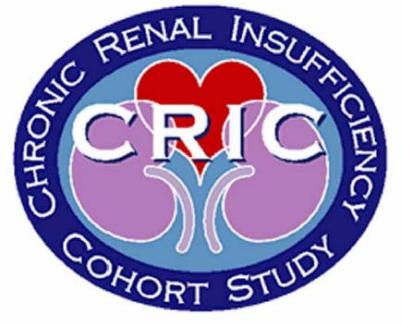

CHRONIC RENAL INSUFFICIENCY COHORT (CRIC) STUDY

Sponsored by the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), Department of Health and Human Services (DHHS)

PROTOCOL—VERSION 3.0

Dated: April 1, 2006

Incorporating Amendment #1, Dated: March 17, 2003
Designated by the indicator: [A1]

Incorporating Amendment #2, Dated: April 20, 2005
Designated by the indicator: [A2]

Incorporating Amendment #3, Dated: December 22, 2005
Designated by the indicator: [A3]

Incorporating Amendment #4, Dated: March 13, 2006
Designated by the indicator: [A4]

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1. INTRODUCTION

1.A. Overview

Chronic Renal Insufficiency (CRI) recently was recognized as a silent epidemic (1) affecting more than 10 million Americans. The burden of morbidity and mortality associated with CRI derives from the frequent progression of CRI toward end-stage renal disease (ESRD) and the disproportionate risk of cardiovascular disease (CVD) in the setting of CRI. CRI is strongly and independently associated with CVD, even after adjustment for traditional CVD risk factors**.** This led to the hypothesis that specific "uremia-related risk factors" augment the rate of CVD in the setting of renal disease and that this causes many patients with progressive renal disease to succumb to fatal cardiovascular events before they need renal replacement therapy.

To gain understanding of the relationship between progressive renal disease and cardiovascular illness, the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK) established the Chronic Renal Insufficiency Cohort (CRIC) Study in 2001. The principal goals of the CRIC Study are to examine risk factors for CRI and CVD events among patients with varying severity of CRI, and develop predictive models that will identify high-risk subgroups with CRI. The latter results will target enrollment of high-risk subjects into future treatment trials and increase application of available preventive therapies. Improved recognition of etiological factors will permit development and testing of interventions to reduce the burden of advanced renal failure and cardiovascular morbidity and mortality.

The overarching aim of the Chronic Renal Insufficiency Cohort (CRIC) Study is to establish an enduring, collaborative CRI research group capable of examining hypotheses concerning disease etiology, diagnosis, health outcomes, and health services utilization among a cohort of patients with CRI. The CRIC research group will include the following components organized to engage in coordinated communication and decision-making.

- Scientific and Data Coordinating Center (SDCC) consisting of a Scientific Coordinating Group and a Data Coordinating Group.
- Seven Clinical Centers (CC)
- NIDDK Project Scientists
- Scientific Advisory Committee
- Ancillary study investigators
- Centralized Diagnostic Image Reading Facilities (CDIRF) for echocardiography, electron beam computerized tomography, and electrocardiography
- Centralized Laboratory (CL) for evaluation of GFR and other biochemical evaluations
- NIDDK Central Repository for DNA and other biological specimens

1.B. Study Hypotheses

The study will address the following hypotheses:

Hypothesis 1. A set of non-traditional risk factors is associated with both progression of CRI and development of end-stage renal disease. (Non-traditional risk factors indicate risk factors that have not yet been well studied in renal disease in contrast to well-studied factors such as blood pressure and proteineuria.)

Hypothesis 2. A set of non-traditional risk factors is associated with CVD events and measures of CVD progression in the setting of CRI.

Hypothesis 3. The risk factors for CRI progression and CVD in the setting of CRI vary by demographic characteristics (age, gender, race/ethnicity) and diabetes status.

Hypothesis 4. The morbidity and complications associated with CRI and its progression diminish global and disease-specific QOL, impair functional status, and increase health resource utilization.

Hypothesis 5. Progression of CRI as estimated by serum creatinine, 1/serum creatinine, and currently available serum creatinine-based formulae may yield biased estimates of the rate of progression of CRI.

1.C. Specific Aims

Each of these five hypotheses motivates a series of specific aims that will be the focus of the CRIC study and are outlined below:

Aims Related to Hypothesis 1.

1. To examine the relationship between the following exposures and the progression of CRI and development of ESRD:
    a. diminished glomerular filtration rate (GFR) at baseline
    b. rapidity of rate of CRI progression
    c. proteinuria
    d. antihypertensive treatment (e.g., ACE inhibition) and blood pressure control
    e. subclinical measures of CVD (EBT)
    f. increased LV mass (Echocardiogram)
    g. poor glycemic control
    h. treatment of dyslipidemia and lipid control
    i. inflammatory cytokines
    j. hemostatic and prothrombotic factors
    k. elevated homocysteine
    l. heavy metal exposure
    m. obesity and other anthropometric measures
    n. abnormalities in nutritional status
    o. selected psychosocial and health-related factors
    p. early referral to subspecialty care

2. To examine the relationships in Aim 1 with respect to the following types of outcomes:
    a. Progression of CRI as defined by slope of change in GFR
    b. Time to ESRD or 50% reduction of GFR

3. Establish the capacity for future biochemical and genetic analyses

Aims Related to Hypothesis 2.

1. To examine the relationship between the development of CVD and exposures listed in Hypothesis 1, as well as:
    a. Anemia
    b. Dysregulation of calcium-phosphate metabolism

2. To examine the relationships in Aim 1 with respect to the following types of outcomes:

   a. Subclinical CVD (coronary calcification by EBT/CT, LV size function by echo, silent ischemia by echo/ECG)
   b. Clinical CVD events (coronary, cerebrovascular, peripheral arterial disease)
   c. CVD-related and all-cause death

3. To explore commonalities of risk factors for both CRI and CVD outcomes in the setting of CRI
4. To establish the capacity for future biochemical and genetic analyses

Aims Related to Hypothesis 3.

1. To examine exposure-outcome relationships among subgroups including those defined by:
   a. Diabetic status
   b. Age
   c. Gender
   d. Race/ethnicity

Aims Related to Hypothesis 4.

1. To evaluate the association of CRI at baseline and rates of progression of CRI with:
   a. Measures of global and disease-specific QOL
   b. Measures of functional status
   c. Health resource utilization

Aims Related to Hypothesis 5.

1. To develop accurate, clinically useful multivariable equations that estimate progression of CRI from routine clinical data in a variety of racial and disease categories
2. To develop additional multivariable equations that provide even more accurate estimates of GFR using data currently acquired through research (for example, using measurements of cystatin C, measurements of lean body mass from DEXA or BIA)
3. To assess the value of these new equations for the prediction of ESRD and CVD events

2. BACKGROUND

2.A. Burden of Chronic Renal Insufficiency

Treated ESRD has increased steadily over the past two decades. In 1998, 323,000 prevalent individuals were treated for ESRD with annual mortality of 20-25% and costs exceeding 20 billion dollars (2). Although previous estimates of the CRI population far exceed the size of the ESRD population (only a subset of individuals with CRI develops ESRD), a true population-based measure of the burden of CRI has been difficult to quantify (3). Strauss et al. (4) examined NHANES II data and estimated that in 1988 there were approximately 650,000 individuals in the US with a serum creatinine (Screat) 2.0 to 8.0 mg/dL. Experience from the Modification of Diet in Renal Disease (MDRD) and the African-American Study of Kidney Disease and HTN (AASK) studies have demonstrated that clinically important reductions in the glomerular filtration rate (GFR) often occurs among individuals with Screats <2.0 mg/dL.

Jones et al. (5) used data from the NHANES III (1988 and 1994) and estimated that approximately 6.2 million and 2.5 million persons ages 12+ years are estimated to have serum creatinine levels $\geq$ 1.5 mg/dL and $\geq$ 1.7 mg/dL, respectively. As shown in Table 1, the prevalence estimates for levels of Screat are strongly related to gender and race. Non-Hispanic black men and women have approximately a 2.5 and 1.5-fold higher prevalence of elevated Screat than their white counterparts. Despite the shortcomings of Screat as a measure of renal function, and the absence of information on cause of renal disease, these data are suggestive of a substantial reservoir of CRI in the US population that is likely disproportionately distributed among blacks and the elderly.

Table 1: % Prevalence (Standard Errors) of Screat by Ethnicity and Gender, NHANES III (5)

	Men			Women		
	Creat $\geq$ 1.5	Creat $\geq$ 1.7	Creat $\geq$ 2.0	Creat $\geq$ 1.5	Creat $\geq$ 1.7	Creat $\geq$ 2.0
Total US	4.98 (0.33)	1.87 (0.19)	0.64 (0.11)	1.55 (0.14)	0.73 (0.11)	0.33 (0.06)
Non-Hispanic White	4.70 (0.42)	1.78 (0.22)	0.55 (0.12)	1.60 (0.17)	0.74 (0.14)	0.30 (0.07)
Non-Hispanic Black	10.79 (0.68)	3.37 (0.31)	1.60 (0.22)	2.64 (0.28)	1.41 (0.19)	0.80 (0.12)
Mexican American	1.46 (0.28)	0.71 (0.15)	0.28 (0.08)	0.55 (0.13)	0.23 (0.07)	0.19 (0.07)

Expanding the definition of CRI to include individuals with proteinuria without elevation in serum creatinine would lead to a substantially larger estimate of the size of the kidney disease population in the United States.

The National Kidney Foundation has recently published clinical practice guidelines proposing a uniform definition and classification of stages of chronic kidney disease (6), that will be of value in assessing the burden of kidney disease and, potentially, risk stratifying afflicted individuals (see Table 2). These definitions incorporate both proteinuria as a marker of kidney damage and GFR estimated from serum creatinine. Using data from NHANES III, estimates of the prevalence of stages of chronic kidney disease and levels of estimated GFR in the US are as shown below in Table 3. These estimates are consistent with the estimates cited above.

Table 2. Definition and Stages of Chronic Kidney Disease

GFR (mL/min/1.73 m²)	With Kidney Damage*		Without Kidney Damage*	
	With HBP**	Without HBP**	With HBP**	Without HBP**
≥90	1	1	"High blood pressure"	"Normal"
60–89	2	2	"High blood pressure with ↓ GFR"	"↓ GFR"[a]
30–59	3	3	3	3
15–29	4	4	4	4
<15 (or dialysis)	5	5	5	5

Shaded area represents chronic kidney disease; numbers designate stage of chronic kidney disease.

* Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies.

** High blood pressure is defined as ≥140/90 in adults and >90th percentile for height and gender in children.

a May be normal in infants and in the elderly.

National Kidney Foundation. K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification and Stratification. Am J Kidney Dis 39: S1-S266, 2002 (suppl 1).

Table 3. Prevalence of Stages of Chronic Kidney Disease and Levels of Kidney Function in the US

Stages of CKD			Levels of Kidney Function		
	N (1000's)*	(%)	GFR (mL/min/1.73 m²)	N (1000's)*	(%)
1	10,500[a] 5,900	5.9[a] 3.3	**≥90**	114,000	64.3
2	7,100[a] 5,300	4.0[a] 3.0	**60–89**	55,300	31.2
3	7,600	4.3	**30–59**	7,600	4.3
4	400	0.2	**15–29**	400	0.2
5	300	0.2	**<15 (or dialysis)**	300	0.2

* Data for Stages 1–4 from NHANES III (1988–1994). Population of 177 million with age ≥20 years. Data for Stage 5 from USRDS (1998), includes approximately 230,000 patients treated by dialysis, and assumes 70,000 additional patients not on dialysis. Percentages total >100% because NHANES III may not have included patients on dialysis. GFR estimated from serum creatinine using MDRD Study equation based on age, gender, race and calibration for serum creatinine.

[a] For Stages 1 and 2, kidney damage was assessed by spot albumin-to-creatinine ratio >17 mg/g (men) or >25 mg/g (women) on one occasion (larger prevalence estimate) or on two measurements (smaller prevalence estimate). Albuminuria was persistent in 54% of individuals with GFR ≥90 mL/min/1.73 m² (n = 102) and 73% of individuals with GFR 60–89 mL/min/1.73 m² (n = 44).

Reference: National Kidney Foundation. K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification and Stratification. Am J Kidney Dis 39: S1-S266, 2002 (suppl 1).

The CRIC Study was conceived and developed by the NIDDK in recognition of the need to investigate the epidemic of CRI and the acknowledgement that existing studies of CRI and ESRD provide an incomplete understanding of the burden of CRI. Morbidity and mortality associated with CRI, often from CVD, make compelling the long-term study of afflicted individuals, because

CRI will often not progress to ESRD due to morbidity from competing illness, a key aspect of the public health importance of this research initiative.

2.B. Risk Factors for Progression of CRI

2.B.1. Overview

It has now been recognized that with advanced forms of renal insufficiency, progressive loss of renal function continues even if initial disease activity abates (7) at least, in part, from hyperfiltration, glomerular capillary hypertension (HTN), and subsequent glomerulosclerosis (8). Earliest clinical evidence for progression is development or worsening of proteinuria which may, itself, be pathogenic (9). Additionally, HTN (10;11), hyperlipidemia (12), and dietary protein (13) act to accelerate the progression of chronic renal disease of varied etiologies. Diabetic CRI deserves particular focus as it accounts for 40% of new cases of ESRD (14) and because of evidence that tight control of blood pressure (BP) and plasma glucose as well as blockade of the renin-angiotensin system all effectively reduce the rate of progression of CRI.

2.B.2. Hypertension (HTN)

The prevalence of HTN in CRI is high, although it is highest in blacks, patients with glomerular diseases, and the elderly (15). Data from the MDRD Study suggest HTN is frequently poorly controlled (16) as compared with JNC-VI targets (17). Evidence linking HTN to progressive renal insufficiency comes from both observational studies and clinical trials. Consistent with observational research (18;19), 20-year follow-up from the Multiple Risk Factor Intervention Trial (MRFIT) (10) demonstrated a graded increase in the risk of ESRD among the 332,000 MRFIT screenees with increasing levels of BP measured at baseline. Clinical trials in both diabetic (20;21) and nondiabetic (22;23) renal disease have demonstrated that antihypertensive therapy slows the rate of progression at least in certain clinical subgroups. Analyses from the MDRD study (16) and the pilot phase of the African-American Study of HTN and Kidney Disease (AASK) (24) suggest that additional renoprotection is safely afforded by target BPs lower than mean arterial pressures of 92 mm Hg, especially in patients with proteinuria.

Growing literature from clinical trials demonstrates the superiority of angiotensin converting enzyme inhibitors (ACEI) in stemming the progression of renal disease in both diabetic and nondiabetic patients (25-27). ACEI have been shown to reduce progression from microalbuminuria to overt proteinuria among patients with insulin-dependent (28) and type II diabetes (29-31). Among patients with CRI from causes other than diabetes, use of ACEI has also been associated with a slower rate of doubling of Screat or ESRD (25).

Despite the marked advancement in our understanding of the role of HTN in the progression of CRI, numerous questions remain. We await additional information on how HTN mediates progression of CRI and why there is an exaggerated effect among African Americans. We also do not know the optimal level of BP for clinical subgroups of patients with CRI and, in particular, those with diabetic renal disease.

2.B.3. Proteinuria

Proteinuria is a negative prognostic factor for numerous types of renal disease. Idiopathic glomerular disorders such as IgA and membranous nephropathy progress

more frequently to ESRD in the presence of high-grade proteinuria. Baseline levels of proteinuria were one of six factors in multivariable analysis in the MDRD Study predicting the rate of CRI progression (32). Further, with increasing baseline levels of proteinuria, there was a progressively larger beneficial effect of BP lowering. The Ramipril Efficacy in Nephropathy (REIN) Study of nondiabetic CRI has also demonstrated a highly significant correlation between proteinuria and decline in GFR (33).

2.B.4. Diet & Other Behaviors

Dietary protein reduction has been a focus in nutritional research in CRI. The MDRD Study examined whether reducing dietary protein slows the progressive decline of GFR in nondiabetic CRI. The overall result failed to demonstrate a beneficial effect of protein reduction (34), but secondary analyses suggested that subgroups may benefit (35). Meta-analyses have reported similar conflicting findings (13;36).

Based on limited data on the relationship between progression of CRI and both lipids and oxidative stress, a focus on these factors is warranted in investigations such as the CRIC study. Finally, obesity has been implicated in the development of some forms of glomerulonephritis and it has been observed that proteinuria improves with weight loss.

Beyond diet, the most well-defined health behavior impacting the course of CRI is smoking (33;37;38). Smoking is a risk factor for ESRD among men with primary renal disease (37) and also has a relationship to elevated Screat in patients without diabetes who are >65 years (38). The relationship between the extent of tobacco use and renal outcomes is unknown, as is the risk reduction that would result from smoking cessation. Even less is known about the impact of alcohol intake.

2.B.5. Glycemic Control

Rates of onset of microalbuminuria and overt proteinuria were decreased among Diabetes Control and Complications Trial patients with type I diabetes randomized to a more intensive glycemic regimen (39). Less clear is the relationship between glycemic control and progression of CRI among patients with type II diabetes.

2.B.6. Lipids

Prevalence of hyperlipidemia is elevated in CRI. Among the few studies published on non-nephrotic patients, approximately 1/3 of patients have elevated LDL, 1/3 depressed HDL, and 1/3 elevated triglycerides (40). Compared to the general population, CRI patients more frequently demonstrate depressed HDL cholesterol with elevated triglycerides despite normal LDL cholesterol (40). Patients with nephrotic syndrome have the highest levels of total and LDL cholesterol and >60% of patients with CRI may have levels of lipoprotein (a) >30 mg/dL.

Hyperlipidemia appears to be a risk factor for progression of CRI in patients with (41) and without diabetic nephropathy (12;42). However, the relationship between the dyslipidemia of CRI on the rate of progression of renal disease is yet to be elucidated. In a small trial in diabetic patients with CRI, lovastatin was associated with reduced progression as assessed by measurement of GFR (43). Other small clinical trials have not reproduced these findings (44). Studies of the importance of apo E2 genotype have provided conflicted results (40).

2.B.7. Vasoactive Compounds, TGF-β, Angiotensin II

Animal studies have demonstrated that numerous cytokines are upregulated in the course of CRI, a cascade of events that appears to be led by the increased concentration of angiotensin II in the kidney. Angiotension II not only leads to glomerular HTN through alteration of intra-renal hemodynamics, but also appears to be an important regulator of cytokines such as transforming growth factor TGF-β, tumor necrosis factor α, nuclear factor kappaB as well as vasoactive compounds such as endothelin and thromboxane (45). Interest in TGF-β arises from evidence that it plays a major role in stimulating extracellular collagen and reducing collagenase production, both of which lead to renal scarring. *In vivo* data have implicated these events in some models of hypertensive renal disease as well as diabetic nephropathy (46). Repeated measures of serum and urinary cytokines from a prospectively followed cohort with CRI represent the next logical step in the clinical research setting.

2.B.8. Genetics

In addition to several single-gene disorders (e.g., polycystic kidney disease), the severity and course of numerous common renal diseases are determined by complex interactions between genetic make-up and environment. These conditions include hypertensive nephrosclerosis, primary glomerular diseases, some forms of vasculitic renal disease, and diabetic nephropathy (47). In particular, there is growing evidence that the progression of these diseases to ESRD is genetically determined (48). Allelic variants responsible for genetic susceptibility may be common polymorphisms that require the coexistence of other genetic or environmental factors to lead to the phenotypic expression of progressive CRI. Polymorphisms that have been evaluated include those related to the renin-angiotensin system, kallikrein, fibrinogenic/fibrinolytic systems, and the Na/H pump. Among the best studied have been the ACE gene polymorphisms, of which the DD allele has been shown to cause progression of CRI. Genetics research will likely turn increasingly to family-based association studies (49;50) to define individual susceptibility to developing progressive renal disease. Such efforts will be feasible to plan using banked specimens of DNA from patients with CRI.

2.B.9. Other

The above list of risk factors is not comprehensive. A variety of other factors have been proposed but are less studied and established. Examples include uric acid, socioeconomic status, anemia, advanced glycation end products and access to health care (nephrologic referral).

2.C. Epidemiology of Cardiovascular Disease (CVD) in End-Stage Renal Disease (ESRD) & CRI

CVD mortality in dialysis patients is >30-fold higher than among the general population (51) and 10-20 fold higher after stratification by age, race, and gender. The 5-12% prevalence of coronary disease in the general population (52) is dwarfed by an estimated 40% prevalence among dialysis patients (53). 40% of incident dialysis patients in 1996 had a history of myocardial infarction (MI) or coronary revascularization (54). Similarly, left ventricular hypertrophy (LVH) has been reported among 20% of the general population (55), but 75% of dialysis patients (56). The clinical relevance of LVH is demonstrated by its independent association with mortality in

hemodialysis patients, much of which occurs after the development of chronic heart failure (CHF) (57;58).
The prevalence of LVH among patients with CRI has been shown to exceed the prevalence in the general population (59-62). Two studies (60;61) have shown an inverse relationship between creatinine clearance and the prevalence of LVH. In a study by Levin et. al. (61), 27% of patients with creatinine clearance > 50 mL/min, 31% with clearance 25-49 mL/min, and 45% of those with clearance < 25mL/min had LVH defined with echocardiography. Similarly, LV mass is directly associated with BP and inversely associated with hemoglobin levels (63). Confirming that these are causal relationships, intervention studies have demonstrated improvement in LVH after treatment of anemia (64) and reduction in BP, especially using ACEI (65). Unknown is the impact of such regression on the occurrence of CHF and other CVD events.

The dearth of follow-up studies complicates estimation of the incidence of CVD in CRI. However, the CV event rate in CRI can be inferred from several clinical trials and cohort studies. In the HTN, Detection, and Follow-up Program, a baseline Screat $\geq$1.7 mg/dL was associated with a 2.2-fold higher adjusted odds of death at eight years compared to baseline Screats <1.7 mg/dL (66). Data from the MDRD Study demonstrated that 25% of first hospitalizations were for CVD (67). Two trials of ACEI for nondiabetic CRI provide data on the combined end-point of sudden death and MI. Among patients with a Screat between 1.5 and 4.0 mg/dL enrolled in a study of benazepril (22), there was a rate of 1% per year, remarkably similar to that observed among patients with >3g/d proteinuria enrolled in the REIN Study (68).

Jungers et al (69) observed a two to three-fold higher rate of MI in CRI compared to patients without CRI. CV events were independently associated with tobacco use, HTN, elevated fibrinogen and homocysteine, and low HDL cholesterol. The Cardiovascular Health Study reported a 71% adjusted elevated rate of mortality among elderly subjects whose baseline Screat was $\geq$1.5mg/dL (70).

Recently, Culleton et al. (71) reported on 6,233 adults in the Framingham Heart Study. The rate of all-cause mortality and CVD was compared between participants with normal and mild CRI (serum creatinine $\geq$136 umol/L in men and $\geq$120 umol/L in women). The adjusted rate ratios for all cause mortality were 1.31 (95% CI: 1.02 – 1.67) for men and 1.08(95% CI: 0.87 – 1.34) for women. The adjusted rate ratios for CVD were 1.06, 95% CI(0.79 – 1.43) for men, and 1.04, 95% CI(0.79 – 1.37) for women. These studies provided compelling evidence for the morbidity risks of CRI beyond simple progression to ESRD. However, full understanding of the relationship between CRI and CVD awaits research that examines biomarkers of putative mechanistic pathways and employs more sensitive tools to detect CVD such as EBT or echocardiography.

2.D. Risk Factors for CVD in CRI

2.D.1. Overview

Clinical trials and cohort studies of patients with renal disease have demonstrated that many of the traditional risk factors for CVD also operate in the CRI population. Furth et al. studied US dialysis patients alive in 1990 and demonstrated that older age, male gender, white race, diabetes, and smoking were independent risk factors for death (72). Analyses of CV risk factors from the HEMO trial found that some (diabetes, smoking, and older age), but not all (e.g., total cholesterol, systolic BP) risk factors were associated with CVD (73). Finally, within ESRD, investigators have described a U-shaped association between survival and both HTN and total cholesterol (74;75). Such

relationships likely represent the interrelationship between incompletely accounted for characteristics such as CHF that both lowers BP and increases the risk of death as well as malnutrition that reduces serum cholesterol while also increasing mortality.

