissue AGE accumulation, has been shown to be an independent predictor of increased mortality in the dialysis population. AGEs are uremic toxins that are formed endogenously under hyperglycaemic, oxidative and inflammatory conditions and exogenously through cigarette smoking and food consumption. Several factors associated with higher SAF have been well-described in the dialysis population; however, the impact of dietary AGE intake on SAF in persons on dialysis has not been previously investigated. We therefore hypothesised that higher dietary AGE intake would be associated with increased SAF in HD and PD populations. However, as described in Chapters 3 and 4, we found that several factors associated with malnutrition were more important determinants of higher SAF than high dietary intake of AGEs in persons on HD and PD, which suggests that correction of malnutrition may result in a decrease in SAF levels, while restriction of dietary AGE intake may put persons on dialysis at risk of developing malnutrition or exacerbate the nutritional status of those who are already malnourished. Further prospective interventional studies are therefore needed to test this hypothesis.

Due to the fact that SAF has proven to be an independent determinant of higher mortality in persons receiving dialysis, there has been a growing interest in SAF as a potentially modifiable risk factor in the dialysis population. Furthermore, it has been reported that an increase in SAF over time also predicts mortality on dialysis; however, few prospective studies have adequately investigated factors that contribute to an increase in SAF over time. The results from the prospective study presented in Chapter 5 are unique because we used multiple SAF measurements over 1 year to derive a more robust trend over time, rather than rely simply on change between two measurements (which is subject to regression to the mean). In addition, we conducted comprehensive dietary and nutritional assessments so that we could study the impact of multiple nutritional factors on change in SAF. Our finding that the development of new or prevalent malnutrition was an independent risk factor for an increase in SAF suggests that strategies to prevent or treat malnutrition may improve SAF, though this should now be tested in prospective studies.

Increased SAF and malnutrition have each been identified in separate prospective cohort studies as important risk factors for higher mortality in dialysis populations, but no studies have investigated these potentially related associations together. This is important because, as described in Chapters 3 and 4, the observed association between higher SAF and malnutrition may in part explain the previously reported association between higher SAF and mortality in dialysis populations. We therefore hypothesised that higher SAF and malnutrition would interact and that this interaction would increase the risk of

mortality. However, the results presented in Chapter 6 showed that increased SAF and malnutrition were each independently associated with higher mortality in persons on dialysis, suggesting that the previously reported association between increased SAF and mortality is not attributable to an association between higher SAF and malnutrition. This strengthens the case for SAF as an independent risk factor for increased mortality in persons receiving dialysis. Interventions to improve survival by reducing SAF should now be tested in prospective trials. In addition, future prospective studies aiming to improve outcomes by decreasing SAF through dietary AGE restriction should be careful to avoid exacerbating malnutrition, which may counteract any beneficial effects.

In light of the afore-mentioned results, we decided to conduct a non-randomised observational proof of principle study to assess the impact of improving nutritional status on SAF by providing intensive individualised nutritional advice rather than a randomised trial of dietary AGE restriction. In this study (Chapter 7), we observed that although dietary AGE intake increased significantly, improvement of nutritional intake and other markers of nutritional status in persons on dialysis with malnutrition who followed personalised dietetic advice was associated with stable SAF levels over time, suggesting that any impact of dietary AGE intake on SAF was outweighed in this population by the greater impact of improved nutritional status. Further interventional studies are therefore needed to confirm these results and to evaluate the impact on long-term outcomes.

8.1 Directions for future research

The work in this thesis has provided evidence that in the dialysis population markers of malnutrition are more important determinants of increased SAF than high dietary AGE intake. Additionally, prevalence and/or development of malnutrition was independently associated with an increase in SAF over time, while dietary AGE intake was not. Moreover, higher SAF and malnutrition were independent predictors of increased mortality and the improvement of nutritional markers in malnourished persons on dialysis by providing individualised dietetic advice resulted in stable SAF levels, despite an increase in dietary intake of AGEs.

Several studies have confirmed that higher SAF is independently associated with increased mortality in the dialysis population^(196, 211, 214); however, there is no published evidence regarding the prognostic value of a decrease in SAF levels on mortality risk. On the other hand, it has been previously reported that change in nutritional status from malnourished to well-nourished is associated with a 65% mortality risk reduction in persons receiving dialysis⁽²⁶⁹⁾, suggesting that interventions to improve nutritional status may in turn improve clinical outcomes. Therefore, we suggest that a long-term, multicentre, randomised and open-label controlled clinical trial to study the effect of a specific dietetic intervention to improve malnutrition, compared to standard/usual care on SAF levels and mortality is designed and conducted in malnourished persons receiving HD and performing PD. To address ethical concerns, it would be important to ensure that participants randomised to the control arm have adequate access to standard dietetic support. This implies that

some in the control arm would also improve their nutritional status meaning that a relatively large number of participants would be required to show a difference between the groups. The hypothesis of this study would be that a specific intensive dietetic intervention would improve nutritional intake and status, which in turn would result in a reduction in SAF levels and, ultimately, improved survival.

Finally, previous small and short-term randomised controlled clinical trials conducted in CKD and PD populations^(228, 241, 242) have reported that following a low AGE diet is associated with a decrease in serum AGE levels; however, none of these trials assessed nutritional status nor measured SAF. It therefore remains unknown whether dietary AGE restriction in the absence of malnutrition would be effective in reducing SAF and improving survival in persons receiving dialysis. Thus, we suggest the design of a long-term, multicentre, randomised and open-label controlled clinical trial to study the impact of dietary AGE restriction compared to regular/usual diet that is habitually high in AGEs, on SAF levels and nutritional status as well as survival in well-nourished persons on dialysis. Reduction of dietary AGE intake would consist of modifying cooking techniques (e.g. steaming or boiling instead of grilling or frying) without changing the quantity, quality or composition of food. A pilot study would be required to test the feasibility and acceptability to patients of this approach. The hypothesis of this study would be that a diet low in AGEs would decrease SAF levels and maintain nutritional status, as well as improve survival. Based on our observation of an association between increased SAF and malnutrition, it would be important to include only

well-nourished patients in such a study and to include regular monitoring of nutritional status in the protocol as well as provision for intervention or withdrawal from the study if nutritional status was observed to decline.

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Appendices

Appendix 2.1 Food frequency questionnaire

Food, type and amounts			AVERAGE USE LAST YEAR										
	TYPE	AMOUNT	N e v e r	1-6 per year	7-11 per year	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2-3 per day
Breakfast foods/Cereals													
Rice Krispies													
Cheerios													
Other:													
Toast													
Waffle	Mix / Frozen												
Pancake	Mix												
Biscuits/Pastries/Snacks													
Biscuit													
Cracker	Wheat / Honey												
Cracker	Cheddar / Sandwich												
Bar	Nutrigrain / Rice												
Bar	Peanut / Chocolate												
Doughnut													
Biscotti													
Cinnamon swirl													
Scone													
Danish													
Muffin													
Pie													
Cake	Icing / NO												
Croissant	Butter / Chocolate												
Potato crisps													
Cheetos													
Corn crisps (Doritos)													
Popcorn													
Pretzel													
Other:													
Fat Circle one													
Almonds	Raw / Roasted												
Cashews	Raw / Roasted												
Walnuts	Raw / Roasted												
Pecans	Raw / Roasted												
Peanuts	Raw / Roasted												
Pistachios													
Other:													
Peanut butter													
Cream cheese	Regular / Soft												
Mayonnaise	Regular / Low fat												

Food, type and amounts				AVERAGE USE LAST YEAR									
	TYPE	AMOUNT	N e v e r	1-6 per year	7-11 per year	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2-3 per day
Fat				Circle one									
Cream	Heavy / Whip / Sour												
Olive													
Salad dressing													
Avocado													
Margarine													
Butter													
Oil													
Deli Meat or Lunchmeats													
Beef, corned brisket													
Beef, frankfurter	Boiled / Grilled												
Ham, deli													
Sausage, beef/pork	Pan fried / BBQ / Microwaved												
Beef, roast													
Beef, salami													
Other:													
Beef													
Minced beef	Pan fried / Grilled / Oven / Other												
Beef, meatballs													
Beef, meatloaf													
Beef, burger	Homemade / Pan fried / Grilled / Oven / Other												
Beef, steak	Pan fried / Grilled / Oven / Other												
Beef, stewed													
Pork													
Bacon	Fried / Microwaved												
Pork, roast													
Pork, chop													
Pork, ribs													
Chicken													
Grilled													
Oven													
Breaded													
Fried													
Roasted													
Boiled / Stewed													
Other:													
Turkey													

Food, type and amounts			AVERAGE USE LAST YEAR										
	TYPE	AMOUNT	N e v e r	1-6 per year	7-11 per year	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2-3 per day
Fish or seafood													
Shrimp													
Crabmeat													
Trout													
Salmon													
Other:													
Tuna, tinned													
Other tinned:													
Other meat:													
Cheese													
Cheddar													
Swiss													
Mozzarella													
Cottage													
Parmesan													
Brie / Feta													
Other:													
Eggs													
Scrambled													
Omelette													
Fried													
Boiled													
Pan fried, margarine													
Pan fried, butter													
Pan fried, oil													
Pan fried, spray													
Combination foods													
Pasta													
Pasta, baked													
Macaroni and cheese													
Salad, Italian pasta													
Roasted potatoes / Hash browns													

Appendix 2.2 The 7-point scale Subjective Global Assessment

HISTORY																				
WEIGHT/WEIGHT CHANGE: 1. Baseline Wt.: _____ (Dry weight from 6 months ago) Current Wt.: _____ (Dry weight today) Actual Wt loss/past 6 mo: _____ %loss: _____ (actual loss from baseline or last SGA) 2. Weight change over past two weeks: _____ No change _____ Increase _____ Decrease																				
DIETARY INTAKE _____ No change _____ (Adequate) _____ No change _____ (Inadequate) 1. Change: Sub optimal intake: _____ Protein _____ Kcal _____ Duration _____ _____ Full liquid: _____ Hypocaloric liquid _____ Starvation _____																				
GASTROINTESTINAL SYMPTOMS <table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left; width: 40%;">Symptom:</th> <th style="text-align: left; width: 30%;">Frequency*:</th> <th style="text-align: left; width: 30%;">Duration€:</th> </tr> </thead> <tbody> <tr> <td>_____ None</td> <td>_____</td> <td>_____</td> </tr> <tr> <td>_____ Anorexia</td> <td>_____</td> <td>_____</td> </tr> <tr> <td>_____ Nausea</td> <td>_____</td> <td>_____</td> </tr> <tr> <td>_____ Vomiting</td> <td>_____</td> <td>_____</td> </tr> <tr> <td>_____ Diarrhoea</td> <td>_____</td> <td>_____</td> </tr> </tbody> </table> *Never, daily, 2-3 times/wk, 1-2 times/wk € >2 weeks, <2 weeks		Symptom:	Frequency*:	Duration€:	_____ None	_____	_____	_____ Anorexia	_____	_____	_____ Nausea	_____	_____	_____ Vomiting	_____	_____	_____ Diarrhoea	_____	_____	
Symptom:	Frequency*:	Duration€:																		
_____ None	_____	_____																		
_____ Anorexia	_____	_____																		
_____ Nausea	_____	_____																		
_____ Vomiting	_____	_____																		
_____ Diarrhoea	_____	_____																		
FUNCTIONAL CAPACITY Description: _____ Duration: _____ _____ No dysfunction _____ _____ Change in function _____ _____ Difficulty with ambulation _____ _____ Difficulty with activity (Patient specific "normal") _____ _____ Light activity _____ _____ Bed/chair ridden with little or no activity _____ _____ Improvement in function _____																				
DISEASE STATE/COMORBIDITIES AS RELATED TO NUTRITIONAL NEEDS Primary diagnosis _____ Comorbidities _____ Normal requirements _____ Increased requirements _____ Decreased requirements _____ Acute metabolic stress: _____ None _____ Low _____ Moderate _____ High																				
PHYSICAL EXAM																				
_____ Loss of subcutaneous fat (below eye, triceps _____ Some areas _____ All areas Biceps, chest) _____ Muscle wasting (temple, clavicle, scapula, ribs, _____ Some areas _____ All areas Quadriiceps, calf, knee, interosseous) _____ Oedema (related to undernutrition/use to evaluate weight change)																				
OVERALL SGA RATING																				
Very mild risk to well-nourished--- 6 or 7 most categories or significant, continued improvement Mild-moderate--- 3, 4, or 5 ratings. No clear sign of normal status or severe malnutrition Severely malnourished--- 1 or 2 ratings in most categories/significant physical signs of malnutrition																				

