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Clinical Investigation of Pharmaceutical and Lifestyle Interventions in Persons with Chronic Kidney Disease

By

ARMIN AHMADI
DISSERTAION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Clinical investigation of pharmaceutical and lifestyle interventions in persons with chronic kidney disease

Chronic kidney disease (CKD) leading to kidney failure and end-stage renal disease (ESRD) is a serious medical condition associated with increased morbidity, mortality, and in particular cardiovascular disease (CVD) risk. CKD is characterized by a range of complex deleterious physiological and metabolic function alterations including disruptions in lipid, amino acid, and carbohydrate metabolism; skeletal muscle dysfunction, insulin resistance, and heightened inflammation/oxidative stress. In addition, concurrent comorbidities such as diabetes, hypertension, and dyslipidemia further exacerbate cardiometabolic risk and worsen patient outcomes. Emerging evidence suggest that dysfunctional mitochondria have a pivotal role in both the development of CKD and its associated comorbidities highlighting it as a promising target for therapeutic intervention. The goal of my work was to comprehensively explore the diverse pathophysiologies linked to CKD, its nutritional implications, and treatment modalities; and the potential to modify health and outcome by pharmacological and non-pharmacological means. By elucidating the interplay between CKD and metabolic derangements, as well as the impact of mitochondrial dysfunction, we aimed to pave the way for novel therapeutic approaches that address management of CKD and improve patient prognosis.

In this dissertation, we have focused on patient-oriented research using metabolic imaging and multi-omics approaches to better understand the pathophysiology of CKD and linking alterations in metabolism with muscle function and physical performance. In doing so, we performed a

comprehensive investigation of metabolic, physiological, and cardiometabolic disturbances in CKD. Chapter 1 provides a comprehensive overview of CKD and CKD-related comorbidities as well as metabolic and physiological complications. Chapter 2 investigates the impact of nicotinamide riboside (NR) and coenzyme Q10 (CoQ10) that target mitochondrial metabolism for their potential effects on systemic markers of mitochondrial metabolism and physical endurance in a sedentary CKD cohort. Chapter 3 and 4 investigate metabolic derangements in non-diabetic moderate-severe CKD. Chapter 3 focuses on identifying altered metabolic pathways contributing to disruptions to insulin response in response to oral glucose tolerance testing in CKD. In chapter 4 we build on the findings from chapter 3 by investigating postprandial incretin hormone levels and their determinants using a standardized OGTT comparing non-diabetic patients with CKD and controls. We separately investigate the association of postprandial circulating incretin hormones with insulin, c-peptide, and glucagon levels during an OGTT by CKD status. Chapter 5 provides a summary of the work performed, concluding remarks, and plans for future studies.

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Chapter 1

Introduction

Chronic kidney disease (CKD) is the 9th leading cause of death in the US. More than 1 in 7 of US adults are estimated to have CKD, that is about 37 million people (1). In the United States, diabetes and high blood pressure are the leading causes of kidney failure, representing about 3 out of 4 new cases (2). Interventions to improve outcomes related to CKD focus on reducing risk, including counseling on lifestyle modifications (i.e. smoking cessation and dietary modifications to reduce proteinuria and aid in weight loss) and interventions aimed at glycemic control, dyslipidemia, and hypertension (3). Despite the current standard of care, there are not any effective treatments/interventions to stop or reverse CKD progression.

CKD is associated with a broad spectrum of complex metabolic and physiological alterations including altered metabolism (lipid, amino acid, mineral and bone), metabolic acidosis, skeletal muscle dysfunction contributing to exercise intolerance, insulin resistance, and inflammation/oxidative stress (4-8). In addition, other comorbidities such as hypertension, dyslipidemia, and diabetes which commonly coexist with CKD further exacerbate these complications leading to worsen patient outcomes and increased risk of cardiovascular disease and mortality (9). The presence of cardiac and renal system dysfunction might lead to accelerated failure of both systems (10). Therefore, there is need to minimize the factors contributing to this "cardio-renal syndrome" in CKD.

CKD also contributes to loss of protein stores with the major target being muscle (11). There are many CKD-driven factors that play a role in muscle metabolism and protein turnover perturbations. The loss of lean body mass and skeletal muscle is considered one of the strongest predictors of morbidity and mortality in this population and other chronic illness (12). Patients with CKD have lower physical function and impaired physical performance contributing to a high prevalence of frailty and mobility disability, and an increased risk for mortality (13). Emerging

evidence suggest that dysfunctional mitochondria have a primary role in the development of CKD as well as in comorbidities related to CKD and underline their role as new therapeutic targets (14). In addition, current studies suggest that mitochondrial dysfunction is the main mechanism leading to decreased physical endurance (15). So, this dissertation focuses on better understanding and addressing the broad spectrum of pathophysiologies associated with kidney disease with a focus on improving mitochondrial metabolism/function in CKD. Chapter 2 investigates the potential benefits of two pharmacological agents on clinically relevant outcomes such as physical endurance, systemic metabolism, and dyslipidemia management in CKD. Chapter 3 and 4 dives into postprandial metabolic and physiological disturbances in CKD by assessing plasma metabolic response, incretin, glucagon, insulin, c-peptide, and glucose homeostasis during a 75g oral glucose tolerance test in a non-diabetic CKD cohort.

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Chapter 2

Randomized crossover clinical trial of coenzyme Q10 and nicotinamide riboside in chronic kidney disease

2.1 Preface

This chapter was originally published in the *Journal of Clinical Investigation Insight*:

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The article has been modified to satisfy the formatting requirements of this dissertation.

2.2 Abstract

Background: Current studies suggest mitochondrial dysfunction is a major contributor to impaired physical performance and exercise intolerance in chronic kidney disease (CKD). We conducted a clinical trial of coenzyme Q10 (CoQ10) and nicotinamide riboside (NR) to determine their impact on exercise tolerance and metabolic profile in patients with CKD.

Methods: We conducted a randomized, placebo-controlled, double-blind, crossover trial comparing CoQ10, NR, and placebo in 25 patients with an estimated glomerular filtration rate (eGFR) of less than 60mL/min/1.73 m^2. Participants received NR (1,000 mg/day), CoQ10 (1,200 mg/day), or placebo for 6 weeks each. The primary outcomes were aerobic capacity measured by

peak rate of oxygen consumption (VO_2 peak) and work efficiency measured using graded cycle ergometry testing. We performed semi targeted plasma metabolomics and lipidomics.

Results: Participants mean age was 61.0 ± 11.6 years and mean eGFR was 36.9 ± 9.2 mL/min/1.73 m^2. Compared with placebo, we found no differences in VO_2 peak (P = 0.30, 0.17), total work (P = 0.47, 0.77), and total work efficiency (P = 0.46, 0.55) after NR or CoQ10 supplementation. NR decreased submaximal VO_2 at 30 W (P = 0.03) and VO_2 at 60 W (P = 0.07) compared with placebo. No changes in eGFR were observed after NR or CoQ10 treatment (P = 0.14, 0.88). CoQ10 increased free fatty acids and decreased complex medium- and long-chain triglycerides. NR supplementation significantly altered TCA cycle intermediates and glutamate that were involved in reactions that exclusively use NAD^+ and $NADP^+$ as cofactors. NR decreased a broad range of lipid groups including triglycerides and ceramides.

Conclusions: Six weeks of treatment with NR or CoQ10 improved markers of systemic mitochondrial metabolism and lipid profiles but did not improve VO_2 peak or total work efficiency.

2.3 Introduction

Patients with chronic kidney disease (CKD) suffer from a loss of functional independence and frailty (1, 2). Comorbid illnesses associated with CKD undoubtedly play a role in impaired muscle function and physical endurance (3). In addition, several lines of evidence suggest that kidney dysfunction itself contributes directly to a reduction in skeletal muscle function and mass (4). Among the mechanisms under investigation (5), disruption of mitochondrial oxidative capacity is

considered to be a central candidate linking kidney disease with skeletal muscle impairment (2, 6). Interventions that target mitochondrial function in CKD have not been evaluated in human studies.

Coenzyme Q10 (CoQ10) is a fat-soluble coenzyme involved in the transfer of electrons from complex I and II to complex III in the mitochondria during oxidative phosphorylation (7). Deficiency in CoQ10 levels has been associated with oxidative stress and impaired mitochondrial function in CKD (8). Plasma concentration of CoQ10 is reduced in patients with CKD compared with healthy controls (9); however, little is known about its treatment effects on mitochondrial metabolism, systemic inflammation, and physical performance. CoQ10 supplementation studies in aged mice have shown enhanced sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α)expression, leading to improved mitochondrial function and inhibition of oxidative stress (10). Improvements in β oxidation of fatty acids and suppressed lipid accumulation have also been reported in diabetic obese mice (11). Among patients with kidney failure, CoQ10 administration has been shown to reduce markers of oxidative stress (9).

Nicotinamide riboside (NR), a precursor to NAD^+, is a crucial cofactor and an electron carrier involved in oxidative metabolism, mitochondrial biogenesis, and redox homeostasis (12). CKD is associated with reduced NAD^+ biosynthesis and increased consumption limiting NAD^+ bioavailability (13). NAD^+ precursor supplementation may have the potential to target metabolic and clinical complications associated with CKD, including dyslipidemia, skeletal muscle dysfunction, impaired amino acid metabolism, and oxidative stress (14, 15). Indeed, administration of NR has been shown to improve insulin sensitivity in obese mice, increase NAD^+ levels leading to augmented mitochondrial SIRT3 activity, and improve muscle endurance along with muscle mitochondrial content (16, 17). Despite the promising results from both rodent and human studies,

the impact of NAD^+ supplements on systemic mitochondrial function and physical performance CKD are still lacking.

Given the central role of mitochondrial dysfunction in the pathogenesis of kidney related skeletal muscle dysfunction (18), we hypothesized that therapies targeting muscle mitochondrial metabolism could improve physical endurance and systemic markers of mitochondrial metabolism in CKD. We conducted a randomized, placebo-controlled, crossover trial to test the impact of CoQ10 and NR on maximal and submaximal physical endurance capacity, work efficiency, and metabolic profiles in patients with CKD.

2.4 Results

Participant characteristics.

A total of 25 participants were enrolled and completed the study (Supplemental Figure 1; supplemental material available online with this article. The mean age of the cohort was 61.0 ± 11.6 years and 40% were female. The mean eGFR was 36.9 ± 9.2 mL/min/1.73 m^2, and 16% of the participants had a diagnosis of diabetes. The median self-reported physical activity of the participants at enrollment was 13 hours per month (IQR [2–30]), and all participants had an Activities of Daily Living (ADL) score of 8 except for 1 participant with an ADL of 7. The average hemoglobin among participants was 12.7 ± 1.8 gm/dL. Characteristics of the participants in this study can be found in Table 1.

Table 1. Participant characteristics of analytic population

Baseline characteristics	*n* = 25
Age (years), mean (SD)	61.0 (11.6)
Male, *n* (%)	15 (60.0)
Race, *n* (%)	
White	20 (80.0)
Asian	3 (12.0)
Black	1 (4.0)
Native Hawaiian/Pacific Islander	1 (4.0)
Hispanic	1 (4.0)
BMI (kg/m^2), mean (SD)	27.7 (5.2)
SBP (mmHg), mean (SD)	129 (22)
eGFR (mL/min/1.73 m^2), mean (SD)	36.9 (9.2)
Screening hemoglobin (g/dL), mean (SD)	12.7 (1.8)
Physical activity past month (hours), median (IQR)	13 (2, 30)
Six-minute walking distance (meters), mean (SD)	414 (69)
ADL score, mean (SD)	8.0 (0.2)
Diabetes, *n* (%)	4 (16.0)
Current smoker, *n* (%)	3 (12.0)
ACE/ARB, *n* (%)	18 (72.0)
Statins, *n* (%)	14 (56.0)
Erythropoietin, *n* (%)	1 (4.0)
Serum total CO_2 (mmol/L), mean (SD)	21.2 (3.4)
Serum hsCRP (mg/dL), mean (SD)	0.3 (0.5)
UACR (mg/g), median (IQR)	35.6 (8.4, 942.7)

CKD was defined as eGFR < 60 mL/min/m^2. SBP, systolic BP; MET, metabolic equivalent; ACE/ARB, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; hsCRP, high-sensitivity CRP.

Six weeks of NR or CoQ10 treatment did not impact physical endurance and cardiorespiratory fitness outcomes compared with placebo.

The mean VO2 peak after placebo was 20.64 ± 4.84 mL/kg/min. Cardiorespiratory fitness (CRF) and VO2 peak were not significantly different with NR and CoQ10 supplementation, with means of 21.38 ± 4.93 mL/min/kg (P = 0.36) and 21.41 ± 4.74 (P = 0.33), respectively (Figure 1A and Table 2). Submaximal efficiency assessed by VO2 at a given submaximal workload was affected

by NR treatment. Mean submaximal absolute VO2 after placebo was 0.70 ± 0.12 L/min at 30 W and 0.99 ± 0.12 L/min at 60 W. Compared with placebo, NR reduced submaximal absolute VO2 at both 30 W to a mean of 0.67 ± 0.1 L/min (P = 0.03) and 60 W to a mean of 0.95 ± 0.14 L/min (P = 0.07) (Figure 1, B and C, and Table 2). However, CoQ10 did not impact submaximal efficiency at 30 W and 60 W (Figure 1, B and C, and Table 2). Mean duration of cycle ergometry after NR and CoQ10 supplementation was 982 ± 317 and 1,012 ± 332, respectively, compared with 1,009 ± 332 seconds with placebo (Figure 1D and Table 2). Physical endurance measured as total work performed during cycle ergometry did not change after NR, with a mean of 57.72 ± 37.29 kJ (P = 0.47) and after CoQ10 with a mean of 61.5 ± 38.35 (P = 0.77) compared with placebo with a mean of 60.32 ± 39.06 (Figure 1E and Table 2). NR and CoQ10 supplementation did not have an impact on total work efficiency, with means of 30.9 ± 18.1 kJ/(L/min) (P = 0.46) and 33.3 ± 15.7 (P = 0.55) compared with placebo with a mean of 32.2 ± 17.5 (Figure 1F and Table 2). There were no alterations in fuel utilization at rest indicated by no change in respiratory exchange ratio (RER) at rest (P = 0.41 and 0.62) and VO2 max (P = 0.44 and 0.87) after NR and CoQ10. However, RER at 30 W and 60 W were increased after NR (P = 0.06 and 0.04) compared with placebo (Table 2). CoQ10 did not have an impact on RER at 30 W or 60 W compared with placebo (P = 0.24 and 0.18) (Table 2). Estimates of differences in physical endurance outcomes, BP, and BW changes comparing NR and CoQ10 to placebo are shown in Supplemental Table 1. Analyses of treatment by study period revealed no evidence for carryover effects among the study interventions.