The Framingham Heart Study's CVD risk score was only weakly associated with baseline renal function and did not predict the elevated event rate in the CRI population (76) suggesting that traditional factors explain some but not all of the elevated risk of CV events with CRI. In contrast, Culleton et al. (71) failed to detect CRI, *per se*, as an independent risk factor for CVD in the Framingham Heart Study cohort (Table 4). These data demonstrate that the rates of diagnosed CVD were greater in the setting of CRI, and that CRI, *per se*, was an independent risk factor for CVD. Inasmuch as some traditional CV risk factors are influenced by CRI, adjustment for them may have attenuated the relationship between CRI and CVD.

Table 4: Rate (per 1000 patient years) of CVD and All-Cause Mortality in the Framingham Heart Study

	Men				Women			
	Normal Creat	CRI	Unadjusted HR	Adjusted HR	Normal Creat	CRI	Unadjusted HR	Adjusted HR
CVD	18.5	21.3	1.17 (0.88-1.57)	1.06 (0.79-1.43)	11.0	25.6	2.19 (1.70-2.83)	1.04 (0.79-1.37)
All-cause mortality	27.5	33.3	1.54 (1.22-1.94)	1.31 (1.02-1.67)	17.6	39.5	2.25 (1.84-2.75)	1.08 (0.87-1.34)

While there is evidence to suggest that many traditional risk factors operate within the CRI population, they alone probably do not account for the excess CV morbidity. These considerations led to a recent National Kidney Foundation Task Force on CVD (77) to emphasize the importance of "uremia-related" risk factors (e.g., proteinuria, elevated Lp(a), etc.) that frequently increase in prevalence or severity as renal function declines (78). Thus, the evaluation of risk factors for CVD in CRI in the proposed cohort study will need to focus on both traditional and novel risk factors, many of which will change in magnitude as underlying renal disease progresses.

2.D.2. Hypertension

The relationship between HTN and CVD has been well demonstrated with respect to coronary heart disease and CHF (79;80), but have been inconsistently identified in ESRD (73;74). The prevalence of HTN in CRI increases as renal function falls and HTN often is not adequately treated (16). Further, systolic BP is a risk factor for CVD in CRI (69). In addition to its high prevalence, the pattern of BP in CRI may also be abnormal. The loss of diurnal variation (normally nocturnal suppression) among individuals with CRI may worsen CV toxicity associated with HTN (59;81). The target values of BP as well as the preferred antihypertensive drugs in CRI with HTN remain to be elucidated.

2.D.3. Lifestyle

Numerous lifestyle characteristics have been implicated in the occurrence of CVD including atherogenic diets and weight gain (82), lack of physical activity (83), and cigarette smoking (84). Few data from patients with CRI are available to examine the importance of these potentially modifiable characteristics among this population. Epidemiological data have shown a protective association between moderate alcohol intake and the risk of coronary heart disease in patients without CRI (85). Whether this relationship applies equally to individuals with CRI has yet to be studied definitively.

2.D.4. Volume Overload & Electrolyte Imbalance

Extracellular fluid overload has been associated with LVH and atherosclerosis. Alterations in calcium, phosphate, and parathyroid hormone metabolism have been associated with elevated rates of CVD morbidity and mortality (86). Clarification of the relationship between divalent ion metabolism and CVD is a highly relevant focus of research because of the plausibility of its role in instigating calcific atherosclerosis and because targeted therapies (phosphate binders and vitamin D analogs) are readily available to improve metabolic abnormalities.

2.D.5. Lipids

The association between total cholesterol and risk of subsequent CVD has been demonstrated by numerous observational studies and a series of randomized clinical trials (RCTs) that have shown decreased CVD rates following reductions in total cholesterol (87) (88), and LDL cholesterol (89) for primary and secondary prevention (90-92). Although evidence from lipid intervention studies in CRI is lacking, a role for hypercholesterolemia in CVD among individuals with CRI is strongly suspected (78),(33). Patients with nephrotic syndrome have a markedly elevated risk of CVD, findings that may arise from the dyslipidemia that is part of the nephrotic syndrome (93). Jungers et al. studied 147 patients with creatinine clearances 20 to 50 ml/min and found HDL cholesterol to be an independent predictor of MI (69;94). Lipoprotein(a) (Lp(a)), composed of an LDL particle bound to an apoprotein, has been associated with CVD in studies of the general public (95-98) and its plasma concentrations are even higher in CRI. Several studies have shown that Lp(a) levels predict fatal CV events in hemodialysis patients (99;100). However, we lack this understanding of the relevance of dyslipidemia in CRI. More data on the relationship of CV outcomes with the magnitude and character of lipid abnormalities observed in CRI are needed to design future clinical studies in this area.

2.D.6. Homocysteine & Oxidant Stress

Plasma homocysteine levels have been linked to the risk of coronary heart disease in prospective studies (101). While average homocysteine levels are 10 umol/L in healthy adults and 15 umol/L in patients with coronary disease, they are 25 to 35 umol/L in patients with ESRD. These two observations led to the hypothesis of an etiological role for homocysteine in the atherogenesis observed among patients with ESRD. Attempts to lower homocysteine with folic acid supplementation in ESRD yielded only small reductions in homocysteine levels (102). Prior to implementing a trial in CRI, the evolution of homocysteine levels and their relationship to CV events in CRI needs to be more fully elucidated.

Animal data and lower observed rates of CVD associated with intake of antioxidant vitamins in observational studies suggest that oxidation of LDL cholesterol may play a role in atherogenesis (103-105). This relationship holds particular interest for CRI because of the higher levels of oxidant stress in these patients compared to the general public (106).

2.D.7. Inflammation

The role of inflammation in the pathogenesis of atherosclerosis is widely accepted (107). Numerous inflammatory markers such as ICAM-1 (108), C-reactive protein (CRP)

(109-111), fibrinogen (109), lipoprotein associated-phospholipase A2 (109) and IL-6 (112) have been associated with CVD. For example, C-reactive protein (CRP) levels were identified to be independent risk factors for both MI and stroke in the Physician's Health Study, improving clinical prediction models based on lipids alone (111). Markers of inflammation are elevated among patients with CRI and ESRD and these have been associated with both CVD-specific and all-cause mortality (113;114), suggesting a potential etiological role in the high rates of morbidity in kidney disease. A number of small studies have examined directly the interrelationship between measures of inflammation and CVD.

2.D.8. Anemia

Although there are few data on the prevalence of anemia in the CRI population, evidence suggests that it is common in advanced CRI. Between 1995 and 1997, 67% of patients beginning dialysis had a hematocrit (HCT) <30% (115). HCT <28% was more common among women, nonwhites, and patients without private insurance (115). The impact of anemia in CRI has only been partially investigated. Several studies indicate that successful treatment of anemia leads to improved energy levels, work capacity, cognitive function, sexual function and quality of life (116). Several promising studies have linked treatment of anemia in CRI to regression of LVH (60;117;118). Levin et al. found a potent association between left ventricular mass index (LVMI) on echocardiograph and anemia among 175 patients with CRI, 38.9% of whom had an elevated LVMI (60). For each 1 g/dL decrease in hemoglobin (HgB), LVMI increased by 6%. In a multivariable analysis, HgB and systolic HTN were the only two independent predictors of LVMI. Larger and more long-term studies are needed to understand the influence of anemia on intermediate and long-term CV outcomes in the setting of CRI.

2.D.9. Sex Hormones

Hormone replacement therapy with exogenous estrogens has been associated with a reduced CVD risk (119;120) in observational studies, but these findings were not confirmed in recent clinical trials of secondary prevention for coronary heart disease (121). Whether CRI alters the effect of hormone replacement on CVD is unknown.

2.D.10. Diabetes & Glucose Metabolism

Diabetes is a well known CVD risk factor. Glucose intolerance and its associated hyperinsulinemia have been implicated in atherogenesis (122) and CVD (123). Insulin resistance has long been known to occur among patients with renal insufficiency (124). However, the epidemiological relationship between this phenomenon and CV events has not been fully evaluated in CRI. Measures such as HbA1c and plasma insulin levels indexed to serum glucose (125) represent important opportunities to examine the relationship between abnormalities of carbohydrate metabolism and CV risk in the proposed cohort study.

2.D.11. Genetics

Extensive evidence has also accumulated for the role of genetics in influencing CV risk factors including lipids, glucose metabolism, prothrombotic factors and HTN. There is also considerable evidence that genetic factors independent of known CV risk factors influence the development of atherosclerotic disease.

The proposed cohort study as well as family association studies (49;50) and other novel study designs will provide an opportunity to study the relationship of CVD with specific genetic markers in a CRI population.

2.E. Summary

Like others (25;78), this review of CVD and CRI-progression among individuals with CRI recognizes many common risk factors and pathogenic pathways. Many aspects of progressive renal disease and CVD among individuals with CRI actually may reflect parts of the same underlying disorder (126). Optimization of the treatment of patients with CRI will depend on identifying strategies that not only stem the progression of CRI, but reduce the excessive burden of CVD in this population. CRIC will provide important information on potential risk factors for progressive CRI and CVD as did studies of the relationship between cholesterol and hypertension to CVD prior to treatment trials of lipid and blood pressure lowering drugs. CRIC is devoted to expanding this knowledge, setting the stage for formulation of hypotheses regarding therapy that will ultimately serve as the basis for a new generation of interventional trials focused on reducing the burden of CRI. The CRIC Study represents a long term commitment to use the tools of observational epidemiology to address research questions regarding etiology, prognosis, therapy, health care services utilization, and quality of life among patients afflicted with both diabetic and non-diabetic CRI. In the CRIC Study, analogous to the Framingham Heart and the ARIC studies, the NIDDK has established a unique resource that will explore long-term consequences of CRI as well as novel hypotheses yet to be formulated. Providing a clinical laboratory for this disease that progresses in a variable, but often protracted time frame, will enable such goals.

3. STUDY METHODS

3.A. Study Design

3.A.1. Overview

The CRIC Study will enroll individuals across the spectrum of severity of renal disease to assure that a sufficient number of patients reach the primary study endpoints. Cohort members will be followed throughout the entire duration of clinical follow-up or until death. We expect that a minority of patients, up to 15-30% based on the experience of the AASK study, will develop ESRD during the study; once this occurs relevant modifications to their continuing follow-up evaluations will be implemented because risk factors that occur prior to ESRD may be very important for the risk of CV events occurring after ESRD treatment begins.

Outcomes regarding progression of renal disease will focus principally on reductions in GFR as well as the occurrence of clinically relevant declines in renal function (e.g., 50% drop in GFR or ESRD). Primary outcomes regarding CVD will focus on clinical events indicative of ischemic heart disease, CHF, stroke, and peripheral vascular disease supplemented by radiographic evidence of progressive CVD (e.g., by echocardiography).

3.A.2. Nested Study Designs for the CRI Cohort Study

The use of biological specimens will be an important component of the CRIC Study. Implementation of efficient nested approaches to cohort research will permit the collection and analysis of these data that are expensive to obtain, from a sample of the study cohort while yielding precise and unbiased estimates of associations between risk factors and outcomes. In this section we discuss nested study designs as well as tradeoffs involved in the choice of how many subjects to include as well as the frequency of data collection.

Nested studies among subsets of cohort members (e.g., case-control or case-cohort studies) are most useful in the examination of hypotheses addressing the relationship between baseline and time-varying characteristics and relatively rare, discrete, clinical outcome events (e.g., CVD and ESRD). These nested approaches permit the efficient pursuit of these hypotheses without the need to obtain costly exposure information (e.g., analysis of blood specimens for biomarkers) for the entire cohort population, and can provide an unbiased estimate of both the relative risk and relative rate of disease (127-129). The principal nested studies include analyses limited to a randomly selected subcohort, the case-control study in which cohort members who develop disease are compared to a sample of individuals who have not yet developed disease (129), and the case-cohort study, in which full exposure and covariate information is obtained from a random sample of subjects in the cohort ("the subcohort") and from all subjects developing the outcome(s) of interest.

Subcohort analyses mirror those that can be performed on a full cohort, but involve less precision because of smaller sample sizes. Nonetheless, often as a result of favorable distributions of exposure measures and common outcome events, subcohort analyses provide sufficient statistical power and preserve study resources. They are particularly

well-suited to the analysis of measurements that cannot be obtained retrospectively (e.g., biomarkers that cannot be assayed from stored specimens and radiographic tests).

Compared to the case-control approach, the case-cohort sampling scheme more readily permits simultaneous unbiased analyses of several outcomes (e.g., ESRD and CVD) using one subcohort that is a random sample of the entire CRI cohort. Further, compared to analyses limited to a subcohort, case-cohort analyses have greater statistical power because of the inclusion of all cases developing in the full cohort. Therefore, we have selected the case-cohort study as our primary approach for conducting nested studies, supplemented by subcohort-only analyses for measures that cannot be obtained retrospectively.

The analysis of biological markers will be obtained on random samples of subjects in the CRI cohort ("subcohort"). In some cases (for case-cohort analyses), we will also obtain additional information from all patients in the entire CRI cohort who develop one of the outcomes of interest, CVD or ESRD, regardless of their inclusion in the subcohorts.

The use of weighted random sampling of the CRI cohort (i.e., case-cohort sampling) has two advantages over case-control sampling. First, to study the evolution of disease through the repeated measure of biological markers, sampling permits the same unbiased analyses as would be performed with the entire cohort. For example, we will be able to study the association of laboratory measures (e.g., fibrinogen) and the rate of decline in renal function (e.g., slope of GFR). Second, in particular for the analysis of the relationship between biological markers and discrete disease events (e.g., CVD and ESRD), this sampling will enable us to use the efficient nested case-cohort study design. For these analyses the subcohort will be compared to cases of disease from among the entire 3000 members of the CRI cohort. We note that, with case-cohort sampling, the probability of selection of members of the subcohort may depend on covariates measured at baseline.

The decision to perform a case-cohort analysis of ESRD and CVD includes how many subjects to include in the subcohort(s), how often and when to measure variables, and when to make these decisions. For laboratory data, decisions may often be made later, because blood samples will be obtained from each subject at each visit and stored. This permits a more constrained approach to laboratory studies that can adaptively be modified during the course of the study. For any fixed number of measurements across the study, there will be tradeoffs between including more subjects in the subcohort and obtaining more measurements per individual. Including more subjects in the subcohort allows more precise estimation of the distribution of the laboratory variables in the CRI population. It also allows more non-cases in case-cohort analyses using these variables, thereby increasing precision of measures of association between them and outcome. However, increasing the size of the subcohort will reduce the number of follow-up measurements possible assuming fixed budgetary resources. Follow-up measurements allow the study of the evolution of those variables over time and permit study of the association of changes in those variables with subsequent ESRD or CVD.

3.A.3. Selection of Subjects for Nested Analyses

As discussed above, certain clinical tests will be performed only in the subcohort. These tests include iothalamate GFR, EBT, and selected laboratory tests. We outline here

some considerations in choosing these subsets for further testing and then provide our strategy.

Subcohort Analyses

For some measurements (including iothalamate GFR, EBT, and assays for specimens which are not stable with freezing), selection of the "subcohort" for the further testing must be made at the time that data or specimens are to be collected or the procedure is to be performed There are several, sometimes competing considerations in the selection of a subcohort, which we discuss here. Ideally, the subcohorts used for different purposes (GFR, EBT, laboratory assays) should be the same, or at least nested within one another if their sizes are different. By maximizing the overlap of subjects tested for multiple factors, this best allows evaluation of the associations of the various factors being subsampled and, thereby, of their joint effects on other outcomes.

Case Cohort Analyses

For selection of subjects for assays where the chemical or other substance is stable when frozen, selection does not need to be made at the time of collection. This allows the use of case-cohort sampling for these measurements. In case-cohort sampling, the information is obtained on all subjects who develop endpoints of interest (ESRD, CVD) and a random sample of the rest of the cohort.

There are competing considerations in the selection of the subcohort for case cohort analyses. Two options include choosing the subcohort as a random sample of the study cohort, or choosing the subcohort based on covariates available at baseline. Choosing for the subcohort a random sample of the whole cohort is simpler; further, it most easily allows generalization to the entire cohort, and most simply allows inference in case-cohort type analyses.

Choosing a subcohort based on baseline levels of measured variables has some potential advantages as well as difficulties. First, a decision must be made regarding what factors are most important in choosing the subcohort. Here, an obvious candidate is estimated GFR (eGFR). Choosing a subcohort in which there are substantial numbers of subjects in both low and high GFR categories will help maximize power to estimate the effect of GFR on various outcomes.

There are several drawbacks with such selection procedures, both conceptual and practical. First, selection of the subcohort to maximize power for certain analyses (e.g., association of baseline GFR with subsequent events by ensuring adequate representation of subjects with low eGFR) may reduce power for other analyses (e.g., slope analyses of repeated measures of GFR, where many subjects with low initial eGFR will develop ESRD and so be removed from subsequent measurement of GFR). In addition, the distribution of eGFR in the population will not be fully known at outset, because study recruitment will be staggered over a 33 month interval; however, selecting subjects for iothalamate GFR and laboratory measures cannot be delayed substantially beyond enrollment.

We will adopt a purposeful, stratified weighted sampling strategy for selection of subjects for the iothalamate GFR and EBT cohorts based on anticipated distribution within the full cohort. During the initial enrollment phase, subjects will be chosen randomly for the iothalamate subcohort. After the enrollment of first 300 subjects, data

will be analyzed to determine the distribution of the important predictor variables (e.g., eGFR) in the study cohort. If the distribution of eGFR appears appropriate (sufficient numbers of subjects with low and high values), we will continue selection of subcohort participants as before. If not, a sampling scheme will be adopted to ensure the adequate distribution of GFR among subcohort members. The scheme will involve random sampling with unequal probabilities of selection; the probabilities of selection will be based on eGFR or other variables, some values of which are poorly represented in the study cohort.

As with enrollment into the study, there will be targets for entry into the subcohort based on age, sex, and race. These ranges will be monitored carefully throughout the enrollment period and adjusted as necessary.

Consent for Participation in Subcohort

We will seek consent for subcohort participation (iothalamate GFR studies and EBT) from the entire cohort, then randomly select participants from those who consent, rather than selecting potential subjects first and seeking consent later. We consider here the implications, positive and negative, of that choice.

One reason for this choice is statistical. Many statistical methods make assumptions about the comparability of subjects with complete data and subjects with partial data; these assumptions are typically poorly justified. When subject selection is performed by a known probability mechanism, the assumptions are justified. This will allow us to use subjects not chosen for the subcohort in various statistical procedures, thus increasing the effective sample size and precision. In particular, the use of new CRIC GFR estimating equations will be much better justified for this larger known group of subjects who would have participated had they been chosen. This approach will also allow for a better understanding of determinants of willingness to participate in iothalamate GFR testing.

The main disadvantage of this approach is the burden it will place on the clinical centers investigators and coordinators. Rather than seeking consent from only enough subjects to obtain 1,000 who consent, this will require seeking consent of the entire cohort, which can be time-consuming. Additionally, seeking consent for procedures which are then not performed may interfere with rapport between the subjects and the study team. This may have a detrimental impact on completeness of follow-up.

The subcohort selection process will be evaluated regularly over the course of study enrollment and varied while remaining random in a process called dynamic adaptive sampling, to ensure that the subcohort accurately reflects the overall cohort enrollment population based on the relative representation of a particular subgroup (i.e., gender, race, and diabetic status). [A2]

Subcohort Selection, Sampling Scheme, and Testing Schedule Variation [A3]

The Scientific and Data Coordinating Center (SDCC) at the University of Pennsylvania has evaluated a different subcohort selection scheme to determine participant eligibility for subcohort tests (I-GFR and cardiac CT/EBT). Previously the determination for subcohort selection and tests, was made based on I-GFR criteria. Prospectively,

eligibility for I-GFR testing and CT/EBT testing will be made independently. For example, a participant who is eligible for GFR testing (and selected based on the statistical subcohort sampling schema), will be scheduled to receive I-GFR testing. This same participant will be evaluated for CT/EBT eligibility and may or may not be selected for this test. Conversely, a participant who is ineligible for I-GFR testing may be eligible and selected for CT/EBT testing.

Participants who have had Coronary Artery Bypass Graft (CABG) surgery, coronary stenting or angioplasty will be excluded from cardiac CT/EBT testing, based on the visible changes introduced by these procedures. Participants whose weight exceeds 300 pounds will be excluded. Participants who were not originally selected for the subcohort will be evaluated and contacted to receive cardiac CT/EBT testing and to participate in that aspect of the CRIC Study.

The possibility of being selected for both tests is described in the Informed Consent Form and explained during the informed consent process. The protocol changes described are in terms of the changes to the test schedule and subcohort selection probability. The changes do not pose additional risk to CRIC Study participants. [A3]

3.B. Study Sites

The seven clinical research centers of the CRIC Study include the University of Pennsylvania, Johns Hopkins University/University of Maryland, Case Western Reserve University, University of Michigan at Ann Arbor, University of Illinois at Chicago, Tulane University Health Science Center, and Kaiser Permanente of Northern California/University of California at San Francisco. The Scientific and Data Coordinating Center for the CRIC Study is located at the University of Pennsylvania.

3.C. Study Patient Population & Sample Distribution

Each of the seven Clinical Centers will plan to enroll approximately 430-500 participants. The final center-specific recruitment approach will take into consideration the observed rate of loss-to-follow-up during the first study year and be chosen to establish the cohort of 3000 CRIC participants who undergo a baseline and the Year 1 follow-up visit. [A2] The estimated rate of dropout during the first year is estimated at 3 – 5 %. This phase will occur over a 33-month period beginning in Year 2 of the overall study calendar.

[A4] Six of seven CRIC clinical research centers will continue to recruit and enroll participants. The CRIC site at Tulane, which was not operational between September and December 2005, has now resumed follow-up activities after spending the last several months attempting to contact study participants.

Each of the six Clinical Centers will plan to enroll an additional 50-80 participants, which will result in approximately 520-550 participants per center. The final center-specific recruitment approach will take into consideration the observed rate of loss-to-follow-up during the first study year and be chosen to establish a cohort of CRIC participants, 3000 of whom successfully participate in the Year 1 follow-up visit. This phase will occur over a 42-month period beginning in Year 2 of the overall study calendar.

The CRIC Study population will include a racially and ethnically diverse group of adult patients with mild-to-moderate CRI, approximately half of whom will have diagnosed diabetes mellitus (DM). Principles underlying the targeted composition of the cohort are:

1. adequate representation of target subgroups (e.g. DM, women)
2. subgroup analysis
3. sufficient representation of subgroup to enable selection of a subcohort capable of addressing needs for developing CRIC GFR estimating equation

The following definitions will be employed for ***identification of diabetes*** in CRIC study participants:

- Type I diabetes: history of ketoacidosis or history of diabetes starting at age < 25 treated with insulin within the first three months of onset. This may misclassify some individuals, but strict classification would require elaborate testing.
- Type II diabetes: established disease can be identified by history that includes prescribed medication, presently or at some time in past.
- Without a clear history in patients with suspected type 2 diabetes, the diagnosis can be established by repeated (two) measurements of either of the following:
    - Random plasma glucose level > 200 mg/dL) plus classic symptoms (polyuria, polydipsia, and unexplained weight loss) OR fasting plasma glucose level > 126 mg/dL.
- Alternatively, a single HbA1c level mean > 2 SDs is highly suggestive of type 2 diabetes but should be confirmed with one of the tests listed above.
- For diagnosis of impaired fasting glucose, a fasting plasma glucose measurement is required (fasting plasma glucose level > 110 mg/dL and < 126 mg/dL (130).

The CRIC Study population will have the following characteristics:

3.C.1. Age Distribution

The following proposed age distribution strives to reconcile the competing goals of ensuring an adequate number of cardiovascular outcome events (benefited by an older age structure) and keeping deaths due to non-renal/CVD etiologies and other non-informative censoring events to an acceptable minimum. (**Table 5**):

Table 5. Proposed Age Distribution in CRIC Study

Age Stratum	Final Proportion of CRIC
21-44 years	25%
45-64 years	50%
65-74 years	25%

3.C.2. Race/Ethnic Distribution

A goal of recruitment is to have broad race/ethnic representation from four major groups (i.e., White, African-American, Latino/Hispanic, and Asian) since much less is known about CRI in Latino/Hispanic and Asian/Pacific Islander subgroups. An extremely small number of Native Americans are available in the source populations at the seven clinical research centers. Based on the estimated distributions of race/ethnicity in the available populations at Clinical Centers, **Table 6** presents initial recruitment goals. As is discussed below, early evaluation of the distributions of sociodemographic characteristics will be conducted to assess variance from target

goals, permitting necessary adaptation of recruitment strategies during the early phase of subject recruitment.

