Exploring the changes in insulin resistance among patients with different degrees of renal failure, and its role in their cardiovascular mortality

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“Of one essence is the human race,
    Thusly has creation put the base.
    One limb impacted is sufficient,
    For all others to feel the mace.
    The unconcern’d with others’ plight,
    Are but brutes with human face.”

Sa’dī Shīrāzī (1184 – 1283 A.D.)
Persian Poet
I would like to dedicate this thesis to my loving husband Amirhossein: without you none of this would have ever been possible.
Abstract

Patients with chronic kidney disease (CKD) are at increased risk of mortality and cardiovascular (CV) disease and those on maintenance haemodialysis (MHD) are at particularly high risk. Insulin resistance (IR) is known as a contributing factor to many of the conditions classified as ‘classic CV risk factors’ and understanding changes in IR is thought to be of key importance in targeting potential medication.

A prospective observational study of individuals with CKD was designed to better establish the relationship between CV risk factors and events, before and after the onset of MHD. In addition, a subgroup study was set up to examine if changes in IR affect CV outcome and mortality.

519 patients were recruited for this study, 210 were on MHD at entry and 309 had CKD stages 3, 4 and 5. Subjects were followed prospectively for 24 months and the relationship between body mass index (BMI), blood pressure (BP), total cholesterol (TC), triglycerides (TG), diabetes status and glycosylated haemoglobin (HbA1c) and CV events analysed in both MHD and non-MHD groups using logistic regression. A small study was also carried out to determine the reliability of HbA1c in MHD subjects. 106 of these subjects were recruited into the subgroup study, where baseline assessment of their IR (using the HOMA-IR model) was carried out as well as other relevant tests.

103 subjects reached an endpoint, and analysis of the whole cohort identified high SBP and diabetes as significant CV risk factors. TC was recognized as a protective factor as lower TC was associated with higher CV risk. The mean HOMA-IR increased through the CKD stages 3-5 before significantly falling in the MHD group. HbA1c showed poor association with glycaemia as measured by use of 48-hour continuous glucose monitors (CGM).

These results suggest that CV risk factors may vary at different stages of CKD. The “reverse” TC result only in the non-dialysis group differs from current theories, where low cholesterol is considered a CV risk factor putatively as a surrogate marker of malnutrition. This study shows that IR increases as renal function deteriorates but is significantly decreased with MHD. In this cohort changes in IR were not predictive of CVE or mortality.
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Statement of Originality

All work presented in this thesis is the result of my own work, unless otherwise stated in the text. All collaborations have been acknowledged in the appropriate place within the text. This work was funded by the King Faisal Foundation.
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Abbreviations

**ACCORD**: Action to control cardiovascular disease in diabetes (trial)

**ACE**: Angiotensin converting enzyme

**ACEI**: Angiotensin converting enzyme inhibitor

**ACTH**: Adrenocorticotropic hormone

**ADA**: American Diabetes Association

**ADVANCE**: Action in diabetes and vascular disease (trial)

**AIDS**: Acquired immune deficiency syndrome

**ARB**: Angiotensin II receptor blocker

**ATP III**: Adult Treatment Programme III

**BMI**: Body mass index

**BP**: Blood pressure

**CAD**: Coronary artery disease

**CAPD**: Continuous ambulatory peritoneal dialysis

**CARDS**: Collaborative Atorvastatin diabetes study

**CGM**: Continuous glucose monitoring

**CHF**: Chronic heart failure

**CHF**: Congestive heart failure

**CI**: Confidence interval

**CIGMA**: Continuous infusion of glucose with model assessment
**CKD:** Chronic kidney disease

**COPD:** Chronic obstructive pulmonary disease

**CRH:** Cortisol releasing hormone

**CRP:** C-reactive protein

**CV:** Cardiovascular

**CVD:** Cardiovascular disease

**CVE:** Cardiovascular event

**DBP:** Diastolic blood pressure

**DCCT:** Diabetes control and complications trial

**ESRD/ESRF:** end-stage renal disease/end-stage renal failure

**GFR/eGFR:** Glomerular filtration rate/estimated GFR

**GH:** Growth hormone

**HbA1c:** Glycosylated haemoglobin

**HD:** Haemodialysis

**HDL:** High-density lipoprotein

**HIV:** Human immunodeficiency virus

**HOMA/HOMA-IR:** Homeostatic model assessment/homeostatic model assessment index of insulin resistance

**Hs-CRP:** high sensitivity c-reactive protein

**ICKTI:** Imperial College kidney and transplant institute

**IGF:** Insulin-like growth factor

**IHD:** Ischaemic heart disease
IL: Interleukin

IST: Insulin sensitivity test

ITT: Insulin tolerance test

Kt/V: Dialysis adequacy

LDL: Low density lipoprotein

LPS: Lipopolysaccharide

MHD: Maintenance haemodialysis

MI: Myocardial infarction

MIA: Malnutrition inflammation atherosclerosis

MICS: Malnutrition-inflammation complex syndrome

OGTT: Oral glucose tolerance test

OR: Odds ratio

PEM: Protein energy malnutrition

PTH: Parathyroid hormone

PVD: Peripheral vascular disease

QUICKI: Quantitative insulin sensitivity check index

RAS: Renin angiotensin system

RRT: Renal replacement therapy

SBP: Systolic blood pressure

SD: Standard deviation

TC: Total cholesterol
TG: Triglycerides

TIBC: Total iron binding capacity

TNF: Tumor necrosis factor

UKPDS: United Kingdom prospective diabetes study

USRDS: United Stated Renal Data System

VADT: Veterans affairs diabetes trial

WHO: World Health Organisation

WLRTC: West London renal and transplant centre
Chapter 1: Introduction

1.1 Cardiovascular Risk Factors in ESRD

1.1.1 Background

Chronic kidney disease (CKD) is a public health problem that has been increasing in parallel to the rise of obesity and subsequent type 2 diabetes [1]. CKD is important because it is primarily a marker for cardiovascular disease (CVD), as even early stage CKD causes an estimated 40-100% increase in risk of cardiovascular events [2]. As CKD progresses, cardiovascular disease remains the main cause of mortality in these patients [3-5], but what is considered as cardiovascular risk factors in this group of patients has been the subject of controversy [6, 7]. These controversies are based on the argument that suggests what constitutes as a cardiovascular risk factor in individuals with normal kidney function, may actually have protective qualities against cardiovascular disease and subsequent mortality in patients who have renal impairment, and more specifically in recipients of renal replacement therapy in the form of haemodialysis [6-10].

One of the main contributors to the rise in CKD is obesity [11-13]. The obesity epidemic that was first noted in the U.S. has now become a pandemic, with more than 1.6 billion overweight and at least 400 million clinically obese people in the world, according to the World health Organisation (WHO) [14]. Over weight and obesity are at the root of many cardiovascular risk factors, including impaired glucose tolerance, type 2 diabetes, dyslipidaemia and hypertension. These conditions are chronic in nature and life-threatening as they are responsible for the expanding epidemics of cardiovascular disease. The association of obesity and cardiovascular disorders (CVD) is well documented and includes hypertension [15], myocardial infarction and stroke [16, 17]. It is these conditions that ultimately cause the increased risk of mortality in obese patients; and why there is a progressive increase in risk of death as adiposity increases above normal [18]. Obesity is also implicated, as an important factor in the emerging spread of chronic kidney disease (CKD) and end-stage renal disease (ESRD) [19]; it has been observed that excess body weight is linked to CKD by two large studies [20, 21], although the exact mechanisms leading to renal damage are still largely unknown.
Another well-established consequence of obesity is type 2 diabetes [22, 23], which is ranked as the leading cause of end-stage renal failure (ESRF) in the developed world [24]. The coexistence of obesity and chronic hyperglycaemia, as a result of impaired glucose tolerance or diabetes [25, 26], dramatically increases risk of cardiovascular disease. Type 2 diabetes alone is responsible for the majority of health costs associated with obesity, and as it is a facilitator of all other consequences of obesity, it has been dubbed ‘the disease of the century’.

Type 2 diabetes is a chronic condition that has many complications; these include macro-vascular disease such as cardiovascular disease (including cardiomyopathy, atherosclerosis and hypertension) and renovascular disease (nephropathy), as well as micro-vascular disease (including retinopathy and peripheral vascular disease). Nephropathy is perhaps one of the most costly complications, as many patients require chronic renal replacement therapies as a result of renal failure. About 30% of patients with diabetes develop nephropathy, about 10% develop renal failure as a cause of this disease, but nearly 44% of all end-stage renal failure (ESRF) cases are due to diabetes [27]. Unfortunately, despite many advances in medicine and renal replacement therapy, the mortality rates for these patients are still high, with 25% first-year mortality rate for patients on maintenance haemodialysis (MHD) and 18% for those on continuous ambulatory peritoneal dialysis (CAPD)[24].

Perhaps the reason behind such high mortality rates in the ESRF population is the fact that mortality risk factors are less well understood. Despite the many studies that have been carried out since the 1960s, modern medicine still looks slightly confused as to what constitutes as a risk factor in these patients. What is known is that more than half of the deaths that occur in this population are attributed to cardiovascular disease, which means that the incidence of fatal cardiovascular events in ESRF is twice as high as the general population (30% vs. 15%) [24, 28].

Identifying the causes and mechanisms of this increased incidence of cardiovascular disease in patients receiving renal replacement therapy will ensure that the required measures are taken to prevent mortality as well as improve patients’ quality of life.
1.1.2 End Stage Renal Failure: Causes

Chronic kidney disease (CKD) is the progressive loss of renal function over months or years. The Kidney Disease Outcomes Quality Initiative guidelines [29] have divided CKD into five stages according to the glomerular filtration rate (GFR);

- **Stage 1**: patient with normal GFR but with some evidence of kidney damage such as microalbuminuria/proteinuria, haematuria, or histological changes
- **Stage 2**: mild CKD with a GFR ranging from 89 to 60 mL/min/1.73 m²
- **Stage 3**: moderate CKD with a GFR ranging from 59 to 30 mL/min/1.73 m²
- **Stage 4**: severe CKD with GFR ranging from 29 to 15 mL/min/1.73 m²
- **Stage 5**: kidney failure when GFR is less than 15 mL/min/1.73 m².

Stage 5 is when renal replacement therapy (RRT) either in the form of dialysis or transplantation has to be considered.

Important notes to keep in mind are that all individuals with a GFR <60 mL/min/1.73m² for 3 months, whether kidney damage is present or not, are classified as CKD; this is because this reduction represents loss of half or more of the normal kidney function in adults. The other point is that all individuals with kidney damage, irrespective of their GFR, are classified as CKD; this is because despite substantial kidney damage, GFR may be maintained at 60 mL/min/1.73 m² or above, and these patients are at increased risk of both of the major outcomes of CKD: kidney failure and cardiovascular disease [29, 30].

As proteinuria is regarded an independent marker for deteriorating renal function and cardiovascular disease, British guidelines append the letter ‘P’ to the stage of CKD in case of significant protein loss [30].

Renal failure is the temporary or permanent loss of normal kidney function. It is categorised into two types: acute and chronic. Acute renal failure (ARF) is abrupt but potentially reversible, while chronic renal failure (CRF) progresses over time and is in most cases permanent. End-stage renal failure (ESRF) is the term used when renal function is permanently lost.
Causes of ESRF can also be classified by the kidney part involved [31]:

- **Vascular:** includes both large vessels, as in renal artery stenosis, and small vessels, as in ischaemic nephropathy, vasculitis and the haemolytic-uraemic syndrome (HUS).

- **Glomerular:** this group is further divided into primary and secondary.
  - Primary: when the problem is initiated within the glomerulus, such as in focal segmental glomerulosclerosis (FSGS) and IgA nephritis.
  - Secondary: when an underlying condition leads to problems within the glomerulus, such as in diabetic nephropathy and Lupus nephritis.

- **Tubulointerstitial:** this includes polycystic kidney disease, reflux nephropathy, and drug-induced chronic tubulointerstitial nephritis.

- **Obstructive:** causes of which include bilateral kidney stones, disease of the prostate and cancer.

The most common cause of ESRF in the world today is diabetes, as it is responsible for 44% of new ESRF cases [32].

**1.1.2.1 Diabetic Nephropathy**

As briefly explained above, diabetic nephropathy is a secondary glomerular disease.

**1.1.2.1.1 Type 1 diabetes:** It has been observed that after the initiation of diabetes in animal models with type 1 diabetes, the nephropathy develops in three stages. The first stage involves renal and glomerular hypertrophy, as well as glomerular hyperperfusion, hyperfiltration and hypertension [33]. In the second stage progressive mesangial expansion occurs and seems to contribute towards proteinuria. In the final stage degrees of glomerular sclerosis occur. In rat models, contrary to human diabetic nephropathy (DN), the animals remain normotensive at this stage. It has been noted that while the superimposition of hypertension accelerates the development of glomerulosclerosis, tight glycaemic control prevents the development of microangiopathy and nephropathy.
1.1.2.1.2 Type 2 diabetes and obesity: By studying the many animal models of type 2 diabetes, the major pathologies have been identified as thickening of the glomerular basement membrane (GBM), mesangial expansion and activation, podocyte injury as well as interstitial inflammatory infiltrate [34-36].

The progression of diabetic nephropathy has been divided into three stages; microalbuminuria (incipient nephropathy), proteinuria with decreasing GFR (overt nephropathy), and ESRD. These stages are illustrated in figure 1.1 below. In type 1 diabetes, two additional stages of glomerular hyperfiltration with enlarged kidneys and early glomerular lesions with basement membrane thickening and normal urinary albumin secretion also exist [37].

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**Figure 1.1** Progression of chronic renal injury; early increase in glomerular filtration rate (GFR) is followed by its decline with increasing proteinuria. Also indicated is the National Kidney Foundation K/DOQI classification of the stages of chronic kidney disease. Figure based on and redrawn from reference [38].

Diabetic nephropathy is currently the leading cause of ESRD in the world, and it accounts for 40% of new ESRD cases in the USA [27]. As the number of adults with type 2 diabetes is progressively increasing and is projected to increase to
approximately 300 million in the year 2025 [39], diabetic ESRD is expected to become even more prevalent in the future.
1.1.3 End Stage Renal Disease: Outcomes

In addition to progressive azotaemia, there are many consequences associated with ESRD and the uraemic syndrome, these include anaemia, osteodystrophy, manifestations in the nervous system and metabolic and endocrine disorders in addition to cardiovascular disease. To better comprehend the complexity of patients with ESRD, I will outline these conditions here.

**Anaemia.** One of the functions of the kidney is to regulate red blood cell production by secreting erythropoietin. Anaemia has been recognised as an important clinical manifestation of progressive renal disease since the 1800s [40, 41]. The degree of anaemia usually increases with the fall of GFR, and tends to plateau by the time patients reach a stage where they require renal replacement therapy [42]. Major causes of anaemia in these patients include:

- Inadequate erythrocyte production: This is the most important cause of anaemia in ESRD; the other factors listed below may add to the severity of anaemia, but are not as instrumental as the decreased production of erythropoietin by the diseased kidneys.

- Shortened erythrocyte survival: It has been observed that the lifespan of erythrocytes reduces from 120 days in normal subjects to 70-80 days in uraemic ones [43]. This has been attributed to both metabolic (decreased activity of sodium and potassium pumps influences shape and rigidity of red blood cells) and mechanical factors (blood loss as explained below). However, more recent studies in well-dialysed patients have shown that RBC lifespan can reach nearly normal levels if blood losses during haemodialysis are prevented [44].

- Blood loss: This can be due to residual blood left in dialysers, vascular access punctures, occasional blood leaks, phlebotomy for routine testing and clotted dialysers.

- Iatrogenic haemolysis: The membranes of the red blood cells in patients on dialysis are sensitive to oxidant drugs and chemicals [45, 46]. If tap water is used in the dialysate, the presence of copper [47], zinc [48], aluminium [49] and nitrates as well as chloramine [50] can lead to haemolysis.
-Splenic dysfunction: A significant number of patients on dialysis have splenomegaly [51, 52] and hypersplenism which leads to removal of red blood cells from the circulation [52].

-Mechanical fragmentation: Red blood cells can also be injured and deformed by mechanical trauma and removed from the circulation [53].

-Inhibition of erythropoiesis: It has been suggested that uraemic serum may have an inhibitory effect on erythroid precursors [54-56].

-Nutritional factors contributing to anemia: Malnutrition is a common consequence of ESRD and dialysis, and is mainly due to anorexia, intercurrent illnesses, dialysate nutrient loss and dietary restrictions [57-59].

**Osteodystrophy of chronic renal disease.** Another major role of the kidneys is to participate in calcium and bone metabolism, by producing 1,25-dihydrocholecalciferol (Calcitriol). Calcitriol is the active form of vitamin D which causes increased absorption of calcium and phosphate in both the gastrointestinal tract and the kidneys. In ESRD, the diseased kidneys cannot fulfil their role and cause bone metabolism dysfunction through three main mechanisms:

-High turnover bone disease: this is the dominant form of osteodystrophy in renal disease. It was initially hypothesised that as patients developed hyperphosphataemia with loss of renal function, they became hypocalcaemic and thus developed secondary hyperparathyroidism. It has since been observed that the decline in serum 1,25-dihydrocholecalciferol is present prior to reduction in serum calcium and correlates inversely with PTH levels [60]. The prolonged half-life of PTH in renal failure and the direct effect of hyperphosphataemia in increasing PTH secretion both contribute to secondary hyperparathyroidism in ESRD [61, 62]. The pathophysiology of secondary hyperparathyroidism in CKD is illustrated in figure 1.2 below.
-Low turnover bone disease: This is due to aluminium toxicity, which is also responsible for dialysis dementia [64]. Aluminium is present in small amounts in normal subjects (10-30 mg), and excess amounts are rapidly cleared through the kidneys. In subjects with renal failure, the gastrointestinal absorption of aluminium is enhanced and the chronic administration of high oral doses leads to significant accumulation and toxicity [65-67]. The deposition of aluminium on bone surfaces causes a decrease in bone formation rate [68-70]. Other metals also associated with low turnover bone disease include iron and strontium (abundant in the water in some areas), both of which are much less common [71-74].

-Mixed uraemic osteodystrophy: As its title implies, this is caused by the combination of both high- and low-turnover bone disease features. The main reason behind it is usually due to severe secondary hyperparathyroidism in poorly nourished individuals with low calcium and/or low phosphate [75].
Nervous system manifestations. Patients with renal failure can exhibit a variety of neurologic disorders, from mild sensorial clouding to delirium and coma. Although many symptoms subside with the onset of successful dialysis, problems such as generalised weakness and peripheral neuropathy may persist. The neurologic disorders that occur within the central nervous system (CNS) as a result of renal failure are referred to as uraemic encephalopathy, which in many cases is present even after renal replacement therapy in the form of dialysis. Dialysis disequilibrium [76], dialysis dementia [77], stroke [78] and sexual dysfunction [79] are usually associated with the onset of the dialysis process. In addition, other conditions such as subdural hematoma, acute stroke, certain electrolyte disorders (hyponatremia, hypernatremia, phosphate depletion, and hypercalcaemia), vitamin deficiencies, hypertensive encephalopathy and drug intoxication may also occur in these patients [75].

Metabolic and endocrine disorders. The increased half-life of insulin and its decreased removal by kidneys causes high plasma levels of circulating insulin, which in turn causes abnormal carbohydrate metabolism. As insulin resistance itself increases the risk of cardiovascular mortality [80-82] and also contributes to hypertension and lipid abnormality [83, 84] which are both known risk factors of cardiovascular disease, it can be argued that it may be the main reason for the accelerated atherogenesis in renal failure. Furthermore, the breakdown of muscle tissue and activation of proteolytic pathways as a result of insulin resistance [85] may be responsible for the increased protein catabolism and malnutrition in CKD patients [86].

Patients with renal failure also have profound dyslipidaemia, which has been noted for a very long time [87] and probably goes hand in hand with the increased prevalence of atherosclerosis in this population.

Malnutrition. About 40 to 50% of ESRD patients are malnourished [88], and this contributes to increased infection, muscle wasting and increased mortality [89]. The causes of malnutrition in ESRD are usually divided into:

1) those increasing nutrient requirements
   - altered lipid and amino acid metabolism,
• impaired glucose tolerance,
• metabolic acidosis [90],
• uraemia,
• inflammation [91]

2) those decreasing food intake

• nausea,
• fatigue,
• anaemia,
• uraemic gastroparesis
• diabetic gastroparesis

In addition to the metabolic disorders mentioned, patients suffering from renal failure also have endocrine problems, such as:

• gonadotropin abnormalities [92, 93],
• hyperprolactinaemia [94],
• low plasma testosterone levels in male HD patients [95, 96],
• low plasma estradiol concentrations which is associated with lower bone density [97-99],
• dysregulation of growth hormone (GH) [100, 101] and insulin-like growth factors (IGFs) [102, 103] which blunts growth in children with renal failure [101, 102],
• elevated plasma vasopressin level which may play a role in hypertension and increased thirst in CKD patients [104, 105],
• mild abnormalities in adrenocorticotropic hormone (ACTH), corticotropin releasing hormone (CRH) and cortisol secretion [106-108],

• decreased plasma concentrations of thyroid hormones [109, 110], which is a reflection of the state of chronic illness and/or malnutrition and most patients appear clinically euthyroid [111],

• abnormalities in parathyroid glands and vitamin D metabolites (as already discussed),

• changes in the Renin-Angiotensin-Aldosterone system (RAS) [112, 113], the importance of which is illustrated by the fact that blocking the system reduces progression and induces partial regression of cardiovascular structural abnormalities and hypertension [114].

These abnormalities all contribute to the complexity of patients with renal failure.

**Cardiovascular Disease.** Long-term dialysis has long been associated with cardiovascular disease, with about a half of all deaths in ESRD patients attributed to cardiovascular disease [5, 24]. This is thought to be partly due to the higher prevalence of many traditional CVD risk factors, such as diabetes and hypertension in this population. It has also been shown that renal failure itself is an independent risk factor for CVD [115] in addition to it causing progression of uraemia-related risk factors; these include anaemia, inflammation, oxidative stress, hypoalbuminaemia and hyperhomocysteinemia [116, 117].

Symptomatic IHD usually results from coronary atherosclerotic disease, but may also be a consequence of myocardial fibrosis or left ventricular hypertrophy (LVH) [118, 119], which may in fact predispose to ischaemia. In renal failure patients, endothelial dysfunction can result from hypertension and flow overload (which increases stress on vascular walls) and the pro-oxidant [120] and chronic inflammatory state [121, 122]. In addition, hyperparathyroidism may cause vascular calcification and medial hypertrophy [123]. Also, small vessel disease, which is prevalent in LVH, diabetes and uraemia, is a common cause of IHD in this population [124]. A summary of the causes of IHD in CKD is illustrated in figure 1.3 below.
Figure 1.3 Schematic representation of the etiology of ischemic heart disease in chronic kidney disease. Figure is based on and redrawn from Diseases of the Kidney and Urinary Tract, 2007 [125]

The specific risk factors of cardiovascular disease among the ESRD population are a controversial topic and one that needs further elucidation. As identifying these risk factors is the main aim of this thesis, this topic will be discussed in detail in later sections.

The majority of patients with ESRD go on to receive renal replacement therapy (RRT) in the form of haemodialysis [24]. Unfortunately, despite advances in the haemodialysis technology, the annual mortality rate for patients on haemodialysis is still unacceptably high, with current mortality rates reported at 20% per year in the US [24] and 18% per year in the UK [126].

In the US more than half of the deaths in ESRD patients are caused by cardiac arrest, acute myocardial infarction (MI) and other cardiac causes. The next major cause is infection, which accounts for about 25%, and the third largest cause is
cerebrovascular disease, which contributes about 6% [24]. In the UK, these figures are as follows: 35% cardiac disease, 20% infection, 13% withdrawal from dialysis, 9% malignancies and 7% from cerebrovascular disease [127].

Cardiac and cerebrovascular disease are usually combined and referred to as one single category of cardiovascular disease.

**1.1.3.1 Conventional Risk Factors**

As mentioned above, the main cause of increased mortality in the ESRD population is cardiovascular events; therefore, the main risk factors for mortality in this population are cardiovascular risk factors. But as previously mentioned, these CV risk factors can be divided into the conventional ones which are similar to those of the general population, and renal-disease-specific risk factors, which will be discussed in the next section.

**Age.** Higher age is associated with an increased risk of CVD and mortality, both in the general population [128] and in patients with ESRD [129].

**Diabetes.** It has been shown repeatedly that diabetes is a CVD risk factor in the general population [130, 131]. There is a conflicting body of evidence regarding diabetes and ESRD, with recent studies claiming no survival benefit among the non-diabetics or the tightly controlled diabetics in contrast to poorly controlled diabetics [132].

**Hypertension.** Hypertension is also a well-established CVD risk factor in the general population [133, 134], and it has been shown that with every 6mmHg reduction in blood pressure (BP), the risk of developing an MI reduces by 14-16% [135]. Hypertension is very common in the ESRD population, with approximately 80% of CKD patients and 86% of dialysis patients suffering from it [124, 136]. Despite available blood pressure reducing agent, only about half of the CKD population [137] and third of the dialysis population in the US have adequately controlled BP [138]. But surprisingly, studies that have looked at the effect of BP on survival, and analysed systolic and diastolic BP separately, have shown a paradoxical relationship in that hypertension predicts longer survival [8]. Despite these observations and on the basis of robust evidence in the general population,
hypertension is considered a CVD risk factor and therefore a risk factor for increased mortality in the ESRD population.

**Smoking.** Smoking is a proven risk factor for CVD in the general population, as it doubles the risk of CVD events [139]. In the CKD population, approximately 25% of patients are either current or former smokers [24], and smoking has been associated with progression of CKD, stroke and development of IHD. In the dialysis population, 30-40% of patients starting dialysis are smokers [24], and this has been associated with an increased risk of mortality (26%) [140].

**Dyslipidaemia.** Increased levels of total cholesterol (TC), LDL-C, lipoprotein(a), and triglycerides (TGs) and decreased HDL levels are associated with CVD in the general population, and this is regarded as an atherogenic lipid profile [141-143]. Such a lipid profile is particularly common in patients with renal disease [144], but there is almost no published data that links dyslipidaemia to CVD in CKD, and conflicting data for the dialysis patients. In the dialysis population, it is low levels of total cholesterol that are associated with increased risk of mortality [7, 145-147], but one has to keep in mind that low cholesterol is also a marker of poor nutrition, which is a prevalent condition linked to increased mortality in this population. A more recent study has shown that while increased cholesterol levels are associated with reduced all-cause mortality in “inflammatory” patients, it increases the risk in “non-inflammatory” individuals [147]. This finding suggests that the inverse association is actually due to the effect of systemic inflammation and malnutrition, not the ‘protective’ qualities of cholesterol.

**1.1.3.2 Renal Failure Specific Risk Factors**

**Homocysteine.** It was first observed that patients suffering from homocystinuria, a rare genetic disorder, have elevated serum Homocysteine (Hcy) as well as accelerated atherosclerosis rates. More recently, observational studies have shown that Hcy levels in the general population are independently associated with CVD [148]. 83% of patients that reach ESRD have Hcy levels above the 90\textsuperscript{th} percentile for the general population [149], and there is limited evidence that this pertains to CVD in both CKD and dialysis patients [150-152].
**Hypocalcaemia.** Hypocalcaemia has been independently associated with both increased mortality [153] and increased development of CVD in the dialysis population [154]. It has been hypothesised that this maybe due to related hyperparathyroidism, which is associated with left ventricular hypertrophy (LVH), dyslipidaemia and myocardial fibrosis, all of which increase mortality [155].

**Hyperphosphataemia.** It has been shown that dialysis patients with hyperphosphataemia have a 41% increased risk of CV death and 20% increased risk of sudden death in comparison to those that are euphosphataemic [156, 157]. This may be due to increased cardiovascular calcification, which is predictive of adverse cardiac events [158]. A retrospective analysis has shown that serum phosphate levels of above 1.13 mmol/L were associated with a significantly increased risk of death in a cohort of CKD patients [159].
1.1.4 Reverse Epidemiology

1.1.4.1 What is Reverse Epidemiology?

The term ‘reverse epidemiology’ has been coined to describe the paradox that conventional CVD risk factors are protective in certain medical conditions [7, 160]. The Framingham Heart Study has identified obesity [161], hypertension [162], diabetes [130] and dyslipidaemia [163] (and several other factors) as cardiovascular risk factors within the general population. Recent studies involving diabetic patients with ESRD requiring dialysis have shown a paradoxical relationship between these ‘traditional’ CVD risk factors and long term survival rates [7, 132, 164-166]. Similar findings have also been reported in other clinically vulnerable populations, namely those with chronic heart failure (CHF), chronic obstructive pulmonary disease (COPD), human immunodeficiency virus (HIV) infection / acquired immune deficiency syndrome (AIDS) and the elderly. Among these extremely high-risk populations, studies have reported that the conventional CVD risk factors, rather than contributing to CVD mortality are actually protective [10, 160, 167, 168]. Taking into account that the components of reverse epidemiology are mainly markers of ‘over-nutrition’, their protective quality in the above mentioned ‘wasting’ conditions is perhaps less surprising. The reverse epidemiology observations suggest that while over-nutrition is bad, under-nutrition is worse, but population studies have failed to show that these findings are limited to those suffering from under/malnutrition.

Although these are mainly observational findings, it is important to note that these findings have more or less been consistent among the at-risk populations mentioned. Therefore, the fact that these findings are counterintuitive to today’s principles of CVD risks should not bias the approach needed to examine their clinical implications. Investigators who oppose the term ‘reverse epidemiology’ do so [169] mainly because such a definition may undermine the complexity of such patients and therefore distract from the important issues in risk factor modification. They argue that it is important to take into account the distinction between association and causation, as well as confounding and bias [169].
1.1.4.2 What are the Known Components of Reverse Epidemiology?

In the maintenance HD population, reverse epidemiology has been observed for the following risk factors, but is not limited to them:

**BMI.** In the general population, a BMI of 20-25 kg/m² is considered ideal, and with every bit of increase from that target, the risk of mortality is also increased [170]. Paradoxically, a BMI below 25 kg/m² has been shown to be a strong predictor of higher mortality in the MHD population, while a high BMI of >25 kg/m² has been correlated with improved survival in the same population [164, 171-177].

**Serum cholesterol.** High total cholesterol, high LDL cholesterol and low HDL cholesterol all increase mortality in the general population [178-180], while high total cholesterol levels have been shown to be predictive of better outcome in MHD patients [6, 146, 181-184].

**Blood pressure.** Hypertension indisputably causes increased cardiovascular and cerebrovascular events, as well as increased mortality in the general population [185-187]. Similarly, a few studies have shown this same relationship between high BP and mortality in the ESRD population [188-191], but larger, more recent studies that looked at pre-dialysis systolic hypertension have failed to show such a relationship [6, 192-197].

**Diabetes.** While diabetes is a well-known risk factor for increased mortality in the general population [130, 198, 199], there is at least one study that shows the same is not true in the MHD population [132]. It has also been observed that the presence of high levels of advanced glycation end products (AGE), which is normally seen in diabetic patients and is associated with higher mortality in the general population, seems to have protective qualities in the MHD population [200].

**Energy and/or protein intake.** This may be associated with obesity and increased mortality in the general population [201, 202], but it has been shown that in the MHD population increased protein intake is correlated with better survival [203-205].
1.1.4.3 What are the Explanations for Reverse Epidemiology?

There are several possible explanations for the reverse epidemiology phenomenon, but the presence of the malnutrition-inflammation complex syndrome (MICS) in dialysis patients seems to be the most plausible one. These observations are not restricted to MHD patients and have also been seen in the elderly population, those with chronic heart failure, cancer, HIV/AIDS and COPD patients [160, 206]. Malnutrition and subsequent cachexia is a common outcome of these conditions and this is the basis for the MICS theory: obesity is bad but malnutrition is worse [207].

1.1.4.3.1 Malnutrition Inflammation Complex Syndrome

Inflammation.

Inflammation is the dynamic response of vascularised tissue to injury [208]. It is a defence reaction of organs to injurious stimuli. It is a protective response which serves to bring defence and healing mechanisms to the site of injury [209], and under normal conditions will resolve the initial injury without any damage to the host. There are several distinct inflammatory pathways, each of which proceeds via a sequence of biologic events. Many of the individual events are controlled by cytokines or other small regulatory molecules, which in this context are called inflammatory mediators. Any given mediator may produce effects directly and also stimulate the production of other mediators, giving rise to an integrated response [210]. The physiological pathways of the inflammatory response to trauma is illustrated in figure 1.4 below.
Inflammation is divided into different categories. The most commonly used categorizations are described in table 1.1.

Figure 1.4 The inflammatory response; each cell is committed to recruiting and/or activating others. Not all interactions are illustrated [211]. (Figure reproduced with permission)
### Basis of classification

<table>
<thead>
<tr>
<th>Extent of inflammation</th>
<th>Length of inflammation</th>
<th>Morphology of inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Local</strong>&lt;br&gt;Local&lt;br&gt;Limited to an area of tissue in the vicinity of its port of entry</td>
<td><strong>Acute</strong>&lt;br&gt;Acute&lt;br&gt;Immediate and early response to injury</td>
<td><strong>Exudative</strong>&lt;br&gt;Exudative</td>
</tr>
<tr>
<td><strong>Metastatic</strong>&lt;br&gt;Metastatic&lt;br&gt;Inflammatory pathogens are transmitted to other organs and tissue</td>
<td><strong>Sub-acute</strong>&lt;br&gt;Sub-acute&lt;br&gt;Longer than acute, but not as prolonged as chronic inflammation</td>
<td><strong>Fibrinous</strong>&lt;br&gt;Fibrinous&lt;br&gt;Serrous</td>
</tr>
<tr>
<td><strong>Generalized</strong>&lt;br&gt;Generalized&lt;br&gt;When the pathogen spreads diffusely throughout the body</td>
<td><strong>Chronic</strong>&lt;br&gt;Chronic&lt;br&gt;State of prolonged inflammation – causes include persistent injury and autoimmune disease</td>
<td><strong>Haemorrhagic</strong>&lt;br&gt;Granulomatous</td>
</tr>
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**Table 1.1** Categorizations of inflammation.

Once inflammation becomes persistent, it may lead to adverse consequences such as decline in appetite, increased protein depletion in tissues, muscle and fat wasting as well as endothelial damage and atherosclerosis [212]. The cause of the chronic inflammatory state present among many dialysis patients has been extensively studied and can be briefly listed as: decreased clearance of pro-inflammatory cytokines, volume overload, oxidative stress, decreased levels of anti-oxidants, deteriorating protein-energy nutritional state and food intake, coexistence of co-morbid conditions, and additional inflammatory factors related to the haemodialysis treatment itself, such as exposure to dialysis tubing, impurities in dialysis water...
and/or dialysate, back-filtration of contaminants, foreign bodies in dialysis access grafts, and intravenous canulae [213].

Activation of pro-inflammatory cytokines (such as TNF-α, IL-1 beta, IL-6 [214, 215] and IL-8) in these patients also causes loss of appetite which is followed by malnutrition [216, 217]. This known overlap between inflammation and malnutrition is addressed as the MICS. There is an ongoing debate about whether the malnutrition aspect of the MICS is a result of inflammation or an independent entity [218-220], and there are convincing arguments on both sides. But what is certain is that an increase in the markers of inflammation, such as serum CRP and pro-inflammatory cytokines, are correlated with adverse CV events in both the general [221, 222] and MHD [115, 223-226] population, as well as CHF patients [227, 228]. Therefore, the inflammation component of MICS could be responsible for the increased prevalence of CVD and mortality in MHD even on its own [229], but when combined with its counterpart, malnutrition, the result may even confound other conventional CVD risk factors, such as obesity, dyslipidaemia, hyperglycaemia and hypertension.

The specific role of inflammation in type 2 diabetes, ESRD and CVD is discussed in section 1.2.2.

Malnutrition.

As briefly discussed, a number of studies have shown that protein-energy malnutrition (PEM) is a predictor of clinical outcome in MHD patients [182, 230, 231]. The same is true for inflammation, which almost always goes hand in hand with PEM and is also a strong predictor of mortality in the MHD population [229, 232-238]. As both CVD and PEM are prevalent among the dialysis population, the common mechanism that leads to both of these conditions may be cytokine release associated with renal failure and the pro-inflammatory state in this population [232, 236]. Activation and increased release of inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor–alpha (TNF-α) lead to loss of appetite as well as muscle proteolysis and ultimately cause atherosclerosis [229, 237]. Because PEM and inflammation are not only strongly associated with each other, but also with survival outcome in the dialysis population,
MICS is the term used to denote both their contributions to ESRD outcome [184]. To emphasise the main consequence of MICS, atherosclerosis, some investigators use the term malnutrition-inflammation atherosclerosis (MIA) when referring to the same phenomenon [233, 237].

Within the renal impaired population the onset of malnutrition can be attributed to increased levels of TNF-alpha and IL-6 which suppress the appetite and decrease food intake [59]. Malnutrition in these individuals means higher susceptibility to infections and an increased inflammatory state, which in turn causes further wasting. From this perspective, the greater the initial body mass index (BMI) the better; because there are higher reserves for protecting against cachexia [239]. Patients who are under-nourished, as characterised by a low BMI, low serum cholesterol or homocystein, are predisposed to infection and/or other inflammatory processes [232], as well as inflammatory diseases [240], which are known to increase both cardiovascular and all-cause mortality, as well as further wasting. The result is a vicious circle that can hardly be stopped and leads the patient to a very poor prognosis. Therefore, conditions that improve the nutritional status of dialysis patients may improve their outcome, which may explain the presence of reverse epidemiology of risk factors among this population, especially when the main components of reverse epidemiology are all related to nutritional status. Even low blood pressure has been shown to be associated with and a manifestation of MICS in MHD patients [193].

Protein-energy malnutrition (PEM) is defined as: “the state of decreased body pools of protein with or without fat depletion or a state of diminished functional capacity, caused at least partly by inadequate nutrient intake relative to nutrient demand and/or which is improved by nutritional repletion” [213]. PEM is known as a common phenomenon in patients on haemodialysis, with a prevalence rate of 18 to 75 percent depending on dialysis modality, patient population origins and nutritional assessment tools [241, 242], and is associated with higher rates of CV mortality [243]. The underlying cause of PEM in MHD patients is not well understood, but there is an increasing list of probable causes which includes: anorexia caused by uraemic toxicity, impaired gastric emptying, emotional and/or psychological disorders, dietary restrictions due to prescription restrictions and social constraints,
inability to acquire or prepare food due to physical incapacity, nutrient loss during dialysis, hyper-catabolism due to co-morbid illness, cardiovascular diseases, diabetic complications, infection and/or sepsis, and other co-morbid conditions [213]. It has been observed that PEM starts well before the start of dialysis, and hypoalbuminaemia, hypercholesterolemia and hypotransferrinaemia develop with the decline of GFR (glomerular filtration rate) [243].

1.1.4.3.2 Role of Adiponectin

Adiponectin, also known as Acrp30, AdipoQ and GPB28, is a 30-kDa adipokine, a cytokine which is exclusively secreted into the plasma by adipose tissue, and is relatively abundant compared to other hormones, accounting for approximately 0.01 percent of total plasma protein [244]. Its role is being extensively explored and it has been shown to increase fatty acid oxidation and cause decreased plasma free fatty acid and triglyceride levels [245]. Adiponectin also improves insulin sensitivity and causes a transient drop in serum glucose levels [245], and is therefore regarded as a key molecule in the metabolic syndrome [246-248]. Its decrease in obesity may be relevant in obesity-linked disorders [249], especially since it is also reduced in type 2 diabetes, dyslipidaemia and CVD [248, 250, 251]. Adiponectin has also been shown to inhibit the inflammatory response in vitro, as well as possess anti-atherogenic properties [252]. Adiponectin may be of clinical relevance and importance in the ESRD population, as it has been shown to have a strong association with many of the aforementioned risk factors for mortality in this group. Adiponectin is inversely associated with body weight [253] and BMI [254], triglycerides [253-255], insulin resistance [253, 255] and blood pressure [254]. It also induces production of other pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-8) by adipose tissue and macrophages [256].

In light of new evidence, hypoadiponectinaemia is being looked at as a novel potential CVD risk factor in patients with renal failure, even those with mild to moderate ESRD [257]. There is now more and more evidence that links hypoadiponectinaemia with increased mortality, CVD and recurrent ischemic heart disease (IHD) in ESRD patients [245, 258, 259].
Adiponectin seems to play a paradoxical role in patients with renal impairment; although its increase is associated with better overall survival and lower CV mortality [245], in the haemodialysis population, plasma adiponectin levels are markedly elevated [260-264]. It has recently been shown that this elevation is not due to over-secretion, but rather accumulation (Adamczak et al. personal unpublished data: measurements of plasma adiponectin concentrations in renal veins and aorta in human subjects confirmed that kidneys play an important role in adiponectin elimination and that the increased plasma adiponectin concentration in CKD is not due to oversecretion). Adiponectin levels have been shown to be inversely correlated with serum CRP levels in MHD patients, and low levels of adiponectin have also been associated with greater mortality among these patients [264]. Interestingly, adiponectin levels are elevated in chronic inflammatory/autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematositis, inflammatory bowel disease and cystic fibrosis [265]. Contrary to the general population, in these patients adiponectin has a positive, rather than negative, correlation with inflammatory markers. A recent study has shown that although plasma adiponectin levels are reduced after successful renal transplants, these levels are still higher than those of healthy controls [266].

High adiponectin has also been shown to be an independent predictor for progression of renal disease [267]. Although it can be argued that this may indicate adiponectin as part of a renal and cardio-protective mechanism that is set in motion to counter critical organ damage [13].

1.1.4.3.3 Other Explanations

Survival bias. There are 10 to 20 million patients with CKD due to irreversible and potentially progressive damage to the kidney in the USA [268], but only an estimated 400,000 people with ESRF [27]; this means that less than 5% of CKD patients go on to develop ESRF. The current explanation for this phenomenon is that most CKD patients don’t live long enough to develop ESRF, as many of these patients suffer severe co-morbidities such as diabetes, hypertension and atherosclerosis. Renal disease in itself is also an independent risk factor for CVD mortality [115, 223]. The other point that has been highlighted is that the population that goes on to develop ESRF has survived many risk factors and is not necessarily
genetically or phenotypically similar to its CKD predecessors, and therefore may not share the same risk factors for mortality [239]. This is similar to the trends observed in patients that reach an advanced age, possibly due to survival advantages [269-271].

Of note also, is that most epidemiological studies treat patients who have started dialysis recently (i.e. incident patients) and those who have been on dialysis for a number of years (i.e. prevalent patients) the same, which leads to a form of selection bias [272]. Because of the high annual mortality rate of 20% in ESRF patients, this form of bias influences longitudinal studies and even clinical trials. However, epidemiological studies that only look at incident dialysis patients have demonstrated the same risk factor reversal [126].

**TNF-α receptors.** As mentioned above, TNF-α, a pro-inflammatory cytokine, is elevated in dialysis patients [273] and may contribute to cachexia, as well as having pro-apoptopic and negative inotropic effects on the CV system [274], which all contribute to poor survival. Soluble TNF-α receptors, also known as IL-1RA, produced by adipose tissue [275], can neutralise the effects of TNF-α by binding to it. Therefore, in theory, obese individuals who have more adipose tissue can subsequently produce more IL-1RA which neutralises the effects TNF-α, and this may contribute to better outcome.