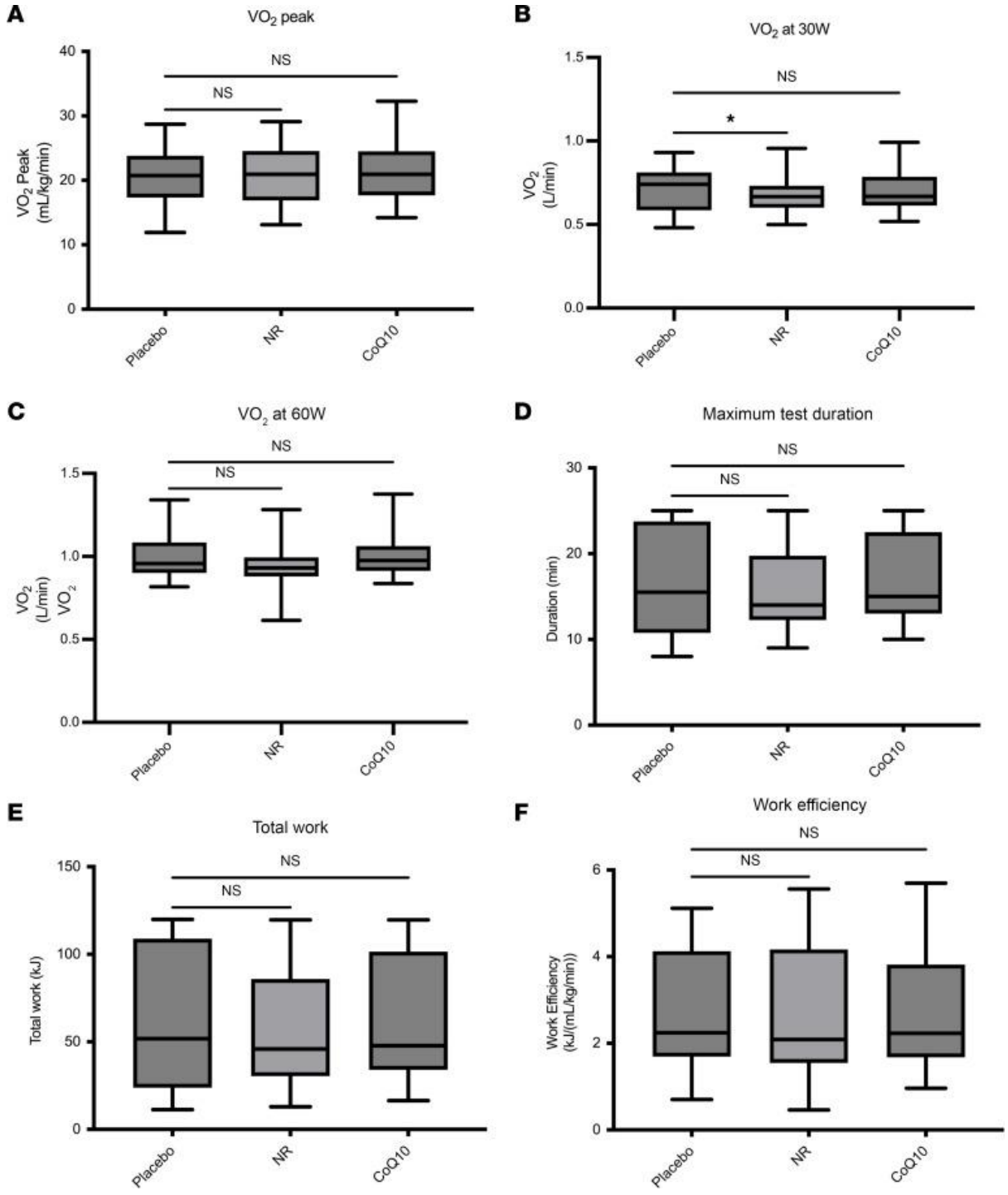

Figure 2.1 No changes in physical endurance and CRF outcomes after NR and CoQ10 treatments compared with placebo (*n* = 25). (**A**) VO_2 peak, (**B** and **C**) absolute VO_2 at 30 and 60 W, (**D**) test duration, (**E**) total work, and (**F**) total work efficiency. Two-way ANOVA was used to compare changes in response to NR and CoQ10 with placebo. The box plots represent median and IQR and the whiskers represent minimum and maximum values. $*P < 0.05$. NS, not significant.

Table 2. Summary of primary and exploratory physical endurance outcomes comparing NR and CoQ10 with placebo

Endpoints	NR vs. placebo (95% CI)	NR vs. placebo *P* value	CoQ10 vs. placebo (95% CI)	CoQ10 vs. placebo *P* value
Primary outcomes				
VO_2 peak, L/min	0.05 (−0.06 to 0.17)	0.36	−0.06 (−0.06 to 0.18)	0.33
VO_2 peak, mL/kg/min	0.68 (−0.93 to 2.3)	0.39	0.67 (−0.32 to 1.67)	0.17
Work efficiency, kJ/(L/min)	−1.33 (−5.04 to 2.37)	0.46	1.56 (−3.79 to 6.92)	0.55
Work efficiency, kJ/(mL/kg/min)	−0.11 (−0.42 to 0.20)	0.47	0.01 (−0.32 to 0.35)	0.91
Exploratory outcomes				
VO_2 at 30 W, L/min	−0.02 (−0.05 to 0.00)	0.03	0.00 (−0.05 to 0.05)	0.95
VO_2 at 30 W, mL/kg/min	−0.31 (−0.79 to 0.16)	0.19	0.02 (−0.53 to 0.59)	0.91
VO_2 at 60 W, L/min	−0.03 (−0.06 to 0.00)	0.07	0.00 (−0.01 to 0.03)	0.35
VO_2 at 60 W, mL/kg/min	−0.28 (−0.83 to 0.26)	0.29	0.17 (−0.24 to 0.59)	0.39
Total work, kJ	2.59 (−10.03 to 4.84)	0.47	1.18 (−7.18 to 9.54)	0.77
Test duration, seconds	−26.52 (−86.65 to 33.61)	0.37	10.67 (−82.49 to 103.8)	0.81
RER at rest	0.01 (−0.02 to 0.05)	0.41	0.00 (−0.02 to 0.04)	0.62
RER at 30 W	0.02 (0.00 to 0.05)	0.06	0.01 (−0.01 to 0.05)	0.24
RER at 60 W	0.02 (0.00 to 0.04)	0.04	0.01 (0.00 to 0.03)	0.18
RER at VO_2 max	0.01 (−0.01 to 0.04)	0.44	0.00 (−0.04 to 0.04)	0.87

Mean difference and 95% CI are shown.

In the subgroup analysis, CRF (relative VO2 peak, mL/kg/min) was higher and RER at 60 W was significantly lower among participants classified as active versus sedentary and good versus poor performers (Supplemental Figure 2, A–D). However, we found no evidence for an impact of NR or CoQ10 on CRF or physical endurance (total work and work efficiency) in these subgroups (Supplemental Figure 2, A and B). Similarly, we found no meaningful or significant impact of NR or CoQ10 supplementation on RER at 60 W in our subgroup analysis (Supplemental Figure 2, C and D). Gender-stratified analysis showed no differential improvements in VO2 peak and RER at 60 W among males and females (Supplemental Figure 2, E and F).

NR or CoQ10 treatment did not change kidney function and inflammatory biomarkers compared with placebo.

Over the 6-week treatment period, we found no meaningful or significant change in BW (Supplemental Table 1) or serum triglyceride associated with NR and CoQ10 supplementation

compared with placebo (P = 0.51 and 0.69, and P = 0.71 and 0.70, respectively). We found no meaningful or statistically significant differences in the urine albumin-to-creatinine ratio (UACR) (P = 0.28 and 0.21) (Supplemental Figure 3A), kidney function biomarkers, including serum creatinine (P = 0.23 and 0.58) and cystatin C (P = 0.08 and 0.87) (Supplemental Figure 3, B and C), and inflammatory biomarker serum C-reactive protein (CRP) (P = 0.21 and 0.12) (Supplemental Figure 3D) after NR and CoQ10, respectively.

CoQ10 supplementation increased plasma free fatty acid and decreased triglycerides.

Three of the 98 tested plasma metabolites decreased in response to CoQ10 treatment compared with placebo at the 0.05 significance level: β-alanine, lactate, and 2-deoxytetronic acid with fold changes of 0.49 (P = 0.01), 0.72 (P = 0.02), and 0.79 (P = 0.03), respectively (data not shown). In contrast, CoQ10 had an impact on the plasma lipid profile, altering 6% (24/394) of the detected lipid species compared with placebo (Supplemental Table 2). These changes predominantly involved free fatty acids (FFAs) (8/24) and triglycerides (7/24). All of the altered FFAs were increased compared with placebo, ranging from an increase of 19% to 40%. Triglycerides showed the opposite pattern with a systematic decrease compared with placebo, ranging from a decrease of 19% to 66% (Supplemental Table 2). The altered triglycerides tended to be long chained with a high degree of unsaturation. Plasma 3-hydroxybutarate was increased (not significantly), with a fold change of 1.41 (P = 0.07) compared with placebo (data not shown).

NR treatment altered NAD+-dependent TCA cycle intermediates while decreasing plasma triglycerides and ceramides.

Compared with placebo, NR supplementation altered 6% (6/98) of the detected metabolites (Table 3). These metabolites included 3 a priori–targeted TCA cycle intermediates: isocitrate, α-ketoglutarate, and malate, which all are involved in reactions that use NAD+ as a cofactor. We found a significant decrease in all 3 TCA cycle intermediates compared with placebo (Table 3). In addition, glutamate, a precursor to α-ketoglutarate that feeds into the TCA cycle using NADP+ as a cofactor, was increased compared with placebo (Table 3). We confirmed a small to medium magnitude of difference on TCA cycle intermediates with effect sizes ranging from –0.37 (95% CI –0.06 to –0.62) (malate) to –0.40 (95% CI 0.1 to –0.71) (isocitrate) after NR supplementation (Table 3).

Table 3. The impact of NR supplementation on plasma metabolites compared with placebo

Metabolites	Fold difference NR/placebo)	Effect size (95% CI)	*P* value	Pathway
1-Methylgalactose	0.33	−0.41 (0.11 to −0.71)	0.007	Pyrimidine nucleotide sugar
Isocitrate[A]	0.90	−0.40 (−0.10 to −0.71)	0.009	TCA cycle
Glutamate[A]	1.07	0.40 (0.09 to 0.71)	0.009	Amino acid
α-Ketoglutarate[A]	0.99	−0.38 (−0.08 to −0.69)	0.012	TCA cycle
Malate[A]	0.99	−0.37 (−0.06 to −0.62)	0.017	TCA cycle
Glycerate	0.85	−0.31 (0.00 to −0.60)	0.045	Amino acid metabolism/Gly, Ser

Fold changes and effect sizes were obtained by linear mixed effects modeling adjusted for fasting status. Only metabolites with $P < 0.05$ are shown. [A]Targeted metabolites.

NR supplementation also had a meaningful impact on the lipid profile, significantly altering 7.5% (30/394) of detected lipid species (Supplemental Table 3). Lipid species were generally decreased with 26 out of 30 altered lipids reduced compared with placebo. We found a significant decrease in all 10 altered triglycerides. These triglycerides tended to be medium chained with a lower degree of unsaturation (Supplemental Table 3). The largest decrease was detected in triglyceride 50:0 with a 52% reduction compared with placebo. Reductions in ceramides, lyso-phosphatidylethanolamine

(LPE), lyso-phosphatidylcholines (LPC), and phosphatidylethanolamines (PE) were also detected compared with placebo (Supplemental Table 3).

Six weeks of CoQ10 or NR supplementation was associated with significant alterations in plasma fatty acyls (fatty acids), glycerolipids (triglycerides and diacylglycerides), glycerophospholipids (PEs, LPEs, LPCs, and PCs), and sphingolipids (ceramides and glucosylceramides).

To investigate systemic plasma lipid profile changes in response to NR and CoQ10, we assessed compositional changes within major lipid classes compared with placebo. We found a systematic increase in fatty acyls and a decrease in glycerolipids after CoQ10 treatment compared with placebo (Figure 2). In comparison, NR supplementation was associated with a systemic decrease in glycerolipids and sphingolipids compared with placebo. NR also decreased plasma glycerophospholipids with the exception of 2 PC species (Figure 2 and Supplemental Table 3).

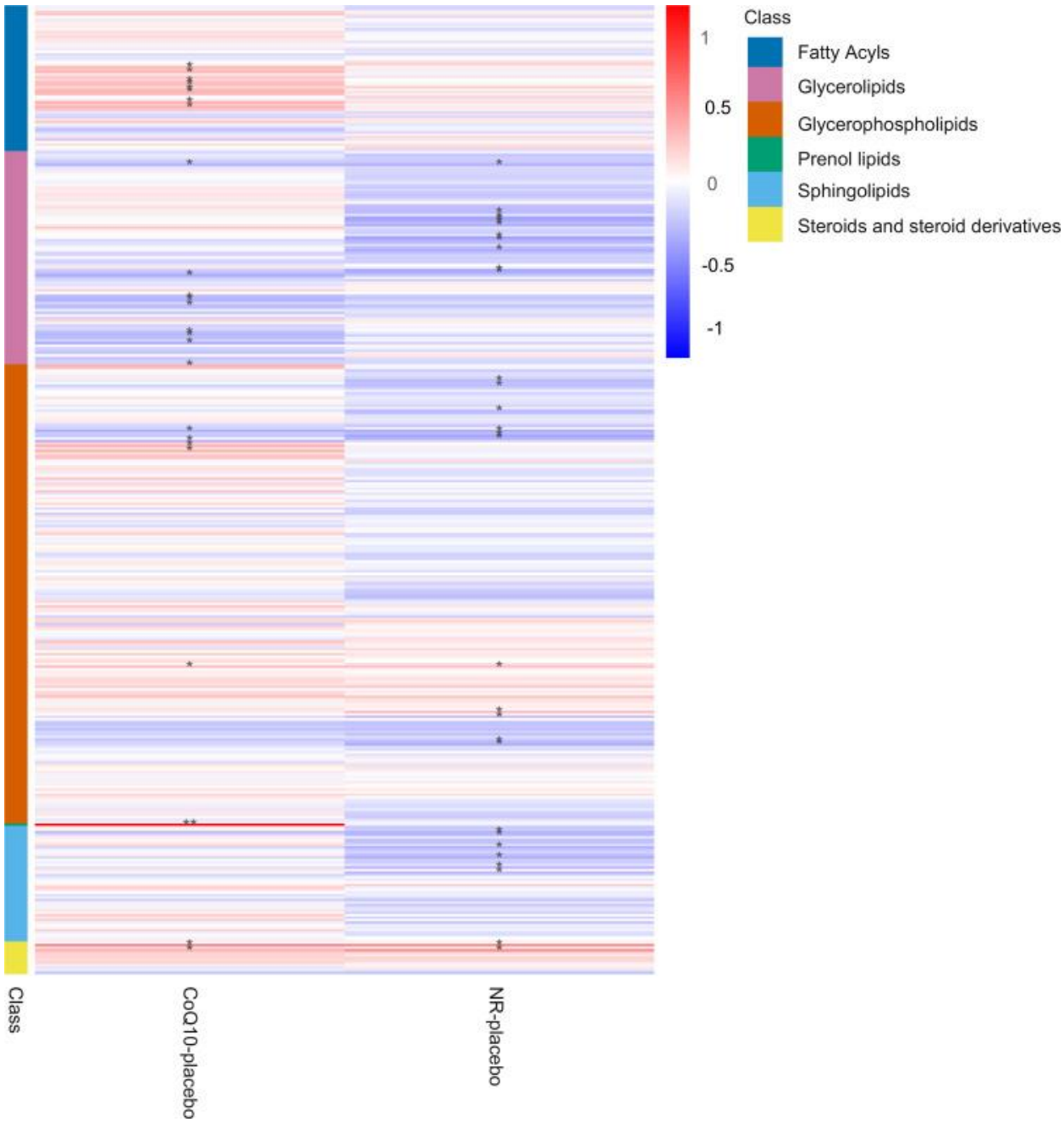

Figure 2.2 Heatmap depicting compositional changes within lipid classes in response to NR and CoQ10 supplementation. The colors of the heatmap are based on effect size obtained from linear mixed effects modeling adjusted for fasting status. *$P < 0.05$, **$P < 0.001$. The lipid classes include fatty acyls (fatty acids), glycerolipids (triglycerides and diacylglycerols), glycerophospholipids (PEs, LPEs, LPCs, and PCs), sphingolipids (ceramides and glycosylceramides), steroids, and steroid lipids (cholesterol and cholesteryl ester).