Table 6. Proposed Racial Target Distribution in CRIC Study [A2]

Racial Group	Final Proportion of CRIC	Adjusted Proportions (June 2004)
White	40%	47.5%
African-American/Black	40%	47.5%
Other	20%	5.0%

To facilitate recruitment of ethnic minorities such as Latino/Hispanics and Asians, key materials will be translated into the most common languages and efforts will be made, if necessary, to have bilingual personnel at relevant clinical centers.

3.C.3. Entry Age Range

The eligible range of age at enrollment will be 21 to 74 years. The lower age limit was chosen since the focus of the CRIC Study is on adults, which is defined by the NIH as 21 years or older for research purposes. The upper age limit of 74 years was chosen to address the role of renal dysfunction in older patients and to increase power for cardiovascular analyses without a significant impact of competing risks and censoring due to death or dropout.

Potential subjects for the CRIC study must fulfill the following criteria:

3.C.4. Age-Based Estimated GFR Inclusion Criteria

The GFR strata were selected based on review of NHANES III data which demonstrate that the distribution of estimated GFR shifts substantially toward lower values with increasing age.

To limit the proportion of patients who experience no significant progression of renal dysfunction, older subjects will be required to have lower levels of GFR at enrollment thereby avoiding enrollment of older patients with age-related decline in GFR (See **Table 5**). The estimated GFR to define eligibility will be evaluated using the simplified MDRD estimating equation:

(GFR (ml/min/1.73m^2) = 186 x [serum Cr (mg/dL)] $^{-1.154}$ x [age] $^{-0.203}$ x [0.742 if female] x [1.212 if black]). (134)

using local laboratory-measured serum creatinine results for the initial screening. Since the variability of assays for serum creatinine performed at local laboratories may be substantial (e.g., serum creatinine measured in the NHANES III laboratory was 0.23 mg/dL higher than the Cleveland Clinic central laboratory)(131), a serum creatinine assay will be performed at the CRIC Central Laboratory to confirm eligibility (**Table 7**). Alternatively, if the overall number of local laboratories used across all seven Clinical Centers is relatively small, a calibration study may be performed against the CRIC Central Laboratory to allow more accurate screening of potentially eligible participants from local laboratory databases.

Table 7. Age-Stratified Entry Estimated GFR Range (132)

Age Stratum	Eligible Estimated GFR Range (ml/min/1.73 m^2)
21-44 years	20-70
45-64 years	20-60
65-74 years	20-50

Therefore, taken together, the proposed goals for the overall CRIC Study population are:

Table 8. Summary of CRIC Study Sample Characteristics

Age Stratum	Eligible Estimated GFR Range (ml/min/1.73 m^2)	No Diabetes	Diabetes
21-44 years	20-70	12.5%	12.5%
45-64 years	20-60	25%	25%
65-74 years	20-50	12.5%	12.5%

There will be target ranges for recruitment based on several key subject-characteristic variables: age, estimated GFR, race, and diabetes. The target ranges for the variables are:

Table 8A: Recruitment Target Ranges [A2]

Variable	Value	Percent of Population
Age (years)	21-44	20-30
	45-64	40-60
	65-74	20-30
Diabetes	No	40-60
	Yes	40-60
GFR	Lower half of range*	40-60
	Upper half of range	40-60
Race/Ethnicity [A2]	White	47.5
	African-American/Black	47.5
	Other	5

* for ages 21-44: <45, for ages 45-64, <40; for ages 65+, <35

These target ranges were identified based on the anticipated distribution of these attributes among the source population at the clinical centers, and the required composition of the cohort so as to be able to achieve the necessary composition of the subcohort. If the observed proportion in a center falls below the target range for the given variable, the center will be directed to increase or decrease recruitment of the under-represented group accordingly. Adherence of each center to the recruitment goals will be monitored monthly at the SDCC. For some attributes such as race, a similar distribution may not be feasible at all study sites. We will strive to achieve the target ranges study-wide by trying to offset variations from the target at individual centers.

3.C.5. Exclusion Criteria

The following table summarizes the proposed exclusion criteria (**Table 9**):

Table 9. CRIC Study Exclusion Criteria

General Exclusion Criteria	
Institutionalized (e.g., prisoner, nursing home resident, skilled nursing facility resident)	Previously received dialysis (peritoneal and/or hemodialysis) lasting more than one month based on patient self-report
Unable or unwilling to provide informed consent	Prior organ or bone marrow transplant; prior renal transplant based on patient self-report
Participant appears unlikely or unable to participate in the required study procedures as assessed by the investigator, study coordinator or designee. [A1]	Received immunosuppressive or other immunotherapy for primary renal disease or systemic vasculitits that affects the kidneys (i.e., anti-GBM, ANCA, SLE, IgA nephropathy, cryoglobulin, etc.) within the past six months before enrollment based on patient self-report. This does not include, for example, use of prednisone for the treatment of reactive airways disease.
NYHA Class III or IV heart failure at baseline	Received chemotherapy or alkylating agents for systemic cancer other than non-melanoma skin cancer within two years prior to enrollment based on patient self report
Known cirrhosis based on patient self-report	Previous diagnosis of multiple myeloma or renal carcinoma based on patient self-report
Known HIV infection and/or AIDS based on patient self-report	Previously diagnosed polycystic kidney disease based on patient self report
Present participation in the AASK Cohort Study	Currently participating in an interventional clinical trial (i.e., primarily trials of therapeutic agents that may have an effect on renal or cardiovascular outcomes as assessed by a Central Adjudication Committee) or in a research study that adds significantly to the participant's burden. Examples that would preclude participation in CRIC are the AASK Cohort or KEEP Study. [A1]
Pregnant women [A2]	
Additional Exclusion Criteria for Participants Undergoing ^{125}I-Iothalamate GFR Testing	
Known iodine allergy	Currently breast feeding, or pregnant based on urine HCG test
Impaired urinary voiding [A2]	Radiation exposure to γ-emitting isotope other than technetium

Additional Exclusion Criteria for Participants Undergoing CT/EBT Testing	
Participants who have had Coronary Artery Bypass Graft (CABG) surgery, coronary stenting or angioplasty [A3]	Participants whose weight is over 300lbs [A3]

As noted in Table 9, other than a known diagnosis of polycystic kidney disease, multiple myeloma, or renal carcinoma, no specific renal diagnoses will be excluded.

However, data will be recorded to define as much as is feasible, the etiology of renal disease.

Women who are pregnant based on urine HCG test will be excluded from the CRIC Study. In addition, women who are screened and enrolled in the cohort study (defined as experiencing the baseline visit) and who subsequently become pregnant will not have physical measurements performed or questionnaires administered during clinical follow-up visits. If a woman is found to be pregnant after screening but before the baseline visit has occurred, she will be withdrawn and can be re-screened approximately one year later. [A2]

Impaired urinary voiding will be evaluated by the investigator in terms of problems emptying bladder, awakening to urinate, incontinence, history of prostate problems and recent urinary tract infection. [A2]

3.C.6. Participant Procedures

Screening Visit

CRIC study participants will be followed for up to six years, depending on the date of enrollment. A pre-screening telephone contact will determine if a subject is potentially eligible to participate. A complete description of recruitment and pre-screening procedures can be found in Sections 4.A.2 and 4.A.3. If interested, participants will be scheduled for a ***screening visit*** during which the following will occur:

- Informed consent process; consent obtained
- Eligibility assessment questionnaire completed
- Contact information provided
- Demographic information recorded
- Non-fasting blood draw (10 cc) for serum creatinine (to calculate eGFR and determine eligibility), and glucose
- Urine dipstick test for presence of glucose, protein, and hematuria

This visit will take approximately 1.5 to 2 hours. During the screening visit, participants who have completed the screening process are provided supplies and instructed about how to collect a 24-hour urine sample. When a participant's study eligibility is confirmed, which is shortly after the screening visit (Visit 2), they are contacted by study personnel who will review the urine collection directions and instruct the participant to collect the 24-hour sample just prior to the next study visit, which is the baseline visit (Visit 3), and bring it with them to this visit. [A2]

Baseline Visit

If a person is eligible according to the information collected during the screening visit, she/he will be scheduled within **3 months** for a baseline visit. [A2] This visit is considered study enrollment during which the following will occur:

- Eligibility assessment confirmed
- Detailed medical history obtained
- Fasting blood draw (130 cc) for the following tests:
  - CBC [Hemoglobin, Hematocrit, WBC, MCV, MCH, MCHC, platelets]

  - Metabolic panel [Albumin, Bicarbonate, Total Bilirubin, Calcium, Carbon Dioxide, Chloride, Creatinine, Glucose, Alkaline Phosphatase, Potassium, Total Protein, Sodium, Aspartate Aminotrasnferase (AST), Alanine Aminotransferase (ALT), Magnesium, Phosphorus, Urea Nitrogen], Total cholesterol, Triglycerides
  - Cystatin C, HbA1C, Homocysteine, Troponin I, iPTH, Fibrinogen and Uric Acid
  - If consent to obtain a research sample for genetic studies was obtained, this blood draw will be used to store this sample.
- Urine assay for creatinine, protein, albumin, urea nitrogen
- Concomitant medication information
- ECG
- Ankle-Brachial Index
- Anthropometric measures (height, weight, mid-abdominal circumference)
- Bioelectrical Impedance Assessment (BIA)
- Assessment questionnaires of dietary intake, physical activity, quality of life, depression, cognitive function, cardiomyopathy [A2] and health resource utilization
- Nail clippings for future study of heavy metal exposure
- Iothalamate - GFR test (if selected for this subcohort)
- A "clean catch" urine sample will be collected at the baseline visit and each annual visit, from all participants. Approximately 100 cc will be reserved for storage. [A2]
- Pulse wave velocity (at baseline and during alternate annual visits) [A2]

This visit will take approximately three to four hours and will occur annually. Participants who are selected for the GFR testing component of the study will spend an additional three to four hours at this visit or during an additional visit, depending on preference. The GFR test will be repeated two and four years after enrollment. One year and four years after enrollment, all participants will also be scheduled for an ECHO test. One year and four years after enrollment, the same subjects who undergo GFR testing will also be scheduled for an EBT test.

During the ***follow-up*** phase, participants will be contacted by ***telephone*** six months after the baseline and annual clinic visits to update contact information, to ascertain interim medical history and potential outcome events, and to assess health resource utilization. Participants may also be contacted at other times during the year to answer additional questions or to join in a new part of this study. (See Appendix A - Participant Visit Schedule for a complete listing of participant procedures and data collection stages).

Annual clinic visits will be scheduled to occur within a range of two months before to two months after the anniversary of the baseline enrollment date. [A2] Participants will experience many of the same procedures as those which occurred at the baseline visit. [See Appendix A – Visit Schedule.]

Tests, questionnaires and physical measures originally scheduled during the Baseline Visit (Visit #3) and annual follow up visits can be completed within a broader time frame than described in the original protocol, without compromising the study integrity.

The range has been re-defined such that any visit may be conducted until the time that the range (or visit window) for the next annual visit begins. This protocol deviation has been evaluated by the investigators and biostatisticians who have determined that this schedule adjustment can be accommodated, noting that the important factors are to collect data at uniform intervals during study participation and to strive to maintain this interval between time points, whenever possible. Therefore, variation in the conduct of telephone contacts, annual visits and the following tests will be permitted: I-GFR, EBT/MSCT, ECG and Echocardiogram. [A3]

3.C.7. CRIC Plus

Changes to the Core CRIC Protocol; Additional Data Collection for Participants Whose Estimated GFR (eGFR) Falls Below 20 ml/min/1.73m² [A3, A4]

The Chronic Renal Insufficiency Cohort (CRIC) study presents a unique opportunity to fill important gaps in knowledge regarding advanced chronic renal insufficiency, including the critical transition to end-stage renal disease (ESRD, defined as the initiation of dialysis or kidney transplant).

Virtually all published studies of incident ESRD patients begin at or after initiation of dialysis and do not have information regarding what transpired during the years prior to dialysis initiation. Conversely, the few prospective studies of chronic renal insufficiency patients have not continued follow-up into ESRD. In the original NIDDK RFA that led to the CRIC study, follow-up of patients after transition into end-stage renal disease was described. However, the original CRIC protocol is not optimized to study patients with advanced chronic renal insufficiency and their transition into ESRD.

With additional resources brought in as a result of the "CRIC-Plus" grant application, the CRIC core protocol will be augmented with more intensive data collection for subjects with advance chronic renal insufficiency (eGFR < 20 ml/min/1.73m^2). Newly enrolled participants will be consented with the revised consent form and participants currently enrolled will be asked to sign the revised consent form which includes the new study procedures applicable only to subjects who progress to estimated GFR < 20 ml/min/1.73m^2.

[A4] To ensure a smooth transition, the CRIC Plus activities will be triggered and apply not only to CRIC participants whose eGFR is observed to fall <20 ml/min/1.73m^2 but also to CRIC subjects with high likelihood of progressing to an eGFR <20 ml/min/1.73m^2 based on the observed and projected trajectory of renal function. Projected likelihood will be estimated using a multivariable regression formula developed for this purpose within CRIC. Relevant CRIC Plus activities include obtaining an additional echocardiogram and measurement of hs-CRP.

The core CRIC protocol is altered for CRIC participant's whose estimated GFR (eGFR) falls below 20 ml/min/1.73m^2 as follows:

All participants will have an echocardiogram when their estimated GFR falls below 20 ml/min/1.73m^2 (within 6 months) If this does not coincide with the core CRIC protocol echocardiogram at Years 1 and 4, an additional echocardiogram will be performed. An

echocardiogram will be scheduled should a patient developed ESRD. CRIC participants whose GFR falls below 20 ml/min/1.73m^2 may have as many as four echocardiograms by the revised core protocol.

For CRIC participants who develop the need for maintenance dialysis, the following additional dialysis-related information will be collected from the dialysis unit chart every 12 months:

1. Presumed cause of ESRD (collected only once)
2. Type of dialysis (hemodialysis vs. peritoneal dialysis with or without a cycler)
3. Information related to type of dialysis facility (e.g., for profit vs. not for profit)
4. Details of dialysis procedure itself (e.g., schedule and dose of dialysis, type of vascular access)
5. Treatment of renal failure complications (e.g., treatment of anemia, secondary hyperparathyroidism)
6. Measures of nutritional status (e.g., albumin, prealbumin, normalized protein catabolic rate)
7. Other routine blood chemistry measurements (e.g., serum potassium or calcium x phosphorus product)
8. Measures of residual renal function
9. Blood pressure (before and after hemodialysis session or at clinic visit for peritoneal dialysis patients)

For CRIC participants who receive kidney transplantation, the following additional transplant data will be collected:

1. Source of transplant (e.g., cadaveric vs. living related vs. living unrelated)
2. Prior duration of dialysis before transplant

Participants will sign the appropriate medical record release forms.

[A4] Newly enrolled participants will be consented using the new consent form that describes these procedures. Participants who are currently enrolled will be asked to sign a revised consent form that reflects the protocol changes associated with CRIC Plus.

The table below summarizes CRIC PLUS protocol elements applicable to participants whose e–GFR falls below 20 ml/min/1.73m^2. [A3, A4]

Begins at CRIC visit when estimated GFR falls <20 ml/min/1.73m^2)		**Study year after a participant's GFR fall < 20 ml/min/1.73m^2**							
		0.5	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**
CRIC CORE PROTOCOL									
Yearly CRIC core protocol data elements	X		x		x		x		x
6-month CRIC core protocol data elements		x		x		x		x	
NEW DATA COLLECTION FOR THOSE WHOSE GFR FALLS < 20 ml/min/1.73m^2									
Echocardiogram	X	A second echocardiogram will be performed if and when the patient develops ESRD							
Tracking of ESRD related variable		For patients who develop ESRD, ESRD related variables will be collected every twelve months							

3.D. Study Data

Extensive data will be collected for the CRIC study and is described in detail in the following four sections: 1) Sociodemographic, comorbidity, treatment, Anthropometric, Psychosocial, Quality of Life and Health Care Resource Utilization Measures, 2) Renal Function Measures, 3) Cardiovascular Measures and 4) Biochemical Measures.

3.D.1. Sociodemographic, Comorbidity, Treatment, Anthropometric, Psychosocial, Quality of Life & Health Care Resource Utilization Measures

Sociodemographic and Medical History

Data on age, gender, detailed race/ethnicity, education and income will be collected. A directed history will be obtained to determine underlying cause of CRI (if known by patient), family history of renal and cardiovascular disease, prior cardiovascular disease and cardiovascular risk factors, coexisting morbidity, and health behaviors (tobacco use, ethanol exposures, illicit drug use).

Blood Pressure Measures [A1]

Blood pressure will be measured at the Screening Visit, repeated at the Baseline Visit and annually thereafter. The procedure involves taking three sequential measurements while the participant is seated followed by one measurement while standing. [A1]

Drug Exposure Data

Participants will be asked to list all prescription and over-the-counter drugs they have taken within 30 days of the clinic visit including dose, frequency, and route of administration.

Assessment of Dietary Intake

The CRIC study will assess dietary intake by administering the current National Cancer Institute (NCI) Dietary History Questionnaire (DHQ). This instrument will yield information on all nutrients and foods of potential interest for CRIC, and also provides limited information on use of dietary supplements. The DHQ consists of 124 food items and includes questions on portion size. The DHQ takes about one hour to complete and was designed to be easy to use in a self-administered format. It can also be administered by an interviewer for subjects who have visual or literacy challenges. The DHQ performance with respect to relative validity is as good or superior to that of other commonly used food frequency questionnaires (FFQ) for most nutrients. Advantages of using the DHQ are that it is public domain, currently supported by the NCI in a package that includes scannable forms, a nutrient database, nutrient calculation software, and can be modified if necessary for study specific needs. NCI is also currently conducting a validation study to evaluate the extent and nature of measurement error associated with protein and energy intake assessed by the DHQ.

Clinical evaluation will include data on weight, height, Body Mass Index (BMI), Subjective Global Assessment (SGA), and evaluation of body composition.

Body Composition will be measured using a weight-based measure such as BMI and will be used along with anthropometric measures (e.g., mid-abdominal circumference).

Anthropometric measures will be performed using standard techniques such as those used in NHANES III. These measures will be supplemented using Bioelectrical Impedance Analysis (BIA) techniques. These measures, along with laboratory values provide opportunity to gain insight into nutritional status of CRI patients which will also likely improve the GFR estimating equation since previous studies did not have these data available.

[A4] Currently BIA is measured at baseline and alternating annual clinic visits following the baseline visit. This amendment proposes to measure BIA at the baseline visit and annually at the clinic visits following the baseline visit. This change will be reflected in the revised visit schedule and revised consent form.

Finally, nutritional intake and body composition data will be used along with laboratory values (cholesterol, creatinine, albumin, bicarbonate) to fully evaluate the nutritional status of CRI patients over time. Additional laboratory values that may be measured for this purpose, some pending ancillary funding, include serum prealbumin, transferrin, CRP and insulin-like growth factor-1 concentrations, total body nitrogen and total body potassium.

Physical Activity

Physical activity will be determined by the use of the MESA Physical Activity Questionnaire. The association between physical activity levels and adverse CVD outcomes has been established, but less is known about the role of physical activity in CKD outcomes.

The modified Kansas City Questionnaire will be used to evaluate physical activity and the presence or worsening of cardiomyopathy symptoms. This questionnaire will be administered to all participants annually. [A2]

Quality of Life

A Quality of Life (QOL) assessment will be performed. Each center will use the KDQOL which includes the SF-36 and dialysis-specific questions, and the MDRD symptom index to capture potential renal-related symptoms.

Health Care Resource Utilization

Health care resource utilization data will be collected using questionnaires developed for the CRIC study, administrative claims data (e.g. in the subset of Medicare-eligible patients) and data obtained directly from CRIC subjects via interval telephone interviews.

Psychosocial Measures

The Beck Depression Inventory will be used to measure depressive symptoms. Cognitive function will be measured using Mini-Mental State Exam (MMSE). Pending ancillary funding, more detailed evaluation of cognitive function will be performed using additional batteries such as Letter Number Sequencing, Digit Symbol Substitution, and Randt New York Stories.

Ancillary Studies Associated with CRIC [A3]

Several ancillary studies have been approved by the CRIC Steering Committee and are being incorporated into the CRIC Study at some but not all clinical centers. Each participating site will submit the ancillary study protocol and revised consent form to their IRB for approval before engaging in ancillary study activities. The table in Appendix G lists the ancillary studies and participating CRIC clinical centers. Appendix G includes a summary of each ancillary study protocol. [A3]

3.D.2. Measurements of Renal Function

Glomerular Filtration Rate (GFR)

Glomerular Filtration Rate (GFR) is the volume of plasma filtered each minute through the glomeruli of both kidneys. An extensive body of clinical and experimental information suggests that GFR is the best overall index of renal function (133-135). In physiologically normal humans, the mean GFR is approximately 125 ml/min/1.73m^2, being slightly lower in women than in men and decreasing progressively with age (133).

GFR is operationally measured as the clearance rate of a filtration marker from the plasma by the kidneys. While urinary clearance of inulin has been widely accepted as the "gold standard" for estimating GFR, its measurement requires an intravenous infusion and a difficult chemical assay. Therefore, other methods for measuring GFR have been developed including measuring urinary clearance of ^{125}I–iothalamate.

In clinical practice and large-scale epidemiological research projects, more convenient methods, such as determinations of serum creatinine and timed urinary creatinine clearance, have been used for diagnosis of renal dysfunction and for monitoring the progression of renal disease (136) (137-142). Factors other than GFR—including generation, tubular secretion, and extrarenal elimination of creatinine—affect serum creatinine concentration. Therefore, use of these serum creatinine or creatinine clearance determinations may not always provide an accurate estimate of renal function (133) (134;135), (139), (140), (142). Cystatin has recently been proposed as a valuable marker of renal function, but has not yet been validated in a large scale epidemiological study. Cystatins comprise a group of proteinase inhibitors, widely distributed in tissues and body fluids that form tight complexes with cysteine proteases. Cystatin C, a secreted molecule of this family, is increased in patients with malignant diseases, and is related to the insufficiency of renal function and appears to be a better marker than creatinine. Cystatins are secreted by many nucleated cells and the serum concentration is not as dependent on muscle bulk and nutritional state as serum creatinine. Serum concentrations of cystatin C have recently been found to be potentially useful markers of renal function that may not have all the drawbacks of serum creatinine and for that reason cystatin C concentrations were incorporated into the CRIC protocol.

Estimating equations based on serum creatinine and other patient parameters (i.e., race, age, sex, and some plasma values) for GFR have been developed and used extensively clinically and in research studies. Unfortunately, these equations have only been calibrated in two large studies of patients with CRI, the MDRD (which had few blacks and diabetics) and the AASK (which had no diabetics and no non-blacks.) The proposed

investigation will give us the opportunity to derive a novel GFR estimating equation in a broadly representative diabetic and non-diabetic CRI population.

In preliminary analyses of data from MDRD and AASK, we found published GFR equations to be inadequate substitutes for ^{125}I-iothalamate GFR for several reasons. Differences between predicted GFR and ^{125}I-iothalamate measures of GFR were often clinically significant. Further, in longitudinal analyses of GFR slope, these differences were correlated with several other variables; consistent with the observation that serum creatinine is influenced by factors other than GFR. Because of this, equation-based analyses may track with factors that affect serum creatinine without affecting GFR. Further, in longitudinal analysis, cross-sectionally derived equations were inadequate for predicting longitudinal changes. For these reasons, we feel that failure to measure GFR using ^{125}I-iothalamate in at least a significant subset of our population would potentially and substantially harm the study's ability to study accurately kidney function, its determinants, and its effects on CVD and other outcomes.

Proteinuria and Microalbuminuria as Risk Factors for Renal Disease Progression

Proteinuria has long been considered an index of the extent and severity of glomerular damage. Recently, a variety of studies have indicated that proteinuria is an important and independent risk factor for the progression of renal disease (140;143;144). In the MDRD trial, urinary protein excretion at baseline was significantly associated with the decline of GFR among patients with non-diabetic renal disease (145). Similarly, in the Ramipril Efficacy in Nephropathy (REIN) study, baseline urinary protein excretion was the most important clinical predictor of decline in GFR and ultimately progression to ESRD in patients with non-diabetic renal disease (146). Proteinuria (or microalbuminuria) has also been documented as an independent risk factor for progressive decline in kidney function among patients with type 1 or type 2 diabetic nephropathy, hypertensive renal disease, and interstitial nephropathies (147-151). Most of the above analyses, however, were conducted either in clinical trials in which patients were highly selected or in small clinical studies. The proposed investigation will examine the relationship between urinary excretion of protein (albumin) and the progression of renal disease in a large population-based study.