Appendix 2.3 Ethical approval documentation

Associations of advanced glycation end-product accumulation and adverse outcomes in peritoneal dialysis and haemodialysis patients and the impact of a dietetic intervention on skin autofluorescence

Draft Version 4.5 Final Version 2.0
4th October 2017

Short title: Skin autofluorescence as a risk marker in people receiving dialysis

Acronym: AGED (**A**dvanced **G**lycation **E**nd-products in **D**ialysis Patients)

Trial Registration: NCT02878317

IRAS Project ID: 200680

Trial Sponsor: University of Nottingham

Sponsor reference: 16050

Funding Source: Internal funds

TRIAL / STUDY PERSONNEL AND CONTACT DETAILS

Sponsor:	University of Nottingham
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Trial / Study Statistician:	Not required, all statistical analysis will be performed by the investigating team
Trial / Study Coordinating Centre:	Renal Unit of the Department of Nephrology Royal Derby Hospital, Uttoxeter Rd, Derby, DE22 3NE

SYNOPSIS

Title	Associations of advanced glycation end-product accumulation and adverse outcomes in peritoneal dialysis and haemodialysis patients and the impact of a dietetic intervention on skin autofluorescence.
Acronym	AGED
Short title	Skin autofluorescence as a risk marker in people receiving dialysis.
Chief Investigator	Professor Maarten Taal
Objectives	Study 1: • To investigate whether the skin autofluorescence (SAF) levels increase over time in prevalent and incident people on peritoneal dialysis (PD) and haemodialysis (HD). • To investigate one-year survival in relation to increased SAF levels. • To determine the most important factors associated with changes in SAF over time. • To investigate the association of increased SAF levels with morbidity, changes in visual function, health-related quality of life and nutritional status. Study 2: • To investigate whether the correction of malnutrition by an intensive nutritional intervention results in a decrease in SAF levels in people on PD and HD.
Trial Configuration	Study 1: longitudinal/prospective. Study 2: observational non-randomized proof of principle study.
Setting	Outpatient secondary care setting.
Sample size estimate	Sample size Study 1: The primary outcome of the study is one-year survival in relation to increased SAF levels in people on HD and PD. With a power of 80%, a two-sided alpha of 5% and an expected hazard ratio of 3.5 and 2.0 in people on PD and HD, respectively, 100 HD and 40 PD participants will be needed. Sample size Study 2: Since this is an observational proof of principle study, it would be reasonable to include 40 dialysis participants (either HD or PD).
Number of participants	Study 1: • 100 participants undergoing maintenance HD treatment (dialysis vintage greater than 3 months). • 40 PD dialysis participants with a dialysis vintage greater than 3 months. • 30 incident participants starting either HD or PD treatment (dialysis vintage $\leq$ 3 months).

	Study 2: 40 malnourished participants on maintenance HD and PD.
Eligibility criteria	HD cohort: • Age: ≥ 18 years (no upper age limit). • At least three dialysis sessions per week for 3 or 4 hours. • Dialysis with biocompatible membranes. • Able to give informed consent. PD cohort: • Age: ≥ 18 years (no upper age limit). • Dialysis with lactate/bicarbonate-buffered solutions with different glucose concentrations as prescribed for routine clinical care. • Able to give informed consent.
Description of interventions	Study 1: no intervention. Study 2: Dietitian supervised nutritional support aimed at improving malnutrition in addition to standard dietetic advice consistent with United Kingdom Renal Association guidelines (Wright and Jones, 2010).
Duration of study	Study 1: Participant recruitment will begin on June 2016 with an anticipated recruitment period of 14 months. Therefore, all baseline data and measurements will be collected and performed between June 2016 and August 2017. <u>Participant Duration:</u> once recruited, all participants will be followed-up for up to five years; consequently, it is expected that the study will be completed by August 2022. Study 2: Participant recruitment will begin in December 2017 with an anticipated recruitment period of 6 months. Therefore, all baseline data and measurements will be collected and performed between December 2017 and June 2018. <u>Participant Duration:</u> once recruited, all participants will be followed-up for 24 months; consequently, it is expected that the study will be completed by June 2020.
Randomization and blinding	Studies 1 and 2: no randomization required.
Outcome measures	Study 1: • <u>Primary outcomes:</u> ▪ Changes in SAF levels over 1 year. ▪ One-year survival.

	• <u>Secondary outcomes:</u> factors associated with the accumulation of AGE, morbidity, biochemistry, visual function, quality of life and nutritional status. Study 2: • <u>Primary outcomes:</u> changes in SAF levels. • <u>Secondary outcomes:</u> biochemistry, visual function, quality of life and nutritional status.
Statistical methods	Intragroup comparisons will be performed using Wilcoxon test or paired t-test for dimensional variables and McNemar test for categorical variables. Intergroup comparisons will be performed using Mann Whitney test or Student t test for continuous variables and χ^2 test or Fisher's exact test for categorical variables. To determine the significance and strength of associations, Pearson's correlation coefficient will be used for continuous variables and Spearman rank for nonparametric variables. Linear regression analysis will be used to identify determinants of advanced glycation end-product (AGE) accumulation. Cox proportional hazards models will be used to investigate the prognostic value of the accumulation of AGE for predicting mortality. A p value ≤ 0.05 will be considered to have statistical significance.

ABBREVIATIONS

AE	Adverse Event
AGE	Advanced glycation end-products
CI	Chief Investigator overall
CKD	Chronic kidney disease
CLF	Collagen linked fluorescence
CML	Carboxymethyl lysine
CRF	Case Report Form
EDTRS	Early Treatment Diabetic Retinopathy Study
ESKD	End stage kidney disease
GCP	Good Clinical Practice
HD	Haemodialysis
ICF	Informed Consent Form
IL-6	Interleukin 6
NHS	National Health Service
PD	Peritoneal dialysis
REC	Research Ethics Committee
R&D	Research and Development department
SAE	Serious Adverse Event
SAF	Skin autofluorescence
SF-36	36-Item Short Form Health Survey
SGA	Subjective global assessment
TNF- α	Tumour necrosis factor- α

TRIAL / STUDY BACKGROUND INFORMATION AND RATIONALE

Chronic kidney disease (CKD) is a global public health problem and is associated with multiple adverse outcomes including reduced survival, especially in people requiring renal replacement therapy (peritoneal dialysis (PD), haemodialysis [HD] and transplantation) (Zhang and Rothenbacher, 2008). Multiple risk factors lead to the development and/or progression of CKD, such as obesity, hyperlipidaemia, glomerulonephritis, intercurrent infections, smoking, type 2 diabetes and hypertension, the two latter being considered as leading causes of CKD worldwide (Zhang and Rothenbacher, 2008; Junaid Nazar et al., 2014).

People on dialysis develop a variety of complications/abnormalities as a result of loss of endocrine or exocrine function of the kidneys, including anaemia, metabolic acidosis, bone and mineral disorders, fluid overload, hypertension, electrolyte disturbances and dyslipidaemia (KDIGO, 2013). In recent years, inflammation, oxidative stress and endothelial dysfunction (other common abnormalities in people on dialysis) have become areas of interest because of their strong relationship with higher rates of cardiovascular morbidity and mortality in people on dialysis (Oleniuc et al., 2012).

Advanced glycation end-products (AGE) are uremic toxins markedly increased in people on dialysis. Formation of AGE starts with a non-enzymatic reaction between proteins and glucose molecules called the Maillard reaction; however, AGE are also formed more rapidly during oxidative stress with the subsequent formation of reactive carbonyl compounds like methyl glyoxal. At this point, AGE synthesis is irreversible and AGE will cross-link with tissue proteins; it seems that collagen in the skin and vascular basement membranes are especially susceptible to AGE accumulation and subsequent injury. AGE also interact with specific AGE receptors that will lead to the activation of systemic inflammation by increasing the release of cytokines and, consequently, exacerbate tissue damage (Arsov et al., 2014). Importantly, AGE are also formed in food during cooking with dry heat at high temperatures such as in frying, grilling or roasting and about 10% of ingested AGE are absorbed.

Skin autofluorescence (SAF) is a relatively new technique that measures the skin accumulation of AGE. It is a non-invasive, operator independent, quick (less than 5 minutes) and easy to perform technique that utilizes the fluorescent properties of AGE, like the extensively used collagen linked fluorescence (CLF) method, and has been validated with specific AGE measurements and CLF in skin biopsies (Oleniuc et al., 2012). It has been reported that SAF is strongly correlated with overall and cardiovascular mortality in people with diabetes and undergoing HD (Hartog et al., 2007; Graff et al, 2014). Several factors have been associated with higher SAF values in people on dialysis in cross-sectional studies, for instance, chronological age in both dialysis modalities, glucose exposure from peritoneal dialysis fluid and dialysis vintage only in people on PD and presence of diabetes in people on HD (McIntyre et al., 2010; Smit et al., 2010).

AGE accumulation is postulated as the one of the modulating factors that drives visual disorders (Kandarakis et al., 2014); the increased accumulation

secondary to hyperglycaemia in diabetes is thought to cause vascular basement membrane thickening and destruction of pericytes from the retinal capillary bed in diabetic retinopathy (Singh et al., 2014). The accumulation of AGE has also been reported to be a mechanism in the deterioration in visual acuity associated with increasing age (Ishibashi et al., 1998). Separately, the accumulation of AGE in other metabolic diseases (e.g. end stage kidney disease [ESKD]) has been found to cause nerve and eye dysfunction. Ocular abnormalities and therefore visual disturbances are reported in people with ESKD but the mechanism for this has not been fully explored or understood. In animal studies, AGE accumulation is seen in the lens, cornea and vitreous humour (Darlene et al., 2011). In humans, systemic AGE levels and visual acuity scoring have not been previously investigated.