**Neurohormonal changes.** It has been observed that obese patients have diminished response of sympathetic nervous system and renin-angiotensin system compared to their lean counterparts [276]. As both of these responses are associated with poor prognosis in conditions of fluid overload [277], this diminished response may be a protective trait in obese MHD patients.

**Haemodynamic state.** Because obese individuals have higher blood pressure [278], they are more tolerant of afterload-reducing antihypertensive medication, such as angiotensin-converting enzyme inhibitors (ACEI) which are known to improve survival [279].

**Competitive risk factors: obesity vs. malnutrition.** Obesity and other conditions associated with over-nutrition (such as hypercholesterolaemia, insulin resistance and type 2 diabetes) are considered risk factors for long-term cardiovascular
mortality in affluent societies [179, 180, 280-283]. On the other hand, in less affluent societies and developing countries, under-nutrition is still causing a shorter life expectancy [284-286]. Because of the increased mortality risk of MHD patients, the long-term effect of risk factors caused by over-nutrition may be overwhelmed by the short-term effects of risk factors of under-nutrition.

**Circulating lipoproteins.** Another hypothesis is that obese individuals have a richer pool of lipoproteins that can actively bind to and remove circulating endotoxins, therefore retarding inflammation and its effects, including subsequent atherosclerosis [207].

**Confounding factors.** Such as low BP and low BMI may be consequences of smoking and/or heart failure [287], which are also associated with increased inflammation [228, 288], malnutrition [289] and mortality [290]. Low BP may also be caused by autonomic neuropathy, which means the subjects may be suffering from other long-term effects of diabetes, as well [291].

**Other hypotheses.** There are several mechanisms through which malnutrition and hypotension contribute towards increased mortality, such as: acute coronary syndrome, autoregulation dysfunction, ischaemia and arrhythmigencity [292, 293]. These factors may be exaggerated in the presence of uraemia [294], and therefore may become more influential than conventional CVD risk factors.

A less well-substantiated hypothesis describes that as obesity and excess weight become more and more prevalent in most industrialized nations, despite the ‘conventional risk factors’ it introduces, these nations are in fact living longer than ever [295]. And as life-expectancy increases effectively hand-in-hand with weight increase in these populations, the question becomes: shouldn’t malnutrition be called the paradoxical risk factor or the ‘reverse epidemiology’?

**Ethnicity and Mortality**

Although African and black Caribbean individuals have higher total mortality rates compared to white individuals in the general population [296-298], among the dialysis population African and black Caribbean individuals have lower annual mortality rates compared to their white counterparts (18% vs. 28%) [27]. In addition,
several other disparities between different ethnic groups within the haemodialysis population have been observed in regards to their long term survival. There seem to be ethnic subgroups within the MHD population that do not exhibit the survival advantage of obesity that is associated with this population; these include white women [172, 299] and Asian-American patients [174, 176, 177]. Also, the usually observed improvement of survival with lower cholesterol levels does not seem to exist in black individuals, as this characteristic is associated with increased mortality among the black MHD population [300]. Although such cultural differences as diet and lifestyle may be contributing causes of these differences, there is currently not enough evidence to rule out biological disparities between races.

The complexity of the mechanism involved in determining outcomes in the maintenance haemodialysis population is illustrated in figure 1.5 below.
Figure 1.5 Potential pathophysiologic mechanisms leading to the reverse epidemiology phenomenon in maintenance dialysis patients. [206] (figure reproduced with permission)
1.1.5 Investigating the role of different risk factors on outcomes: designing a Risk Engine

Identifying generalizable risk factors for cardiovascular disease is a task that cannot be achieved through experimental studies in animal models or cross-sectional or case-control studies in small groups of patients, although these types of studies can lend insight to mechanisms of disease. To successfully identify such risk factors, large, rigorous, prospective studies in relevant clinical populations are needed. In the field of CVD, the Framingham Study [301] is the model population-based cohort study that has identified what are considered conventional cardiovascular risk factors that have been widely confirmed by subsequent randomised controlled trials [302, 303]. Unfortunately, such large prospective studies are rare among the ESRD population [129, 304], thus the reliance on data from retrospective cohorts, national registries, cross-sectional and case-control studies which provide less conclusive information. Conducting reliable prospective studies among the ESRD population is a difficult task because of the confounding effect of existing cardiovascular disease prior to the onset of ESRD, which is common among this population.

The UK Prospective Diabetes Study (UKPDS) Risk Engine is another model population-based study for type 2 diabetes-specific risk factors [198]. The UKPDS risk calculator includes HbA1c and traditional CVD risk factors and while earlier versions calculated coronary heart disease risk and stroke risk separately, in the latest version equations have been derived that directly estimate CVD risk [305]. This novel equation has been validated by the Collaborative Atorvastatin Diabetes Study (CARDS) [306] to have good predictive ability.

The Framingham and UKPDS risk equations both had an excellent ability for correctly identifying individuals who would develop CVD, which means they both had high sensitivity. However, both equations have poor specificity, with the Framingham risk equation having a specificity of 30% and the UKPDS Risk Engine a specificity of 31% [307]. The ideal risk engine would have both a high sensitivity and a high specificity in recognising risk factors.
1.2 Insulin Resistance and Inflammation in ESRD

1.2.1 Role of Insulin Resistance

Insulin resistance is a feature in many conditions that are regarded as well-known CVD risk factors, such as obesity, hypertension, dyslipidaemia and type 2 diabetes. As these are the same risk factors involved in the reverse epidemiology phenomenon, insulin resistance may be the link that can explain these observations. To do that, it is important to understand what insulin resistance is, how it develops, and how it is affected by renal failure and subsequent haemodialysis.

Insulin is a hormone with many effects; it influences amino acid uptake, protein synthesis, proteolysis, lipolysis, hepatic triglyceride output, glucose uptake, glycogen synthesis and gluconeogenesis. Sensitivity to insulin is therefore crucial for it to regulate these effects. Insulin sensitivity and resistance is usually defined by an individual’s response to an oral or IV glucose or insulin stimulus. Individuals who develop insulin resistance have an abnormal response to a glucose challenge or demonstrate impaired glucose tolerance, have elevated fasting glucose and/or overt hyperglycaemia, or reductions in insulin action after an IV administration of insulin [308]. Insulin resistant individuals in general tend to be overweight or obese, sedentary and consume a diet high in total or saturated fats [84, 309-311].

The obesity pandemic of recent decades is at the root of the increased incidence of insulin resistance and the metabolic syndrome, which have led to an increase in CVD and type 2 diabetes as well as ESRD [12, 81, 83, 312, 313]. Once individuals become insulin resistant, normoglycaemia is initially achieved by modest increases in beta-cell mass and/or an increase in insulin secretion [314]. If, over time, this capacity is lost type 2 diabetes will occur, and if it can be maintained in the long-term, type 2 diabetes is prevented despite the presence of hyperinsulinaemia.

Overt insulin resistance is the main characteristic of the metabolic syndrome, a concurrence of visceral obesity, dyslipidaemia, hyperglycaemia, and hypertension. The term ‘metabolic syndrome’ was first used in 1977 by Haller to describe the clustering obesity, diabetes, hyperlipidaemia, hyperuricaemia and hepatic steatosis and their additive effect on atherosclerosis [315]. A decade later, in 1988, Reaven
suggested that insulin resistance is the common aetiology for impaired glucose tolerance (IGT), hyperinsulinaemia, hypercholesreolaemia, hypertriglyceridaemia and hypertension, which together he referred to as Syndrome X to stress the unknown aspects of this syndrome [316]. In 1989 Kaplan recognised central obesity as an important factor in the syndrome described by Reaven [317]. The presence of chronic subclinical inflammation in subjects with metabolic syndrome was identified in the year 2000 by Festa et al [318] and increased levels of CRP, TNF-alpha, IL-6 and IL-8, and decreased levels of the cardio-protective anti-inflammatory adipokine adiponectin were subsequently described [253, 255, 319, 320]. As mentioned previously (section 1.4.3.2), adiponectin may be an inducer of other pro-inflammatory cytokines, and its levels are elevated in classic chronic inflammatory/autoimmune disease. But in the metabolic syndrome, adiponectin levels correlate negatively rather than positively with inflammatory markers, and are reduced. That is why presently, it is of great interest to know whether the primary proinflammatory defect in adipose tissue is in fact the reduction in adiponectin or whether this reduction is secondary to the inflammatory state already present [265, 321, 322].

1.2.1.1 Insulin Resistance in Type 2 Diabetes

Most patients with type 2 diabetes have insulin resistance and metabolic syndrome before its onset, and in fact, in 75 to 85% of these patients insulin resistance, hyperinsulinaemia, dyslipidaemia and obesity precede the disease[323]. In addition, it has been observed that the risk for developing type 2 diabetes is increased fivefold in individuals with the metabolic syndrome in comparison with those without [324]. And of all the components of the metabolic syndrome, hyperinsulinaemia is by far the strongest predictor for progression to type 2 diabetes [325].

1.2.1.2 Insulin Resistance and ESRD

One of the characteristics of the uraemic state is insulin resistance, which is accompanied by hyperinsulinaemia, glucose intolerance and dyslipidaemia [326-329]. Although insulin resistance is well studied and quite well understood in the general population, the same cannot be said for insulin resistance in ESRD. It has long been established that insulin resistance and the conditions that usually follow, i.e. hypertension, obesity, dyslipidaemia and type 2 diabetes, are among the leading
causes of CV mortality. They are also known as leading causes of chronic kidney disease (CKD) and renal failure [27, 330].

The renal clearance of insulin is significantly greater than the GFR due to significant uptake and degradation of insulin in the peritubular epithelial and endothelial cell membranes. Peritubular insulin uptake increases as renal function deteriorates, and insulin clearance is maintained until the GFR reaches 15-20 ml/min. From this point on, insulin clearance falls rapidly [331]. The accumulation of uraemic toxins is thought to cause an inhibition of the insulin degradation system, especially by the liver which is responsible for clearing about 50% of the insulin secreted into the portal system, causing increased half-life of insulin [331]. Theoretically, both of these mechanisms are reversible through dialysis, but whether insulin resistance improves with the commencement of dialysis is yet to be confirmed.

Other causes of insulin resistance in ESRD include physical inactivity [332], and the accumulation of adipokines in uraemic plasma such as TNF-alpha [333, 334]. The increase in these molecules may be responsible for the presence of insulin resistance, especially in non-obese ESRD patients. These factors are summarized in table 1.2.

<table>
<thead>
<tr>
<th>Contributing factors to insulin resistance in ESRD</th>
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<tbody>
<tr>
<td>Reduced renal clearance (peritubular clearance maintained until GFR reaches 15-20 ml/min)</td>
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<tr>
<td>Increased half-life (uraemia causes reduced hepatic degradation of insulin)</td>
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<tr>
<td>Accumulation of insulin resistance inducing adipokines</td>
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<tr>
<td>Physical inactivity due to illness</td>
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Table 1.2 Contributing factors to insulin resistance in ESRD

Assessment of insulin sensitivity using more modern techniques such as the euglycaemic hyperinsulinaemic clamp has allowed researchers to document diminished insulin-stimulated glucose uptake by extrahepatic tissue [335, 336]. Although insulin sensitivity seems to be reduced early on in the natural history of
renal failure, in fact when GFR is still within the normal range, it is not problematic in most patients as the pancreatic beta-cells continue to secrete enough insulin to overcome this state, thus leading to hyperinsulinaemia [337]. But as renal failure progresses, with the anaemia, acidosis and hyperparathyroidism in many, the beta-cells fail to secrete sufficient insulin to counter the reduced insulin sensitivity which leads to impaired glucose tolerance thereby becoming glucose intolerant and hyperglycaemic [338-340]. Current literature suggests that the increased intracellular calcium concentration due to PTH imbalance may contribute to the insulin secretion impairment in renal failure [341, 342], especially since parathyroidectomy in patients with secondary hyperparathyroidism appears to ameliorate insulin secretory defects in pre-haemodialysis patients [343, 344].

Insulin resistance in the early stages of CKD has been implicated as a cause, rather than a consequence, of CKD, in the general population. Chen et al. have shown a strong, positive, significant, and dose-response relationship between insulin resistance, insulin level and risk of CKD among non-diabetic subjects [345]. Other studies, mostly prospective, have also shown that non-diabetic nephropathies are more likely to reach ESRD if the subject has diabetes [346-348]. Although data on the relationship between insulin resistance and non-diabetic CKD is sparse, several small studies have shown the presence of insulin resistance in non-diabetic CKD patients [349-351], and one prospective study has shown that insulin resistance appears earlier than microalbuminuria in non-diabetic subjects [352]. These findings suggest that early detection and correction of insulin resistance may benefit patients in delaying the onset of CKD, even in non-diabetic patients. But to definitively establish the causal effect of insulin resistance in CKD and ESRD warrants further studies.

1.2.1.3 Insulin Resistance and Cardiovascular Outcomes
Insulin resistance clusters CVD risk factors such as hypertension, dyslipidaemia and glucose intolerance. Several large epidemiological prospective studies on CVD have shown insulin resistance occurs alongside other risk factors for atherosclerosis and CVD, including hypertension, dyslipidaemia and glucose intolerance or type 2 diabetes. Many of these studies have also shown hyperinsulinaemia and other indices of IR to be associated with CVD (listed in table 1.3).
<table>
<thead>
<tr>
<th>IR associated with:</th>
<th>References</th>
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<tbody>
<tr>
<td>Prevalent atherosclerosis</td>
<td>Folsom et al, 1994 [353]</td>
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<td>Agewall et al, 1995 [354]</td>
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<td>Bavenholm et al, 1995 [355]</td>
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<td>Kahn et al, 1995 [356]</td>
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<td>Bressler, 1996 [80]</td>
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<td></td>
<td>Howard et al, 1996 [82]</td>
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<td></td>
<td>Haffner et al, 1998 [357]</td>
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<tr>
<td>Incident CHD</td>
<td>Despres et al, 1996 [358]</td>
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<tr>
<td>Incident stroke</td>
<td>Pyorala et al, 1998 [359]</td>
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<tr>
<td>Risk of death from CHD</td>
<td>Welborn and Weare, 1979 [360]</td>
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<td></td>
<td>Rosselin et al, 1985 [361]</td>
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<td>Grandits et al, 1994 [362]</td>
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**Table 1.3** Conditions known to be associated with increased insulin resistance

Current evidence suggests that a quantified value of insulin resistance, such as the homeostatic model assessment index of insulin resistance or HOMA-IR (detailed in section 1.6.4) is an independent predictor of cardiovascular disease in patients with type 2 diabetes [363] as well as in individuals without diabetes [364, 365].

The clustering of CVD and insulin resistance with other CVD risk factors synergistically increases the risk of atherosclerosis, as was shown by Neaton et al that CVD risk increases with each added factor [366]. It is therefore expected that the effect of insulin resistance on CV mortality is dependent on these other risk factors, as it is the underlying mechanism for their clustering. Since this landmark study, it has also been shown that insulin resistance is an independent risk factor for CVD in ESRD patients without diabetes [367]. As the role of insulin resistance in predicting CVD in non-diabetic ESRD patients is independent of BMI, and BMI seems to be negatively associated with CVD and mortality in the ESRD population,
it would be safe to assume that insulin resistance and adiposity per se have different roles in CV death in the ESRD population [368].

1.2.1.4 Insulin Resistance and Inflammation in ESRD

Interaction between insulin resistance and inflammation was first suggested in the 1990s. Insulin resistance may also be a consequence of inflammation, and chronic inflammation has also been shown to be an independent risk factor for CV mortality in the general population [369, 370]. To what extent chronic inflammation would influence insulin resistance in non-diabetic patients is not known, however, in non-diabetic ESRD patients, the effects of insulin resistance could be attributed to the presence of the inflammatory state induced by uraemia. However, the Shinohara et al. study showed that the increased insulin resistance was independent of C-reactive protein, and also that there is no significant association between insulin resistance and CRP [368].

1.2.1.5 Insulin Resistance and MHD

Although there have been recent studies looking at HOMA-IR values among MHD patients [371, 372], and other studies looking at insulin resistance in patients with mild to moderate kidney failure [32, 337] we have not been able to find any prospective study looking at HOMA-IR values and how they change with progression of renal failure in patients with and without diabetes. What would be even more interesting would be to see whether whatever effect ESRD and MHD have on insulin resistance is reversed once patients successfully receive a renal transplant, but unfortunately the effects of transplantation on insulin resistance are confounded by the effects of the immunosuppressive drugs [373], this will be a more difficult task.

1.2.1.6 Quantification of Insulin Resistance

The concept of insulin resistance is the lack of insulin’s ability to regulate its functions following a stimulus (e.g. meal ingestion). Its measurement, therefore, must describe the circumstances in which it is assessed as well as the methodology used [374]. A number of different methods have been described for quantifying insulin resistance for clinical assessment; listed below is a simplified list of these
methods with a short summary of the methodology used in each case, with a summary in table 1.4.

Model assessments:

**Homeostasis Model Assessment (HOMA).** This method was developed by Robert Turner at the Oxford University in the late 1970s [375], and refined to its current nature in 1998 by Jonathan Levy et al [376]. A measurement of insulin resistance is derived from fasting plasma glucose and insulin concentrations, using the formula:

\[
\text{HOMA index} = \frac{\text{insulin (mU/L)} \times \text{glucose (mmol/L)}}{22.5}
\]

Its simplicity and practicality make it the most widely used method for estimating insulin resistance, and it has been shown to correlate well with the euglycaemic clamp technique in both the general population and those with renal impairment [377]. To achieve a more accurate insulin measurement to incorporate into the above formula, 3 insulin measurements are usually obtained at five minute intervals; this is because insulin secretion is pulsatile and the average of the three measurements is a more accurate indication of true insulin levels. It has, however, been argued that in studies where HOMA values are being compared within a certain population, a single sample could be enough [378].

**Continuous Infusion of Glucose with Model Assessment (CIGMA).** This is a steady-state mathematical model where following a low-dose, constant rate glucose infusion over one hour, samples are taken to measure glucose and insulin at 50, 55 and 60 minutes into infusion [379].

**Minimal Model.** This is essentially an intravenous glucose tolerance test that is followed by injecting a bolus dose of insulin. It requires two canulae and frequent blood sampling (12 or 22 samples, depending on the protocol), and may take up to four hours [380]. Although it has also been validated against the gold standard, which is the euglycaemic clamp (below) [381], its difficult and time-consuming nature reduce its practicality in clinical research.

 Clamp techniques:
**Euglycaemic Clamp.** This method is known as the gold standard of insulin quantification and uses an exogenous infusion of insulin to maintain a hyperinsulinaemic level while simultaneously infusing sufficient glucose to ‘clamp’ its level at fasting concentration (5 mmol/L) [382]. The only problem with this technique is its difficulty; it takes two hours to perform, requires the use of two intravenous lines, retrograde cannulation of a vein as well as the use of a calibrated pump, all of which make it quite impractical for clinical research purposes.

**Hyperglycaemic Clamp.** In this method, glucose is infused intravenously at a variable rate to clamp its concentration at a predetermined plasma glucose concentration, and plasma samples are obtained toward the end of the infusion to be assayed for insulin. Insulin sensitivity is calculated by dividing glucose amount required to maintain the hyperglycaemic clamp by mean insulin concentration over the last 20-30 minutes of the test [382-384].

**Insulin infusion techniques:**

**Insulin Tolerance Test (ITT).** This method measures the decay of plasma glucose after a bolus dose of insulin is injected. Although it has been validated against the euglycaemic clamp [385], this technique is cast as inferior because counter-regulatory hormones are invoked, as well as the profound risk of hypoglycaemia [372].

**Insulin Sensitivity Test (IST).** In this method, glucose, insulin and somatostatin are continuously infused over a period of 150-180 minutes at a constant rate. The mean plasma concentration of glucose over the last 30 minutes of the test is calculated and used as a reflection of the insulin sensitivity [386, 387].

**Other:**

**Oral Glucose Tolerance Test (OGTT).** This test has long been used for the diagnosis of diabetes, but can also be used to derive an estimate of insulin resistance. This is done by calculating an estimate of insulin resistance based on plasma insulin concentration 2 hours after a 75g oral glucose load [384].
<table>
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<tr>
<th>Quantification method</th>
<th>References</th>
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<tbody>
<tr>
<td>Homeostasis Model Assessment (HOMA)</td>
<td>Turner et al, 1979 [375]</td>
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<td>Levy et al, 1998 [376]</td>
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<tr>
<td>Continuous Infusion of Glucose with Model Assessment (CIGMA)</td>
<td>Hosker et al, 1985 [379]</td>
</tr>
<tr>
<td>Minimal Model</td>
<td>Bergman et al, 1987 [380]</td>
</tr>
<tr>
<td>Euglycaemic Clamp</td>
<td>DeFronzo et al, 1979 [382]</td>
</tr>
<tr>
<td>Hyperglycaemic Clamp</td>
<td>Defronzo et al, 1979 [382]</td>
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<td></td>
<td>Matthews and Hosker, 1989 [383]</td>
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<td>Nijpels et al, 1994 [384]</td>
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<tr>
<td>Insulin Tolerance Test (ITT)</td>
<td>Gelding et al, 1994 [385]</td>
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<td>Akinmokun et al, 1992 [388]</td>
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<tr>
<td>Insulin Sensitivity Test (IST)</td>
<td>Heine et al, 1985 [386]</td>
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<td></td>
<td>Yeni-Komshian et al, 2000 [387]</td>
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<tr>
<td>Oral Glucose Tolerance Test (OGTT)</td>
<td>Nijpels et al, 1994 [384]</td>
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*Table 1.4* Common techniques for quantifying insulin resistance.
1.2.2 Role of Inflammation

The basic background for the role of inflammation in the MHD population has been discussed in section 1.1.4.3.1.

1.2.2.1 Inflammation in Type 2 Diabetes

The first observations that linked diabetes and inflammation were made over a century ago by Ebstein, who demonstrated that using high doses of sodium salicylate reduces glycosuria in the diabetic patient [389], and were confirmed by Williamson 25 years later [390]. And although subsequent trials in the mid 20th century reconfirmed these findings [391-393], it wasn't until the 1990s that the mechanisms behind these findings were elucidated [394-399].

It was first shown in 1993 that TNF-alpha, a pro-inflammatory cytokine produced by adipose tissue and over-produced in obesity, was able to induce insulin resistance [395, 399]. Within a few years, the concept of adipose tissue as a site for cytokine production was well-established, and the list included leptin, IL-6, resistin, and adiponectin, to name a few [398, 400-404]. TNF-alpha, IL-6 and other cytokines induce and sustain the sub-acute inflammatory state that is present in obesity, and they activate intracellular pathways that lead to the development of insulin resistance and subsequent type 2 diabetes [405]. Studies in insulin-resistant groups other than those with diabetes, i.e. individuals with obesity and hypertension, have lent further support to the adverse effect of TNF-alpha in the development of insulin resistance [406, 407]. Figure 1.6 illustrates the potential mechanisms through which these cytokines induce an inflammatory response.
Figure 1.6 Potential cellular mechanisms for activating inflammatory signalling [408]. (Figure reproduced with permission)

Not only has inflammation been linked to the key features of insulin resistance syndrome [409-412], the presence of chronic low-grade inflammation is probably the main cause of CVD in insulin resistant individuals [405].

1.2.2.2 Inflammation and ESRD

Current evidence shows that CRP levels are elevated in 30-50% of predialysis, MHD and CAPD patients, which indicates an inflammatory state [413]. As this is
present in predialysis patients as well, the inflammatory response is probably aided by factors not related to the dialysis process, such as residual renal function, ethnicity, gender and age [414]. Loss of renal function has been shown to be associated with elevated serum cytokine levels [415], and creatinine clearance has a positive correlation with a number of cytokines, including IL-1, IL-6 and TNF-alpha, and their soluble receptors in the predialysis population [416-418].

That said, the presence of the inflammatory state is primarily found in patients undergoing the dialysis procedure, whether in the form of MHD or CAPD [419, 420]. In addition to the factors mentioned above, the inflammatory response in the dialysed population is enhanced by non-biocompatible membranes, non-sterile dialysates and the back-leak of dialysate across the dialysis membrane [421-423].

1.2.2.3 Inflammation and Cardiovascular Outcome

The association between inflammation and CVD is well documented in both renal and non-renal patients. Evidence suggests that CRP alone may contribute to atherogenesis, as it is deposited in the arterial wall of early atherosclerotic lesions [424], it is co-localized with complement in heart tissue during acute MI [425], and it can induce adhesion molecule expression, which means it has a direct pro-inflammatory effect on human endothelial cells [426]. IL-6 is also regarded as a pro-atherogenic cytokine, as it mediates the attachment and migration of leukocytes across endothelial surfaces by stimulating soluble intercellular adhesion molecule 1 (sICAM-1) [427], induces hepatic production of CRP [428] which is also directly involved in atherogenesis, as well as other metabolic, endothelial and coagulant mechanisms [429]. Other acute-phase reactants with known direct role in atherogenesis are serum amyloid A (SAA), Lp(a), and fibrinogen [430-432]. Figure 1.7 illustrates the role of inflammation in the different stages of atherosclerosis.
Figure 1.7 Participation of inflammation in all stages of atherosclerosis. A, Leukocyte recruitment to the nascent atherosclerotic lesion. Blood leukocytes adhere poorly to the normal endothelium. When the endothelial monolayer becomes inflamed, it expresses adhesion molecules that bind cognate ligands on leukocytes. Selectins mediate a rolling, or saltatory, interaction with the inflamed luminal endothelium. Integrins mediate firmer attachment. Proinflammatory cytokines expressed within atheroma provide a chemotactic stimulus to the adherent leukocytes, directing their migration into the intima. Inflammatory mediators such as Macrophage Colony Stimulating Factor (M-CSF) can augment expression of macrophage scavenger receptors leading to uptake of modified lipoprotein particles and formation of lipid-laden macrophages. M-CSF and other mediators produced in plaques can promote the replication of macrophages within the intima as well. B, T lymphocytes join macrophages in the intima during lesion evolution. These leukocytes, as well as resident vascular wall cells, secrete cytokines and growth factors that can promote the migration and proliferation of Smooth Muscle Cells (SMCs). Medial SMCs express specialized enzymes that can degrade the elastin and collagen in response to inflammatory stimulation. This degradation of the arterial extracellular matrix permits the penetration of the SMCs through the elastic laminae and collagenous matrix of the growing plaque. C, Ultimately, inflammatory mediators can inhibit collagen synthesis and evoke the expression of collagenases by foam cells within the intimal lesion. These alterations in extracellular matrix metabolism thin the fibrous cap, rendering it weak and susceptible to rupture. Cross-talk between T lymphocytes and macrophages heightens the expression of the potent procoagulant tissue factor. Thus, when the plaque ruptures, as shown here, the tissue factor induced by the inflammatory signaling triggers the thrombus that causes most acute complications of atherosclerosis [433]. (Figure reproduced with permission)
In the ESRD population, it is thought that the accumulation of pro-inflammatory and proatherogenic cytokines, such as TNF-alpha and IL-6, with deteriorating renal function is a key mechanism for CVD [418, 434, 435].
1.2.2.4 Quantification of Inflammation

There are numerous measurable markers of inflammation, but perhaps the most widely used marker by clinicians is C-reactive protein or CRP. CRP is a protein that is synthesised by the liver in response to adipokines and found in the blood [436, 437]. It is an acute-phase reactant that rises up to 50,000 times during acute inflammation. Normal levels of CRP are less than 5 mg/L. To detect low and intermediate levels of CRP more accurately a high-sensitivity CRP (hs-CRP) test is used.

Adiponectin is also a quantifiable marker of inflammation, although adiponectin’s role in inflammation is quite complex. While adiponectin is classified as an anti-inflammatory molecule, its levels are elevated in individuals with high inflammatory scores [438].

Other markers of inflammation include monocyte chemo-attractant protein 1 (MCP-1), TNF-alpha, IL-1, IL-6, IL-8, ferritin, white blood cell count, ceruloplasmin, Serum amyloid A, haptoglobin, orosomucoid, salic acid, fibrinogen and complement factors among many others.
1.3 Glycaemic Control in ESRD

As the leading cause of ESRD is diabetes, and nearly half of the ESRD population suffers from this condition [24], adequate glycaemic control is a key component in the effective treatment of this at-risk population.

1.3.1 Targets for Glycaemic Management

Even in the non-CKD population, there seems to be more and more controversy surrounding this topic. Recently published data from large randomised controlled trials such as the Action to Control Cardiovascular Risk in Diabetes study (ACCORD) [439], the Action in Diabetes and Vascular Disease study (ADVANCE) [440], and the Veterans Affairs Diabetes Trial (VADT) [441] have only added to the dispute on 'how low is too low?' when it comes to glycaemic control.

Early intervention to lower glycaemia and HbA1c in both type 1 and type 2 diabetic patients is regarded as beneficial; this was reconfirmed recently by both the UKPDS [198] and the DCCT [442] follow-up studies. Lower HbA1c lowers the incidence of both macrovascular disease, i.e. CVD, and microvascular disease, which in turn improves survival and reduces mortality.

In late intervention, however, it is very important to achieve as low a level of glycaemia as possible without pushing the patient toward hypoglycaemia, which in fact increases mortality [443]. Aiming to achieve HbA1c values close to that of the general population (<6.5%) has been associated with increased mortality and no benefit to CVD [439], and even targeting long-established target of <7.0% in the diabetic population has been shown harmful in those with long standing type 2 diabetes [441]. Even trials that claim to show improved survival with lower HbA1c levels [440], only do so marginally.

There are several guidelines in regards to glycaemic targets in the general population; the American Diabetes Association (ADA) recommends aiming to achieve an HbA1c of less than 7.0%, while its European equivalent, the European Association for the Study of Diabetes (EASD) targets HbA1c values of below 6.5% [444]. Although concerns have been raised over the safety of these targets in light of the recent new evidence, the guidelines have not been altered by either party, yet.
As there are no specific glycaemic guidelines for the ESRD population, the same is taken to apply to this group of patients. And, the subject of glycaemic control is an equally controversial one in ESRD and MHD patients. No solid evidence can show beneficial effects of tight glycaemic control on diabetic nephropathy in CKD stages 3 to 5. However, clinicians are always urged to pursue tight glycaemic control in order to reduce development and progression of other diabetic complications, such as retinopathy, neuropathy and macroangiopathy\[445\]. And although there is evidence that associates poor glycaemic control with increased mortality in the MHD population \[446, 447\], there is also data from large epidemiological studies that shows glycaemic control has no effect on survival and outcome of such patients \[132\].

1.3.2 Tools for Assessment of Glycaemic Management

Perhaps the most widely used tool for assessing patients’ glycaemic management is the HbA1c, or glycosylated haemoglobin. Glycosylated haemoglobin is the product of the irreversible non-enzymatic glycation of one or both amino-terminal valines of the beta-haemoglobin chain \[448\]. The reason it is widely used is that it shows plasma glucose values averaged over the half-life of the red blood cells (RBC), which is about 50-55 days in the general population. But an important fact to keep in mind is that it is believed that the major proportion of the HbA1c value is attributed to the younger RBCs, those two to four weeks old, and that the oldest RBCs, those aged 90-120 days only attribute about 10% to total HbA1c values \[449\].

In addition to the HbA1c, most clinicians also use random glucose values as measured by a finger prick test carried out by the patients or a healthcare professional. Finger-prick results are much less reliable, because they not only depend on the manufacturer of the monitor in use, but also the time in relationship to medication and food, the age of the strips used and the expertise of the person carrying out the test that effect the results.

The most reliable method for assessing glycaemic management may become continuous glucose monitoring. Continuous glucose monitors (CGM) are usually portable devices that can be worn by the patients without restricting their physical mobility and daily routine. They generally measure interstitial glucose every few
minutes and record the results in their internal chip, or transmit them wirelessly to another device; a mobile phone, a computer or an insulin pump. Emergence and spread of use of CGM for assessing glycaemic control, has the potential to revolutionise the monitoring of diabetic patients.

### 1.3.3 The Reliability of HbA1c

Generally, HbA1c has proven to be an invaluable tool in assessing long-term glycaemic control. However, certain conditions have long been known to decrease this reliability as they either interfere with the HbA1c assay, or effect RBC survival and in turn cause a ‘disproportionate’ HbA1c value. ESRD causes both of the above; uraemia interferes with assays as it produces carbamylated haemoglobin which caused problems with older HPLC assays of HbA1c [450], and it reduces RBC half-life and therefore produces under-estimated HbA1c results [451]. But, in the absence of any other method for a long-term estimation of glycaemic control, clinicians continue to use the HbA1c, despite its known shortcomings in the ESRD population. Small studies have even shown that contrary to its theoretical problems, the HbA1c assay is sufficiently reliable in the ESRD population [452]. The ADAG (A1c Derived Average Glucose Study Group) investigators recently reported [453] that HbA1c levels can be converted to average glucose levels in T2DM. However, CKD patients were excluded from this study and it is possible that the metabolic fluctuations seen around haemodialysis may weaken the relationship between HbA1c and average glucose.

In keeping with the recent concerns surrounding the reliability of HbA1c in subjects with ESRD, glycated albumin has been proposed as a surrogate marker of glycaemic control in patients with renal anaemia and in receipt of erythropoietin [454, 455]. Recent reports have shown improved survival among subjects with lower glycated albumin levels [456].

### 1.3.4 The Role of Continuous Glucose Monitors

One of the alternative methods of glycaemic assessment to HbA1c is continuous glucose monitoring. First introduced in the 1990s, continuous glucose monitoring systems are becoming more and more advanced and common. They can usually stay on for 3 to 7 days (depending on the system used), and they measure and
record glucose values about every 3-5 minutes. CGMs usually measure interstitial glucose, not plasma glucose, and this does mean that it does not really reflect real-time plasma glucose. The lag-time differs according to the system used and the technique that system uses to pick up glucose signals. At present, it is thought that systems that use the microdialysis technique are most accurate, as they have the shortest lag time. Another problem in measuring interstitial glucose as opposed to plasma glucose is that the devices lose their accuracy as the glucose level drops to hypoglycaemia level, and because of the discussed lag time are not optimal for picking up imminent hypoglycaemia, and generally over-estimate the duration of hypoglycaemia as the lag-time increases during recovery [457]. Nevertheless, many studies have shown that using a CGM to guide diabetes treatment significantly improves HbA1c and diabetes control [458-461].
1.4 Hypothesis

1) Patients on haemodialysis have different cardiovascular risk factors compared to CKD patients.

2) Although the MHD population is known to be insulin resistant, insulin sensitivity is improved once patients commence haemodialysis compared to the preceding late CKD stages.

3) HbA1c may not be an accurate measurement of glycaemic control among the haemodialysed population with diabetes, thus warranting new methods for assessing glycaemic control in this population.
1.5 Aims and Objectives

Aim 1:

To re-assess the 'reverse epidemiology' hypothesis by identifying cardiovascular risk factors in a cohort of patients with a spectrum of renal impairment, mainly before and after haemodialysis.

Objectives:
To identify and categorise patients within the ICKTI into the following groups: predialysis patients (those with CKD stages 3, 4 and 5 who are not yet on renal replacement therapy) and patients on haemodialysis. Both groups will have patients with and without diabetes. To document their baseline phenotypic, demographic and biochemical data and monitor predefined cardiovascular events during the 24-month follow-up period. To calculate the risk associated with baseline data and compare the results between and with event rates in the general population.

Aim 2:

To evaluate insulin resistance and inflammation at different stages of ESRD, and to examine its relationship, if any, to degree of renal disease, mode of treatment and cardiovascular events.

Objectives:
To quantify insulin resistance among patients within the groups mentioned above by use of the homeostatic model assessment (HOMA) technique, and evaluate inflammation by measuring plasma adiponectin and high sensitivity CRP in a subset of the aforementioned cohort. To compare results for before and after haemodialysis groups.

Aim 3:

To re-assess the glycaemic control in patients with diabetes and on haemodialysis.

Objectives:
To identify and recruit a subgroup of diabetic patients on MHD, fit them with continuous glucose monitoring devices and look at glycaemic profiles and identify factors that affect it. Also, to compare mean blood glucose values over 48 hours with relevant HbA1c values to determine the accuracy of HbA1c in this group.
Chapter 2: Methods

2.1 Identifying Cardiovascular Risk Factors in ESRD

The study is a prospective case-controlled cohort study.

2.1.1 Ethics

The study was approved by the Hammersmith Hospital Research Ethics Committee (Registration Number: 06/Q0406/148). As the data being used was retrieved from existing hospital records and databases, written informed consent was not necessary for enrollment into the database. At 24 months, patients were sent questionnaires to complete and return in order to detect endpoints that may not have been recorded in hospital records. A copy of the questionnaire is supplied in the appendix (6.3.4).

2.1.2 Study objectives

To identify and categorise patients within the ICKTI into the following groups: pre-dialysis patients (those with CKD stages 3, 4 and 5 who are not yet on renal replacement therapy) and patients on haemodialysis. Both groups will have patients with and without diabetes. To document their baseline phenotypic, demographic and biochemical data and monitor predefined cardiovascular events during the 24-month follow-up period. To calculate the risk associated with baseline data and compare the results between and with event rates in the general population.

2.1.3 Subjects

Subjects were recruited from the Imperial College Kidney & Transplant Institute (ICKTI), which is sited at Hammersmith Hospital in London. This is where renal services for the whole of north-west London has been centralised, and forms the hub of a network connecting the major hospitals across this sector of London; these include Hammersmith, Charing Cross, Northwick Park, St Charles, Central Middlesex, Watford, Ealing, West Middlesex and Ashford Hospitals. The WLRTC provides renal care for a population of 3.5 million people residing in north-west London, and renal replacement therapy to approximately 2000 patients (data from internal audit, March 2007), making it the largest programme of its kind in Europe. For the purposes of this study, patients were mainly recruited from Hammersmith and Charing Cross hospitals.
**Inclusion Criteria:** Competent adults (age 18 or greater) within the ICKTI; individuals with an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73m², with and without diabetes; patients currently receiving renal replacement therapy in the form of maintenance haemodialysis, with and without diabetes.

**Exclusion Criteria:** Age less than 18; eGFR > 60 mL/min/1.73m²; RRT other than maintenance haemodialysis (haemodiafiltration, continuous ambulatory peritoneal dialysis (CAPD)); and known pregnancy were prospectively determined exclusion criteria.

Subjects were identified from a central index of patient records and clinic notes and recruited subjects were enrolled into a database where relevant clinical data was recorded. Subjects were then prospectively monitored for 24 months from date of entry into the database. The list of documented characteristics can be found below. Recruitment, data collection and follow-up of subjects was facilitated by volunteer medical students.

**2.1.3.1 Demographics:** Hospital number: The unique number assigned to each subject for identifying and retrieving information from hospital records.

Hospital site: As patients were recruited from multiple sites within ICKTI, the specific location was recorded for reference.

Date of birth: As specified within the subjects' hospital records. This was recorded both for identification purposes as well as to calculate subjects' age.

Gender: Also recorded as specified within the subjects' hospital records.

Ethnicity: This factor was retrieved from the subjects' hospital records, which is based on a self-reported questionnaire. The results were reclassified to fewer groups with larger numbers to facilitate statistical calculations. The reclassification method is outlined below in figure 2.1.
Figure 2.1 Classification of ethnicity in the cohort, based on the data collect by the Trust at admission.

2.1.3.2 Baseline Characteristics: Body mass index (BMI): Most subjects had their weights recorded at every clinic appointment. Heights were also recorded in most cases. Where either weight, height or both were missing for a subject, the aim was to ascertain this information the subject’s next scheduled clinic visit.

Diabetes status: Ascertained from subjects’ clinic notes. For diabetic subjects, the date of first diagnosis and type of diabetes (type 1 or type 2) was also recorded.

Smoking status: As reported by the patient and recorded in clinic notes.
Cause of renal failure: As recorded in clinic notes; in some cases renal biopsies had been obtained while others were either based on the physicians’ deduction from patients’ history or unknown.

Stage of ESRD: This was classified as CKD 3 (eGFR 59 to 30 mL/min/1.73 m²), CKD 4 (eGFR 29 to 15 mL/min/1.73 m²), CKD 5 (eGFR < 15 mL/min/1.73 m²), and MHD if receiving maintenance haemodialysis.

Dialysis adequacy: For subjects who were on MHD, dialysis adequacy, as measured by the Kt/V formula (where K=dialyser clearance (L/min), t=time (min) and V=body volume of urea (L). Kt/V is a dimensionless number, as (L/min x min)/L=1) was obtained from hospital notes as a measurement of quality of dialysis [462].

Existing co-morbidities: Other existing conditions and diseases were retrieved from hospital and clinic notes. Of special priority were pre-existing cardiovascular conditions.

Current medication: A list of the patients’ medication at entry into database was obtained from their hospital records and clinic notes.

Blood pressure: The patients’ last recorded blood pressure at entry into the study was ascertained from patients’ notes and recorded in the database. In case of haemodialysed subjects, both pre- and post-dialysis blood pressure was recorded.

2.1.3.3 Subject Classification: To observe changes throughout the course of end stage renal disease, patients were classified into groups of those losing kidney function and those on haemodialysis:

1-the pre-dialysis group: this group comprises of patients with CKD stages 3, 4 and 5.

2-the on dialysis group: this group includes patients who are currently receiving maintenance haemodialysis at an ICKTI unit.
2.1.4 Laboratory analysis

The most recent biochemistry and blood count results for patients were used to ascertain the following factors for each subject at entry into the study.

**Haemoglobin:** measurements were performed in the hospital’s routine haematology laboratory using a XE2100 auto-analyzer (Sysmex, Toa Medical Electronics, Kobe, Japan) running a variation of the CyMet-haemoglobin absorbtimetric method.

**Total cholesterol:** measurements were performed by the hospital’s routine clinical biochemistry laboratory using the Architect c Systems™ (Abbott Diagnostics, Illinois, USA). The assay uses an enzymatic methodology where cholesterol esters are hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. The free cholesterol is then oxidized and hydrogen peroxide is released, which goes on to form a chromophore (quinoneimine dye) which is quantitated.

**High density lipoprotein (HDL) cholesterol:** measurements were performed by the hospital’s routine clinical biochemistry laboratory using the Architect c Systems™ (Abbott Diagnostics, Illinois, USA). The assay uses an accelerator selective detergent to accelerate the reaction of cholesterol oxidase with non-HDL unesterified cholesterol and dissolves HDL cholesterol selectively. The reagent consists of a chromogenic coupler that develops colour for the quantitative determination of HDL cholesterol.

**Triglycerides:** measurements were performed by the hospital’s routine clinical biochemistry laboratory using the Architect c Systems™ (Abbott Diagnostics, Illinois, USA). The assay uses an enzymatic methodology where triglycerides are hydrolyzed to free fatty acids and glycerol. The glycerol is phosphorylated to produce glycerol-3-phosphate, which is then oxidized to produce hydrogen peroxide (H2O2) which reacts with reagents to produce a red coloured dye. The absorbance of this dye is proportionate to the concentration of triglycerides in the sample.

**Glycosylated haemoglobin (HbA1C):** The HbA1c measurements were performed in the hospital’s clinical biochemistry laboratory using a DCCT-aligned HA-8160 HbA1c auto-analysers (A.Menarini Diagnostics). This analyzer is not subject to
interference by urea as this reverse-phase cation exchange high-performance liquid chromatography method provides good separation of HbA1c from carbamylated HbA1.