Oral supplementation with 1,000 mg/day of NR and 1,200 mg/day of CoQ10 was well tolerated and elicited no serious adverse effects.

Twenty-three of the 25 participants consumed greater than 75% of NR, CoQ10, and placebo pills administered. We observed no difference in treatment-associated adverse events compared with placebo (Supplemental Table 4). During the trial, a total of 13 adverse events were reported by the participants, with 6 during NR, 3 during CoQ10, and 4 during placebo supplementation. The adverse events were counted from the start of the treatment until the end of the washout period.

2.5 Discussion

In a randomized crossover trial testing 6 weeks of NR and CoQ10 treatments in patients with CKD, neither therapy improved the primary study outcomes of physical endurance measured by maximal aerobic capacity (VO2 peak) and total work efficiency. Exploratory analysis, however, revealed that 6 weeks of NR improved submaximal exercise efficiency by reducing absolute VO2 at 30 W and 60 W with a concomitant increase in RER, suggesting better efficiency in carbohydrate utilization at submaximal intensity. NR and CoQ10 treatments led to mechanistically plausible impacts on TCA cycle intermediates and lipid metabolites: CoQ10 increased plasma FFAs and decreased highly unsaturated medium- and long-chain triglycerides, and NR significantly altered plasma metabolites that are involved in reactions that use NAD+ or NADP+ as a cofactor, including 3 TCA cycle intermediates and glutamate. In addition to changes in plasma metabolites, NR supplementation resulted in a reduction in a wide range of lipid species, including triglycerides, ceramides, LPEs, LPCs, and PEs, compared with placebo. The treatments were

generally well tolerated over the duration of the study. The observed impacts on the metabolic profile suggest early beneficial changes in systemic mitochondrial metabolism and lipid profile that argue for future trials with a longer treatment duration.

Our findings are consistent with previous studies that also show no physical performance-enhancing effect from short-term CoQ10 or NR supplementation across a wide range of ages and physical fitness levels. A prior study of NR supplementation involving older males showed improved skeletal muscle NAD+ levels and a reduction in circulating inflammatory cytokines after 21 days but no changes in grip strength or mitochondrial bioenergetics (19). Similarly, a 14-day CoQ10 supplementation among trained and untrained individuals showed reduction in plasma oxidative stress levels but no changes in anaerobic or aerobic capacity and ventilatory threshold (20). Negligible changes in metabolic substrate use indicated by comparable RER measurements at resting and 60 W after CoQ10 supplementation are also in agreement with previous findings. A randomized trial of patients with myalgia showed no changes in RER after 8 weeks of CoQ10 supplementation (21).

Contrary to previous studies, we detected changes in VO2 and RER at submaximal workloads (22, 23) in our post hoc exploratory analyses. In particular, we detected increased RER at 30 W and 60 W, suggesting enhanced carbohydrate utilization after NR supplementation. Higher submaximal RERs coincided with lower absolute VO2 at submaximal workload, suggesting improved submaximal exercise efficiency. It is known that the NAD+/NADH ratio regulates oxidative metabolism through sirtuins (24). A possible explanation for the enhanced carbohydrate utilization with NR supplementation is improved SIRT1 activity that directly regulates mitochondrial function and biogenesis through PGC-1α (25). Studies have shown that NAD+ supplementation

modulates SIRT1 activity (16, 26, 27), leading to improved glucose utilization via improved oxidative metabolism. A long-term (5 months) NR supplementation study among BMI-discordant twins showed increased muscle mitochondrial biogenesis linked to upregulation of SIRT1, TFAM, MFN2, and NRF1 — all genes involved in glucose metabolism (27). The changes in multiple TCA cycle intermediates may support improved efficiency of energy metabolism underlying the increased submaximal RER with NR supplementation. This suggests that short-term NR supplementation may lead to improved energy expenditure from carbohydrates without impacting fat oxidation during light- to moderate-intensity exercise in CKD; however, further studies confirming this observation are needed. Together, our data along with published studies show that solely targeting mitochondrial dysfunction with short-term pharmacologic interventions does not counteract CKD-associated exercise intolerance. The complementary effects of CoQ10 and NR on lipid metabolism motivates future studies testing the combination of these supplements added to a structured exercise program to improve exercise tolerance in patients with CKD.

CoQ10 supplementation in our study was associated with metabolic changes suggesting improved mitochondrial β oxidation known to be disrupted in CKD. CKD is associated with adverse metabolic complications, including dyslipidemia (28), amino acid and protein catabolism (29), and insulin resistance (30), contributing to impaired mitochondrial energy metabolism (14, 31). Prior studies have demonstrated CoQ10 supplementation reduces lipid peroxidation, a maker of oxidative stress, in patients with kidney failure (32). Impaired mitochondrial β oxidation and consequent lipid accumulation contributes to inflammation, oxidative stress, and insulin resistance in CKD (14, 33). Several lines of evidence suggest CoQ10 favorably impacts β oxidation. First, we observed biologically relevant alterations in the structure and amount of plasma FFAs and triglycerides after CoQ10 supplementation (Supplemental Table 2 and Figure 2). Resting-state

plasma FFA concentration is directly linked to the peak fat oxidation rate (34, 35). Further studies are needed to assess whether these changes in resting FFA reflect changes in insulin sensitivity. Second, CoQ10 supplementation resulted in a systemic decrease of medium- and long-chain triglycerides that accumulate in CKD and are hallmarks of impaired β oxidation (36). This also confirms previous studies showing reduction of serum triglycerides in response to CoQ10 supplementation (37). The reductions in longer triglycerides with high numbers of double bonds with CoQ10 treatment in our study suggests improved β oxidation. Finally, while not statistically significant ($P = 0.07$), there was a meaningful 41% increase in the plasma level of an important ketone body elevated in fatty acid oxidation, 3-hydroxybutyrate levels, a marker for β oxidation rate (38), with CoQ10. Our findings are consistent with prior in vitro and in vivo studies in mice showing improved β oxidation after CoQ10 supplementation (11, 39, 40). Our results build on findings from previous studies confirming that CoQ10 supplementation improves the lipid profile in CKD by increasing β oxidation and decreasing plasma medium- and long-chain triglycerides. These findings motivate future trials involving longer durations of CoQ10 supplementation investigating long-term improvements in fatty acid oxidation and reduction in medium- and long-chain triglycerides. These future studies have clinical implications for dyslipidemia management in CKD and alleviating its metabolic and physiological complications such as insulin resistance, impaired energy metabolism, and oxidative stress.

We found evidence that NR supplementation changed plasma levels of our targeted TCA cycle intermediates, suggesting improved systemic mitochondrial metabolism. Sufficient NAD+ levels are needed for a wide range of anabolic and catabolic pathways, including glycolysis, TCA cycle, oxidative phosphorylation, fatty acid oxidation, and pentose phosphate pathway (41). Diminished NAD+ biosynthesis results in depleted NAD+ levels in CKD, contributing to impairment in

energy-producing metabolic pathways (13, 42). We found significant alterations in 3 targeted TCA cycle intermediates (isocitrate, α-ketoglutarate, and malate) that exclusively use NAD+ as a cofactor. In addition, glutamate, which is an anapleurotic precursor of α-ketoglutarate that uses NADP+ as a cofactor, was also significantly altered. Our results in patients with CKD agree with prior studies of NR supplementation in murine models of aging. A study using muscle stem cells from aged mice showed that NR supplementation increases the expression of genes associated with TCA cycle and oxidative phosphorylation in addition to increased oxidative respiration, mitochondrial membrane potential, and ATP production (43). Further evidence for an impact of NR on mitochondrial function comes from a recent study of 12-week NR supplementation in heart failure patients with reduced ejection fraction, showing an increased mitochondrial respiration and decreased proinflammatory cytokine expression in PBMCs (44). Future studies are needed to assess mitochondrial metabolism impairments associated with reduced NAD+ and the impact of NAD+ precursor supplementation on ex vivo mitochondrial bioenergetics and oxidative phosphorylation capacity in CKD.

NR treatment led to changes in the lipidomic profile, decreasing a broad range of plasma lipid species including triglycerides. CKD is known to be associated with disordered lipid metabolism with lower kidney function and greater albuminuria associated with impairments in β oxidation and higher levels of ceramides, triglycerides, and phosphatidylcholines (33, 45, 46). These lipid class alterations have been associated with biologic pathways of oxidative stress, insulin resistance, and inflammation in patients with CKD (47–49). NAD+ is a crucial cofactor needed for β oxidation of fatty acids. NAD+ deficiency in CKD contributes to impaired fatty acid β oxidation resulting in intracellular accumulation of lipids, including enrichment of triglycerides high in polyunsaturated fatty acids, which are thought to upregulate de novo biosynthesis of

triglycerides and phospholipids via increased mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) signaling (36, 50, 51). Although total triglyceride levels remained unchanged with NR supplementation, there was evidence of metabolically favorable changes, particularly in reductions in subclasses of triglycerides that were predominantly short and medium chained with a high degree of saturation. Consistent with another prior study of NAD+ precursors in humans (44), our findings suggest that NR supplementation improved lipid metabolism by enhancing catabolism of shorter more saturated triglycerides without a significant impact on longer polyunsaturated triglycerides. However, future studies are needed to better understand the underlying mechanism of NAD+ supplementation on improved triglyceride metabolism.

In addition to reductions in triglycerides, NR treatment led to reductions in biologically relevant ceramides (a sphingolipid), LPEs, LPCs, and PEs (glycerophospholipids). The presence and severity of kidney disease is associated with increased ceramide levels known to adversely impact metabolic health (52). A recent large prospective cohort study showed that PEs, triglycerides, and ceramides are strongly associated with increased risk of CKD (53). Ceramides are considered lipotoxic, promoting lipid accumulation, insulin resistance, mitochondrial dysfunction, impaired β oxidation, inflammation, and apoptosis (54–56). Similarly, elevated LPE, LPC, and PE levels have also been implicated in cardiovascular disease, diabetes, and neurodegenerative disease (57–59). In our study, NR supplementation improved the plasma lipid profile by reducing lipotoxic species in CKD. Future studies are needed to investigate the underlying mechanism of improved CKD-associated dyslipidemia with increased NAD+ bioavailability and its long-term effect on kidney function and other clinical outcomes. Key candidates for improved mitochondrial and lipid metabolism are NAD+-dependent mitochondrial sirtuins (and their downstream effectors)

involved in mitochondrial biogenesis, mitochondrial dynamics, fatty acid oxidation, and oxidative stress defense (60).

This study had notable strengths and limitations. First, we used an efficient and rigorous double dummy, placebo-controlled, randomized crossover trial design and physiologically relevant measures of endurance exercise capacity and substrate utilization during graded cycle ergometry testing. Second, we applied semitargeted metabolomics and lipidomics profiling and accounted for fasting status changes to identify changes in mitochondrial and lipid metabolism after NR and CoQ10 supplementation. However, this study had several limitations. First, the sample size was small and treatment duration was short, limiting evaluation to early potential treatment effects. This may have limited our ability to detect differences in muscle and exercise tolerance requiring longer-term treatment. Second, our study partially coincided with the start of the COVID-19 pandemic, leading to inevitable lifestyle changes among participants during the study period. We were unable to reliably track the impact this may have had on habitual physical activity. Third, a number of study participants were, on average, more active than the general CKD population who are, on average, much more sedentary (61). Fourth, we did not account for multiple comparisons in our lipidomics analyses. Nevertheless, our exploratory lipidomics analysis detected meaningful effects of CoQ10 and NR supplementation on specific biologically relevant functional classes of lipids known to be associated with CKD and demonstrated to have adverse effects on the kidney function. Finally, we did not have intracellular or tissue-specific (i.e., skeletal muscle) readouts of NAD+ or CoQ10 before and after NR or CoQ10 supplementation to confirm higher intracellular levels of NAD+ or CoQ10. Nonetheless, we did confirm a significant increase in plasma CoQ10 levels through our lipidomics analysis with an effect size of 1.3 (95% CI of 0.92 to 1.72) (P = 1.44 × 10–11) compared with placebo. Plasma and skeletal muscle CoQ10 levels have been shown to

have a significant correlation in previous studies (62). Similarly, an NR supplementation study among older adults (70–80 years old) showed that 1,000 mg daily supplementation of NR for 21 days significantly elevated NAD+ metabolome in the skeletal muscle (19).

In contrast to the benefits of exercise in patients with CKD, our findings demonstrate NR or CoQ10 supplementation alone does not lead to improved maximal physical performance. Clinical trials of exercise training in persons with nondialysis CKD have shown significant improvements in maximal aerobic capacity, physical performance, and physical functioning (63, 64). Future studies combining exercise with these treatments are needed to detect synergy in improvement of metabolic health and exercise tolerance in patients with CKD given the overlapping mechanisms of exercise and NAD+ precursor supplementation. Animal studies demonstrate NAD+-mediated mitochondrial hormetic response (mitohormesis), a well-known mediator of exercise-induced adaptations (65), through activation of SIRT1 and SIRT3 modulating mitochondrial fitness following NAD+ precursor supplementation (66, 67). In addition, a prior clinical trial in elderly adults combining 4 months of an antioxidant shown to induce SIRT1 and mitochondrial biogenesis (68) with exercise training demonstrated increases in muscle strength and physical endurance via 6-minute walking distance compared with exercise alone (69). Further human studies are needed to investigate the signaling systems involved in regulation of mitochondrial homeostasis via NAD+ precursor supplementation and if they enhance mitohormetic effects improving adaptation to exercise training in CKD.

In conclusion, short term CoQ10 and NR supplementation in patients with moderate to severe CKD resulted in biologically plausible changes in mitochondrial metabolism and the plasma lipid profile. Metabolic changes were more pronounced for the plasma lipidome than the metabolome.

CoQ10 treatment led to improved β oxidation resulting in a systemic increase in plasma FFAs and a decrease in complex triglycerides while NR supplementation altered levels of TCA cycle intermediates and resulted in a broad decrease of plasma lipid species, including lipotoxic subclasses of sphingolipids. These findings add to the body of preclinical evidence supporting the efficacy of NR and CoQ10 for improving markers of mitochondrial metabolism and the lipid profile in persons with CKD not treated with dialysis. Given the distinct beneficial impacts of NR and CoQ10 on plasma metabolome and lipid profile, future studies of longer duration are needed to investigate the potential synergistic effects of a combination therapy of NR and CoQ10 in sedentary patients with CKD.