Because of the adverse outcomes strongly associated with higher levels of SAF, several options focused on reducing the accumulation of AGE have been proposed. One of these promising interventions is the reduction of dietary AGE; it has been suggested that cooking techniques that avoid very high temperatures such as poaching, steaming, stewing and boiling can significantly reduce the AGE content of food when compared to frying, broiling, grilling and roasting (Uribarri et al., 2010); nevertheless, most of the evidence regarding dietary modifications to reduce exogenous AGE is of low quality and therefore further studies are required (Kellow and Savage, 2013).

Nevertheless, analysis of baseline data from Study 1 has identified strong associations between higher SAF and malnutrition whereas no correlations were observed between higher SAF and high dietary AGE intake. Correction of malnutrition may therefore represent a more important dietary intervention to reduce accumulation of AGE in people receiving dialysis. We further reasoned that placing people with malnutrition on a restrictive diet may worsen their malnutrition and we have therefore adapted our original research plan to include an observational study to assess the impact of correcting malnutrition on SAF by providing a dietitian supervised nutritional support intervention, which essentially involves the usual/standard dietetic care/advice provided by the NHS with some additional supervision, follow-up and approved dietary supplements (also provided by the NHS), rather than a randomised trial of dietary AGE restriction.

The results from the present project will benefit people on dialysis because they may demonstrate that the correction of malnutrition decreases the SAF levels in this population. Published observational studies suggest that reduction of SAF levels will in turn be associated with a reduction in the high morbidity and mortality rates associated with chronic dialysis and, consequently, healthcare costs. Improved survival and reduced comorbidity would also be expected to improve the quality of life of people on dialysis.

TRIAL / STUDY OBJECTIVES AND PURPOSE

PURPOSE

The purpose of the present project is to investigate the association between AGE accumulation and adverse outcomes (mainly mortality) and the impact of a dietary intervention on SAF levels in people on HD and PD.

PRIMARY OBJECTIVES

Study 1:

- To investigate whether the SAF levels increase over time in prevalent and incident HD and PD participants.
- To investigate one-year survival in relation to increased SAF levels.

Study 2:

- To investigate whether the correction of malnutrition by an intensive nutritional intervention results in a decrease in SAF levels in people on PD and HD.

SECONDARY OBJECTIVES

Study 1:

- To determine the most important factors associated with change in SAF over time.
- To investigate the association of increased SAF levels with morbidity, health-related quality of life and nutritional status.
- To investigate the association between SAF levels and changes in visual function.

Study 2:

- To investigate the effect of an intensive nutritional intervention on the improvement of health-related quality of life and nutritional markers.

DETAILS OF DEVICE(S)

No products will be used in the present project.

Description

The medical device that will be used in the present project is a validated “AGE reader Standard Unit (SU)”. The AGE reader is a medical device used in clinical research which can non-invasively measure tissue accumulation of AGE through a fluorescent technique called SAF.

The AGE reader SU illuminates a skin surface of 1 cm², guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation ≈370 nm). Light from the skin is measured with a spectrometer (AVS-USB2000, Avantes Inc., Eerbeek, The Netherlands) in the 300-600 nm range, using a 200-µm glass fibre. SAF will be measured as the ratio between emission and excitation, calculated in arbitrary units (AU), by dividing the intensity of the fluorescent light coming from the skin (measured as area under the curve [AUC] of fluorescent wave lengths between 420 and 600 nm) by the intensity of the emitted light (measured as AUC of wave lengths between 300 and 420 nm) multiplied by 100. For each participant, three measurements will be performed on the volar surface of the lower arm at ≈10 cm below the elbow, ensuring that the area has normal skin without visible vessels, scars or other abnormalities. For HD participants, only the non-fistula arm will be used and evaluations will take place prior to dialysis sessions (Siriopol et al., 2015). This technique is non-invasive and there is no risk of harm to the participant. Measurements take only a few minutes.

The AGE reader SU will be used by Daniela Viramontes Horner (PhD student) following user instructions provided by the manufacturer.

Manufacture

The name of the manufacturer is DiagnOptics Technologies BV (Aarhusweg 4-9, Groningen, The Netherlands)

The AGE reader SU is a distributed CE marked product covered by the "CE Marking of Conformity Certificate", reference number: 2085393CE01, delivered by DEKRA Certification B.V., Arnhem, The Netherlands, Notified Body Identification Number 0344, and conforms to the required technical documentation, in accordance with Annex VII of the "EC-Directive", the Council Directive 93/42/EEC of 14 June 1993, concerning medical devices.

The AGE reader SU will be used in the present project in accordance with this CE marking.

Packaging and labelling

Storage, dispensing and return

The AGE reader SU will be stored securely, in a locked room, or locked cupboard or cabinet. Access to the medical device will be limited to the study staff and investigators.

Placebo

No placebo products will be used in the present project.

Known Side Effects

The AGE-Reader has been extensively tested in thousands of adults for several years without any adverse effects. The AGE Reader has CE approval (2085393CE01) to be used as a medical device and thus has passed the rigid safety standard. The amount of ultraviolet light exposure is actually far below the safety limits.

Reference source:

AGE reader. Cardiovascular risk assessment.

<http://www.diagnoptics.com/age-reader/> [Accessed on 12th May, 2016]

TRIAL / STUDY DESIGN

TRIAL / STUDY CONFIGURATION

Studies 1 and 2 will be single-centre.

Study 1: longitudinal/prospective.

Study 2: observational non-randomized proof of principle study where malnourished dialysis participants will receive a dietitian supervised intensive nutritional support.

Primary endpoints

Study 1:

- Changes in SAF levels after 12 months of follow-up.
- One-year survival.

Study 2: changes in SAF levels after 6 months of a dietetic intervention (i.e. intensive nutritional support).

Secondary endpoints

Study 1:

- Factors associated with the accumulation of AGE.
- Association of SAF levels with morbidity, biochemical variables (haemoglobin, haemoglobin A1C (HbA1C), urea, creatinine, potassium, phosphate, calcium, sodium, albumin, total proteins, cholesterol, triglycerides, and intact parathyroid hormone), visual function, circulating AGE levels (carboxymethyl lysine [CML] and pentosidine), inflammatory biomarkers (tumour necrosis factor- α [TNF- α], interleukin 6 [IL-6], C reactive protein [CRP] and others) health-related quality of life and nutritional status (energy, protein and fat intake, dietary AGE intake, anthropometry and subjective global assessment [SGA]).

Study 2:

- Association of SAF levels with morbidity, biochemical variables (haemoglobin, HbA1C, urea, creatinine, potassium, phosphate, calcium, sodium, albumin, total proteins, cholesterol, triglycerides, and intact parathyroid hormone), visual function, circulating AGE levels (CML and pentosidine), inflammatory biomarkers (TNF- α , IL-6, CRP and others) health-related quality of life and nutritional status (energy, protein and fat intake, dietary AGE intake, anthropometry and SGA).

Safety endpoints

There will not be any safety endpoints for these studies.

Stopping rules and discontinuation

Participant Withdrawal

Participants will be withdrawn from the study if they withdraw their consent at any time. Participants may be withdrawn from the study at the discretion of the Investigator. Participants will be made aware that withdrawal will not affect their future care. Participants who withdraw will be asked for permission to retain data already collected for use in the final analysis.

If participants fail to attend for their study visits, a member of the research team will try to contact them by telephone within 24 hours of their appointment and by written correspondence within a week of the missed appointment. Multiple attempts at contact will be made by the research team over a period of a month before stating that a participant has been lost to follow-up.

Participants will be withdrawn from the study if they:

- Withdraw their consent at any point.
- Become pregnant.
- Decline to comply with the dietetic intervention (Study 2 only).

Criteria for terminating the study

Study 1 is observational and will therefore only be terminated if it becomes clear that for unforeseen reasons the study is not feasible.

The intervention in Study 2 is not expected to be associated with any adverse effects. Study 2 will therefore only be terminated if it becomes clear that for unforeseen reasons the study is not feasible.

RANDOMIZATION AND BLINDING

Maintenance of randomization codes and procedures for breaking code

TRIAL/STUDY MANAGEMENT

The Chief Investigator (CI; Professor Maarten Taal) has overall responsibility for the study and shall oversee all study management. The data custodian will be the CI.

The study will be coordinated from the University of Nottingham campus at the Royal Derby Hospital. Data will be collected at the Renal Unit of the Department of Nephrology at the Royal Derby Hospital (Derby Teaching Hospitals NHS Foundation Trust) and stored at the University of Nottingham.

Daniela Viramontes Horner (PhD student), Dr. Nick Selby and Professor Maarten Taal are responsible for study planning, recruitment, consenting, data collection, handling and interpretation.

DURATION OF THE TRIAL / STUDY AND PARTICIPANT INVOLVEMENT

Study Duration:

Study 1:

Participant recruitment will begin on June 2016 with an anticipated recruitment period of 14 months. Therefore, all baseline data and measurements will be collected and performed between June 2016 and August 2017.

Participant Duration: once recruited, all participants will be followed-up for up to five years; consequently, it is expected that the study will be completed by August 2022.

Study 2:

Participant recruitment will begin in December 2017 with an anticipated recruitment period of 6 months. Therefore, all baseline data and measurements will be collected and performed between December 2017 and June 2018.

Participant Duration: once recruited, all participants will be followed-up for 24 months; consequently, it is expected that the study will be completed by June 2020.

End of the Trial

The end of studies 1 and 2 will be when long term follow-up has been completed.

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

Potential participants on HD and PD will be recruited from the Renal Unit of the Department of Nephrology at the Royal Derby Hospital. The initial details of the study and participant information sheet will be provided by the usual care team (which may include the researcher). Participants will then be given at least 24 hours to consider whether they wish to participate, as well as ask any questions about the study, before being re-contacted by the investigators.

If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the study, the participant information sheets, and consent forms, but the consent forms and information sheets will not be available in other languages.

It will be explained to the potential participant that entry into the study is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

The participant's general practitioner will not be informed about the study because there are no implications for other aspects of treatment.

Eligibility criteria

Inclusion criteria

HD cohort:

- Age: ≥ 18 years (no upper age limit).
- At least three dialysis sessions per week for 3 or 4 hours.
- Dialysis with biocompatible membranes.
- Able to give informed consent.

PD cohort:

- Age: ≥ 18 years (no upper age limit).
- Dialysis with lactate/bicarbonate-buffered solutions with different glucose concentrations as prescribed for routine clinical care.
- Able to give informed consent.

Exclusion criteria

- Does not wish to participate.
- Renal transplant.
- Pregnancy or breast feeding or intending pregnancy.
- Expected survival less than one year (for Study 1).

Expected duration of participant participation

Participants will be participating in the Study 1 for five years and in the Study 2 for 24 months.

Removal of participants from therapy or assessments/Participant Withdrawal

Participants will be withdrawn from the study if they withdraw their consent at any point, receive a renal transplant or decline to comply with the dietetic intervention (Study 2). Female participants will be withdrawn from the study if they become pregnant.

Participants may be withdrawn from the study either at their own request or at the discretion of the Investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and, with their consent, may still be used in the final analysis.