**Urea:** Serum urea tests were performed by the hospital’s routine clinical biochemistry laboratory using an Architect ci8200 multi-channel analyser (Abbott Diagnostics, Illinois, USA). This method uses an enzymatic methodology where urea is hydrolysed to ammonia and carbon dioxide; ammonia is then converted to glutamate and water with the concurrent oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD). Two moles of NADH are oxidized for every mole of urea present.

**Creatinine:** measurements were performed by the hospital’s routine clinical biochemistry laboratory using the Architect c Systems™ (Abbott Diagnostics, Illinois, USA). The assay uses alkaline picrate to form a creatinine-picrate complex. The rate of increase in absorbance at 500nm due to the formation of this complex is directly proportionate to the concentration of creatinine in the sample.

**Ferritin:** measurements were performed by the hospital’s routine clinical biochemistry laboratory using the Architect c 8000® System (Abbott Diagnostics, Illinois, USA). The assay uses a latex reagent coated with rabbit IgG anti-ferritin. When a sample containing ferritin is mixed with the reagent, agglutination occurs and its degree is directly proportionate to the concentration of ferritin in the sample and is determined by the decrease of transmitted light caused by the aggregates.

**Total iron binding capacity (TIBC):** measurements were performed by the hospital’s routine clinical biochemistry laboratory using the Architect c 8000® System (Abbott Diagnostics, Illinois, USA). The assay uses ferric chloride saturating solution to bind all available apotransferrin binding sites with iron and alumina adsorbent to remove excess iron from the serum mixture. The mixture is then analysed for total iron using the iron assay and the result is multiplied by the dilution factor of 3 to compensated for dilution of the serum by the saturating solution.
**C-reactive protein (CRP):** measurements were performed by the hospital’s routine clinical biochemistry laboratory using the Architect c Systems™ (Abbott Diagnostics, Illinois, USA). The assay uses an immuneturbidimetric methodology where anti-C-reactive protein antibody is adsorbed to latex particles and reacts with CRP to result in agglutination. This agglutination is detected as an absorbance change, where the magnitude of the change is proportionate to the quantity of CRP in the sample.

**Calcium:** measurements were performed by the hospital’s routine clinical biochemistry laboratory using the Architect c Systems™ (Abbott Diagnostics, Illinois, USA). The assay uses Arsenazo III dye which reacts with calcium in an acid solution to form a blue-purple complex. The colour developed is measured at 660nm and is proportionate to the calcium concentration in the sample.

**Phosphate:** measurements were performed by the hospital’s routine clinical biochemistry laboratory using the Architect c Systems™ (Abbott Diagnostics, Illinois, USA). The assay uses ammonium molybdate which reacts with inorganic phosphate to form a heteropolyacid complex, and the absorbance at 340nm is directly proportionate to the inorganic phosphate level in the sample.

**Glucose:** measurements were performed by the hospital’s routine clinical biochemistry laboratory using the Architect c Systems™ (Abbott Diagnostics, Illinois, USA). The assay uses hexokinase to phosphorylate glucose into glucose-6-phosphate (G-6-P) which is oxidized with concurrent reduction of nicotinamide adenine dinucleotide (NAD) to nicotinamide adenine dinucleotide reduced (NADH). One micromole of NADH is produced for every micromole of glucose consumed.

The subjects within the database were routinely monitored at 12 month intervals for predefined endpoints, which are listed below. This was derived mainly from the Trust’s centralised database (ICHIS) as well as from clinic notes, discharge summaries, telephone enquiries and death certificates.
2.1.5 Endpoints

The study endpoints were divided into primary and secondary endpoints.

**Primary endpoints:** Any cardiovascular event was classified as a primary endpoint. Cardiovascular events were defined as: myocardial infarction, aortocoronary bypass, percutaneous transluminal coronary angioplasty, angiographically verified stenosis of the coronary arteries, stroke, or a symptomatic stenosis of the peripheral arterial vessels (carotids, aorto-iliac, or femoral arteries). Admission for any of the above during the course of the study was considered a primary endpoint. In addition, admission to hospital with a primary diagnosis of ‘chronic ischaemic heart disease’ or any other pre-existing condition that exasperated during the monitoring period was constituted as a primary endpoint. In the event of multiple events during the monitoring period, the first event was used for the statistical calculations.

**Secondary endpoint:** Death due to all causes was categorised as the secondary endpoint. As subjects may have had a cardiovascular event prior to this, it is possible for subjects to reach both a primary and secondary endpoint within the study timeframe.
2.2 Measuring Insulin Resistance and Inflammation

There are a number of techniques for quantifying insulin resistance (See section 1.6.4). For the purposes of this study the homeostatic model assessment (HOMA) technique was used.

The reasons for choosing the HOMA technique and its principles have already been discussed. The protocol that was designed for this study is described here.

2.2.1 Ethics

The study was approved by the Hammersmith Hospital Research Ethics Committee (Registration Number: 06/Q0406/148) and written informed consent obtained in all cases. A copy of the patient invitation letter and information sheet, as well as the consent form are supplied in the appendix (section 6.3)

2.2.2 Study objectives

To explore the relationship between insulin resistance, adiponectin and hs-CRP with cardiovascular events in a group of subjects with varying degrees of renal impairment; to compare levels of insulin resistance, hs-CRP and total adiponectin before start of MHD with their levels 8 weeks into MHD.

2.2.3 Subjects

106 subjects agreed to come in after an overnight fast to participate in the study. They were recruited from the haemodialysis and renal outpatient units at Hammersmith and Charing Cross hospitals. Subjects included patients with CKD 3, CKD 4, CKD 5 (not on HD) and on MHD. Both diabetic and non-diabetic subjects were included in the analysis.

To analyse changes in insulin resistance, hs-CRP and total adiponectin levels before and after MHD, a number of subjects scheduled to start MHD were identified and approached, 7 of who agreed to participate in this subgroup study. They had their first sample taken on the day of their first haemodialysis session, prior to its initiation. All subjects gave informed written consent to repeat the procedure 8 weeks into MHD. Of the seven subjects, one was transplanted before reaching 8
weeks, one died before reaching 8 weeks, and four refused to take part on the day. Only one patient successfully completed both pre- and post-dialysis sampling.

2.2.4 Exclusion criteria

The exclusion criteria were prospectively defined as: age less than 18, eGFR > 60 mL/min/1.73m², RRT other than maintenance haemodialysis (haemodiafiltration, continuous ambulatory peritoneal dialysis (CAPD)), and known pregnancy.

2.2.5 Blood samples and laboratory analysis

For the insulin measurements three blood samples were taken at five minute intervals after an overnight fast of at least 12 hours. The samples were drawn into Lithium Heparin (LH) tubes to inhibit coagulation and enable plasma separation. The samples were chilled on ice and centrifuged immediately at 500g (3500 rpm) for 10 minutes. The plasma was then separated and each sample was stored as 3 aliquots of > 0.5 ml at -80 degrees Celsius. At the end of the study period, one aliquot from each sample was sent to the laboratory to be assayed for insulin levels; the samples were analysed in two parts by the same laboratory to minimise inter assay errors. The insulin assays were done using the Abbott Axsym; this is a well established fully automated immunometric assay employing an enzyme label and microparticle separation; % coefficients of variation are 4-6% across the diagnostic range of the assay and the functional sensitivity is 0.5mlU/l.

For the glucose measurement needed for the HOMA calculation, blood was drawn into fluoride oxalate tubes and immediately sent to the chemical pathology department for analysis. A number of medical students volunteered to help with recruitment and sample collection.

As almost half of the population studied were haemodialysis patients who are usually quite ill and have already undergone many invasive procedures, to minimise the discomfort of taking three consecutive blood samples, samples were taken out of the haemodialysis circuit. To make sure that this would not compromise our results I had to make sure that no insulin was being dialysed out. This was done by testing the outgoing dialysate fluid for insulin. As expected, no insulin was detected in this fluid and thus samples were collected from the dialysis circuit.
As mentioned in the section above, 9 plasma aliquots of > 0.5 ml were stored for each subject (3 samples per subject, 3 aliquots per sample) at -80 °C. One of these aliquots was used to measure total adiponectin and high sensitivity CRP levels in each subject. Adiponectin was measured by ELISA (RayBiotech, Norcross, GA, USA) and performed according to manufacturers’ instructions. This assay employs an antibody specific for human adiponectin coated on a 96 well plate. Standards or diluted serum were added to the microtitre wells and adiponectin present in the sample bound to the immobilised antibody. Following incubation the wells were washed and biotinylated anti-human adiponectin antibody added. Following a further incubation unbound biotinylated antibody was washed away and HRP streptavidin was added to the wells. After further incubation the wells are washed and bound HRP visualised using TMB substrate solution. The colour that developed is proportional to the amount of adiponectin present in the sample. After incubation the reaction is stopped by the addition of sulphuric acid and the optical density of the reactive solution was read at 450nm. The concentration of adiponectin was calculated by reference to a standard curve of known adiponectin concentration. The assays were carried out by the Translational Research Laboratory at the Kennedy Institute of Rheumatology of Imperial College London.

CRP was measured by a high sensitivity CRP ELISA (Kalon Biological, Guildford UK) and performed according to manufacturers’ instructions. This assay employs an antibody specific for CRP coated on a 96 well plate. Standards or diluted serum were added to the microtitre wells and CRP present in the sample bound to the immobilised antibody. Following incubation the wells are washed and alkaline phosphatase labelled anti-human CRP antibody was added. After further incubation the wells were washed and bound alkaline phosphatase visualised using 4-nitrophenylphosphate substrate solution. The colour that developed is proportional to the amount of CRP present in the sample. After incubation the reaction was stopped by the addition of EDTA solution and the optical density of the reactive solution was read at 405nm. The concentration of CRP was calculated by reference to a standard curve of known CRP concentration. The assays were carried out by the Translational Research Laboratory at the Kennedy Institute of Rheumatology of Imperial College London.
2.3 Assessing Glycaemic Control in ESRD

A 48-hour continuous glucose monitoring device called the GlucoDay® S from Menarini Diagnostics (Florence, Italy) was used to assess glycaemic control in haemodialysed patients with type 2 diabetes (figure 2.2). This device measures and records glucose values every 3 minutes for up to 48 hours. It uses a microdialysis mechanism that is delivered via a microfibre that is inserted into the peri-umbilical subcutaneous adipose tissue. The biosensor, which consists of a 20µm membrane of cellulose acetate, a nylon net with the immobilised glucose oxidase enzyme and a polycarbonate membrane [463].

Figure 2.2 Photograph of the GlucoDay® S continuous glucose monitor device (left) and subject wearing the device with the supplied belt and pouch (right).

2.3.1 Ethics

The study was initially approved by the Hammersmith Hospital Research Ethics Committee in 2002 for continuous glucose monitoring in free living people with type 2 diabetes treated with insulin and on a low glycaemic index diet (Registration Number: 2002/6260). A substantial amendment application was put forward and approved by the same committee to extend the project to include haemodialysed subjects. Written informed consent obtained in all cases. Copies of the amendment, patient information sheet and consent form are available in the appendix (section 6.5).

2.3.2 Study objectives

To compare glucose profiles from days on and off dialysis using 48-hour
CGM in type 2 diabetic patients; to examine the association between self-reported food intake and the CGM values; to evaluate glycaemic assessment obtained using 48-hour CGM in type 2 diabetic MHD patients.

2.3.3 Subjects

Nineteen (14 male) Type 2 diabetic subjects were recruited from the MHD program at Imperial College Kidney and Transplant Institute (ICKTI). Subjects were dialysed against a <2 g/l glucose containing dialysate (equivalent to <11.1 mmol/l of glucose) for 4-5½ hours either during the morning, afternoon or early evening. Inclusion criteria were a stable haemoglobin (Hb) level, defined as <10% change in Hb value and no blood transfusion in the preceding 3 months, a stable dose of erythropoietin and no haemoglobinopathy. History of cardiovascular disease was established as documented ischaemic heart disease (history of myocardial infarction, revascularisation procedure or angiographically proven coronary disease), cerebrovascular disease (history of cerebrovascular accident or transient ischaemic attack) or peripheral vascular disease (history of amputation due to gangrene, revascularisation procedure or angiographically or doppler proven peripheral vascular disease).

Subjects were fitted with the device (figure 2.2) at the prior to their dialysis session and instructed to keep a complete diary of their diet, physical activity, medication and finger prick test results (at least one). A copy of the food diary is available in the appendix (section 6.4.4). The device was taken out before the start of the next session of dialysis, 48 hours later. As patients are heparinised during dialysis, the device was fitted before and also removed before the session to minimise the risk of bleeding.

2.3.4 Exclusion criteria

Exclusion criteria were prospectively defined as: Type 1 diabetes, inter current illness, changes to medication regimen during the monitoring period or occurrence of prolonged hypoglycaemia.
2.3.5 Blood samples and laboratory analysis

Blood samples were taken for HbA1c, haemoglobin, albumin and urea at the start of dialysis. The HbA1c measurements were performed in the hospital’s clinical biochemistry laboratory using a DCCT-aligned HA-8160 HbA1c auto-analyser (A. Menarini Diagnostics). This analyzer is not subject to interference by urea as this reverse-phase cation exchange high-performance liquid chromatography method provides good separation of HbA1c from carbamylated HbA1.

The haemoglobin measurements were performed in the hospital's routine haematology laboratory using a XE2100 auto-analyzer (Sysmex, Toa Medical Electronics, Kobe, Japan) running a variation of the CyMet-haemoglobin absorbtimetric method.

Serum urea and serum albumin tests were performed on an Architect ci8200 multi-channel analyser (Abbott Diagnostics, Illinois, USA).

2.3.6 Glucose profiles

The 48-hour glucose profiles were quantified using dedicated software (GlucoDay®S Data Presentation Software) as the area under the 3-minute glucose curve (AUC) and the mean glucose value. The time periods studied were the first 24-hour period starting the first hour of dialysis (day on dialysis) and the 24-hour period ending one hour prior to the next dialysis session (day off dialysis). The 6-hour nocturnal periods from midnight to 6:00 a.m. for each of these 24-hour periods were also examined in order to examine the effect of dialysis. Hypoglycaemia, defined as a continuous glucose reading <2.5 mmol/l for more than 30 minutes, was identified from the CGM profiles. Subjects were questioned regarding symptoms of hypoglycaemia at the end of the CGM period.

2.3.7 Patient diaries and dietetic analysis

On the first day subjects were given a 48-hour diary to record the exact time and amount of food, drink and medications taken during the entire CGM monitoring period, together with any episodes of symptomatic hypoglycaemia and all capillary blood glucose results. Completed food diaries were checked during a dietary consultation with a registered dietitian. Food portions were verified using a pictorial
food atlas (MAFF Publications 1997). Comparisons of dietary intake during the 24-hour periods on and off dialysis were performed by a data-analyst blinded to the study using the Dietplan 6 software package (Forestfield Software). Daily energy requirement was calculated at 30-35 kCal/kg ideal body weight (Renal Association 2002). A registered dietician helped with dietary data analysis.
2.4 Statistical Calculations

All data was exported into PASW Statistics versions 14-17 software (SPSS for Windows, Release 17.0.2 [11 March 2009], Copyright 1993-2007 Polar Engineering and Consulting). The Shapiro-Wilk test was used to test normality of data and comparisons of means were carried out using Student’s t-test, ANOVA, and non-parametric test were applicable.

Power calculations to determine the required sample size for the cohort are mainly based on Van Belle’s book, Statistical Rules of Thumb [464], which states that “in logistic regression situations about 10 events per variable are necessary in order to get reasonably stable estimates of the regression coefficients.” As the current mortality rate for patients on haemodialysis in the UK is reported to be 20% annually [465], according to this rule, if we are looking to analyse 8 prognostic factors, we will need a sample population that will give us 80 events, in this case death. With a 20% annual mortality rate, an initial population of at least 220 haemodialysis patients are needed to achieve 80 events in 2 years (calculations: 20% die in year 1, 0.8 x 220=176 left \(\rightarrow\) 20% of those left die in year 2, 0.8 x 176=141 left \(\rightarrow\) =79 deaths). The present database holds 210 haemodialysed subjects and 308 non-haemodialysed subject for comparison.

Since it is more likely that subjects develop a non-fatal cardiovascular event [27], this outcome was categorised as the primary endpoint; this ensures that the study produces significant results as it is powered to produce results for the less common outcome of death, which is categorised as the secondary endpoint for this study.

Logistics model was used to calculate odds ratios associated with each studied risk factor for primary and secondary endpoints. Odds ratios were calculated for BMI, total cholesterol, triglycerides, systolic and diastolic blood pressure and diabetes status. The calculations were carried out for the two subgroups within the whole population as well; the CKD and MHD groups. Logistic model was also used to calculate odds ratios associated with HOMA-IR, adiponectin and hs-CRP for primary and secondary endpoints in the group of patients they were measured in. These calculations were also done for the two subgroups within this population; the CKD and MHD groups.
There are also different statistical methods of analysing this data: logistic regression (which is used to express the present results), which calculates an odds ratio which is independent of time and Cox regression, which calculates hazard ratios in relation to time from start of disease to endpoint. The reason logistic regression is used to express the results of the present study is that although time of endpoint is recorded for all subjects, time of start of disease was impossible to record in some cases, as CKD is a gradual process that develops, in some instances, over very long periods of time. Also, as many patients had multiple co-morbidities such as diabetes and pre-existing cardiovascular disease, the definition of the 'start of disease' was too broad to accommodate these subjects; i.e. start of which disease? Nevertheless, Cox regression was carried out to make sure that its results were not contradictory to those achieved by logistic regression. To that end, time of start of disease was defined as time of recruitment into the present study; therefore the start date for all subjects was recorded as March 10\textsuperscript{th}, 2007. The results of Cox regression analysis were very similar, and in some cases identical, to that of logistic regression. Neither the direction of effect nor the statistical significance calculated were different from those achieved by logistic regression. It was therefore decided to use the logistic regression results to express the risks, as this method was scientifically more suitable for the present dataset in light of the above explanation. The Cox regression analysis was used to draw the cumulative hazard plots for the risk factors, in which case continuous variables (such as BMI, BP, TC, TG, HOMA-IR, hs-CRP and total adiponectin) were transformed into categorical variables of above or below a certain cut-off point (either the upper limit of normal range or the median within the cohort).

Statistical help and advice was sought from the Imperial College Statistical Advisory Service, who were actively involved in the statistical calculations for this part of the study. The final report from the statistician is available in the appendix (Appendix II).

The CGM data was also exported into SPSS versions 14.0 to 17.0.2 software (SPSS for Windows, Release 14.0.0 to 17.0.2 [5 September 2005 to 11 March 2009], LEAD Technologies, Inc.) and tested for normality using the Shapiro-Wilk test. All normally distributed data is expressed as mean and standard deviation (SD) and non-normally distributed data as median and range. All comparisons of the
glycaemic profiles and dietary intake between days on and off dialysis was analysed using paired Student's t-tests.

Linear regression analysis was used to assess the relationship between laboratory HbA1c, and weekly EPO dose, serum urea and serum albumin. Linear regression was also used to look at relationships between different risk factors throughout the study.

The level of significance was defined as p<0.05 throughout the study.
Chapter 3: Results

3.1 Cardiovascular Risk Factors in ESRD

3.1.1 Distributions and comparison of subgroups

A total of 518 patients were recruited into the database. Of these, 210 subjects are classified as MHD subjects as they were on HD at recruitment, and 308 are categorised as CKD as they were CKD 3 or above and not on HD at recruitment. The characteristics of this cohort are as follows.

3.1.1.1 Age. Birth date was recorded for 518 subjects in the database. The minimum recorded age is 22 years while the maximum is 98 years old. The mean age is 66 with 15.0 years standard deviation.

The mean age for CKD subjects not on haemodialysis is 67 years ± 14.7 years with the same range as the whole population, while in the haemodialysis group the mean age is 64 years ± 15.3 years with a slightly narrower range of 24 to 94 years (table 3.1.1).

<table>
<thead>
<tr>
<th></th>
<th>CKD (n=308)</th>
<th>MHD (n=210)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>67±14.7 (years)</td>
<td>64±15.3 (years)</td>
</tr>
<tr>
<td>GENDER (M/F &amp;M%)</td>
<td>187/121 (60.7%)</td>
<td>113/97 (53.8%)</td>
</tr>
<tr>
<td>DM</td>
<td>59.4%</td>
<td>43.3%</td>
</tr>
<tr>
<td>ETHNICITY</td>
<td>37.7% White</td>
<td>32.4% White</td>
</tr>
<tr>
<td></td>
<td>13.6% Black</td>
<td>30.0% Asian</td>
</tr>
<tr>
<td></td>
<td>12.3% Asian</td>
<td>23.8% Black</td>
</tr>
<tr>
<td></td>
<td>7.6% Other</td>
<td>10.0% Other</td>
</tr>
<tr>
<td></td>
<td>25.7% Undisclosed/ Missing</td>
<td>3.3% Undisclosed/ Missing</td>
</tr>
</tbody>
</table>

*Table 3.1.1 Demographic details at recruitment for the CKD and MHD subgroups*

3.1.1.2 Gender. Gender was recorded for all subjects in the database, and of the 518 subjects, 301 were male. This means the cohort consists of 42% female and
58% male subjects. In the CKD group 187 subjects (60.7%) are male while in the HD group 113 subjects (53.8%) are male (figure 3.1.1 and table 3.1.1).

![Male to female ratio within the CKD, MHD and the whole cohort of ESRD patients recruited.](image)

**Figure 3.1.1** Male to female ratio within the CKD, MHD and the whole cohort of ESRD patients recruited. The CKD group (n=308) consists of 60.7% males, the MHD group (n=210) 53.8%, and the whole group (n=518) 58% males.

### 3.1.1.3 Diabetes Status

Diabetes status is unknown for eleven subjects in the database and of the remaining subjects, 275 patients (53.0%) have diabetes. In the CKD group, 10 subjects have unknown status and 183 subjects (59.4%) have diabetes. In the HD group only one subject has unknown diabetic status and of the remaining 210 subjects, 91 (43.3%) have diabetes (figure 3.1.2 and table 3.1.1).
Figure 3.1.2 Ratio of subjects with and without diabetes within the CKD, MHD and the combined cohort of ESRD patients recruited. The percentage of subjects with diabetes in the CKD group (n=308) is 59.4%, in the MHD group (n=210) 43.3%, and in the whole group (n=518) 53%.

3.1.1.4 CKD Stage. The 518 subjects on the database are divided into different groups in regard to their diagnosed CKD stage at recruitment. Subjects on haemodialysis are categorised as CKD stage 5. As being stage 3 or above was one of the inclusion criteria, only one CKD stage 2 subject is present in the database, whose results are deleted from analysis. 169 subjects are stage 3, 99 subjects are stage 4 and 247 subjects are stage 5. Of the subjects in the stage 5 group, 210 are recipients of maintenance haemodialysis at recruitment, which leaves 37 subjects (7% of entire cohort) with virtually no kidney function (eGFR below 15 mL/min/1.73 m²) and no form of renal replacement therapy (figure 3.1.3).
Figure 3.1.3 Number of subjects in each group within the cohort of ESRD patients recruited. The CKD group (n=308) is broken down by CKD stage into CKD 3 (n=170), CKD 4 (n=99) and CKD 5 (n=37), while the MHD group is a single group (n=210).

3.1.1.5 Dialysis Adequacy. As stated above, of the 518 subjects within the database, 210 were on MHD at recruitment into the study. Dialysis adequacy, as measured by Kt/V was recorded for 166 (79%) of these. The mean Kt/V was 1.73 units (range 0.92 – 2.80) ± 0.34, which is well above the target (1.3 units [29]).

3.1.1.6 Ethnic Distribution. Ethnicity is recorded for 502 subjects (96.7% of the entire cohort). Of these 35.5% are of white background (British, Irish, or any other white), 19.5% are Asian (Indian, Pakistani, Bangladeshi, or any other Asian), 17.7% are of black background (African, Caribbean, or any other black), 7.5% are of other ethnic background, 0.8% (4 subjects) have mixed background, and 15.8% have not disclosed their ethnicity to hospital records.

In the CKD group ethnicity is recorded for 292 subjects (94.8% of entire group), and the distribution of present ethnicities in regards to frequency is slightly different from the whole cohort. The most prevalent ethnicity is still white (37.3%), but unlike the whole group analysis, the second is black (13.6%) and third is Asian (12.3%). In
comparison, the HD group has the biggest proportion of Asians (30.0%), although it still ranks second by only a small amount from the white population (32.4%). In the HD group, the blacks make up 23.8% of the population, which is again a bigger proportion than the previously mentioned groups (figure 3.1.4 and table 3.1.1).

**Figure 3.1.4 Ethnic distribution in each group within the cohort of ESRD patients recruited.** The CKD group (n=308) consists of 115 (37.3%) white subjects, 42 (13.6%) black African and black Caribbean, 38 (12.3%) Asian, 22 (7.6%) other ethnic background, and 75 (25.7%) subjects with unknown or undisclosed ethnic background. The MHD group (n=210) consists of 68 (32.4%) white subjects, 50 (23.8%) black African and black Caribbean, 63 (30%) Asian, 21 (10%) other ethnic background, and 7 (3.3%) subjects with unknown or undisclosed ethnic background.
The characteristics of the study population in regards to the cardiovascular risk factors that were aimed to be analyzed are described at this point. In order to better understand the differences between the haemodialysed and the non-haemodialysed populations, these characteristics are broken down for the CKD and MHD groups. Analysis of each factor is summarized at the end of each section as a 2 panel figure consisting of box plots comparing distribution of the specific factor within the different subgroups (CKD and MHD as well as CKD 3, CKD 4, CKD 5 and MHD), and significant statistical differences are marked by asterisks (*). Comparison tables are also available for each factor in the appendix III.
3.1.1.7 Body Mass. Weight and height values at entry into study were recorded for 476 subjects in the database and BMI was calculated accordingly. The minimum recorded BMI was 15 kg/m$^2$ and the maximum recorded value is 54.3 kg/m$^2$. The mean ± SD for BMI in this cohort is 27.5 ± 6.4 kg/m$^2$.

Comparing the CKD group and the MHD group, it is evident that the MHD group is lighter than the CKD group with the mean BMI at 25.3 ± 5.9 kg/m$^2$ (range: 15.0 – 44.0) for the MHD group (available for 201 subjects, 95.7%) and 29.1 ± 6.2 kg/m$^2$ (range: 16.3 – 54.3) for the CKD group (available for 263 subjects, 85.3%). The comparison of means (ANOVA) between these two groups shows statistically significant lower BMI in the MHD group with a p-value of <0.001. Analysis of BMI means between the different CKD stages, where MHD is classified as the final stage, showed mean BMI ± SD for CKD 3 at 29.9 ± 6.2 kg/m$^2$, for CKD 4 at 29.1 ± 6.3 kg/m$^2$ and for CKD 5 at 26.0 ± 5.2 kg/m$^2$. Comparison of means (ANOVA) is still statistically significant in these subgroups (figure 3.1.5, and supplementary table 1 available in appendix III).
Figure 3.1.5 Box and whisker plot comparing BMI value distribution. Panel A compares BMI in CKD and MHD groups (p<0.001) and panel B compares BMI between different CKD stages and MHD (p<0.001). Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers. Horizontal bars indicate comparison of means between groups and ** indicates statistically significant finding.
3.1.1.8 Blood Pressure. Last recorded value for both systolic and diastolic blood pressure were extracted from hospital records and recorded in the database. In the case of MHD patients, pre-dialysis blood pressure was used. Mean ± SD (range) for systolic blood pressure (available for 422 subjects, 81.3%) is 144.2 ± 22.4 (87.0 – 229.0) mmHg and for diastolic blood pressure (available for 414 subjects, 79.8%) is 77.2 ± 13.5 (47.0 – 123.0) mmHg.

In the CKD group BP values were present for 241 subjects (78.2%), where the mean systolic BP (SBP) is 138.0 ± 18.1 mmHg (range: 93.0 – 199.0) and the mean diastolic BP (DBP) is 73.9 ± 12.1 mmHg (range: 47.0 – 123.0). In the MHD group BP values are available for 172 subjects (81.9%), and the mean systolic BP is 152.4 ± 25.0 mmHg (range: 87.0 – 229.0) while the mean diastolic BP is 81.7 ± 14.2 mmHg (range: 48.0 – 120.0). The comparison of means (ANOVA) between these two groups shows statistically significant higher SBP and DBP in the MHD group with a p-value of <0.001 for both analyses. Analysis of SBP means between the different CKD stages, where MHD is classified as the final stage, showed mean SBP ± SD for CKD 3 at 137.2 ± 17.0 mmHg, for CKD 4 at 137.9 ± 18.9 mmHg and for CKD 5 at 143.1 ± 21.0 mmHg. Comparison of means (ANOVA) is still statistically significant in these subgroups with p<0.001 (figure 3.1.6 and supplementary table 2 available in appendix III). Analysis of mean DBP between the different CKD stages, where MHD is classified as the final stage, showed mean SBP ± SD for CKD 3 at 73.0 ± 11.7 mmHg, for CKD 4 at 74.7 ± 12.6 mmHg and for CKD 5 at 76.5 ± 12.1 mmHg. Comparison of means (ANOVA) is still statistically significant in these subgroups with p<0.001 (figure 3.1.7 and supplementary table 3 available in appendix III).
Figure 3.1.6 Box and whisker plot comparing systolic BP value distribution. Panel A compares SBP in CKD and MHD groups (p<0.001) and panel B compares SBP between different CKD stages and MHD (p<0.001). Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers. Horizontal bars indicate comparison of means between groups and ** indicates statistically significant finding.
Figure 3.1.7 Box and whisker plot comparing diastolic BP value distribution. Panel A compares DBP in CKD and MHD groups (p<0.001) and panel B compares DBP between different CKD stages and MHD (p<0.001). Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers. Horizontal bars indicate comparison of means between groups and ** indicates statistically significant finding.
3.1.1.9 **Lipids.** To look at serum lipid status, data was collected in the form of total cholesterol, HDL cholesterol and triglycerides. Unfortunately, some physicians still prefer requesting total cholesterol and triglycerides instead of a complete lipid profile, which is why HDL values were only found for 310 subjects (60%). Total cholesterol values were present for 462 subjects (89%) and the mean total cholesterol for the entire cohort is 4.2 ± 1.3 mmol/l (range: 0.1 – 13.5), while triglyceride values are available for 448 subjects (86.5%) and the mean TG for the cohort is 1.78 ± 0.98 mmol/l (range: 0.31 – 6.0).

Looking at lipid profiles in the CKD group, the mean total cholesterol (available for 86% of the group) is 4.5 ± 1.5 mmol/l (range: 2.1 – 13.5). In the MHD group, the mean total cholesterol (available for 93% of the group) is 3.8 ± 0.95 mmol/l (range: 0.1 – 6.5). The comparison of mean TC (ANOVA) between these two groups shows statistically significant lower TC in the MHD group with a p-value of <0.001. Analysis of mean TC between the different CKD stages, where MHD is classified as the final stage, showed mean TC ± SD for CKD 3 at 4.4 ± 1.3 mmol/l, for CKD 4 at 4.7 ± 1.7 mmol/l and for CKD 5 at 4.3 ± 1.6 mmol/l. Comparison of means (ANOVA) is still statistically significant in these subgroups with p<0.001 (figure 3.1.8 and supplementary table 4 available in appendix III).
**Figure 3.1.8** Box and whisker plot comparing total cholesterol value distribution. Panel A compares TC in CKD and MHD groups (p<0.001) and panel B compares TC between different CKD stages and MHD (p<0.001). Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers. Horizontal bars indicate comparison of means between groups and ** indicates statistically significant finding.
Looking at lipid profiles in the CKD group, the mean TG (available for 85%) is 1.86 ± 1.06 mmol/l (range: 0.5 – 6.0). In the MHD group, the mean TG (available for 88%) is 1.67 ± 0.85 mmol/l (range: 0.31 – 5.09). The comparison of TG means (ANOVA) between these two groups is not statistically significant with a p-value of 0.051. Analysis of mean TG between the different CKD stages, where MHD is classified as the final stage, showed mean TG ± SD for CKD 3 at 1.81 ± 0.99 mmol/l, for CKD 4 at 1.92 ± 1.12 mmol/l and for CKD 5 at 1.88 ± 1.17 mmol/l. Comparison of means (ANOVA) is not statistically significant in these subgroups with p = 0.261 (figure 3.1.9 and supplementary table 5 available in appendix III).
Figure 3.1.9 Box and whisker plot comparing triglycerides value distribution. Panel A compares TG in CKD and MHD groups (p=0.051) and panel B compares TG between different CKD stages and MHD (p=0.216). Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers. Horizontal bars indicate comparison of means between groups and ** indicates statistically significant finding.
**3.1.1.10 Glycaemic Control.** Glycaemic control as assessed by glycosylated haemoglobin (HbA1c) was recorded for subjects with diabetes at entry into study. Of the 275 subjects with diabetes in the cohort, 258 (94%) have a corresponding HbA1c value. The mean HbA1c in the total population is 7.3% ± 1.9% (range: 0.9% – 14.8%).

The CKD group has 183 subjects with diabetes (59.4%) and 171 (93.4%) of these subjects have a corresponding HbA1c value. The mean HbA1c in the CKD population is 7.5% ± 2.0% (range: 0.9% – 14.8%), where mean haemoglobin level is 12.1 ± 1.6 g/dL (range: 7.2 – 16.5). In the MHD group, of the 91 subjects with diabetes (43.3% of MHD group) 86 (94.5%) have a corresponding HbA1c value. The mean HbA1c in the MHD population is 6.8% ± 1.6% (range: 4.5% – 13%), where mean haemoglobin level is 11.4 ± 1.4 g/dL (range: 7.6 – 15.3). The comparison of HbA1c means (ANOVA) between these two groups shows that the MHD group has significantly lower HbA1c with a p-value of 0.003. Analysis of mean HbA1c between the different CKD stages, where MHD is classified as the final stage, showed mean HbA1c ± SD for CKD 3 at 7.4 ± 1.7 %, for CKD 4 at 7.8 ± 2.4 % and for CKD 5 at 7.0 ± 2.3 %. Comparison of means (ANOVA) is still statistically significant in these subgroups with p = 0.009 (figure 3.1.10 and supplementary table 6 available in appendix III).
Figure 3.1.10  Box and whisker plot comparing HbA1c value distribution. Panel A compares HbA1c in CKD and MHD groups (p=0.003) and panel B compares TG between different CKD stages and MHD (p=0.009). Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers. Horizontal bars indicate comparison of means between groups and ** indicates statistically significant finding.
3.1.1.11 Summary of group comparisons

Comparison of mean BMI, blood pressure, total cholesterol, triglycerides and HbA1c between the CKD and MHD group revealed that the MHD group has significantly lower BMI, total cholesterol and HbA1c and significantly higher systolic and diastolic blood pressure. Only triglyceride levels were not significantly different between the two groups (table 3.1.2).

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Comparison of means in subgroups</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>CKD &gt; MHD</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>CKD &lt; MHD</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>CKD &lt; MHD</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>CKD &gt; MHD</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>CKD ~ MHD</td>
<td>p=0.051</td>
</tr>
<tr>
<td>HbA1c</td>
<td>CKD &gt; MHD</td>
<td>p=0.003</td>
</tr>
</tbody>
</table>

*Table 3.1.2* Summary of comparison of mean values for each risk factor in the two subgroups of CKD and MHD. ‘>’ indicates higher mean values in the CKD subgroup while ‘<’ indicates higher mean values in the MHD population, and ‘~’ indicates no statistically significant difference.
3.1.2 Analysis of Primary Endpoint

As the primary endpoint, predefined cardiovascular events data was gathered for subjects over the two year follow up period and risk analysis was carried out in regard to each of the predetermined risk factors. These included: myocardial infarction, aortocoronary bypass, percutaneous transluminal coronary angioplasty, angiographically verified stenosis of the coronary arteries, stroke, or a symptomatic stenosis of the peripheral arterial vessels (carotids, aorto-iliac, or femoral arteries), admission to hospital with a primary diagnosis of ‘chronic ischaemic heart disease’ or any other pre-existing condition that exasperated during the monitoring period.

These analyses were carried out for the whole population as well as separately for CKD and MHD groups. The results in this section are described in that specific order: whole cohort, CKD group and then MHD group and are expressed as odds ratio (OR), 95% confidence interval (CI) and p-values. As mentioned in section 2.4, hazard ratios were also calculated for each risk factor using Cox regression analysis, and the results were in agreement with and very similar to logistic regression results (odds ratios). Although logistic regression was ultimately chosen for data presentation as it was statistically better suited to this population, calculated hazard ratios were used to draw the cumulative hazard plots presented in this section.

Over the course of the 24-month follow up period, a total of 103 subjects reached a primary endpoint, bringing the event rate to nearly 20%. Of these, 47 subjects were in the CKD group and 56 subjects were in the MHD group, meaning the CV event rate in the CKD group was 15.3% while in the MHD group this was at 26.7% (figure 3.1.11).
Figure 3.1.11 Follow-up data for each group within the cohort of ESRD patients recruited. In the combined cohort (n=518) 103 (19.9%) subjects had a CVE (primary endpoint) and 59 (11.4%) subjects died (secondary endpoints). The CKD group (n=308) had 47 (15.3%) CVEs and 21 (6.8%) deaths, while the MHD group (n=210) had 56 (26.7%) CVEs and 38 (18.1%) deaths.

Results for each risk factor were calculated with and without adjusting for the confounding effect of pre-existing cardiovascular disease, but as this had negligible effect on the results, the adjusted results are not mentioned here (available in appendix section II).
3.1.2.1 Body Mass. The risk associated with increased BMI and CV events was calculated for the whole cohort using logistic regression. This did not show a significant impact of BMI on CV events, with an odds ratio (OR[BMI]) of 1.017 with the 95% confidence interval (CI) of OR between 0.984 and 1.050 and a p-value of 0.318.

In the CKD group, OR(BMI) = 1.037 with 95% CI of 0.988 and 1.088, p-value = 0.137, and in the MHD group, OR(BMI) = 1.047 with 95% CI of 0.997 and 1.100, p-value = 0.066. Figure 3.1.12 shows the distribution of BMI in CKD and MHD subgroups, categorised by whether they developed a CVE or not. Although BMI has not reached significance in predicting CV events in either group, the trends seems to be in the same direction, although as the lower limit of the CI is below 1 (even if slightly) means that we cannot conclude that this is in line with that of the general population where increased BMI increases the risk of CV events.
Figure 3.1.12 Box and whisker plot comparing BMI distribution between subjects who developed CVE and those who did not by subgroup. Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers.

To illustrate the effect of BMI on CVE risk, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). The subjects were classified as either overweight or not overweight, using the cut off point of 25 kg/m$^2$. Figure 3.1.13 demonstrates the lack of effect of BMI on CVE risk. These plots could not be drawn for the CKD and MHD subgroups due to the much smaller numbers.
Figure 3.1.13 Cumulative hazard plot for CVE in the whole cohort over time stratified by BMI above or below 25 kg/m². There is no statistically significant difference between the two groups.
3.1.2.2 Blood Pressure. Both systolic and diastolic blood pressure were analysed to calculate the risk associated with each in regards to CV events.

For systolic BP in the combined population, significant impact was seen on CV events, with an odds ratio (OR[SBP]) of 1.011 with the 95% confidence interval (CI) of OR between 1.002 and 1.021 and a p-value of 0.023. This can be interpreted as greater risk of CV events with increasing systolic BP in the group as whole, which follows the trend of the general population.

When broken down for the CKD and MHD groups, however, systolic BP loses significant association with CV events, as in the CKD group, OR(SBP) = 1.012 with 95% CI of 0.993 and 1.031, p-value = 0.217, and in the MHD group, OR(SBP) = 1.001 with 95% CI of 0.989 and 1.014, p-value = 0.830. As the lower limit of the CI is less than 1, we cannot conclude that increase in systolic BP leads to an increase in CV events.

For diastolic BP in the combined population, no significant impact was seen on CV events, with an odds ratio (OR[DBP]) of 1.004 with the 95% confidence interval (CI) of OR between 0.989 and 1.021 and a p-value of 0.560.

In the CKD group, OR(DBP) = 1.000 with 95% CI of 0.972 and 1.028, p-value = 0.981, and in the MHD group, OR(DBP) = 0.988 with 95% CI of 0.967 and 1.010, p-value = 0.299. The fact that the OR in the MHD group is below 1 indicates that diastolic blood pressure may be inversely associated with CV events, and although this result is not significant its direction is important and in line with reverse epidemiology. Figure 3.1.14 shows the distribution of SBP and DBP in CKD and MHD subgroups, categorised by whether they developed a CVE or not.
**Figure 3.1.14** Box and whisker plot comparing SBP and DBP distribution between subjects who developed CVE and those who did not in the CKD (panel A) and MHD (panel B) subgroups. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.
To illustrate the effect of SBP and DBP on CVE risk, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). The subjects were classified as either hypertensive or not, with 140 mmHg as the upper limit of normal for SBP and 90 mmHg as the upper limit for DBP. Figure 3.1.15 demonstrates the hazard plots for CVE in regards to SBP and DBP. These plots could not be drawn for the CKD and MHD subgroups due to the much smaller numbers.
Figure 3.1.15 Cumulative hazard plot for CVE in the whole cohort over follow-up time in months stratified by SBP cut off of 140.0 mmHg (panel A) and DBP cut off of 90.0 mmHg (panel B). Subjects with higher SBP have a significantly higher risk of CVE (p=0.023).
3.1.2.3 Lipids. Both total cholesterol (TC) and triglycerides (TG) were analysed to calculate the risk associated with each in regards to CV events.

For TC in the combined population, significant impact was seen on CV events, with an odds ratio (OR[TC]) of 0.681 with the 95% confidence interval (CI) of OR between 0.550 and 0.844 and a p-value of <0.001. This means that increase in TC reduces the risk of CV events; in other words, increased TC or hypercholesterolaemia is protective against CV events. This is contrary to a rich body of literature that identifies hypercholesterolaemia as a definite CV risk factor in the general population.

When broken down for the two groups, in the CKD group, OR(TC) = 0.589 with 95% CI of 0.419 and 0.828, p-value = 0.002, and in the MHD group, OR(TC) = 0.924 with 95% CI of 0.676 and 1.262, p-value = 0.619. This means that the trend observed in the whole group is probably led by the effect of TC on CV events in the CKD group, as this subgroup demonstrates significant reverse relationship with CV events, while the MHD subgroup does not. However, the fact that the OR in the MHD group is also below 1, and the lower limit of the CI is well below 1, means we cannot conclude that increase in TC leads to an increase in CV events in this subgroup.

For TG in the combined population, no significant impact was seen on CV events, with an odds ratio (OR[TG]) of 0.897 with the 95% confidence interval (CI) of OR between 0.717 and 1.123 and a p-value of 0.344.