Proteinuria and Microalbuminuria as Risk Factors for CVD

The prevalence of microalbuminuria and proteinuria is increased in patients with diabetes (152) or hypertension (153). A meta-analysis of prospective cohort studies among type II diabetic patients suggests that the presence of microalbuminuria at baseline is associated with about a two-fold increase in the risk of CVD events (154). However, this may well be an overestimate since only one of the 6 studies which reported relative risks adjusted for confounding variables. Microalbuminuria has also been associated with an increased risk of CAD among nondiabetic individuals (155) and with an increased risk of LVH and CHD among patients with essential hypertension (153). However, since microalbuminuria is strongly associated with a number of established risk factors for CVD (for example, increased LDL-cholesterol and blood pressure), whether microalbuminuria is itself an independent risk factor for CVD remains uncertain. The proposed study provides an excellent opportunity to examine the independent relationship between microalbuminuria (proteinuria) and risk of CVD after adjustment for established CVD risk factors among patients with CRI.

3.D.2.a. Renal Function Measurements

Glomerular Filtration Rate

Glomerular filtration rates will be estimated using a number of different approaches. In a subset of patients (see below), GFR will be measured as the renal clearance of ^{125}I-iothalamate after its subcutaneous injection (156;157). A total of 1000 patients will undergo ^{125}I-iothalamate GFRs at years 0, 2, and 4. We have selected ^{125}I-iothalamate because it is comparable to inulin as a filtration marker and can be assayed accurately and precisely in a central laboratory (156;157). All voided urine will be collected, and after an equilibration period of 60 to 90 minutes, the clearance period will begin. Plasma samples will be taken at the beginning and end of each of at least 4 carefully timed clearance periods of 30 minutes duration. GFR will be expressed per 1.73 m^2 of body surface area (BSA), as well as without indexing to BSA. To decrease variability in ^{125}I-iothalamate clearance results due to dietary protein intake (158), time of day (159), and posture (160), all measurements will be performed with participants who have consumed only a low protein (<10 gm) meal [A2], in the supine or sitting position, and at approximately the same time of day.

Given the prognostic and generalizability limitations of current GFR estimating equations, we plan to derive a GFR estimating equation that is based on easily obtained demographic and biochemical data and that will be applicable to the entire CRIC Study population. This equation will be validated both cross-sectionally and longitudinally against ^{125}I-iothalamate GFR. We also hope the equation to be predictive of events of renal dysfunction. Because this equation will be derived in diabetic and non-diabetic patients across a representative range of demographic characteristics and a wide degree of renal dysfunction, our goal is that it will be broadly generalizable.

Additional proxy measures of GFR to be obtained yearly will include serum cystatin, serum creatinine, and creatinine clearance. Serum and urinary creatinine will be measured using the kinetic Jaffe method. UV/P creatinine will be measured simultaneously during the iothalamate clearance periods to compute creatinine clearance (thus eliminating collection variation as a problem in directing comparing GFR with CrCl). A secondary creatinine clearance will be obtained using the 24-hour urine collection and a single measurement of serum creatinine.

Proteinuria

Twenty-four-hour urinary excretion of total protein and albumin will be measured annually. In addition, urinary protein, albumin (by specific immunoassay) and creatinine concentrations will be measured from a spot specimen to calculate the urine protein/creatinine ratio and urine albumin/creatinine ratio. These will be measured at least annually.

Renal Size and Morphology

Tentatively, renal length and renal volume will be estimated in the subset of patients undergoing EBT at years 1 and 4 of follow-up. In addition, feasibility of measuring renal artery calcium scores is under exploration.

3.D.2.b. Renal Outcome Measures

Primary outcome: The slope of GFR is the primary outcome.

Secondary outcomes:

1. Onset of ESRD (start of chronic dialysis or renal transplantation) or development of GFR < 15 ml/min/1.73m^2. This will be time-to-event analysis. Key here is careful consideration of GFR at entry into cohort study by stratification or multivariate analysis.
2. "Significant loss of renal function" defined as 50% decline or 25 ml/min/1.73 m^2 decline in GFR from baseline. This will also be a time-to-event analysis that will take into account baseline GFR.
3. Composite clinical outcome defined by the occurrence of either 50% decline, or
   25 l/min/1.73 m2 decline in GFR from baseline, or onset of ESRD.
4. Slope of change in proteinuria over time as assessed by spot urine protein/urine creatinine ratio (UP/Cr). We will also assess for development of new microalbuminuria (20-200 mg albumin/gm creatinine), new overt proteinuria -UP/Cr > 0.22 (or > 300 mg/d of proteinuria) or new significant proteinuria UP/Cr > .66 (or >1 gm/d of proteinuria).

"Renal-specific" death will not be used because of the difficulty of defining this outcome, the wide variability in assignment of this definition, and the usual application of renal replacement therapy. Few patients appear to die directly of renal disease; the only clear examples might be hyperkalemia or pericardial tamponade. Therefore, only all-cause death will be captured as a "renal end-point."

3.D.3. Measurement of Cardiovascular Disease

3.D.3.a. Overview of Rationale for Cardiovascular Testing

The cardiovascular testing strategy for the CRIC Study was developed to enable evaluation of the broad spectrum of vascular disease (i.e., coronary heart disease, cerebrovascular disease, and peripheral arterial disease) and its relationship to CRI. The selection and timing of cardiovascular testing for the CRIC protocol was the result of lengthy deliberations by the Cardiovascular Measurement Subcommittee of the CRIC Steering Committee, aided by thoughtful contributions from an expert panel composed of John Crouse III, MD (Wake Forest University), Philip Greenland, MD, (Northwestern University), Terri Manolio, PhD, (NHLBI) and Paul Ridker, MD, (Harvard University). The testing strategy was refined further by the Steering Committee, and the approved protocol is outlined in Figure 1, Section 3.D.4.

Measures of subclinical cardiovascular disease, as was anticipated in the RFA, will have several complementary roles in the CRIC Study, as outlined below:

- **Role as Outcome Measurement:** It was anticipated that the number of discrete clinical cardiovascular events in a cohort of the proposed size followed for six years would be too few to support all of the specific aims of the study. For this reason, inclusion of subclinical CV outcome measures are

required to address the core CRIC Study hypotheses and specific aims regarding the progression of CVD in the setting of CRI.

- **Provide Insight into Pathophysiological Mechanisms:** Certain targeted subclinical CV measures may provide novel insight into potential mechanisms explaining why CRI patients may be at increased CVD risk (e.g. EBT and the role of calcium-phosphorus dysregulation in the development of subclinical and clinical CVD).
- **Role as Risk Factors for Progression of CRI and Subsequent Clinical CV Events:** In addition to the role of CV tests as outcomes measures and providing insights into pathophysiologic mechanisms, it was recognized that measures may also serve as risk factors for subsequent events, thus allowing the evaluation of novel hypotheses regarding progression of CVD in patients with CRI and potentially better risk stratification in this high-risk population.

The selection of the specific measures included in the protocol took into consideration the scientific scope of the CRIC Study, its scientific rationale, issues of statistical analyses/power, assessment of patient burden, and associated costs for obtaining and evaluating the CV measures. Plans for each of the components of the proposed testing strategy as well as the rationale and limitations for each are first summarized briefly, followed by an overview of the schedule of tests.

3.D.3.b. Cardiovascular Tests

Electrocardiography (ECG)

Twelve-lead electrocardiograms will be performed in all participants annually [A1] in order to assess non-invasively the presence of cardiac rhythm and conduction disorders, atrial and ventricular arrhythmias, Q-wave myocardial infarction (MI) (including, therefore, silent MI), and electrocardiographic left ventricular hypertrophy (LVH).

Experience in the general population: ECGs have been used in essentially all clinical trials of coronary disease for confirmation of a condition of interest (e.g., acute myocardial infarction, unstable angina with ischemic changes, atrial fibrillation), to identify prior silent myocardial infarction, and for possible risk stratification. Epidemiologically, the prognostic value of ECG findings for subsequent CV events has been established in many large longitudinal cohorts such as the Framingham Heart Study, Cardiovascular Health Study, and the Atherosclerosis Risk In Communities (ARIC) Study (161-167).

Experience in the CRI population: Electrocardiographic abnormalities found on standard 12-lead ECGs have been evaluated more often in the end-stage renal disease population receiving dialysis compared with patients who have mild-to-moderate chronic renal insufficiency (148;168-170). In the literature on ESRD, the focus has primarily been on electrolyte abnormalities and identification of acute ischemia surrounding dialysis. Few studies have examined the potential electrocardiographic predictors of adverse clinical outcomes in the setting of CRI.

The challenges related to ECGs relate primarily to obtaining centralized and standardized reading. Standardization of ECG tracings will be assured through the use of

a training of technicians and uniform ECG equipment. An experienced central reading center will interpret all studies.

Role — ECGs will be used as an outcome variable for specific electrocardiographic abnormalities (e.g., silent myocardial infarction, arrhythmias, conduction disorders) as well as a risk factor or covariate for subsequent cardiovascular outcomes.

Transthoracic Echocardiography

A transthoracic echocardiogram will be obtained in all participants at the year 1 visit and again four years following enrollment. Echocardiography offers the ability to investigate parameters of cardiac structure, systolic and diastolic function, and hemodynamics. A "limited" transthoracic echocardiogram (171) will be performed, which will include the following components:

- 2-D directed M-Mode measurement of the left atrium and aorta
- 2-D measurement of the aortic annulus (as part of stroke volume calculation)
- 2-D directed M-Mode measurement of left ventricular (LV) mass
- LV volumes and ejection fraction from 2-D measurements of LV end-diastolic and end-systolic areas (Simpson's Method, "Method of Discs")
- LV inflow (E wave and A wave) to assess simple measurements of LV diastolic filling
- Flow velocity integral of the LV outflow (as part of stroke volume calculation)
- Heart rate (as part of formula to determine cardiac output)

Experience in the general population: The Framingham Heart Study demonstrated that echocardiographic LVH identifies a population at high risk for cardiovascular disease and predicts an increased risk of cardiovascular morbidity and death (172;173). Echocardiography is now a mainstay in clinical cardiology due to its versatility, safety, and affordability. Echocardiography provides enormous opportunities for diagnosis and prognosis that complements and exceeds clinical evaluation.

Experience in the CRI population: The ESRD population has been studied extensively with echocardiography and exhibits a variety of characteristic abnormalities of structure and systolic/diastolic function (174). Several of these have been demonstrated to be amenable to therapy (e.g. erythropoietin) and others have been demonstrated to be potent predictors of future cardiovascular events (e.g. sudden death). We lack systematic and definitive information regarding the epidemiology, development, and impact of LVH in the CRI population, an area to which the CRIC study will be able to contribute. However, the available limited evidence suggests that the prevalence of LVH increases in association with declining renal function (175-177). In a prospective study, Levin found significant progression of LVH after one year (defined as >20% increase in LVMI or as an absolute increase of >20g/m^2) in a CRI population.

The major challenges of echocardiography are related to cost and the challenge of maintaining standardization across sites. Standardized training for echocardiography technicians will be implemented to ensure standardization and quality of data. An experienced central reading center will interpret all studies as well as assure standardized interpretation.

Role — Echocardiography will serve all three functions in the CRIC study depending on the specific analysis: outcome variable, potential mediating variable, and as a prognostic risk factor (e.g., development and progression of LVH, level of and changes in ventricular function, changes in chamber size and structure).

Coronary Calcium Assessment by Electron Beam Tomography (EBT)

Electron beam tomography to measure coronary artery calcification will be performed in one third of the participants after one year of follow-up and again at 4 years (in the same subcohort as those undergoing iothalamate GFR measurements). EBT is a sensitive method to detect the presence, anatomical location, and extent of coronary calcification. Relationships between the extent of EBT-defined calcium measurement and the extent of coronary disease defined histologically, by coronary angiography and by intravascular ultrasound have been shown (178).

Experience in the General Population: The EBT calcium score has been shown to have prognostic value with respect to the development of cardiac endpoints. Several studies have found that the presence of coronary artery calcification is associated with a measurable risk of a definable ischemic cardiac event developing over a relatively brief period and that this risk increases in direct proportion to the EBT calcium score (67;179-181). For these reasons, EBT is receiving increased recognition as a potential means to diagnose subclinical coronary disease and facilitate risk stratification in non-CRI populations (182).

Experience in the CRI Population: In a study of 49 hemodialysis patients, Braun noted that coronary-artery calcification detected by EBT was much more common than among normal subjects of the same age and sex (183). More recently, Goodman et al. demonstrated marked coronary calcification in very young individuals on dialysis that progressed rapidly over time (184). However, there is a lack of experience regarding the use of EBT to detect severity of and changes in coronary calcification with the CRI population. One of the hypotheses within the CRIC study is that the excess cardiovascular risk associated with progressive renal dysfunction is mediated, in part, through dysregulation of calcium-phosphate metabolism and subsequent enhanced vascular calcification.

Careful attention will be focused on maintaining standardization and quality control. Further, EBT and the other CV testing modalities will need to be evaluated again over time as technological advances occur.

Role — The role of EBT is one of a potential clinical predictor that may be useful clinically as well as a measure that may reflect metabolic effects of CRI on the vasculature (e.g. mechanism of disease). Coronary arterial calcification assessment by EBT will be used to provide important insights into pathophysiologic mechanisms of excess cardiovascular risk associated with progressive renal dysfunction. It will also serve as an outcome variable for the progression of subclinical coronary heart disease and secondarily, as a prognostic risk factor for clinical cardiovascular events in the setting of CRI.

[A2] EBT is a sensitive method to detect the presence, anatomical location, and extent of coronary calcification. Electron beam tomography to measure coronary artery calcification will be performed in one third of the CRIC Study participants after one year

of follow-up and again at 4 years (in the same subcohort as those undergoing iothalamate GFR measurements). The introduction of Mechanical Multi-Slice Spiral (MSCT) Scanners with shorter rotation times presents additional options for cardiac imaging with conventional CT scanners. New MSCT scanners offer the possibility of high quality cardiac imaging with good reproducibility. In addition, thin slice scanning protocols [4 – 16 images] can improve the performance of these scanners.

The CRIC Study Steering Committee, in consultation with Matthew Budoff, MD, Division of Cardiology, Harbor-UCLA Medical Center, has decided that for the purposes of studies conducted within CRIC, EBT or MSCT may be used to assess coronary calcium. Dr. Budoff, who is collaborating with the CRIC Study as the Principal Investigator of the EBT Central Reading Center, has recommended that EBT or MSCT scanning can be conducted on CRIC Study participants. There will be no significant difference in information obtained from these different modalities.

Ankle-Brachial Index (ABI)

Ankle-Brachial Index (ABI) will be performed in all participants at baseline and annually. ABI provides a measure of overt or clinically occult peripheral arterial disease. Moreover, it is an established predictor of cardiovascular events and death (185-188). It is therefore useful as a predictor of events, a measure of vascular outcomes and a marker of mechanism of vascular disease in the CRI population. ABI is inexpensive and requires minimal training. While peripheral arterial disease is a well known macrovascular complication of diabetes, the role of progressive renal dysfunction on development and exacerbation of this disease in the presence or absence of diabetes has not been well studied and is an area where the CRIC Study will contribute.

The principal limitation of ABI is its interpretation in the setting of medial artery calcification (particularly in diabetics).

Roles—The ABI will serve as a main outcome variable for the development of significant peripheral arterial disease, as well as a potential risk factor for the development of clinical cardiovascular disease (i.e., coronary heart disease and cerebrovascular disease).

Aortic Pulse Wave Velocity (APWV) [A2]

Measures of Aortic Pulse Wave Velocity (APWV) in CKD patients could improve upon the predictive power of blood pressure measurement for CKD and CVD endpoints. In a related subset of CKD patients on hemodialysis, measurement of APWV provides independently predictive information on the potential for target damage, including heart failure, stroke and CV death when compared to systolic, diastolic or pulse pressure measurements.[1,2]

Evaluating a large body of cardiovascular outcome evidence over the last decade suggests that diastolic blood pressure is a strong predictor in younger (< 50 year old) patients. Between the ages of 50-59 no single blood pressure measure (systolic, diastolic or pulse pressure) has clear dominance, and beyond 59 years of age the systolic blood pressure and more recently the pulse pressure becomes particularly predictive of CV events.[3] The diastolic pressure is related more to the systemic vascular resistance than stiffness, the systolic pressure more to arterial stiffness, and the pulse

pressure appears to be the blood pressure measure most related to arterial stiffness. Thus, with brachial arterial blood pressure measures, the more closely they center on arterial stiffness, the more predictive of CV endpoints they become.

While the predictive value of brachial blood pressure in predicting CV events is well established, it shortcomings are perhaps best summarized in the following quote from the Medical Research Council Trial of Hypertension in 1985 (which, at the time, was the single largest randomized controlled trial in hypertension, analyzing data on >85,000 patient years of follow-up.) "The trial has shown that if 850 mildly hypertensive patients are given active antihypertensive drugs for one year about one stroke will be prevented. This is an important but an infrequent benefit. …More than 95% of the control patients remained free of any cardiovascular event during the trial.[4]" We treat most hypertensive patients to prevent target organ damage in a subset. Thus, knowledge of the blood pressure, even in conjunction with other CV risk factors as in this MRC trial, has room for improvement in that other vascular measures (such as those proposed in this ancillary study) potentially could add to the assessment of CV event likelihood and provide a means by which we can better assess CKD progression and CV risk in patients.

Participants will be asked to undergo two noninvasive tests that evaluate how blood vessels adapt to each heartbeat. Three ECG leads are placed on the torso while the participant is lying on his/her back. Distance is measured from the suprasternal notch to the carotid and femoral arteries and a probe measures the pulse at these points and the radial artery. The probe is adjusted until an acceptable waveform has been generated and measurements have been acquired which will take approximately 15 minutes. The device used is FDA approved for research but results are not typically used to manage health care. There are no known risks associated with this test.

APWV References:

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CV Measures Considered but not Included in the CRIC Protocol:

Carotid Artery Intima Medial Thickness by Ultrasound

Carotid artery intima medial thickness (IMT) as assessed by carotid ultrasound has been shown to be an important and reliable predictor of CVD outcomes in populations without renal disease. The use of carotid IMT was discussed extensively by the Cardiovascular Measures Subcommittee and the CRIC Steering Commiteee for possible inclusion in the main protocol. However, the decision was made not to include this as a core measurement tool for several important reasons including recent findings from the ARIC Study showing that changes in carotid IMT associated with various cardiovascular risk factors including CRI took many years to detect, the lack of proven association between regression in IMT and improved CVD outcomes, and the high cost. However,

given that it is a direct measure of non-coronary atherosclerosis and the relative lack of data on its utility within CRI populations, it is anticipated that carotid IMT will likely be evaluated via an ancillary study at selected centers.

Ambulatory Blood Pressure Monitoring

In view of its emerging potential value as a prognostic predictor, the inclusion of ambulatory blood pressure monitoring was carefully evaluated by the Cardiovascular Measures Subcommittee. The decision was made not to include it as a core measurement tool because of several logistical considerations, including participant burden (time committed to wearing the device and the need for a second visit), cost, and the lack of availability at a several of the clinical centers. However, it is anticipated that ABPM will be evaluated via an ancillary study at selected centers with established expertise in this area.

Vascular/Endothelial Function Measures

Abnormalities in vascular/endothelial function are related both to atherosclerotic and non-atherosclerotic processes and are associated with adverse CVD and non-CVD outcomes. Relatively crude measures of aortic vascular stiffness (e.g., calculated pulse pressure) have been shown to be predictors of mortality in dialysis patients as well as in populations without significant renal disease. Advances in technology have begun to make possible evaluation of vascular stiffness and other aspects of endothelial function in various vascular beds (e.g., pulse wave velocity). The different options for assessing vascular/endothelial function were carefully evaluated by the Cardiovascular Measures Subcommittee. The decision was made not to include a specific measure as part of the core protocol because of the lack of currently accepted standardized measures, logistical concerns, and high associated cost. However, the high potential for advances in this area and the important role that the CRIC Study can play in this field was clearly recognized by the Cardiovascular Measures Subcommittee. Investigators will pursue potential measures using the ancillary study mechanism.

3.D.3.c. Schedule of Cardiovascular Exams

The panel of cardiovascular testing will include electrocardiography (ECG), echocardiography and coronary arterial calcification assessment by electron beam tomography (EBT). The schedule of these studies is summarized in the following table. Timing of tentatively planned ^{125}I-iothalamate GFR clearances is included as well.

Figure 1. Time (Years) Since Enrollment

Enrollment	Year 1	Year 2	Year 3	Year 4
ECG (all subjects)	**ECG** [A1] (all subjects)	**ECG** [A1] (all subjects)	**ECG** [A1] (all subjects)	**ECG** (all subjects)
	ECHO (all subjects)			**ECHO** (all subjects)
	EBT (1/3 of subjects)			**EBT** (1/3 of subjects)
GFR (1/3 of subjects)		**GFR** (1/3 of subjects)		**GFR** (1/3 of subjects)

In order to systematically gather CV endpoint data, clinical centers will query the patient at each interim contact on possible CV hospitalizations as well as key outpatient CV tests (to include cardiac catheterization, or noninvasive cardiac functional assessment with echo, MUGA, or nuclear perfusion imaging).

3.D.3.d. Clinical Cardiovascular Outcome Definitions

Cardiac Death:

1. Sudden Death (SD): Sudden loss of consciousness leading to unexpected death within one hour of onset in a previously stable patient. Includes patients who were comatose and then died after attempted resuscitation.
2. Post Resuscitation: Death from complications post arrest in patients with intervening consciousness.
3. Definite Myocardial Infarction (MI): Death which occurs more than 60 minutes from the onset of symptoms, occurs during or before the hospitalization for the MI and is related to a cardiac complication (e.g. CHF, arrhythmia, shock) or non-cardiac complication (e.g. pulmonary embolus) of the acute event. MI is documented by pathologic findings or by two of the following three criteria: clinical, electrocardiographic and biomarkers of cardiac necrosis (CPK-MB and/or troponin). If the patient has a documented MI then dies "suddenly" while making an otherwise normal recovery the cause of death will be classified as "Definite MI".
4. Possible Myocardial Infarction: Typical clinical setting with chest pain or other findings suggestive of Acute MI in the absence of diagnostic biomarker or ECG changes.
5. Congestive Heart Failure (intractable HF): Death from intractable congestive heart failure not associated with an acute event.
6. Procedural Death: Death during or prior to discharge which directly resulted from multi-system organ failure due to cardiogenic shock. Patients who are taken to surgery as a heroic lifesaving measure may not be classified as a surgical death according to the opinion of the reviewers.
7. Primary Intractable Serious Arrhythmia: Must be documented arrhythmia witnessed on a monitor.
8. Other Cardiovascular: Death in which there is evidence of a primary cardiac etiology, which cannot be classified as Definite MI, Congestive Heart Failure, SD, etc. (e.g. peripheral arterial related death, endocarditis).

Non-Cardiac Death:

Death in which there is no evidence of a primary cardiac etiology as noted above.

1. Procedural Death: Death during or prior to discharge from surgery NOT directly resulted from primary cardiogenic shock. Patients who are taken to surgery as a heroic lifesaving measure may not be classified as a surgical death according to the opinion of the reviewers.
2. Hemorrhagic death due to hemodynamic collapse secondary to blood loss.
3. Sepsis: Infection-related death secondary to multi-system organ failure following sepsis.
4. Cerebrovascular: Death secondary to intractable cerebral anoxia or cerebrovascular accident (ischemic stroke or intracranial hemorrhage).

5. Primary Respiratory failure: Death due to primary respiratory failure in the absence of infection.
6. Pulmonary Embolus
7. Non-Cardiac Death - Other (specify)

Unknown Death:

Patients out of human contact for 24 hours in which circumstances of death were unknown.

Myocardial Infarction:

Myocardial Infarction (MI) is defined as: Typical rise and gradual fall (troponin) or typical rise and rapid fall (CPK-MB) of cardiac enzymes to >2X upper limit of the normal range (ULN) in combination with one of the following:

- Symptoms of myocardial ischemia
- Other clinical manifestations of myocardial ischemia (e.g. CHF or new ventricular tachyarrhythmias)
- ECG changes compatible with ischemia or infarction (ST depression or elevation or new or presumed new Q waves or new or presumed new LBBB) (189)

"Aborted" myocardial infarction in the absence of enzyme elevation: Typical ischemic symptoms with ST elevation > 1mm in ≥2 contiguous ECG leads of >30 minutes duration successfully treated with thrombolytics or percutaneous coronary intervention (PCI) within three hours of onset of symptoms. (190-192), (193-195)

Cardiac testing evidence of new infarction to include any of the following:

- New fixed perfusion abnormality on nuclear perfusion imaging as compared to prior examination with corroborating wall motion abnormality
- New akinesis or dyskinesis of a myocardial region on functional evaluation (echocardiography or MUGA) as compared to prior exam.
- Newly occluded coronary artery on coronary angiography in comparison to prior coronary angiogram may be considered as supporting evidence but by itself does not necessarily constitute definitive evidence of a myocardial infarction.
- New pathologic Q waves in ≥ 2 contiguous leads on ECG as compared to prior ECG.