If participants fail to attend for their study visits, a member of the research team will try to contact them by telephone within 24 hours of their appointment and by written correspondence within a week of the missed appointment. Multiple attempts at contact will be made by the research team over a period of a month before stating that a participant has been lost to follow-up.

With participant consent, data that is collected prior to withdrawal will be kept and analysed as part of the data set. The date, time and reason for participant withdrawal will be documented on the case report form (CRF).

To ensure attainment of adequate numbers of participants for the analysis to be valid, the research team will attempt to recruit other participants to replace any who withdraw from the study.

Informed consent

The process for obtaining participant informed consent will be in accordance with the Research Ethics Committee (REC) guidance, Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced.

The research team have both training and experience in assessing participant capacity and gaining informed consent in the clinical context.

Detailed information will be provided on the study to ensure that participants understand the purpose and potential risks involved. The study will not involve participants who are unable to represent their own interests or are particularly susceptible to coercion.

All participants will provide written informed consent. The Informed Consent Form will be signed and dated by the participant before they enter the study. The Investigator will explain the details of the study and provide a Participant Information Sheet, ensuring that the participant has sufficient time to consider participating or not. The Investigator will answer any questions that the participant has concerning study participation.

The decision regarding participation in the study is entirely voluntary. The Investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care.

Informed consent will be obtained from each participant before they undergo any interventions (including physical examination and history taking) related to the study. One copy of this will be kept by the participant, one will be kept by the Investigator, and a third will be retained in the participant's hospital records.

Should there be any subsequent substantial amendment to the final protocol, which might affect a participant's participation in the study, continuing consent

will be obtained using an amended Consent Form which will be signed by the participant.

TRIAL / STUDY TREATMENT AND REGIMEN

Study 1 - longitudinal/prospective study

100 participants undergoing maintenance HD treatment (dialysis vintage greater than 3 months), 40 PD participants with a dialysis vintage greater than 3 months and up to 30 incident participants starting either HD or PD treatment (dialysis vintage less than 3 months) will be recruited from the Renal Unit of the Department of Nephrology at the Royal Derby Hospital and will be followed-up for five years.

Data collection and study procedures/assessments

Once participants have given their informed consent, relevant participant characteristics will be extracted from electronic data captured in medical records and from a self-report questionnaire at baseline and will include demographic characteristics, educational level, occupation status, family history, duration of renal replacement therapy, dialysis adequacy, aetiology of CKD, medical history (e.g. history of cardiovascular disease, diabetes and hypertension), current co-morbidities, alcohol history, smoking status and current medication. For the PD participants, information regarding the type of PD and the volume of the different PD solutions will also be recorded.

The accumulation of AGE will be assessed by measuring SAF using a validated Autofluorescence Reader (AGE Reader, DiagnOptics, Groningen, The Netherlands) at baseline, 3, 6, 9 and 12 months of follow-up. The method has been previously described by Meerwaldt et al. (2005). As described in the study by Siriopol et al. (2015), "the autofluorescence reader illuminates a skin surface of 1 cm², guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation ≈370 nm). Light from the skin is measured with a spectrometer (AVS-USB2000, Avantes Inc., Eerbeek, The Netherlands) in the 300-600 nm range, using a 200-μm glass fibre". SAF will be measured as the ratio between emission and excitation, calculated in arbitrary units (AU), by dividing the intensity of the fluorescent light coming from the skin (measured as area under the curve [AUC] of fluorescent wave lengths between 420 and 600 nm) by the intensity of the emitted light (measured as AUC of wave lengths between 300 and 420 nm) multiplied by 100. For each participant, three measurements will be performed on the volar surface of the lower arm at ≈10 cm below the elbow, ensuring that the area has normal skin without visible vessels, scars or other abnormalities. For HD participants, only the non-fistula arm will be used and readings will be undertaken within the first hour of HD treatment (Siriopol et al., 2015).

Routine blood/laboratory tests will be performed at baseline and monthly and will include haemoglobin, HbA1C, urea, creatinine, potassium, phosphate, calcium, sodium, albumin, total proteins, cholesterol, triglycerides and intact parathyroid hormone. Pre- and post-dialysis blood samples (10 mL) will be taken at baseline, 3, 6, 9 and 12 months of follow-up and centrifuged to obtain at least 2 mL of serum and 2 mL of plasma which will be frozen and stored at -80°C for subsequent analysis of circulating AGE levels (CML and pentosidine) and markers of inflammation (TNF-α, IL-6, CRP and others).

Nutritional assessments will be performed at baseline and at 6 and 12 months of follow-up and will include: three 24-h dietary recalls to assess energy, fat and protein intake, a validated food frequency questionnaire to evaluate dietary AGE intake; the 7-point scale SGA for the classification of the nutritional status (normal, mild-moderate malnutrition and severe malnutrition) and anthropometric measurements and indexes (weight, height, mid-arm circumference, triceps skinfold thickness, body mass index, hand-grip strength and mid-arm muscle area).

Participants will have all aspects of their visual acuity assessed at baseline and 12 months. Those on HD will be tested before and after a single mid-week dialysis session. Participants will initially be asked a screening questionnaire to assess whether or not they note a change in their visual acuity or experience visual disturbances peri dialysis. The assessments will include near, distance, contrast and colour vision assessed using the Jaeger near vision chart, standardized Early Treatment Diabetic Retinopathy Study (EDTRS) testing, Pelli-Robson Contrast Sensitivity Chart, and Ishihara Color Vision Test, respectively. Participants will be asked to bring their glasses or wear their contact lenses on the study day to assess best corrected visual acuity.

Health-related quality of life will be evaluated at baseline and at 6 and 12 months of follow-up with the 36-Item Short Form Health Survey (SF-36) and EQ5D questionnaire.

After the first year of the study, SAF readings and questionnaires will be repeated once a year for up to five years.

Study 2 - Observational non-randomised proof of principle study

Starting December 2017, 40 malnourished (i.e. SGA scores from 1 to 5) participants on maintenance HD and PD will be recruited from the Renal Unit of the Department of Nephrology at the Royal Derby Hospital and then will be followed-up for 24 months. Therefore, Study 2 will be running alongside Study 1. Depending on the results of the longitudinal/prospective study, we will decide to include incident HD and PD participants as well.

Description of intervention

Once the identified malnourished participants have given their informed consent, they will receive intensive dietitian supervised nutritional support with the aim of improving their malnutrition. In addition, participants will receive standard dietary advice for people on dialysis based on the Nutritional Guidelines in CKD published by the Renal Association in March 2010 in the UK (Wright and Jones, 2010) and will include the following: energy (35 kcal/kg/day) and protein intake (1.2 g/kg/day), as well as potassium, phosphate and sodium restriction, according with biochemical blood parameters.

Adherence to the intervention will be evaluated by means of three 24-h dietary recalls every month (1 dialysis day, 1 non-dialysis day and 1 weekend day for the HD participants and 2 weekdays and 1 weekend day for the PD participants).

Data collection and study procedures/assessments

Relevant participant characteristics will be extracted from electronic data captured in medical records and from a self-report questionnaire at baseline and will include demographic characteristics, educational level, occupation status, family history, duration of renal replacement therapy, dialysis adequacy, aetiology of CKD, medical history (e.g. history of cardiovascular disease, diabetes and hypertension), current co-morbidities, alcohol history, smoking status and current medication. For the PD participants, information regarding the type of PD and the volume of the different PD solutions will also be recorded.

The accumulation of AGE will be assessed by measuring SAF using a validated Autofluorescence Reader (AGE Reader, DiagnOptics, Groningen, The Netherlands) at baseline, 3 and 6 months of follow-up according to the method described above.

Routine blood/laboratory tests will be performed at baseline and monthly and will include haemoglobin, HbA1C, urea, creatinine, potassium, phosphate, calcium, sodium, albumin, total proteins, cholesterol, triglycerides and intact parathyroid hormone. Pre- and post-dialysis blood samples (10 mL) will be taken at baseline, 3 and 6 months of follow-up and centrifuged to obtain at least 2 mL of serum and 2 mL of plasma which will be frozen and stored at -80°C for subsequent analysis of circulating AGE levels (CML and pentosidine) and markers of inflammation (TNF- α , IL-6, CRP and others).

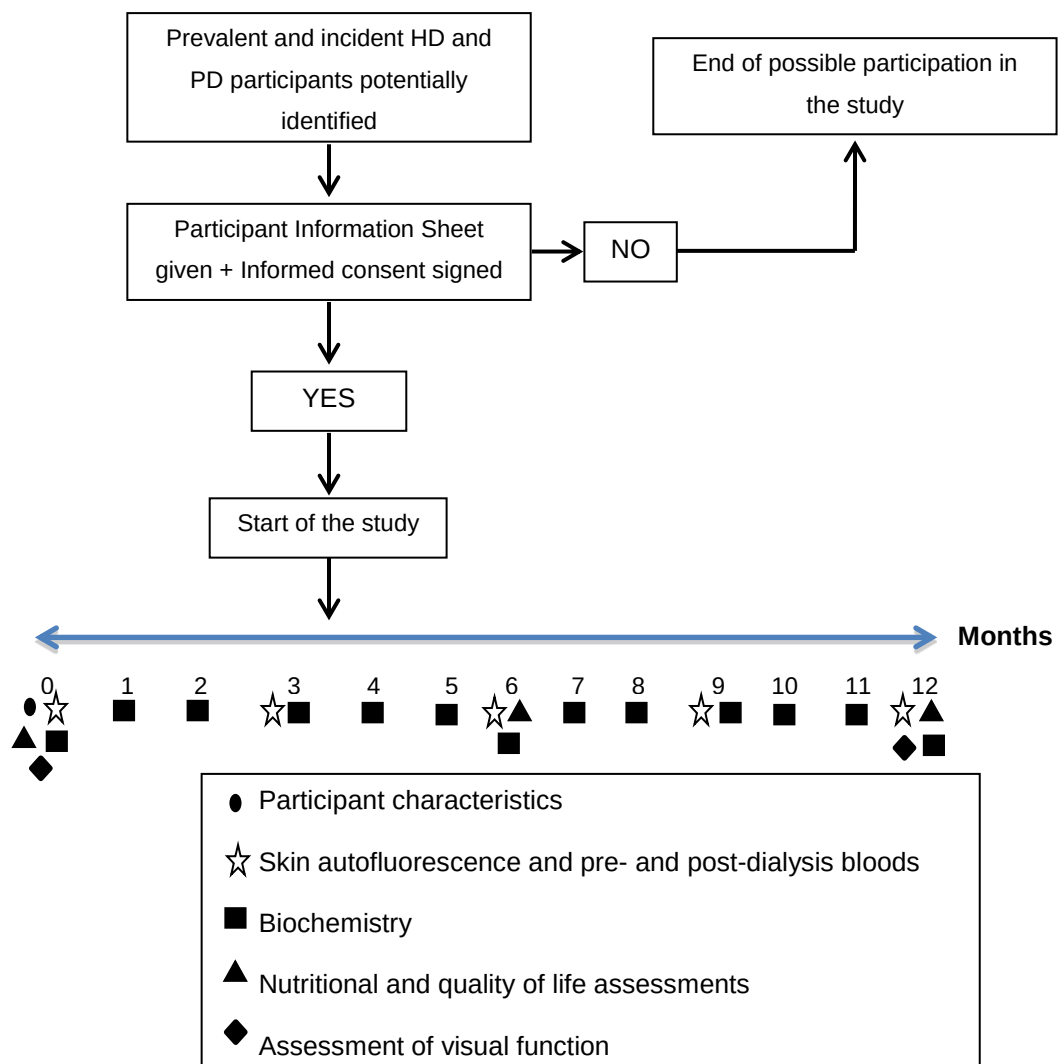
Nutritional assessments will be performed at baseline and at 6th month of treatment and will include: three 24-h dietary recalls to assess energy, fat and protein intake, a validated food frequency questionnaire to evaluate dietary AGE intake, the 7-point scale SGA for the classification of the nutritional status (normal, mild-moderate malnutrition and severe malnutrition) and anthropometric measurements and indexes (weight, height, mid-arm circumference, triceps skinfold thickness, body mass index, hand-grip strength and mid-arm muscle area).