When broken down for the two groups, in the CKD group, OR(TG) = 0.921 with 95% CI of 0.679 and 1.248, p-value = 0.594, and in the MHD group, OR(TG) = 0.952 with 95% CI of 0.667 and 1.357, p-value = 0.784. The fact that the OR below 1 in both groups means that we cannot conclude that increase in TG leads to an increase in CV events these subgroups. In fact, TG seems to be mimicking the trend of TC in both groups, with stronger tendency toward being protective in the CKD group and less so but in the same direction in the MHD group. Figure 3.1.16 shows the distribution of TC and TG in CKD and MHD subgroups, categorised by whether they developed a CVE or not.
Figure 3.1.16 Box and whisker plot comparing TC and TG distribution between subjects who developed CVE and those who did not in the CKD (panel A) and MHD (panel B) subgroups. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.
To illustrate the effect of TC and TG on CVE risk, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). The subjects were classified as either above or below the median value for TC (4.00 mmol/l) and TG (1.53 mmol/l) in the cohort. Figure 3.1.17 demonstrates the hazard plots for CVE in regards to TC and TG. These plots could not be drawn for the CKD and MHD subgroups due to the much smaller numbers.
Figure 3.1.17 Cumulative hazard plot for CVE in the whole cohort over follow-up time in months stratified by TC cut off of 4.00 mmol/l (panel A) and TG cut off of 1.53 mmol/l (panel B). Subjects with higher TC have a significantly lower risk of CVE (p<0.001).
It is important to rule out the effect of lipid-lowering drugs, specifically statins, when looking at outcomes in relation to lipid levels. In the present study, to rule out the effects of statin therapy, the differences between the MHD and CKD groups in relation to statin use and lipid levels were analysed. The results showed similar TC and TG levels in those on statin therapy and not on statin therapy in both groups (Figure 3.1.18).
Figure 3.1.18 Box and whisker plot of TC (panel A) and TG (panel B) values in the CKD and MHD group in regards to statin use, where TC and TG values are expressed as mmol/l. Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers. Distribution is almost identical in the different subgroups.
Furthermore, the hazard ratio for CVE was plotted over time stratifying by presence or absence of statin therapy to look at its effect on the primary endpoint (figure 3.1.19). It is evident by looking at this plot that statin therapy does not have a significant effect on CVE in this cohort (Hazard Ratio: 1.145, 95%CI: 0.715, 1.834, p=0.574 for CVE).

**Figure 3.1.19** Cumulative hazard plot for CVE in the whole cohort over follow-up time in months stratified by statin use. There was no statistically significant difference between the two groups in regard to risk of CVE.
3.1.2.4 Diabetes. The risk associated with the presence of diabetes and CV events was significant, with an odds ratio (OR[DM]) of 1.622 with the 95% CI between 1.074 and 2.449 and a p-value of 0.021 in the whole cohort.

In the CKD group, OR(DM) = 1.488 with 95% CI of 0.792 and 2.795, p-value = 0.217, and in the MHD group, OR(DM) = 2.538 with 95% CI of 1.417 and 4.546, p-value = 0.002. This shows that in the MHD group, the presence of diabetes increases the risk of CV events significantly. Similarly, the CKD group results imply that the presence of diabetes increases the risk of CV events, although this finding does not reach statistical significance and the lower limit of the 95% CI is below 1, which means that this result is inconclusive.

To illustrate the effect of diabetes on CVE risk, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). Figure 3.1.20 demonstrates the hazard plot for CVE in regards to diabetic status. This plot could not be drawn for the CKD and MHD subgroups due to the much smaller numbers.

![Cumulative hazard plot for CVE in the whole cohort over follow-up time in months stratified by diabetes status. Subjects with diabetes have a significantly higher risk of CVE (p = 0.021).]

Figure 3.1.20 Cumulative hazard plot for CVE in the whole cohort over follow-up time in months stratified by diabetes status. Subjects with diabetes have a significantly higher risk of CVE (p = 0.021).
3.1.2.5 Summary of regression analyses for primary endpoint

Regression analyses were carried out to determine odds ratios (OR) for CVE in regard to the risk factors in question: BMI, blood pressure, total cholesterol, triglycerides and diabetes. In the combined cohort, high systolic BP had a significant effect in increasing the risk of CVE, which is similar to observations in the general population. The other significant result in this group was the effect of increasing TC in reducing the risk of CVE, which is in contrast to what is observed in the general population and can be described as reverse epidemiology. A summary of the regression analyses in the whole cohort is available in table 3.1.3 below.

<table>
<thead>
<tr>
<th>Combined Population Data</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.017</td>
<td>0.984, 1.050</td>
<td>0.318</td>
<td>-</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>1.011</td>
<td>1.002, 1.021</td>
<td>0.023*</td>
<td>Classic</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>1.004</td>
<td>0.989, 1.021</td>
<td>0.560</td>
<td>-</td>
</tr>
<tr>
<td>Total Cholesterol (TC)</td>
<td>0.681</td>
<td>0.550, 0.844</td>
<td>&lt;0.001**</td>
<td>Reverse</td>
</tr>
<tr>
<td>Triglycerides (TG)</td>
<td>0.897</td>
<td>0.717, 1.123</td>
<td>0.344</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.622</td>
<td>1.074, 2.795</td>
<td>0.021*</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.1.3 Summary of regression analyses results for primary endpoint (CVE) in the combined cohort, looking at the effects of all the factors studied.

In the CKD subgroup the only statistically significant finding was the effect of increasing TC in reducing the risk of CVE, which is again contrary to what is observed in the general population and can be described as reverse epidemiology. A summary of the regression analyses in the CKD subgroup is available in table 3.1.4.
Table 3.1.4 Summary of regression analyses results for primary endpoint (CVE) in the CKD subgroup, looking at the effects of all the factors studied.

In the MHD subgroup the only statistically significant finding was the effect of presence of diabetes which significantly increases the risk of CVE. This is similar to observations in the general population, and can therefore be described as classic epidemiology. A summary of the regression analyses in the MHD subgroup is available in table 3.1.5 below.

Table 3.1.5 Summary of regression analyses results for primary endpoint (CVE) in the MHD subgroup, looking at the effects of all the factors studied.
3.1.3 Analysis of Secondary Endpoint

Mortality was defined as our secondary, or perhaps better to say exploratory, endpoint. Date of death as well as cause of death data was gathered for subjects over the two year follow up period and risk analysis was carried out in regard to each of the predetermined risk factors. Again, these analyses were carried out for the whole population as well as separately for CKD and MHD groups. The results in this section are described in that specific order: whole cohort, CKD group and then MHD group and are expressed as odds ratio (OR), 95% confidence interval (CI) and p-values.

Over the course of the 24-month follow up period, a total of 59 subjects reached a primary endpoint, bringing the death rate to 11.4%. Of these, 21 subjects were in the CKD group and 38 subjects were in the MHD group, meaning the death rate in the CKD group was 6.8% while in the MHD group this was at 18.1% (see figure 3.1.11).

Results for each risk factor were calculated with and without adjusting for the confounding effect of pre-existing cardiovascular disease, but as this had negligible effect on the results, the adjusted results are not mentioned here.
3.1.3.1 **Body Mass.** The mortality risk associated with increased BMI was calculated for the whole cohort using logistic model. This did not show a significant impact for BMI on mortality, with an odds ratio (OR[BMI]) of 0.982 with the 95% confidence interval (CI) of HR between 0.937 and 1.028 and a p-value of 0.428 in the combined cohort.

In the CKD group, OR(BMI) = 1.028 with 95% CI of 0.955 and 1.105, p-value = 0.465, and in the MHD group, OR(BMI) = 0.994 with 95% CI of 0.934 and 1.059, p-value = 0.856. As evident, BMI did not reach significance in predicting mortality in either group, and because the OR for the MHD group is below 1 while the OR for the CKD group is above 1, we cannot conclude that the relationship between BMI and mortality in the two groups is in a similar direction. Figure 3.1.21 shows the distribution of BMI in CKD and MHD subgroups, categorised by whether they developed a CVE or not.

*Figure 3.1.21 Box and whisker plot comparing BMI distribution between subjects who died and those who did not by subgroup. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.*
To illustrate the effect of BMI on risk of mortality, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). The subjects were classified as either overweight or not overweight, using the cut off point of 25 kg/m$^2$. Figure 3.1.22 demonstrates the lack of effect of BMI on risk of mortality. This plot could not be drawn for the CKD and MHD subgroups due to the much smaller numbers.

Figure 3.1.22 Cumulative hazard plot for mortality in the whole cohort over time stratified by BMI above or below 25 kg/m$^2$. There is no statistically significant difference between the two groups.
3.1.3.2 Blood Pressure. Both systolic and diastolic blood pressure were analysed to calculate the risk associated with each in regards to mortality.

For systolic BP in the whole cohort, the calculations showed an odds ratio (OR[SBP]) of 1.010 with the 95% confidence interval (CI) of OR between 0.997 and 1.023 and a p-value of 0.146. This shows no significant impact of systolic BP on mortality in the cohort.

When broken down for the CKD and MHD groups, however, systolic BP gains significant association with mortality in the CKD group, with OR(SBP) = 0.958 and 95% CI of 0.926 and 0.990, p-value = 0.011, in the direction opposite that of the general population. In the MHD group, OR(SBP) = 1.013 with 95% CI of 0.998 and 1.029, p-value = 0.095, and although this result does not reach significance, its importance is in its direction, which is opposite that of the CKD group and therefore in line with that of the general population.

For diastolic BP in the whole cohort, no significant impact was seen on mortality, with an OR(DBP) of 0.985 with the 95% confidence interval (CI) of OR between 0.962 and 1.009 and a p-value of 0.230.

In the CKD group, OR(DBP) = 0.929 with 95% CI of 0.879 and 0.982, p-value = 0.010, which is reflecting the results of systolic BP in this group. In the MHD group, OR(DBP) = 0.986 with 95% CI of 0.958 and 1.014, p-value = 0.327, this result does not reach significance (as for systolic BP in this group), but of note is that its direction is not similar to that of systolic BP in this group. Figure 3.1.23 shows the distribution of SBP and DBP in CKD and MHD subgroups, categorised by whether they reached a secondary endpoint or not.
Figure 3.1.23  Box and whisker plot comparing SBP and DBP distribution between subjects who died and those who did not in the CKD (panel A) and MHD (panel B) subgroups. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.
To illustrate the effect of SBP and DBP on risk of mortality, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). The subjects were classified as either hypertensive or not, with 140 mmHg as the upper limit of normal for SBP and 90 mmHg as the upper limit for DBP. Figure 3.1.24 demonstrates the hazard plots for death in regards to SBP and DBP. These plots could not be drawn for the CKD and MHD subgroups due to the much smaller numbers.
Figure 3.1.24 Cumulative hazard plot for mortality in the whole cohort over follow-up time in months stratified by SBP cut off of 140.0 mmHg (panel A) and DBP cut off of 90.0 mmHg (panel B). There is no statistically significant difference between the two groups for either factor.
3.1.3.3 Lipids. Both total cholesterol (TC) and triglycerides (TG) were analysed to calculate the risk associated with each in regards to mortality.

For TC in the combined cohort, significant impact was seen on mortality, with an OR(TC) of 0.635 with the 95% confidence interval (CI) of OR between 0.466 and 0.864 and a p-value of 0.004. This suggests that increase in TC reduces the risk of mortality, which is similar to the finding in regard to CV risk, and contrary to what is seen in the general population.

In the CKD group, OR(TC) = 0.500 with 95% CI of 0.284 and 0.880, p-value = 0.016, and in the MHD group, OR(TC) = 0.864 with 95% CI of 0.576 and 1.296, p-value = 0.480. This shows that what we see in the whole group is probably being led by the CKD group, as this group shows significant lowering of mortality risk with increased TC, while the MHD group does not follow this trend.

For TG in the combined cohort, a significant impact was seen on mortality, with an odds ratio (OR[TG]) of 0.504 with the 95% confidence interval (CI) of OR between 0.322 and 0.788 and a p-value of 0.003. This means that increased TG are associated with lower risk of death in our study population.

When TG is broken down in the two groups, in the CKD group, OR(TG) = 0.468 with 95% CI of 0.219 and 0.998, p-value = 0.049, and in the MHD group, OR(TG) = 0.546 with 95% CI of 0.310 and 0.964, p-value = 0.037. As evident from these results, increased TG in both groups leads to a decreased risk of mortality. Figure 3.1.25 shows the distribution of TC and TG in CKD and MHD subgroups, categorised by whether they developed a secondary endpoint or not.
Figure 3.1.25 Box and whisker plot comparing TC and TG distribution between subjects who died and those who did not in the CKD (panel A) and MHD (panel B) subgroups. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.
To illustrate the effect of TC and TG on risk of mortality, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). The subjects were classified as either above or below the median value for TC (4.00 mmol/l) and TG (1.53 mmol/l) in the cohort. Figure 3.1.26 demonstrates the hazard plots for CVE in regards to TC and TG. These plots could not be drawn for the CKD and MHD subgroups due to the much smaller numbers.
Figure 3.1.26 Cumulative hazard plot for mortality in the whole cohort over follow-up time in months stratified by TC cut off of 4.00 mmol/l (panel A) and TG cut off of 1.53 mmol/l (panel B). Subjects with higher TC have significantly lower risk of death (p=0.004) and subjects with higher TG also have a lower risk of death (p=0.003).
Furthermore, the hazard ratio for death was plotted over time stratifying by presence or absence of statin therapy to look at its effect on the secondary endpoint (figure 3.1.27). It is evident by looking at this plot that statin therapy does not have a significant effect on CVE in this cohort (Hazard Ratio: 1.540, 95%CI: 0.780, 3.041, p=0.213 for mortality).

**Figure 3.1.27** Cumulative hazard plot for mortality in the whole cohort over follow-up time in months stratified by statin use. There is no statistically significant difference between the two groups in regard to risk of death.
3.1.3.4 Diabetes. The risk associated with the presence of diabetes and mortality was significant, with an OR(DM) of 1.869 with the 95% CI between 1.047 and 3.334 and a p-value of 0.034.

For diabetes, in the CKD group OR(DM) = 3.846 with 95% CI of 1.101 and 13.433, p-value = 0.035, and in the MHD group OR(DM) = 2.032 with 95% CI of 0.997 and 4.146, p-value = 0.051. This shows that while in the CKD group, the presence of diabetes increases the risk of mortality significantly, the MHD group results lose statistical significance and fall just below the cut-off point, implying that the presence of diabetes does increase the risk of mortality in this group, as well.

To illustrate the effect of diabetes on risk of death, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). Figure 3.1.28 demonstrates the hazard plot for mortality in regards to diabetic status. This plot could not be drawn for the CKD and MHD subgroups due to the much smaller numbers.

![Figure 3.1.28](cumulative_hazard_plot.png)

**Figure 3.1.28** Cumulative hazard plot for mortality in the whole cohort over follow-up time in months stratified by diabetes status. Subjects with diabetes have a significantly higher risk of death (p = 0.034).
3.1.3.5 Summary of regression analyses for secondary endpoint

Regression analyses were carried out to determine odds ratios (OR) for mortality in regard to the risk factors in question: BMI, blood pressure, total cholesterol, triglycerides and diabetes. In the combined cohort, high TC had a significant effect in reducing the risk of death, which is in contrast to what is observed in the general population and can be described as reverse epidemiology. The same situation applied to TG, where its increase resulted significant lowering of risk of death. The other significant result in this group was the effect of presence of diabetes in increasing the risk of death, which is in similar to what is observed in the general population and can be described as classic epidemiology. A summary of the regression analyses in the whole cohort is available in table 3.1.6 below.

<table>
<thead>
<tr>
<th>Combined Population Data</th>
<th>Odds Ratio (OR)</th>
<th>95 % Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.989</td>
<td>0.937, 1.028</td>
<td>0.428</td>
<td>-</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>1.010</td>
<td>0.997, 1.023</td>
<td>0.146</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.985</td>
<td>0.962, 1.009</td>
<td>0.230</td>
<td>-</td>
</tr>
<tr>
<td>Total Cholesterol (TC)</td>
<td>0.635</td>
<td>0.466, 0.864</td>
<td>0.004**</td>
<td>Reverse</td>
</tr>
<tr>
<td>Triglycerides (TG)</td>
<td>0.504</td>
<td>0.322, 0.788</td>
<td>0.003**</td>
<td>Reverse</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.869</td>
<td>1.047, 3.334</td>
<td>0.034*</td>
<td>Classic</td>
</tr>
</tbody>
</table>

Table 3.1.6 Summary of regression analyses results for secondary endpoint (death) in the combined cohort, looking at the effects of all the factors studied.

In the CKD subgroup, both high systolic and diastolic BP had a significant effect in decreasing the risk of death, which is in contrast to observations in the general population, and therefore can be described as reverse epidemiology. Increasing TC and TG also both decrease the risk of death in this group, which is also in contrast to what is observed in the general population and can be described as reverse epidemiology. The final significant result in the CKD group was the effect of presence of diabetes which increased the risk of death, which is similar to what is
observed in the general population and can be described as classic epidemiology. A summary of the regression analyses in the CKD subgroup is available in table 3.1.7.

<table>
<thead>
<tr>
<th>CKD group (non-MHD)</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.028</td>
<td>0.955, 1.105</td>
<td>0.465</td>
<td>-</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.958</td>
<td>0.926, 0.990</td>
<td>0.001**</td>
<td>Reverse</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.929</td>
<td>0.879, 0.982</td>
<td>0.010**</td>
<td>Reverse</td>
</tr>
<tr>
<td>Total Cholesterol (TC)</td>
<td>0.500</td>
<td>0.284, 0.880</td>
<td>0.016*</td>
<td>Reverse</td>
</tr>
<tr>
<td>Triglycerides (TG)</td>
<td>0.468</td>
<td>0.219, 0.998</td>
<td>0.049*</td>
<td>Reverse</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.846</td>
<td>1.101, 13.433</td>
<td>0.035*</td>
<td>Classic</td>
</tr>
</tbody>
</table>

*Table 3.1.7* Summary of regression analyses results for secondary endpoint (death) in the CKD subgroup, looking at the effects of all the factors studied.

In the MHD subgroup, increasing TG resulted lowering of risk of mortality, which is contrary to what is observed in the general population, and therefore can be described as reverse epidemiology. The result for this effect was not statistically significant for TC in the MHD group. The other statistically significant finding in the MHD group was the effect of presence of diabetes which significantly increased the risk of death. This is similar to observations in the general population, and can therefore be described as classic epidemiology. A summary of the regression analyses in the MHD subgroup is available in table 3.1.8.
<table>
<thead>
<tr>
<th>MHD group</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.994</td>
<td>0.934, 1.059</td>
<td>0.856</td>
<td>-</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>1.013</td>
<td>0.998, 1.024</td>
<td>0.095</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.986</td>
<td>0.958, 1.014</td>
<td>0.327</td>
<td>-</td>
</tr>
<tr>
<td>Total Cholesterol (TC)</td>
<td>0.864</td>
<td>0.576, 1.296</td>
<td>0.480</td>
<td>-</td>
</tr>
<tr>
<td>Triglycerides (TG)</td>
<td>0.546</td>
<td>0.310, 0.964</td>
<td>0.037*</td>
<td>Reverse</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.032</td>
<td>0.997, 4.146</td>
<td>0.051(*)</td>
<td>Trend: Classic</td>
</tr>
</tbody>
</table>

**Table 3.1.8** Summary of regression analyses results for secondary endpoint (death) in the MHD subgroup, looking at the effects of all the factors studied.
3.1.3.6 Other risk factors

*Ethnicity.* The ethnic diversity of the present cohort allows for detailed analysis of the effects of ethnicity on both primary and secondary endpoints. Cox regression analysis for effects of ethnicity on CVE shows the ‘Black’ ethnic group to have the highest risk of developing CVEs (Hazard Ratio=6.154, 95%CI: 2.141, 17.684, p=0.001), while the ‘White’ ethnic group has the highest risk for mortality (Hazard Ratio=10.558, 95%CI: 1.388, 80.314, p=0.023) (figure 3.1.29).

It is interesting that in this population, the White ethnic group has the lowest risk of CVE but the highest risk of mortality, and this is almost completely opposite the Black ethnic group, who have the highest risk for CVE but the lowest risk for mortality (after Mixed and Unknown ethnic groups).
Figure 3.1.29 Cumulative hazard ratio for CVE (panel A) and mortality (panel B) in the whole cohort over follow-up time in months stratified by ethnicity; statistically significant HRs for CVE are: White: HR=5.075, 95%CI:1.813,14.205, p=0.002, Black: HR=6.154, 95%CI: 2.141, 17.684, p=0.001, Asian: HR=5.220, 95%CI: 1.798, 15.151, p=0.002, Other: HR=5.307, 95%CI: 1.634, 17.235, p=0.005 and the only statistically significant HRs for mortality are for the White group: HR=9.135, 95%CI: 2.191, 38.080, p=0.002 and for the Other group: HR=9.074, 95%CI: 2.105, 39.112, p=0.003.
When the population is broken down into the subgroups, regression analyses show that the risk of CVE is higher among the Black ethnic group in comparison to the White ethnic group in both the CKD and MHD groups. Figure 3.1.30 illustrates risk of CVE stratified by ethnicity (significant hazard ratios available in the figure legend).
Figure 3.1.30 Cumulative hazard ratio for CVE in the CKD (panel A) and MHD (panel B) subgroups over follow-up time in months stratified by ethnicity; statistically significant HRs for the CKD group are: White: HR=5.672, 95%CI:1.702, 18.890, p=0.005, Black: HR=6.419, 95%CI: 1.766, 23.328, p=0.005, and there are no statistically significant HRs for the MHD group.
White ethnicity has the highest risk of mortality in both CKD and MHD groups, although the risk is greater in the MHD group, and Black ethnicity has much lower risk in both groups in comparison. Figure 3.1.31 illustrates risk of mortality stratified by ethnicity (significant hazard ratios available in the figure legend).
Figure 3.1.31 Cumulative hazard ratio for mortality in the CKD (panel A) and MHD (panel B) subgroups over follow-up time in months stratified by ethnicity; the statistically significant HR for the CKD group is the White group: HR=10.558, 95%CI:1.388, 80.314, p=0.023 and there are no statistically significant hazard ratios for mortality in the MHD group.
3.2 Sub-cohort Study: Insulin Resistance and Inflammation in ESRD

A subgroup of the cohort (dubbed ‘the sub-cohort’) was recruited and asked to donate blood samples for quantification of insulin resistance, inflammation and total adiponectin. 106 subjects were recruited, 56 from the CKD subgroup and 50 from the MHD subgroup. While insulin resistance was quantified by the HOMA-IR model in all of these subjects, hs-CRP and total adiponectin levels were only available for 92 of the subjects, 47 of which belonged to the CKD subgroup and 44 were from the MHD subgroup.

3.2.1 Distributions and comparison of subgroups

3.2.1.1 Insulin Resistance (HOMA-IR). Insulin resistance was quantified in a subgroup of patients using the HOMA model (explained in section 1.2.1.6). The aim was to measure insulin resistance in 50 CKD patients, 50 MHD patients and 10 patients about to start MHD, where the latter group would be re-measured after 8 weeks into HD.

HOMA-IR was measured for 106 subjects in total. The overall mean ± SD (range) HOMA-IR was 2.08 ± 2.02 (0.40 – 11.50); 56 subjects were in the CKD group where the overall mean ± SD (range) HOMA-IR was 2.63 ± 2.31 (0.40 – 11.50), and 50 subjects were in the MHD group whose overall mean ± SD (range) HOMA-IR was 1.50 ± 1.46 (0.40 – 6.60). Comparison of mean HOMA-IR between CKD and MHD groups was significant, with p = 0.004. HOMA-IR values were also compared between subjects with and without diabetes. In the diabetic group, mean ± SD HOMA-IR was 2.94 ± 2.34 and in the non-diabetic group mean ± SD HOMA-IR was 1.22 ± 1.13. Comparison of mean HOMA-IR between the two groups was significant, with p < 0.001. Comparison of mean HOMA-IR between the different CKD stages, where MHD is classified as the final stage, showed mean HOMA-IR ± SD for CKD 3 at 1.78 ± 1.30, for CKD 4 at 2.52 ± 2.73 and for CKD 5 at 3.57 ± 2.33. Comparison of means (by Kruskal-Wallis test, as data not normally distributed in the smaller subgroups) is statistically significant in these subgroups, p=0.001 (figure 3.2.1 and supplementary table 7 available in appendix III).
A)

B)
Figure 3.2.1 Box and whisker plot comparing HOMA-IR value distribution. Panel A compares HOMA-IR in CKD and MHD groups (p=0.004) and panel B compares HOMA-IR between subjects with and without diabetes (p<0.001) and panel C compares HOMA-IR between different CKD stages and MHD (p=0.001). Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers. Horizontal bars indicate comparison of means between groups and ** indicates statistically significant finding.
3.2.1.2 Inflammation (hs-CRP). High sensitivity C-reactive protein (hsCRP) was used to quantify inflammation for 92 subjects in total. Of these, 47 subjects were classified into the CKD subgroup and 44 into the MHD subgroup.

The mean value ± SD for hs-CRP in the whole sub-cohort was 8.92 ± 18.95 mg/l with a range of 0.17 mg/l to 157.13 mg/l. In the CKD subgroup within the sub-cohort, the mean value ± SD for hs-CRP was 5.62 ± 11.35 mg/l with a range of 0.19 mg/l to 72.47 mg/l, while in the MHD subgroup of the sub-cohort it was 12.64 ± 24.37 mg/l with a range of 0.37 mg/l to 157.13 mg/l. The difference between the CKD and MHD groups within the sub-cohort was not statistically significant, with p = 0.103. Comparison of mean hs-CRP between the different CKD stages, where MHD is classified as the final stage, showed mean hs-CRP ± SD for CKD 3 at 7.97 ± 17.57 mg/l, for CKD 4 at 5.43 ± 7.62 mg/l and for CKD 5 at 2.98 ± 3.28 mg/l. Comparison of means is statistically significant in these subgroups, p=0.003 (figure 3.2.2 and supplementary table 8 available in appendix III). As hs-CRP was not normally distributed in this sub-cohort (Shapiro-Wilk test, p<0.001), the Kruskal Wallis non-parametric test was used to compare means between subgroups (instead of the ANOVA, which assumes normality). For comparison between the larger subgroups, the student's t-test was used [466].
Figure 3.2.2 Box and whisker plot comparing hs-CRP value distribution. Panel A compares hs-CRP in CKD and MHD groups (p=0.103) and panel B compares hs-CRP between different CKD stages and MHD (p=0.003). Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers. Horizontal bars indicate comparison of means between groups and ** indicates statistically significant finding.
3.2.1.3 Total Adiponectin. Serum total adiponectin was measured for 92 subjects in total. Of these, 47 subjects were classified into the CKD subgroup and 44 into the MHD subgroup.

The mean value ± SD for total adiponectin in the whole sub-cohort was 46.56 ± 25.73 ug/ml with a range of 9.3 ug/ml to 150.00 ug/ml. The CKD group within the sub-cohort had a mean ± SD of 47.92 ± 25.78 ug/ml with a range of 10.80 ug/ml to 149.10 ug/ml, while the MHD group within the sub-cohort had a mean ± SD of 45.08 ± 26.20 ug/ml with a range of 9.30 ug/ml to 150.00 ug/ml. The difference between the CKD and MHD groups within the sub-cohort was not statistically significant, with p = 0.861. Comparison of mean total adiponectin between the different CKD stages, where MHD is classified as the final stage, showed mean total adiponectin ± SD for CKD 3 at 39.67 ± 32.54 ug/ml, for CKD 4 at 50.81 ± 21.48 ug/ml and for CKD 5 at 54.05 ± 20.64 ug/ml. Comparison of means is between the CKD stages did not reveal statistically significant difference when the MHD group was included as the final stage of CKD (p = 0.060), but the difference was statistically significant when the comparison was made only between CKD stage 3 to 5, excluding MHD (p = 0.041) (figure 3.2.3 and supplementary table 9 available in appendix III).
Figure 3.2.3 Box and whisker plot comparing total adiponectin value distribution. Panel A compares total adiponectin in CKD and MHD groups ($p=0.861$) and panel B compares total adiponectin between different CKD stages ($p=0.041$) as well as with MHD ($p=0.060$). Box and whiskers indicate median and interquartile range, while outliers are shown with corresponding identification numbers. Horizontal bars indicate comparison of means between groups and ** indicates statistically significant finding.
3.2.1.4 Summary of group comparisons

Comparison of mean HOMA-IR, hs-CRP and total adiponectin between the CKD and MHD groups within the sub-cohort revealed that the MHD group has significantly lower HOMA-IR, and other factors did not differ significantly between the two groups (table 3.2.1).

<table>
<thead>
<tr>
<th>Potential Risk Factor</th>
<th>Comparison of means in subgroups</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>CKD &gt; MHD</td>
<td>p=0.004</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>CKD ~ MHD</td>
<td>p=0.103</td>
</tr>
<tr>
<td>Total Adiponectin</td>
<td>CKD ~ MHD</td>
<td>p=0.861</td>
</tr>
</tbody>
</table>

Table 3.2.1 Summary of comparison of mean values for each potential risk factor in the two subgroups of CKD and MHD within the sub-cohort. ‘>’ indicates higher mean values in the CKD subgroup while ‘<’ indicates higher mean values in the MHD population, and ‘~’ indicates no statistically significant difference.
3.2.2 Analysis of Primary Endpoint

The follow-up data gathered for the cohort study was used for the subjects in the sub-cohort study to determine the effect of HOMA-IR, hs-CRP and total adiponectin on the primary endpoint, predefined cardiovascular events. These events included: myocardial infarction, aortocoronary bypass, percutaneous transluminal coronary angioplasty, angiographically verified stenosis of the coronary arteries, stroke, or a symptomatic stenosis of the peripheral arterial vessels (carotids, aorto-iliac, or femoral arteries), admission to hospital with a primary diagnosis of ‘chronic ischaemic heart disease’ or any other pre-existing condition that exasperated during the monitoring period.

These analyses were carried out for the whole sub-cohort as well as separately for CKD and MHD groups within the sub-cohort. The results in this section are described in the same order as in section 3.1.2 (risk analysis of primary endpoints in the entire population studied): whole sub-cohort, CKD subgroup and then MHD subgroup and are expressed as odds ratio (OR), 95% confidence interval (CI) and p-values. As mentioned in section 2.4, hazard ratios were also calculated for each risk factor using Cox regression analysis, and the results were in agreement with and very similar to logistic regression results (odds ratios). Although logistic regression was ultimately chosen for data presentation as it was statistically better suited to this population, calculated hazard ratios were used to draw the cumulative hazard plots presented in this section.

Over the course of the 24-month follow up period, a total of 23 subjects (out of 106) reached a primary endpoint in the sub-cohort, bringing the event rate to 21.6%. Of these, 12 subjects were in the CKD subgroup (out of a total 55 subjects in this group) and 11 subjects were in the MHD subgroup (out of the total 51 subjects in this group), meaning the CV event rate was almost identical between these two subgroups, with the CKD group event rate at 21.8% and the MHD group event rate at 21.6% (figure 3.2.4).
Figure 3.2.4 Follow-up data for each group within the sub-cohort. In the combined sub-cohort (n=106) 23 (21.6%) subjects had a CVE (primary endpoint) and 6 (5.6%) subjects died (secondary endpoints). The CKD subgroup (n=55) had 12 (21.8%) CVEs and 3 (5.5%) deaths, while the MHD subgroup (n=51) had 11 (21.6%) CVEs and 3 (5.9%) deaths.
3.2.2.1 HOMA-IR. The odds ratio (OR) for cardiovascular event in regard to HOMA-IR in the sub-cohort was calculated at 1.171, with a 95% CI of 0.937 and 1.464, and a p-value of 0.165; this result is not significant and we cannot conclude whether an increase in HOMA-IR value causes an increase or decrease in the risk of suffering a CV event.

In the CKD group within the sub-cohort, the odds ratio (OR) for cardiovascular events in regard to HOMA-IR was 1.315, with a 95% CI of 0.963 and 1.796 and a p-value of 0.085; although statistically insignificant, this result suggests that an increase in HOMA-IR value may cause an increase in the risk of suffering a CV event. The odds ratio (OR) for CVE in regard to HOMA-IR in the MHD group within the sub-cohort was 0.996, with a 95% CI of 0.655 and 1.513 and a p-value of 0.986; this result is inconclusive on whether an increase in HOMA-IR value causes an increase or a decrease in the risk of suffering a CV event.

Figure 3.2.5 shows the distribution of HOMA-IR in sub-cohort, categorised by whether they developed a CVE or not.
Figure 3.2.5 Box and whisker plot comparing HOMA-IR distribution between subjects who developed CVE and those who did not by subgroup. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.

To illustrate the effect of HOMA-IR on CVE risk, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). The subjects were classified as either above or below the cut off point of 1.35, which is the median for HOMA-IR in the sub-cohort. Figure 3.2.6 demonstrates the lack of effect of HOMA-IR on CVE risk in the whole sub-cohort, as well as in the CKD and MHD groups within the sub-cohort.
Figure 3.2.6 Cumulative hazard plot for CVE the whole sub-cohort (panel A), the CKD subgroup (panel B) and MHD subgroup (panel C) stratified by HOMA-IR level above or below the cut-off point (median value for the whole sub-cohort=1.35). There are no statistically significant differences between any of the groups.
3.2.2.2 hs-CRP. As the values for hs-CRP were not normally distributed, the log transformation of the value was used to look at the effect of hs-CRP value on endpoints. Normality was determined by using the Shapiro-Wilk test.

The odds ratio (OR) for cardiovascular event in regard to log-hsCRP in the sub-cohort was 1.485, with a 95% CI of 0.982 and 2.246, and a p-value of 0.061; although this result is not significant the trend suggests that an increase in hs-CRP value may increase the risk of CV events. In the CKD group within the sub-cohort, the odds ratio (OR) for cardiovascular event in regard to log-hsCRP was 2.008 with a 95% CI of 1.005 and 4.010 with a p-value of 0.048; this is a borderline statistically significant result that suggests there may be an increased risk of CV events in subjects with increased hs-CRP levels. The odds ratio (OR) for cardiovascular event in regard to log-hsCRP in the MHD group within the sub-cohort was 1.273, with a 95% CI of 0.717 and 2.262 with a p-value of 0.409; this statistically insignificant result means that an increase in hs-CRP value cannot be associated with either an increase or a decrease in the risk of suffering a CV event.

Figure 3.2.7 shows the distribution of hs-CRP in the sub-cohort, categorised by whether subjects developed a CVE or not.
Figure 3.2.7 Box and whisker plot comparing hs-CRP distribution between subjects who developed CVE and those who did not by subgroup. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.

To illustrate the effect of hs-CRP on CVE risk, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). The subjects were classified as either above or below the cut off point of 4.27 mg/l, which is the median for hs-CRP in the sub-cohort. Figure 3.2.8 demonstrates the lack of effect of hs-CRP on CVE risk in the whole sub-cohort and the MHD subgroup, and also the presence of an effect in the CKD subgroup.
Figure 3.2.8 Cumulative hazard plot for CVE in the whole sub-cohort (panel A), the CKD subgroup (panel B) and MHD subgroup (panel C) stratified by hs-CRP level above or below the cut-off point (median value for the whole sub-cohort=4.27 mg/dl). Subjects with higher hs-CRP have significantly higher risk of CVE in the CKD subgroup (p=0.048). Other differences are not significant.
3.2.2.3 Total Adiponectin. The odds ratio (OR) for cardiovascular event in regard to total adiponectin in the sub-cohort was 0.982, with a 95% CI of 0.959 and 1.005, with a p-value of 0.118; this result is not significant and we cannot conclude whether an increase in total adiponectin causes an increase or decrease in the risk of suffering a CV event. In the CKD group within the sub-cohort, the odds ratio (OR) for cardiovascular event in regard to total adiponectin was 0.979, with a 95% CI of 0.944 and 1.014 with a p-value of 0.240. As this result is statistically insignificant, it suggests no association between increased total adiponectin levels and CV events. The odds ratio (OR) for cardiovascular event in regard to total adiponectin in the MHD group within the sub-cohort was 0.984, with a 95% CI of 0.954 and 1.014 with a p-value of 0.292; this means that an increase in total adiponectin value cannot be associated with either an increase or a decrease in the risk of suffering a CV event.

Figure 3.2.9 shows the distribution of total adiponectin in the sub-cohort, categorised by whether subjects developed a CVE or not.
To illustrate the effect of total adiponectin on CVE risk, the cumulative hazard was plotted using the Cox regression calculations (available in appendix II). The subjects were classified as either above or below the cut off point of 45.65 ug/ml, which is the median for total adiponectin in the sub-cohort. Figure 3.2.10 demonstrates the lack of effect of total adiponectin on CVE risk in the whole sub-cohort, as well as in the CKD and MHD groups within the sub-cohort.
Figure 3.2.10 Cumulative hazard plot for CVE the whole sub-cohort (panel A), the CKD subgroup (panel B) and MHD subgroup (panel C) stratified by total adiponectin level above or below the cut-off point (median value for the whole sub-cohort=45.65 ug/mL). There are no statistically significant differences between any of the groups.
3.2.2.4 Summary of regression analyses for primary endpoint

Regression analyses were carried out to determine odds ratios (OR) for CVE in regard to the potential risk factors in question: HOMA-IR, hs-CRP and total adiponectin. In the whole sub-cohort, none of the factors studied had a significant effect on CVE. A summary of the regression analyses in the whole sub-cohort is available in table 3.2.2 below.

<table>
<thead>
<tr>
<th>Combined Population Data</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA2-IR</td>
<td>1.171</td>
<td>0.937, 1.464</td>
<td>0.165</td>
<td>-</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.982</td>
<td>0.959, 1.005</td>
<td>0.118</td>
<td>-</td>
</tr>
<tr>
<td>Log hs-CRP</td>
<td>1.485</td>
<td>0.982, 2.246</td>
<td>0.061</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.2.2 Summary of regression analyses results for primary endpoint (CVE) in the combined group, looking at the effects of the factors studied.

In the CKD group within the sub-cohort, the only statistically significant finding was the effect of increasing inflammation as quantified by log hs-CRP in increasing the risk of CVE, which is similar to what is observed in the general population and can be described as classic epidemiology. A summary of the regression analyses in the CKD group is available in table 3.2.3 below.

<table>
<thead>
<tr>
<th>CKD Group (non-MHD)</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA2-IR</td>
<td>1.315</td>
<td>0.963, 1.796</td>
<td>0.085</td>
<td>-</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.979</td>
<td>0.944, 1.014</td>
<td>0.240</td>
<td>-</td>
</tr>
<tr>
<td>Log hs-CRP</td>
<td>2.008</td>
<td>1.005, 4.010</td>
<td>0.048*</td>
<td>Classic</td>
</tr>
</tbody>
</table>

Table 3.2.3 Summary of regression analyses results for primary endpoint (CVE) in the CKD subgroup, looking at the effects of the factors studied.
In the MHD group within the sub-cohort, none of the factors studied had a significant effect on CVE. A summary of the regression analyses in the whole cohort is available in table 3.2.4 below.

<table>
<thead>
<tr>
<th>MHD Group</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA2-IR</td>
<td>0.996</td>
<td>0.655, 1.513</td>
<td>0.986</td>
<td>-</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.984</td>
<td>0.954, 1.014</td>
<td>0.292</td>
<td>-</td>
</tr>
<tr>
<td>Log hs-CRP</td>
<td>1.273</td>
<td>0.717, 2.262</td>
<td>0.409</td>
<td>-</td>
</tr>
</tbody>
</table>

*Table 3.2.4* Summary of regression analyses results for primary endpoint (CVE) in the MHD subgroup, looking at the effects of the factors studied.
3.2.3 Analysis of Secondary Endpoint

The follow-up data gathered for the cohort study was used for the subjects in the sub-cohort study to determine the effect of HOMA-IR, hs-CRP and total adiponectin on the secondary endpoint, death. These analyses were carried out for the whole sub-cohort as well as separately for CKD and MHD groups within the sub-cohort. The results in this section are described in the same order as in section 3.1.3 (risk analysis of secondary endpoints in the entire population studied): whole sub-cohort, CKD subgroup and then MHD subgroup and are expressed as odds ratio (OR), 95% confidence interval (CI) and p-values. As mentioned in section 2.4, hazard ratios were also calculated for each risk factor using Cox regression analysis, and the results were in agreement with and very similar to logistic regression results (odds ratios). Number of deaths was too small in the sub-cohort to draw the hazard plots for mortality.

Over the course of the 24-month follow up period, a total of 6 subjects (out of 106) reached a primary endpoint in the sub-cohort, bringing the death rate to 5.6%. Of these, 3 subjects were in the CKD subgroup (out of a total 55 subjects in this group) and 3 subjects were in the MHD subgroup (out of the total 51 subjects in this group), meaning the death rate was almost identical between these two subgroups, with the CKD group death rate at 5.5% and the MHD group event rate at 5.9% (figure 3.2.4).
3.2.3.1 HOMA-IR. The odds ratio (OR) for mortality in regard to HOMA-IR in the sub-cohort was calculated at 1.146 with a 95% CI of 0.823 and 1.597 and a p-value of 0.419, which is inconclusive in regards to its effect on mortality. In the CKD group within the sub-cohort, the OR for mortality in regards to HOMA-IR is 1.415 with a 95% CI of 0.909 and 2.201 and a p-value of 0.123, which is also not statistically significant but the trend suggests that an increase in HOMA-IR increases the risk of death in this group. In the MHD group within the sub-cohort, the OR is 0.866 with a 95% CI of 0.374 and 2.001 and a p-value of 0.736, which is also inconclusive of HOMA-IR's effect on mortality in the MHD subgroup.

Figure 3.2.11 shows the distribution of HOMA-IR in the sub-cohort, categorised by whether they subjects died or not.

![Box and whisker plot comparing HOMA-IR distribution between subjects who died and those who did not by subgroup. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.](image)

**Figure 3.2.11** Box and whisker plot comparing HOMA-IR distribution between subjects who died and those who did not by subgroup. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.
3.2.3.2 *hs-CRP*. As the values for hs-CRP were not normally distributed, the log transformation of the value was used to look at the effect of hs-CRP value on endpoints. Normality was determined by using the Shapiro-Wilk test.

As for the effect of log-hsCRP on mortality, the OR was 1.032 with a 95% CI of 1.000 and 1.064 and a p-value of 0.051, which is statistically insignificant, but the strong trend again suggests that greater hs-CRP levels are associated with an increased risk of death in the sub-cohort. In the CKD group within the sub-cohort, the OR for effect of log-hsCRP on mortality is 1.025 with a 95% CI of 0.936 and 1.123 with a p-value of 0.592. In the MHD group within the sub-cohort, the OR is 1.797 with a 95% CI of 0.715 and 4.518 with a p-value of 0.213. These results are statistically insignificant in both subgroups, which mean that hs-CRP level is not associated with risk of death in either group.

Figure 3.2.12 shows the distribution of hs-CRP in the sub-cohort, categorised by whether they subjects died or not.

![Figure 3.2.12](image)

*Figure 3.2.12* Box and whisker plot comparing hs-CRP distribution between subjects who died and those who did not by subgroup. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.
3.2.3.3 Total Adiponectin. As for the effect of total adiponectin on mortality, the OR is 1.031 with a 95% CI of 1.004 and 1.058 with a p-value of 0.024, which means that an increase in total adiponectin is associated with an increased risk of death in this study population. In the CKD group, the OR for the effect of total adiponectin on mortality is 1.016 with a 95% CI of 0.961 and 1.074 with a p-value of 0.575, which suggests that there is no association between an increase in total adiponectin and the risk of death. In the MHD group, however, the OR is 1.042 with a 95% CI of 1.001 and 1.083 with a p-value of 0.041, which is statistically significant and implies that an increase in total adiponectin level is associated with an increase in the risk of death in the MHD group of our study population.