Because of differences in the natural history and prognosis, peri-or postprocedural MI's (definitions below) will be recorded separately from "native" MI's.

Following PCI one of the following criteria must be met:

- Typical rise and gradual fall (troponin) or typical rise and rapid fall (CPK-MB) of cardiac enzymes to > 2X ULN.
- New pathologic Q waves in ≥ 2 contiguous leads.

Following CABG, one of the following criteria must be met:

- Typical rise and gradual fall (troponin) or typical rise and rapid fall (CPK-MB) of cardiac enzymes to > 5X ULN.
- New pathologic Q waves in ≥ 2 contiguous leads.

Acute Coronary Syndrome:

Hospitalization for ischemic chest pain or equivalent ischemic symptom occurring at rest or with minimal exertion, lasting for >5 minutes and associated with any of the following:

- ST segment depression ≥ 1 mm in two or more contiguous leads
- Symmetric t-wave inversion >1.5 mm amplitude in ≥2 contiguous leads.

Evidence of noncardiac causes of chest pain and/or angiographic demonstration of absence of significant CAD (>50% obstruction of at least one artery or branch) and/or noninvasive testing demonstrating lack of ischemia will serve to exclude the diagnosis of ACS.

Hospitalization for Congestive Heart Failure:

Hospital admission (including prolonged "observation unit" admissions of >24 hours) for new or worsening congestive heart failure will be defined as the presence of a syndrome characterized clinically by breathlessness, pulmonary congestion, effort intolerance, fluid retention and peripheral hypoperfusion. These clinical signs and symptoms must represent a clear abrupt change from the normal clinical state of the patient (i.e., baseline status at screening or preinfusion). Episodes of hospitalized heart failure will be adjudicated as to whether the CHF is characterized principally as heart failure with reduced systolic function (based on proximate assessment of LV systolic function), heart failure with preserved systolic function (based on demonstration of intact LV systolic function), or unclassified.

During the index hospital stay, the above symptoms and signs must be accompanied by failing cardiac output as determined by peripheral hypoperfusion (in the absence of clear cut underlying sepsis or hypovolemia) or peripheral or pulmonary edema which requires intravenous therapy (diuretics, inotropes, or vasodilators). Supportive documentation of reduced cardiac index, rising pulmonary capillary wedge pressures, falling oxygen saturation and end organ hypoperfusion, if available, will be assessed. These criteria are consistent with the Framingham Heart Study clinical criteria for heart failure used in other epidemiological cohort studies.

Pulmonary Edema:

In order to standardize criteria used to deem an adverse event as pulmonary edema, the following four conditions must be met:

1. Abrupt change (clear departure for clinical state that is norm for the subject)
2. Presence of respiratory distress (tachypnea ≥ 24, hypoxia, diaphoreses)
3. Evidence of pulmonary edema (rales ≥ 1/3 bilaterally; alveolar or interstitial infiltrates on CXR that are clear departure from baseline)
4. IV diuretic therapy

Serious Cardiac Arrhythmia:

Serious cardiac arrhythmias are defined as the presence of a sustained cardiac rhythm disturbance as noted below. It will be attempted to determine if the arrhythmia is primary or secondary.

Examples include:

- Ventricular Tachycardia
- Torsade de Pointes
- Ventricular Fibrillation
- AICD discharge (must state the underlying initiating rhythm)
- Symptomatic Bradycardia
- Complete Heart Block

Atrial Fibrillation/Flutter

- Supraventricular Tachycardia

Cerebrovascular Endpoints:

Ischemic stroke is defined as a fixed (>24 hours) neurologic deficit not explained by another etiology (i.e., primary hemorrhage, trauma, infection, vasculitis, etc.). Confirmatory imaging studies are not essential to the clinical diagnosis of a stroke. Further description will be based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) and the CARE Study Classification of Subtypes of Acute Ischemic Stroke:

1. Large artery atherosclerosis (embolism/thrombosis)*
2. Cardioembolism (high-risk/medium-risk)*
3. Small-vessel occlusion (lacunae)*
4. Stroke of other determined etiology*
5. Stroke of undetermined etiology
    - Two or more causes identified
    - Negative evaluation
    - Incomplete evaluation

*Possible or probable depending on results of ancillary studies.

Cerebrovascular revascularization procedures will include surgery or percutaneous interventions in the cerebrovascular circulation.

Intracranial hemorrhage is defined as a fixed (>24 hours) neurologic deficit due to a primary intracranial hemorrhage (i.e., intracerebral, subarachnoid, subdural hematoma) that is confirmed by neuroimaging (CT or MRI) or pathology.

Peripheral Vascular Disease:

Peripheral vascular endpoints will include:

- Amputation due to vascular disease
- Peripheral surgical or percutaneous revascularization.

3.D.4. Biochemical Measures

A number of conditions dictate the performance of assays for biological measures at the time blood samples are obtained. These include the need for assessing eligibility (serum creatinine at eligibility and baseline visits), the inability to perform assays on specimens stored for prolonged periods of time (e.g., fibrinogen, PTH, troponin I), and the desire to provide immediate feedback to subjects and their physicians to enhance recruitment and retention (e.g., metabolic panel, homocysteine). It is anticipated that most other biochemical measures will be made in a subcohort of subjects selected for nested studies. (See Nested Studies Section 3.E.6) All subjects will provide annual fasting blood, urine and nail specimens providing the capability of performing core and ancillary biochemical assays as frequently as annually on specimens that will be collected and stored in the CRIC Central Laboratory. See **Appendix F** for a listing of confirmed and proposed biochemical measures.

Glycemic Control

HbA1C concentrations will be measured at baseline as part of the core study for all participants. In subsequent years, this measure will be repeated on diabetic patients.[A1] Pending ancillary funding, insulin and advanced glycation endproducts (AGE), may also be measured as markers of glucose control among CRIC subjects with diabetes mellitus.

Lipids and Lipoproteins

Total cholesterol, triglycerides, HDL and LDL cholesterol [A1] will be obtained annually as part of the core study. Pending ancillary funding, Lipoprotein (a) and Apolipoprotein-B will also be measured.

Markers of Inflammation

Pending ancillary funding, inflammatory markers including hs-CRP and sICAM will be assayed.

Nutritional Status

Nutritional status will be assessed using a combination of clinical evaluation, measurement of estimated body composition and biochemical markers. Serum creatinine, albumin, bicarbonate, calcium, total cholesterol and triglyceride values and homocysteine will be used to evaluate nutritional status. Pending ancillary funding and storage capability, additional laboratory values may be measured for this purpose such as serum prealbumin, vitamins A, B6, B12, C, E, and folate, zinc, C-reactive protein, transferrin, CRP and insulin-like growth factor-1 concentrations, carotenoids, total body nitrogen and urinary electrolyte excretion.

Hemostatic/Prothrombotic Factors

Fibrinogen will be measured at baseline [A1] in all subjects as part of the core study. Pending ancillary funding, plasminogen activator inhibitor-1 (PAI-1) may be measured as a marker of hemostatic/prothrombotic activity.

Measures of Myocyte Injury

Troponin I will be measured at baseline [A1] in all subjects as part of the core study. Troponin, a direct measure of myocyte injury, is generally considered to be the most sensitive and specific measurement available (189). Though most experts in the field consider it to be close to 100% sensitive and specific for cardiac ischemia in the right clinical setting, as troponin should not be found in the bloodstream, this is certainly an overstatement in the renal-failure population, and even less is known about its utility within an ambulatory CRI population. .

Oxidative Stress

Pending ancillary funding, oxidative stress will be estimated by measurement of urinary isoprostanes using one of the aliquots from the outpatient urine collection.

Cytokines

Measures of TGF-alpha and TGF-beta will be obtained on stored specimens pending ancillary funding. Significant information on the role of cytokines, particularly TGF-beta in diabetes, testifies to their utility in predicting worsening CKD. TNF-alpha is less well understood, but appears to be important in CVD progression.

Endothelial Function

Pending ancillary funding, endothelial function will be assessed by the determination of plasma concentration of asymmetric dimethyl-arginine (ADMA).

Fibrosis

Pending ancillary funding, fibrosing predisposition will be assessed by measurement of serum concentrations of procollagen-1.

Heavy Metal Toxicity

Pending ancillary funding, exposure to heavy metals (e.g., lead and cadmium) and body accumulation will be assessed through assay of trace metal concentrations in toenail clippings.

Iron Status

Pending ancillary funding, iron status will be assessed by serum iron and iron-binding capacity and serum ferritin concentrations. The importance of anemia and iron status has been demonstrated in both CKD and CVD.

Other Biochemical Measures

Electrolytes (Sodium, Potassium, Chloride, Bicarbonate), BUN, Creatinine, Glucose, AST, ALT, Total Bilirubin, Total Protein, Calcium, and CBC will be measured annually. A urine dipstick will be performed annually for qualitative glucose, protein, and hematuria determinations. Magnesium, Phosphorous, Uric acid, and iPTH will be measured at baseline. [A1]

Genotyping

Specific genotype assays will be developed during the course of this study, likely in conjunction with ancillary proposals targeting specific gene-based questions. At the

outset of the study buffy coat white cells will be isolated and stored frozen in the NIDDK repository.

3.D.5. Obtaining Interval Clinical Outcomes

Recognizing the importance of accurately describing and quantifying interim clinical events, the CRIC study has developed an outline for the collection, verification, and cumulative reporting of this information.

3.D.5.a. Potential Data Sources and Ascertainment Strategy

Data Sources. The participant contact pattern includes annual clinic visits and interim phone calls at least every six months. These contacts will be primary sources for obtaining information on potential interim clinical events. For selected sites that have access to electronic administrative or billing records, regular searches of those data sources will be performed (e.g., monthly or quarterly) for their center's study participants. We will also explore the feasibility of using additional databases (e.g., Medicare A claims, National Death Index) to identify outcomes, although it is clearly recognized that there is often a long delay (e.g., 12-18 months or longer) in getting access to these files, which are updated annually and require additional governmental approval. The implications of HIPAA regulations on access to these data sources are also largely unknown at this time.

Outcome Search Strategy. For potential clinical events that led to hospitalization, we will acquire information from the study subject or their self-assigned proxy regarding the dates (admission and discharge) and hospital (name, address, phone number) where the patient was hospitalized. The scope of medical record acquisition is limited to the following events: hospitalizations, outpatient cardiac catherization, outpatient ECHO (MUGA or nuclear perfusion imaging), new onset heart failure, extended (>24 h) ER or observation unit stay.

For potential clinical events that did not lead to hospitalization, we will acquire information from the study subject or their self-assigned proxy regarding the date of the potential event and the location where the patient received treatment (e.g., outpatient clinic/surgicenter, address, phone number, and treating physician).

For deaths, we will contact the study subject's listed proxies/other contacts to determine if death occurred (as well as the date and possible cause). In the event that we are unable to reach the contacts, we will also investigate the feasibility of contacting the subject's listed primary care physician, if known, via letter followed by a phone call. As noted above, in the case of deaths that cannot be confirmed using the approach described above, we will explore whether the National Death Index may serve as a backup data source to get nearly complete data on mortality for CRIC. See Appendix E for additional information.

3.E. Statistical Analysis

3.E.1. General Methods for Statistical Analysis

A brief overview of some of the statistical methods that may be used at the time of analysis, both for descriptive purposes and in more comprehensive analysis of the primary research questions, is summarized in the following sections. For a large study

with many exposures or predictors, several outcome variables, and many scientific questions of interest, it is not possible to detail analyses of each question. Nonetheless, the types of analyses described here are likely to be used to answer many of the questions related to the main specific aims. We consider here the principal methodological issues that are likely to arise in addressing these aims, and approaches for dealing with those issues.

We consider analyses designed for prediction of outcomes as well as those designed to elucidate mechanisms of disease progression, although the issues are intimately related. We also discuss separately analyses relating baseline characteristics of the cohort to clinical outcomes as well as those that will incorporate time-varying characteristics (e.g., lipid levels, BP etc.) that will be measured during follow-up. Earlier we described for these latter analyses nested designs such as the case cohort approach, that will permit us to address etiological questions with respect to time-varying measures, but that will not demand collection of costly time-varying data such as cytokine levels on the entire cohort.

Standard descriptive statistics will be used to describe baseline characteristics and follow-up measures, both overall and within comparison subgroups. Summary statistics such as means, medians, standard deviations, and ranges will be produced for measured variables. Frequencies will be tabulated for categorical and ordinal variables. Graphical methods will be used extensively to examine distributions, identify potential influential points, and guide in data transformations if warranted. For outcomes collected longitudinally, and to examine associations among various measures, scatterplots and grouped boxplots will be produced to examine assumptions of linearity, symmetry, and homoscedascity.

3.E.2. Analysis during the Adaptive Recruitment Phase

The study has several recruitment goals regarding the distribution of various groups, including the distribution of serum creatinine or estimated GFR, the prevalence of diabetes, age and other characteristics. These measures may be available in advance from databases available at each center; others will be available only at the time of the initial clinic visit.

During the course of recruitment, we will examine repeatedly the distribution of age, race, eGFR, and diabetes among subjects who are recruited. If, after completion of the recruitment of 300 subjects, the distributions of these variables are close to the target ranges specified above, recruitment will continue as previously. If not, subjects with some levels of these variables will be oversampled and others undersampled.

3.E.3. Failure-Time Analyses

Two of the main endpoints in the study are failure-times: CVD events and the development of clinically important events of renal dysfunction (e.g., ESRD and 50% declines in GFR). We will examine the associations of various baseline predictors with these failure-time events using standard methods: Kaplan-Meier curves, log-rank tests, and the Cox proportional hazards model. For estimating the effects of single factors, we will use the proportional hazards model to adjust for possible confounding by other variables. We will also use the model for predictive purposes. In each case, the variable and model selection procedures will be tailored to the question at hand (196;197).

We will use the usual diagnostic procedures (e.g., including time by covariate interaction terms, examining residual plots, etc.) to assess the adequacy of the fit of the model.

We will also consider models including time-varying factors as predictors. We will use the proportional hazards model with time-varying covariates for this purpose. In these models, we will be cognizant of the different roles variables play; in particular, in analyses designed to examine the associations of a predictor/explanatory factor with failure, we will not include variables intermediate on the causal pathway from the factor to the outcome in the model.

In some cases, we will be concerned with estimating the joint effects of treatments or exposures received at different times on the rate of failure (e.g., the levels of glucose control measured at different times). To do this, we will use marginal structural models and structural nested models, which can control for the confounding effect of a time-varying covariate without inappropriately adjusting for it as an intermediate variable.

3.E.4. Repeated Measures & Slope Analyses

Several study outcomes will be repeated measures of continuous or categorical variables. For these analyses, we will use standard mixed effects growth curve models. These models allow for both the estimation of individual subjects' slopes and intercepts and for the comparison of groups defined by baseline or time-varying covariates. Regression diagnostics will be used for all models to assess model adequacy and examine potential outlying or influential datapoints. The sample sizes/power calculations outlined earlier in this protocol were based on detecting associations between baseline measures and slopes. Where necessary, we will supplement these analyses with marginal models estimated by generalized estimating equations. Both approaches account for the non-independence of data within repeated measurements of individual subjects.

It is expected that a substantial proportion of patients may withdraw prior to the final assessment at five years of follow-up. In addition, length of follow-up will differ among patients depending on the time of study entry. The statistical methods for longitudinal data analysis outlined above allow for staggered entry and differential lengths of follow-up among patients. However, careful attention will be paid to the varying length of follow-up among patient subgroups, especially those defined by baseline and time-varying covariates associated with the outcomes of interest, in order to evaluate any potential bias introduced by differential follow-up among patient subgroups. The characteristics of patients without complete follow-up will be examined, and the drop-out mechanism will be modeled. All attempts will be made to keep missing data to a minimum and all available data on all patients will be used for the primary analyses.

3.E.5. Special Considerations for the Study of Progression of Renal Disease

The progression and severity of renal disease play a central role in the design and analysis of the study. As such, the severity and progression of renal disease will be considered both as a predictor and as an outcome variable. As outcomes, the progression of renal disease may be analyzed using slope-based analyses or event-based analyses. As has also been discussed elsewhere, both types of analyses have advantages

and drawbacks (198). Since we plan to perform both types of analysis for examining these outcomes, we provide some further discussion.

Slope-based analysis uses all available information on GFR or serum creatinine and so is likely to provide more power to detect differences between groups. Thus, it is likely to be of particular importance in studying subtle changes in renal function, and in studying the subgroup of subjects in the CRI who begin with higher levels of GFR.

Nonetheless, drop-out poses problems, especially for slope-based analysis. There are two likely reasons for drop-out from slope analysis: drop-out because there is (essentially) no GFR to measure (i.e., because of development of ESRD), and loss to follow-up for other reasons (including refusal to do GFR). These are, in fact, two very different situations; in the former, one can assume that GFR is less than some threshold; in the latter, we know nothing about GFR; thus, this type of dropout affects both slope and time-to-event analysis.

When a variable like GFR is known to be below some threshold, methods for censored data may be used. One can use a censored regression model (known in survival analysis as the accelerated failure time model) to model the association of time and other predictor variables with GFR in an appropriate fashion. Mixed effects versions of these models have been developed (199) for dealing with the correlation of observations within subject. We will employ these methods. Further, one can use robust variance estimates/GEE to estimate the variance of the parameters taking into account the multiple observations of GFR within subjects.

In time-to-event analysis, the development of ESRD will be an event and so, its incorporation in the analysis poses no problem. Normally, time-to-event analysis has substantially reduced power and precision compared with repeated measures/slope analysis. The power of event-based analysis will be enhanced by including as events declines of 50% in GFR or predicted GFR. Such event-based analyses will, like slope analyses, be limited by error, both systematic and random, in the measurement of GFR. For all time-to-event alanyses, it will be important to consider GFR at entry into cohort since that may be a more powerful determinant of outcome than factors influencing GFR decline.

For actual drop-out, where no information on GFR is available, both repeated measures and failure- time approaches are acceptable. In time-to-event analysis, we consider a subject censored at the time that GFR information becomes unavailable; standard methods for dealing with censoring in survival data may then be applied.

If post-baseline covariates are associated with GFR and dropout, we may have dependent censoring, and standard approaches will be biased. Inverse probability weighting approaches may be applied to deal with dependent censoring in both failure-time and repeated measures/slope analysis (200).

[A2] Over the course of the study, participants who begin dialysis or receive a kidney transplant will be asked to provide additional information in the form of a brief questionnaire that asks focused questions about the procedure and their preparation and experience. To whatever extent possible, these participants will be followed according to the study protocol.

3.E.6. Nested Analyses

Several important study variables, including iothalamate GFR, certain radiologic measures, and some measures of serum chemistry, will be performed only on a subset of study subjects. In the case of GFR and radiologic measures, the tests will only be available from a preselected random sample of the study cohort, the subcohort. For certain serum chemistry measures, these variables will be available from the subcohort and from all subjects who develop either of the primary endpoints of interest: cardiovascular events and ESRD. We consider these in turn.

For measures like GFR which are only available in a predefined subset, our main analyses will be similar to those of other repeated measures. Thus, where these variables are outcomes, we will use the same modeling strategies as before (i.e., mixed effects models), including only study subjects in the subcohort in the modeling. Similarly, we will use these variables as predictor variables for other outcomes (e.g., failure-time outcomes) in the analysis. For these analyses, we will also consider multiple imputation (201) for dealing with missing data on these variables in subjects not in the subcohort. The approach can improve efficiency of the analyses by including more subjects; however, it can also lead to bias if the models for imputing missing values are incorrectly specified.

For GFR, we will perform event-based analyses to supplement consideration of GFR as a continuous, repeated measures outcome. In particular, we will consider subjects to have failed if they either develop ESRD or experience a drop of 50% in GFR, after adjusting for baseline GFR. These analyses will be supplemented by analyses in which an estimated GFR criterion is substituted for the directly measured iothalamate GFR criterion; the estimates will derive from prediction equations using CRIC data and analogous to MDRD equations; these analyses can include all study subjects, not just those in the subcohort, which can increase efficiency, but are potentially subject to additional bias.

For laboratory measures where blood samples are stored and frozen, information can be obtained not only on the subcohort but also from all subjects developing the endpoints of interest. Standard analysis of these data to look at the associations of predictor variables and outcomes will use inverse probability weighting methods as described in Chen (202) as with full cohort data, relative hazards are estimated. The efficiency of this approach can be improved by including all subjects who have the laboratory measure performed in the analysis, using weighting methods. Thus, in an analysis where ESRD is the endpoint, subjects in the subcohort, subjects developing ESRD, and subjects who develop CVD events even if they fall in neither of the former categories, can be included in the analysis (the last group is not included in traditional case-cohort analysis).

3.E.7. Center Effects

There may be systematic differences among the centers in subject mix and methods of treatment; some of these differences will be measured imperfectly, by other variables recorded by the study. Because of this, we will need to account for center in the analysis in order to get appropriate estimates of standard errors, and possibly to control for confounding by center; this will hold both for failure-time and repeated measures outcomes. We will consider several ways to account for center in survival models; by

fitting mixed- or fixed-effects models including separate center-specific coefficients, by fitting marginal models not including center-specific coefficients, and by using models that condition or stratify on center. For prediction in a wider population, the centers will not themselves be of interest; thus, we will rely primarily on marginal models using the robust variance estimator.

3.E.8. Measurement Error

Some variables will inevitably be measured with error; this can result in bias in measurement of the associations of these variables with various outcomes. In the simplest settings, this measurement error is thought to dilute these associations and so has been termed "regression dilution." To deal with this problem, certain laboratory variables will be selected for measurement of replicated values near baseline. Other may be selected for comparison against a "gold standard." Appropriate methods (e.g., regression calibration) for reducing the bias due to measurement error will then be employed (203)

3.F. Sample Size & Power Considerations

3.F.1. Overview

The actual power to detect specified associations will depend on the particular outcome of interest (e.g., CVD events, slope of change in GFR, etc.), the underlying rate or progression of disease, the distribution of risk factors in our study population, the alpha error we are willing to tolerate, and the proportion of our cohort members who are included in the analysis. For example, analyses including coronary calcification data will have 1,000 of the 3,000 cohort members contributing data. For other subgroup analyses, we may have yet smaller sample sizes available. Power can be calculated for each of the types of analyses we envision. We focus this discussion on the analyses that will have the least power to detect differences in outcomes. First, we consider traditional cohort analyses incorporating time to event (e.g., CVD and ESRD) analyses, followed by nested case-cohort analyses and, finally, address analyses focusing on the difference in the slope of continuous measures such as GFR.

3.F.2. Time to Event Analyses

Data on the event rate of CVD in patients with CRI are available from several sources (69;71;204). Jungers et al. (69) estimated that the rate of myocardial infarction ranges from 0.62 to 2.78% per year among men, and 0.16 to 1.27%/year among women, depending on age. Consistent with these findings, others (204) reported a rate of CVD in subjects with mild CRI of 2.3%/year, where CVD included coronary heart disease, CHF, and stroke. A separate report by this same investigator (71) reported an incidence of cardiac ischemia, MI, and cardiac mortality of 3% per year among Framingham study subjects with more than trace proteinuria, and 2% per year among those with trace proteinuria. This was similar to the rate of 3.5% per year for the combined outcome of MI, stroke, or CV death in the MicroHOPE study of individuals with diabetes mellitus and either prior CVD or one additional CV risk factor. Finally, among the subset of 980 subjects in the HOPE trial with CRI (approximately one third with diabetes mellitus) and prior CVD or an additional CV risk factor, the rate of the composite CV outcome was 5.8% per year.

Data on the rate of ESRD among individuals with CRI suggest an event rate of similar magnitude to that reported for CVD. For example, in a study of benazapril (22) to slow the progression of CRI, there was a 3%/year rate of ESRD; similar to the average rate reported from the MDRD, study A.