Participants will have all aspects of their visual acuity assessed at baseline and 6 months. Those on HD will be tested before and after a single mid-week dialysis session. Participants will initially be asked a screening questionnaire to assess whether or not they note a change in their visual acuity or experience visual disturbances peri dialysis. The assessments will include near, distance, contrast and colour vision assessed using the Jaeger near vision chart, standardized EDTRS testing, Pelli-Robson Contrast Sensitivity Chart, and Ishihara Color Vision Test respectively. Participants will be asked to bring their glasses or wear their contact lenses on the study day to assess best corrected visual acuity.

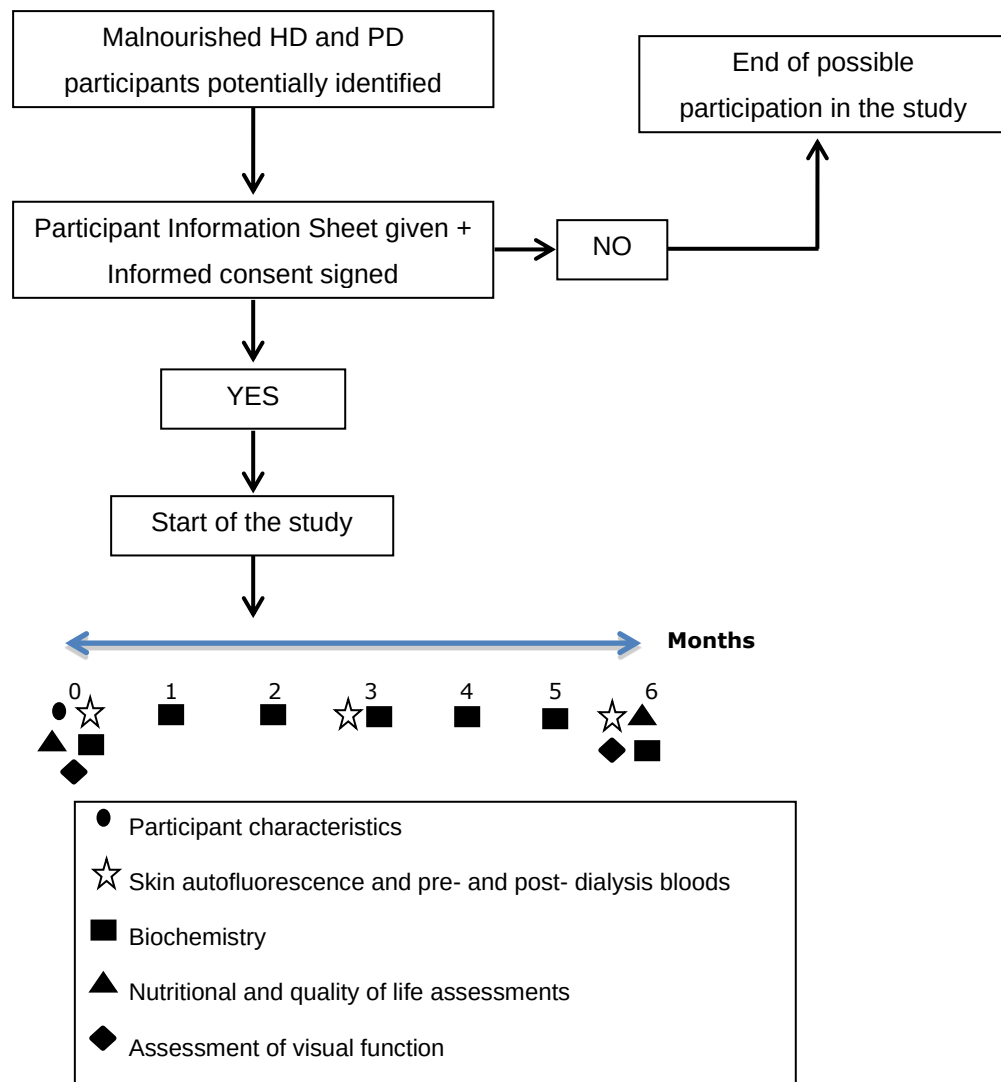
Health-related quality of life will be evaluated at baseline and at 6th month of follow-up with the SF-36 and EQ5D questionnaire.

After the first 6 months of the study, SAF readings and questionnaires will be repeated once every 6 months for up to 2 years.

Schematic diagram of study procedures and stages – Study 1 (timeline showing only the first year of study)



Schematic diagram of study procedures and stages – Study 2 (timeline showing only the first 6 months of the study)



Compliance

The Study 1 (prospective/longitudinal) involves SAF measurements and visual function, nutritional and quality of life assessments during one year of follow-up. Therefore, there is no need for an assessment of participant compliance with the study protocol. Should the participant not complete the study day, this will be classified as a withdrawal rather than non-compliance (see Section **Participant Withdrawal**).

Compliance with the intervention of Study 2 will be evaluated by means of three 24-h dietary recalls every month (1 dialysis day, 1 non-dialysis day and 1 weekend day for the HD participants and 2 weekdays and 1 weekend day for the PD participants).

Criteria for terminating trial

Study 1 is observational and will therefore only be terminated if it becomes clear that for unforeseen reasons the study is not feasible.

The intervention in Study 2 is not expected to be associated with any adverse effects. Study 2 will therefore only be terminated if it becomes clear that for unforeseen reasons the study is not feasible.

TRANSPORT AND STORAGE OF THE TISSUES

Blood samples (10 mL) will be collected at intervals as described and centrifuged. At least 2 mL of serum and 2 mL of plasma will be stored at -80°C for future analysis.

Samples and data will be stored in a linked anonymised format. Samples will be labelled using a combination of study reference, unique study identifier and cross referenced with location code numbers to permit accurate linkage to study data and the consent form.

The master database will be held by the CI in a password encrypted file.

Where participants do not agree to the future use of the samples, they will be destroyed in accordance with the Human Tissue Act, 2004.

LABORATORY ANALYSES

Routine blood/laboratory tests will be analysed in a local NHS laboratory at the Royal Derby Hospital, which is serviced and managed according to national standards and governance frameworks. No DNA or RNA extraction will occur as part of this study. Assays for CML and inflammatory markers will be conducted in research laboratories at the University of Nottingham.

STATISTICS

Methods

Statistical analysis will be conducted by Daniela Viramontes Horner (PhD student), Professor Maarten Taal and Dr. Nick Selby. Data management and statistical analyses will be performed using statistical software SPSS version 22.0.

Data will be presented as mean $\pm$ standard deviation, median (percentiles 25%-75%), or percentages, as appropriate.

To compare baseline versus final evaluations, Wilcoxon test or paired t-test will be used in the case of dimensional variables, and McNemar test in the case of categorical variables. Intergroups comparisons will be performed using Mann Whitney test or Student t test for continuous variables and χ^2 test or Fisher's exact test for categorical variables. To determine the significance and strength of associations, Pearson's correlation coefficient will be used for analyses of associations between continuous variables and Spearman rank for nonparametric variables. Linear regression analysis will be used to identify determinants of AGE accumulation. Cox proportional hazards models will be used to investigate the prognostic value of the accumulation of AGE for predicting mortality. A p-value less than or equal to 0.05 will be considered to have statistical significance.

Sample size calculation of Study 1 was performed by using the software nQuery Advisor v.6.0.

Sample size Study 1:

The primary outcome for sample size determination is one-year survival in relation to increased SAF levels in people on HD and PD. With a power of 80%, a two-sided alpha of 5% and an expected hazard ratio of 3.5 and 2.0 in people on PD and HD, respectively (based on similar findings of the studies of Kimura et al. [2014] and Siriopol et al. [2015]), 100 HD and 40 PD participants will be needed.

Sample size Study 2

Since this is a proof of principle study, it would be reasonable to include 40 dialysis participants (either HD or PD).

Assessment of efficacy

Primary endpoints

Study 1:

- Mean change in SAF levels (AU) from baseline to 12 months of follow-up.
- One-year survival.

Study 2:

Mean change in SAF levels (AU) after 6 months of a dietitian supervised intensive nutritional support intervention

Secondary endpoints

Study 1:

- Regression coefficients for identifying factors associated with the accumulation of AGE.
- Correlation coefficients for analysing the association of SAF levels with morbidity, biochemistry, circulating AGE levels, inflammatory biomarkers, visual function, health-related quality of life and nutritional status.

Study 2:

- Changes in biochemistry, circulating AGE levels, inflammatory biomarkers, visual function, health-related quality of life and nutritional status.

Assessment of safety

Tolerability and safety of the nutritional support intervention during the proof of principle study (Study 2) will be assessed by direct interview. If any serious adverse event (SAE) is reported during the study, it will be recorded on an appropriate SAE report form.

Procedures for missing, unused and spurious data

All SAF measurements, biochemistry, nutritional and quality of life assessments for Study 1 and 2 will be used in the statistical analysis, including data from participants who did not complete the entire study protocol.

Definition of populations analysed

Study 1:

Full Analysis set: All participants included in the protocol for whom at least one post-baseline assessment of the primary endpoint is available.

Per protocol set: All participants in the Full Analysis set who are deemed to have no major protocol violations that could interfere with the objectives of the study.

Study 2:

Full Analysis set: All randomised participants, who participated in at least one treatment and for whom at least one post-baseline assessment of the primary endpoint is available.

Per protocol set: All participants in the Full Analysis set who are deemed to have no major protocol violations that could interfere with the objectives of the study.

ADVERSE EVENTS

The occurrence of an adverse event as a result of participation within this study is not expected and as such no adverse event data will be collected.

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

The study will not be initiated before the protocol, informed consent forms and participant information sheets have received approval / favourable opinion from the REC, and the respective NHS Research & Development (R&D) department. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of GCP, and the Department of Health Research Governance Framework for Health and Social care, 2005.

INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent will be in accordance with the REC guidance, GCP and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Trial Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the study.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No study-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

RECORDS

Case Report Forms

Case Report Forms (CRFs) will be the primary data collection instrument. All data requested on the CRF will be recorded. Data collected includes inclusion and exclusion criteria, demographic characteristics, educational level, occupation status, family history, duration of renal replacement therapy, dialysis adequacy, aetiology of CKD, medical history (e.g. history of cardiovascular disease, diabetes and hypertension), current co-morbidities, alcohol history, smoking status, current medication, type of PD, volume of PD solutions SAF measurements, routine blood tests, circulating AGE levels, inflammatory biomarkers, visual function, nutritional and quality of life assessments.