2.6 Methods

Study design and procedures

CoQ10 and NR in CKD (CoNR trial) is a placebo-controlled, double-blind, randomized crossover trial with 3 arms: placebo, CoQ10, and NR (Clinicaltrials.gov NCT03579693). The trial was conducted from November 2018 to April 2021. There were 3 phases in the study, each 42 (6 weeks) days separated by a 1-week washout period. Subjects received 1,000 mg/day of NR (Niagen) or 1,200 mg/day of CoQ10 (Tishcon) for 6 weeks (Supplemental Figure 1). The doses and study duration were chosen based on the review of studies in the literature evaluating biological activity, safety, and tolerability (70, 71). A previous randomized, crossover trial of NR at 1,000 mg daily for 6 weeks demonstrated improvements in increasing NAD+-related metabolites coinciding with improvements in systolic BP (70). At all treatment periods, each participant took the same amount

of identical-looking tablets. Participants, study physician, assessors, and study staff were blinded to the treatment and sequence allocation of participants.

Adherence to the study treatment was assessed by phone call from the study coordinator midway through each treatment arm to confirm adherence and inventory method to assess remaining pills at the end of the study. Participants were asked to bring in unused study pills at each visit to return to The University of Washington Investigational Drug Services (IDS), which managed the study drugs. Adherence was defined as consumption of at least 75% of the prescribed regimen.

There were 4 study visits in total for this trial: 1 baseline visit followed by 3 more at the end of each treatment period. The baseline visit was the same as visits 2, 3, and 4, with an addition of medical history and anthropometrics testing. Participants underwent baseline evaluation including a detailed assessment of demographics, smoking history, medical history, medication inventory, and vital signs. Independent functional status was obtained using an ADL score. These were collected using the Lawton-Bowdy Instrumental Activities of Daily Living (IADL) scale (72) coding responses as 0 and 1 summed to a total score of 8, with 8 being the most independent and 0 being the least independent. To avoid introducing any potential bias into the study, the participants were asked to avoid making any changes to their habitual physical activity during the trial. All concomitant medications taken by the participants within 4 weeks prior to study enrollment were recorded. In addition to the prescribed medication, over-the-counter medications (i.e., vitamins, herbal remedies) were also recorded. The participants were also asked to fast at least 6 hours before each visit and abstain from caffeine intake, smoking, and exercising 48 hours before bicycle ergometry testing. Alcohol and recreational or street drug use was also recorded for interpretation and documentation of observed participant health status. These were verified by the

study coordinators the day of testing. During each visit, blood and cycle ergometry data were collected for each participant.

A brief screening process to evaluate eligibility criteria and to obtain informed consent took place approximately 1 month before the baseline visit. The screening visit included verification of inclusion and exclusion criteria, measuring vital signs, anthropometrics, 6-minute walking distance, screening labs (renal panel and complete blood count), and bicycle ergometry practice to familiarize participants with the equipment. Qualified participants were randomized to a supplementation sequence (Supplemental Figure 1).

Study population

Study participants were selected from a larger observational cohort study of muscle energetics in CKD: the Muscle Mitochondrial Energetics and Dysfunction (MEND) study (6). Additional participants were recruited from nephrology clinics at the University of Washington System, including Harborview Medical Center and University of Washington Medical Center. Inclusion criteria were ages 30–79, eGFR of less than 60mL/min/1.73 m2 (using the creatinine-based 2012 Chronic Kidney Disease Epidemiology Collaboration equation), and a 6-minute walking distance of less than 550 meters. Exclusion criteria included insulin-dependent diabetes, treatment with dialysis, kidney transplantation, and weight of over 300 lb. A total of 25 participants were recruited for the study (Table 1). The last participant exited the study in April 2021.

Physical endurance measurements

The primary outcomes of the study were changes in physical exercise endurance measured by aerobic capacity (VO2 peak), total work, and work efficiency. We also measured RER via cycle

ergometry (Lode Corival Cycle Ergometer, MGC Diagnostics Ultima CardiO2 pulmonary exercise system). These measurements were obtained in a dedicated exercise research center at the Fred Hutchinson Cancer Center using cycle ergometry measuring oxygen uptake starting at 0 W at 60 rotations per minute (rpm) and increasing it by 30 W every 3 minutes until exhaustion. If participants were to complete 120 W for 3 minutes, they would continue pedaling at 120 W until exhaustion. A mean rate of perceived exertion (RPE) of greater than 17 (Borg scale) and a mean RER of 1.109 ±.099 were achieved across all visits, suggesting maximal effort was given by the participants (73, 74). The VO2 was measured as 30-second averages. The VO2 peak was defined as the highest VO2 obtained during the last 30 seconds of the exercise testing. Total work performed (kJ) was calculated as follows: (W × seconds)/1,000. Work efficiency during exercise testing was calculated as follows: total work/VO2 peak. RER at rest, 30 W, 60 W, and max workload were measured as the last 30-second averaged RER of each specific stage (rest, 30 W, 60 W, and VO2 max).

Metabolic profiling

We evaluated changes in plasma metabolites and circulating lipid species through semitargeted metabolomics and lipidomics profiling approaches using gas chromatography coupled to time-of-flight mass spectrometry (GC TOF-MS) and liquid chromatography coupled to quadrupole time-of-flight mass spectrometer-charged surface hybrid (LC QTOF CHS), respectively, as described before (75, 76). A priori–selected metabolites that use NAD+/NADP+ as cofactors were targeted for plasma metabolites based on their anticipated response to the study treatments. These metabolites include pyruvate, malate, lactate, isocitrate, glutamate, glucose-6-phosphate, and α-

ketoglutarate. Both platforms were performed at West Coast Metabolomics Center. A total of 98 plasma metabolites and 394 lipid species were detected across all 4 visits.

Statistics

Physical endurance. We used ANOVA to estimate sequence effects, treatment effects, and period effects on physical performance outcomes. The coefficient of the interaction term between the treatment (NR, CoQ10, and placebo) and time point (before versus after) was used to estimate the treatment effect for each intervention. The impact of treatment on BW, kidney function biomarkers, and cystatin C was estimated using the method. To estimate treatment effect in the stratified analysis of physical endurance outcomes, the interaction term of activity level (active versus sedentary) and treatment or performance (poor versus good performers) and treatment were used. Treatment effects in the gender-stratified comparison were estimated using the interaction term of sex (male versus female) and treatment. We tested for possible carryover effects of the study interventions using the interaction of study period and treatment. One-way ANOVA and 2-way ANOVA were performed using GraphPad Prism 9.0. A P value of less than 0.05 was considered significant for all analyses unless stated otherwise.

Subgroup analysis. Participants were grouped into the "active" category if they were physically active for at least 300 minutes (moderate intensity) per month, according to their self-reported online survey. This threshold was picked based on the recommendation of the Physical Activity Guidelines for Americans (2nd edition) for our participants' age demographics with chronic conditions (77). The second grouping strategy was based on participants' performance during their baseline cycle ergometry testing. The participants who completed 3 minutes at all stages of the exercise protocol from 0 W to 120 W were included, which equals a minimum of 15 minutes total

test duration and were labeled as "good performers." The participants who were unable to complete the last stage of 120 W and therefore had a test duration less than 15 minutes were labeled "poor performers."

Metabolomics and lipidomics. Plasma metabolites were available for 25 participants for all 4 visits except for 1 participant who missed a post-CoQ10 visit. All metabolites were checked for normality. To eliminate systematic variation during sample preparation, raw metabolites were normalized using Systematic Error Removal Using Random Forest (SERRF) (78). Differences in plasma metabolites were assessed using a linear mixed modeling approach with random intercepts in which SERRF-normalized metabolites were regressed on treatment type (placebo versus CoQ10 or NR), time point (before versus after), and the interaction of the 2, additionally adjusted for fasting status. Fold changes were calculated using the mean ratio of postsupplementation (CoQ10 or NR) over placebo. To quantify the magnitude of impact on the plasma metabolic and lipid profile, we determined the effect size using standardized mean difference (79). An effect size of 0.5 is considered a medium effect and an effect size above 0.8 is considered a large effect. Linear mixed effects modeling was also used to perform carryover analysis to confirm negligible lingering effects from each supplementation period. Data are presented as mean ± SD unless otherwise stated. Statistical analysis was performed using R 3.6.1 (80).

Power calculations. Sample size was calculated based on a pilot, randomized, controlled trial study of patients with CKD demonstrating the effect of a 12-month combined resistance and aerobic exercise program resulting in an increase of 5.7 mL/kg/min VO2 peak with a standard deviation of the difference in VO2 peak on bicycle ergometry of 2.1 mL/kg/min (81). The probability is 92% that the study will detect a treatment difference at a 2-sided 0.05 significance

level, if the true difference between treatments is 2.1 (1 SD) mL/kg/min. This assumes that the standard deviation of the difference in the response variables is 2.1 mL/kg/min. The same power calculation was employed for each treatment.

Study approval

The procedures in the study and model informed consent forms were reviewed by the University of Washington Human Subjects Division (HSD). All participants provided written informed consent. The study was approved by the ethical review board of University of Washington (STUDY00004998).

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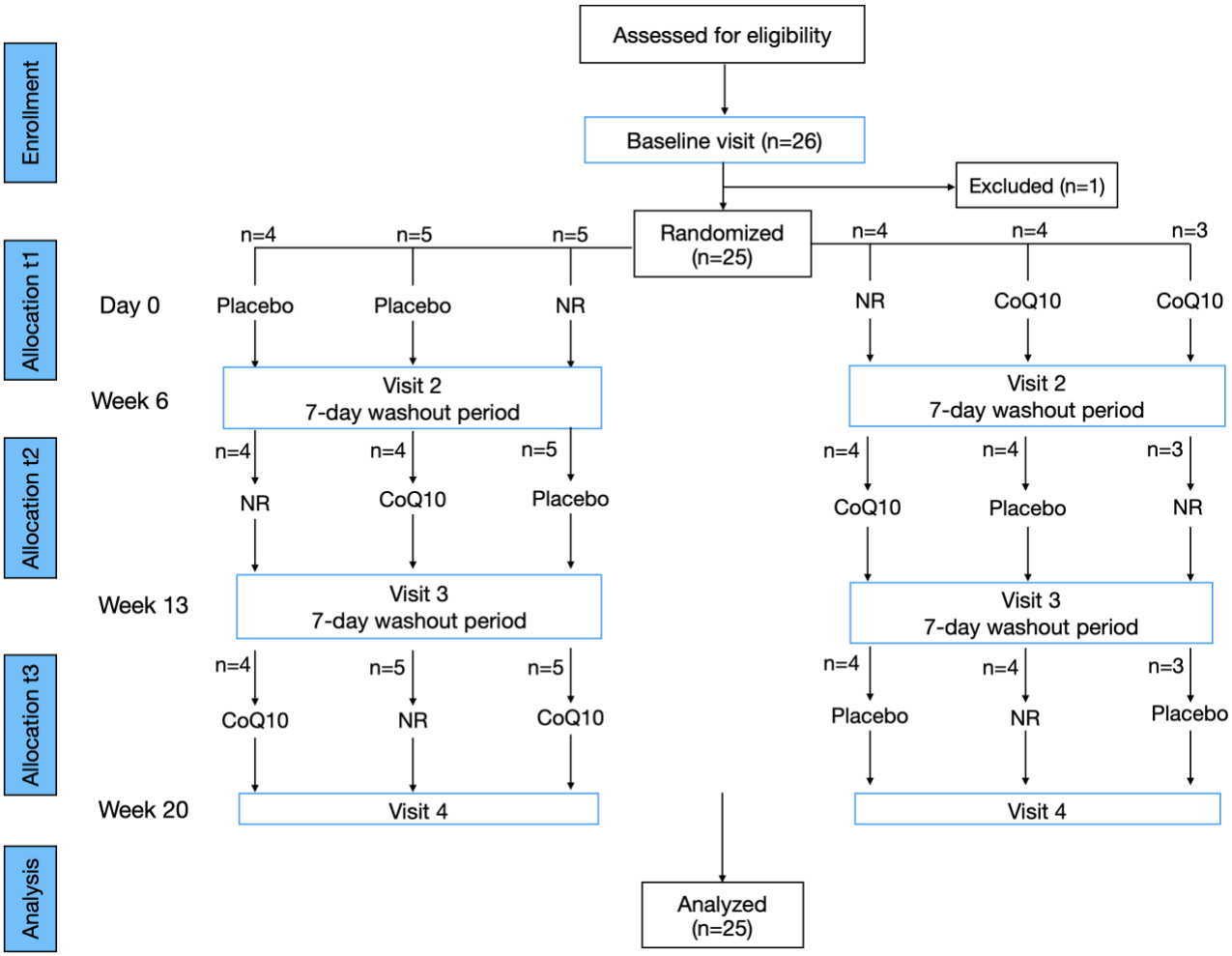
2.9 Supplemental materials

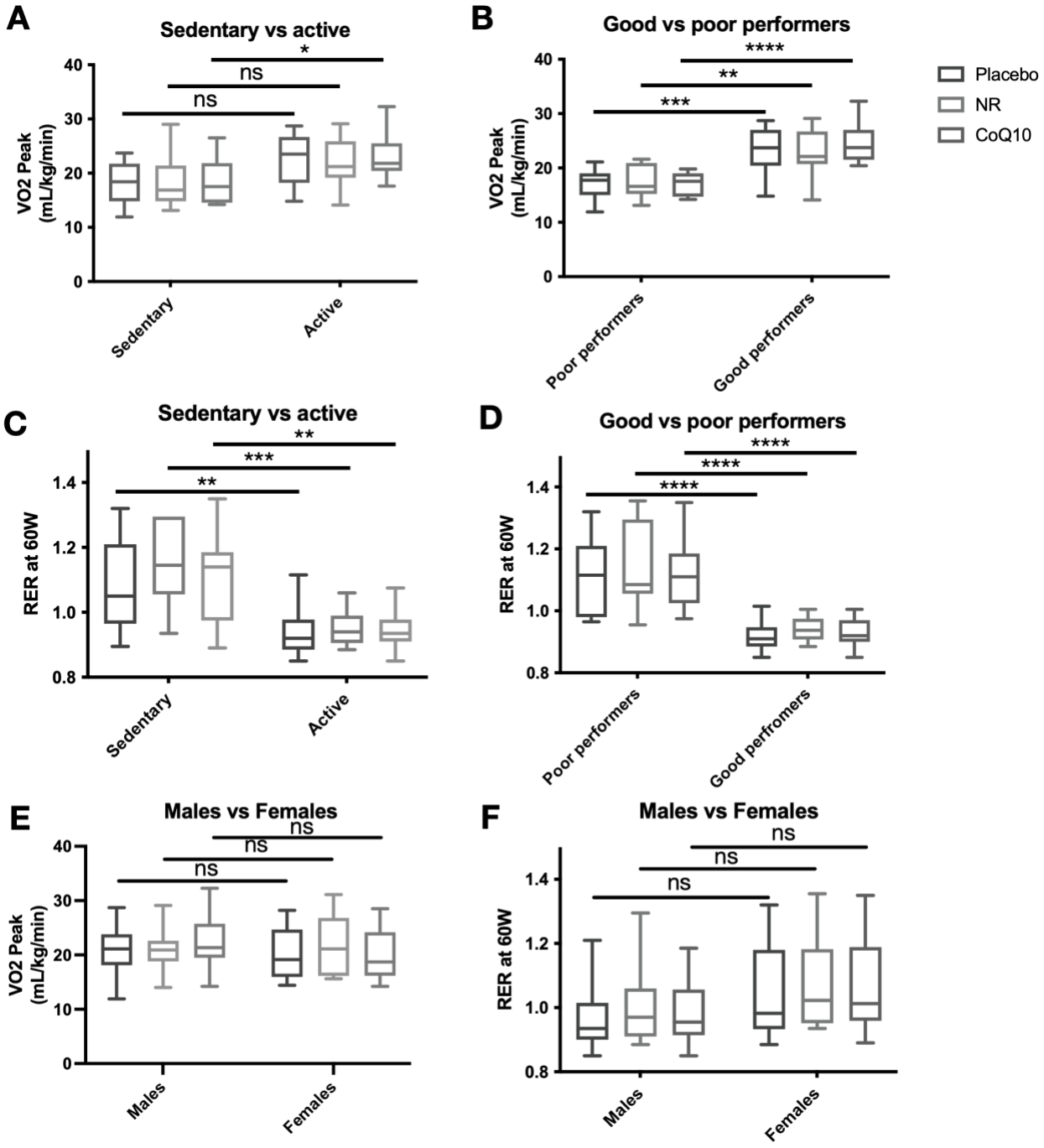
Supplemental Figure 1.1 Study design and sample size. Given the efficient crossover design of the study, all 25 participants received each supplement by the end of the study.