Based on these data, we estimated the detectable hazard ratio for a range of analyses that include all or subsets of the CRI cohort, a range of exposure prevalence, a risk of outcome events in the non-exposed group of either 0.02/year or 0.04/year or 0.06/year, an alpha error of 0.05, and 80% power (Table 10) using a log-rank test. A potential loss of follow-up of 4% per year was incorporated within the calculations. Under the assumptions of a recruitment period of 33 months, and additional follow-up time of 42 months, the detectable hazard ratios in Table 10 were computed under 80% power requirements. For the entire cohort of 3,000 subjects, using the most conservative estimate of 2% for the incidence of CVD events per year (conservative because it is the lower range reported in the literature, and because our sample will be enriched with subjects with diabetes who are at higher risk), we will be able to detect a hazard ratio of 1.65 if the prevalence of the exposure is only 10%. For a subset analysis with 1,500 subjects, relevant to the subset analyses of the diabetes-specific subgroups, we will be able to detect hazard ratios of 1.95 for an exposure with prevalence 10%, and 1.57 for exposure prevalence 50%. Finally, if we examine a subgroup with only 500 subjects, we will be able to detect hazard ratios of 2.82 for an exposure prevalence of 0.10, and 2.08 for an exposure prevalence of 0.50.

Table 10. Minimum Detectable Hazard Ratio From Proportional Hazard Analysis, Alpha error=0.05, Power=0.80; Loss-of-follow-up Rate=4%

#Subjects in Analysis	Exposure Prevalence	Length of Follow-up								
		42 months			66 months			90 months		
		Event risk/yr in non-exposed group			Event risk/yr in non-exposed group			Event risk/yr in non-exposed group		
		0.02	0.04	0.06	0.02	0.04	0.06	0.02	0.04	0.06
200	0.1	4.31	3.21	2.78	3.76	2.89	2.55	3.45	2.70	2.42
200	0.5	2.97	2.30	2.05	2.64	2.11	1.91	2.45	2.00	1.83
300	0.1	3.52	2.71	2.38	3.12	2.46	2.20	2.88	2.32	2.10
300	0.5	2.50	2.01	1.81	2.25	1.86	1.71	2.11	1.78	1.65
500	0.1	2.82	2.25	2.02	2.54	2.07	1.89	2.38	1.97	1.81
500	0.5	2.08	1.74	1.60	1.91	1.64	1.52	1.81	1.57	1.48
1,000	0.1	2.20	1.84	1.68	2.02	1.72	1.60	1.92	1.65	1.55
1,000	0.5	1.72	1.50	1.41	1.61	1.43	1.35	1.54	1.39	1.32
1,500	0.1	1.95	1.67	1.55	1.81	1.58	1.48	1.73	1.52	1.44
1,500	0.5	1.57	1.40	1.32	1.48	1.34	1.28	1.43	1.31	1.26
3,000	0.1	1.65	1.46	1.38	1.55	1.40	1.33	1.50	1.36	1.30

Extending the CRIC for an additional 2 yrs. (66 mos.) or 4 yrs. (90 mos.) beyond current plans, improves detectable risk ratios only slightly. For example, for all diabetics (N = 1,500), assuming an annual event risk of 4%, the detectable hazard ratio only improves from 1.67 to 1.52 for a risk factor with exposure prevalence = 10%, by this extension of

follow-up for 4 additional years. A small gain in power with longer follow-up is related to attenuation of cohort (4% lost-to-follow-up per year).

3.F.3. Case-Cohort Analyses

Case-cohort analyses will be performed with subsets of our entire cohort without substantial loss of power compared to the full cohort analysis. Table 11 shows the power for case-cohort analysis, assuming that 1,000 subjects (1/3 of the total study subjects) are in the subcohort that has additional testing. The bottom two rows show the minimum detectable hazard ratios when associations apply to the entire study cohort. Other rows apply for associations within smaller subsets of the study cohort (e.g., diabetics).

Table 11. Minimum Detectable Hazard Ratio from Case-Cohort Analysis, Alpha error=0.05, Power=0.80, Loss-of-follow-up=4% Subcohort 1/3 of Total Cohort (1000 subjects)

#Subjects in subgroup for analysis	Exposure Prevalence	Event Risk per Year in Non-exposed Group		
		0.02	0.04	0.06
200	0.1	5.10	4.02	3.64
200	0.5	3.39	2.71	2.48
300	0.1	4.09	3.28	3.00
300	0.5	2.80	2.31	2.13
500	0.1	3.20	2.64	2.42
500	0.5	2.28	1.95	1.82
1,000	0.1	2.43	2.08	1.93
1,000	0.5	1.83	1.62	1.54
1,500	0.1	2.12	1.85	1.74
1,500	0.5	1.66	1.49	1.43
3,000	0.1	1.75	1.57	1.50
3,000	0.5	1.44	1.33	1.29

These analyses are conservative because most of the laboratory measures to be analyzed in the case-cohort analyses will be continuous, whereas the formulas shown here are for binary predictor or exposure variables, which tend to yield lower power.

3.F.4. Analyses of Slope of Lothalamate GFR & Predicted GFR

We plan to measure iothalamate GFR values at baseline, two and four years after enrollment. We are interested in detecting the difference of slope (ml/min per 1.73m^2 per year) in GFR between exposed and unexposed subgroups within the CRIC cohort. The calculation of detectable differences in slope depends on several factors; the standard deviation of GFR measured cross-sectionally at the same point in time, the correlation of repeated measures within subjects, sample size, exposure prevalence, the number and timing of GFR measures, the alpha error, and power. We used AASK data to obtain an estimate the standard deviation of GFR of 12.9 ml/min per 1.73m^2. Also using AASK data, we estimate the correlation between repeated measurements to be 0.732.

Table 12 displays the detectable slope differences (ml/min/1.73 m^2/year) at various total sample sizes and exposure prevalences, assuming two-sided hypothesis testing at

the 5% level (188). Total is the total number of subjects recruited at the beginning of the study. An anticipated rate of loss to follow-up of 4% per year is accounted for in the calculation of the detectable slope differences assuming no replacement of lost subjects. For a proposed iothalamate-GFR-subcohort size of 1,000, an exposure prevalence of 0.1, iothalamate-GFR measures at baseline, 2, and 4 years of follow-up, we will be able to detect slope differences of at least 0.76 ml/min/ 1.73m^2 per year with 80% power.

Table 12. Detectable Iothalamate GFR Slope Differences Under Specified Parameters: Exposure Prevalence=0.1,0.5; Annual Loss-of-follow-up = 4%

Time (Years)	Total	Exposure prevalence=0.1		Exposure prevalence=0.5	
		80% Power	90% Power	80% power	90% Power
(0, 2, 4)	1,500	0.62	0.71	0.37	0.43
(0, 2, 4)	1,000	0.76	0.88	0.45	0.53
(0, 2, 4)	800	0.85	0.98	0.51	0.59
(0, 2, 4)	600	0.98	1.13	0.59	0.68
(0, 2, 4)	500	1.07	1.24	0.64	0.74

3.F.5. Analysis of Slope in Coronary Calcification

A total of 1,000 subjects will undergo measurement of coronary calcium scores at one and four years of follow-up. Since there are no preliminary data to estimate the standard deviation of calcium score and correlation between repeated measurements, we examined several studies in the literature such as Goodman and Tamashiro (184), (205). We selected the median standard deviation of 605 among these studies to perform the sample size calculation using a moderate correlation of 0.5 between repeated measurements. Table 13 presents the minimum detectable slope differences at various total sample sizes and values of exposure prevalence, assuming two-sided hypothesis testing at the 5% level. Total is the total number of subjects recruited at the beginning of the study. Loss of follow-up of 4% per year is accounted for in the calculation of the detectable slope differences assuming no replacement of lost subjects. For our proposed subcohort size of 1,000, even if the prevalence of exposure is 0.1, we can detect slope difference in coronary calcium score of at least 63.270 per year with 80% power.

Table 13. Detectable Slope Difference in Coronary Calcification Under Specified Parameters; Exposure Prevalence=0.1,0.5; Annual Loss-of-follow-up = 4%

Time		Total	Exposure Prevalence=0.1		Exposure Prevalence=0.5	
			80% power	90% power	80% power	90% power
1	4	4,000	31.640	36.612	18.984	21.967
1	4	3,500	33.822	39.137	20.293	23.482
1	4	3,000	36.536	42.277	21.922	25.366
1	4	2,500	40.020	46.309	24.012	27.785
1	4	2,000	44.751	51.784	26.851	31.070
1	4	1,500	51.670	59.789	31.002	35.873
1	4	1,000	63.270	73.213	37.962	43.928
1	4	800	70.738	81.854	42.443	49.113
1	4	600	81.681	94.517	49.009	56.710
1	4	500	89.528	103.597	53.717	62.158

4. HUMAN SUBJECT CONSIDERATIONS

4.A. CRIC Participant Considerations

4.A.1. Recruitment

Sources of participants will vary from center to center. Likely sources include computerized searches of databases, hand searches of medical records of health care providers, referrals from health care providers other than CRIC investigators, and the patient panels of CRIC investigators. The recruitment goal is challenging, namely, 3,000 participants who complete not only the baseline visit but also the first annual follow-up visit. Each of the seven Clinical Centers will plan to enroll approximately 430-500 participants. The final center-specific recruitment approach will take into consideration the observed rate of loss-to-follow-up during the first study year and be chosen to establish the cohort of 3000 CRIC participants who undergo a baseline and the Year 1 follow-up visit. [A2] To account for loss of recruitment and anticipated incomplete follow-up at the Tulane site, each of the six Clinical Centers will plan to enroll an additional 50-80 participants, approximately 520–550 participants. [A4]

In this setting, we anticipate that most centers will need to secure access to one or more large databases that can identify individuals with an elevated serum creatinine and that can also sort on other variables (e.g. age, gender, presence or absence of diabetes). We also anticipate that most centers will attempt to recruit participants from clinics that are enriched with individuals who might be eligible (e.g. nephrology clinics and diabetes clinics) as well as general internal medicine clinics. Other recruitment sources might include flyers and brochures placed in medical facilities as well as advertisements and articles in print media.

The process of securing local physician approval and contacting the potential screenee will depend on prevailing guidelines of local IRBs, the requirements of each medical facility and the governmental HIPAA Guidelines which will become effective in April 2003. Typically, screenees will first learn of the study from an invitational letter signed by the local principal investigator and/or personal physician. Occasionally, some individuals may learn of the study during a routine encounter with a health care provider who has agreed to assist in CRIC recruitment. Those individuals who express preliminary interest in the study will have a pre-screening telephone interview to confirm eligibility. Those who remain interested will be scheduled for the in-person screening visit at which point we will secure written informed consent.

4.A.2. Screening & Enrollment

The screening and enrollment process will require two visits. The visits are structured such that essential information required to assess eligibility is acquired prior to the conduct of more intensive and time-consuming procedures during the subsequent baseline visit. [See Appendix A]

The screening visit will be a brief in-person visit to obtain informed consent for the entire protocol and sociodemographic characteristics, confirm study eligibility, and provide participants with additional information about the study. A non-fasting blood specimen, spot urine and blood pressure will be obtained. A questionnaire assessing eligibility will be completed and contact information will be collected. If found to be

eligible for the study, participants will be instructed in completing a Food Frequency Questionnaire, and receive directions for collecting a 24-hour urine sample.

Those persons who are eligible and interested after the screening visit will be invited to attend the baseline visit. The completion of this visit defines enrollment in the cohort study. Key eligibility criteria will be reviewed and confirmed, anthropometric measures will be collected, resting blood pressure and heart rate will be obtained, medical and family history will be collected, medications used in the past 30 days will be collected, a urine sample will be obtained, a fasting blood specimen (approximately 130 cc) will be drawn, ABI and BIA will be measured, the 24 hour urine sample will be collected, toenail clippings will be obtained, and a 12-lead ECG will be performed. A urine pregnancy test will be performed in all women of childbearing age chosen for the subcohort before initiating the iothalamate GFR testing. Iothalamate-GFR testing will be performed in the subcohort. A series of questionnaires concerning quality of life, dietary assessment, psychosocial characteristics and physical activity will be conducted at this time. Assistance will be provided to participants who experience difficulty completing questionnaires.

4.A.3. Informed Consent

The consent process may differ somewhat by clinical center according to local IRB guidelines. The informed consent document will be structured such that it enables potential participants to indicate which aspects of study they may not be willing to engage in. This form will cover all aspects of screening, baseline testing and subsequent follow-up visits.

A separate section and signature page will be required for consent to collect a blood sample for genetic testing and storage of DNA. Participants may enroll in CRIC but refuse to sign the genetic consent form without consequence to study eligibility. See Appendix B.

The Health Insurance Portability and Accountability Act (HIPAA) became effective on April 14, 2003 and will affect the CRIC research study with regard to the use or disclosure of protected health information. Each institution may hold different requirements regarding HIPAA. Some institutions may request that this HIPAA language be inserted into the appropriate sections of the informed consent form; other institutions may require a stand alone document.

Participants will be asked to sign an additional form on an annual basis authorizing study personnel to request and review their medical records. This will permit access to medical record information which will enable investigators to inspect medical information and documents associated with reported clinical events.

4.A.4. Participant Follow-Up

Participants will be scheduled to return annually for the tests and procedures listed on the visit schedule in Appendix A. The burden of a lengthy annual visit can be diminished by staggered scheduling of tests and procedures, in order to accommodate the schedule and convenience of participants. At the six month interval between clinic visits, participants will be contacted by phone. During this contact, participants will be asked to provide information about study outcomes (e.g. onset of dialysis), and update

general health and contact information. The purpose of these contacts will be to reinforce the importance of continued study involvement and potentially to collect selected questionnaire data, thereby reducing subject burden during annual in-person visits. Regular contact with subjects will also be facilitated through the use of newsletters about the study and its results, as well as recognition of important dates (e.g. birthdays, holidays). It is anticipated that over the course of the study, approximately 3 - 5% of participants will drop out each year. A recruitment approach based on the observed rate of loss-to-follow-up will be used to achieve a baseline cohort of 3000 participants who undergo a baseline and Year 1 follow-up visit. [A2] To that end, a somewhat larger number of subjects will be enrolled into the study at the baseline visit, since some subjects in the study will inevitably be lost to follow-up.

4.A.5. Participant Retention

Retention of participants is central to the internal validity of the study and will be an extraordinarily high priority of the investigators and staff. A key element is a pleasant, attentive and responsive staff that provides a reasonably flexible visit schedule. Other clinical center features that promote high retention rates include local tracking systems; frequent staff meetings; free and convenient parking; personal contacts through birthday cards, holiday cards, sympathy cards and flowers; small gifts at visits; and modest monetary incentives. For people lost-to-follow up, we will use search services and the National Death Index if indicated. To facilitate these searches, we will ask participants to provide their social security number at the time of enrollment. This information will be kept in a secure file at the Clinical Centers with access restricted only to the Clinical Center Principal Investigator and necessary personnel.

Participants selected for GFR and EBT testing will invest additional time and energy in the CRIC study. Recognizing this commitment, each center may offer additional incentives to these participants in order to make complying with this section of the protocol as positive and convenient as possible.

4.A.6. Participant Withdrawal

It is anticipated that over the course of time, CRIC participants may withdraw from the study. This may occur officially by formal notification from the participant to the investigator, or unofficially when a participant cannot be reached via the usual methods of contact. Every effort will be made to acquire complete data on all participants.

Participants who relocate to an area from which it is no longer feasible to travel to the clinical center will be asked to permit study personnel to contact them annually for a telephone contact follow-up. Centers may offer inducements to participants who drop-out or relocate in the form of additional travel reimbursement in return for their continued participation.

4.B. Clinical Management of Participants

Investigators recognize the obligation and importance of reporting research information to the health care providers of participants. It is essential that investigators report clinically actionable information to the appropriate provider in a timely fashion. A procedure for reporting such information from participating reading centers to CRIC investigators will be implemented and described in the Manual of Procedures.

4.B.1. Transmission of Study Findings & Response Time

As results become available, they will be sent to participants and their primary care physicians. Permission to forward this information will be obtained during the consent process at the time of study entry. The following results will be included in the reports: BP measurements, estimated body mass index, ankle brachial index, chemistries (including metabolic panel), CBC, homocysteine, estimated GFR using the modified MDRD equation, and ^{125}I-GFR and calcium score as available. The schedule for reporting CRIC lab and physical measures to participants and their health care provider has been revised to reflect the accumulation of lab test results from several laboratories that require approximately 6 – 8 weeks for the initial (primary) report and 4 – 5 months for tests acquired at a later date or batch tested. [A2] Similar reports will be provided after subsequent examinations. Central clinical interpretation of the ECG has been deferred; however, it will be evaluated for urgent alert conditions within 72 hours. [A2] Timeliness in reporting findings is dictated by the multi-center nature of the study and its complexity. It is important to note that information from tests listed above may be less than the full complement received during tests performed for clinical evaluation.

In addition, for subjects undergoing EBT, information on non-cardiac findings (primarily lung and liver abnormalities) will not be available since these non-cardiac CT fields will be blocked and not reviewed by the central EBT reading laboratory. This approach is identical to the policy recently employed by the NHLBI-sponsored Mutli-Ethnic Study of Atherosclerosis (MESA) study that is using EBT in a large cohort. The primary reason for this approach is that the purpose of the EBT is only to exam coronary calcification and cardiac structure (other potential, scientifically relevant measures may include visceral/subcutaneous fat, vertebral bone mineral density, and renal size) and to remain ethically consistent.

4.B.2. Alert Findings

Participants and their physicians will be notified as soon as possible if potentially serious medical problems are identified during any of the examinations. Alerts will be defined as immediate or urgent. (**Appendix D** categorizes alert findings and optimal response time for reporting results.)

Immediate Alerts:

Immediate alerts are medical emergencies which are encountered or discovered at the time of the study visit such as hypertensive emergencies or chest pain. These alerts will be evaluated by a physician who will determine the appropriate disposition. Immediate notification of the participant's physician should be accomplished by telephone, prior to the participant leaving the clinic. A follow-up letter documenting information discussed by phone should also be sent to the participant's physician.

Urgent Alerts:

Certain laboratory tests, such as potassium and glucose, will be performed centrally and results will not be known immediately. For this reason, certain significantly abnormal results are classified as urgent. Urgent alerts require notification of the primary care physician within 24 hours of receipt of the results.

4.B.3. Referral of Participants Lacking a Primary Care Provider

Every effort will be made to identify a primary care physician for each participant. In cases in which participants are unable to identify a primary physician, the participant will be offered assistance in finding one. Each clinical center will establish a referral plan to accommodate participants with and without insurance. Clinical centers will develop a list of subsidized health centers for participants who are under or uninsured and when necessary, clinical centers will direct the participant to social service agencies. Each clinical center will submit their referral plan to the Clinical Management Subcommittee for review.

4.B.4. Standards of Care

Guidelines outlining current medical standards of clinical care will be provided to all identified primary care physicians. Participants will receive an explanation of these guidelines in conjunction with their test results. Participants will be encouraged to discuss results with their primary care physicians. Clinical center personnel and investigators will be accessible to answer additional questions.

4.C. Ethical Issues

4.C.1. Potential Risks to Participants

A minimal physical risk to participants arises from the diagnostic procedures for the collection of blood specimens, the measurement of GFR, and the collection of diagnostic imaging data. For the former, which requires venipuncture of approximately 130 cc blood annually, there is minimal risk of hematoma or infection. Implementation of the GFR studies using ^{125}I-Iothalamate involves minimal radiation exposure risk from this test. The dose of ^{125}I-Iothalamate, given throughout the study, is between 25 and 35 micro-curies. Pregnant women are excluded from the study and all pregnant and breast feeding women are excluded from GFR testing. The risk of electrocardiography and echocardiography are nil as they do not involve exposure to ionizing radiation. Participants are exposed to small amounts of radiation from an EBT exam. All pregnant and breast feeding women are excluded from EBT testing. There is a potential risk of loss of confidentiality with respect to study data and arising from the plans to store DNA and other biological specimens in a central NIH-based repository. Subjects are protected from these risks as described below.

4.C.2. Risk/Benefit Assessment

While there is no immediate direct benefit to the individual participants of this study, there is considerable potential benefit to future patients and to society as a whole if predisposing factors for progression of CRI and development of CVD are identified from this study. Identification of such factors would potentially lead to interventions that may reduce the burden of chronic renal failure. Finally, laboratory and radiographic data obtained as part of this study is made available to participants' treating physicians. Conceivably, this information, which is additional to that obtained in the course of usual care, may provide diagnostic insights to treating physicians that would benefit study participants directly (e.g. treatment of elevated cholesterol).

4.C.3. Gender and Minority Inclusion

This is a multi-center study drawing a clinical population from seven institutions across the United States. We estimate the race/ethnic composition of participants to be approximately 47.5% White/Caucasian, 47.5% being African American, 5% Latino/Hispanic, Asian/Pacific Islander and Other. We plan to enroll approximately equal numbers of men and women.

4.C.4. Informed Consent

Each clinical center will prepare an informed consent form following the guidelines of their local Institutional Review Board (IRB) and applicable regulations for Informed Consent. The informed consent form must be **signed** and **dated by the participant** prior to initiation of any study related activity and at a minimum, contain a description of the potential risks, benefits, expense to the participant, and alternative treatment. [See Appendix B].

Participants are asked to sign and date the informed consent form prior to the start of the screening visit protocol. This form provides consent for both the screening, testing and follow-up procedures. An additional consent form may be required for blood samples drawn and stored for genetic studies. Prior to signing the informed consent, the Research Coordinator will review the details of the consent form orally with the participant, and answer any questions that the participant has concerning participation in the study. The original signed consent form is stored in the participant study file at the clinical center, while a copy of the signed consent form is given to the participant. Specifically, the following must be accomplished during the informed consent process:

- The participant must be informed that participation in the study is **voluntary** and that refusal to participate will involve no penalty or loss of benefits or negative impact on their medical care.
- The participant must be informed of the **purpose** of the study and that it involves **research**.
- The participant must be informed of any **alternative procedures,** if applicable.
- The participant must be informed of any reasonably foreseeable **risks**.
- The participant must be informed of any **benefits** from the research.
- An outline of safeguards to protect participant **confidentiality** must be included, as well as an indication of the participant's right to withdraw without penalty. This should be balanced with a discussion of the effect withdrawals have on the study, and the responsibility a participant has, within limits, to continue in the study if he or she decides to enroll.
- The participant must be informed **whom to contact** for information about research subjects' rights, information about the research study, and in the event of research-related injury.
- The participant must be informed as to whether or not **compensation** is offered for participation in the study and/or in the event of a medical injury.
- The participant must be informed that he/she will be notified of any significant **changes** in the protocol that might affect their willingness to continue in the study.

4.C.5. Confidentiality

Protection of participants depends on the joint activities of all Clinical Centers as well as the SDCC. Extensive efforts will be made to ensure that participants' confidentiality is maintained. Each participant is assigned a unique study identification number and is never tracked through the study by name, social security number, medical record number, or other personal identifier. A log of the participant names, participant ID numbers, and pertinent registration information (e.g. home address, telephone number, and emergency contact information) is maintained in a locked area at each clinical site. The staff at the SDCC does not have access to this log. Only the participant ID number and initials are given to the SDCC staff and entered into the study database. Any communication between the SDCC and clinical sites regarding participant data occurs via the participant ID number. Any forms or documents sent to the SDCC, IRB or Regulatory Authorities will have all personal information removed.

Authorized representatives of the Sponsor, the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), National Institute of Health (NIH), participating clinical institution, as well as the IRB, have access to and may copy both medical records and records from participation in this study consistent with the policy of the NIH Certificate of Confidentiality. Such access is necessary to insure the accuracy of the findings and the safety and welfare of participants. If any publication or presentations result from this research, participants will not be identified by name or other personal identifier. All research reports, articles, and presentations will report only aggregate findings.

5. STUDY ORGANIZATION

5.A. Clinical Centers

The participating Clinical Centers will have primary responsibility for developing the study protocol, recruiting a sufficient number of study participants, maintaining high rates of follow-up and data collection, obtaining data of high quality, and interpreting, presenting, and publishing findings from the study.

- Johns Hopkins University
  Baltimore, MD
  Principal Investigator: Lawrence J. Appel, M.D., M.P.H.
- Case Western Reserve University, University Hospitals of Cleveland
  Cleveland, OH
  Principal Investigator: Mahboob Rahman, M.D. [A1]
- Kaiser Permanente of Northern California
  Oakland, CA
  Principal Investigator: Alan S. Go, M.D.
- Tulane University Health Science Center
  New Orleans, LA
  Principal Investigator: Jiang He, M.D., Ph.D.
- University of Illinois at Chicago
  Chicago, IL
  Principal Investigator: James P. Lash, M.D.
- University of Michigan at Ann Arbor
  Ann Arbor, MI
  Principal Investigator: Akinlolu O. Ojo, M.D., Ph.D.
- University of Pennsylvania Medical Center
  Philadelphia, PA
  Principal Investigator: Raymond R. Townsend, M.D.