Each participant will be assigned a study identity code number for use on CRFs, other study documents and the electronic database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and final two digits from their year of birth.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Study Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the study, in accordance with regulatory requirements and for follow-up as required.

CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Trial Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.

The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

Sample Labelling

Each participant will be assigned a study identity code number for use on the samples, consent forms and other study documents and the electronic database. The identifier will include subject number, participant initials (of first and last names separated by a hyphen or a middle name initial when available) and final two digits from their year of birth.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and records. A CRF may also completely serve as its own source data. Only study staff as listed on the Delegation Log shall have access to study documentation other than the regulatory requirements listed below.

Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall made be available at all times for review by the CI, Sponsor's designee and inspection by relevant regulatory authorities (e.g. DH, Human Tissue Authority).

DATA PROTECTION

All study staff and investigators will endeavour to protect the rights of the study's participants to privacy and informed consent, and will adhere to the Data Protection Act, 1998. The CRF will only collect the minimum required information for the purposes of the study. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the study staff and investigators and relevant regulatory authorities (see above). Computer held data including the study database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).

Information about the study in the participant's medical records/hospital notes will be treated confidentially in the same way as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

Insurance and indemnity for study participants and study staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but study participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

TRIAL CONDUCT

Study conduct may be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the study; Trial Delegation Log; CVs of study staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); AE recording and reporting; accountability of study materials and equipment calibration logs.

The Study Coordinator/Academic Supervisor, or where required, a nominated designee of the Sponsor, shall carry out a site systems audit at least yearly and an audit report shall be made to the Trial Steering Committee.

TRIAL DATA

Monitoring of study data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Study Coordinator/Academic Supervisor, or where required, a nominated designee of the Sponsor, shall carry out monitoring of study data as an ongoing activity.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the study risk assessment) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the study database will be checked. Where corrections are required these will carry a full audit trail and justification.

Study data and evidence of monitoring and systems audits will be made available for inspection by REC as required.

RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Research Code of Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at

least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and study documents held by the CI on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all study databases and associated meta-data encryption codes.

DISCONTINUATION OF THE TRIAL BY THE SPONSOR

The Sponsor reserves the right to discontinue this study at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Trial Steering Committee and Data Monitoring Committee as appropriate in making this decision.

STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files. Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

If information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher will discuss this with the CI and where appropriate report accordingly.

Data generated as a result of this study will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

The results of this study will be submitted to peer-reviewed journals for publication as soon as data analysis is completed. The results will also be presented at national and international scientific conferences. Participants will not be identified in any publications. Participants will be contacted either by email or a letter sent to their addresses about the findings of the research (unless participants advise the research team that they do not wish to be contacted). In addition, participants will be informed of the results of the study via a departmental research newsletter, which is made available to all people on dialysis. The results of this study will form part of PhD thesis for Daniela Viramontes Horner. The study has been registered on www.clinicaltrials.gov (Trial registration number: NCT02878317)

USER AND PUBLIC INVOLVEMENT

Currently a patient focus group including people on HD is being established.

STUDY FINANCES

Funding source

This study is funded by internal funds from the University of Nottingham and Department of Renal Medicine, Royal Derby Hospital.

Participant stipends and payments

Participants will not be paid to participate in the study. Travel expenses will be offered for any hospital visits in excess of usual care. The majority of study assessments will be timed to take place at the time of routine visits for dialysis treatment or assessment.

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Participant Information Sheet
(Draft Version 5.0 / Final version 1.0: 14/07/2016)

Title of Study: Association of advanced glycation end-product accumulation/skin autofluorescence and adverse outcomes in peritoneal dialysis and haemodialysis patients (Study 1).

Name of Researcher(s): Professor Maarten Taal (Chief Investigator); Dr. Nick Selby (Co-investigator); Daniela Viramontes Horner (PhD Student).

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?

The purpose of the present study is to investigate the association between the accumulation of advanced glycation end-products (AGEs) in the skin and adverse outcomes in people receiving haemodialysis (HD) and peritoneal dialysis (PD).

AGEs are compounds that form naturally in our bodies from the chemical reaction of sugars with proteins. If the concentration of AGEs becomes excessive in our bloodstream, they can cause damage to almost every tissue and organ in our bodies. More importantly, AGEs are also found naturally in foods and the content of AGEs in foods is increased considerably by using cooking methods that require high temperatures, such as frying, roasting and grilling.

Why have I been invited?

You are being invited to take part because you are undergoing HD or PD and have met all the requirements to be included in this study. We are inviting up to 170 participants like you to take part.

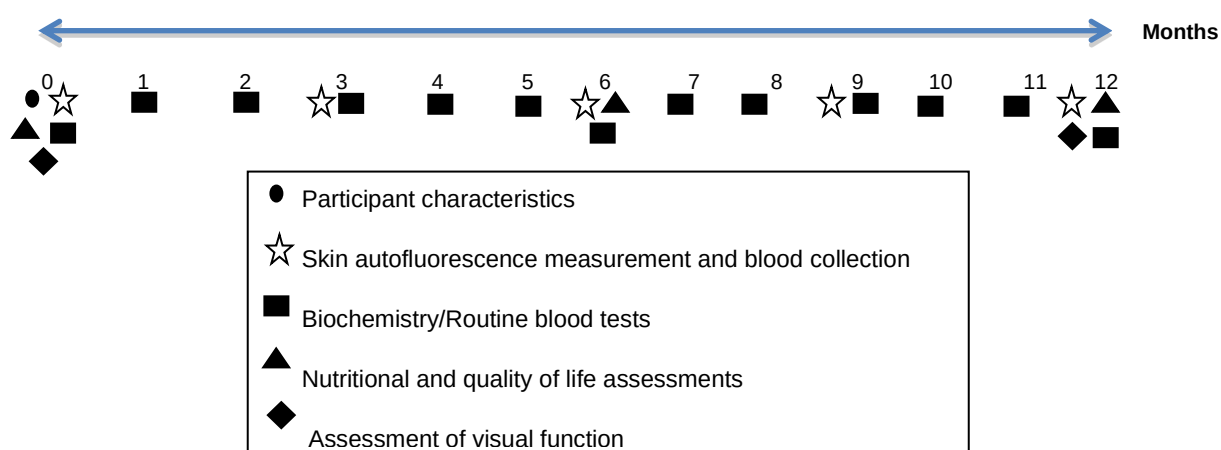
Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This would not affect either your legal rights or the quality of your future medical care.

What will happen to me if I take part?

The research team will measure the accumulation of advanced glycation end-products (AGEs) in your skin using a quick (less than five minutes) and painless technique called skin autofluorescence. This involves placing your forearm on a piece of equipment that shines a light on the skin and measures the amount of light that is reflected back. In addition, the research team will ask you information regarding your age, educational level, socioeconomic status, current medication, smoking status, medical and alcohol history. We will also ask you to complete several nutritional and quality of life questionnaires, as well as measurements of your weight, height, arm circumference and skinfold thickness, simple tests of your eyesight and routine blood tests. We will repeat the skin autofluorescence measurements every 3 months for 1 year and will ask you to complete the questionnaires again after 6 and 12 months. Study assessments will be arranged to take place when you attend for dialysis or routine clinic visits. We will collect the results of the blood tests that you usually have done every month as part of your treatment. We will also collect and store approximately two teaspoons of your blood every 3 months for 1 year to measure AGE levels and do some additional blood tests to help us investigate the effects of AGEs on the body. If you are on haemodialysis we will take the blood during your usual treatment so that no extra needle puncture will be required and if you are on peritoneal dialysis we will try to take the blood at the same time as your routine blood tests. The blood specimens will be stored in a freezer until they are

analysed in a laboratory. If you agree, we will also store some of the remaining blood for future research. If you do not agree to the future use of the samples, they will be destroyed in accordance with the Human Tissue Act, 2004. In the diagram shown below, you will find a timeline for the first year of the study. After the first year of the study, we will repeat the skin autofluorescence readings and questionnaires once a year. We will collect information from your medical records about your health and any hospital admissions you have for a total of 5 years.



Expenses and payments

You will not be paid to participate in this study. Study assessment will usually be arranged to take place when you attend for dialysis or routine clinic visits but if you have to make an extra visit for the study, travel expenses will be offered.

What are the possible disadvantages and risks of taking part?

This study does not involve any intervention or new treatment and there are therefore no specific risks associated with taking part. However, if you experience anything untoward, the research team will record it and you will be closely monitored until it has resolved.

What are the possible benefits of taking part?

This study does not involve any intervention or new treatment and there is therefore unlikely to be any specific benefit. The study will however help us to better understand the role of advanced glycation end-products in health problems

experienced by people on dialysis and may identify ways in which these could be improved.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers contact details are given at the end of this information sheet.

If you remain unhappy and wish to complain formally, you can do this by contacting the Patient Advisory & Liaison Service (PALS) at:

Freephone: 0800 7837691

Email: pals@derbyhospitals.nhs.uk

Office phone: 01332 785156

Will my taking part in the study be kept confidential?

We will follow ethical and legal practice and all information about you will be handled in confidence.

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for 6-12 months after the end of the study so that we are able to contact you about the findings of

the study (unless you advise us that you do not wish to be contacted). All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

What will happen if I don't want to carry on with the study?

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and with your permission, this information may still be used in the project analysis.

Involvement of the General Practitioner/Family doctor (GP)

Your GP will not specifically be informed about your involvement because it will not have any impact on your treatment.

What will happen to the results of the research study?

The results of this study will be submitted to scientific journals for publication as soon as data analysis is completed. The results will also be presented at conferences. You will not be identified in any publications. However, participants will be informed of the results of the study via a departmental research newsletter, which is made available to all people on dialysis at Royal Derby Hospital. The results of this study will form part of PhD thesis for Daniela Viramontes Horner.

Who is organising and funding the research?

This research is being organised by the University of Nottingham and is being funded by the University and Royal Derby Hospital.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by East Midlands – Nottingham 1 Research Ethics Committee.

Further information and contact details

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Participant Information Sheet
(Draft Version 6.5 / Final version 2.0: 04/10/2017)

Title of Study: Associations of advanced glycation end-product accumulation and adverse outcomes in peritoneal dialysis and haemodialysis patients and the impact of a dietetic intervention on skin autofluorescence (Study 2).

Name of Researcher(s): Professor Maarten Taal (Chief Investigator); Dr. Nick Selby (Co-investigator); Daniela Viramontes Horner (PhD Student).

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?

Advanced glycation end-products (AGEs) are compounds that form naturally in our bodies from the chemical reaction of sugars with proteins. If the concentration of AGEs becomes excessive in our bloodstream, they can cause damage to almost every tissue and organ in our bodies. Our previous research shows that people who become under-nourished while receiving dialysis tend to have higher AGE levels in their skin. It therefore seems likely that improving nutrition will result in a decrease in AGE levels, but this has not previously been studied.