Figure 3.2.13 shows the distribution of total adiponectin in the sub-cohort, categorised by whether they subjects died or not.

![Box and whisker plot comparing total adiponectin distribution between subjects who died and those who did not by subgroup. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.](image)

**Figure 3.2.13** Box and whisker plot comparing total adiponectin distribution between subjects who died and those who did not by subgroup. Box and whiskers indicate median and interquartile range, while outliers are shown as circles.
3.2.2.4 Summary of regression analyses for secondary endpoint

Regression analyses were carried out to determine odds ratios (OR) for mortality in regard to the potential risk factors in question: HOMA-IR, hs-CRP and total adiponectin. In the whole sub-cohort, increasing total adiponectin had a statistically significant effect on increasing the risk of death. As adiponectin is thought to have protective qualities, this result seems to be in contrast to what is seen in the general population, and therefore can be described as reverse epidemiology. However, as adiponectin is a complex substance with many regulatory effects, its increased level maybe a consequence of other conditions that increase mortality, rather than a cause for mortality. A summary of the regression analyses in the whole sub-cohort is available in table 3.2.5 below.

<table>
<thead>
<tr>
<th>Combined Population Data</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA2-IR</td>
<td>1.146</td>
<td>0.823, 1.597</td>
<td>0.419</td>
<td>-</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>1.031</td>
<td>1.004, 1.058</td>
<td>0.024*</td>
<td>Reverse (?)</td>
</tr>
<tr>
<td>Log hs-CRP</td>
<td>1.032</td>
<td>1.000, 1.064</td>
<td>0.051(*)</td>
<td>Trend: Classic</td>
</tr>
</tbody>
</table>

Table 3.2.5 Summary of regression analyses results for secondary endpoint (death) in the whole sub-cohort, looking at the effects of the factors studied.

In the CKD group within the sub-cohort, none of the factors studied had a significant effect on mortality. A summary of the regression analyses in the whole sub-cohort is available in table 3.2.6.
In the MHD group within the sub-cohort, the only statistically significant finding was the effect of increasing total adiponectin on increasing the risk of death. Again, this finding may be categorized as reverse epidemiology, although the physiologic nature of adiponectin makes it difficult to interpret these results. A summary of the regression analyses in the whole cohort is available in table 3.2.7 below.

**Table 3.2.6** Summary of regression analyses results for secondary endpoint (death) in the CKD subgroup, looking at the effects of the factors studied.

<table>
<thead>
<tr>
<th>CKD Group (non-MHD)</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA2-IR</td>
<td>1.415</td>
<td>0.909, 2.201</td>
<td>0.123</td>
<td>-</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>1.016</td>
<td>0.961, 1.074</td>
<td>0.575</td>
<td>-</td>
</tr>
<tr>
<td>Log hs-CRP</td>
<td>1.025</td>
<td>0.9366, 1.123</td>
<td>0.592</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 3.2.7** Summary of regression analyses results for secondary endpoint (death) in the MHD subgroup, looking at the effects of all the factors studied.

<table>
<thead>
<tr>
<th>MHD Group</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA2-IR</td>
<td>0.866</td>
<td>0.374, 2.001</td>
<td>0.736</td>
<td>-</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>1.042</td>
<td>1.001, 1.083</td>
<td>0.041*</td>
<td>Reverse (?)</td>
</tr>
<tr>
<td>Log hs-CRP</td>
<td>1.797</td>
<td>0.715, 4.518</td>
<td>0.213</td>
<td>-</td>
</tr>
</tbody>
</table>
3.2.4 Pre- & Post Haemodialysis Group Analysis

As explained in section 2.2.2, one of the objectives of the sub-cohort study was to compare levels of insulin resistance, hs-CRP and total adiponectin before start of MHD with their levels 8 weeks into MHD. Unfortunately, this part of the study was not completed as the follow-up data was not available for the 7 subjects recruited. Nonetheless, the results available are presented in this section.

3.2.4.1 HOMA-IR. HOMA-IR was measured for 7 subjects on the day of their first HD session, right before initiation of HD. These values were constituted as ‘pre-dialysis’ HOMAs, where the post-dialysis HOMAs would be measured in 8 weeks. Unfortunately, of the 7 subjects with ‘pre-dialysis’ values, only one has a ‘post-dialysis’ value. One patient was transplanted before reaching the 8 week target, one died and 4 did not agree to re-testing, although they had initially given written informed consent and understood that the study required them to complete both parts.

For the pre-dialysis values, the overall mean ± SD (range) HOMA-IR was 3.40 ± 2.54 (0.80 – 7.60). Table 3.2.8 outlines the data for this group.
<table>
<thead>
<tr>
<th>Case</th>
<th>Pre-dialysis HOMA-IR</th>
<th>Post-dialysis HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>0.8</td>
<td>Did not agree</td>
</tr>
<tr>
<td>Case 2</td>
<td>1.6</td>
<td>Did not agree</td>
</tr>
<tr>
<td>Case 3</td>
<td>2.8</td>
<td>Did not agree</td>
</tr>
<tr>
<td>Case 4</td>
<td>7.6</td>
<td>Did not agree</td>
</tr>
<tr>
<td>Case 5</td>
<td>4.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Case 6</td>
<td>1.2</td>
<td>Transplanted</td>
</tr>
<tr>
<td>Case 7</td>
<td>5.7</td>
<td>Died</td>
</tr>
</tbody>
</table>

*Table 3.2.8* HOMA-IR measurements for the pre- and post-dialysis study. Only one subject has a post-dialysis HOMA-IR value.

**3.2.4.2 hs-CRP.** hs-CRP was available for 5 subjects on the day of their first HD session, right before initiation of HD. These values were constituted as pre-dialysis hs-CRP, where the post-dialysis hs-CRP would be measured in 8 weeks. Unfortunately, of the 5 subjects with pre-dialysis values, only one has a post-dialysis value, as the other 4 did not agree to re-testing, although they had initially given written informed consent and understood that the study required them to complete both parts.
For the pre-dialysis values, the overall mean ± SD (range) hs-CRP was 1.43 ± 1.32 mg/l (0.28 – 3.49 mg/l). Table 3.2.9 outlines the data for this group.

<table>
<thead>
<tr>
<th>Case</th>
<th>Pre-dialysis hs-CRP</th>
<th>Post-dialysis hs-CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>0.28</td>
<td>n/a</td>
</tr>
<tr>
<td>Case 2</td>
<td>0.48</td>
<td>n/a</td>
</tr>
<tr>
<td>Case 3</td>
<td>3.49</td>
<td>n/a</td>
</tr>
<tr>
<td>Case 4</td>
<td>0.97</td>
<td>n/a</td>
</tr>
<tr>
<td>Case 5</td>
<td>1.93</td>
<td>7.08</td>
</tr>
</tbody>
</table>

*Table 3.2.9* hs-CRP measurements for the pre- and post-dialysis study. Only one subject has a post-dialysis hs-CRP value.

**3.2.4.3 Total Adiponectin.** Total adiponectin was measured for 5 subjects on the day of their first HD session, right before the beginning of HD. These values were constituted as pre-dialysis total adiponectins, where the post-dialysis total adiponectins would be measured in 8 weeks. Unfortunately, of the 5 subjects with pre-dialysis values, only one has a post-dialysis value, as the other 4 did not agree to re-testing, although they had initially given written informed consent and understood that the study required them to complete both parts.

For the pre-dialysis values, the overall mean ± SD (range) total adiponectin was 69.78 ± 15.90 ug/ml (45.2 – 84.0 ug/ml). Table 3.2.10 outlines the data for this group.
<table>
<thead>
<tr>
<th>Case</th>
<th>Pre-dialysis adiponectin</th>
<th>Post-dialysis adiponectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>84.0</td>
<td>n/a</td>
</tr>
<tr>
<td>Case 2</td>
<td>64.6</td>
<td>n/a</td>
</tr>
<tr>
<td>Case 3</td>
<td>72.2</td>
<td>n/a</td>
</tr>
<tr>
<td>Case 4</td>
<td>82.9</td>
<td>n/a</td>
</tr>
<tr>
<td>Case 5</td>
<td>45.2</td>
<td>29.6</td>
</tr>
</tbody>
</table>

**Table 3.2.10** Total adiponectin measurements for the pre- and post-dialysis study. Only one subject has a post-dialysis total adiponectin value.
3.3 Glycaemic Control in ESRD

3.3.1 Continuous Glucose Monitoring

20 (14 male) subjects were recruited and three were subsequently excluded; one due to repeated hypoglycaemia during both monitoring periods that were caused by underlying acute illness, one due to CGM technical failure and the third was confirmed as a type 1 after the monitoring period.

The age, duration of diabetes and years of dialysis [mean ± SD, (range)] of the 17 (13 male) subjects included were 61.5 yrs ± 8.8 yrs, (42-79); 18.8 ±7.6 yrs (4-30) and 4± 2.6 yrs (0.5-10.2), respectively. Previous CVD history, diabetic medications, erythropoietin dose, HbA1c, haemoglobin and urea values are given in the table below.
<table>
<thead>
<tr>
<th>No</th>
<th>Age (yrs)</th>
<th>M/F</th>
<th>Yrs of diabetes</th>
<th>Urea (mmol/L)</th>
<th>CVD (Y/N)</th>
<th>Medication</th>
<th>Hb (g/dL)</th>
<th>HbA1c (%)</th>
<th>Yrs on dialysis</th>
<th>Erythropoietin dose (µg/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53</td>
<td>M</td>
<td>20</td>
<td>5.1</td>
<td>Y</td>
<td>Gliclazide, 40mg BD</td>
<td>13.4</td>
<td>5.1</td>
<td>1.2</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>M</td>
<td>18</td>
<td>19.3</td>
<td>Y</td>
<td>Gliclazide, 80mg OD</td>
<td>11.3</td>
<td>5.3</td>
<td>3.7</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>M</td>
<td>6</td>
<td>15.1</td>
<td>Y</td>
<td>Diet</td>
<td>12.9</td>
<td>6</td>
<td>1.3</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>M</td>
<td>16</td>
<td>22.3</td>
<td>Y</td>
<td>Gliclazide, 80mg OD</td>
<td>9.9</td>
<td>6</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>M</td>
<td>20</td>
<td>23.0</td>
<td>Y</td>
<td>Humulin M3 (8u BD)</td>
<td>14.6</td>
<td>6.4</td>
<td>3.6</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>M</td>
<td>18</td>
<td>28.2</td>
<td>Y</td>
<td>Gliclazide, 40mg BD</td>
<td>15.1</td>
<td>6.5</td>
<td>3.7</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>65</td>
<td>M</td>
<td>24</td>
<td>14.0</td>
<td>Y</td>
<td>Novorapid (10u am),</td>
<td>12.1</td>
<td>6.6</td>
<td>2.8</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glargine (35u pm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>68</td>
<td>M</td>
<td>26</td>
<td>14.0</td>
<td>Y</td>
<td>Novomix 30 (10u BD)</td>
<td>10.9</td>
<td>5.6</td>
<td>7.7</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>M</td>
<td>30</td>
<td>17.9</td>
<td>Y</td>
<td>Mixtard 30 (10u, 8u)</td>
<td>12</td>
<td>6.7</td>
<td>5.4</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>67</td>
<td>M</td>
<td>18</td>
<td>24.0</td>
<td>Y</td>
<td>Gliclazide, 40mg BD</td>
<td>13.1</td>
<td>6.7</td>
<td>10.2</td>
<td>60</td>
</tr>
<tr>
<td>11</td>
<td>58</td>
<td>F</td>
<td>9</td>
<td>17.0</td>
<td>N</td>
<td>Mixtard 50 (23u, 24u)</td>
<td>9.3</td>
<td>7.4</td>
<td>0.5</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>53</td>
<td>M</td>
<td>13</td>
<td>26.7</td>
<td>Y</td>
<td>Gliclazide, 160mg BD</td>
<td>11.7</td>
<td>7.9</td>
<td>2.7</td>
<td>40</td>
</tr>
<tr>
<td>13</td>
<td>79</td>
<td>M</td>
<td>22</td>
<td>21.9</td>
<td>Y</td>
<td>Mixtard 30 (18u, 12u)</td>
<td>14</td>
<td>8</td>
<td>6.8</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>42</td>
<td>M</td>
<td>26</td>
<td>16.0</td>
<td>Y</td>
<td>Mixtard 30 (18u, 12u)</td>
<td>13.4</td>
<td>8.5</td>
<td>3.9</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>65</td>
<td>F</td>
<td>30</td>
<td>13.2</td>
<td>Y</td>
<td>Mixtard 30 (6u BD)</td>
<td>13.8</td>
<td>7.3</td>
<td>6.2</td>
<td>50</td>
</tr>
<tr>
<td>16</td>
<td>65</td>
<td>F</td>
<td>4</td>
<td>14.5</td>
<td>Y</td>
<td>Gliclazide, 160mg BD</td>
<td>12.4</td>
<td>8.9</td>
<td>3.3</td>
<td>30</td>
</tr>
<tr>
<td>17</td>
<td>54</td>
<td>F</td>
<td>19</td>
<td>17.8</td>
<td>N</td>
<td>Novomix 30 (16u, 10u)</td>
<td>11.7</td>
<td>9.2</td>
<td>1.7</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 3.3.1 Clinical details of 17 subjects whose CGM data was included in the final analysis. Abbreviations used: CVD, documented history of vascular disease defined as ischaemic heart disease (history of MI, revascularisation procedure or angiographically proven coronary disease), cerebrovascular disease (history of CVA or TIA) or peripheral vascular disease (history of amputation due to gangrene, revascularisation procedure or angiographically/Doppler proven peripheral vascular disease).
**HbA1c values.** The HbA1c (Mean ±SD) was 6.9 ± 1.2%, (range 5.1-9.2 %), with 7 subjects having an HbA1c of ≤6.5% (Table 1).

Linear regression analysis between HbA1c and erythropoietin dose, serum albumin and urea were not significant, (r²=0.17, p=0.0995; r²=0.161, p=0.536 and r²=0.163, p=0.533, respectively.)

**Haemoglobin values.** The mean haemoglobin was 12.4 ± 1.6 g/L (range 9.3-15.1).

### 3.3.1.1 Glycaemic Data

**Analysis of glycaemic profiles.** The 24-hour AUC glucose values and mean 24-hour CGM data were significantly higher on the day off dialysis than the day on dialysis (5932.1 ± 2673.6 vs. 4694 ± 1988 mmol/3min/L, p=0.022 and 12.6 ± 5.6 mmol/L vs. 9.8 ± 3.8 mmol/L, p=0.013, respectively), Figure 3.3.1. The difference in the 24-hour mean glucose levels for the day off dialysis to the day on dialysis ranged from -2.1 to 10.4mmol/L.
Figure 3.3.1 CGM data for day on (Day 1) and day off dialysis (Day 2), expressed as area under curve (AUC) glucose (A) and mean glucose (B) for each 24 hour period. Data for individual subjects are represented as triangles connected by lines. The mean ± SD for each 24 hour period is represented as a square. A. Mean ± SD area under the 3 minute glucose curve for the whole study group was 5932.1 ± 2673.6 mmol.3min/L on the day off dialysis vs. 4694 ± 1988 mmol.3min/L on the day on dialysis, p=0.022. B. Mean ± SD CGM glucose values for the whole group were 12.6 ± 5.6 mmol/L on the day off dialysis vs. 9.8 ± 3.8 mmol/L on the day on dialysis, p=0.013.
The AUC glucose profiles and the mean glucose values for the 6-hour nocturnal period from midnight to 6 am were significantly higher for the second than the first night (1541 ± 834 vs. 1137 ± 529 mmol/3min/L, p<0.05; and 12.9 ± 7.0 vs. 9.5 ± 4.4 mmol/L p<0.05, respectively). With a median 6-hour mean nocturnal glucose difference of 4.2mmol/L (range -8.5 to 17.1 mmol/L), Figure 3.3.2.
Figure 3.3.2 Nocturnal CGM data for the 6 hour period from midnight to 6 am for day on (Night 1) and day off dialysis (Night 2) expressed as area under curve (AUC) glucose (A) and mean glucose (B). Data for individual subjects are represented as triangles connected by lines. The mean ± SD for each 24 hour period is represented as a square. A. Mean ± SD area under the 3 minute glucose curve for the whole study group was 1541 ± 834 for the night of the day off dialysis (night 2) vs. 1137 ± 529 mmol.3min/L for the night of the day on dialysis (night 1), p<0.05. B. Mean ± SD CGM glucose values for the whole group were 12.9 ± 7.0 mmol/L on night 2 vs. 9.5 ± 4.4 mmol/L on night 1.
**Analysis of hypoglycaemia.** Four of the 17 subjects had CGM recordings of below 2.5 mmol/L for more than 30 minutes; in 3, this occurred in the first 24-hour monitoring period. Examination of individual CGM profiles showed that 14/17 subjects reached their glucose nadir (range 1.38-9.81 mmol/L) within the first 24 hours, with 10/17 having their lowest reading within 12 hours of starting dialysis. No subject reported any episode of symptomatic hypoglycaemia.

**Analysis of measured and predicted HbA1c values.** Comparison was made between measured and predicted HbA1c values, where predicted HbA1c was calculated using the mean glucose value derived from CGM data and incorporated into the DCCT published formula. The mean ± SD measured HbA1c was significantly less than the mean ± SD predicted HbA1c (6.9 ± 1.2% vs. 8.6 ± 2.3%, p< 0.006), Figures 3.3.3.

![Figure 3.3.3](Image)  
*Figure 3.3.3 Linear regression plot showing the relationship between measured HbA1c and overall mean glucose from the 48-hour CGM period (blue); the reference line has been drawn in green, showing what the projected results look like based on the DCCT-derived formula.*
3.3.1.2 Dietary Data

**Analysis of the food diaries.** Two subjects failed to complete their 48-hour food diaries (subjects 6 and 15). Analysis of the 15 completed diaries showed no significant difference between recorded dietary intakes for the day on dialysis and the day off dialysis (1636 ± 603 kCal vs. 1702 ± 559 kCal, respectively, p = 0.596). There was no trend towards greater food intake on either day, with 7 subjects recording a greater calorie intake during the day on dialysis verses 8 the day off dialysis, although the average meal frequency was greater on the non-dialysis day (3 meals on dialysis days vs. 4 meals on non-dialysis days). The timing of the dialysis shift did not appear to influence the energy intake.

The total energy intake for each subject was significantly lower, both on dialysis days (mean 1636kCal/day) and off dialysis days (mean 1702kCal/day), than the estimated mean energy requirement (2000kCal/day), p = 0.01, (data not shown). An equal number of patients reported better perception of appetite on dialysis days and non-dialysis days, Figure 3.3.4.
**Figure 3.3.4** Daily calorie intake for study subjects on dialysis and non-dialysis days is shown together with the calculated recommended daily intake for individuals.
Chapter 4: Discussion

4.1 Cardiovascular Risk Factors in ESRD

Chronic kidney disease has become an increasing public health concern, as the population of patients suffering from this condition is rapidly rising as a result of the obesity pandemic and subsequent type 2 diabetes. What concerns clinicians and scientists about this group is the high incidence of cardiovascular disease which contributes greatly to the high mortality rate seen in this population. Identifying the cause of this increased risk for developing cardiovascular disease has been a priority for decades, but the rapidly evolving nature of modern medicine and the dynamic demographics of the condition mean that to date cardiovascular risk factors in the ESRD population is the subject of much debate and controversy. A number of large, retrospective population based studies have been carried out on this population [5, 7, 132, 181, 467], and they have produced at times contradictory results. The lack of prospective studies looking at large groups of subjects with ESRD means not being able to distinguish the consequence from the cause. This has led to different theories in regards to morbidity and mortality risk factors in the ESRD population, one of which is the reverse epidemiology theory. Reverse epidemiology suggests that certain cardiovascular risk factors may have a reversed role in patients with ESRD, meaning they may actually be protective. For example, while obesity and high BMI is a well-known CVD risk factor in the general population, it is favourable in comparison to low BMI for the prolonged survival of patients on dialysis.

When addressing CVD risk factors in the ESRD population, it is important to distinguish between the different groups within the ESRD population; those who are receiving renal replacement therapy and those who are not. The latter is the CKD group, which is divided into stages 1-5, where stages 1 and 2 have normal kidney function as measured by GFR, therefore loss of function starts at stage 3. The RRT group comprises of transplanted patients and those on dialysis, and the dialysis group is broken down into two main groups of peritoneal dialysis (CAPD) and haemodialysis (HD).
This study aimed to identify the role of four well-known cardiovascular risk factors for the general population in the ESRD population; obesity, hypertension, dyslipidaemia and diabetes. The ESRD population we chose to study was CKD patients with stages 3, 4 and 5 and MHD patients, as the first group has gradually deteriorating kidney function while the second group theoretically has gradual improvement of ‘artificial’ kidney function.

Once recruitment was completed, an initial analysis of the population was carried out to compare the two groups: CKD and MHD. In terms of event rates, the MHD group had higher CVE and death rates compared to the CKD group, which is expected. The total death rate for two years in the MHD group is 18.1%, which is approximately half of the published UK haemodialysis mortality rates of 18-20% [330], and this is almost three times more than the mortality rate in the CKD group (table 4.1.1 below; also see figure 3.1.11).

Figure 4.1.1 Classifications within the ESRD population.
Table 4.1.1 Number of endpoints reached in the CKD and MHD subgroups over the 24 months follow-up period.

<table>
<thead>
<tr>
<th></th>
<th>CKD</th>
<th>MHD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No event</td>
<td>240 (77.9%)</td>
<td>116 (55.2%)</td>
<td>356 (68.7%)</td>
</tr>
<tr>
<td>CVE</td>
<td>47 (15.3%)</td>
<td>56 (26.7%)</td>
<td>103 (19.9%)</td>
</tr>
<tr>
<td>Death</td>
<td>21 (6.8%)</td>
<td>38 (18.1%)</td>
<td>59 (11.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>308 (100%)</td>
<td>210 (100%)</td>
<td>518 (100%)</td>
</tr>
</tbody>
</table>

Effect of Risk Factors on CV events and mortality

The higher incidence of cardiovascular disease and mortality in CKD and MHD patients is well documented [24, 468, 469], but it is the cause of this prevalence that has been subject to much debate. Researchers’ theories on the roots of these problems have been as dynamic as the treatments used, and the rapid modernisation of medicine and dialysis techniques has led to new answers as well as new questions.

Classically, vascular calcification has been recognised as one of the main causes of cardiovascular mortality in both the CKD and the MHD population, as it promotes arterial stiffness, left ventricular hypertrophy, cardiovascular events and mortality [469-473]. Other than the ESRD-specific risk factors for vascular calcification, which is mainly mineral metabolism abnormalities and extreme PTH levels [474-476], the remaining risk factors reported for vascular calcification is largely similar to those for cardiovascular disease in general: aging, hypertension, diabetes, dyslipidaemia and obesity [477, 478]. As discussed in the introduction, large epidemiological studies in the MHD population have shown a phenomenon referred to as ‘reverse epidemiology’, which suggests these ‘classic’ CV risk factors have a ‘reversed’ role in this population [7]. According to the reverse epidemiology theory, what is perceived as a CV risk factor in one population, may have protective qualities in another population; more specifically that while markers of over-nutrition are considered CV risks in the general population, they may be associated with
improved survival in the ESRD population who is specially at risk of malnutrition [59, 184].
4.1.1 BMI

Body mass index (BMI) is the most widely used tool for assessing weight status in populations, and although it does not calculate the percentage of body fat, its ease of use and practicality means individuals can be categorised as either underweight, ideal weight, overweight or obese within a few minutes by using two common measurements that most people are already aware of; weight and height (BMI = weight (kg) / height $^2$ (m$^2$)).

However, how accurately obesity, which is fundamentally excess body fat, can be measured and categorised by BMI is a subject of controversy. Although most of our understanding of the effects of obesity on health is based on our BMI-based definition of obesity, epidemiologists have concluded that BMI is an inadequate measurement for cardiovascular risk of obesity [479], and some have gone further and demanded that BMI be abandoned [480]. Detailed studies have been carried out to look at the correlations between BMI and other measurements of body fat, such as waist to hip ratio and abdominal visceral fat measured by computed tomography, and their results have shown that BMI correlates very well with both measurements and thus some obesity experts see no reason for replacing BMI with other measurements of obesity [481], although this conclusion is still heavily debated.

With the increasing prevalence of worldwide obesity, and in light of an ever increasing body of evidence that links obesity and overweight to CVD, cancer and mortality, it is at the very least intriguing to ponder the possibility of favourable effects of increased BMI. As postulated before (section 1.1.4.3.1), the ‘protective’ effect of increased BMI in the MHD population that has been observed in some studies is most probably relative to the potentially lethal effect of malnutrition; in other words, while obesity is bad, malnutrition is worse. Nevertheless, it should not be forgotten that the observations that lead to reversed BMI effect theories in this population are all either cross-sectional or retrospective, and therefore inconclusive. The present study set out to prospectively follow a mixed representation of the ESRD population to the end of producing more reliable data on the true effect of BMI on CV events and mortality.
In the CKD group, of the 308 subjects BMI values were recorded for 263; the mean BMI in this group was $29.1 \pm 6.2 \text{ kg/m}^2$. In the MHD group, of the 210 subjects BMI values were recorded for 201 patients, and the mean BMI was $25.3 \pm 5.9 \text{ kg/m}^2$. The difference between BMI in the two groups is statistically significant, with a $p$-value of $<0.001$. The lower mean BMI in the MHD group is consistent with data from similar populations [7, 482]. Taking into account that the MHD population is the one with greater risk of CV events and mortality, this data may be interpreted as showing that lower BMI is a greater risk factor for CVE and mortality, therefore giving weight to the reverse epidemiology theory.

The fact that this MHD population is lighter in weight than its CKD counterpart is not unexpected; not only do MHD patients lose the accumulated fluid due to kidney failure, but they have also been known to be prone to loss of appetite and in severe cases, malnutrition [483, 484]. Also, the physical limitations that follow being dialysed for 4-5 hours, 3 times a week added to what is in many, if not most, cases long travel hours and waiting times, means that these patients also have limited access to food for a total of 1 day a week (average 6 hours a day, 3 times a week adds up to 18 hours a week – which is about one ‘awake’ day). And while a mean BMI of 25.3 kg/m$^2$ may seem healthy at first glance, it must be kept in mind that only those with BMIs greater than the mean will benefit from fewer meals (88 patients, 50 of them male). For the other 122 patients, it is important to maintain their body weight in order to prevent weakness that may be followed by infections and/or malnutrition.

In terms of risk for cardiovascular events (CVE) and mortality, the result of logistic regression analysis for BMI is summarized in the table below (table 4.1.2).
Table 4.1.2 Summary of 2-year follow up results in regards to the effect of BMI on CVE and mortality.

As evident above, 24-months of prospective CVE and mortality data on a total of 518 subjects was unable to reach significant results in terms of the ability of BMI in predicting CVE and/or mortality in any of the groups studied.

There are different ways of expressing the effect of BMI on endpoints; one is to look at the effect of every single incremental increase in relation to the endpoint in question (which is what is shown above). Some studies look at the effect of different classifications, where subjects are divided into 4 groups of underweight, ideal weight, overweight and obese and the effect of each category on endpoints is analysed, and some just look at the effect of obesity, in which case subjects are categorised into two groups of obese and non-obese and endpoints are analysed in relation to that. In the population studied here, dividing subjects into four groups produced small numbers in each group; the subjects were therefore classified as either overweight or not overweight, using the cut off point of 25 kg/m$^2$. When the cumulative hazard ratio for CVE and mortality are plotted for the two groups, the lack of effect of BMI is readily seen, as shown see figure 3.1.13.

In the general population, the risk of death consistently increases as BMI rises, regardless of age, gender, race and medical history [170, 485, 486], although the effect of increasing BMI is more pronounced in certain groups. As for similar studies in the MHD population, Fleischmann et al. report a 30% reduced risk of mortality for
every increment increase in a population of 1346 MHD patients [173], and Abbott et al. observed a similar improvement in survival in BMI values above 30 kg/m² (adjusted hazard ratio of 0.89) for 1675 MHD patients which could not be reproduced for their 1662 CAPD patients [175]. These findings were confirmed by large historical cohort studies using the USRDS data on haemodialysed patients [172, 177, 273], and by the Dialysis Outcomes and Practice Patterns Study (DOPPS), which was a large prospective study of 9714 patients on HD across the globe, that showed increasing BMI was associated with better survival in all subgroups of patients [164].

The fact that the results of the present study did not reach significance can mean that the effect of BMI is too weak to reach significance in a population of this size and/or with this length of follow-up period, which in itself is a finding contrary to both the general population and the ESRD population. The fact that the subjects in this study are a mixture of both CKD and MHD could cause a mixed effect on mortality for different risk factors in the two groups, and when the results are broken down into subgroups, the smaller number may be contributing to the insignificance of the results. Of note in these results is the opposite direction of the OR for CVE and mortality in both the combined and the MHD population; while the OR above 1 for effect of BMI on CVE in all subgroups indicates higher BMI may be associated with higher event rate, the OR below 1 for effect of BMI on mortality in the MHD group can mean a favourable effect of BMI on survival, which is also observed in the combined population calculations. Of course, as none of these observations reach statistical significance, these theories cannot be verified unless the population is followed for a greater period of time and more endpoints are reached in all groups, which may push the results toward statistical significance.

What remains is that despite its known short comings, BMI is still the most widely used measure for body size both in the general population and in ESRD despite the fact that it does not distinguish between fat mass and lean mass, nor does it give information on fat distribution. Beddhu et al. showed that in dialysis patients with similar BMI, those with lower muscle mass (urinary creatinine ≤ 0.55 g/day) had a higher risk of both cardiovascular and all-cause mortality [487]. In another study by Honda et al. protein-energy wasting measured by a subjective global assessment of
nutrition was shown to be equally prevalent in underweight, ideal weight and overweight ESRD subjects [488]. Honda’s study did not show a significant effect for BMI on mortality, perhaps due to their small size of 328 patients, but protein-energy malnutrition was associated with increased mortality in all groups. Perhaps the ideal way to deal with this problem is to combine BMI, waist circumference, and nutritional assessment for a more refined prognostic element. Once such a measurement has been calculated for ESRD patients, clinical trials can determine whether weight loss can be effective in improving survival in some ESRD patients, and whether weight gain can mean the same in other ESRD patient.

In summary:

- BMI does not appear to have a significant effect on either CVE or mortality in this population

- Interpretation of the trend (although weak) in the MHD population suggests the effect of increasing BMI on CVE is in the same direction as that of the general population (classic epidemiology), but its effect on mortality is in the opposite direction to that of the general population suggesting that higher BMI may have a favourable effect on survival in the MHD population (reverse epidemiology).
4.1.2 BP

When measuring BP, the force of blood against blood vessels’ walls is being measured; this means that both fluid overload and stiffness and/or narrowing of vessels can cause increased BP [489]. Patients with kidney disease often suffer at least one, if not both, of these conditions, and many patients suffer kidney disease as a result of existing essential or secondary hypertension. It is therefore not surprising that the majority of the subjects on the database either had high BP or were on antihypertensive medication. The importance of monitoring and controlling BP in kidney disease is well-known; not only is hypertension an easily treated condition with adverse effects on the cardiovascular system, but the kidneys are also at risk of further damage, in addition to the fact that kidney disease can also cause hypertension.

BP has been widely investigated and shown to be directly correlated to CV mortality in the general population [162, 185, 490-494] (see figure 4.1.2). However, in the ESRD population, specifically in dialysed patients, BP has been among the controversial components of the reverse epidemiology phenomenon [7, 8] and many investigators have observed the link between low pre-dialysis BP and increased mortality [165, 195, 495-497].
Figure 4.1.2 Ischamic heart disease mortality rate in each decade versus usual BP at start of that decade. Figure reproduced with permission from Lewington S, et al [498].
It is known that BP values in MHD patients differ depending on the time it’s measured in relation to dialysis, and although predialysis BP is the measure most often used to address BP in MHD patients, it is not known whether it is better correlated with long-term outcome compared to intradialysis or post-dialysis BP [499-501]. The effect of BP on CVE and/or mortality in the haemodialysed population is heavily debated; some observational studies have shown that hypertension is a risk factor for increased CV morbidity and mortality in long-term follow up of MHD patients [502-505], while some researchers have shown that higher BP is associated with better survival and that hypotension is the risk factor in their MHD subjects [506-510], highlighting BP as one of the factors of reverse epidemiology in this population. The lack of controlled trials to show survival benefit of BP control, either pharmacologic or through dialysis, adds to the inconclusivity of the present data.

An important factor to keep in mind is that in populations with severe cardiac disease, low BP is usually a marker of severity of disease, and therefore it may not be surprising to find it correlates with increased morbidity and mortality [7]. Therefore, the discrepancy between the above mentioned observations may be due to the number of patients with severe CVD in each study cohort.

To look at the effect of BP on CVE and mortality in this study population, mean SBP and DBP were compared between the subgroups, and logistic regression analysis was carried out for each population as well.

In the CKD group, of the 308 subjects BP values were recorded for 241; the mean SBP ± SD in this group was 138.0 ± 18.1 mmHg and the mean DBP ± SD was 73.9 ± 12.1 mmHg. In the MHD group, of the 210 subjects BP was recorded for 172 patients, and the mean SBP was 152.4 ± 25.0 mmHg and mean DBP was 81.7 ± 14.2 mmHg. The difference between both SBP and DBP in the two groups is statistically significant, with a p-value of <0.001 for both factors. The higher mean BP in the MHD group, the one with greater risk of CVE, suggests that this is in line with the general population and not in keeping with reverse epidemiology; the group with the higher BP has the higher CVE rate. Similar observations have reported
increased BP in the MHD population compared to both the general population and the CKD population [511, 512].

In terms of risk for cardiovascular events (CVE) and mortality, the result of logistic regression analysis for SBP and DBP is summarized in the table 4.1.3 below.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Endpoint</th>
<th>Population</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>CVE</td>
<td>Combined</td>
<td>1.011</td>
<td>1.002, 1.021</td>
<td>0.023*</td>
<td>Classic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD</td>
<td>1.012</td>
<td>0.993, 1.031</td>
<td>0.217</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MHD</td>
<td>1.001</td>
<td>0.989, 1.014</td>
<td>0.830</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mortality</td>
<td>Combined</td>
<td>1.010</td>
<td>0.997, 1.023</td>
<td>0.146</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD</td>
<td>0.958</td>
<td>0.926, 0.990</td>
<td>0.001**</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MHD</td>
<td>1.013</td>
<td>0.998, 1.024</td>
<td>0.095</td>
<td>-</td>
</tr>
<tr>
<td>DBP</td>
<td>CVE</td>
<td>Combined</td>
<td>1.004</td>
<td>0.989, 1.021</td>
<td>0.560</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD</td>
<td>1.000</td>
<td>0.972, 1.028</td>
<td>0.981</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MHD</td>
<td>0.988</td>
<td>0.967, 1.010</td>
<td>0.299</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mortality</td>
<td>Combined</td>
<td>0.985</td>
<td>0.962, 1.009</td>
<td>0.230</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD</td>
<td>0.929</td>
<td>0.879, 0.982</td>
<td>0.010**</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MHD</td>
<td>0.986</td>
<td>0.958, 1.014</td>
<td>0.327</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.1.3 Summary of 2-year follow up results in regards to the effect of BP on CVE and mortality.

As evident above, 24-months of prospective CVE and mortality data on a total of 518 subjects showed mixed results for the relationship between BP and CVE and/or mortality in the different groups studied.

The regression results for SBP show that in terms of its effect on the primary endpoint, CVE, no significant effect is seen in the two subgroups, whereas the combined population shows a statistically significant increase in CVE with increasing SBP. We can therefore assume that the reason no significance is achieved in the two subgroups is due to the smaller number of subjects, and the
fact that the OR in both groups is above 1.0, meaning that the direction of the effect is similar to that observed in the combined population, is in line with that theory. This means that not only is higher SBP a risk factor for CVE in the ESRD population, but it also suggests that there are no discrepancies between the MHD population, the CKD population and the general population.

For the effect of SBP on mortality, the secondary endpoint, the only significant result was seen in the CKD group, where a statistically significant OR of less than 1.0 means that mortality risk is reduced with increasing SBP in this group. Surprisingly, the OR for the MHD population is above 1.0, suggesting that the direction of the effect of SBP on mortality is different in MHD and in line with the general population where increased BP is associated with increased risk of mortality (although this finding is not statistically significant).

Looking at the DBP regression analysis for its effect on CVE, no significant result was observed in any of the groups. Perhaps the only noteworthy observation here is the OR value below 1.0 in the MHD group; although lacking statistical significance, this result suggests the possibility of a reversed relationship, where increasing DBP lowers the risk of CVE in the MHD population. In his meta-analysis Agarwal has reported that in such studies where investigators are looking at the role of BP on CVE and mortality, analysing SBP and DBP separately leads to the observation that high SBP is a strong predictor of CVE while DBP may exhibit an inverse relationship [513]. This is exactly what this study’s data has shown.

As for the effect of DBP on mortality, the results are similar to SBP in that the only statistically significant finding is that increasing DBP lowers the risk of mortality in the CKD group. However, of note here is that the OR for the effect of DBP on mortality in both the combined population and the MHD group is below 1.0, and again although not statistically significant, in the same ‘reversed’ direction as the effect in the CKD group.

As 49.9% of the entire cohort studied have pre-existing CVD other than hypertension, and in the CKD group, a total of 161 patients (52.3%) have pre-existing CVD while in the MHD group there are 98 patients (46.7%) with pre-existing CVD, it can be hypothesized that there are a greater number of patients with severe
CVD and/or heart failure in the CKD group, which would explain why this group is benefiting from increased BP. The lower than 1.0 OR for DBP in the MHD group is explained by Agarwal’s observations that DBP retains an inverse relationship with CVE and mortality when considered as an independent factor in the MHD population [513].

In summary:

- Systolic BP shows a significant effect on CVE in the combined cohort which is in the same direction as that of the general population (classic epidemiology).

- In the CKD subgroup, increased systolic and diastolic BP are both significantly associated with decreased odds of death, which is in the opposite direction of the general population (reverse epidemiology)
4.1.3 Lipids

While a complete lipid profile is needed for full insight into patients’ balance (or lack of) of clinically important lipids, i.e. LDL cholesterol, HDL cholesterol, total cholesterol and triglycerides, it has long been established that high levels of serum cholesterol and triglycerides increase the risk of developing CVD in the form of coronary heart disease (CHD) and therefore subsequently increase mortality [163, 492, 514, 515]. Since the onset of the obesity pandemic, these markers of over-nutrition are fast rising in an increasing proportion of patients. But it has also been reported that in the ESRD population, specifically in the MHD population, high cholesterol levels are associated with improved survival [7, 166, 516-518]. It is unlikely, although not improbable, that this observation can be attributed to unknown protective effects of high TC in the ESRD population. As low TC and TG levels are indicative of under-nutrition, or in severe cases malnutrition, it is more likely that the previously mentioned observed favourable effect of high TC is due to the relative survival advantage of over-nutrition to under- or malnutrition.

In the cohort studied here, the effect of TC and TG on both CVE and mortality were analysed by comparison of means between subgroups, as well as logistic regression analyses in both groups.

Of the 308 subjects in the CKD group, 265 had recorded TC and TG values; the mean TC \(\pm SD\) in this group was 4.5 \(\pm\) 1.5 mmol/l and the mean TG \(\pm SD\) was 1.86 \(\pm\) 1.06 mmol/l. In the MHD group, of the 210 subjects lipids were recorded for 196 patients, and the mean TC \(\pm SD\) was 3.8 \(\pm\) 0.95 mmol/l and mean TG \(\pm SD\) was 1.67 \(\pm\) 0.85 mmol/l. The difference between mean TC in the two groups is statistically significant, with a p-value of <0.001, but the p-value for the difference of mean TG in the two groups is just above the cut-off for statistical significance at 0.051. The lower mean TC and TG in the MHD group, the group with higher CVE risk, suggests that this is may be a ‘reverse epidemiology’ observation; the group with the lower lipid levels has the higher CVE and mortality rate. As mentioned above, several other studies have reported similar observations.

The lower TC and TG in the MHD group is expected to an extent, as BMI was also lower in the MHD group; however, while the significant difference in BMI between
the two groups did not seem to have conclusive effects on endpoints, logistic regression analyses for TC and TG reveal significant effects on both CVE and mortality (Table 4.1.4).

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Endpoint</th>
<th>Population</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>CVE</td>
<td>Combined</td>
<td>0.681</td>
<td>0.550, 0.844</td>
<td>&lt;0.001**</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD</td>
<td>0.589</td>
<td>0.419, 0.828</td>
<td>0.002**</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MHD</td>
<td>0.924</td>
<td>0.676, 1.262</td>
<td>0.619</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mortality</td>
<td>Combined</td>
<td>0.635</td>
<td>0.466, 0.864</td>
<td>0.004**</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD</td>
<td>0.500</td>
<td>0.284, 0.880</td>
<td>0.016*</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MHD</td>
<td>0.864</td>
<td>0.576, 1.296</td>
<td>0.480</td>
<td>-</td>
</tr>
<tr>
<td>TG</td>
<td>CVE</td>
<td>Combined</td>
<td>0.897</td>
<td>0.717, 1.123</td>
<td>0.344</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD</td>
<td>0.921</td>
<td>0.679, 1.248</td>
<td>0.594</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MHD</td>
<td>0.952</td>
<td>0.667, 1.357</td>
<td>0.784</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mortality</td>
<td>Combined</td>
<td>0.504</td>
<td>0.322, 0.788</td>
<td>0.003**</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD</td>
<td>0.468</td>
<td>0.219, 0.998</td>
<td>0.049*</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MHD</td>
<td>0.546</td>
<td>0.310, 0.964</td>
<td>0.037*</td>
<td>Reverse</td>
</tr>
</tbody>
</table>

**Table 4.1.4** Summary of 2-year follow up results in regards to the effect of lipids on CVE and mortality.

The table 4.1.4 shows that 24-months of prospective CVE and mortality data on a total of 518 subjects exhibit mixed results for the relationship between TC and TG and CVE and/or mortality in the different groups studied.

The regression results for TC show that in terms of its effect on the primary endpoint, CVE, no significant effect is seen for the MHD group, whereas the combined population and the CKD group both show a statistically significant decrease in CVE risk with increasing TC. This result is counter intuitive and in contrast to what is observed in the general population, where higher TC is synonymous with higher risk of CVE. What is interesting here is that this observation of ‘reverse epidemiology’ is seen in the CKD group and not the MHD group. Although the OR for the MHD group is below 1.0, indicating that the
relationship may be reversed here as well, it is much higher than the OR for the CKD group. We can therefore assume that the reason significance is observed in the combined population is the strong effect in the CKD group, which has a larger number of subjects. This could mean that the effect of TC on CVE in the ESRD population is reversed compared to the general population; the data produced by this study is insufficient in determining whether this effect is only present in the non-dialysed CKD population or also in the MHD population. Longer follow-up of the cohort may lead to significant results in the MHD population.