5.B. Scientific & Data Coordinating Center (SDCC)

The Scientific Data Coordinating Center (SDCC) contributes content area expertise and shares in scientific leadership of the research network through the Steering and Planning Committee as it identifies and prioritizes specific areas of investigation and subsequently refines and modifies the CRIC Research Group's scientific direction and emerging scientific knowledge. The SDCC assists in protocol development and preparation of scientific publications. The SDCC has the major responsibility of creating a database and data collection systems for the Clinical Centers, ongoing evaluation of data quality and performance monitoring of the Clinical Centers, and statistical analyses of the data.

University of Pennsylvania School of Medicine
Center for Clinical Epidemiology and Biostatistics
Philadelphia, PA
Principal Investigator: Harold I. Feldman, M.D., M.S.C.E.
Co-Principal Investigator: J. Richard Landis, Ph.D.

5.C. Steering Committee

The primary governing body of the study is the Steering Committee, comprised of each of the Principal Investigators of the Clinical Centers and the Principal Investigators of the SDCC, and the NIDDK Project Scientist. Dr. Feldman from the University of Pennsylvania is the Chair of the Steering Committee. The Steering Committee develops policies for the study pertaining to access to patient data and specimens, ancillary studies, performance standards, and publications and presentations. They develop the study protocol and meet to discuss the progress of the study and to consider problems arising during its conduct. The Steering Committee may establish subcommittees on such topics as recruitment, measurement of renal function, risk factor assessment for renal disease and cardiovascular disease, cardiovascular studies, quality control, and publications and ancillary studies. Small working groups may be established to prepare manuscripts and presentations.

5.D. Study Subcommittees

The following subcommittees have been established to address specific study issues.

- Clinical Management Subcommittee
- Cardiovascular Measures Subcommittee
- Economic Subcommittee
- Genetic Studies Subcommittee
- Glomerular Filtration Rate (GFR) Subcommittee
- Informed Consent Subcommittee
- Nutrition Subcommittee
- Outcome Adjudication Subcommittee
- Primary and Ancillary Studies Subcommittee
- Renal Function Subcommittee
- Study Design/Forms Review Subcommittee
- Quality Control Committee
- Publication Committee

5.E. Scientific Advisory Committee

An independent group of experts in areas such as nephrology, cardiology, preventive medicine, epidemiology, nutrition, ethics, health economics, and biostatistics who are not otherwise involved in the study have been recruited by the NIDDK to evaluate the proposed protocol and periodically review the progress of the study.

5.F. Proposed Participating Laboratories

Central GFR Laboratory
Cleveland Clinic Foundation
Cleveland, OH
Principal Investigator: Phillip Hall, M.D.

Glomerular filtration rate (GFR) is measured at each Clinical Center by a trained technician. The Central GFR Laboratory at the Cleveland Clinic Foundation implements and coordinates the procedures for measurement of GFR, including GFR sample receipt and counting, GFR calculation,

and result reporting. The Clinical Centers perform the GFR test and ship the processed samples together with the GFR procedures form to the Central GFR Laboratory.

Centralized Biochemistry Laboratory
University of Pennsylvania
Philadelphia, PA
Principal Investigator: Daniel Rader, M.D.
Consultant: Paul Ridker, M.D.

The Central Biochemistry Laboratory (CBL) has the responsibility for processing and tracking all biological samples generated as part of the study and for collaborating with the SDCC to develop a Specimen Management and Tracking System for the Cohort Study.

5.G. Proposed Participating Imaging Centers

ECG
Wake Forest University
Winston-Salem, NC
Principal Investigator: Ronald J. Prineas, M.D., Ph.D.

Standard 12-lead electrocardiograms (ECG) are recorded, using GEMSIT MAC 1200 electrocardiographs in the clinics for electronic transmission of ECGs to the Central ECG Reading Center (CERC).

EBT
Los Angeles County Harbor-UCLA Medical Center
Torrence, CA
Principal Investigator: Mathew Jay Budoff, M.D., F.A.C.C.

Coronary calcium is assessed in relation to the risk of future cardiac events, and from repeated scans, the progression of coronary calcium is related to baseline risk factors and risk of future events.

Echocardiography
University of Pennsylvania
Philadelphia, PA
Principal Investigator: Martin St. John Sutton, M.B.B.S.

The initial echocardiography study establishes baseline characteristics, which can be correlated in prospective fashion to cardiovascular events. Interrelationships with other clinical and laboratory predictors can also be made. Ultimate comparison of follow-up echocardiographic data obtained allows determination of those parameters with significant change over time. Additionally, the relationship of the time course and magnitude of change to both the incidence of CV events and other clinical variables of interest is assessed.

5.H. CRIC Study Policies

5.H.1. Ancillary Study Policy

To enhance the value of the CRIC Study, the Steering Committee welcomes proposals from individual investigators to carry out ancillary studies. Nevertheless, to protect the integrity of the CRIC Study, such ancillary studies must be reviewed and approved by the Primary and Ancillary Studies Committee and the Steering Committee before their

inception or submission of a proposal for external funding consideration. The group has reviewed and approved a comprehensive study policy that clearly defines requirements and describes a process for review and approval of individual proposals. See Appendix C for a listing of the currently funded ancillary studies.

[A1] An ancillary study is one based on information from CRIC Study participants in an investigation or analysis which is relevant to, yet not described in the CRIC protocol, and derives support from non-CRIC study funds. A typical ancillary study will propose the collection of additional data not collected or analyzed as part of the routine CRIC Study data set. Ancillary studies may be submitted by investigators within the CRIC Study or investigators without a prior relationship to the CRIC Study. Ancillary studies require external funding. Examples include studies funded by investigator-initiated NIH research awards (R series awards, K series awards, and other career development awards) or grants from academic institutions or private sources (e.g. private foundations, pharmaceutical companies, etc.). Any ancillary study must have sufficient funding to cover the costs incurred by the CRIC Study Clinical Centers and Laboratories (e.g. to process or ship samples), and by the Scientific Data Coordinating Center.

5.H.2. Publication & Presentations

It is the policy of the CRIC Study that preparation of all publications or presentations, other than materials prepared for local publicity purposes, must be assigned by the Steering Committee to specifically appointed writing committees, and that all such materials must be reviewed and approved by the Committee before publication. A formal policy has been being developed.

5.H.3. Access to Study Data & Specimens

The Steering Committee will authorize access to study data and disposition of specimens. Investigators must submit a proposal requesting approval to access CRIC study data/specimens. A formal policy is being developed to direct this process. The CRIC Study will collaborate with the NIDDK to participate in their Central Repository for study specimens and data.

6. STUDY MANAGEMENT

6.A. Clinical Center Responsibilities

It is the responsibility of each clinical center to conduct the study according to the study protocol and applicable regulatory guidelines. Conduct of particular aspects of the study may be delegated to qualified personnel; however, it is the responsibility of each clinical center principal investigator to oversee the overall study management. The clinical center study staff must be trained in study procedures.

Each clinical center is responsible to screen, recruit, enroll and retain a designated number of study participants. It is the responsibility of the clinical center study staff to assess their accrual, ensure participant confidentiality, maintain appropriate study documentation, enter and transfer data in a timely manner, and participate in the CRIC study meetings and conference calls.

6.A.1. Institutional Review Board

It is the responsibility of the Principal and Co-Investigator(s) at each site to provide their Institutional Review Board (IRB) with all pertinent materials and consent documents. Approval of the protocol, the informed consent form, and data collection forms/questionnaires must be obtained and forwarded to the University of Pennsylvania, SDCC prior to screening or enrolling participants. The Investigator also maintains the responsibility of initiating protocol re-approval, notification of protocol and/or consent form changes and termination of the study according to IRB requirements. The SDCC monitors submission and annual renewal of these documents.

6.A.2. Record Retention

Investigators maintain, on-site, in an orderly fashion, for a proscribed period of time, and make available to the sponsor or the sponsor's representative, the following documents: the signed study protocol, amendments, informed consent documents, and approval letters from the IRB, CRFs, all primary source documentation, and all letters of correspondence. The SDCC maintains all study records for a period in accordance with their internal Standard Operating Procedures (SOP) and applicable regulations.

6.B. SDCC Responsibilities

The SDCC is responsible for the overall study management, document control and distribution, study communications, data management and analysis. The SDCC is responsible for establishing a database, developing a web-based data transmission system, assessing data quality and completeness throughout the study, and providing general assistance to the Clinical Centers to maintain long-term participation of the cohort study subjects. The SDCC also performs analyses as suggested by the Clinical Centers, Central Laboratories and Central Reading Centers, as well as proposes original analyses to the collaborative group for their consideration. The SDCC prepares periodic reports on the progress of the study, including data quality control, and interim and final results to the Steering Committee, the NIDDK and the group of external advisors. The SDCC is responsible for arranging meetings and conference calls of the Steering Committee, meetings of the external advisors, and performing other administrative functions necessary to coordinate the efficient operation of the collaborative study group. The SDCC has established, via subcontracts, Central Laboratories and Reading Centers, as deemed necessary by the study protocol. They also provide administrative coordination for the Central Repository to be

established and directly supported by the NIDDK, to store genetic material and other biological specimens obtained from cohort study participants.

6.B.1. Personnel Training

The SDCC will conduct a personnel training session prior to initiation of the protocol. This comprehensive training session will include all aspects of the protocol and Manual of Procedures (MOP) implementation such as staff-participant interaction, specimen handling, and data collection and entry procedures. Periodic conference calls and training sessions will be conducted to maintain standard application of procedures. All new personnel will be required to participate in a study training session.

6.B.2. Study Monitoring

The SDCC has developed written standard operating procedures (SOP) to ensure that all aspects of the study are conducted in a standard and uniform manner. These procedures are organized into a Manual of Procedures (MOP), which complies with the protocol, GCP and applicable regulatory requirements. A data monitoring plan and schedule will be developed to assess protocol adherence. This plan will be presented to the Scientific Advisory Committee for approval prior to implementation.

6.B.3. Quality Assurance

The Scientific and Data Coordinating Center (SDCC) will include a comprehensive Quality Assurance (QA) Plan in the Manual of Procedures that will consist of the following activities:

- **Personnel Training and Certification:** Prior to cohort study enrollment a comprehensive training session will be conducted with all study personnel that will encompass all aspects of the study including communication, principles of Good Clinical Practice, study implementation and procedures, data entry and verification, test and specimen conduct and transfer.
- **Clinical Protocol and MOP Adherence and Auditing Activities:** The SDCC will request and verify specific information from clinical and reading centers to ensure the application of study procedures as they apply to participant safety, required intervals for timely conduct of procedures, appropriate documentation of data and specimens and compliance with SOPs. This information will take the form of a written report and may be acquired during clinical site monitoring visits.
- **Site Monitoring:** Site monitoring may include a proportional assessment of information transfer from source documents (patient charts, lab reports, images) to Case Report Forms (CRF). A certain percentage of data will be reviewed to assess the accuracy of this process at each center. This information will take the form of a written report.
- **Database Auditing:** A comparison of a certain percentage of data written on CRFs to that entered into the electronic database provides information that describes and quantifies the accuracy of the data entry process and use of the data management system by personnel at each clinical center. This information will take the form of a written report.
- **Database Administration and Network Security:** The SDCC has Standard Operating Procedures established for authorizing and documenting secure access to the study website, documents and electronic Data Management

System (DMS). These procedures ensure that only authorized personnel are able to view, access and modify study data.

- **Data Reporting:** A set of standard reports will be developed to describe study activities that include accrual, study progress, and data quality. These reports will be developed using ORACLE REPORTS and provided to investigators, NIDDK and designated committees as appropriate.
- **Preparation and Integrity of Analysis Datasets:** The SDCC Database Administrator will create a set of standard data access descriptor/view files, which will be used in the generation of SAS analysis datasets. As datasets are extracted from the main study database, they can be utilized separately from direct database processing and thereby protect the integrity of the data.

6.B.4. CRIC Website Development & Maintenance

The SDCC will develop a study website for study-wide communication management, data and document management, and activity management and coordination. The CRIC website provides general information to the public, single-point restricted access to tools and information for investigators and clinical center study personnel including study resources, communication tools, as well as data entry and management tools. It provides an additional level of restricted access for SDCC study personnel.

6.C. Data Management

The SDCC provides overall coordination, logistical support, and implementation for all aspects of the study protocol including data collection, data processing, tracking of participant recruitment, tracking of specimens, training, quality assurance, and statistical analysis. The Clinical Research Computing Unit (CRCU), through its clinical data management, project management, and software systems develops, places into the field, and maintains a state-of-the-art world wide web-based data system that accommodates all scientific study data and permits tracking and coordination of all CRIC network activities within the framework of multidisciplinary project teams. The CRCU Technical Director has overall responsibility to establish the technical strategies and monitor the implementation activities within the SDCC and at the seven participating clinical centers. The Biostatistics Analytical Center (BAC) provides all statistical programming and data management needs in the course of analyzing study data.

6.C.1. Electronic Data Management Systems (DMS)

The CRIC study DMS has been developed as sets of applications using Oracle forms, which resides upon the Oracle Relational Data Base Management System (RDBMS.) The DMS has been developed and maintained by trained and experienced Oracle developers and certified Oracle Database Administrators (DBA) permanently employed by the University of Pennsylvania. Project specific standard operating procedures (SOP) have been developed by the SDCC to ensure that DMSs are utilized by project personnel in a consistent manner. A distributed DMS is utilized for data submission by the CRIC study. Emphasis is placed upon utilizing to the greatest degree possible a 'paperless data collection method.' When possible, data collected by the Clinical Center Coordinator is entered directly from the source document or participant to a data entry screen, bypassing the intermediate step of transcribing the data to a CRF.

6.C.2. Security

Access to the CRIC DMS is limited to users authorized to perform data entry functions by the SDCC. Security of the computing environment is administered by assignment of a unique username and password.

6.C.3. Standard Reports

In order to evaluate enrollment, the SDCC will generate monthly reports that describe overall study accrual by site and demographic characteristics of the population.

On a quarterly basis, the SDCC will generate reports that represent the study data collected as well as assessment of that data. The following list represents data and quality reports:

- Study accrual: overall, by site, compared to target
- Demographic characteristics of the population
- Participants screened versus enrolled
- Reasons for screening failure
- Enrollment and withdrawal reports
- Contact completion rates [Follow-up visits expected v. completed]
- Timely data entry reports

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APPENDIX A—VISIT SCHEDULE REVISED 04/01/2006

CRIC Visit Schedule	PRE-SCREEN	SCREEN-ING	BASE-LINE	6 MOS.	12 MOS.	18 MOS.	24 MOS.	30 MOS.	36 MOS.	42 MOS.	48 MOS.	54 MOS.	60 MOS.
Type of Contact	PHONE [V1]	VISIT [V2]	VISIT [V3]	PHONE [V4]	VISIT [V5]	PHONE [V6]	VISIT [V7]	PHONE [V8]	VISIT [V9]	PHONE [V10]	VISIT [V11]	PHONE [V12]	VISIT [V13]
Eligibility Assessment	X												
Informed Consent		X											
Medical Record Consent		X			X		X		X		X		X
Contact Information		X		X	X	X	X	X	X	X	X	X	X
Labs: Serum Creatinine, Serum Glucose		X											
Demographic Information		X											
Eligibility Confirmation		X	X										
Medical History [CV, Renal, Health Behaviors]			X		X		X		X		X		X
Genetic Blood Sample			X		X		X		X		X		X
Labs: CBC, Metabolic Panel, Lipids[†]			X		X		X		X		X		X
Urinary Assay: 24 Hour Urine		X	X		X		X		X		X		X
Urine sample collection [A2]			X		X		X		X		X		X
Blood Pressure		X	X		X		X		X		X		X
Ankle Brachial Index & Anthropometric Measures			X		X		X		X		X		X
Bioelectrical Impedance Assessment [BIA] [A4]			X		X		X		X		X		X
Nail Clippings			X		X		X		X		X		X
ECG			X		X		X		X		X		X
Echocardiogram[‡]					X						X		
EBT or MSCT (1/3 Subcohort Participants)					X						X		
I-GFR (1/3 Subcohort Participants)			X				X				X		
Pulse Wave Velocity Measure [alternating annual visits] [A2]			X				X				X		
Physical Activity Assessment			X				X				X		
Concomitant Medications			X	X	X	X	X	X	X	X	X	X	X
MDRD Symptom Index			X		X		X		X		X		X
KDQOL - Quality of Life Questionnaire			X		X		X		X		X		X
Diet History Questionnaire			X				X				X		
Beck Depression Inventory			X				X				X		
Cognitive Function Testing [Mini Mental Status Exam]			X				X				X		
Cardiomyopathy Questionnaire – KCQ [A2]					X		X		X		X		X
Recent Medical History – Event Information			X	X	X	X	X	X	X	X	X	X	X

[†] hs-CRP will be measured when a patient develops observed or projected eGFR < 20 ml/min/1.73m^2 **[A3 and A4]**
[‡] Additional Echocardiogram may be performed if observed or projected eGFR < 20 ml/min/1.73m^2 **[A3 and A4]**

APPENDIX B—INFORMED CONSENT TEMPLATE

An informed consent template is attached to this protocol. It includes separate signature pages for comprehensive study consent and genetic sampling. It is anticipated that each center will customize the informed consent document to represent the specific investigator contact information, compensation to participants and other center-specific information. Institutional Review Boards may also request content changes to the informed consent which reflect their policies, however, the essential consent elements should not be altered.

APPENDIX C—CRIC ANCILLARY STUDY TABLE (REVISED) [A3]

Ancillary Study Proposals - Approved and Funded			
INVESTIGATOR AND INSTITUTION		**TOPIC**	**TYPE**
	Cheryl A. M. Anderson, PhD, MPH University of Pennsylvania	Nutrition Assessment In Chronic Renal Insufficiency Cohort (CRIC) Study	Minority Supplement to SDCC CRIC Study Award
	Andrew S. Levey, M.D. Tufts University School of Medicine	Development and Validation of GFR Prediction Equations for Clinical	RO1
	Julio C. Vijil, Jr., M.D. University of Illinois at Chicago	Training/Career Application: Hispanic Recruitment in the CRIC Study	Minority Supplement to SDCC CRIC Study Award
	Michael Shlipak, M.D. University of California, San Francisco	Heart Failure Detection and Progression in Kidney Disease	RO1
	Sylvia Rosas, M.D. University of Pennsylvania	Novel Cardiovascular Risk Factors in Patients with Renal Insufficiency	R21
	Raymond Townsend, M.D. University of Pennsylvania	Pulse Wave Velocity in Chronic Kidney Disease	RO1
	Mary Leonard, M.D., MSCE University of Pennsylvania	Structural effects of renal bone disease in CRI	RO1
	Bruce M. Robinson, M.D. University of Pennsylvania	Associations of Insulin Resistance with Atherosclerotic Cardiovascular Disease (ASCVD) and with Progression of Chronic Renal	K23
	James P. Lash, MD University of Illinois at Chicago	HCRIC- Hispanic Chronic Renal Insufficiency Study	RO1
	Eve Van Cauter, PhD University of Illinois at Chicago	Sleep Disturbances as a Non-traditional Risk Factor in CKD	RO1
	Kristine Yaffe, MD University of California, San Francisco	Cognitive Function in Chronic Renal Insufficency	RO1
	Juan E. Grunwald, MD, University of Pennsylvania.	Diabetic and Hypertensive Retinopathy and Chronic Renal	RO1
	Muredach Reilly MB University of Pennsylvania	Genetic Epidemiology of Sub-clinical Atherosclerosis in Chronic Renal	RO1
	Chi-yuan Hsu, MD, MSc University of California	CRIC Plus- Study of advanced chronic renal insufficiency	RO1

APPENDIX D—TABLE OF CLINICAL ALERT VALUES AND CRIC RESPONSE TIME

FINDING	ALERT VALUE	OPTIMAL RESPONSE TIME
Systolic BP > 180 or Diastolic > 110	Urgent or Immediate*	NA
"Acute Distress" (includes chest pain or other signs or symptoms constituting an emergency)	Immediate	NA
Potassium ≥ 6 mEq/L or ≤ 3.0 mEq/L	Urgent	Within 24 hours of receipt of report+
Sodium <125 mEq/L or >155 mEq/L	Urgent	Within 24 hours of receipt of report+
Total Bicarbonate <15 mEq/L or > 40 mEq/L	Urgent	Within 24 hours of receipt of report+
Calcium <6.5 or >13.5 mg/dL	Urgent	Within 24 hours of receipt of report+
Glucose < 50 mgdL or > 350 mg/dL	Urgent	Within 24 hours of receipt of report+
Creatinine doubling from last value	Urgent	Within 24 hours of receipt of report+
CBC Hb < 7 gm/dL	Urgent	Within 24 hours of receipt of report+
ECG: Acute MI Heart Rate < 45 or >120 Ventricular Tachycardia Atrial Fibrillation Atrial Flutter Mobitz Type II 2nd degree heart block 3rd degree heart block Complete left bundle branch block [A1]	Urgent	Local ECG review within 72 hours^ [A2]
ECHO abnormalities identified by technician and/or reading center: Severe aortic stenosis Aortic dissection Vegetation Tumor Cardiac tamponade LV thrombosis	Immediate	Local review by technician within 24 hours/central reading 8 weeks

*as assessed by clinical evaluation

+ Receipt of report at originating clinical center

^ Local review for the presence of urgent ECG alerts has been increased from 24 to 72 hours.

APPENDIX E—COLLECTION OF DATA IN RELATION TO DEATH, AND SPECIFIC CARDIAC, RENAL, AND OTHER CLINICAL OUTCOME EVENTS

The detection and verification of specific diagnoses related to the discovery of intercurrent illnesses and major medical events that occur to CRIC participants over the course of their involvement in the cohort study will be guided by a specific set of policies and procedures. They are modeled on successful tracking and documentation of incident events occurring between study visits and phone contacts in related studies such as ARIC and MESA. Information will be acquired from participants during annual clinic visits and telephone communication. This initial report from participants will be documented via standard cohort follow-up procedures on Case Report Forms and revised based on medical record review at regular intervals.

The process of event confirmation will be labor intensive and require the oversight of study personnel experienced in methods of medical record abstraction. It will entail the following elements:

- Initial notification of event investigation
- Obtaining access to hospital medical records
- Acquiring hospital discharge index information, if available
- Abstraction of medical records
- Confirmation of diagnoses from primary care physician, if necessary
- Identification and acquisition of death certificates
- Investigation of deaths occurring outside of the hospital setting
- Creating a summary of event investigation information
- Review by Outcome Adjudication Subcommittee
- Event determination by Committee
- Compilation of verified events into standard report

The following list defines the medical event information that will be acquired, confirmed and categorized:

I. RENAL OUTCOME MEASURES

A. Primary outcome: The slope of GFR is the primary outcome.

B. Secondary outcomes:

1. Onset of ESRD (start of chronic dialysis or renal transplantation) or development of GFR < 15 ml/min/1.73m^2. This will be time-to-event analysis. Key here is careful consideration of GFR at entry into cohort study by stratification or multivariate analysis.
2. "Significant loss of renal function" defined as 50% decline or 25 ml/min/1.73 m^2 decline in GFR from baseline. This will also be a time-to-event analysis that need to take into account baseline GFR.
3. Composite clinical outcome defined by the occurrence of either 50% decline, or 25 l/min/1.73 m2 decline in GFR from baseline, or onset of ESRD (start of chronic dialysis or renal transplantation).
4. Slope of change in proteinuria over time as assessed by spot urine protein/urine creatinine ratio (UP/Cr). We will also assess for development of new microalbuminuria (20-200 mg albumin/gm creatinine), new overt proteinuria -UP/Cr > 0.22 (or > 300 mg/d of proteinuria) or new significant proteinuria UP/Cr > .66 (or >1 gm/d of proteinuria).