The purpose of this study is to investigate whether improving your nutrition by providing personalised advice from a dietitian and nutritional support (which includes dietary supplement drinks) results in a decrease in the accumulation of

advanced glycation end-products (AGEs) in the skin of people receiving haemodialysis (HD) and peritoneal dialysis (PD).

Why have I been invited?

You are being invited to take part because you are undergoing HD or PD and assessment by a dietitian shows that you would benefit from additional support to improve your nutrition. We are inviting 40 people like you to take part.

Do I have to take part?

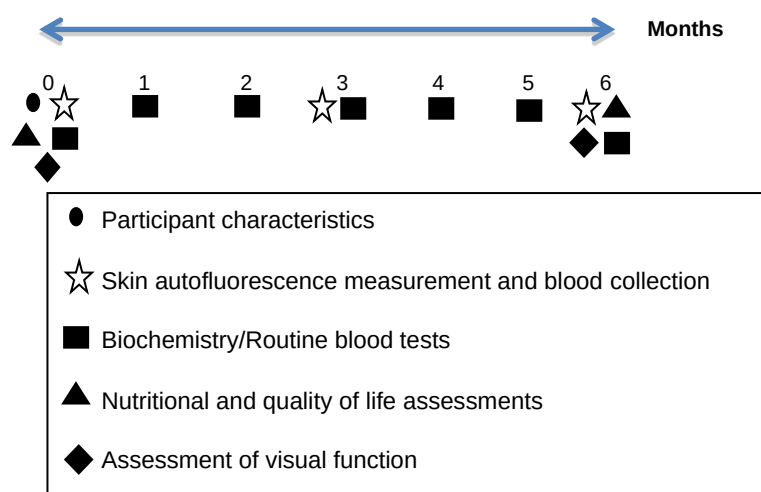
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This would not affect either your legal rights or the quality of your future medical care.

What will happen to me if I take part?

Once you have agreed to take part in this study, you will be asked to take dietary supplement drinks for up to **6 months** and will receive advice regarding your diet by an experienced dietitian. You will receive precise oral and written instructions on how and when to take the supplements and how to keep to your diet.

The research team will measure AGEs in your skin through a quick (less than five minutes) and painless technique called skin autofluorescence before you start taking the diet supplements. This involves placing your forearm on a piece of equipment that shines a light on the skin and measures the amount of light that is reflected back. In addition, the research team will ask you information regarding your age, educational level, occupational status, current medication, smoking status, medical and alcohol history. We will also ask you to complete several diet and quality of life questionnaires, as well as measurements of your weight, height, arm circumference and skinfold thickness, simple tests of your eyesight and routine blood/laboratory tests. We will repeat the skin autofluorescence measurements every 3 months for 6 months and will ask you to complete the questionnaires again after 6 months. Study assessments will be

arranged to take place when you attend for dialysis or routine clinic visits. We will collect the results of the blood tests that you usually have done every month as part of your treatment. We will also collect and store approximately two teaspoons of your blood every 3 months for 6 months to measure AGE levels and do some additional blood tests to help us investigate the effects of AGEs on the body. If you are on haemodialysis we will take the blood before and after your usual treatment so that no extra needle puncture will be required and if you are on peritoneal dialysis we will try to take the blood at the same time as your routine blood tests. The blood specimens will be stored in a freezer until they are analysed in a laboratory. If you agree, we will also store some of the remaining blood for future research. If you do not agree to the future use of the samples, they will be destroyed in accordance with the Human Tissue Act, 2004. In the diagram shown below, you will find a timeline for the 6 months of the study. We will collect information from your medical records about your health and any hospital admissions you have for a total of 2 years.



Expenses and payments

You will not be paid to participate in this study. Study assessments will usually be arranged to take place when you attend for dialysis or routine clinic visits but if you have to make an extra visit for the study, travel expenses will be offered.

What are the possible disadvantages and risks of taking part?

This study will involve taking a dietary supplement and following your normal diet; therefore, this intervention is not expected to be associated with any risks. However, if you experience anything untoward, the research team will record

this and you will be closely monitored until it has resolved. Participants will be monitored regularly to ensure that they are taking the supplements.

What are the possible benefits of taking part?

This study involves a dietitian supervised intervention (dietary advice and supplements) that may help to improve the nutritional status of your body. The study will also help us to better understand the role of advanced glycation end-products in health problems experienced by people on dialysis and may identify ways in which these could be improved.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this by contacting the Patient Advisory and Liaison Service (PALS) at:

Freephone: 0800 7837691

email: pals@derbyhospitals.nhs.uk

Office phone: 01332 785156

Will my taking part in the study be kept confidential?

We will follow ethical and legal practice and all information about you will be handled in confidence.

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a

password protected database. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for 6-12 months after the end of the study so that we are able to contact you about the findings of the study (unless you advise us that you do not wish to be contacted). All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

What will happen if I don't want to carry on with the study?

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and with your permission, this information may still be used in the project analysis.

Involvement of the General Practitioner/Family doctor (GP)

Your GP will not specifically be informed about your involvement because it will not have any impact on your treatment.

What will happen to the results of the research study?

The results of this study will be submitted to scientific journals for publication as soon as data analysis is completed. The results will also be presented at conferences. You will not be identified in any publications. However, participants will be informed of the results of the study via a departmental research newsletter, which is made available to all people on dialysis at Royal Derby Hospital. The results of this study will form part of PhD thesis for Daniela Viramontes Horner.

Who is organising and funding the research?

This research is being organised by the University of Nottingham and is being funded by the University and Royal Derby Hospital.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by East Midlands – Nottingham 1 Research Ethics Committee.

Further information and contact details**Professor Maarten Taal**

Professor of Medicine and Honorary Consultant Nephrologist

Division of Medical Sciences and Graduate Entry Medicine, University of Nottingham, Derby Medical School, Uttoxeter Rd, Derby, DE22 3NE

Phone: 01332 789344

Email: m.taal@nottingham.ac.uk;

maarten.taal1@nhs.net

Dr Nick Selby

Associate Professor and Honorary Consultant Nephrologist

Royal Derby Hospital, Uttoxeter Rd, Derby, DE22 3NE

Phone: 01332 789344

Email: nicholas.selby@nottingham.ac.uk

Daniela Viramontes Horner, MRes Dietetics

Student, PhD in Medicine and Health

Royal Derby Hospital, Uttoxeter Rd, Derby, DE22 3NE

Phone: 01332 788262

Email: mzxdrv@nottingham.ac.uk

East Midlands - Nottingham 1 Research Ethics Committee

The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

06 July 2016

Professor Maarten Taal
Division of Medical Sciences and Graduate Entry Medicine
University of Nottingham, Derby Medical School
Uttoxeter Rd, Derby
DE22 3NE

Dear Professor Taal

Study title:	Association of advanced glycation end-product accumulation and adverse outcomes in peritoneal dialysis and haemodialysis patients and the impact of a dietetic intervention on skin autofluorescence
REC reference:	16/EM/0243
Protocol number:	15050
IRAS project ID:	200680

Thank you for responding to the Favourable opinion with conditions letter. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 23 June 2016

Documents received

The documents received were as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Interview schedules or topic guides for participants [AGEs Food choice list]	1.0	01 July 2016
Participant consent form [Consent form Study 1]	1.0	01 July 2016
Participant consent form [Consent form Study 2]	1.0	01 July 2016
Participant information sheet (PIS) [PIS Study 1]	1.0	01 July 2016
Participant information sheet (PIS) [PIS Study 2]	1.0	01 July 2016
Validated questionnaire [Dietary questionnaire for the assessment of AGE intake]		

Approved documents

The final list of approved documentation for the study is therefore as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering letter on headed paper [Cover letter]		24 May 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [University of Nottingham Clinical Trials Insurance]		21 July 2015
Interview schedules or topic guides for participants [AGEs Food choice list]	1.0	01 July 2016
IRAS Application Form [IRAS_Form_24052016]		24 May 2016
IRAS Application Form XML file [IRAS_Form_24052016]		24 May 2016
IRAS Checklist XML [Checklist_01062016]		01 June 2016
Letter from sponsor [Letter from sponsor]	1.0	23 May 2016
Non-validated questionnaire [Screening questionnaire for visual acuity assessment]		
Participant consent form [Consent form Study 1]	1.0	01 July 2016
Participant consent form [Consent form Study 2]	1.0	01 July 2016
Participant information sheet (PIS) [PIS Study 1]	1.0	01 July 2016
Participant information sheet (PIS) [PIS Study 2]	1.0	01 July 2016
Research protocol or project proposal [Final protocol]	1.0	17 May 2016
Summary CV for Chief Investigator (CI) [CV Prof. Maarten Taal]		21 March 2016
Summary CV for student [Daniela Viramontes Horner (PhD Student)]	1.0	19 May 2015
Summary CV for supervisor (student research) [Prof. Maarten Taal (Principal academic supervisor)]		21 March 2016
Summary CV for supervisor (student research) [Dr. Nick Selby (Second Academic Supervisor)]		24 November 2015
Validated questionnaire [EQ5D quality of life questionnaire]	1.0	17 May 2016
Validated questionnaire [36-Item Short Form Health Survey (SF-36)]	1.0	17 May 2016
Validated questionnaire [7-point scale SGA]	1.0	17 May 2016
Validated questionnaire [24 h dietary recall questionnaire]		
Validated questionnaire [Visual functioning questionnaire]		
Validated questionnaire [Dietary questionnaire for the assessment of AGE intake]		

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

16/EM/0243

Please quote this number on all correspondence

Yours sincerely



Andrea Graham
REC Manager

E-mail: NRESCommittee.EastMidlands-Nottingham1@nhs.net

Professor Maarten Taal
 Division of Medical Sciences and Graduate Entry Medicine
 University of Nottingham, Derby Medical School
 Uttoxeter Rd, Derby
 DE22 3NE

27 July 2016

Dear Professor Taal

Letter of HRA Approval

Study title:	Association of advanced glycation end-product accumulation and adverse outcomes in peritoneal dialysis and haemodialysis patients and the impact of a dietetic intervention on skin autofluorescence
IRAS project ID:	200680
Protocol number:	15050
REC reference:	16/EM/0243
Sponsor	The University of Nottingham

I am pleased to confirm that HRA Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. Please read Appendix B carefully, in particular the following sections:

- Participating NHS organisations in England – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- Confirmation of capacity and capability - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.

- Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria) - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The document “After Ethical Review – guidance for sponsors and investigators”, issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the After Ethical Review document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at <http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/>.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please email the HRA at hra.approval@nhs.net. Additionally, one of our staff would be happy to call and discuss your experience of HRA Approval.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at <http://www.hra.nhs.uk/hra-training/>

Your IRAS project ID is **200680**. Please quote this on all correspondence.

Yours sincerely

Natalie Wilson

Assessor

Email: hra.approval@nhs.net

Copy to:

Ms Angela Shone, University of Nottingham, Sponsor contact

Ms Teresa Grieve, Derby Hospital NHS Foundation Trust, Lead R&D contact

Miss Daniela Viramontes Homer, University of Nottingham, PhD student

Appendix A - List of Documents

The final document set assessed and approved by HRA Approval is listed below.