For the effect of TC on mortality, the secondary endpoint, the results seem to reflect that of the effect of TC on CVE; significant results seen in the CKD group as well as the combined cohort, where a statistically significant OR of less than 1.0 means that mortality risk is reduced with increasing TC in these groups. Again, the OR for the MHD population is also below 1.0 here, but larger in comparison to the OR for mortality in the CKD group. These results indicate that reverse epidemiology of TC exist in the CKD cohort, but not at present in the MHD group. Whether the statistically insignificant result in the MHD group is due to smaller sample size and/or not enough follow-up for TC’s weaker ‘reversed’ effect to exhibit can only be addressed by longer follow-up of the cohort. With time and as more subjects reach endpoints, the effect of TC on mortality should either reach significance with an OR of less than 1.0, or the OR may go above 1.0 and the results may remain statistically insignificant, in which case we can postulate that either TC does not affect mortality in the MHD population, or that its effect is in the same direction as the general population.

Looking at the TG regression analysis for its effect on CVE, no significant result was observed in any of the groups. But interestingly, the OR value observed is below 1.0 in all groups; although lacking statistical significance, this suggests the possibility of a reversed relationship, such as that seen for TC in relation to CVE, where increasing TG lowers the risk of CVE in the ESRD population.

The results for the effect of TG on mortality are quite interesting; the entire cohort and both subgroups show statistically significant OR of well below 1.0, meaning increasing TG lowers the risk of mortality in all groups. This result is clearly ‘reverse’ in comparison to the general population, where increased TG is a risk factor for
mortality [141, 519]. The importance of this result maybe in that it suggests that with enough endpoints, all the results in this block (effect of lipids on endpoints) would reach statistical significance. The fact that significant ‘reversed’ results are observed in both the CKD and the MHD group also shows that as far as reverse epidemiology of TG is concerned, there are no discrepancies between ESRD subgroups, and that whatever the reason for this reversed result, it is not being corrected through dialysis. If one can now find the missing link that causes this reversal of effect in the ESRD population – perhaps a mediator with effects on TG that cannot be dialysed out – one can then design future trials that would focus on enhancing survival in this population.

It is important to rule out the effect of lipid-lowering drugs, specifically statins, when looking at outcomes in relation to lipid levels. Many researchers have observed statins’ favourable effects on survival in the general population, but studies such as the Deutsche Diabetes Dialyse Studie (4D) trial [520] and the more recent ‘A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Haemodialysis: An Assessment of Survival and Cardiovascular Events’ (AURORA) trial [521] have shown that while statins lower lipid cholesterol levels in patients on MHD, they have no effect on CVE or mortality in this population. In the present study, to rule out the effects of statin therapy, the differences between the MHD and CKD groups in relation to statin use and lipid levels were analysed. The results showed similar TC and TG levels in those on statin therapy and not on statin therapy in both groups as shown in figure 3.1.18).

Risk analysis for effect of statins on CVE and mortality showed no significant results. The fact that statin therapy has no effect on endpoints in the present cohort means that the reversed effect of TC and TG on mortality and the reversed effect of TC on CVE are not linked to favourable anti-inflammatory side-effects of statins [522-524]. Keeping that in mind, it seems that the profound negative effect of low TC and TG on mortality can be attributed to under-nutrition and its subsequent adverse outcomes, such as infection. Also, since there is usually a relatively high prevalence of malignancy in the ESRD population [525] it is possible that the low lipid levels are secondary to cancer cachexia in this group, further explaining the higher mortality in the lower lipids levels group. However, this does not seem to be
the case in the present population, as the prevalence of malignancy is 8% in the whole cohort, 7% in the CKD group and 9% in the MHD group; although this is more than twice the prevalence in the UK general population which is estimated at 3.2% \[526\], it is almost the same as the prevalence of cancer in the population of 65 and over living with cancer, which is estimated at 7.5% \[527\]; and as the mean age for this cohort is 66 (±15) years, it is almost in line with the general population.

In summary:

- The effect of TC and TG in this population is reversed in certain subgroups
- Increasing TC reduces the risk of both CVE and mortality in the CKD population, but not in the MHD population
- Increasing TG reduces the risk of mortality in both the CKD and the MHD group, but has no effect on CVE in either group
- We can therefore postulate that because the ‘reversed’ effect of TC on both endpoints is only present in the CKD group and not the MHD group, the cause of this reversal is removed through dialysis
- TG has similar effects in both groups and is significantly affecting mortality but not CVE, suggesting a different mechanism to TC for its effects on outcome
4.1.4 Diabetes and Glycaemic control.

In the MHD group 43.3% of the subjects have diabetes, compared to 59.4% in the CKD group (the prevalence of diabetes in the UK ESRD population is about 23% [330, 528]). While the incidence of diabetes in the MHD group is similar to that of other ESRD populations (44% in the US MHD population according to USRDS), it may be over-represented in the CKD group. This is most likely a sampling error led by the fact that patient records are kept in greater detail for patients with diabetes and therefore more likely to be included in this database. Patients with diabetes and kidney disease have comprehensive records in both the renal and diabetes departments, and are also seen at closer intervals; therefore a bias in consecutive sampling is difficult to avoid. The greater number of patients with diabetes in the CKD group compared to the MHD group may also be a result of more diabetic patients either not progressing to later stages of CKD, or dying before they start haemodialysis. To rule out each of these theories, large numbers of patients must be prospectively followed through the different stages and onto dialysis.

Logistic regression analysis for the effect of diabetes on endpoints revealed that diabetes had significant ‘traditional’ results in all subgroups, except for CVE in the CKD group where it failed to reach significance (see table 4.1.5). This means that diabetes is a risk factor for both CVE and mortality in the MHD population, as well as for mortality in the CKD population. Its effect on CVE in the CKD population is unclear at present, and only further follow-up can show whether diabetes has an adverse effect on CVE outcome in the CKD population. Although the present literature shows no evidence at the moment that diabetes in itself may be protective against CVE in the MHD population and therefore a component of reverse epidemiology, there are studies that show diabetes has no effect on CVD outcomes in this group [132] [529].
When it comes to glycaemic control within the diabetic ESRD population, Williams et al. showed a reversed relationship between HbA1c levels and CVE and mortality in the MHD population [132], while most observe better outcomes with tighter control [446, 530, 531]. Regression analyses were not carried out for HbA1c effects on CVE and mortality because of the smaller numbers in the diabetic subgroups and the fact that the study was not powered to analyse HbA1c.

HbA1c is the most widely used marker of glycaemic control in patients with diabetes. Among the 183 CKD patients with diabetes, 171 had a recent HbA1c value recorded (within the past 6 months). The mean HbA1c in this group was 7.5%±2.0% (range: 0.9%-14.8%), slightly above the ADA recommendation of <7% [532]. The mean HbA1c of the MHD group, however, was 6.8%±1.6% (range: 4.5%-13%) in the 86 subjects with recent HbA1c values out of the 91 with diabetes. Comparison of mean HbA1c between the two groups showed significance, with a p-value of 0.003. It was also observed that with the progression of CKD stage in figure 3.1.10 (from 3 to 4 to 5 and onto MHD), mean HbA1c values dropped, and this decrease was statistically significant (p=0.001). Other studies have described similar observations in this population [529, 533].

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Population</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Significance (p-value)</th>
<th>Classic / Reverse Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVE</td>
<td>Combined</td>
<td>1.622</td>
<td>1.074, 2.795</td>
<td>0.021*</td>
<td>Classic</td>
</tr>
<tr>
<td></td>
<td>CKD</td>
<td>1.488</td>
<td>0.792, 2.795</td>
<td>0.217</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MHD</td>
<td>2.538</td>
<td>1.417, 4.546</td>
<td>0.002**</td>
<td>Classic</td>
</tr>
<tr>
<td>Mortality</td>
<td>Combined</td>
<td>1.869</td>
<td>1.047, 3.334</td>
<td>0.034*</td>
<td>Classic</td>
</tr>
<tr>
<td></td>
<td>CKD</td>
<td>3.846</td>
<td>1.101, 13.433</td>
<td>0.035*</td>
<td>Classic</td>
</tr>
<tr>
<td></td>
<td>MHD</td>
<td>2.032</td>
<td>0.997, 4.146</td>
<td>0.051(*)</td>
<td>Trend: Classic</td>
</tr>
</tbody>
</table>

*Table 4.1.5 Summary of 2-year follow up results in regards to the effect of diabetes on CVE and mortality.*
This improvement of glycaemic control with progression of kidney failure can be explained by the lowering of BMI through the stages which suggests calorie loss which can contribute to better glycaemic control. Secondly, it is probable that patients with higher HbA1c’s who are poorly controlled do not go on to MHD, which may be because they do not survive long enough to go on to RRT or that they stabilize in the CKD stage and do not progress.

In summary:

- Diabetes increases the risk of both CVE and mortality in the combined cohort
- In the CKD subgroup, diabetes significantly increases the risk of mortality, but not CVE
- In the MHD subgroup, diabetes significantly increases the risk of CVE, with a trend towards increasing the risk of mortality, as well
- Glycaemic control also seems to be important, with the trend showing increasing risk of both CVE and mortality with HbA1c above 7.0% in the combined population
4.1.5 Other risk factors

There are many other identified risk factors for both CVE and mortality, several of which are mentioned in the introduction. This study is not powered to look at all the contested risk factors in renal disease, but it is important to look at the effect of important factors that may be altering survival outcomes.

Ethnicity

Analysis of the effect of ethnicity on CVE suggested the ‘Black’ ethnic group had the highest risk of developing CVEs (HR=6.154, 95%CI: 2.141, 17.684, p=0.001), while the ‘White’ ethnic group has the highest risk for mortality (Hazard Ratio=10.558, 95%CI: 1.388, 80.314, p=0.023) (figure 3.1.17). This agrees with the present literature on the effect of ethnicity on survival in the ESRD population which reports better survival outcomes in the Asian ethnic group [534] and the ethnic groups that are categorised as Black [535-538] (see figure 4.1.3).

Figure 4.1.3 Kaplan-Meier survivor function for unadjusted survival on haemodialysis by race/ethnicity among patients with ESRD for at least three months. Figure reproduced with permission [538]
When the population is broken down into the subgroups, regression analyses show that White ethnicity has the highest risk of mortality in both CKD and MHD groups, although the risk is greater in the MHD group, and Black ethnicity has much lower risk in both groups in comparison (figure 3.1.20). This finding is in agreement with present literature, where Black ethnicity is shown to be associated with better survival on haemodialysis [27]. However, the risk of CVE is higher among the Black ethnic group in comparison to the White ethnic group in both the CKD and MHD groups (figure 3.1.19).

Interestingly, the Asian ethnic group seem to have a relatively low risk of CVE in the CKD group, but the highest risk of CVE in the MHD group. One explanation for this finding may be that the Asian subjects in the MHD group have more co-morbidities compared to their CKD counterparts. Another noteworthy point is that while the ethnic group categorised as ‘Mixed’ consists of a relatively small proportion of patients (about 1% of the whole cohort), they show the highest risk for CVE in the CKD population – the lack of a line corresponding to Mixed ethnicity in the hazard plots of the MHD group is due to the fact that no subjects in this group were categorised as having Mixed ethnicity.

Unfortunately, the small sample size in each ethnic subgroup prevented me from analysing effects of each risk factor on endpoints within these subgroups. As mentioned in the introduction, present literature implies that the reverse effect of cholesterol on survival in the haemodialysed population does not exist among Black individuals on MHD [300]. Longer follow-up period resulting increased number of events may allow further investigation of this point in the present cohort.

**Under-nutrition**

Poor nutrition seems to be essentially at the centre of all hypotheses surrounding reversed epidemiology findings, and it often goes hand in hand with inflammation to form the malnutrition-inflammation complex syndrome or the MICS. While the effects of inflammation will be looked at in detail in the next section, it is worth noting the nutritional discrepancies between the different groups.
Perhaps one of the questions most in need of an answer in the ESRD population is: what is the best marker for nutrition in this population? In the general population, BMI and lipid profile are the main surrogates of nutrition used in day to day practice. Using the same markers in the present ESRD population would tell us that the MHD population is less nourished than the CKD group, as their BMI, TC and TG is lower. But this comparison does not distinguish between those with ‘over-nutrition’ and ‘under-nutrition’.

**Co-morbidities**

ESRD is a long-term complication that is almost always secondary to a pre-existing condition, and patients with ESRD often suffer multiple long term conditions which are referred to as co-morbidities. When looking at the CVE and mortality rate in this population, it is important to keep in mind the effect of these co-morbidities. Up to five co-morbidities were recorded for each subject on the database, and although all statistical analyses were adjusted for co-morbidities, because the majority of subjects had at least one co-morbidity the results did not differ greatly before and after adjusting. But this does not mean the presence of co-morbidities cannot be used to explain outcomes.

The most recurring co-morbidities in subgroups within the present cohort are listed in table 4.1.6 below. Subjects may have any number of co-morbidities at the same time, which is why the numbers are only to show the frequency of occurrence and do not add up to the total number of patients in the subgroups. Diabetes is not categorised as co-morbidity, as it is analysed as an independent risk factor in this study.
Table 4.1.6 Frequency of most common co-morbidities within the cohort studied. Pre-existing CVD remained the most prevalent condition even after deletion of hypertension (HTN), which may be a consequence of renal failure in some subjects.

The most prevalent co-morbidity in this cohort is pre-existing CVD, and notably hypertension. However, it is difficult to distinguish between hypertension that was present before onset of kidney disease and hypertension that is caused by renal failure; pre-existing CVD remains the most prevalent co-morbidity even when HTN was deleted. This is important because subjects who start dialysis with diagnosed CVD have a 57% greater risk of developing non-fatal MI and 14% greater risk for cardiac death [539].

The second most common condition in the MHD population is hyperparathyroidism, which is an outcome of ESRD (section 1.3). The rate of malignancy in the CKD group is 6.8%, which is acceptable considering the prevalence rate of malignancy in the UK population over 65 years old is reported at 7.5% [540] and the mean age for this group is 67 years. The prevalence rate in the MHD group is 10%, which is
higher than that of the CKD group, especially considering that this is a younger group, with a mean age of 64 years. Whether the higher prevalence of cancer in the MHD group is due to an increased incidence of malignancy as a result of the haemodialysis process itself, or secondary to other conditions that are common in this population (for example inflammation) is unknown. Specifically designed randomised controlled trials are needed to address this question and therefore reduce cancer rates, and ultimately mortality rates, in this population.

In summary:

- Ethnicity may have an effect on CVE and mortality outcome in both the CKD and MHD population, with White race at highest risk of mortality in both subgroups and Black race at highest risk of CVE in both subgroups

- A marker of malnutrition specific to the ESRD population is needed to better establish its role in CVE and mortality in this population, as surrogate markers such as BMI, TC and TG can be misinterpreted in this population

- The ESRD population is a complex one where subjects usually suffer multiple comorbidities that all possibly contribute to the high incidence of CVE and mortality in this population
4.2 Insulin Resistance and Inflammation in ESRD

A subgroup of subjects from the entire cohort were recruited to form a ‘sub-cohort’ from whom blood samples were taken and stored for further analysis; insulin resistance (HOMA-IR), inflammation (hs-CRP) and total adiponectin levels were quantified. This sub-cohort consists of 105 subjects, 55 of them are categorised as CKD (CKD 3=17, CKD 4=20, CKD 5=18) and 51 are on MHD. While insulin resistance quantification was carried out on all of the 106 subjects, inflammation and adiponectin measurements were available for 92 subjects. As with the previous part of the study, the mean values were compared between the subgroups to observe any significant differences between the groups.

As this sub-cohort is part of the bigger cohort, 24-month follow-up data is also available for these subjects (table 4.2.1 below, also see figure 3.2.4).

<table>
<thead>
<tr>
<th></th>
<th>CKD</th>
<th>MHD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No event</td>
<td>40 (72.7%)</td>
<td>37 (72.5%)</td>
<td>77 (72.6%)</td>
</tr>
<tr>
<td>CVE</td>
<td>12 (21.8%)</td>
<td>11 (21.6%)</td>
<td>23 (21.7%)</td>
</tr>
<tr>
<td>Death</td>
<td>3 (5.5%)</td>
<td>3 (5.9%)</td>
<td>6 (5.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>55 (100%)</td>
<td>51 (100%)</td>
<td>106 (100%)</td>
</tr>
</tbody>
</table>

*Table 4.2.1* Number of endpoints reached in the CKD and MHD subgroups within the sub-cohort.
4.2.1 Insulin Resistance

Insulin resistance is a known predictor of CVE and cardiovascular death in the general population [358, 360, 541-543]. Insulin resistance is a common link between obesity, hypertension, dyslipidaemia and diabetes, and therefore it is important to understand its behaviour in ESRD. Patients with chronic kidney disease develop insulin resistance, and this is due to loss of kidney function [331, 544], insulin resistance inducing adipokines such as TNF-alpha and leptin [333, 334, 545], reduced secretion due to increased intracellular calcium (caused by PTH imbalance) [341, 342], and physical inactivity [332]. It has also been shown that insulin resistance can be the cause, rather than consequence, of CKD in non-diabetic subjects [345, 347, 350, 352].

To quantify insulin resistance, the HOMA of insulin resistance (HOMA-IR) index was used, where a fasting glucose sample and 3 consecutive fasting insulin samples (5 minutes apart) were incorporated into the HOMA calculator (©The University of Oxford 2004, available to download from http://www.dtu.ox.ac.uk). In addition to HOMA-IR, HOMA of beta-cell function (%B) and HOMA of insulin sensitivity (%S) were also calculated; %B and %S are expressed as percentages of a normal reference population. As the distribution of HOMA-IR, %B and %S were not normal (Shapiro-Wilk test of normality p-values<0.001) in the subgroups of the sub-cohort, the Mann-Whitney U test was used to compare means between the subgroups that had relatively small numbers of subjects, and a one-way ANOVA test was used to compare means between the CKD and MHD subgroups.

The calculations revealed that there was statistically significant difference between HOMA-IR and %S means between all subgroups (CKD and MHD, and with diabetes and without within each group – see table 4.2.2 below), but the difference between mean %B values did not reach statistical significance in any of the subgroups. The fact that HOMA-IR and %S behave similarly is due to the nature of the measure; %S is simply the reciprocal of HOMA-IR [378]. Having said that, it is important to keep in mind that while %S values may be used in subjects on insulin therapy, its use has not been validated among patients receiving exogenous insulin. As for %B, it cannot be used for subjects on insulin therapy as it measures beta-cell functionality based on insulin secretion, which cannot be accurately measured in
subjects receiving exogenous insulin [378]. Therefore, the only index that can be used in all subgroups is HOMA-IR.

<table>
<thead>
<tr>
<th></th>
<th>HOMA2-IR</th>
<th>%B</th>
<th>%S</th>
<th>Mann-Whitney U Results</th>
<th>ANOVA Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (105)</td>
<td>2.08±202</td>
<td>121.61±112.59</td>
<td>99.0±75.80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CKD-all (55)</td>
<td>2.63±2.31</td>
<td>140.81±145.12</td>
<td>73.57±59.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CKD-DM (33)</td>
<td>3.29±2.56</td>
<td>133.22±154.20</td>
<td>51.68±36.42</td>
<td>P(IR)= 0.003 P(%B)= 0.135 P(%S)= 0.004</td>
<td>P(IR)= 0.004 P(%B)= 0.076 P(%S)&lt; 0.001</td>
</tr>
<tr>
<td>CKD-non-DM (22)</td>
<td>1.65±1.46</td>
<td>152.19±133.03</td>
<td>106.41±71.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MHD-all (50)</td>
<td>1.50±1.46</td>
<td>101.68±55.35</td>
<td>126.08±83.43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MHD-DM (19)</td>
<td>2.45±1.85</td>
<td>96.46±74.13</td>
<td>82.79±80.28</td>
<td>P(IR)= 0.001 P(%B)= 0.142 P(%S)= 0.001</td>
<td>-</td>
</tr>
<tr>
<td>MHD-non-DM (31)</td>
<td>0.91±0.70</td>
<td>104.87±40.99</td>
<td>152.62±74.74</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.2.2 Summary of HOMA indices within the different subgroups of the ‘sub-cohort’; comparison of means between the CKD and MHD groups is carried out using a one-way ANOVA test, and the comparison of means between the subgroups within the subgroups is carried out using the Mann-Whitney U test, which is a non-parametric test that compares means between two independent groups that are not normally distributed, as is the case here.

Comparing HOMA-IR between CKD and MHD group shows significantly higher HOMA-IR in the CKD group (p=0.004); in the MHD group, the diabetic patients predictably have significantly higher mean HOMA-IR. Of note is that the mean HOMA-IR in the non-diabetic subjects in the MHD group is below 1.0; this means that it is lower than general population with ‘normal’ insulin sensitivity. This suggests that while loss of kidney function causes insulin resistance, haemodialysis may correct the situation, and subjects’ insulin resistance returns to normal, and maybe
even below that. The cause of this lower insulin resistance than the general population, if true, may be a result of low BMI and an under-nourished state in these subjects. These results are comparable to those reported by other similar studies, where insulin resistance was shown to improve on dialysis [546-548]. However, it is important to note that the use of HOMA-IR has not been specifically validated in haemodialysed subjects; to attempt to overcome this as much as possible, I drew the blood samples for HOMA analysis from the patients right before they started the dialysis process.

Regression analyses were carried out to look at the effect of HOMA-IR on CVE and mortality, as with other risk factors in the whole cohort. Logistic regression did not show a significant effect for IR on CVE or mortality in the combined cohort or its subgroups.

The fact that CKD subjects have markedly elevated IR compared to the general population seems to go hand in hand with the fact that these subjects have higher incidence of CVE, for which IR is a proven risk factor of in the general population. However, as the MHD population has an even higher incidence of both CVE and mortality, it would be expected that if anything, they should be more insulin resistant. The fact that this is not the case may be due to a ‘reversed’ effect of IR on endpoints in the MHD population, and this hypothesis is in part confirmed by the OR below 1.0 for CVE and death in the MHD group, although this result is not statistically significant, the trend is toward a reverse effect. Longer follow-up may lead to significant results in this subgroup, which can then be used to support the hypothesis.

In summary:

- Insulin resistance as measured by HOMA-IR increases with decreasing renal function (increasing CKD stage), but returns to near normal levels in the MHD population

- In this population, increased HOMA-IR did not increase risk of CVE or mortality in the combined cohort or either subgroup
4.2.2 Inflammation

It was earlier explained that inflammation may induce an insulin resistant state and therefore be potentially responsible for CVD in insulin resistant individuals [405] (section 1.2.2.1). Inflammation is also present in both pre-dialysis CKD and on dialysis [414] and this is thought to be caused by a number of factors, including the accumulation of serum cytokines [415]. However, inflammation is primarily a concern for the dialysis population, and it has been used to explain the high CVD rate in this population [549, 550]. The logical assumption would then be that because inflammation is high in dialysis, so is insulin resistance. As discussed in the previous section, in this sub-cohort study insulin resistance was lower in the dialysis population, and that in itself is contrary to that logic. To see if the relationship between inflammation and insulin resistance is similar to that of the general population, a reliable marker of inflammation was measured in the sub-cohort, as well. CRP is an acute phase reactant that is commonly used as a marker of systemic inflammation, and a high sensitivity CRP test was used for this study, as this test allows for the more accurate measurement of CRP at lower levels.

Hs-CRP was measured in 92 subjects within the sub-cohort; 48 in the CKD subgroup and 44 in the MHD subgroup. As hs-CRP was not normally distributed in this sub-cohort (Shapiro-Wilk test, p<0.001), the Kruskal Wallis non-parametric test was used to compare means between subgroups (instead of the ANOVA, which assumes normality). For comparison between the larger subgroups, the student’s t-test was used [466]. These tests showed that hs-CRP significantly increased with loss of renal function, and was higher among the MHD population.

Looking at the effect of inflammation on endpoints, logistic regression was carried out for the whole sub-cohort, as well as the CKD and MHD subgroups. Log hs-CRP was used in the analyses, as hs-CRP was not a normally distributed variable. Hs-CRP showed a statistically significant effect in increasing the risk of CVE considerably in the CKD subgroup. It also showed significantly increased risk of mortality for the whole sub-cohort. Hs-CRP did not show a significant effect on mortality in the subgroups, or on CVE in the MHD group. Keeping in mind that the MHD group has significantly higher hs-CRP (p=0.003), this may mean that the MHD population have become resistant to the effects of the chronic inflammatory state on
the cardiovascular system. On the other hand, these results might be related to the relatively small sample size or the relatively short follow-up time, and may change with longer follow-up and more endpoints.

It is interesting that the MHD population studied here has a more inflammatory state compared to the CKD population, and is more insulin sensitive at the same time. Given the rich body of literature citing inflammation as an important factor in the pathogenesis of insulin resistance (as described in sections 1.2.1.4 and 1.2.2.1), perhaps the coexistence of these two conditions in the MHD population can also be classified as reverse epidemiology.

In summary:

- Hs-CRP levels in this population are highly elevated in some patients, but do not seem to form a pattern in regard to CKD stage and/or MHD status
- Increased hs-CRP is associated with increased risk of CVE in the CKD subgroup, but not in the MHD subgroup
- Increased hs-CRP seems to increase the risk of mortality in the combined cohort
4.2.3 Adiponectin

Adiponectin is thought to be important in regulating both insulin resistance and inflammation (detailed in section 1.1.4.3.2); it ‘improves’ both conditions and has been shown to be cardio-protective. Total adiponectin was measured to see whether it retained these qualities in the ESRD population and also if it was correlated with either insulin resistance or inflammation in this sub-cohort.

Comparison of mean total adiponectin levels between the different subgroups shows that total adiponectin consistently increases with progression of renal failure (p=0.041) but is lower in MHD, although it is still higher than the level in CKD stage 3. When comparing the means between the whole CKD subgroup and the MHD subgroup, the difference is not statistically significant, although the mean total adiponectin in both groups is well above the normal range in the general population (which is reported to be an estimated 10 ± 5 ug/mL [246, 551] in normal weight non-diabetic individuals). The fact that the difference between mean total adiponectin levels in CKD and MHD subgroups is not significant could explain why the CVE rates between these two subgroups are similar (21.8% in the CKD group and 21.6% in the MHD group).

To look at the effect of total adiponectin on endpoints, logistic regression analyses were performed in the whole sub-cohort, as well as the CKD and MHD subgroups. Interestingly, total adiponectin did not have a statistically significant effect on CVE in the whole sub-cohort or the CKD and MHD subgroups, but it did have a negative effect on mortality in the whole group and in the MHD subgroup; increasing total adiponectin increased the risk of death in these groups. This result is counterintuitive as adiponectin is thought to be protective. This finding may be secondary to the inflammatory state that is present in these individuals (adiponectin is anti-inflammatory and therefore increases in inflammatory states), or it may be due to reduced clearance by the kidneys. Another explanation may be that although total adiponectin is elevated, the isoforms circulating in these subjects have different physiological properties than those in the general population.

The fact that increased total adiponectin is associated with increased risk of mortality in this sub-cohort agrees with the limited literature on the subject. In non-ESRD subjects, the cardio-protective nature of adiponectin is mostly observed in
cross-sectional studies [552-554], and prospective studies such as that carried out by Kistorp et al. showed that high, rather than lower, adiponectin is associated with greater all-cause mortality rate [168]. A few cross-sectional studies on subjects with type 1 diabetes have also shown an increased prevalence of CVD and retinopathy with higher adiponectin levels [555-557]. Although experimental evidence strongly confirms the role of adiponectin in reducing CVD risk, epidemiologic studies have not yet reached a conclusive role for the molecule. In the CKD population, both non-dialysed and dialysed, studies have observed a cardio-protective nature for adiponectin [558-560], but none showed adiponectin to be similarly associated with all-cause mortality.

Menon et al. used frozen samples and follow-up data from the Modification Diet in Renal Disease Study [561] to look at the effect of adiponectin on CV death and all-cause mortality in the cohort, which consisted of subjects with CKD stage 3 and 4. Their results showed a direct relationship between adiponectin and all-cause as well as CV mortality [562]. The results of the present study confirm these findings.

In summary:

- Total adiponectin levels increase with decreasing renal function but are not normalised with haemodialysis
- Total adiponectin levels are similarly significantly higher than normal in the CKD and MHD group
- In this population, higher adiponectin is associated with increased mortality in the combined group as well as in the MHD subgroup, but has no significant effect on CVE
4.3 Glycaemic Control in Haemodialysis

The need to balance glycaemic targets to avoid hypoglycaemia with the risks of microvascular disease from hyperglycaemia requires accurate glycaemic assessment. This is especially so for diabetic patients on MHD who have a high prevalence of microvascular and macrovascular disease and are at an increased risk of asymptomatic hypoglycaemia during HD [563]. Continuous subcutaneous glucose monitors are ideally suited for diabetic patients on MHD, as unlike the HbA1c they can examine short-term glycaemic changes around the time of dialysis and are unaffected by urea, RBC life-span and RBC production.

The subjects in this study were typical of the UK type 2 diabetes MHD population with a long duration of diabetes (18.8 ±7.6 years) and high prevalence rates of established vascular disease (15/17). This preliminary study suggests that CGM offers clinically useful data for such a high risk group.

4.3.1 Reliability of HbA1c

The need to set the appropriate glycaemic targets for type 2 diabetic patients with a high CVD risk was highlighted by the Action to Control Cardiovascular Risk in Diabetes study (ACCORD) [439] and the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) [442] randomized controlled trials. These studies recruited subjects at high risk of CVD and neither showed any CVD benefit from targeting HbA1c levels below 6.5% and 7%, respectively. The ACCORD study actually showed a small increase in overall mortality when targeting HbA1c below 6.5%. That low HbA1c levels may not confer survival benefit in ESRD was also suggested by a one year follow-up study of 23,000 American diabetic subjects [132]. By contrast good glycaemic control prior to dialysis does appear to have some CVD benefit [446]. Thus, it may be necessary for HbA1c targets originally based on low CVD non-CKD populations to be re-evaluated for diabetic haemodialysis patients.

The HbA1c is a measure of the irreversible non-enzymatic glycation product of one or both NH₂-terminal valines of the beta-hemoglobin chain. In ESRF the HbA1c assay can be affected by interference from carbamylated haemoglobin formed from urea-derived isocyanate that accumulates in uraemia [448]. However advances in
reverse-phase cation exchange HPLC analyzers, as used in this study, allow for greater haemoglobin peak separation [564].

Shortened red blood cell (RBC) life-span or increased RBC production [451] can occur in ESRD and both can falsely lower HbA1c values by reducing the RBC glycaemic exposure time. However, a study of 23 MHD patients on regular erythropoietin therapy and with stable haemoglobin values, concluded that it was the ambient glucose concentration rather than RBC life-span that was the major determinant, as no correlation between RBC life-span and HbA1c measured by either immunoassay or HPLC was shown [452]. Starting or increasing erythropoietin treatment could, by increasing RBC production, falsely lower HbA1c values by increasing the proportion of younger RBCs and thereby reducing glucose exposure time. In the present study all subjects had stable haemoglobin values and their erythropoietin doses had remained constant over the preceding 3 months.

In this study of stable diabetic MHD out-patients the HbA1c value obtained using a methodology unaffected by ureamia and the predicted HbA1c derived from 48 hour CGM profiles were discordant by 1.7% (6.9 ± 1.2% vs. 8.6 ± 2.3%). The lack of correlation between the laboratory HbA1c value and the haemoglobin concentration, erythropoietin dose, urea or serum albumin suggests that these variables do not explain the observed differences in the laboratory HbA1c values and the predicted HbA1c. However the sample size may have been too small to detect weak associations between laboratory HbA1c and these variables.

It is highly likely that the degree to which the HbA1c under-reported glycaemic profiles in these subjects led to a false sense of clinical reassurance concerning their diabetic management, and prevented changes to their diabetic medications.

The clinical implications of this study are that HbA1c analyses that are not affected by carbamylated haemoglobin may still under-report HbA1c in stable MHD patients.

**4.3.2 Effect of dialysis on glycaemia**

The present study showed over a 2 day period that the GlucoDay®S CGM device recorded significantly higher glucose profiles on the day off dialysis than the day on dialysis and that the overall level of glycaemia was higher than the laboratory
HbA1c suggested.

The CGM glucose values during the first 24-hour monitoring period (day of dialysis), including the six-hour nocturnal period were significantly less than the second 24-hour monitoring period. During the hours of midnight to 6.00am, the only common time all subjects were resting and not eating, the magnitude of this difference ranged from -8.5 to 17.1 mmol/L, with a median difference of 4.2 mmol/L. These differences in glucose profiles were not explained by the difference in 24-hour energy intake, changes in medication or dialysis shift. However, there are potential limitations over the accuracy of the food diaries, as data was collected over 48 hours only and was self-reported. The food data did highlight that all subjects were likely to be malnourished as they consumed less than their recommended intake.

The CGM data also showed that 4 subjects had hypoglycaemia, <2.5 mmol/L over 30 minutes or more, and that this occurred within 24 hours of dialysis in 3 subjects. The lowest glucose recording for all subjects was within the first 24-hour period, with the majority being within 12 hours of dialysis.

The differences between the days on and off dialysis also suggest that for some patients’ increases in medication doses should be given on the days off dialysis to improve control, while hypoglycaemic therapy may need to be reduced for the day on dialysis or delayed according to the time of dialysis.

Larger studies on MHD populations will be required to determine if data from CGM should be used for medication adjustments around dialysis days in order to optimise glycaemic control and avoid hypoglycaemia.

In summary:

- HbA1c may not be a reliable tool for assessing glycaemic control in diabetic subjects on MHD, as it often underestimates glycaemia

- Glycaemic profiles of haemodialysed diabetic subjects are significantly different on dialysis and on off-dialysis days

- Patients are at risk of hypoglycaemia within the first 12 hours of being dialysed
• The calorific intake of subjects in this study was significantly lower than their recommended daily intake
4.3 Study Limitations

This is a cohort study, where patients with ESRD are prospectively followed and the endpoints under investigation are CVE and mortality. Ideally, in a cohort study subjects are recruited at a single point in treatment or disease and followed over time, whereas in this study subjects were recruited from all ages, with differing degrees of renal failure, and differing durations of diabetes and haemodialysis. While this is more practical as it provides us with data in a shorter duration of follow-up, the limitation caused by this is that our cohort may be representing survivors on haemodialysis or with diabetes, and therefore biased. Subjects that have survived longer on MHD and have fewer or less serious co-morbidities may be over-represented and this would lower the risks associated with endpoints. Similarly, in the CKD population, subjects with longstanding CKD 3 or 4 may have stabilized and therefore not have an equal risk for endpoints compared to their counterparts. If these points were to be taken into account for a future study, such a study would need a much longer follow-up duration in order to produce results, which would perhaps be impractical.

The other limitation is that because patients were recruited from an already existing patient population, the data used is the information collected for patient care in practice, which is limited in both nature and quality. Although this type of study is valuable in that it uses readily available data and therefore considerably reduces costs, the data collected is not necessarily all of what is needed to answer specific research questions, risk factor data may be missing for some patients. However, the fact that laboratory tests were done at a centralised laboratory means that their results are quality controlled and standardised. Lastly, this study did not capture any silent MIs.

The fact that the serum samples were a one-off sample and follow-up samples were not taken limits this study. Follow-up samples would have helped establish whether increase or decrease in the measured factors leads to better outcomes. The study was initially designed to recruit 20 subjects right before starting MHD to collect samples from, and take follow-up samples from 8 weeks into MHD. Unfortunately, most of the subjects approached did not agree to take part in the study within the given timeframe, and of the few who did (a total of 7), only one actually completed
the study with a follow-up sample. Once this part of the study is carried out, individual changes in insulin resistance, inflammation and adiponectin can be correlated with outcomes, and a clearer role can be described for these factors in ESRD.

Studies designed to look at effects of different factors on survival outcomes usually require follow-up over a long period of time; examples of such studies include the Framingham Study, the UKPDS and the EDIC/DCCT study. As the mortality rate is quite high in the ESRD and especially in the dialysed population, such very long studies are impractical. For this patient population, with an annual mortality rate of around 20%, a follow-up period of five years is practical and can reveal useful additional information, as the majority of patients would have reached an endpoint by then. Longer follow-up time means that more patients are likely to fall out of ICKTI’s care and therefore be lost to follow-up. Revisiting the present database at months 36, 48 and 52 is recommended to continue follow-up and maximise the use of its data. The implementation of the NHS Care Records Services (CRS) in the near future (scheduled for launch at Imperial College Healthcare NHS Trust in mid-2010) means that detailed follow-up of patients will most likely become less difficult, and even if patients move out of the Trust’s care, an integrated comprehensive digital recording database will be at hand to monitor mortality.
**Chapter 5: Conclusions**

**5.1 Cardiovascular risk factors in ESRD**

In the combined cohort, high systolic blood pressure, diabetes and low total cholesterol were identified as risk factors for cardiovascular events. BMI, diastolic blood pressure and triglycerides had no significant effect on risk of cardiovascular events. Risk factors for all-cause mortality were slightly different, with diabetes, low total cholesterol and low triglycerides as significant risk factors for mortality. BMI and systolic and diastolic blood pressure did not have a significant effect on risk of death in this cohort.

In the CKD subgroup, the only significant risk factor identified for cardiovascular events was low total cholesterol. BMI, systolic and diastolic blood pressure, triglycerides and diabetes had no significant effect on risk of cardiovascular events in the CKD subgroup. The risk factors for all-cause mortality in the CKD subgroup were identified as: diabetes, low systolic and diastolic blood pressure, low total cholesterol and low triglycerides. BMI did not have a significant effect on risk of death in the CKD subgroup.

In the MHD subgroup, only diabetes was a significant risk factor for cardiovascular events. BMI, systolic and diastolic blood pressure, total cholesterol and triglycerides had no significant effect on risk of cardiovascular events in the MHD subgroup. The only risk factor for mortality in this subgroup was low triglycerides. The presence of diabetes also had a strong trend towards increasing risk of death in this population, but the result did not reach statistical significance. BMI, systolic and diastolic blood pressure and total cholesterol had no significant effect on risk of death in the MHD subgroup.

These results confirm the study’s first hypothesis: ‘patients on haemodialysis have different cardiovascular risk factors compared to CKD patients’. However, although it was initially thought that ‘reverse epidemiology’ is present in the MHD population, in the cohort studied here it is the CKD population that shows aspects of reverse epidemiology (BP, TC and TG), and this reversal of risk factors was overall more evident for all-cause mortality risk, and less so for cardiovascular risk.
5.2 Insulin resistance and inflammation in ESRD

In the sub-cohort study, it was observed that Insulin resistance as measured by HOMA-IR increases with decreasing renal function (increasing CKD stage), but returns to near normal levels in the MHD population.

None of the potential risk factors thought to be involved in cardiovascular event risk, i.e. insulin resistance (HOMA-IR), inflammation (hs-CRP) and total adiponectin, showed a significant effect on risk of cardiovascular events in this study. However, elevated hs-CRP and total adiponectin levels had a significant effect on increasing the risk of death. HOMA-IR did not affect risk of mortality in this group.

In the CKD group within the sub-cohort, high hs-CRP was identified as a cardiovascular risk factor. HOMA-IR and total adiponectin did not have a significant effect on cardiovascular risk in this group. None of the factors studied here showed a significant effect on risk of mortality in the CKD group within the sub-cohort.

In the MHD group within the sub-cohort, none of the potential risk factors studied had a significant effect on risk of cardiovascular events. High total adiponectin was identified as a predictor of mortality in this group, while HOMA-IR and hs-CRP had no significant effect on risk of death in this MHD subgroup.

The results confirm the study’s second hypothesis: ‘although the MHD population is known to be insulin resistant, insulin sensitivity is improved once patients commence haemodialysis compared to the preceding late CKD stages’. Moreover, these results show that changes in insulin resistance are independent of changes in hs-CRP and total adiponectin. They also suggest that insulin resistance may not be an important predictor of outcome in the ESRD population.
5.3 Glycaemic control on haemodialysis

This part of the study showed that HbA1c may not be a reliable tool for assessing glycaemic control in diabetic subjects on MHD, as it often underestimates glycaemia even in subjects with stable haemoglobin. Furthermore, the study identified a potentially dangerous pattern in the glycaemic profile of diabetic subjects on MHD: that mean glucose values are consistently significantly lower on dialysis days compared to non-dialysis days. This effect seems to be independent of food intake, as there was no significant difference in calorific intake between on and off dialysis days in this population.

It was also observed that the majority of subjects had their lowest glucose levels within the first 12 hours of being dialysed, which can put these subjects at an increased risk of asymptomatic hypoglycaemia during this time. Furthermore, it was observed that the total calorific intake of subjects in this study was significantly lower than their recommended daily intake, which suggests better dietetic input may be beneficial for this population.

These results confirm the study’s third hypothesis: ‘HbA1c may not be an accurate measurement of glycaemic control among the haemodialysed population with diabetes, thus warranting new methods for assessing glycaemic control in this population’. Given the significant variability in glycaemic profiles of this population, it is perhaps better not to use long term estimates of glycaemia, but rather more frequent short and medium term indicators of circulating glucose, which may help better tailor hypoglycaemic treatment for diabetic subjects on MHD.
5.4 Future Research

The present study is based primarily on 24-month follow up data of the cohort described. Longer follow up time will lead to more endpoints, and the statistical analyses will become more powerful as number of endpoints increase. Most ‘risk engine’ studies run for a minimum of 5 years; with the 20% cardiovascular event rate over 24 months, the ideal amount of time for this study to follow its subjects would be 10 years, or until all subjects have reached an endpoint. Lack of statistical significance for some risk factors may then be interpreted more robustly.

As this study was observational in design, it did not explore potential mechanisms for its findings. Further research is needed to identify potential regulatory substances that may be involved in causing the discrepancy between risk factors in the CKD and MHD populations.

To determine whether the increased insulin resistance observed in the CKD population is a result of accumulation of insulin due to loss of kidney function or the result of a metabolic change in the subjects, a further study could be designed to look at insulin mediated glucose uptake of adipocytes in these subjects. Adipocytes could be donated by renal transplant recipients, which would include both CKD and MHD patients and allow for comparison of adipocyte behaviour in the two populations.

To further explore the effect of haemodialysis on glycaemia, future research is needed to compare glycaemic profiles of diabetic and non-diabetic subjects to establish whether the variability observed in the diabetic population in this study is present in the non-diabetic population, and if so whether the non-diabetic haemodialysed patient is at risk of haemodialysis induced asymptomatic hypoglycaemia. Further studies should also be designed to identify better markers for glycaemia, as HbA1c may not be a reliable marker in these subjects.
References


40. Bright, R., Cases and observations, illustrative of renal disease accompanied with the secretion of albuminous urine. Guys Hosp Rep 1836. 1(338).


428. RÉGis, B. and K. Bernard, C-reactive protein levels as a direct indicator of interleukin-6 levels in humans in vivo. 1992. p. 982-983.


Assessing Glycemic Control in Maintenance Hemodialysis Patients With Type 2 Diabetes

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OBJECTIVE — Optimizing glycemic control in diabetic patients undergoing maintenance hemodialysis requires accurate assessment. We hypothesize that 1) 48-h continuous glucose monitoring (CGM) provides additional information that would provide the AIC measurement and 2) glycemic profiles differ significantly between day on and day off dialysis.

RESEARCH DESIGN AND METHODS — With the use of GlucoDay 5, 48-h CGM was performed in 19 type 2 diabetic subjects undergoing hemodialysis to capture 2 consecutive 24-h periods on and off dialysis. Energy intake was calculated using food diaries. AIC was assessed by a high-performance liquid chromatography method.