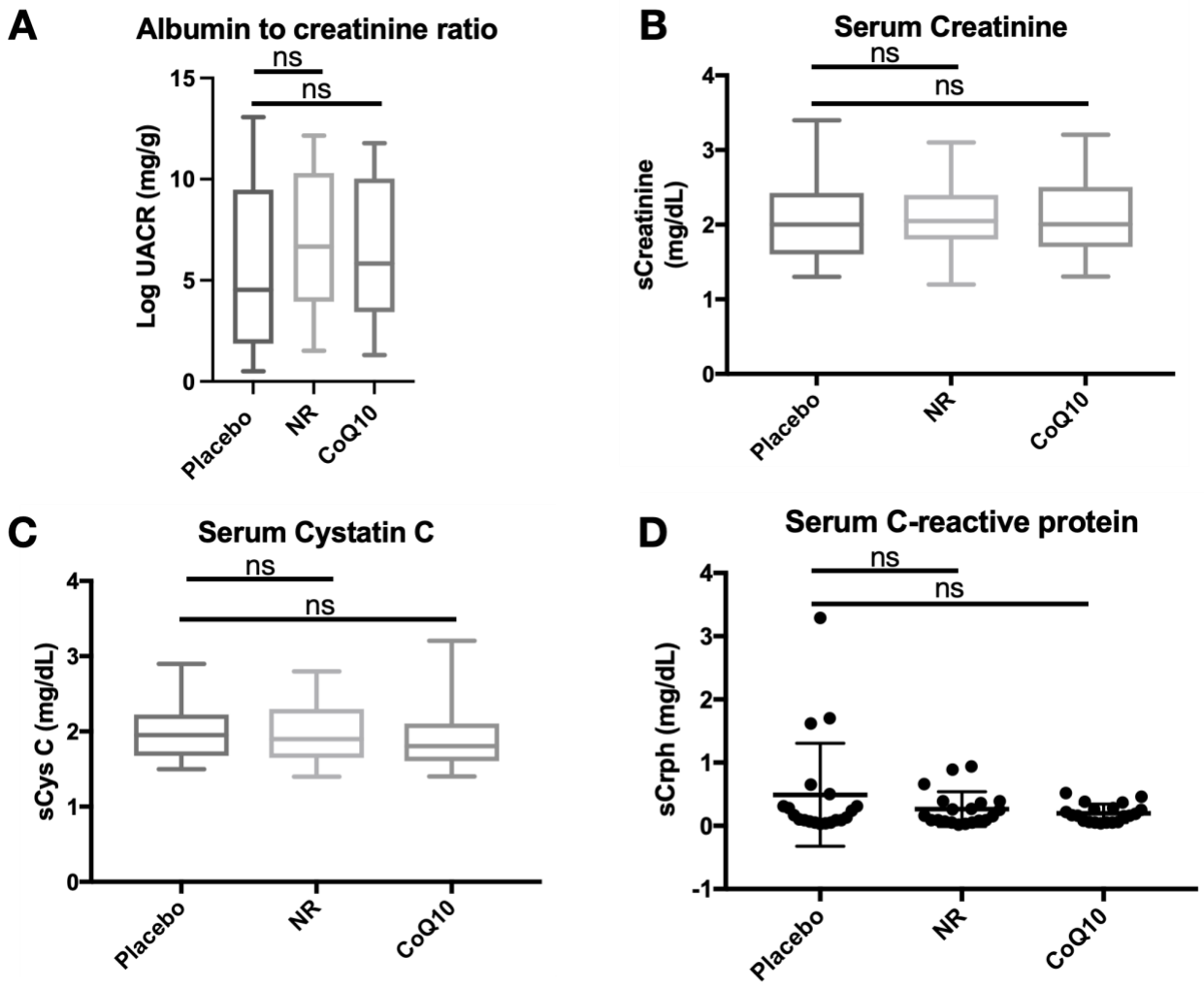
Supplemental Table 1. Estimates of difference in physical endurance outcomes comparing CoQ10 and NR to placebo. Mean difference and 95% CI are shown below.

Endpoints	NR vs. placebo (95% CI)	NR vs. placebo P-value	CoQ10 vs. placebo (95% CI)	CoQ10 vs. placebo P-value
Body weight, kg	-0.37 (-1.52 to 0.78)	0.51	0.74 (-3.11 to 4.64)	0.69
Systolic blood pressure at 30W, mmHg	5.48 (-4.01 to 14.98)	0.24	3.31 (-5.92 to 12.54)	0.46
Diastolic blood pressure at 30W, mmHg	2.81 (-2.31 to 7.93)	0.26	-1.51 (-5.69 to 2.66)	0.45
Systolic blood pressure at 60W, mmHg	4.32 (-4.29 to 12.95)	0.30	2.62 (-8.32 to 13.58)	0.62
Diastolic blood pressure at 60W, mmHg	3.76 (-1.77 to 9.29)	0.16	-0.33 (-6.22 to 5.55)	0.90
VO_2 peak, L/min	0.05 (-0.06 to 0.17)	0.36	-0.06 (-0.06 to 0.18)	0.33
VO_2 peak, mL/kg/min	0.68 (-0.93 to 2.3)	0.39	0.67 (-0.32 to 1.67)	0.17
VO_2 at 30W, L/min	-0.02 (-0.05 to 0.00)	0.03	0.00 (-0.05 to 0.05)	0.95
VO_2 at 30W, mL/kg/min	-0.31 (-0.79 to 0.16)	0.19	0.02 (-0.53 to 0.59)	0.91
VO_2 at 60W, L/min	-0.03 (-0.06 to 0.00)	0.07	0.00 (-0.01 to 0.03)	0.35
VO_2 at 60W, mL/kg/min	-0.28 (-0.83 to 0.26)	0.29	0.17 (-0.24 to 0.59)	0.39
Total work, kJ	2.59(-10.03 to 4.84)	0.47	1.18(-7.18 to 9.54)	0.77
Work efficiency, kJ/(L/min)	-1.33 (-5.04 to 2.37)	0.46	1.56 (-3.79 to 6.92)	0.55
Work efficiency, kJ/(mL/kg/min)	-0.11(-0.42 to 0.20)	0.47	0.01(-0.32 to 0.35)	0.91
Test Duration, seconds	-26.5 (-86.7 to 33.6)	0.37	10.7 (-82.5 to 103.8)	0.81
RER at rest	0.01(-0.02 to 0.05)	0.41	0.00 (-0.02 to 0.04)	0.62
RER at 30W	0.02 (0.00 to 0.05)	0.06	0.01 (-0.01 to 0.05)	0.24
RER at 60W	0.02(0.00 to 0.04)	0.04	0.01(0.00 to 0.03)	0.18
RER at VO_2 max	0.01 (-0.01 to 0.04)	0.44	0.00 (-0.04 to 0.04)	0.87

Supplemental Figure 1.2 Stratified analysis of changes in cardiorespiratory fitness and RER at 60W comparing higher vs lower performers and males vs females during cycle ergometry (n=25). (A and C) VO_2 peak and RER at 60W comparison between sedentary (n=9) vs active (n=16) participants. (B and D) VO_2 peak and RER at 60W comparison between good (n=15) vs poor (n=10) performers. (E and F) VO_2 peak and RER at 60W comparison between males (n=15) and females (n=10). Bar graphs represent median (IQR), and the whiskers represent minimum and maximum values. ****P<0.0001,***P <0.001, **P < 0.01, *P < 0.05.



Supplemental Figure 1.3 Kidney function and inflammatory biomarkers are not impactive by NR or CoQ10 treatment. (A) log-transformed urine albumin to creatinine ratio, (B) serum creatinine, (C) serum cystatin C, and (D) serum C-reactive protein. Bar graph represents mean, and the error bars represent SD for (A and D). Box plots represent median (IQR), and the whiskers represent minimum and maximum values for (B and C).



Supplemental Table 2. Differences in plasma lipid profile in response to CoQ10 treatment compared to placebo.

Lipids	Fold difference (CoQ10/placebo)	Effect size (95% CI)	P-value
CE 16:0	1.24	0.53 (0.21 to 0.85)	0.001
TG 59:3	0.60	-0.39 (-0.70 to 0.08)	0.012
TG 54:4	0.78	-0.3 (-0.70 to 0.07)	0.013
PC 30:0	1.26	0.38 (0.07 to 0.68)	0.015
LPE 18:1	0.78	-0.38 (-0.68 0.07)	0.015
FA 18:2 (linoleic acid)	1.40	0.36 (0.05 to 0.66)	0.020
TG 56:4	0.76	-0.36 (-0.66 to -0.05)	0.022
LPC 14:0	1.17	0.35 (0.05 to 0.66)	0.022
PC 31:1	1.20	0.36 (0.04 to 0.66)	0.022
PC 16:0/9:0 CHO	0.87	-0.35 (-0.66 to -0.04)	0.024
FA 20:3 (homo-gamma-linolenic acid)	1.24	0.35 (0.04 to 0.65)	0.024
FA 20:2 (eicosadienoic acid)	1.29	0.35 (0.04 to 0.65)	0.024
CE 18:1	1.14	0.35 (0.04 to 0.66)	0.025
FA 14:1 (physeteric acid)	1.31	0.34 (0.03 to 0.64)	0.029
PC P-36:5 or PC O-36:6	1.26	0.34 (0.02 to 0.64)	0.032

DAG 38:6	0.83	-0.33 (-0.64 to -0.02)	0.033
TG 56:3	0.51	-0.32 (-0.63 to -0.02)	0.036
TG 58:4	0.34	-0.32 (-0.62 to -0.01)	0.038
FA 12:0 (lauric acid)	1.30	0.30 (0.01 to 0.60)	0.045
TG 58:8	0.70	-0.31(-0.62 to -0.01)	0.045
TG 56:6	0.81	-0.31 (-0.61 to 0.00)	0.046
FA 18:1 (oleic acid)	1.35	0.30 (0.00 to 0.60)	0.047
FA 17:0 (margaric acid)	1.19	0.30 (0.00 to 0.60)	0.047
FA 17:1	1.30	0.30 (0.00 to 0.60)	0.047

Fold changes and effect sizes were obtained by linear mixed effects modeling adjusted for fasting status. Only lipids with $P<0.05$ are shown. CE; cholesterol ester, FA; fatty acid, DAG, diacylglycerol; PC, phosphatidylcholine; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; TG, triacylglycerol.

Supplemental Table 3. The impact of NR supplementation on plasma lipid profile compared to placebo.

Lipids	Fold difference (NR/placebo)	Effect size (95% CI)	P-value
CE 18:1	1.22	0.53 (0.21 to 0.85)	0.001
CE 16:0	1.24	0.53 (0.20 to 0.84)	0.001
TG 49:1	0.62	-0.42 (-0.73 to -0.11)	0.007
LPE 20:4	0.86	-0.42 (-0.73 to -0.10)	0.008
TG 51:1	0.66	-0.41(-0.72 to -0.10)	0.009
TG 49:2	0.72	-0.40 (-0.71 to -0.09)	0.011
LPE 18:1	0.77	-0.39 (-0.69 to -0.08)	0.013
TG 54:1	0.56	-0.38 (-0.69 to -0.07)	0.014
Cer d42:0	0.85	-0.38 (-0.69 to -0.07)	0.016
PE 38:5	0.83	-0.38(-0.68 to -0.06)	0.016
TG 52:1	0.59	-0.37 (-0.68 to -0.06)	0.017
Cer d40:0	0.86	-0.37 (-0.67 to -0.05)	0.019
TG 54:2	0.60	-0.36 (-0.67 to -0.05)	0.021
TG 51:2	0.73	-0.36 (-0.66 to -0.04)	0.022
TG 50:0	0.48	-0.35 (-0.65 to -0.04)	0.024

Cer d34:0	0.82	-0.35 (-0.65 to -0.04)	0.026
Cer d44:1	0.91	-0.35 (-0.65 to -0.03)	0.027
DG 38:6	0.82	-0.34 (-0.65 to -0.03)	0.027
LPE 22:6	0.88	-0.34 (-0.65 to -0.03)	0.027
Cer d34:1	0.92	-0.33 (-0.64 to -0.02)	0.032
PC P-44:4 or PC O-44:5	1.07	0.32 (0.01 to 0.63)	0.037
GlcCer d38:1	0.92	-0.32 (-0.63 to -0.01)	0.037
PE 38:4 Isomer B	0.87	-0.32 (-0.63 to -0.01)	0.038
PE 34:1	0.79	-0.32 (-0.62 to -0.01)	0.039
TG 48:3	0.70	-0.31(-0.62 to -0.01)	0.041
LPC 18:0	0.89	-0.31(-0.62 to -0.01)	0.042
LPC 22:4	0.71	-0.31(-0.61 to -0.01)	0.042
PC P-36:5 or PC O-36:6	1.24	0.31 (0.00 to 0.62)	0.046
LPC 18:1	0.90	-0.30 (-0.61 to -0.00)	0.047
TG 49:3	0.77	-0.31 (-0.61 to -0.01)	0.048

Fold changes and effect sizes were obtained by linear mixed effects modeling adjusted for fasting status. Only lipids with $P<0.05$ are shown. CER; ceramide, DAG, diacylglycerol; PE; phosphatidylethanolamine; LPC, Lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; TG, triacylglycerol.

Supplemental Table 4. Treatment-emergent adverse events ordered by total frequency (n=25). Treatment emergent adverse events were counted from the start of treatment until the end of the washout period.

Adverse events	CoQ10	NR	Placebo
Upper respiratory illness	1 (4%)	1 (4%)	1 (4%)
Nausea	0 (0%)	1 (4%)	1 (4%)
Atrial fibrillation	1 (4%)	0 (0%)	0 (0%)
Cramping	0 (0%)	1 (4%)	0 (0%)
Hyperglycemia	0 (0%)	1 (4%)	0 (0%)
Hypotension	0 (0%)	0 (0%)	1 (4%)
Palpitations	0 (0%)	1 (4%)	0 (0%)
Right bundle branch block	1 (4%)	0 (0%)	0 (0%)
Stiff neck	0 (0%)	1 (4%)	0 (0%)
Swollen arm	0 (0%)	0 (0%)	1 (4%)

Chapter 3

Chronic kidney disease is associated with attenuated plasma metabolome response to oral glucose tolerance testing

3.1 Preface

This chapter was originally published in the *Journal of Renal Nutrition*:

Ahmadi A, Huda MN, Bennett BJ, et al. Chronic Kidney Disease is Associated With Attenuated Plasma Metabolome Response to Oral Glucose Tolerance Testing. *J Ren Nutr*. 2023.