II. CARDIOVASCULAR OUTCOME MEASURES

Cardiac Death:

1. Sudden Death (SD): Sudden loss of consciousness leading to unexpected death within one hour of onset in a previously stable patient. Includes patients who were comatose and then died after attempted resuscitation.
2. Post Resuscitation: Death from complications post arrest in patients with intervening consciousness.
3. Definite Myocardial Infarction (MI): Death which occurs more than 60 minutes from the onset of symptoms, occurs during or before the hospitalization for the MI and is related to a cardiac complication (e.g. CHF, arrhythmia, shock) or non-cardiac complication (e.g. pulmonary embolus) of the acute event. MI is documented by pathologic findings or by two of the following three criteria: clinical, electrocardiographic and biomarkers of cardiac necrosis (CPK-MB and/or troponin). If the patient has a documented MI then dies "suddenly" while making an otherwise normal recovery the cause of death will be classified as "Definite MI".
4. Possible Myocardial Infarction: Typical clinical setting with chest pain or other findings suggestive of Acute MI in the absence of diagnostic biomarker or ECG changes.
5. Congestive Heart Failure (intractable HF): Death from intractable congestive heart failure not associated with an acute event.
6. Procedural Death: Death during or prior to discharge which directly resulted from multi-system organ failure due to cardiogenic shock. Patients who are taken to surgery as a heroic lifesaving measure may not be classified as a surgical death according to the opinion of the reviewers.
7. Primary Intractable Serious Arrhythmia: Must be documented arrhythmia witnessed on a monitor.
8. Other Cardiovascular: Death in which there is evidence of a primary cardiac etiology, which can not be classified as Definite MI, Congestive Heart Failure, Sudden Death, etc. (e.g. peripheral arterial related death, endocarditis).

Non-Cardiac Death:

Death in which there is no evidence of a primary cardiac etiology as noted above.

1. Procedural Death: Death during or prior to discharge from surgery NOT directly resulted from primary cardiogenic shock. Patients who are taken to surgery as a heroic lifesaving measure may not be classified as a surgical death according to the opinion of the reviewers.
2. Hemorrhagic death due to hemodynamic collapse secondary to blood loss.
3. Sepsis: Infection-related death secondary to multi-system organ failure following sepsis.
4. Cerebrovascular: Death secondary to intractable cerebral anoxia or cerebrovascular accident (ischemic stroke or intracranial hemorrhage).
5. Primary Respiratory failure: Death due to primary respiratory failure in the absence of infection.
6. Pulmonary Embolus
7. Non-Cardiac Death Other (specify)

Unknown Death: Patients out of human contact for 24 hours in which circumstances of death were unknown.

Myocardial Infarction:

Myocardial Infarction (MI) is defined as: Typical rise and gradual fall (troponin) or typical rise and rapid fall (CPK-MB) of cardiac enzymes to >2xupper limit of the normal range (ULN) in combination with one of the following:

- Symptoms of myocardial ischemia
- Other clinical manifestations of myocardial ischemia (e.g. CHF or new ventricular tachyarrhythmias)
- ECG changes compatible with ischemia or infarction (ST depression or elevation or new or presumed new Q waves or new or presumed new LBBB) (189)

"Aborted" myocardial infarction in the absence of enzyme elevation: Typical ischemic symptoms with ST elevation > 1mm in ≥2 contiguous ECG leads of >30 minutes duration successfully treated with thrombolytics or percutaneous coronary intervention (PCI) within three hours of onset of symptoms. (190-192), (193-195)

Cardiac testing evidence of new infarction to include any of the following:

- New fixed perfusion abnormality on nuclear perfusion imaging as compared to prior examination with corroborating wall motion abnormality
- New akinesis or dyskinesis of a myocardial region on functional evaluation (echocardiography or MUGA) as compared to prior exam.
- Newly occluded coronary artery on coronary angiography in comparison to prior coronary angiogram may be considered as supporting evidence but by itself does not necessarily constitute definitive evidence of a myocardial infarction.
- New pathologic Q waves in ≥ 2 contiguous leads on ECG as compared to prior ECG.

Because of differences in the natural history and prognosis, peri-or postprocedural MI's (definitions below) will be recorded separately from "native" MI's.

Following PCI one of the following criteria must be met:

- Typical rise and gradual fall (troponin) or typical rise and rapid fall (CPK-MB) of cardiac enzymes to > 2X ULN.
- New pathologic Q waves in ≥ 2 contiguous leads.

Following CABG, one of the following criteria must be met:

- Typical rise and gradual fall (troponin) or typical rise and rapid fall (CPK-MB) of cardiac enzymes to > 5X ULN.
- New pathologic Q waves in ≥ 2 contiguous leads.

Acute Coronary Syndrome:

Hospitalization for ischemic chest pain or equivalent ischemic symptom occurring at rest or with minimal exertion, lasting for >5 minutes and associated with any of the following:

- ST segment depression ≥ 1 mm in two or more contiguous leads
- Symmetric t-wave inversion >1.5 mm amplitude in ≥2 contiguous leads.

Evidence of noncardiac causes of chest pain and/or angiographic demonstration of absence of significant CAD (>50% obstruction of at least one artery or branch) and/or noninvasive testing demonstrating lack of ischemia will serve to exclude the diagnosis of ACS.

Hospitalization for Congestive Heart Failure

Hospital admission (including prolonged "observation unit" admissions of >24 hours) for new or worsening congestive heart failure will be defined as the presence of a syndrome characterized clinically by breathlessness, pulmonary congestion, effort intolerance, fluid retention and peripheral hypoperfusion. These clinical signs and symptoms must represent a clear abrupt change from the normal clinical state of the patient (i.e., baseline status at screening or preinfusion). Episodes of hospitalized heart failure will be adjudicated as to whether the CHF is characterized principally as heart failure with reduced systolic function (based on proximate assessment of LV systolic function), heart failure with preserved systolic function (based on demonstration of intact LV systolic function), or unclassified.

During the index hospital stay, the above symptoms and signs must be accompanied by failing cardiac output as determined by peripheral hypoperfusion (in the absence of clear cut underlying sepsis or hypovolemia) or peripheral or pulmonary edema which requires intravenous therapy (diuretics, inotropes, or vasodilators). Supportive documentation of reduced cardiac index, rising pulmonary capillary wedge pressures, falling oxygen saturation and end organ hypoperfusion, if available, will be assessed. These criteria are consistent with the Framingham Heart Study clinical criteria for heart failure used in other epidemiological cohort studies.

Pulmonary Edema

In order to standardize criteria used to deem an adverse event as pulmonary edema, the following four conditions must be met:

1. Abrupt change (clear departure for clinical state that is norm for the subject)
2. Presence of respiratory distress (tachypnea ≥ 24, hypoxia, diaphoreses)
3. Evidence of pulmonary edema (rales ≥ 1/3 bilaterally; alveolar or interstitial infiltrates on CXR that are clear departure from baseline)
4. IV diuretic therapy

Serious Cardiac Arrhythmia

Serious cardiac arrhythmias are defined as the presence of a sustained cardiac rhythm disturbance as noted below. It will be attempted to determine if the arrhythmia is primary or secondary.

Examples include:

- Ventricular Tachycardia
- Torsade de Pointes
- Ventricular Fibrillation
- AICD discharge (must state the underlying initiating rhythm)
- Symptomatic Bradycardia
- Complete Heart Block
- Atrial Fibrillation/Flutter
- Supraventricular Tachycardia

Cerebrovascular Endpoints

Ischemic stroke is defined as a fixed (>24 hours) neurologic deficit not explained by another etiology (i.e., primary hemorrhage, trauma, infection, vasculitis, etc.). Confirmatory imaging studies are not essential to the clinical diagnosis of a stroke. Further description will be based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) and the CARE Study Classification of Subtypes of Acute Ischemic Stroke:

1. Large artery atherosclerosis (embolism/thrombosis)*
2. Cardioembolism (high-risk/medium-risk)*
3. Small-vessel occlusion (lacunae)*
4. Stroke of other determined etiology*
5. Stroke of undetermined etiology
    a.) Two or more causes identified
    b.) Negative evaluation
    c.) Incomplete evaluation

*Possible or probable depending on results of ancillary studies.

Cerebrovascular revascularization procedures will include surgery or percutaneous interventions in the cerebrovascular circulation.

Intracranial hemorrhage is defined as a fixed (>24 hours) neurologic deficit due to a primary intracranial hemorrhage (i.e., intracerebral, subarachnoid, subdural hematoma) that is confirmed by neuroimaging (CT or MRI) or pathology.

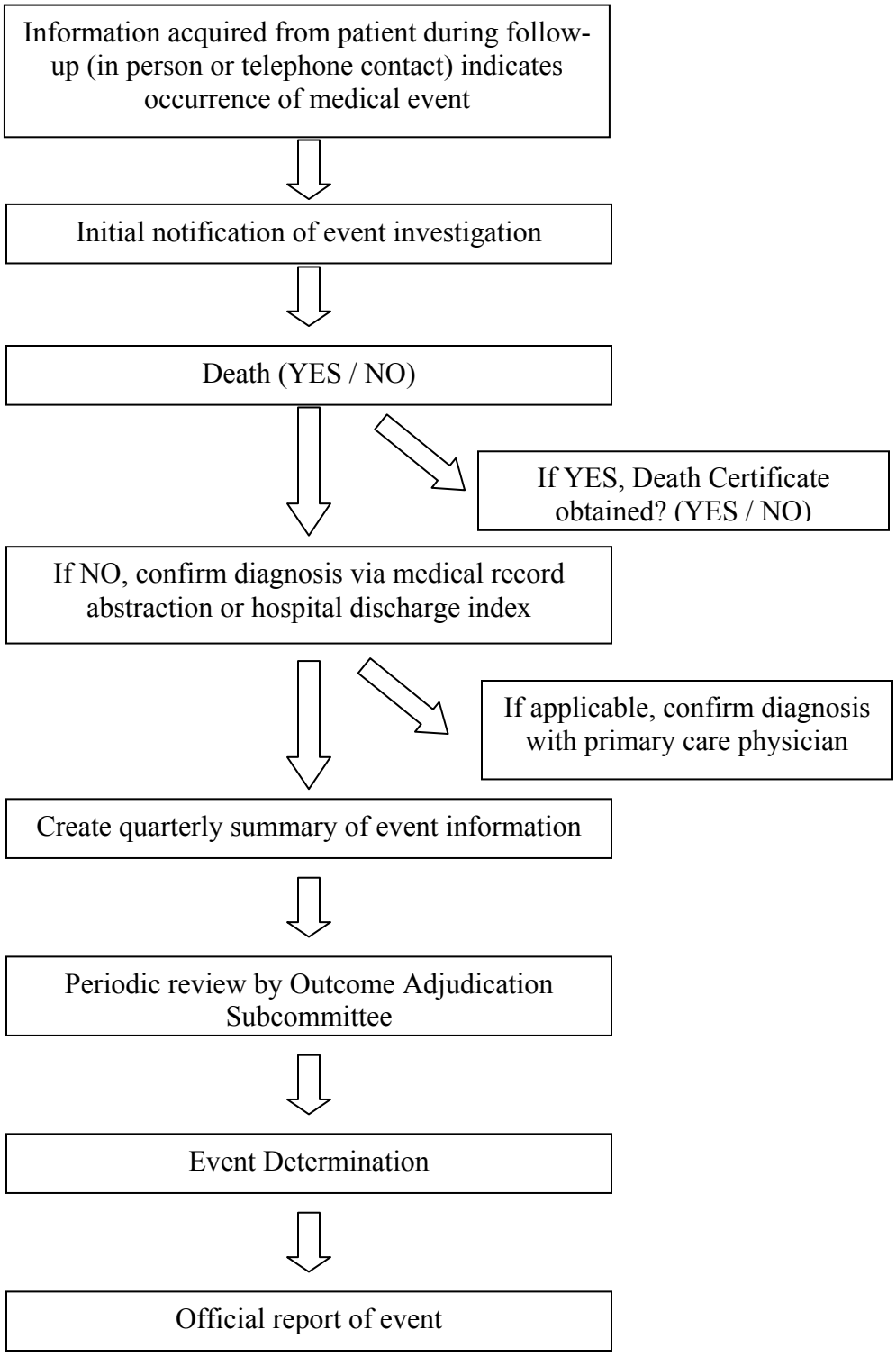
Peripheral Vascular Disease

Peripheral vascular endpoints will include:

- Amputation due to vascular disease
- Peripheral surgical or percutaneous revascularization.

Event Determination: Specific Case Report Forms and procedures will be described in the study Manual of Procedures. The following diagram represents a brief outline of the process.

EVENT INVESTIGATION PROCESS OUTLINE



APPENDIX F—CRIC Core and Potential Biochemical Measures

Core Serum Measures	Proposed Serum Measures [Pending Ancillary Funding]
Metabolic panel including: • Albumin • Bicarbonate • Total Bilirubin • Calcium • Carbon Dioxide • Chloride • Creatinine • Glucose • Alkaline Phosphatase • Potassium • Total Protein • Sodium • Aspartate Aminotrasnferase (AST) • Alanine Aminotransferase (ALT) • Total Cholesterol • Triglycerides • Urea Nitrogen CBC including: • Hemoglobin • Hematocit • WBC (with differential) • Platelet Count • MCV • MCH • MCHC In addition: • Magnesium • Phosphorus • Cystatin C • HbA1C • Homocysteine • Troponin I • iPTH • Fibrinogen • Uric Acid	• Advanced Glycation Endproducts (AGE) • Lipoprotein (a) • Apolipoproteins • LDL Cholesterol • HDL Cholesterol • Inflammatory marker CRP/hs-CRP • Inflammatory marker sICAM • Vitamins A, B6, B12, C, E • Folate • Zinc • Transferrin • Insulin • Insulin-like Growth Factor-1 • Carotenoids • Total Body Nitrogen • Plasminogen Activator Inhibitor (PAI-1) • TGF – Alpha and Beta • TNF – Alpha • Asymmetric Dimethyl-arginine (ADMA) • Procollagen - 1 • Iron and Iron-binding Capacity • Ferritin Concentration • Renin • Aldosterone • Prealbumin **Proposed Measure [Pending Ancillary Funding]** • Heavy Metal Accumulation [from nail clipping]
Core Urinary Measures	**Proposed Urinary Measures [Pending Ancillary Funding]**
• Creatinine • Protein • Albumin • Urea nitrogen	• Urinary Isoprostanes • Sodium • Potassium

APPENDIX G—ANCILLARY STUDY SUMMARIES [A3]

1. Retinopathy in Chronic Renal Insufficiency (RCRIC) Cohort Study

Since many of the CRIC Study participants are at high risk of developing significant retinopathy due to diabetes mellitus, systemic hypertension and other vascular diseases, it is important to assess the ocular condition. In addition, because both diabetic and hypertensive retinopathy have been shown to be associated with CRI and CVD, identification of these abnormalities in CRIC participants may improve predictive models aimed at identifying high risk subgroups with CRI.

The RCRIC research project will be an ancillary study to the CRIC study in which a single set of fundus photographs is obtained on CRIC participants either during their baseline visit or an annual visit marking 1, 2, or 3 years of participation in the CRIC Study. This spread over the years of participation in the CRIC study is due to the staggered recruitment of the CRIC study.

The RCRIC project will recruit 2200 volunteers from the 3000 subjects participating in the CRIC Study across the United States. Recruitment is planned to start in March 2006 and end in February 2007. Ages of the participants will be between 21 and 76 years at the time of enrollment. All participants will have some degree of renal insufficiency, half of the participants will have Diabetes Mellitus, and about 75% will have systemic hypertension.

The RCRIC is a multicenter study that will be carried out in six Clinical Centers: 1) University of Pennsylvania Medical Center, Philadelphia, PA. 2) University of Maryland, Baltimore, MD. 3) University Hospitals of Cleveland, Cleveland, OH. 4) University of Michigan, Ann Arbor, MI. 5) University of Illinois, Chicago, IL. 6) Kaiser Permanente of California, Oakland, CA. It will be the responsibility of each clinical center to conduct the study according to the study protocol and applicable regulatory guidelines which will include the approval of this project by the IRB of each of the institutions involved. The Fundus Reading Center of the RCRIC study will be located in the Department of Ophthalmology at the University of Pennsylvania, Philadelphia, PA.

In all CRIC Study participants who agree to take part in our Retinopathy in CRI ancillary study, one set of fundus photography will be performed in both eyes with a non-mydriatic fundus camera that does not require pupillary dilatation.

A CRIC coordinator will present the purposes and detailed protocol of the ancillary study to all CRIC participants. Following signature of the consent form, the CRIC coordinator will obtain fundus photographs of the disk and macula of both eyes. Dilatation of the pupils is not necessary. Fundus photography will be obtained in a darkened room. To induce a natural dilatation of the pupil, a period of dark adaptation of about five minutes will precede photography. Time required to complete the photography will be about ten minutes.

A letter with information about the findings observed in the fundus photographs will be sent to the patients. Patients that have fundus findings that require treatment will be advised to seek a complete eye examination by their Ophthalmologist of choice. This ancillary study will pose minimal risks to participants.

2. Cognitive Function in Chronic Renal Insufficiency

Cognitive impairment is common in patients with end-stage renal disease (ESRD) although the etiology remains unclear. Both dialysis and renal transplantation appear to reverse these deficits. Correction of anemia with erythropoietin has been shown to improve cognitive function in

anemic persons with ESRD. However, these interventions only partially correct the cognitive deficits associated with ESRD. Furthermore, the role of co-morbid conditions associated with both cognitive impairment and renal failure has not been well studied.

This study proposes to administer a battery of cognitive tests to participants enrolled in 3 of the 7 CRIC sites. These tests would be administered at the first possible annual CRIC clinic visit, or if necessary, at a separate study visit conducted close to the annual CRIC visit, and repeated at each annual visit thereafter. Given that age is the biggest risk factor for both cognitive impairment and for much comorbidity, cognitive testing will be obtained only on CRIC participants over age 55 years.

The choice of cognitive tests is driven by scientific goals, feasibility, ease of training and administration, prior use in CRI patients, and minimizing the burden to CRIC participants and CRIC staff. The current proposed battery of 5 tests would take approximately 40 minutes to administer.

- **Trails A and B (5 minutes):** measure visuospatial scanning, sequential processing, motor speed, executive function, and attention.
- **Category Fluency (2 minutes):** measures verbal production, semantic memory, and language with higher scores indicating better performance.
- **Modified Mini Mental Status Exam (3MS) (10-15 minutes):** a brief, general cognitive battery with components for orientation, concentration, language, praxis, and immediate and delayed memory. Of note, this is not the same as the MMSE but provides complementary information and is more sensitive than the traditional MMSE. The 3MS is increasingly becoming a widely used cognitive test in epidemiological studies of aging. Using the 3MS in addition to the already collected MMSE is slightly redundant but provides expanded cognitive domains and would allow for inter-conversion with the shorter MMSE.
- **Buschke Selective Reminding Test (10-15 minutes):** The Buschke is a well-established validated test of verbal memory with immediate and delayed components.
- **Boston Naming (5 minutes):** a brief test of naming and language.

CRIC participants will be approached by the CRIC Study coordinator and will be asked to participate in the cognitive function ancillary study. Following signature of the appropriate consent form the tests will be administered by the CRIC coordinator at the time of the regular CRIC visit.

Participation in this study poses no additional risks.

3. Sleep Disturbances as a Non-Traditional Risk Factor for CKD

Sleep disturbances and poor quality sleep are common in patients with chronic kidney disease. Among patients with chronic renal failure, the prevelance of sleep apnea syndrome is estimated to be between 30-80%, which is much higher than the 2-4% prevalence in the general population. Additionally, approximately 80% of dialysis patients experience symptoms of restless legs syndrome. Thus, CKD patients may be at a greater risk of developing co-morbidities, such as cardiovascular disease and stroke that are known to be associated with impaired sleep. Furthermore, higher quality of life was significantly associated with lower daytime sleepiness in patients on hemodialysis.

There is also evidence to suggest that sleep disturbances will have an adverse effect on kidney function. Indeed, the hormones of the renin-angiotensin-aldosterone system exhibit large diurnal variations that are dependent on sleep.

Both plasma renin activity (PRA) and aldosterone levels are markedly elevated during sleep. The nocturnal increase in PRA and aldosterone levels is markedly blunted by acute total sleep deprivation and in conditions of abnormal sleep architecture. This blunting of the sleep-related increase in renin and aldosterone could play a role in the pathophysiology of chronic kidney disease.

The present study therefore seeks to explore the role of decreased sleep duration and/or quality as a risk factor for the progression of chronic renal insufficiency and the development of cardiovascular disease in CKD.

CRIC Study participants will be asked to sign the appropriate section of the informed consent document to indicate their willingness to participate in this ancillary study. The goal is to study a total sample size of 800. Collection of data will involve the distribution by mail of a well-validated wrist activity monitor (Actiwatch, Mini-Mitter Co. Inc. Bend, OR), and of validated questionnaires assessing sleep duration, sleep quality and depression (Karolinska Sleep Log, Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, Berlin and Center for Epidemiologic Studies Depression questionnaires).

Patients will wear an Actiwatch on their wrist for 5 days continuously to record sleep. They will wear a second Actiwatch on one leg at night only to screen for periodic leg movements and obtain a measure of frequency and amplitude of leg movements.

Finally, they will complete the sleep log and questionnaires, and mail all instruments back to investigators. Two separate sleep assessments will be performed in each patient two years apart. Additionally we will request that insulin and C-reactive protein (CRP) be measured on a small aliquot (2 ml) of existing blood from the blood draw performed upon enrollment as well as on the blood drawn during the last year of the CRIC study period. This will not require an additional blood draw.

Participation in this study poses no additional risks.

4. Genetics of Atherosclerosis in Chronic Kidney Disease

Atherosclerotic cardiovascular disease (CVD) is the major cause of death in patients with end-stage renal disease (ESRD). The incidence and prevalence of ESRD in the United States continues to rise and is directly related to a much larger and expanding population of patients with less severe chronic renal insufficiency (CRI). The risk of atherosclerotic CVD also appears to be increased in CRI compared to the general population. This may be due in part a higher prevalence of established atherosclerotic CVD risk factors in addition to unidentified factors that are likely to have a strong genetic basis. The genetic basis of atherosclerotic CVD has been established through family based heritability and linkage studies and candidate gene-association studies in multiple populatiuons.

The **metabolic syndrome (**METSYN**),** a clustering of cardiovascular risk factors characterized by central obesity, insulin resistance, dyslipidemia and a pro-inflammatory state, is increasingly prevalent in our society. The prevalence of MS is increased in CRI. In fact, insulin resistance, raised triglycerides and low HDL are well recognized features of both CRI and ESRD and are

believed to contribute to the progression of renal disease in addition to atherosclerotic CVD events. Low grade activation of innate immune **inflammation**, a feature of the METSYN, is common a finding in CRI and ESRD. Epidemiologic and basic science studies suggest that activation of innate immunity is a proximal event in the METSYN and is likely to contribute to its complications including diabetes, atherosclerotic CVD and possibly CRI. The mechanism of innate immunity activation in the METSYN is unknown but appears to have a strong genetic basis and to be directly influenced by the amount of central adipose tissue, a rich source of inflammatory adipocytokines. Thus, the METSYN and innate inflammation are major, non-traditional, candidate pathways for the promotion of atherosclerotic CVD in CRI and genes in these pathways may represent novel risk factors and therapeutic targets for CVD in CRI.

This study will will examine multiple CVD traits that provide mechanistic insight into our primary analyses of clinical CVD. Thus, we will determine the relationship of tagSNPs and estimated haplotypes in innate immune (Aim 1) and insulin resistance (Aim 2) genes (N=52) to (a) inflammatory and insulin resistance biomarkers, (b) coronary artery calcification (CAC), a direct measure of atherosclerosis, and (c) the risk of CVD. Further, we utilize re-sequencing data, generated by NIH-sponsored programs, to select tagSNPs in candidate genes for genotyping in the full cohort.

We propose to do additional EBT scans at 4 clinical centers (University of Pennsylvania, University of Michigan, Tulane University and Kaiser/Permanente). At these sites, we will invite all eligible subjects to undergo EBT scanning at their next or most convenient visit. Participants who are over the weight limit for the EBT scanner (>300lbs) or who have had procedures which interfere with the ability to read the EBT results (angioplasty with stent placement or coronary artery bypass surgery) will not be invited to have an EBT scan. The EBT scans will be read at the central reading center for the study and results will be provided to the participant and their physician.

For all CRIC subjects who have consented to genetic testing, DNA will be isolated from samples acquired during annual visits. High throughput genotyping will be performed for SNPs in genes related to inflammation and insulin resistance.

Additional time for EBT testing would be the main additional burden to both Clinical Center staff and their participants. The amount of time will depend upon the site and the waiting time at the EBT scanning facility. The total time of the scan is only about 15 minutes but this may have to be performed at a separate visit.

For all CRIC subjects who have consented to genetic testing, SNPs in genes related to inflammation. These samples are already collected and therefore this portion of the study does not involve any additional burden to participants.