RESULTS — CGM data were available for 17 subjects (13 male) with a mean (range) age of 61.3 years (42–79 years) and diabetes duration of 16.8 years (4–30 years). The 24-h CGM area under the glucose curve and 24-h mean glucose values were significantly higher during the day off dialysis than on dialysis: 5.3 ± 0.7 (4.6 ± 0.8) mmol/L. The AIC on day off was 0.022, and 12.6 ± 3.6 vs. 9.8 ± 3.8 mmol/L, P = 0.013, respectively, independent of energy intake. Asymptomatic hypoglycemia occurred in 4 subjects, within 24 h of dialysis, and the glucose nadir in 14 subjects occurred within 24 h of dialysis.

CONCLUSIONS — Glucose values are significantly lower on dialysis days than on nondialysis days despite similar energy intake. The risk of asymptomatic hypoglycemia was highest within 24 h of dialysis. Physicians caring for patients undergoing hemodialysis need to be aware of this phenomenon and consider enhanced glycemic monitoring after a hemodialysis session. CGM provides glycemic information in addition to AIC, which is potentially relevant to clinical management.

Diabetic nephropathy is the leading cause of end-stage renal failure (ESRF) (1), representing 30–45% of the U.K. and U.S. (2) populations undergoing long-term maintenance hemodialysis. The patients typically are elderly type 2 diabetic patients with established micro- and macrovascular disease (3). Hypoglycemia is common because of impaired renal glucose reabsorption, malnutrition, and the increased half-life of insulin and hypoglycemic agents (4). The annual morality among diabetic patients undergoing hemodialysis is high and is predominantly due to cardiovascular disease (CVD) (2).

Intensive glycemic management delays progression of microvascular disease (5–8) and improves malnutrition (9), however, large randomized controlled trials show no mortality benefit in high-risk groups with CVD (7,10). Hypoglycemic events increase with intensive treatment and in the presence of CVD can cause fatal dysrhythmia (11). U.K. diabetes guidelines recommend that this provided by the AIC measurement and 2) glycemic profiles differ significantly between day on and day off dialysis.

In patients without ESRF, the AIC value is routinely used to assess long-term glycemic control, and assays are standardized to those used in the Diabetes Control and Complications Trial (11). There is a strong correlation between AIC values and the weighted mean glucose values of the preceding 2–3 months (12). The validity of the AIC measurement in patients with ESRF undergoing hemodialysis depends on the methodology (13). A number of factors may influence the assay including altered red blood cell (RBC) life span and metabolic and cellular factors (10). Potential metabolic factors are interference from carboxymethylated hemoglobin formed in uremia and acetylated hemoglobin formed from long-term aspirin use (17).

A limitation of the AIC value in patients undergoing hemodialysis is that it is not informative regarding glycemic control on the days on and off dialysis. In the U.K., maintenance hemodialysis is typically given in a hospital setting three times a week, with sessions lasting 4–5 h. The CGM devices that measure glucose every 3 min using a biosensor and a subcutaneous microbore cannula are, in contrast, ideally suited to examine the effect of dialysis on glucose profiles over a 48-h period. Thus, in the present study we test the hypotheses 1) that 48-h CGM provides additional, clinically relevant, information that would provide the AIC measurement in patients undergoing hemodialysis and 2) that 24-h glucose profiles are different on the day that includes a dialysis session compared with those on a day that does not.

RESEARCH DESIGN AND METHODS — The study was approved by the Hammersmith Hospital Research Ethics Committee, and written consent was obtained from all subjects. ACGM data were collected over 3 consecutive days, with the first day serving as a control day and the second and third days serving as the dialysis days. The AIC measurement was performed by a high-performance liquid chromatography method.
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*Documented history of vascular disease defined as ischemic heart disease (history of myocardial infarction, revascularization procedure or angiographically proven coronary disease), cerebrovascular disease (history of cerebrovascular accident or transient ischemia attack) or peripheral vascular disease (history of amputation due to gangrene, revascularization procedure, or peripheral vascular disease proven angiographically or by Doppler ultrasonography). F: female; M, male.

Informed consent was obtained (Registration No. 2002/6260). Study objectives were to compare glucose profiles from days on and off dialysis using 48-h CGM in type 2 diabetic patients, to examine the association between self-reported food intake and CGM values, and to evaluate glycemic assessment obtained using 48-h CGM in type 2 diabetic patients undergoing maintenance hemodialysis.

Nineteen (14 male) type 2 diabetic subjects were recruited from the maintenance hemodialysis program at Imperial College Kidney and Transplant Institute (ICKT). Dialysis was performed against a <2 mmol/l glucose-containing dialysate for 4 -5.5 h during the morning, afternoon, or early evening. Inclusion criteria were a stable hemoglobin level, defined as a <10% change in hemoglobin value and no blood transfusion in the preceding 3 months, a stable dose of erythropoietin, and no hemoglobinopathy. A history of CVD was established as documented ischemic heart disease (history of myocardial infarction, revascularization procedure, or angiographically proven coronary disease), cerebrovascular disease (history of cerebrovascular accident or transient ischemia attack), or peripheral vascular disease (history of amputation due to gangrene, revascularization procedure, or peripheral vascular disease proven angiographically or by Doppler ultrasonography).

**Blood samples**

Blood samples were taken at the start of dialysis for measurement of A1C, hemoglobin, albumin, and urea.

**CGM**

Day 1. Subjects attended ICKTI, and before they started dialysis a GlucoDay S CGM device from A Menarini Diagnostics (Florence, Italy) (18) was fitted and placed in a pouch to be worn around the waist. The CGM biosensor was calibrated retrospectively using capillary blood glucose testing as advised by the manufacturer.

Day 3 (48 h later). Subjects attended ICKTI, and before their dialysis session the CGM device was removed. The data were downloaded to a computer using dedicated software (GlucoDay S Data Preanimation Software).

**Exclusion criteria**

Exclusion criteria were prospectively defined as type 1 diabetes, intercurrent illness, changes to medication regimen during the monitoring period, or occurrence of prolonged hypoglycemia.

**Patient diaries**

On day 1, subjects were given a 48-h diary to record the exact time and amount of food, drink, and medications taken during the entire CGM monitoring period, together with any episodes of symptomatic hypoglycemia and all capillary blood glucose results.

**Laboratory analysis**

A1C measurements were performed in the hospital's clinical biochemistry laboratory using a Diabetes Control and Complications Trial-aligned HbA1C Autoanalyzer (A. Menarini Diagnostics). This analyzer is not subject to interference by urea, as this reverse-phase cation exchange high-performance liquid chromatography (HPLC) method provides good separation of A1C from carboxamidated hemoglobin A1. Hemoglobin measurements were performed in the hospital's routine hematology laboratory using a XE2100 autoanalyzer (Sysmex, Kobe, Japan) running a variation of the CyMet hemoglobin absorbometric method. Serum urea and serum albumin tests were performed on an Architect ci8200 multichannel analyzer (Abbott Diagnostics, North Chicago, IL).
Assessment of glycemic control

The 24-h glucose profiles were quantified, using dedicated software (glucomark Data Presentation Software), as the area under the 3-min glucose curve (AUC) and the mean glucose value. The time periods studied were the first 24-h period starting the first hour of dialysis (day on dialysis) and the 24-h period ending 1 h before the next dialysis session (day off dialysis). The 6-h nocturnal periods from midnight to 6:00 a.m. for each of these 24-h periods were also examined to determine the effect of dialysis. Hypoglycemia, defined as a continuous glucose reading <2.5 mmol/L for >30 min, was identified from the CGM profiles. Subjects were questioned regarding symptoms of hypoglycemia at the end of the CGM period.

Dietetic analysis

Completed food diaries were checked during a dietary consultation with a registered dietitian. Food portions were verified using a pictorial food atlas (19). Comparisons of dietary intake during the 24-h periods on and off dialysis were performed by a data analyst blinded to the study using the Dietplan software package (Forefield Software). The daily energy requirement was calculated to be 30–35 kcal/kg ideal body weight (20).

Statistical analysis

The CGM data were exported into SPSS software (version 14; SPSS for Windows; LEAD Technologies) and tested for normality using the Shapiro-Wilk test.

All normally distributed data are expressed as means ± SD and nonnormally distributed data are expressed as median (range). All comparisons of the glycemic profiles and dietary intake between days on and off dialysis were analyzed using paired Student’s t tests. Linear regression analysis was used to assess the relationship between laboratory A1C and weekly erythropoietin dose, serum albumin, and urea levels. The level of significance was defined as P < 0.05.

RESULTS — Nineteen (14 male) subjects were recruited, and 2 were subsequently excluded, one because of repeated hypoglycemia during both monitoring periods and one because of CGM technical failure. The age, duration of diabetes, and years of dialysis (mean ± SD) of the 17 (13 male) subjects included were 63.5 ± 8.8 years (42–79 years), 18.8 ± 7.6 years (4–30 years), and 4 ± 2.6 years (0.5–10.2 years), respectively. Previous CVD history, diabetes medications, erythropoietin dose, A1C, hemoglobin, and urea values are given in Table 1.

A1C values

The A1C (mean ± SD) was 6.9 ± 1.2% (range 5.1–9.2%), with seven subjects having A1C ≤6.5% (Table 1). Results of linear regression analysis among A1C and weekly erythropoietin dose, serum albumin, and urea were not significant (r² = 0.17, P = 0.0995; r² = 0.161, P = 0.536; and r² = 0.163, P = 0.533, respectively).

Hemoglobin values

Mean ± SD hemoglobin was 12.4 ± 1.6 g/dl (range 9.3–15.1 g/dl).

Analysis of glycemic profiles

The 24-h AUC glucose values and mean 24-h CGM data were significantly higher the day off dialysis than the day on dialysis (5.632 ± 2.673.6 mmol·3 min⁻¹·1⁻¹ vs. 4.604 ± 1.988 mmol·3 min⁻¹·1⁻¹ on the day off dialysis vs. 4.604 ± 1.988 mmol·3 min⁻¹·1⁻¹ on the day on dialysis, P = 0.0223). Mean ± SD CGM glucose values for the whole group were 12.6 ± 5.6 mmol/L on the day off dialysis vs. 9.8 ± 3.8 mmol/L on the day on dialysis (P = 0.013).

Analysis of hypoglycemia

Four of the 17 subjects had CGM recordings of <2.5 mmol/L for >30 min; in 3 subjects, this recording occurred in the
Figure 2.—Nocturnal CGM data for the 6-h period from midnight to 6:00 A.M. for day on (night 1) and day off (night 2) dialysis, expressed as AUC glucose (A) and mean glucose (B). — data for individual subjects; mean ± SD for each 24-h period. A: Mean ± SD area under the 3-min glucose curve for the whole study group was 1,541 ± 834 mmol·3 min⁻¹·l⁻¹ for the night of the day off dialysis (night 2) vs. 1,137 ± 529 mmol·3 min⁻¹·l⁻¹ for the night of the day on dialysis (night 1) (P < 0.05). B: Mean ± SD CGM glucose values for the whole group were 12.9 ± 7.0 mmol/l on night 2 vs. 9.5 ± 4.4 mmol/l on night 1.

first 24-h monitoring period. Examination of individual CGM profiles showed that 14 of 17 subjects reached their glucose nadir (range 1.38–9.81 mmol/l) within the first 24 h, with 10 of 17 having their lowest reading within 12 h of starting dialysis. No subject reported any episode of symptomatic hypoglycemia.

Analysis of the food diaries
Two subjects failed to complete their 48-h food diaries (subjects 6 and 15). Analysis of the 15 completed diaries showed no significant difference between recorded dietary intakes for the day on dialysis and the day off dialysis (1,636 ± 603 vs. 1,702 ± 559 kcal, respectively, P = 0.596). There was no trend toward greater food intake on either day, with 7 subjects recording a greater calorie intake during the day on dialysis versus 8 during the day off dialysis. The timing of the dialysis shift did not appear to influence the energy intake (data not shown). The total energy intake for each subject was significantly lower, both on dialysis days (mean 1,636 kcal/day) and off dialysis days (mean 1,702 kcal/day), than the estimated mean energy requirement (2,000 kcal/day) (P = 0.01; data not shown).

Medications
No subject recorded a change in frequency or dosing of medications, including insulin, on the 2 days.

CONCLUSIONS—The need to balance glycemic targets to avoid hypoglycemia with the risks of microvascular disease from hyperglycemia requires accurate glycemic assessment. This is especially so for diabetic patients undergoing maintenance hemodialysis, who have a high prevalence of microvascular and macrovascular disease and an increased risk of asymptomatic hypoglycemia during hemodialysis (21). The use of continuous subcutaneous glucose monitors is ideally suited for diabetic patients undergoing hemodialysis because, unlike AIC measurements, they can examine short-term glycemic changes around the time of dialysis and are unaffected by urea, RBC life span, and RBC production.

The need to set the appropriate glycemic targets for type 2 diabetic patients with a high CVD risk was highlighted by the Action to Control Cardiovascular Risk in Diabetes (ACCORD) (7) and the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) (8) randomized controlled trials. These studies recruited subjects at high risk of CVD, and neither showed any CVD benefit from targeting AIC levels <6.5 and 7%, respectively. The ACCORD study actually showed a small increase in overall mortality when AIC <6.5% was targeted. That low AIC levels may not confer survival benefit in ESFR was also suggested by a 1-year follow-up study of 23,000 American diabetic subjects (22). In contrast, good glycemic control before dialysis does appear to have some CVD benefit (23). Thus, it may be necessary for AIC targets originally based on low-risk populations without chronic kidney disease to be reevaluated for diabetic patients undergoing hemodialysis.

The AIC is a measure of the irreversible nonenzymatic glycation product of one or both NH2-terminal valines of the β-hemoglobin chain. In ESFR, the AIC assay can be affected by interference from carboxymethylated hemoglobin formed from urea-derived isocyanate that accumulates in uremic (24). However, advances in reverse-phase cation exchange HPLC analyzers, as used in this study, allow for greater hemoglobin peak separation (25). Shortened RBC life span or increased RBC production (16) can occur in ESFR, and both can falsely lower AIC values by reducing the RBC glycemic exposure time. However, a study of 23 patients undergoing hemodialysis who were receiving regular erythropoietin therapy and had stable hemoglobin values concluded that the ambient glucose concentration rather than RBC life span was the major determinant, as no correlation between RBC life span and AIC measured by either immunossay or HPLC was shown (26). Starting or increasing erythropoietin treatment could, by increasing RBC pro-
duction, falsely lower AIC values by increasing the proportion of younger RBCs and thereby decreasing glucose exposure time. In the present study, all subjects had stable hemoglobin values and their crythroidin doses had remained constant over the preceding 3 months.

Furthermore, the AIC value may be less informative in the type 2 diabetic population undergoing maintenance hemodialysis and may be easily translated into mean glycemic values than in other populations. The AIC Derived Average Glucose Study Group (ADAG) investigators recently reported (27) that AIC levels can be converted to average glucose levels in type 2 diabetic patients. However, patients with chronic kidney disease were excluded from this study, and it is possible that the metabolic fluctuations seen with hemodialysis may weaken the relationship between AIC and average glucose. CGM is one alternative to AIC for assessing glycemia.

The present study showed that over a 2-day period the Clucoday5 CGM device recorded significantly higher glucose profiles on the day of dialysis than the day on dialysis. The CGM glucose values during the first 24-h monitoring period (day of dialysis), including the 6-h nocturnal period, were significantly less than those for the second 24-h monitoring period. During the hours of midnight to 6:00 a.m., the only common time all subjects were resting and not eating, the magnitude of this difference ranged from 8.5 to 17.1 mmol/L, with a median difference of 4.2 mmol/L. These differences in glucose profiles were not explained by the difference in 24-h energy intake, changes in medication, or dialysis shift. However, there are potential limitations regarding the accuracy of the food diaries, as data were collected over 48 h only and were self-reported. The food data did highlight the fact that all subjects were likely to be malnourished because they consumed less than their recommended intake.

The CGM data also showed that 4 subjects had hypoglycemia (<2.5 mmol/L over >30 min) and that this occurred within 24 h of dialysis in 3 subjects. The lowest glucose recording for 14 subjects was within the first 24-h period, with the majority being within 12 h of dialysis. Thus, the results of our study suggest that type 2 diabetic patients undergoing maintenance hemodialysis, who already have a very high risk of developing morbidity and mortality, may have an increased risk of hypoglycemia in the 24-h period after a dialysis session. Current renal practice includes assessment of glycemic control by blood glucose measurements while the patient is undergoing hemodialysis. However, physicians caring for these patients need to be aware of this phenomenon and consider enhanced monitoring for these patients who may develop hypoglycemia several hours after they have left the dialysis unit.

The subjects in this study were typical of the U.K. type 2 diabetic population with a long duration of diabetes (18.8 ± 7.6 years) and high prevalence rates of established vascular disease (15 of 17 subjects) who are undergoing hemodialysis. Our preliminary study suggests that CGM offers clinically useful data for such a high-risk group. Larger studies on populations undergoing hemodialysis will be required to determine whether data from CGM should be used for medication adjustments around dialysis days to optimize glycemic control and avoid hypoglycemia.

In summary, as glycemic targets become redefined to avoid overaggressive management in individuals with high CVD risk, it is important that the measurements of glycemic control in patients undergoing hemodialysis are as informative as possible.

Acknowledgments — We are grateful for support from the National Institute for Health Research Biomedical Research Centre funding scheme. S.K.-A. is funded by the King Faisal Foundation. J.J.O.T. is a British Heart Foundation intermediate fellow. This study was supported by the Biomedical Research Centre at Imperial College’s Academic Health Science Centre.

No potential conflicts of interest relevant to this article were reported.

We thank A Menarini Diagnostics for providing us with the CGM monitors.


References


CGM assessment in dialysis


Assessing Glycemic Control in Maintenance Hemodialysis Patients With Type 2 Diabetes

Response to Kazempour-Ardehli et al.

We read with great interest the article by Kazempour-Ardehli et al. (1), studying the glycemic control in maintenance hemodialysis patients with type 2 diabetes. It was particularly interesting because we recently published an article on the same topic and found quite similar results while using another device for continuous glucose monitoring (2).

Two important points must be acknowledged in this regard: 1) The study by Kazempour-Ardehli et al. did not evaluate the accuracy criteria of GlucoDay in this special population with brittle diabeties. By contrast, in our work (2), we showed that the continuous glucose monitoring system is an accurate tool in type 2 hemodialysis patients: the relative absolute difference between glucose levels determined by the glucose meter and that determined by the continuous glucose monitoring system did not differ significantly between type 2 hemodialysis patients and nonhemodialysis type 2 diabetic control subjects (9.2 ± 10.5 vs. 8.2 ± 7.6; P = 0.165). 2) The glucose content in the dialysate is clearly different in the two articles. It is interesting that in the article by Kazempour-Ardehli et al., the dialysate contained a low-glucose concentration, while the dialysate glucose concentration in our study was at 5.5 mmol/l (100 mg/dl). This could explain the clear discrepancy between the incidences of hypoglycemia, which were quite frequent in the article by Kazempour-Ardehli et al. and occurred very rarely in our population. We think that hypoglycemia should be considered life threatening in this very fragile population and could be prevented by dialysate with adequate glucose concentration.

To conclude, this article (1) and the very recently released study we conducted (2) both showed the importance of the assessment of glycemic control in type 2 diabetic hemodialysis patients.

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Acknowledgments—No potential conflicts of interest relevant to this article were reported.

References
Assessing Glycemic Control in Maintenance Hemodialysis Patients With Type 2 Diabetes

Response to Riveline and Hadjadji

In response to the comment by Riveline and Hadjadji [1] on the work presented in our article “Assessing Glycemic Control in Maintenance Hemodialysis Patients With Type 2 Diabetes” [2], we would like to acknowledge the following:

1) Due to the abundance of existing evidence on the accuracy of continuous glucose monitoring (CGM) in patients with diabetes and the lack of interference from urea metabolites with the GlucoDay (data provided by manufacturer), we did not set out to prove the accuracy of CGM in hemodialyzed patients. It is reassuring to see the accuracy of CGM confirmed by the authors in their study [3].

2) We have reviewed the glucose concentrations in the dialysate fluids used for the study population and have identified that the following correction must be made regarding these concentrations: most patients were using a dialysate solution with 2 g/l glucose (not 2 mmol/l as stated in the article), which translates to 200 mg/dl or 11.1 mmol/l, while a smaller number were using a dialysate solution with 1 g/l glucose, which is equivalent to 100 mg/dl or 5.5 mmol/l (the same as that used for the study by Riveline et al. [3]). However, this does not change the fact that we did observe postdialysis hypoglycemic episodes, and of the three patients who had profound hypoglycemia (<2.5 mmol/l), two were on the dialysate fluid with 2 g/l glucose. We agree with the authors that relative hypoglycemia is a dangerous side effect that must be prevented in this at-risk population. To this end, we would like to highlight that providers of dialysate solutions in the U.K. are looking to standardize the glucose concentration for all dialysates at 1 g/l (equivalent to 5.5 mmol/l), while the evidence from our study shows that this may not be high enough to prevent postdialysis asymptomatic hypoglycemia.

In conclusion, we would like to thank the authors for their insight and helpful comments and further emphasize the importance of closely monitoring glycemic control in hemodialyzed patients with diabetes.

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Gary Frost, MD
Jeremy J.O. Turner, DM, MRCPI

Acknowledgments—No potential conflicts of interest relevant to this article were reported.

References
Appendix II: Statistical analysis of effect of risk factors on endpoints

Summary of study objectives

PART 1 Analysis plan
1. To compare the risk of occurrence of any CV events between levels of:
   a. BP, lipid (cholesterol or HDL), diabetes status and BMI
   b. If diabetes status is important, subanalysis of diabetic subjects will be conducted using HbA1c
2. To compare the risk of time to first CV event between levels of:
   a. BP, lipid (cholesterol or HDL), diabetes status and BMI
   b. If diabetes status is important, subanalysis of diabetic subjects will be conducted using HbA1c
3. To compare the risk of time to all-cause death event between levels of:
   a. BP, lipid (cholesterol or HDL), diabetes status and BMI
   b. If diabetes status is important, subanalysis of diabetic subjects will be conducted using HbA1c

PART 2 Analysis plan
4. Subgroup analysis of primary dataset (n_{total}=518) by haemodialysis status (n_{Y}=210, n_{N}=307, n_{missing}=1): To compare the risk of occurrence of any CV events (during the 2-year follow up) between levels of SBP, DBP, total cholesterol, triglycerides, diabetes status and BMI
5. Subgroup analysis by haemodialysis status: To compare the risk of occurrence of death (during the 2-year follow up) between levels of SBP, DBP, total cholesterol, triglycerides, diabetes status and BMI
6. Analysis of HOMA dataset: To compare the risk of occurrence of any CV events (during the 2-year follow up) between levels of adiponectin and HOMA-IR. Analysis will be repeated with adjustment for confounding by CKD stage.
7. Analysis of HOMA dataset: To compare the risk of occurrence of death (during the 2-year follow up) between levels of adiponectin and HOMA-IR. Analysis will be repeated with adjustment for confounding by CKD stage.

PART 3 Analysis plan
8. Analysis of HOMA dataset: To compare the risk of occurrence of any CV events (during the 2-year follow up) between levels of HOMA-IR and CRP. Analysis will be repeated with adjustment for confounding by CKD stage.
9. Analysis of HOMA dataset: To compare the risk of occurrence of death (during the 2-year follow up) between levels of HOMA-IR and CRP. Analysis will be repeated with adjustment for confounding by CKD stage.
10. Analysis of HOMA dataset: Subgroup analysis by haemodialysis status: To compare the risk of occurrence of any CV events (during the 2-year follow up) between levels of adiponectin, HOMA-IR and CRP.

11. Analysis of HOMA dataset: Subgroup analysis by haemodialysis status: To compare the risk of occurrence of death (during the 2-year follow up) between levels of adiponectin, HOMA-IR and CRP.

12. To compare the risk of occurrence of death (during the 2-year follow up) between levels of SBP, DBP, total cholesterol, triglycerides, diabetes status and BMI
## Results

### Objective 1: Results of “end of 2 year” CVE risk analysis (logistic model)

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>Odds ratio (OR) for 1 unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariable analysis or Adjusted for ANYCONDIS</td>
<td>FAS (N=518)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>FAS (n=410)</td>
<td>1.011</td>
<td>(1.002,1.021)</td>
<td>0.023</td>
</tr>
<tr>
<td>SBP adjusted</td>
<td>FAS (n=410)</td>
<td>1.010</td>
<td>(1.001,1.020)</td>
<td>0.035</td>
</tr>
<tr>
<td>DBP</td>
<td>FAS (n=402)</td>
<td>1.004</td>
<td>(0.989, 1.021)</td>
<td>0.560</td>
</tr>
<tr>
<td>DBP adjusted</td>
<td>FAS (n=402)</td>
<td>1.005</td>
<td>(0.989,1.022)</td>
<td>0.531</td>
</tr>
<tr>
<td>TC</td>
<td>FAS (n=449)</td>
<td>0.681</td>
<td>(0.550,0.844)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC adjusted</td>
<td>FAS (n=449)</td>
<td>0.709</td>
<td>(0.570,0.882)</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL</td>
<td>FAS (n=301)</td>
<td>0.550</td>
<td>(0.281,1.060)</td>
<td>0.074</td>
</tr>
<tr>
<td>HDL adjusted</td>
<td>FAS (n=301)</td>
<td>0.577</td>
<td>(0.298,1.118)</td>
<td>0.103</td>
</tr>
<tr>
<td>BMI</td>
<td>FAS (n=464)</td>
<td>1.017</td>
<td>(0.984,1.050)</td>
<td>0.318</td>
</tr>
<tr>
<td>BMI adjusted</td>
<td>FAS (n=464)</td>
<td>1.015</td>
<td>(0.983,1.048)</td>
<td>0.374</td>
</tr>
<tr>
<td>DIABETIC</td>
<td>FAS (n=495)</td>
<td>1.622</td>
<td>(1.074,2.449)</td>
<td>0.021</td>
</tr>
<tr>
<td>DIABETIC adj’d</td>
<td>FAS (n=495)</td>
<td>1.506</td>
<td>(0.989,2.293)</td>
<td>0.056</td>
</tr>
</tbody>
</table>

Note: ANYCONDIS is binary: “0” if no comorbidities and “1” if 1 or more comorbidities.

SBP=systolic blood pressure (mmHg), DBP=diastolic blood pressure (mmHg), TC=total cholesterol (units?), HDL=high density lipoprotein (units?), BMI=body mass index (kg/m²), DIABETIC=status indicator for having diabetes, HBA1C=HbA1c.

### Comments:
- Negligible confounding by adjusting for any comorbidity (ANYCONDIS).
- Effect of TC is not intuitive. What is happening in this study population?
- I did not attempt to build a CVE risk (glm) model with multiple covariates because such a model would definitely include TC, which is nonsensical.
<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>X increment</th>
<th>Odds ratio (OR) for X unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
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<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>DAS (N=274)</td>
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<tr>
<td>SBP</td>
<td>FAS (n=410)</td>
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<td>(1.016,1.23)</td>
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<tr>
<td>DBP adjusted</td>
<td>FAS (n=402)</td>
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<td>(0.894,1.240)</td>
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<td>TC</td>
<td>FAS (n=449)</td>
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<td>0.681</td>
<td>(0.550,0.844)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC adjusted</td>
<td>FAS (n=449)</td>
<td>1</td>
<td>0.709</td>
<td>(0.570,0.882)</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL</td>
<td>FAS (n=301)</td>
<td>1</td>
<td>0.550</td>
<td>(0.281,1.060)</td>
<td>0.074</td>
</tr>
<tr>
<td>HDL adjusted</td>
<td>FAS (n=301)</td>
<td>1</td>
<td>0.577</td>
<td>(0.298,1.118)</td>
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<td>BMI</td>
<td>FAS (n=464)</td>
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<td>1.085</td>
<td>(0.924,1.275)</td>
<td>0.318</td>
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<tr>
<td>DIABETIC adj’d</td>
<td>FAS (n=495)</td>
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<td>(0.989,2.293)</td>
<td>0.056</td>
</tr>
<tr>
<td>HBA1C</td>
<td>DAS (n=224)</td>
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<td>(0.905,1.224)</td>
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<tr>
<td>HBA1C adjusted</td>
<td>DAS (n=224)</td>
<td>1</td>
<td>1.055</td>
<td>(0.907,1.227)</td>
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</table>
### Objective 2: Results of 2 year follow up time to CVE analysis (Cox proportional hazards model)

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>Hazard ratio (HR)</th>
<th>95% CI of HZ</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANYCONDIS</td>
<td>FAS (N=518)</td>
<td>1.010</td>
<td>(1.000,1.020)</td>
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<td>DAS (N=274)</td>
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<tr>
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<td>(0.991,1.020)</td>
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<td>DBP</td>
<td>FAS (n=402)</td>
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<td>(0.990,1.020)</td>
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</tr>
<tr>
<td></td>
<td>FAS (n=402)</td>
<td>1.000</td>
<td>(0.990,1.020)</td>
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<td>DIABETIC</td>
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<td>(1.000,2.070)</td>
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<td>HBA1C</td>
<td>DAS (n=224)</td>
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<td>(0.920,1.180)</td>
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<tr>
<td></td>
<td>DAS (n=224)</td>
<td>1.040</td>
<td>(0.923,1.180)</td>
<td>0.500</td>
</tr>
</tbody>
</table>

Note: ANYCONDIS is binary: “0” if no comorbidities and “1” if 1 or more comorbidities. SBP=systolic blood pressure (mmHg), DBP=diastolic blood pressure (mmHg), TC=total cholesterol (units?), HDL=high density lipoprotein (units?), BMI=body mass index (kg/m²), DIABETIC=status indicator for having diabetes, HBA1C=HbA1c.
**Objective 3: Results of 2 year follow up time to DEATH analysis (Cox proportional hazards model)**

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>Hazard ratio (HR)</th>
<th>95% CI of HZ</th>
<th>p-value</th>
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<tbody>
<tr>
<td>ANYCONDIS</td>
<td>FAS (N=518)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DAS (N=274)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>FAS (n=421)</td>
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<td>(0.997,1.020)</td>
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<tr>
<td>SBP adjusted</td>
<td>FAS (n=421)</td>
<td>1.010</td>
<td>(0.996,1.020)</td>
<td>0.190</td>
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<tr>
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<td>FAS (n=413)</td>
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<td>(0.964,1.010)</td>
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<tr>
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<td>(0.964,1.010)</td>
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<tr>
<td>TC adjusted</td>
<td>FAS (n=461)</td>
<td>0.675</td>
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<td>HDL</td>
<td>FAS (n=309)</td>
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<tr>
<td>HDL adjusted</td>
<td>FAS (n=309)</td>
<td>0.404</td>
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<td>(0.942,1.030)</td>
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<tr>
<td>BMI adjusted</td>
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<td>(0.941,1.030)</td>
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<td>0.033</td>
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<tr>
<td>DIABETIC adjusted</td>
<td>FAS (n=507)</td>
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<td>(0.974,2.960)</td>
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<tr>
<td>HBA1C</td>
<td>DAS (n=232)</td>
<td>0.973</td>
<td>(0.816,1.160)</td>
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<tr>
<td>HBA1C adjusted</td>
<td>DAS (n=232)</td>
<td>0.972</td>
<td>(0.815,1.160)</td>
<td>0.750</td>
</tr>
</tbody>
</table>

Note: ANYCONDIS is binary: “0” if no comorbidities and “1” if 1 or more comorbidities.

SBP=systolic blood pressure (mmHg), DBP=diastolic blood pressure (mmHg), TC=total cholesterol (units?), HDL=high density lipoprotein (units?), BMI=body mass index (kg/m²), DIABETIC=status indicator for having diabetes, HBA1C=HbA1c.

Comments:
- Negligible confounding by adjusting for any comorbidity (ANYCONDIS).
- Effect of TC is not intuitive. What is happening in this study population?
- I did not attempt to build a CVE or DEATH survival model with multiple covariates because such a model would definitely include TC, which is nonsensical.
Objective 4: Results of subgroup analysis by haemodialysis status for “end of 2 year” CVE risk analysis (logistic model)

Analysis sets recap
- FAS n=518
- FAS with observed CVE outcome n=506

Let define analysis sets:
- Patients on haemodialysis (HAS-Y) n=210
- Patients not on haemodialysis (HAS-N) n=307
- Patients with unknown haemodialysis status n=1

Finally, let define HAS-Y and HAS-N with observed CVE outcome:
- Patients on haemodialysis and with observed CVE outcome (HAS-Y) n=208
- Patients not on haemodialysis with observed CVE outcome (HAS-N) n=298

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>X increment</th>
<th>Odds ratio (OR) for X unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>HAS-Y (N=210)</td>
<td>1</td>
<td>1.001 (0.989,1.014)</td>
<td>0.830</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HAS-N (N=307)</td>
<td>1</td>
<td>1.012 (0.993,1.031)</td>
<td>0.217</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>HAS-Y (N=171)</td>
<td>1</td>
<td>0.988 (0.967,1.010)</td>
<td>0.299</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HAS-N (N=231)</td>
<td>1</td>
<td>1.000 (0.972,1.028)</td>
<td>0.981</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>HAS-Y (N=194)</td>
<td>1</td>
<td>0.924 (0.676,1.262)</td>
<td>0.619</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HAS-N (N=255)</td>
<td>1</td>
<td>0.589 (0.419,0.828)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>HAS-Y (N=183)</td>
<td>1</td>
<td>0.952 (0.667,1.357)</td>
<td>0.784</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HAS-N (N=253)</td>
<td>1</td>
<td>0.921 (0.679,1.248)</td>
<td>0.594</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>HAS-Y (N=201)</td>
<td>1</td>
<td>1.047 (0.997,1.100)</td>
<td>0.066</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HAS-N (N=263)</td>
<td>1</td>
<td>1.037 (0.988,1.088)</td>
<td>0.137</td>
<td></td>
</tr>
<tr>
<td>DIABETIC</td>
<td>HAS-Y (N=207)</td>
<td>1</td>
<td>2.538 (1.417,4.546)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HAS-N (N=288)</td>
<td>1</td>
<td>1.488 (0.792,2.795)</td>
<td>0.217</td>
<td></td>
</tr>
</tbody>
</table>
Note: SBP=systolic blood pressure (mmHg), DBP=diastolic blood pressure (mmHg), TC=total cholesterol (units?), TG=Triglycerides (units?), BMI=body mass index (kg/m$^2$), DIABETIC=status indicator for having diabetes.

Comments:
We have trawled through the data with many tests without controlling the type 1 error anywhere. All analyses are therefore exploratory and need to be confirmed with a more focussed study.

There is some evidence for interaction with haemodialysis status in effect of diabetic status. There seems to be some evidence for interaction in effect of TC, although this is probably driven by the few high TC values seen in the non-haemodialysis group.
Objective 5: Results of subgroup analysis by haemodialysis status for “end of 2 year” death risk analysis (logistic model)

Analysis sets recap
- FAS n=518
- FAS with observed death outcome n=518

Let define analysis sets:
- Patients on haemodialysis (HAS-Y) n=210
- Patients not on haemodialysis (HAS-N) n=307
- Patients with unknown haemodialysis status n=1

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>X increment</th>
<th>Odds ratio (OR) for X unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>HAS-Y (n=180)</td>
<td>1</td>
<td>1.013</td>
<td>(0.998,1.029)</td>
<td>0.095</td>
</tr>
<tr>
<td>SBP</td>
<td>HAS-N (n=240)</td>
<td>1</td>
<td>0.958</td>
<td>(0.926,0.990)</td>
<td>0.011</td>
</tr>
<tr>
<td>DBP</td>
<td>HAS-Y (n=172)</td>
<td>1</td>
<td>0.986</td>
<td>(0.958,1.014)</td>
<td>0.327</td>
</tr>
<tr>
<td>DBP</td>
<td>HAS-N (n=240)</td>
<td>1</td>
<td>0.929</td>
<td>(0.879,0.982)</td>
<td>0.010</td>
</tr>
<tr>
<td>TC</td>
<td>HAS-Y (n=196)</td>
<td>1</td>
<td>0.864</td>
<td>(0.576,1.296)</td>
<td>0.480</td>
</tr>
<tr>
<td>TC</td>
<td>HAS-N (n=264)</td>
<td>1</td>
<td>0.500</td>
<td>(0.284,0.880)</td>
<td>0.016</td>
</tr>
<tr>
<td>TG</td>
<td>HAS-Y (n=185)</td>
<td>1</td>
<td>0.546</td>
<td>(0.310,0.964)</td>
<td>0.037</td>
</tr>
<tr>
<td>TG</td>
<td>HAS-N (n=261)</td>
<td>1</td>
<td>0.468</td>
<td>(0.219,0.998)</td>
<td>0.049</td>
</tr>
<tr>
<td>BMI</td>
<td>HAS-Y (n=203)</td>
<td>1</td>
<td>0.994</td>
<td>(0.934,1.059)</td>
<td>0.856</td>
</tr>
<tr>
<td>BMI</td>
<td>HAS-N (n=272)</td>
<td>1</td>
<td>1.028</td>
<td>(0.955,1.105)</td>
<td>0.465</td>
</tr>
<tr>
<td>DIABETIC</td>
<td>HAS-Y (n=209)</td>
<td>1</td>
<td>2.032</td>
<td>(0.997,4.146)</td>
<td>0.051</td>
</tr>
<tr>
<td>DIABETIC</td>
<td>HAS-N (n=297)</td>
<td>1</td>
<td>3.846</td>
<td>(1.101,13.433)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Note: SBP=systolic blood pressure (mmHg), DBP=diastolic blood pressure (mmHg), TC=total cholesterol (units?), TG=Triglycerides (units?), BMI=body mass index (kg/m²), DIABETIC=status indicator for having diabetes.

Comments:
We have trawled through the data with many tests without controlling the type 1 error anywhere. All analyses are therefore exploratory and need to be confirmed with a more focussed study.
Objective 6: Results of HOMA dataset analysis for “end of 2 year” CVE risk analysis (logistic model)

Note: HOMA dataset does not contain any CVE or DEATH variables. Need to merge in this data from the primary dataset.

Analysis sets
- HOMA FAS n=107
- HOMA FAS overlapping with primary dataset n=95
- HOMA FAS overlapping with primary dataset and with observed CVE outcome n=88
- HOMA FAS overlapping with primary dataset and with observed CVE outcome+CKD known n=88

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>X inc</th>
<th>Odds ratio (OR) for X unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariable analysis or Adjusted for CKD stage and/or interaction</td>
<td>FAS (N=107)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>FAS (n=74)</td>
<td>1</td>
<td>0.982</td>
<td>(0.959,1.005)</td>
<td>0.118</td>
</tr>
<tr>
<td>Adiponectin+CKD</td>
<td>FAS (n=74)</td>
<td>1</td>
<td>0.980</td>
<td>(0.956,1.004)</td>
<td>0.097</td>
</tr>
<tr>
<td>Adiponectin+CKD stage+interaction</td>
<td>FAS (n=74)</td>
<td>1</td>
<td>CKD3: 0.989</td>
<td>(0.934,1.045)</td>
<td>0.713</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD4: 0.963</td>
<td>(0.912,1.018)</td>
<td>0.181</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD5: 0.983</td>
<td>(0.955,1.012)</td>
<td>0.242</td>
</tr>
<tr>
<td>HOMA</td>
<td>no data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA +CKD</td>
<td>no data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA +CKD stage+interaction</td>
<td>no data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: CKD is trinary: stage “3”, “4” or “5”.
Model for Adiponectin+CKD include CKD as categorical variables.
Model for Adiponectin+CKD+ interaction includes CKD as categorical variables and interactions with adiponectin.
Objective 7: Results of HOMA dataset analysis for “end of 2 year” death risk analysis (logistic model)

Note: HOMA dataset does not contain any CVE or DEATH variables. Need to merge in this data from the primary dataset.

Analysis sets
- HOMA FAS n=107
- HOMA FAS overlapping with primary dataset n=95
- HOMA FAS overlapping with primary dataset and with observed death outcome n=95
- HOMA FAS overlapping with primary dataset and with observed death outcome+CKD known n=94

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>X inc</th>
<th>Odds ratio (OR) for X unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA</td>
<td>FAS (N=107)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>FAS (n=81)</td>
<td>1</td>
<td>1.031</td>
<td>(1.004,1.058)</td>
<td>0.024</td>
</tr>
<tr>
<td>Adiponectin+CKD</td>
<td>FAS (n=80)</td>
<td>1</td>
<td>1.041</td>
<td>(1.004,1.079)</td>
<td>0.029</td>
</tr>
<tr>
<td>Adiponectin+CKD stage+interaction</td>
<td>FAS (n=80)</td>
<td>CKD3: 1.000</td>
<td>(0.000,1e75)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD4: 1.025</td>
<td>(0.927,1.135)</td>
<td>0.626</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD5: 1.043</td>
<td>(1.003,1.085)</td>
<td>0.036</td>
<td></td>
</tr>
</tbody>
</table>

Note: CKD is trinary: stage “3”, “4” or “5”.
Model for Adiponectin+CKD include CKD as categorical variables.
Model for Adiponectin+CKD+ interaction includes CKD as categorical variables and interactions with adiponectin.
Objective 8: Results of HOMA dataset analysis for “end of 2 year” CVE risk analysis (logistic model)

Note: new HOMA dataset (“15.07.09 Data Sheet for statistical analysis with ALL HOMAs.xlsx”) contains an additional variable “endpoint”. This variable has factor levels = (“CVE”, “dead”, “no data”, “no event”). It is a mixture of CVE events and DEATH events and will not identify overlap between these two events in the same subject. As such this variable cannot be used on its own. I will need to cross-check this variable with the primary dataset.

<table>
<thead>
<tr>
<th>HOMA</th>
<th>cve</th>
<th>no cve</th>
<th>&lt;NA&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVE</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>dead</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>no data</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>no event</td>
<td>0</td>
<td>59</td>
<td>4</td>
</tr>
<tr>
<td>&lt;NA&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Analysis sets

- HOMA FAS n=107
- HOMA FAS overlapping with primary dataset n=95
- HOMA FAS overlapping with primary dataset and with observed CVE outcome n=88
- HOMA FAS overlapping with primary dataset and with observed CVE outcome+CKD+HDx known n=88
- HOMA FAS not overlapping with primary dataset and with observed CVE outcome n=4
- HOMA FAS not overlapping with primary dataset and with observed CVE outcome+CKD+HDx n=4

For consistency with analysis in Objective 6, the analysis in Objective 8 will be based on the n=88, i.e. not n=88+4.
<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>X inc</th>
<th>Odds ratio (OR) for X unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin+CKD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin+CKD stage+interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA</td>
<td>FAS (n=87)</td>
<td>1</td>
<td>1.171</td>
<td>(0.937,1.464)</td>
<td>0.165</td>
</tr>
<tr>
<td>HOMA + CKD</td>
<td>FAS (n=87)</td>
<td>1</td>
<td>1.158</td>
<td>(0.925,1.449)</td>
<td>0.200</td>
</tr>
<tr>
<td>HOMA + CKD stage+interaction</td>
<td>FAS (n=87)</td>
<td>1</td>
<td>CKD3: 1.778</td>
<td>(0.100,31.70)</td>
<td>0.695</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD4: 1.543</td>
<td>(0.815,2.922)</td>
<td>0.183</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD5: 1.042</td>
<td>(0.784,1.385)</td>
<td>0.777</td>
</tr>
<tr>
<td>logCRP</td>
<td>FAS (n=74)</td>
<td>1</td>
<td>1.485</td>
<td>(0.982, 2.246)</td>
<td>0.061</td>
</tr>
<tr>
<td>logCRP +CKD</td>
<td>FAS (n=74)</td>
<td>1</td>
<td>1.523</td>
<td>(0.994, 2.334)</td>
<td>0.053</td>
</tr>
<tr>
<td>logCRP + CKD stage+interaction</td>
<td>FAS (n=74)</td>
<td>1</td>
<td>CKD3: 1.701</td>
<td>(0.538, 5.371)</td>
<td>0.365</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD4: 2.307</td>
<td>(0.809, 6.583)</td>
<td>0.118</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD5: 1.319</td>
<td>(0.793, 2.192)</td>
<td>0.286</td>
</tr>
</tbody>
</table>

Note: CKD is trinary: stage “3”, “4” or “5”. logCRP= log transform of CRP values.
Model for HOMA + CKD include CKD as categorical variables.
Model for HOMA + CKD + interaction includes CKD as categorical variables and interactions with HOMA.
Model for logCRP + CKD include CKD as categorical variables.
Model for logCRP + CKD + interaction includes CKD as categorical variables and interactions with logCRP.
Objective 9: Results of HOMA dataset analysis for “end of 2 year” DEATH risk analysis (logistic model)

Note: new HOMA dataset (“15.07.09 Data Sheet for statistical analysis with ALL HOMAs.xlsx”) contains an additional variable “endpoint”. This variable has factor levels = (“CVE”, “dead”, “no data”, “no event”). It is a mixture of CVE events and DEATH events and will not identify overlap between these two events in the same subject. As such this variable cannot be used on its own. I will need to cross-check this variable with the primary dataset.