The article has been modified to satisfy the formatting requirements of this dissertation.

3.2 Abstract

Objective: CKD is associated with decreased anabolic response to insulin contributing to protein-energy wasting. Targeted metabolic profiling of oral glucose tolerance testing (OGTT) may help identify metabolic pathways contributing to disruptions to insulin response in CKD.

Methods: Using targeted metabolic profiling, we studied the plasma metabolome response in 41 moderate-to-severe non-diabetic CKD patients and 20 healthy controls at fasting and 2 hours after an oral glucose load. We used linear mixed modeling with random intercepts, adjusting for age, sex, race/ethnicity, body weight and batch to assess heterogeneity in response to OGTT by CKD status.

Results: Mean eGFR among CKD participants was 38.9±12.7 ml/min per 1.73 m^2 compared 87.2±17.7 ml/min per 1.73 m^2 among controls. Glucose ingestion induced an anabolic response resulting in increased glycolysis products and a reduction in a wide range of metabolites including amino acids, TCA cycle intermediates, and purine nucleotides compared to fasting. Participants with CKD demonstrated a blunted anabolic response to OGTT evidenced by significant changes in 13 metabolites compared to controls. The attenuated metabolome response predominant involved mitochondrial energy metabolism, vitamin B family, and purine nucleotides. Compared to controls, CKD participants had elevated lactate: pyruvate (L:P) ratio and decreased GTP:GDP ratio during OGTT.

Conclusion: Metabolic profiling of OGTT response suggests a broad disruption of mitochondrial energy metabolism in CKD patients. These findings motivate further investigation into the impact of insulin sensitizers and mitochondrial targeted therapeutics on energy metabolism in patients with non-diabetic CKD.

3.3 Introduction

Chronic kidney disease (CKD) is a public health burden worldwide with the estimated global prevalence of 14% affecting over 650 million people globally (1). Insulin resistance (IR) is one of the very early metabolic alterations in CKD increasing with severity of kidney disease (2). IR is considered a cardio-metabolic risk factor correlated with increased systemic inflammation (3) and oxidative stress (4). Multiple pathophysiologic features of CKD contribute to IR in CKD including chronic inflammation (5), altered gut microbiome (6), oxidative stress (7), metabolic acidosis (8),

and accumulation of uremic toxins (6). However, evidence for the biologic basis for IR in humans with CKD is lacking.

Although tissue insensitivity to insulin is the main contributor to IR, changes in insulin secretion and degradation have also been established in CKD (9). In particular, a recent study using the gold-standard high-dose hyperinsulinemic-euglycemic clamp to investigate peripheral insulin sensitivity showed disturbances in insulin clearance as a principle characteristic distinguishing patients with non-diabetic CKD to controls (10). This study demonstrated profound alterations in the plasma metabolome response to insulin in patients with CKD compared to controls. However, understanding of the biologic basis for IR in humans with CKD at more physiologic, endogenous levels of insulin, such as that elicited by OGTT remain unknown.

OGTT provides estimates of IR (11) but also captures additional physiologic processes by stimulating gut-derived incretin hormones known to augment insulin secretion (12, 13). Metabolomic profiling of the response to OGTT may reveal specific metabolites and metabolic pathways underlying impaired glucose homeostasis in CKD thus identifying therapeutic targets for improving insulin sensitivity in this vulnerable population. We performed targeted plasma metabolic profiling comparing non-diabetic patients with moderate-severe CKD to healthy controls during an oral glucose challenge. We hypothesize that CKD, in the absence of diabetes is associated with impaired plasma metabolic response to oral glucose challenge compared to controls. Our goal was to identify specific metabolic alterations in non-diabetic CKD to better understand the metabolic and biological basis for glucose intolerance and IR in CKD.

3.4 Materials and Methods

Study Population and Study design: The Study of Glucose and Insulin in Renal Disease (SUGAR) is a cross-sectional study of glucose and insulin metabolism in moderate-to-severe nondiabetic CKD. Among the 98 recruited participants, 95 had adequate plasma samples collected for metabolomics, and of these, 61 had plasma samples before and after OGTT (10). Sixty-one participants were included from the SUGAR study where 41 of them were moderate to severe non-diabetic CKD patients (eGFR < 60 ml/min per 1.73 m^2) and 20 healthy controls (eGFR > 60 ml/min per 1.73 m^2). Plasma metabolites were measured after overnight fasting, 2h after a 75g oral glucose load for OGTT and during the clamp as reported previously (15). Plasma biomarkers of kidney function and inflammation were measured in the fasting blood. A detailed description of the study design and distribution of the clinical phenotypes between CKD patients and healthy controls has been reported earlier (10).

Metabolic profiling: Targeted metabolomics based in a liquid chromatography-tandem mass spectroscopy (LC-MS/MS) platform was performed at the Northwest Metabolomics Research Center as described previously (14). Briefly, blood samples were prepared by protein precipitation (methanol), centrifugation (21,694 g for 10 minutes at 4 °C), drying and reconstitution. The LC-MS/MS analyses were performed using an Agilent 1260 LC (Agilent Technologies) with a SeQuant ZIC-cHILIC column (150 × 2.1 mm × particle size 3.0 μm, Merck KGaA), which was coupled to a AB Sciex QTrap 5500 MS (AB Sciex) system equipped with a standard electro-spray ionization (ESI) source. Each sample was injected two times for analysis in positive and negative ionization modes (2 μl and 10 μl respectively). The flow rate was at 0.3 ml/min. Metabolites identities were determined by spiking pooled serum samples with mixtures of standard compounds. Pooled serum samples were used as quality control samples and were run after every

10 biological samples to monitor for instrument drift and signal normalization if needed. A total of four isotope-labeled internal standards were used to monitor sample preparation. A total of 88 metabolites were detected pre and post OGTT. More details for sample preparation, sample collection times, and reagents has been described earlier (15).

Statistical analysis: Plasma metabolite data were available from 61 subjects at fasting and 2h post-oral glucose load. Raw metabolite data were converted to ratio of 2h post-oral glucose load and corresponding fasting values and checked for batch effect of the ratio values. All clinical and metabolite data were checked for normality. To account for drift of sample preparation batches, raw metabolite data was normalized using Systematic Error Removal Using Random Forest (SERRF)(16). To account for the correlation of measurements within participants, we examined the fold changes associated with the OGTT procedure via a linear mixed model with random intercepts, regressing the log transformed SERRF normalized metabolite on the sample type (during OGTT versus fasting sample), adjusting for age, sex, race/ethnicity, body weight and batch. To evaluate whether the effect of the oral glucose challenge procedure differed between CKD and non-CKD participants, we used linear mixed effect modeling with random intercepts where SERRF normalized metabolites were regressed on a sample type (OGTT vs fasting), CKD status, and their interaction, additionally adjusting for covariates listed above. Data are presented as means ± SD unless otherwise indicated. Statistical analysis was performed using R 3.6.1 for windows release (17). P-values were adjusted for multiple comparisons using the "Benjamini Hochberg" approach, and an adjusted p-value <0.05 was considered significant for all analyses unless stated otherwise.

Pathway-associated metabolite sets enrichment and metabolite pathway analysis were performed using MetaboAnalystR v4.0 (18) with the KEGG human metabolite database (19). Pathway

analysis using SERRF normalized pre OGTT and post OGTT metabolite level was used to evaluate metabolic changes in response to OGTT in the entire cohort. Significantly altered metabolic pathway by CKD status was determined by comparing the changes in metabolite levels calculated by subtracting log-transformed post OGTT SERRF normalized values to pre OGTT time points.

Differences in insulin sensitivity (Matsuda index) between CKD and controls were evaluated by Mann-Whitney U test using GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA). Differences in plasma metabolites including GTP:GDP ratio and L:P ratio as well as differences in insulin secretion between fasting and 2h post-oral glucose load were evaluated using ANOVA for multiple hypothesis testing (Bonferroni).

Metabolite data were checked for excessive missing values by using the R package WGCNA's (20) "goodSampleGenes" test. The correlation between kidney biomarkers was determined by Spearman correlation. The ratio of plasma metabolites post oral glucose to pre glucose challenge was used for correlation network analysis using WGCNA R package. A soft threshold approach was used with a power of 6 (based on scales free topology) in a WGCNA default unsigned network with dynamic tree cutting (deep split = 2) and a min Module Size = 3 as parameters for the dynamic tree cut function (21). The module eigengene, defined as the first principal component (PC) of a module's metabolite concentration matrix, was used to calculate the Spearman correlation between a metabolite module and kidney biomarkers.

3.5 Results

Characteristics of the study participants. The study included 41 CKD participants with a mean eGFR of 38.9±12.7 ml/min per 1.73 m^2, and 20 control subjects with a mean eGFR of 87.2±17.7

ml/min per 1.73 m^2. The mean age was 61.2±12.9 years, 44% were women, and 22% were self-reported Black (Table 1). Compared with controls, participants with CKD had higher body weight, lower fat-free mass, higher BMI, and daily calorie intake, and greater plasma inflammatory markers.

Table 1. Demographic characteristics by CKD status of analytic population (n=61).

	Controls N=20	**Chronic Kidney disease** N=41
Demographics:		
Age (yr), mean (SD)	60.3 (12.9)	61.6 (14.3)
Female, number (%)	9 (45)	18 (44)
Race/ethnicity, number (%)		
White	16 (80)	28 (68)
Black	4 (20)	10 (24)
Asian/Pacific Islander	0 (0)	3 (7)
Physical characteristics, mean (SD)		
Weight (Kg)	85.9(21.4)	89.5(20.3)
Fat-free mass (kg)	54.3(11.9)	51.9(11.5)
BMI, mean (SD)	28.7(6.43)	30.2(6.16)
Dietary data		
Total calorie intake (kcal), mean (SD)	1987.1(615.3)	1819.0(573.1)
Daily protein intake (g), mean (SD)	75.0(23.1)	74.4(29.8)
Laboratory data:		
eGFR (mL/min/1.73m2), mean (SD)	87.2 (17.7)	38.9 (12.7)
Serum creatinine (mg/dL), median (IQR)	0.9 (0.81-1.0)	1.67 (1.50-1.80)
Serum cystatin C (mh/dL), median (IQR)	0.89 (0.68-0.97)	1.49 (1.40-1.81)

Urine albumin excretion rate (mg/24 hours), median (IQR)	5 (3.4-7.8)	127.15 (20.57-422.85)
CRP (mg/dL), median (IQR)	0.11 (0.05-0.25)	0.23 (0.13-0.27)
IL-1β (pg/mL), median (IQR)	0.09 (0.05-0.14)	0.12 (0.08-0.15)
IL-6 (pg/mL), median (IQR)	0.84 (0.56-1.19)	0.99 (0.83-1.51)
IFN-ɣ (pg/mL), median (IQR)	2.24 (1.09-4.15)	3.26 (2.59-5.62)
TNFa (pg/mL), median (IQR)	1.49 (0.75-1.95)	2.53 (2.23-2.85)
Baseline medication:		
Antihypertensive medication, number (%)	8 (40.0)	31 (86.1)
RAASi	4 (20)	22 (61.11)
Diuretic	2 (10)	15 (41.6)
Statin	3 (15)	14 (38.9)
Beta blocker	2 (10)	12 (33.3)
CCBs	2(10)	18 (50)

Abbreviations: CB, calcium channel blocker; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IQR, interquartile range; RAASi, renin-angiotensin-aldosterone system inhibitor; SD, standard deviation. Chronic kidney disease was defined as estimated glomerular filtration rate < 60 mL/min per 1.73 m^2; controls as ≥ 60 mL/min per 1.73 m^2.

Oral glucose challenge (OGTT) was associated with a significant reduction in a wide range of metabolites primarily amino acids, purine nucleotides, and dicarboxylic acids. After adjusting for age, sex, race/ethnicity, body weight, and LC-MS batch, 65% (58/88) of the detected plasma metabolites were significantly altered post oral glucose challenge in the overall cohort (Table 2 and Supplemental Table 1). The largest reductions in individual metabolite concentrations post OGTT were observed for linoleic acid, ADP, and nicotinamide with a percent change of -82%, -79%, and -63%, respectively (Table 2). Only 6% (5/88) of the detected metabolites significantly increased post OGTT compared to fasting state. These metabolites included erythrose, glucose,

and kynurenate with a percent change of +53%, +28%, and +17% respectively (Supplemental Table 2).

Table 2. Differences in plasma metabolites during OGTT, compared with the fasting state.

Metabolite	Fold change between the OGTT/fasting states (95% CI)	p-value
Linolenic acid	0.18 (0.15, 0.21)	< 0.001
ADP	0.21 (0.14, 0.32)	< 0.001
Niacinamide	0.37 (0.29, 0.48)	< 0.001
Malonic acid	0.38 (0.28, 0.51)	< 0.001
Taurine	0.49 (0.41, 0.58)	< 0.001
Aspartic acid	0.51 (0.43, 0.6)	< 0.001
Adenylosuccinate	0.53 (0.44, 0.65)	< 0.001
iso-Leucine	0.55 (0.5, 0.6)	< 0.001
Leucine	0.56 (0.51, 0.61)	< 0.001
Citrulline	0.58 (0.53, 0.62)	< 0.001
PGE	0.6 (0.53, 0.69)	< 0.001
Succinate	0.61 (0.55, 0.68)	< 0.001
Tyrosine	0.65 (0.61, 0.69)	< 0.001
Sorbitol	0.66 (0.6, 0.72)	< 0.001
Methionine	0.67 (0.62, 0.71)	< 0.001
Cystathionine	0.7 (0.59, 0.84)	< 0.001
Glutamic acid	0.7 (0.6, 0.82)	< 0.001
2-Hydroxyglutarate	0.7 (0.62, 0.78)	< 0.001
Serine	0.71 (0.66, 0.76)	< 0.001
MethylSuccinate	0.72 (0.67, 0.78)	< 0.001

Top 20 based on p-value and fold change are shown in the table. Fold changes are adjusted to age, sex, race, body weight and batch effect. The fold changes are compared to the fasting state. For example, Nicotinamide has fold change of 0.37 meaning it had a 63% reduction post OGTT compared to the fasting state.

Insulin secretion and sensitivity measured during OGTT did not differ among participants with and without CKD. We evaluated plasma insulin concentrations measured at 0, 10, 20, 30, 60, 90, and 120 minutes after glucose ingestion and found no significant differences between CKD and controls (Figure 1A). Similarly, the Matsuda index estimation of insulin sensitivity was not significantly different in CKD compared to controls (p-value=0.08) (Figure 1B). The median Matsuda index in CKD and controls was 3.93 (IQR of 2.44, 5.63) and 3.70 (IQR of 2.30, 9.13) respectively.