<i>Document</i>	<i>Version</i>	<i>Date</i>
Interview schedules or topic guides for participants [AGEs Food choice list]	1.0	01 July 2016
IRAS Application Form [IRAS_Form_24052016]		24 May 2016
Non-validated questionnaire [Screening questionnaire for visual acuity assessment]		
Other [Schedule of Events]	1	26 July 2016
Other [Statement of Activities]	1	26 July 2016
Participant consent form [Consent for Study 1]	5	14 July 2016
Participant consent form [Consent form Study 2]	5	14 July 2016
Participant information sheet (PIS) [PIS Study 1]	5	14 July 2016
Participant information sheet (PIS) [PIS Study 2]	5	14 July 2016
Research protocol or project proposal [Final protocol]	1.0	17 May 2016
Validated questionnaire [EQ5D quality of life questionnaire]	1.0	17 May 2016
Validated questionnaire [36-Item Short Form Health Survey (SF-36)]	1.0	17 May 2016
Validated questionnaire [7-point scale SGA]	1.0	17 May 2016
Validated questionnaire [Dietary questionnaire for the assessment of AGE intake]		
Validated questionnaire [24 h dietary recall questionnaire]		
Validated questionnaire [Visual functioning questionnaire]		
Validated questionnaire [Dietary questionnaire for the assessment of AGE intake]		

Appendix B - Summary of HRA Assessment

This appendix provides assurance to you, the sponsor and the NHS in England that the study, as reviewed for HRA Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England to assist in assessing and arranging capacity and capability.

For information on how the sponsor should be working with participating NHS organisations in England, please refer to the, participating NHS organisations, capacity and capability and Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria) sections in this appendix.

The following person is the sponsor contact for the purpose of addressing participating organisation questions relating to the study:

Daniela Viramontes Homer (Tel: 01332 788262; Email: Daniela.Viramonteshorner@nottingham.ac.uk)

HRA assessment criteria

Section	HRA Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	Minor amendments have been made to the PIS and Consent forms post REC to ensure that they conform to HRA Standards.
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	This is a non-commercial study, single site study where the sponsor is not the participating organisation. A Statement of Activities has been submitted by Sponsor. Sponsor is using a separate agreement for use between themselves and participating NHS organisations. Sponsor is using a modified template agreement where several amendments have been made. Sponsor has confirmed that a MTA is not required.

Section	HRA Assessment Criteria	Compliant with Standards	Comments
4.2	Insurance/indemnity arrangements assessed	Yes	Where applicable, independent contractors (e.g. General Practitioners) should ensure that the professional indemnity provided by their medical defence organisation covers the activities expected of them for this research study
4.3	Financial arrangements assessed	Yes	Sponsor will not provide participating NHS organisations with funding.
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	Sponsor has confirmed that no patient identifiable data will be leaving the participating NHS organisation.
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	
6.3	Devices – MHRA notice of no objection received	Not Applicable	
6.4	Other regulatory approvals and authorisations received	Not Applicable	

Participating NHS Organisations in England

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.

This is a non-commercial, single site study. There is only one site-type involved in the research. Participants will be identified by their usual dialysis care team. Interested individuals will be handed a copy of the PIS. The researcher will then be contacted by the dialysis team. A range of study activities and procedures will take place at participating NHS organisations.

The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. For NIHR CRN Portfolio studies, the Local LCRN contact should also be copied into this correspondence. For further guidance on working with participating NHS organisations please see the HRA website.

If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England which are not provided in IRAS or on the HRA website, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net. The HRA will work with these organisations to achieve a consistent approach to information provision.

Confirmation of Capacity and Capability

This describes whether formal confirmation of capacity and capability is expected from participating NHS organisations in England.

Participating NHS organisations in England **will be expected to formally confirm their capacity and capability to host this research.**

- Following issue of this letter, participating NHS organisations in England may now confirm to the sponsor their capacity and capability to host this research, when ready to do so. How capacity and capability will be confirmed is detailed in the *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* section of this appendix.
- The [Assessing, Arranging, and Confirming](#) document on the HRA website provides further information for the sponsor and NHS organisations on assessing, arranging and confirming capacity and capability.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and the minimum expectations for education, training and experience that PIs should meet (where applicable).

Sponsor has indicated that the Chief Investigator (CI) is responsible for research activity at the participating NHS organisation. A local PI and local collaborator is not required.

Sponsor has determined that the main researcher (PhD student) should complete GCP training as the study is a Randomised Controlled Trial.

GCP training is not a generic training expectation, in line with the [HRA statement on training expectations](#).

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken.

Sponsor has indicated that the researcher (PhD student) employed by the University of Nottingham will have an Honorary Clinical Contract in place with the participating NHS organisation.

If the researcher (PhD student) does not have an Honorary Clinical Contract in place, an enhanced CRB, plus appropriate barred list check, occupational health clearance and Honorary Research Contract will be required.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England to aid study set-up.

The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.

East Midlands - Nottingham 1 Research Ethics Committee

The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

14 November 2017

Professor Maarten Taal
Division of Medical Sciences and Graduate Entry Medicine
University of Nottingham, Derby Medical School
Uttoxeter Rd, Derby
DE22 3NE

Dear Professor Taal

Study title:	Association of advanced glycation end-product accumulation and adverse outcomes in peritoneal dialysis and haemodialysis patients and the impact of a dietetic intervention on skin autofluorescence
REC reference:	16/EM/0243
Protocol number:	16050
Amendment number:	1
Amendment date:	13 October 2017
IRAS project ID:	200680

The above amendment was reviewed at the meeting of the Sub-Committee held in correspondence on 13 November 2017.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Notice of Substantial Amendment (non-CTIMP) [AmendmentForm 13.10.17.pdf]	1	13 October 2017
Participant consent form [Consent form study 2. Skin autofluorescence as a risk marker in people receiving dialysis v5.5 04.10.2017.doc]	5.5	04 October 2017
Participant information sheet (PIS) [PIS Study 2. Skin autofluorescence as a risk marker in people receiving dialysis v6.5 04.10.2017.docx]	6.5	04 October 2017
Research protocol or project proposal [PROTOCOL. Skin autofluorescence as a risk marker in people receiving dialysis v4.5 04.10.2017.docx]	4.5	04 October 2017

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our Research Ethics Committee members' training days – see details at <http://www.hra.nhs.uk/hra-training/>

16/EM/0243: Please quote this number on all correspondence

Yours sincerely



Ms Ellen Milazzo

Chair

E-mail: NRESCommittee.EastMidlands-Nottingham1@nhs.net

Enclosures:

List of names and professions of members who took part in the review

East Midlands - Nottingham 1 Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 13 November 2017

Committee Members:

<i>Name</i>	<i>Profession</i>	<i>Present</i>
Mr Oliver Matias	Clinical Trials Researcher	Yes
Ms Ellen Milazzo (Chair)	Development and Change Management Consultant	Yes

Also in attendance:

<i>Name</i>	<i>Position (or reason for attending)</i>
Mr George R. Martin	REC Assistant (Minutes)

From: TOTENHOFER, Ashley (HEALTH RESEARCH AUTHORITY)
ashley.totenhofer@nhs.net
Sent: 13 December 2017 14:58
To: Maarten Taal; sponsor@nottingham.ac.uk
Cc: Daniela Viramontes Horner
Subject: RE: IRAS 200680. Confirmation of Amendment Assessment

Dear Professor Taal

Further to the below, I am pleased to confirm **HRA Approval** for the referenced amendment.

You should implement this amendment at NHS organisations in England, in line with the conditions outlined in your categorisation email.

Please contact hra.amendments@nhs.net for any queries relating to the assessment of this amendment.

Kind regards

Ashley

Ashley Totenhofer
Technical Assurance Officer
Health Research Authority

HRA Centre Manchester | 3rd Floor, Barlow House | 4 Minshull Street,
Manchester | M1 3DZ T. 0207 104 8017 E ashley.totenhofer@nhs.net W.
www.hra.nhs.uk

From: NRESCCommittee.EastMidlands-Nottingham1@nhs.net
[mailto:NRESCCommittee.EastMidlands-Nottingham1@nhs.net]
Sent: 18 October 2017 16:41
To: m.taal@nottingham.ac.uk; sponsor@nottingham.ac.uk
Subject: IRAS 200680. Amendment confirmation of REC Validation, categorisation and implementation information

Amendment Confirmation of REC Validation, Categorisation and Implementation Information

Dear Professor Taal,

Thank you for submitting an amendment to your project. Please find attached a copy of the REC validation letter for the submitted amendment.

If you have participating NHS/HSC organisations in any other UK nations we will forward the information to the relevant national coordinating function(s).

Please note that you may only implement changes described in the amendment notice.

What Happens Next?

When available, please forward any other regulatory approvals that are expected for this amendment to hra.amendments@nhs.net. However, you do not need to forward the REC favourable opinion as we will be able to access this through our systems.

Information Specific to Participating NHS Organisations in England

You should now share your notice of amendment and, if applicable, amended documents, together with this email, with all participating NHS organisations in England. In doing so, you should include the [NHS R&D Office, LCRN](#) (where applicable) as well as the local research team. A template email to notify participating NHS organisations in England is provided on the [HRA website](#).

The participating NHS organisations in England should prepare to implement this amendment.

Your amendment will be reviewed by the REC, as per the attached letter. In parallel to this, an assessment against [HRA standards](#) will take place.

Once the REC Favourable Opinion is issued, any other regulatory approvals are in place and the HRA assessment has been successfully completed, you will receive an email confirming that your amendment has HRA Approval.

You may implement your amendment at all participating NHS organisations in England 35 calendar days from the day on which you provide the organisations with this email and your amended documents (or as soon as the participating NHS organisation confirm that you may implement, if sooner), so long as you have HRA Approval for your amendment by this date. **NHS organisations do not have to confirm they are happy with the amendment.** If HRA Approval is issued subsequent to this date, you may implement following HRA Approval.

You may not implement the amendment at any participating NHS organisations in England that requests additional time to assess, until it confirms that it has concluded its assessment.

You may not implement at any participating NHS organisation in England that declines to implement the amendment.

IRAS Project ID:	200680
Short Study Title:	Skin autofluorescence as a risk marker in people receiving dialysis
Date complete amendment submission received:	13 October 2017
Amendment No./ Sponsor Ref:	1
Amendment Date:	13 October 2017
Amendment Type:	Substantial
Outcome of HRA Assessment	HRA Approval for the amendment is pending. The HRA will separately confirm HRA Approval for the amendment by email.
Implementation date in NHS organisations in England	35 days from date amendment information together with this email, is supplied to participating organisations (provided HRA Approval for the amendment is in place and conditions above are met)
For NHS/HSC R&D Office information	
Amendment Category	A

If you have any questions about the ethical review of this amendment, please do not hesitate to contact me.

If you have any questions relating to the wider HRA approval process, please direct these to hra.approval@nhs.net.

If you have any questions relating this amendment in one of the devolved administrations, please direct these to the relevant [national coordinating function](#).

Additional information on the management of amendments can be found in the [IRAS guidance](#).

Please do not hesitate to contact me if you require further information.

Kind regards

Mr George R. Martin
REC Assistant
Health Research Authority

Ground Floor | Skipton House | 80 London Road | London | SE1 6LH
E. hra.amendments@nhs.net
W. www.hra.nhs.uk