<table>
<thead>
<tr>
<th>PRIMARY</th>
<th>alive</th>
<th>dead</th>
<th>&lt;NA&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVE</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>dead</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>no data</td>
<td>5</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>no event</td>
<td>61</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>&lt;NA&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Analysis sets
- HOMA FAS n=107
- HOMA FAS overlapping with primary dataset n=95
- HOMA FAS overlapping with primary dataset and with observed DEATH outcome n=95
- HOMA FAS overlapping with primary dataset and with observed DEATH outcome+CKD+HDx known n=94
- HOMA FAS not overlapping with primary dataset and with observed DEATH outcome n=2
- HOMA FAS not overlapping with primary dataset and with observed DEATH outcome+CKD+HDx n=2

For consistency with analysis in Objective 6, the analysis in Objective 8 will be based on the n=88, i.e. not n=88+4.
<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>X inc</th>
<th>Odds ratio (OR) for X unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariable analysis or Adjusted for CKD stage and/or interaction</td>
<td>FAS (N=107) (Actual no. subjects in analysis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>See analysis objective 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin+CKD</td>
<td>FAS (n=94)</td>
<td>1</td>
<td>1.146</td>
<td>(0.823, 1.597)</td>
<td>0.419</td>
</tr>
<tr>
<td>HOMA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA +CKD</td>
<td>FAS (n=93)</td>
<td>1</td>
<td>1.143</td>
<td>(0.812, 1.609)</td>
<td>0.442</td>
</tr>
<tr>
<td>HOMA +CKD stage+interaction</td>
<td>FAS (n=93)</td>
<td>1</td>
<td>CKD3: 1.000</td>
<td>(0.000, infinity)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD4: 1.276</td>
<td>(0.762, 2.137)</td>
<td>0.354</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD5: 1.058</td>
<td>(0.667, 1.678)</td>
<td>0.810</td>
</tr>
<tr>
<td>logCRP</td>
<td>FAS (n=81)</td>
<td>1</td>
<td>1.032</td>
<td>(1.000, 1.064)</td>
<td>0.051</td>
</tr>
<tr>
<td>logCRP +CKD</td>
<td>FAS (n=80)</td>
<td>1</td>
<td>1.035</td>
<td>(0.992, 1.080)</td>
<td>0.109</td>
</tr>
<tr>
<td>logCRP +CKD stage+interaction</td>
<td>FAS (n=80)</td>
<td>1</td>
<td>CKD3: 1.000</td>
<td>(0.000, infinity)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD4: 1.084</td>
<td>(0.914, 1.287)</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CKD5: 1.033</td>
<td>(0.993, 1.074)</td>
<td></td>
</tr>
</tbody>
</table>

Note: CKD is trinary: stage “3”, “4” or “5”.
Model for HOMA+CKD include CKD as categorical variables.
Model for HOMA +CKD+ interaction includes CKD as categorical variables and interactions with HOMA.
Model for CRP+CKD include CKD as categorical variables.
Model for CRP +CKD+ interaction includes CKD as categorical variables and interactions with CRP.
Objective 10: Results of subgroup analysis by haemodialysis status for HOMA dataset analysis for “end of 2 year” CVE risk analysis (logistic model)

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>X inc</th>
<th>Odds ratio (OR) for X unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAS-N (N=55)</td>
<td>1</td>
<td>0.979</td>
<td>(0.944, 1.014)</td>
<td>0.240</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>HAS-Y (N=51)</td>
<td>1</td>
<td>0.984</td>
<td>(0.954, 1.014)</td>
<td>0.292</td>
</tr>
<tr>
<td></td>
<td>HAS-N (n=31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>HAS-Y (n=43)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA</td>
<td>HAS-N (n=38)</td>
<td>1</td>
<td>1.315</td>
<td>(0.963, 1.796)</td>
<td>0.085</td>
</tr>
<tr>
<td>HOMA</td>
<td>HAS-Y (n=49)</td>
<td>1</td>
<td>0.996</td>
<td>(0.655, 1.513)</td>
<td>0.986</td>
</tr>
<tr>
<td>logCRP</td>
<td>HAS-N (n=31)</td>
<td>1</td>
<td>2.008</td>
<td>(1.005, 4.010)</td>
<td>0.048</td>
</tr>
<tr>
<td>logCRP</td>
<td>HAS-Y (n=43)</td>
<td>1</td>
<td>1.273</td>
<td>(0.717, 2.262)</td>
<td>0.409</td>
</tr>
</tbody>
</table>
Objective 11: Results of subgroup analysis by haemodialysis status for HOMA dataset analysis for “end of 2 year” DEATH risk analysis (logistic model)

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>X inc</th>
<th>Odds ratio (OR) for X unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>HAS-N (N=55) (n=37)</td>
<td>1</td>
<td>1.016</td>
<td>(0.961, 1.074)</td>
<td>0.575</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>HAS-Y (N=51) (n=43)</td>
<td>1</td>
<td>1.042</td>
<td>1.001,1.083)</td>
<td>0.041</td>
</tr>
<tr>
<td>HOMA</td>
<td>HAS-N (N=44) (n=44)</td>
<td>1</td>
<td>1.415</td>
<td>(0.909, 2.201)</td>
<td>0.123</td>
</tr>
<tr>
<td>HOMA</td>
<td>HAS-Y (N=49) (n=49)</td>
<td>1</td>
<td>0.866</td>
<td>(0.374, 2.001)</td>
<td>0.736</td>
</tr>
<tr>
<td>CRP</td>
<td>HAS-N (N=37) (n=37)</td>
<td>1</td>
<td>1.025</td>
<td>(0.936, 1.123)</td>
<td>0.592</td>
</tr>
<tr>
<td>CRP</td>
<td>HAS-Y (N=43) (n=43)</td>
<td>1</td>
<td>1.797</td>
<td>(0.715, 4.518)</td>
<td>0.213</td>
</tr>
</tbody>
</table>
**Objective 12: Results for “end of 2 year” death risk analysis (logistic model)**

Analysis sets recap
- FAS n=518
- FAS with observed death outcome n=518

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Analysis set</th>
<th>X increment</th>
<th>Odds ratio (OR) for X unit increase in covariate</th>
<th>95% CI of OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>FAS (n=421)</td>
<td>1</td>
<td>1.010</td>
<td>(0.997, 1.023)</td>
<td>0.146</td>
</tr>
<tr>
<td>DBP</td>
<td>FAS (n=413)</td>
<td>1</td>
<td>0.985</td>
<td>(0.962, 1.009)</td>
<td>0.230</td>
</tr>
<tr>
<td>TC</td>
<td>FAS (n=461)</td>
<td>1</td>
<td>0.635</td>
<td>(0.466, 0.864)</td>
<td>0.004</td>
</tr>
<tr>
<td>TG</td>
<td>FAS (n=447)</td>
<td>1</td>
<td>0.504</td>
<td>(0.322, .788)</td>
<td>0.003</td>
</tr>
<tr>
<td>BMI</td>
<td>FAS (n=475)</td>
<td>1</td>
<td>0.982</td>
<td>(0.937, 1.028)</td>
<td>0.428</td>
</tr>
<tr>
<td>DIABETIC</td>
<td>FAS (n=507)</td>
<td>1</td>
<td>1.869</td>
<td>(1.047, 3.334)</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Note: SBP=systolic blood pressure (mmHg), DBP=diastolic blood pressure (mmHg), TC=total cholesterol (units?), TG=Triglycerides (units?), BMI=body mass index (kg/m²), DIABETIC=status indicator for having diabetes.

Results are marginal over the subgroup results in Objective 5.
Appendix III: Comparison of means

**Supplementary Table 1:**

<table>
<thead>
<tr>
<th></th>
<th>Mean BMI (kg/m²)</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>27.5</td>
<td>6.4</td>
<td>-</td>
</tr>
<tr>
<td>CKD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29.1</td>
<td>6.2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>29.9</td>
<td>6.2</td>
<td>3, 4, 5, &amp; MHD</td>
</tr>
<tr>
<td>4</td>
<td>29.1</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>26.0</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>MHD</td>
<td>25.3</td>
<td>5.9</td>
<td></td>
</tr>
</tbody>
</table>

**Supplementary Table 1** Comparison of mean BMI values in different subgroups of the study cohort. Mean BMI values, expressed as kg/m², and standard deviations (SD) are shown for the entire cohort (‘combined’), the CKD group as a whole (‘CKD’), the subsets of different CKD stages (CKD3, CKD 4 and CKD 5) and MHD groups. p-values are derived from comparisons of means carried out using the ANOVA test.

**Supplementary Table 2:**

<table>
<thead>
<tr>
<th></th>
<th>Mean SBP (mmHg)</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>144.2</td>
<td>22.4</td>
<td>-</td>
</tr>
<tr>
<td>CKD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>138.0</td>
<td>18.1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>137.2</td>
<td>17.0</td>
<td>3, 4, 5 &amp; MHD &lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>137.9</td>
<td>18.9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>143.1</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>MHD</td>
<td>152.4</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

**Supplementary Table 2** Comparison of mean systolic BP values in different subgroups of the study cohort. Mean SBP values, expressed as mmHg, and standard deviations (SD) are shown for the entire cohort (‘combined’), the CKD group as a whole (‘CKD’), the subsets of different CKD stages (CKD3, CKD 4 and CKD 5) and MHD groups. P-values are derived from comparisons of means carried out using the ANOVA test.
**Supplementary Table 3:**

<table>
<thead>
<tr>
<th></th>
<th>Mean DBP (mmHg)</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>77.2</td>
<td>13.5</td>
<td>-</td>
</tr>
<tr>
<td>CKD Total</td>
<td>73.9</td>
<td>12.1</td>
<td>-</td>
</tr>
<tr>
<td>CKD 3</td>
<td>73.0</td>
<td>11.7</td>
<td>3, 4, 5 &amp; MHD &lt;0.001</td>
</tr>
<tr>
<td>CKD 4</td>
<td>74.7</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>CKD 5</td>
<td>76.5</td>
<td>12.1</td>
<td>CKD &amp; MHD &lt;0.001</td>
</tr>
<tr>
<td>MHD</td>
<td>81.7</td>
<td>14.2</td>
<td></td>
</tr>
</tbody>
</table>

**Supplementary Table 3** Comparison of mean diastolic BP values in different subgroups of the study cohort. Mean DBP values, expressed as mmHg, and standard deviations (SD) are shown for the entire cohort ('combined'), the CKD group as a whole ('CKD'), the subsets of different CKD stages (CKD3, CKD 4 and CKD 5) and MHD groups. P-values are derived from comparisons of means carried out using the ANOVA test.

**Supplementary Table 4:**

<table>
<thead>
<tr>
<th></th>
<th>Mean TC (mmol/l)</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>4.2</td>
<td>1.3</td>
<td>-</td>
</tr>
<tr>
<td>CKD Total</td>
<td>4.5</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>CKD 3</td>
<td>4.4</td>
<td>1.3</td>
<td>3, 4, 5 &amp; MHD &lt;0.001</td>
</tr>
<tr>
<td>CKD 4</td>
<td>4.7</td>
<td>1.7</td>
<td>CKD &amp; MHD &lt;0.001</td>
</tr>
<tr>
<td>CKD 5</td>
<td>4.3</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>MHD</td>
<td>3.8</td>
<td>0.95</td>
<td></td>
</tr>
</tbody>
</table>

**Supplementary Table 4** Comparison of mean total cholesterol values in different subgroups of the study cohort. Mean TC values, expressed as mmol/l, and standard deviations (SD) are shown for the entire cohort ('combined'), the CKD group as a whole ('CKD'), the subsets of different CKD stages (CKD3, CKD 4 and CKD 5) and MHD groups. P-values are derived from comparisons of means carried out using the ANOVA test.
**Supplementary Table 5:**

<table>
<thead>
<tr>
<th></th>
<th>Mean TG (mmol/l)</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>1.78</td>
<td>0.98</td>
<td>-</td>
</tr>
<tr>
<td>CKD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.86</td>
<td>1.06</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.81</td>
<td>0.99</td>
<td>CKD &amp; MHD =0.216</td>
</tr>
<tr>
<td>4</td>
<td>1.92</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.88</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>MHD</td>
<td>1.67</td>
<td>0.85</td>
<td></td>
</tr>
</tbody>
</table>

**Supplementary Table 5** Comparison of mean triglyceride values in different subgroups of the study cohort. Mean TG values, expressed as mmol/l, and standard deviations (SD) are shown for the entire cohort (combined), the CKD group as a whole (CKD), the subsets of different CKD stages (CKD3, CKD 4 and CKD 5) and MHD groups. P-values are derived from comparisons of means carried out using the ANOVA test.

**Supplementary Table 6:**

<table>
<thead>
<tr>
<th></th>
<th>Mean HbA1c (%)</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>7.3</td>
<td>1.9</td>
<td>-</td>
</tr>
<tr>
<td>CKD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7.5</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>7.4</td>
<td>1.7</td>
<td>CKD &amp; MHD =0.009</td>
</tr>
<tr>
<td>4</td>
<td>7.8</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.0</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>MHD</td>
<td>6.8</td>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

**Supplementary Table 6** Comparison of mean HbA1c values in different subgroups of the study cohort. Mean HbA1c values, expressed as %, and standard deviations (SD) are shown for the entire cohort ('combined'), the CKD group as a whole ('CKD'), the subsets of different CKD stages (CKD3, CKD 4 and CKD 5) and MHD groups. P-values are derived from comparisons of means carried out using the ANOVA test.
Supplementary Table 7:

<table>
<thead>
<tr>
<th></th>
<th>CKD-All (55)</th>
<th>CKD 3 (17)</th>
<th>CKD 4 (20)</th>
<th>CKD 5 (18)</th>
<th>MHD (50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>2.63±2.31</td>
<td>1.78±1.30</td>
<td>2.52±2.73</td>
<td>3.57±2.33</td>
<td>1.50±1.46</td>
</tr>
<tr>
<td>Kruskal-Wallis</td>
<td></td>
<td></td>
<td></td>
<td>P=0.001</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-test</td>
<td></td>
<td></td>
<td></td>
<td>P=0.002</td>
<td></td>
</tr>
</tbody>
</table>

Supplementary Table 7 Summary of HOMA-IR ± SD in the CKD and MHD subgroups, as well as the subgroups within the CKD group; the Kruskal Wallis test was used to compare means between the CKD 3, CKD 4, CKD 5, and MHD subgroups, while the student t-test was used to compare means between the CKD and MHD groups.
**Supplementary Table 8:**

<table>
<thead>
<tr>
<th></th>
<th>Hs-CRP</th>
<th>Log hs-CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (92)</td>
<td>8.92±18.94</td>
<td>0.55±0.59</td>
</tr>
<tr>
<td></td>
<td>(0.17-157.13)</td>
<td>(-0.77-2.20)</td>
</tr>
<tr>
<td>CKD-All (47)</td>
<td>5.62±11.35</td>
<td>0.36±0.57</td>
</tr>
<tr>
<td></td>
<td>(0.19-72.47)</td>
<td>(-0.72-1.86)</td>
</tr>
<tr>
<td>CKD 3 (16)</td>
<td>7.97±17.57</td>
<td>0.39±0.66</td>
</tr>
<tr>
<td></td>
<td>(0.19-72.47)</td>
<td>(-0.72-1.86)</td>
</tr>
<tr>
<td>CKD 4 (18)</td>
<td>5.43±7.62</td>
<td>0.42±0.53</td>
</tr>
<tr>
<td></td>
<td>(0.49-31.98)</td>
<td>(-0.31-1.50)</td>
</tr>
<tr>
<td>CKD 5 (13)</td>
<td>2.98±3.28</td>
<td>0.23±0.52</td>
</tr>
<tr>
<td></td>
<td>(0.22-11.56)</td>
<td>(-0.66-1.06)</td>
</tr>
<tr>
<td>MHD (44)</td>
<td>12.64±24.37</td>
<td>0.79±0.51</td>
</tr>
<tr>
<td></td>
<td>(0.37-157.13)</td>
<td>(-0.43-2.20)</td>
</tr>
</tbody>
</table>

Kruskal Wallis Results

P=0.003

T-test Results

P=0.103

P=0.232

**Supplementary Table 8** Comparison of means ± SD (range) for markers of inflammation for the CKD and MHD subgroups, as well as the subgroups within the CKD group; the Kruskal Wallis test was used to compare means between the CKD 3, CKD 4, CKD 5, and MHD subgroups, while the student t-test was used to compare means between the CKD and MHD groups.
**Supplementary Table 9:**

<table>
<thead>
<tr>
<th></th>
<th>All (92)</th>
<th>CKD-All (47)</th>
<th>CKD 3 (16)</th>
<th>CKD 4 (18)</th>
<th>CKD 5 (13)</th>
<th>MHD (44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total adiponectin</td>
<td>46.56 ± 25.73</td>
<td>47.92 ± 25.78</td>
<td>39.67 ± 32.54</td>
<td>50.81 ± 21.48</td>
<td>54.05 ± 20.64</td>
<td>45.08 ± 26.20</td>
</tr>
<tr>
<td>(ug/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kruskal-Wallis</td>
<td>-</td>
<td>-</td>
<td>p=0.041*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>p=0.060</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T-test</td>
<td>-</td>
<td></td>
<td>p=0.861</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Supplementary Table 9** Comparison of means ± SD (range) for adiponectin for the CKD and MHD subgroups, as well as the subgroups within the CKD group; the Kruskal Wallis test was used to compare means between the CKD 3, CKD 4, CKD 5, and MHD subgroups, while the student t-test was used to compare means between the CKD and MHD groups.
Appendix IV: Ethics documents

Supplement 1: Letter of invitation

Re: Study Ref Number: 06/Q0406/148

Short Title: CV Risk Factors & Insulin Resistance in Renal Impaired Patients

Dear

I am writing to invite you to take part in a research programme connected with your treatment at the West London Renal and Transplant Centre. Please find enclosed a patient information sheet giving more details. If you have any questions, please do not hesitate to contact us. I look forward to meeting you at your forthcoming clinic appointment.

Yours sincerely

Dr Sara Kazempour A.

Clinical Research Fellow

Invitation letter version 1.2, 27.11.2006

Supplement 2: Patient information sheet
INFORMATION SHEET for RESEARCH PARTICIPANTS

Version 1.3, 27.11.2006

Study title

Identifying cardiovascular risk factors in diabetic and non-diabetic patients with different degrees of renal impairment and measuring their insulin sensitivity by using the HOMA (Homeostatic Model Assessment) index.

Invitation Paragraph

You are being invited to take part in a research study in which we are looking at the role of risk factors for heart disease in renal patients. We are investigating this because there have been some investigations that suggest risk factors for heart disease in renal patients may be different than for other people. Before you decide whether or not to participate it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

If you decide to take part, please let us know beforehand if you have been involved in any other study during the last year. If you decide not to take part your treatment will not be affected by your decision. You are free to withdraw at any time without explanation and your subsequent treatment will not be affected.
What is the purpose of the study?

The purpose of this study is to investigate whether there are differences in risk factors for heart disease in patients with compromised kidney function and the general population. We hope this will help us understand how to prevent heart disease more effectively in patients with kidney disease. The study will take about twenty minutes of your time while we discuss whether you would like to take part and formally seek your consent. You will then be seen between one and three times over the next two years, each time for about twenty minutes, where three small fasting blood samples will be taken at five minute intervals. This will be done at the same time as your routine clinic visit and you will not be asked to make any additional trips to the hospital.

Why have I been chosen?

We have chosen to ask you to take part in this study, because you have kidney disease and you do/do* not have diabetes. We are asking 850 people to take part in this initial study. (* delete as appropriate).

Do I have to take part?

It is up to you 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, you will also be given a copy of the consent form. If you do decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

If you do decide to take part we would like your permission to take your height, weight and waist circumference and access your medical records for your latest laboratory test results. We will also ask you to fast for half a day before coming in at your next clinic visit; if you are diabetic and are taking insulin, we will ask you to delay your morning dose of insulin while you are fasting. Where we will take three small (5ml, about one teaspoon full) tubes of blood from you while you are having the routine blood tests that you will need as part of
your care. You may be asked to permit us to repeat this one or two times over the next two years.

This is a **Prospective study** which means that we will collect the data we need over the next two years and calculate the risk of heart disease associated with each factor. In addition to identifying risk factors for heart disease in people with impaired kidney function, we will also be measuring insulin resistance, which is a condition that regularly develops with kidney disease and can be a cause of heart disease in these people.

**What do I have to do?**

You do not need to make any alterations to your lifestyle to take part in this study. You should continue taking any medicines you are on and continue the rest of your life as normal on the day of the clinic appointment.

**What are the side effects of taking part?**

The only side effect that may arise from taking part in this study is due to fasting. This will be minimised by exclusion of anyone not medically fit enough, and limitation of length of fast. If you are diabetic, we will also ask you to delay your morning dose of insulin while you are fasting, so that there will be no increased chance of having a hypo. The volume of blood we are taking is well within the range of what is normally taken at a routine diagnostic blood test.

**What are the possible disadvantages and risks of taking part?**

As stated above there are no obvious risks to taking part in this study.

**What are the possible benefits of taking part?**

There is no benefit to you personally from taking part in this study. The study is aimed at helping us identify risk factors for heart disease among people with reduced kidney function.

**What if there is a problem?**
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2. In case you feel the need to make a complaint, you can contact: 020 8383 4682.

**Will my taking part in this study be kept confidential?**

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it and you would not be identified in any publications emanating from the study.

**Contact for further information**

For further information contact Dr Sara Kazempour, Department of Endocrinology, Hammersmith Hospital. Tel 020 8383 4682 or email s.kazempour@imperial.ac.uk.

*This completes Part 1 of the Information Sheet.*

**If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.**

**Part 2**

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

As explained before, if you decide not to take part your treatment will not be affected by your decision. You are free to withdraw at any time without explanation and your subsequent treatment will not be affected.

**What if there is a problem?**

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you.
Complaints:

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions 020 8383 4682. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure (or Private Institution). Details can be obtained from the hospital.

Notifying your GP

With your permission if you decide to take part in this study we would like to inform your GP. This will help keep your GP fully informed and also give him or her the opportunity to raise any concerns you may have on your behalf.

What will happen to any samples I give?

During the course of this study, we will be asking you to allow us to take up to three blood samples over two years. These samples will be used to measure your body’s sensitivity to insulin, a hormone that regulates blood sugar. The data obtained will be recorded, and any excess samples will be destroyed.

Will any genetic tests be done?

No.

What will happen to the results of the research study?

We will aim to publish the results of this study in a medical journal. We will also use the results to direct further research in the area.

Who is organising and funding the research?

The research is organised by Dr Jeremy Turner and Imperial College. The organisation funding this research is the King Faisal Foundation.

Who has reviewed the study?
The study has been reviewed by the Hammersmith research ethics committee as well as the Imperial College Higher Degrees Research Committee.

Thank you for taking time to read this and if you decide to take part in the study, thank you, again.

Dr Sara Kazempour
Supplement 3: Consent form

Study Number: 06/Q0406/148
Patient Identification Number for this trial:

CONSENT FORM
(version 1.3, 27.11.2006)

Title of Project:
Identifying cardiovascular risk factors in diabetic and non-diabetic patients with different degrees of renal impairment and measuring their insulin sensitivity by using the HOMA (Homeostatic Model Assessment) index.

Name of Researcher: Dr Sara Kazempour Ardebili

1. I confirm that I have read and understand the information sheet dated ...27/11/2006.... (version ...1.3...) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐

3. I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

☐

4. I agree to my GP being informed of my participation in the study.

☐

5. I agree to take part in the above study.

☐

________________________________________  ______________________  __________________
Name of Patient                            Date                        Signature

________________________________________  ______________________  __________________
Name of Person taking consent
(if different from researcher)             Date                        Signature

________________________________________  ______________________  __________________
Researcher                                Date                        Signature
Supplement 4: Follow-up questionnaire

Study Registration Number: 06/Q0406/148
Questionnaire Version 1.2

Title of study: Identifying cardiovascular risk factors in diabetic and non-diabetic patients with different degrees of renal impairment and measuring their insulin sensitivity by using the HOMA (Homeostatic Model Assessment) index.

What is the purpose of the study?
The purpose of this study is to investigate whether there are differences in risk factors for heart disease in patients with compromised kidney function and the general population. We hope this will help us understand how to prevent heart disease more effectively in patients with kidney disease. This part of the study will take about 5 minutes of your time while we ask you to provide us with some information.

Why have I been chosen?
We have chosen to ask you to take part in this study, because you have kidney disease. We are asking 550 people to take part in this part of the study.

Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognized from it and you would not be identified in any publications emanating from the study.
If you have any questions in regards to either this questionnaire or the study in general, please contact Dr Sara Kazempour at Hammersmith Hospital on 020 8383 4682 or by email: Sara.KazempourArdebili@imperial.nhs.uk.
Regards,
Dr S Kazempour

Please continue to the next page.
Instructions: Please tick the appropriate answer to the questions below and return this sheet in the pre-paid, self-addressed envelope provided.

Questions:

1- Have you been admitted to the hospital in the past two years? Please tick:

◊ Yes
◊ No

If no, please proceed to question 4.

2- What was/were the reason/s for your hospital stay?

3- Have you been admitted to the hospital to have any procedures performed on your heart?

If yes, what? Please tick:

◊ CABG (Coronary Artery Bypass Graft)
◊ Coronary angioplasty / PTCA (Percutaneous Transluminal Coronary Angioplasty)
◊ Any other interventions that treat coronary heart disease: (please specify)

4- Have you been admitted for or diagnosed with any of the following in the past two years? Please tick:

◊ Stroke
◊ TIA (Transient Ischaemic Attack)
◊ MI
◊ Any other condition relating to the heart or vascular system: (please specify)

Finally, please write your name and/or hospital number in the space below. Again, we assure you that this data will be kept confidential and will be anonymised prior to analysis.

First name:

Last name:

Hospital number:

Thank you for answering these questions.
Supplement 5: Amendment to previous ethics application

NOTICE OF SUBSTANTIAL AMENDMENT

For use in the case of all research other than clinical trials of investigational medicinal products (CTIMPs). For substantial amendments to CTIMPs, please use the EU-approved notice of amendment form (Annex 2 to ENTR/CT1) at http://eudract.emea.eu.int/document.html#guidance.

To be completed in typescript by the Chief Investigator in language comprehensible to a lay person and submitted to the Research Ethics Committee that gave a favourable opinion of the research (“the main REC”). In the case of multi-site studies, there is no need to send copies to other RECs unless specifically required by the main REC.


<table>
<thead>
<tr>
<th>Details of Chief Investigator:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name:</strong></td>
</tr>
</tbody>
</table>
| **Address:** | Department Diabetes and Endocrinology  
| | Hammersmith Hospital  
| | Du Cane Road  
| | London  
| | W12 0NN |
| **Telephone** | 0208 383 4604 |
| **E-mail:** | a.dornhorst@imperial.ac.uk |
| **Fax:** | |

<table>
<thead>
<tr>
<th>Full title of study:</th>
<th>Quantifying the 24 hour glycaemic response of a low glycaemic index diet in free living people with type 2 diabetes treated with insulin: a pilot study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name of main REC:</strong></td>
<td>Hammersmith and Queen Charlotte’s &amp; Chelsea hospitals</td>
</tr>
<tr>
<td><strong>REC reference number:</strong></td>
<td>2002/6260</td>
</tr>
<tr>
<td><strong>Date study commenced:</strong></td>
<td>2002</td>
</tr>
<tr>
<td><strong>Protocol reference (if applicable), current version and date:</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Amendment number and date:</strong></td>
<td>Version 5 24th July 06</td>
</tr>
</tbody>
</table>
Type of amendment (indicate all that apply in bold)

(a) Amendment to information previously given on the REC application form

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

If yes, please refer to relevant sections of the REC application in the “summary of changes” below.

(b) Amendment to the protocol

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

If yes, please submit either the revised protocol with a new version number and date, highlighting changes in bold, or a document listing the changes and giving both the previous and revised text.

(c) Amendment to the information sheet(s) and consent form(s) for participants, or to any other supporting documentation for the study

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

If yes, please submit all revised documents with new version numbers and dates, highlighting new text in bold.

Is this a modified version of an amendment previously notified to the REC and given an unfavourable opinion?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

Summary of changes

Briefly summarise the main changes proposed in this amendment using language comprehensible to a lay person. Explain the purpose of the changes and their significance for the study. In the case of a modified amendment, highlight the modifications that have been made.

If the amendment significantly alters the research design or methodology, or could otherwise affect the scientific value of the study, supporting scientific information should be given (or enclosed separately). Indicate whether or not additional scientific critique has been obtained.

A recent audit undertaken within our Trust on the diabetic care of patients receiving haemodialysis showed their diabetic ‘control,’ as assessed using the standard biochemical indicator of control (HbA1c), was better than expected. In addition the frequency of reported hypoglycaemic episodes was less than expected.

We would like to assess diabetic haemodialysis patient’s glycaemic control now using a
To see whether the standard HbA1c measurement in these patients provides an accurate reflection of their day to day blood glucose control. We also wish to assess whether any dietary changes on the days of dialysis is affecting overall glycaemic control.

This amendment differs from the original protocol in a number of ways:

1. The continuous monitor used will be a ‘Glucoday’ rather than the previously used ‘minimed monitor’. The Glucoday monitor although made by a different manufacturer it is used clinically in a similar manner to that of the minimed monitor.
2. The patients will only be required to wear the monitor for one 48 hour period, this will cover one day on dialysis and an adjacent day when they are not on dialysis.
3. The patients will be asked to complete a food diary, during the time they are being monitored.
4. The subjects will be recruited from the haemodialysis units in the west London region (as covered by the West London Renal and Transplant Centre, Hammersmith Hospital).

Any other relevant information

Applicants may indicate any specific ethical issues relating to the amendment, on which the opinion of the REC is sought.

List of enclosed documents

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Information Sheet for Research Participants</td>
<td>5</td>
<td>25th July 2006</td>
</tr>
<tr>
<td>Participant Consent Form</td>
<td>5</td>
<td>25th July 2006</td>
</tr>
<tr>
<td>Letter to GP</td>
<td>1</td>
<td>25th July 2006</td>
</tr>
<tr>
<td>Documentation of Changes to Protocol</td>
<td>-</td>
<td>25th July 2006</td>
</tr>
</tbody>
</table>
Supplement 6: Patient information sheet for CGM study

INFORMATION SHEET FOR RESEARCH PARTICIPANTS
You will be given a copy of this Information Sheet

Title of Project:
Quantifying the 48-hour glycaemic control in diabetic patients on haemodialysis who are eating a low glycaemic index diet as appropriate for diabetes and kidney disease.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following the information carefully and discuss it with friends, relatives, and your doctor, if you wish. Ask us if there is anything that is not clear or if you would like more information.

We will be happy to let you have a copy of the leaflet entitled “Medical Research and You” published by Consumers for Ethics in Research (CERES). This leaflet gives more information about medical research and looks at some questions you may want to ask. If you would like a copy please ask.

What is the purpose of the study?

We know that being on haemodialysis effects people in many different ways. We are interested in looking at how it may affect your blood sugar control. Diet is important in patients who have kidney failure and diabetes. Low glycaemic index starchy foods such as pasta, fruit and beans are reported to help low glucose levels after a meal. There is no data in people outside of the laboratory situation, going about their normal living or indeed on dialysis. There is a new meter that can sense blood sugar every couple of minutes for 2 days. For the first time, this allows the possibility of determining the effect of diet when people are at home and on dialysis. We believe that the introduction of a low glycaemic index diet, which contains many slowly absorbed starchy foods, will improve blood glucose control over 24-hours.

What is being tested?

We know that low glycaemic index carbohydrates like those that you are encouraged to eat as part of your usual diabetic and renal diet help to lower blood sugars. There is little information on the effect of this advice when given verbally and the effect on blood sugars when people have a free choice of what to eat. We are particularly interested in seeing how haemodialysis affects your choice of foods on the days you are being dialysed and those days you are not, and how these food choices may affect your blood sugars.

Why have I been chosen?

We have asked you to take part in this study because you have diabetes and are receiving haemodialysis. We are keen to discover more about the affects that haemodialysis and a low glycaemic diet have on your blood sugar levels. We want
to see if you blood sugars are different during a day you are being dialysed compared with a day you are not being dialysed, and if any difference in blood sugar on the dialysis days can be explained by differences in the choice of carbohydrates you eat on day you are dialysed.

**What is involved and what will I have to do?**

The study lasts for 2 days. On the 1st day we will fit a blood glucose monitor that detects the level of glucose in the blood every couple of minutes. You will have a special electrode placed under the skin on your tummy and this may hurt a little while it is placed. You should not feel it once it is in. The investigators are happy to show you the device before you decided whether to take part or not. It is not any more painful than a normal blood sugar test. You will then wear this machine for 48 hours. The monitor will be fitted when you attend for your dialysis and removed at your next session. You will also be asked to fill in a food diary for the 48 hours while you are wearing the monitor.

**Are there any risks in taking part?**

There are no risks, other than a small amount of discomfort when the glucose sensor is placed.

**What are the possible benefits of taking part?**

We hope that the information from this study will give an indication whether the results from laboratory tests can be duplicated in people who have a free choice of food. This information will help decide the best dietary and treatment advice for people with diabetes on haemodialysis, and whether this advice should be different on dialysis and non-dialysis days.

**What happens when the research study stops?**

We will give all people who took part in the study a copy of the results.

**What if something goes wrong?**

In the event of your suffering any adverse effects, as a consequence of your participation in this study, you will be compensated through the Imperial College School of Medicine’s “No Fault” Compensation Scheme.

**Will my taking part in this study be kept confidential?**

All information, which is collected about you during the course of the research, will be kept strictly confidential. Any information about you, which leaves the hospital, will have your name and address removed so that you cannot be recognised from it. We will seek your permission to tell your GP about the study before you start on the research project.
What will happen to the results of the research study?

The results of the research study will be published in a peer-reviewed journal. In addition, they will be available to everyone who took part in the study within six months of the study finishing. Individuals will not be identified in any report/publication.

Who is organising and funding the research?

The research is organised by the Department of Diabetes and Kidney Medicine at Hammersmith Hospital.

Who has reviewed the study?

The Hammersmith Hospitals Trust Research Ethics Committee has reviewed the study.

Contact for Further Information

If you wish to talk the study through or wish to raise a concern please write or ring Dr Anne Dornhorst or Dr Sara Kazempour at the Department of Diabetes, Hammersmith Hospital, Du Cane Road, London, W12 0HS. Telephone No: 0208 383 1000.

Thank you for volunteering to take part in this study!
Supplement 7: Consent form for CGM study

PARTICIPANT CONSENT FORM

Title of Project:
Quantifying the 48-hour glycaemic control in diabetic who are eating a low glycaemic index diet as appropriate for diabetes.

The participant should complete the whole of this sheet him or herself.

(Please tick each statement if it applies to you)

- I have read the Information Sheet for Patients and Healthy Volunteers
- [ ]

- I have been given the opportunity to ask questions and Discuss this study.
- [ ]

- I have received satisfactory answers to all my questions.
- [ ]

- I have received enough information about the study.
- [ ]

The study has been explained to me by:
Prof/Dr/Mr/Mrs/Ms____________________________

- I understand that I am free to withdraw from the study, at any time, without having to give a reason for withdrawing and without affecting my future medical care.
- [ ]

- I agree to take part in this study.
- [ ]

Signed……………………………………..Date…………………

(Name in Block Capitals)………………………………………...

Investigator’s signature…………………………..Date………….

(Name in Block Capitals)………………………………………...
Thank you for agreeing to fill out this food diary.

This record is designed to obtain accurate information about the type and quantity of food that you eat.

Please answer the General Question section and then go on to the Food Record.
GENERAL QUESTIONS

Which type of bread do you **usually** eat?
- White
- Brown
- Granary
- Wholemeal
- None

Do you **usually** buy large or small loaves, sliced or unsliced?
- Large
- Small
- Sliced
- Unsliced

If you eat any type of biscuit regularly, please specify which brands?
__________________________________________________________________

Which type of milk do you **usually** use?
- Full cream milk (silver top)
- Semi-skimmed milk (red striped top)
- Skimmed milk (blue top)
- None

How much milk do you **usually** use?
- 1-2 pints daily
- ½-1 pint
- ¼-½ pint
- None

How many tablespoons of milk do you take in tea and coffee?

______ tablespoons milk in a cup of tea / coffee

Which brand of butter or margarine do you buy?
______________________________

What do you do with the visible fat on your meat?
- Eat most of the fat
- Eat as little as possible
- Eat some of the fat
- Don't eat meat

Do you drink alcoholic drinks? Y / N

If the answer is Yes, please indicate how many units you drink per week?

______

1 unit = ½ pint beer/lager OR 1 glass wine OR 1 tot spirit.
FOOD RECORD

Read through these instructions and the example carefully once or twice before you start. We would like you to record, as accurately as possible, what you eat and drink for 3 days as explained by your doctor.

Please record ALL food and drink consumed. Record at the time of eating and NOT from memory at the end of the day. Keep this record sheet with you throughout the day. You should include all meals and snacks, plus sweets, drinks etc. When recording food eaten at meals, please include any sauces, dressing or extras eg: gravy, salad dressing, pickles, as well as the main food. If you do not eat a particular meal or snack simply draw a line across the page at this point.

Guidelines for describing food & drink:

1. Give as many details as possible about the type of food you eat:
   a) State brand name where applicable
      eg: 'Princes' sardines in tomato sauce OR 'Sainsbury's' half-fat Edam cheese.
   b) Name the type of biscuit, cake or cereal
      eg: Rich Tea, Madeira, Branflakes.
   c) Name the type of cheese, fish or meat
      eg: Cheshire cheese, haddock fillet, pork chop.

2. Suggestions for recording quantity of food and drink:
   a) For many foods such as vegetables, cereals and some fruit a household measure is adequate, state the number of teaspoons (tsp) or tablespoons (tbsp) or cups, and whether level, rounded or heaped.
   b) All convenience foods have their weight on the packaging and this can be quoted
      eg: 150g carton Ski raspberry yoghurt OR ½ 15 oz can baked beans.
   d) Cheese, fish, meat. When possible, pleaseweigh your portions of these foods. Otherwise describe as well as you can.
      eg: 2 large thin slices ham OR 2 small lamb chops (no fat eaten) OR Medium fillet of cod grilled with 1 tsp flora OR Cube of cheddar cheese the size of a matchbox.

Remember to include everything you eat and drink including snacks and nibbles. Please do not change what you normally eat just because you are filling in this record.

Look at the example of how to fill in your record - you may find this helpful.

THANK YOU VERY MUCH FOR YOUR HELP
<table>
<thead>
<tr>
<th>TIME</th>
<th>DETAILS OF FOOD &amp; DRINK</th>
<th>QUANTITY EATEN</th>
<th>Leave Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.45am</td>
<td>Tea with Skimmed milk</td>
<td>1 cup</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 tbsp</td>
<td></td>
</tr>
<tr>
<td>9am</td>
<td>Branflakes (Kellogg’s) Skimmed milk for cereal &amp; drinks</td>
<td>3 heaped tbsp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wholemeal bread (large loaf) Flora extra light margarine</td>
<td>½ pint</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coffee</td>
<td>1 medium slice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 tsp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 mugs</td>
<td></td>
</tr>
<tr>
<td>11.10am</td>
<td>Coffee with skimmed milk Apple (eaten with skin)</td>
<td>1 mug</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 tbsp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 medium</td>
<td></td>
</tr>
<tr>
<td>1.15pm</td>
<td>Sandwiches: wholemeal bread (Allinsons) large loaf, sliced</td>
<td>4 slices</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flora extra light margarine Ham (no fat) Tomato Banana Diet Tango</td>
<td>4 level tsp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 thin slices</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 large</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 large</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 can (330ml)</td>
<td></td>
</tr>
<tr>
<td>TIME</td>
<td>DETAILS OF FOOD &amp; DRINK</td>
<td>QUANTITY EATEN</td>
<td>Leave Blank</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>----------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>4.30pm</td>
<td>Low Calorie squash made with concentrated squash</td>
<td>1 glass</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KP roasted salted peanuts</td>
<td>25g pkt</td>
<td></td>
</tr>
<tr>
<td>715pm</td>
<td>Chicken &amp; mushroom casserole (home-made with skimmed milk in the sauce)</td>
<td>4 heaped tbsp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jacket potato</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Broccoli, boiled</td>
<td>1 apple sized</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shape raspberry yoghurt</td>
<td>3 tbsp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Half mineral water/half natural orange juice</td>
<td>1 x 150g tub</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tea with skimmed milk</td>
<td>1 glass</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 cup</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 tbsp</td>
<td></td>
</tr>
<tr>
<td>8pm</td>
<td>Nothing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.40pm</td>
<td>Ovaltine made with:</td>
<td>1 mug</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ovaltine</td>
<td>1 tsp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ordinary silver top milk, the rest water</td>
<td>½ mug</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rich Tea biscuits (Sainsburys).</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
# DIETARY RECORD SHEET

**DAY 1:** ______________________  **DATE:** ______________________

<table>
<thead>
<tr>
<th>Time</th>
<th>Details of Food &amp; Drink</th>
<th>Quantity Eaten</th>
<th>Leave Blank</th>
</tr>
</thead>
</table>


<table>
<thead>
<tr>
<th>Time</th>
<th>Details of Food &amp; Drink</th>
<th>Quantity Eaten</th>
<th>Leave Blank</th>
</tr>
</thead>
</table>

DAY 2: ______________________ DATE: _____________________
<table>
<thead>
<tr>
<th>Time</th>
<th>Details of Food &amp; Drink</th>
<th>Quantity Eaten</th>
<th>Leave</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Blank</td>
</tr>
</tbody>
</table>

DAY 3: ______________________   DATE: _____________________

dietrec.doc/c:diets/JUN02