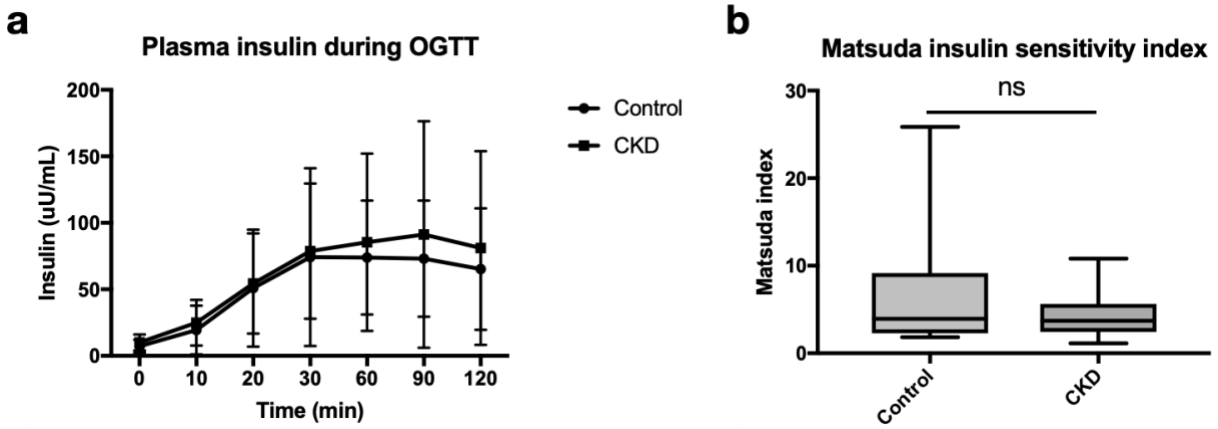


Figure 3.1 Measurements of insulin secretion and insulin sensitivity comparing CKD (n = 40) and controls (n = 20) during OGTT. (A) Plasma insulin concentration. Data points represent means and error bars represent 95% CI. (B) Matsuda index. One-way ANOVA for multiple comparison testing (A) and Mann-Whitney U test (B) were used.

CKD attenuates the plasma metabolome response to glucose challenge in metabolites from predominantly the vitamin B family, TCA cycle intermediates, and purine nucleotides. After adjustment, changes in 15% (13/88) of detected plasma metabolites were significantly altered between CKD and controls post OGTT (Table 3 and Supplemental Table 2). Overall, CKD was associated with higher plasma levels of these metabolites such as succinate, inositol, nicotinamide, and glucose in response to OGTT compared to control. A notable exception to this was kynurenate. These metabolic changes in response to OGTT are not driven by alterations in the fasting state

concentrations of these metabolites in CKD versus controls (Supplemental Table 3). Except for IMP, the rest of significantly altered metabolites among CKD participants in response to OGTT were either similar or lower during fasting compared to controls (Supplemental Table 3). In general, controls had on average a greater decline in plasma metabolites in response to the oral glucose challenge compared to participants with CKD. The largest difference in response to the glucose challenge comparing CKD and controls were observed in ADP, glycochenodeoxycholate, and IMP. The ratio in fold change comparing CKD to control for these metabolites were 3.7, 3.6, and 3.1 respectively (Table 3).

Table 3. Differences in plasma metabolites response post oral glucose tolerance test by chronic kidney disease status.

Metabolite	Fold change between OGTT/fasting (95% CI) in	Fold change between OGTT/fasting (95% CI) in	p-value for interaction	Pathway
	Control	CKD		
Succinate	0.48 (0.37, 0.62)	0.78 (0.71, 0.85)	< 0.001	TCA Cycle
Taurine	0.41 (0.28, 0.6)	0.75 (0.66, 0.85)	0.0032	Amino acids metabolism/Sulfur metabolism
Adenylosuccinate	0.41 (0.26, 0.65)	0.84 (0.72, 0.98)	0.0037	Nucleotide/Purine metabolism
ADP	0.17 (0.07, 0.42)	0.63 (0.47, 0.86)	0.0063	Nucleotide/Purine metabolism
Biotin	0.74 (0.59, 0.93)	1.02 (0.94, 1.1)	0.011	Vitamins
Niacinamide	0.35 (0.2, 0.6)	0.7 (0.58, 0.84)	0.019	Vitamins
Glycochenodeoxycholate	0.47 (0.16, 1.35)	1.69 (1.18, 2.42)	0.025	Bile acid metabolism
Inositol	0.92 (0.85, 0.99)	1 (0.98, 1.03)	0.026	Glucose/inositol metabolism

Kynurenate	1.07 (0.82, 1.4)	0.78 (0.71, 0.85)	0.026	Amino Acid metabolism/Try
IMP	0.18 (0.07, 0.48)	0.55 (0.39, 0.76)	0.037	Nucleotide/Purine metabolism
Uridine	0.69 (0.49, 0.96)	0.99 (0.89, 1.11)	0.043	Nucleotide/Pyrimidine metabolism
Glucose	1.07 (0.88, 1.31)	1.34 (1.25, 1.43)	0.043	Glycolysis/sugar
GDP	0.73 (0.59, 0.89)	0.9 (0.84, 0.97)	0.05	Nucleotide/Purine metabolism

Fold changes between OGTT and fasting states by CKD status are adjusted for age, sex, race, weight, and batch. Fold changes are compared to the fasting state. For example, a fold change of 0.78 indicates a 22% reduction in metabolite level. p-value for interaction test the heterogeneity in fold change by CKD status.

The response to the oral glucose challenge demonstrates a broad disruption of amino acid and mitochondrial energy metabolism in CKD patients. In the entire cohort, oral glucose challenge impacted alpha-Linoleic acid metabolism (p-value=3.84 x 10^{-31}), aminoacyl-tRNA biosynthesis (p-value=1.15 x 10^{-26}) and arginine biosynthesis (p-value=2.66 x 10^{-25}) (Figure 2A). In addition, metabolic pathways involving amino acid metabolism such as branch chain amino acids (valine, leucine, isoleucine), phenylalanine, and histidine metabolism were also impacted. Compared to controls, participants with CKD demonstrated significant aberrations in alpha-Linoleic acid metabolism (p-value=2.91 x 10^{-5}), nicotinamide metabolism (p-value=5.18 x 10^{-5}), and arginine biosynthesis (p-value=1.96 x 10^{-4}) (Figure 2B).

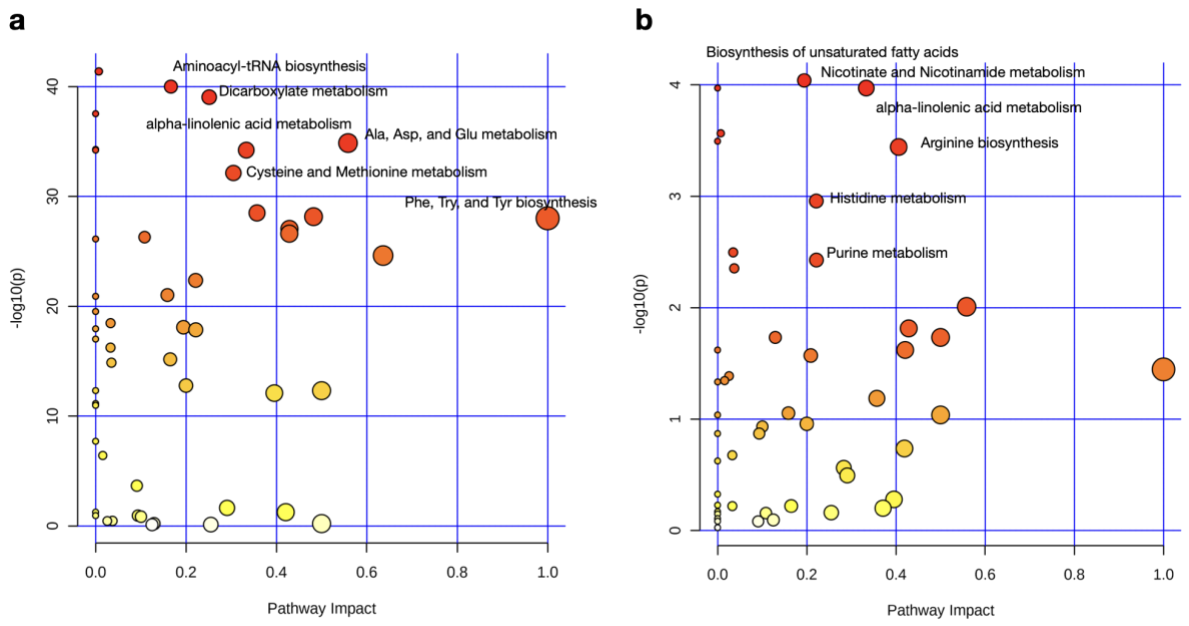


Figure 3.2 Pathway analysis of changes with oral glucose tolerance test (**A**) in the overall cohort (n=61) and (**B**) comparing CKD (n=41) with controls (n=20). The size and color of the nodes represent pathway impact value and p-value, respectively.

CKD is associated with greater lactate to pyruvate (L:P) ratio, an indicator of mitochondrial respiratory chain impairment. An elevated lactate to pyruvate ratio suggests respiratory chain dysfunction (22-24). L:P ratio at fasting and post-OGTT was higher in participants with CKD compared to controls (Supplemental Figure 1A). The median L:P ratio at fasting was 28.9 (IQR of 26.2, 31.2) for controls compared to 36.7 (IQR of 29.3, 42.3) among participants with CKD (p-value=0.0282). The high L:P ratio was sustained post OGTT with a median of 32.8 (IQR of 30.7, 37.2) in CKD in contrast to 29.5 (IQR of 27.8, 32.7) in controls (p-value=0.771) (Supplemental Figure 1A). The difference in L:P ratio is mostly driven by an increase in pyruvate levels however, lactate (fold change (FC), of 1.15 vs 1.20) and pyruvate (FC of 1.08 vs 1.34) elevations were not meaningfully different in CKD compared to controls during OGTT (Supplemental Table 2).

CKD is associated with disruption of GTP:GDP ratio post-OGTT. The GTP:GDP ratio is known to reflect the ATP:ADP ratio (25, 26). We looked at the GTP:GDP ratio during fasting and OGTT

separately. The median GTP:GDP ratio at fasting was similar in CKD and controls (p-value=0.767) (Supplemental Figure 1B). After OGTT, it is expected that the GTP and ATP levels will increase and ADP and GDP levels will decrease, thus resulting in greater GTP:GDP and ATP:ADP ratios (25, 27). As expected, the GTP:GDP ratio increased during OGTT compared to fasting in both groups with the median of 3.11 to 3.50 in controls (p-value=0.0236) and 3.01 to 3.13 in CKD (p-value=0.207); however, the ratio did not increase meaningfully in participants with CKD compared to controls. Participants with CKD had a significantly higher post-OGTT median GTP:GDP value of 3.13 [IQR of 2.77, 3.52] compared to 3.50 [IQR of 3.11, 3.89] in controls (p-value=0.039) (Supplemental Figure 1B).

Changes in plasma metabolites in response to OGTT is associated with CKD signature and inflammation markers. We performed weighted gene co-expression network analysis (WGCNA) to better understand how closely related metabolite groups associate with CKD. We identified 8 metabolite modules in response to glucose load. Of the total 8 metabolite modules, those indicated in blue and green modules were significantly correlated with CKD signature and known CKD-associated inflammation markers (Figure 3A, 3B, and 3C). Green module positively correlated with CKD status, plasma cystatin C, and plasma creatinine and negatively correlated with eGFR (Figure 3B). Some notable metabolites from the green module include uric acid, kynurenate, and malonic acid (Supplemental Table 4). The blue module also positively correlated with plasma cystatin C and plasma creatinine (Figure 3C). Both modules also positively correlated with plasma TNF-a (Figure 3C).

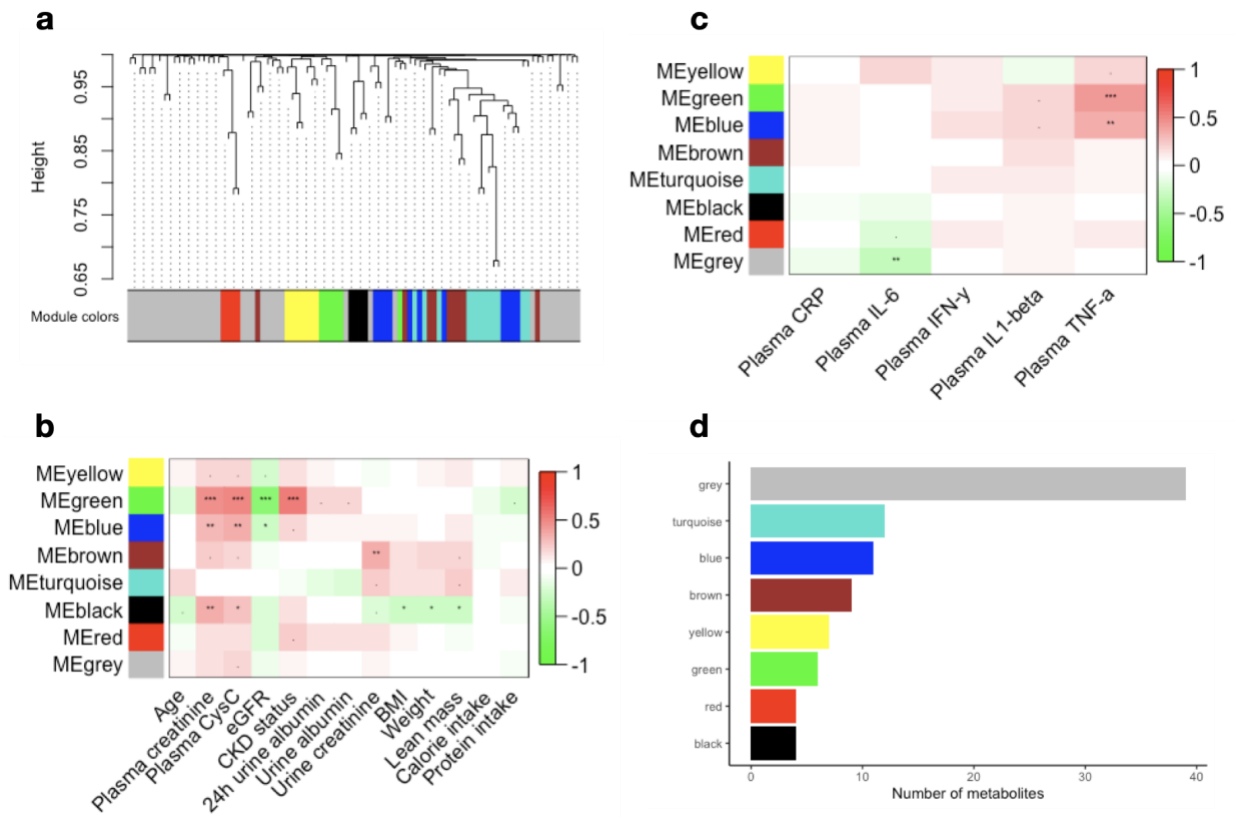


Figure 3.3: **Changes in the plasma metabolic profile due to an oral glucose load is associated with kidney disease and inflammatory markers**. a) Identified metabolite modules. Correlation between plasma metabolite modules and b) plasma biomarkers of kidney disease and c) inflammation markers. Each row in the table corresponds to a module, and each column represents a kidney biomarker, as indicated. The correlation between kidney and inflammatory biomarkers and metabolite modules were determined using spearman correlation. The correlation coefficient is color-coded as indicated in the color key legend (red = positive and green = negative correlation) "***" = p-value<0.001, "**" = p-value<0.01, "*" = p-value<0.05, "." = p-value<0.10. (d) The number of metabolites in each of the metabolite-module.

3.6 Discussion

Using targeted metabolic profiling, we identified several attenuated biological and metabolic pathways in response to an oral glucose challenge in the overall cohort with evidence of marked

heterogeneity by CKD status. First, we observed that compared to the fasting state OGTT resulted in a significant decrease in a wide range of metabolites including purine nucleotides and amino acids in the overall cohort. Second, patients with CKD demonstrate an attenuated plasma metabolome response to OGTT compared to controls independent of alterations in the fasting state. We observed an attenuated plasma response to OGTT particularly in vitamin B family members, mitochondrial energy metabolism, and purine metabolism. Together, our findings suggest CKD is associated with suppressed anabolic response to glucose challenge consistent with prior findings of impaired anabolic response to insulin in this population (15).

The application of metabolomics to the OGTT demonstrated several insights into the metabolic response to a glucose challenge. Consistent with previous studies, our cross-sectional data on 2-hour changes pre and post OGTT supports the known anabolic actions of insulin in promoting glycolysis and oxidative phosphorylation while suppressing proteolysis (28, 29). In addition to TCA cycle intermediates, we observed a decrease in all detected amino acids and their metabolites suggesting suppression of proteolysis and cataplerosis in the overall cohort that was comparable in magnitude to high-dose insulin clamp testing in a prior study (Table 2).

We observed significant heterogeneity by CKD status in the plasma metabolome response to glucose load revealing broad disruption in energy metabolism. In animal models, CKD leads to a post-insulin receptor defect in insulin signaling (30) contributing to disruption of lipid (31), carbohydrate (32), and protein metabolism (33) impacting global energy metabolism (34). We identified CKD-associated disruptions in GDP, ADP, and succinate; all metabolites involved in the TCA cycle. Despite equivalent levels at fasting compared to controls, these metabolites were significantly higher in CKD compared to controls post-OGTT suggesting an impaired insulin response to activate the TCA cycle (Table 3). These findings are also consistent with previous

study demonstrating diminished TCA cycle activity in patients with non-diabetic CKD (35). Our findings underscore the importance of IR associated with metabolic defects in CKD and its potential contribution to mitochondrial dysfunction. This association is supported by a recent study suggesting that muscle specific insulin receptor knockout in a murine model impaired mitochondrial respiration, decreased ATP production, and increased reactive oxygen species (36).

One prominent finding suggesting mitochondrial dysfunction in CKD was an elevated lactate-to-pyruvate (L:P) ratio in CKD compared to controls. The L:P ratio is known to be in near equilibrium with the $NADH/NAD^+$ ratio with an elevated L:P ratio serving as an indicator for impaired mitochondrial function and TCA cycle disorder (22, 37). A L:P ratio of >30 has been suggestive of respiratory chain dysfunction (24). Increased levels of pyruvate, lactate, and L:P ratio has also been shown in patients with acute kidney failure (38). Glycolysis products, lactate and pyruvate, were increased in both groups in response to OGTT signaling glycolysis activation. Lactate and pyruvate however were also both more elevated in CKD compared to the controls suggesting impaired TCA cycle and oxidative phosphorylation upon glycolytic activation (Supplemental Table 2). We also observed an elevated (L:P>30) L:P ratio in CKD compared to controls both at fasting and post-OGTT suggesting mitochondrial metabolism dysfunction (Supplemental Figure 1A). Taken together our findings suggest persons with CKD have impaired mitochondrial metabolism evidenced by metabolic profiles suggesting blunted activation of TCA cycle, redox imbalance, as well as disruption in the mitochondrial respiratory chain.

CKD was associated with disruption in purine and pyrimidine metabolism, a crucial source of necessary energy and cofactors needed for bioenergetics and biomolecular demands of metabolism (39). Purines are found in biomolecules such as ATP, GTP, cyclic AMP, NADH, and coenzyme A (40). We noted several indicators of impairment in the purine nucleotide cycle. First, four

metabolites involved in purine nucleotide cycle GDP, ADP, adenylosuccinate, and IMP all had attenuated reduction in response to the glucose challenge in CKD compared to controls (Table 3). Fluctuations in ATP, ADP, and AMP and corresponding changes in GTP and GDP are regulated by purine nucleotide cycle, particularly in skeletal muscle (41, 42). The purine cycle also enhances glycolysis and the TCA cycle via enhancing the rate of glycolysis by activating phosphofructokinase (PFK) and production of fumarate (43, 44). Second, to broadly assess disruptions in its regulatory role of energy molecules during glycolytic changes, we investigated changes in GTP:GDP ratios at fasting and during OGTT in both groups. Despite similar fasting GTP:GDP ratios in CKD compared to controls, CKD patients had lower GTP:GDP ratio post-OGTT suggesting an impairment in the purine nucleotide cycle interfering with energy generation. These findings in the plasma are hypothesis generating and motivate interrogation in human tissues to confirm a mechanistic link between CKD and impaired energy metabolism specifically focusing on the impact of CKD on ATP:ADP ratio and purine and pyrimidine metabolism.

CKD was also associated with disruption of metabolites in the vitamin B family with OGTT. Vitamin B family members are essential for metabolism involved in major metabolic pathways acting as cofactors in catabolic and anabolic pathways including carbohydrate, protein, and fat metabolism (45). We found a decrease in vitamin B family derivatives in response to OGTT in both groups. Among vitamin B family members, the most significant decrease post OGTT was observed in nicotinamide (a form of vitamin B3), important in various oxidation/reduction reactions. Compared to controls where nicotinamide levels profoundly decreased in response to OGTT, the levels of nicotinamide in participants with CKD remained elevated (Table 3) consistent with our prior findings using insulin clamp testing (15). Our data confirms findings from these other studies showing nicotinamide and other NAD^+ catabolites accumulating in uremic patients

suggesting an insulin mediated defect to use nicotinamide during anabolic reactions. Dynamic changes in other B vitamins were also detected post-OGTT (Supplemental Table 2). Together, the attenuated response of vitamin B family members post-OGTT in CKD versus controls points toward an impairment in activating insulin mediated anabolic pathways involving accumulation of these cofactors in patients with CKD.

There were several metabolic profile changes in response to the glucose challenge characteristic of CKD (46) and levels of CKD associated inflammation markers identified using WGCNA analysis (47). A module consisting of malonate, leucic acid, IMP, kynurenate, uric acid and inositol had a significant positive correlation with plasma creatinine, plasma cystatin C, CKD status, and a significant negative correlation with eGFR (Figure 3B). This module also had a significant positive correlation with TNF-a levels (Figure 3C). Interestingly, uremic retention solutes and uremic toxins including inositol, malonate, and uric acid make up a considerable number of correlating metabolites in this module. Inositol is involved in energy metabolism and considered a uremic retention solute. It was attenuated post-OGTT in the CKD group compared to controls, and part of the green module correlating with CKD status and TNF-a levels. Serum inositol levels have been negatively correlation with GFR (48, 49) and implicated to have an adverse impact on renal progression particularly among patients with type 2 diabetes where higher myo-inositol levels associates with greater likelihood of progression to end stage renal disease (ESRD) (50). In summary, WGCNA analysis suggests uremic toxins are strongly associated with disruption in response to glucose challenge in CKD compared to controls and linked to inflammatory markers in our cohort.

Our study has several notable strengths and limitations. First, we assessed dynamic changes in response to glucose challenge and accounted for several potential confounding factors by adjusting

for age, sex, race, body weight, and batch in our analysis. Second, we applied targeted metabolic profiling to assess a broad range of attenuated metabolic pathways impacted by physiological effects of insulin and identified its potential disturbed mechanisms in CKD. Our study also had some notable limitations. First, we did not investigate changes in human tissue relying instead on changes in the plasma metabolome. Changes in the plasma metabolome may not correlate with tissue or organ-specific metabolic alterations. Second, plasma ATP measurements were not available during fasting and OGTT. We relied on GTP:GDP ratios as a reflection of the changes in ATP:ADP. Third, we can't rule out the potential impact of residual confounding by differences in unmeasured characteristics between CKD and controls. Finally, we are unable to precisely identify the potential differences in the incretin induced secretion of insulin between our two groups. Further studies are necessary to interrogate the association of reduced activation of TCA cycle, redox imbalance, and electron transport chain efficiency in human tissues especially in the skeletal muscle.

In summary, numerous plasma metabolites are altered in response to oral glucose challenge in CKD. The response to glucose challenge in CKD is associated with disruption in plasma levels of TCA cycle intermediates, purine nucleotide cycle metabolites, and vitamin B family members relative to controls. These alterations highlight a remarkable overlap in the metabolic pathways, the magnitude, and the pattern of changes in plasma metabolites compared to our hyperinsulinemic-euglycemic clamp study among CKD. Both procedures resulted in similar changes in vitamin B family including nicotinamide and biotin. In addition, impairment in taurine metabolism were also replicated during glucose challenge. Overall, both studies highlight impairments in TCA cycle intermediates, amino acid metabolism, purine metabolism, and vitamin B family as the consequence of blunted insulin anabolic response in CKD. Together, our findings

from response to OGTT and insulin clamp point to abnormal mitochondrial energy metabolism as the main mechanism of the impaired anabolic response to insulin in CKD. This abnormal metabolic profile in response to glucose challenge adds to prior studies indicating the IR in CKD is predominantly characterized by a depressed anabolic response and disruption in energy metabolism. Given recent evidence for clinical benefits of insulin sensitizers, there is an urgent need for future studies interrogating the pleotropic effects of insulin sensitizers on mitochondrial energy metabolism and anabolism in CKD.

3.7 Practical Application

In this paper, we show that the response to glucose challenge in CKD is associated with disruption in plasma levels of TCA cycle intermediates, purine nucleotide cycle metabolites, and vitamin B family members relative to healthy controls. This abnormal metabolic profile in response to glucose challenge adds to prior studies indicating the insulin resistance in CKD is predominantly characterized by a depressed anabolic response and disruption in energy metabolism. Given recent evidence for clinical benefits of insulin sensitizers, there is an urgent need for future studies interrogating the pleotropic effects of insulin sensitizers on mitochondrial energy metabolism and anabolism in CKD.

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Supplemental table 1. Differences in plasma metabolites in response to glucose load compared to fasting. Results are from a regression SERRF normalized metabolites on sample type (OGTT vs fasting) adjusted for age, sex, race, weight, and batch. A p-value of <0.05 was used to determine significance (in bold). The fold change is in response to oral glucose is the adjusted fold change associated with OGTT compared to fasting state (e.g., a fold change of 1.28 indicates a 28% increase in metabolite levels).

Metabolite Name	Fold change between OGTT/fasting (%95 CI)	p-value
Hippuric acid	3.28 (2.28, 4.73)	**< 0.001**
Erythrose	1.53 (1.2, 1.94)	**< 0.001**
Glycochenodeoxycholate	1.52 (0.94, 2.45)	0.089
Glycocholate	1.39 (0.94, 2.07)	0.1
Glucose	1.28 (1.17, 1.39)	**< 0.001**
Kynurenate	1.17 (1.04, 1.31)	**0.0076**
Creatine	1.16 (1.02, 1.32)	**0.022**
lactate	1.13 (0.98, 1.3)	0.091
Xanthine	1.11 (0.99, 1.24)	0.063
Cystamine	1.11 (0.91, 1.35)	0.29
Glucoronate	1.09 (0.95, 1.25)	0.24
Pyruvate	1.09 (0.92, 1.29)	0.31
Pyridoxal-5-P	1.04 (0.88, 1.24)	0.64
Aminoisobutyrate	1.03 (0.94, 1.14)	0.52
Carnitine	1.02 (0.96, 1.08)	0.58
L-Kynurenine	1.02 (0.93, 1.12)	0.67
Trimethylamine	1.01 (0.94, 1.07)	0.88
Betaine	1.01 (0.93, 1.1)	0.77
Urate	1 (0.95, 1.05)	0.9
5-Hydroxytryptophan	0.99 (0.94, 1.04)	0.73
Oxalic acid	0.99 (0.86, 1.13)	0.83
Uridine	0.97 (0.79, 1.19)	0.78
F16BP	0.95 (0.91, 1)	**0.03**
Inositol	0.95 (0.91, 0.98)	**0.0028**
Cystine	0.95 (0.88, 1.02)	0.14
Choline	0.95 (0.87, 1.04)	0.29
1-Methyladenosine	0.94 (0.89, 0.99)	**0.026**

Hypoxanthine	0.94 (0.83, 1.07)	0.36
Glycerate	0.94 (0.8, 1.1)	0.41
Fumaric acid	0.92 (0.83, 1.02)	0.12
Biotin	0.92 (0.82, 1.03)	0.14
Dimethylglycine	0.92 (0.79, 1.06)	0.25
Citraconic acid	0.92 (0.74, 1.14)	0.46
GTP	0.91 (0.87, 0.96)	**< 0.001**
Creatinine	0.91 (0.87, 0.96)	**< 0.001**
Propionate	0.91 (0.77, 1.08)	0.27
Allantoin	0.9 (0.81, 1)	**0.049**
Alanine	0.88 (0.81, 0.96)	**0.005**
Chenodeoxycholate	0.88 (0.81, 0.96)	**0.0037**
Homovanilate	0.88 (0.77, 1)	0.058
Glutamine	0.87 (0.81, 0.93)	**< 0.001**
D-Leucic acid	0.87 (0.72, 1.06)	0.17
G6P	0.86 (0.81, 0.92)	**< 0.001**
Melatonin	0.86 (0.67, 1.11)	0.25
Lysine	0.85 (0.8, 0.91)	**< 0.001**
Shikimic acid	0.85 (0.74, 0.99)	**0.032**
Histidine	0.84 (0.79, 0.88)	**< 0.001**
Adipic acid	0.84 (0.72, 0.96)	**0.014**
TMAO	0.84 (0.6, 1.16)	0.29
Glycine	0.83 (0.77, 0.9)	**< 0.001**
Pentothenate	0.83 (0.73, 0.94)	**0.0036**
Oxaloacetate	0.81 (0.74, 0.89)	**< 0.001**
GDP	0.8 (0.74, 0.88)	**< 0.001**
Tryptophan	0.78 (0.73, 0.84)	**< 0.001**
Aconitate	0.78 (0.72, 0.84)	**< 0.001**
Glyceraldehyde	0.78 (0.69, 0.9)	**< 0.001**
Oxypurinol	0.78 (0.67, 0.9)	**< 0.001**
Proline	0.77 (0.72, 0.83)	**< 0.001**
Asparagine	0.75 (0.71, 0.79)	**< 0.001**
Arginine	0.75 (0.66, 0.85)	**< 0.001**

Valine	0.74 (0.69, 0.8)	< **0.001**
Ornithine	0.74 (0.68, 0.82)	< **0.001**
Threonine	0.74 (0.68, 0.8)	< **0.001**
Phenylalanine	0.73 (0.69, 0.77)	< **0.001**
Guanidinoacetate	0.73 (0.66, 0.8)	< **0.001**
N-AcetylGlycine	0.73 (0.64, 0.83)	< **0.001**
Hydroxyproline	0.73 (0.61, 0.88)	< **0.001**
MethylSuccinate	0.72 (0.67, 0.78)	< **0.001**
Serine	0.71 (0.66, 0.76)	< **0.001**
IMP	0.71 (0.47, 1.07)	0.098
2-Hydroxyglutarate	0.7 (0.62, 0.78)	< **0.001**
Glutamic acid	0.7 (0.6, 0.82)	< **0.001**
Cystathionine	0.7 (0.59, 0.84)	< **0.001**
Methionine	0.67 (0.62, 0.71)	< **0.001**
Sorbitol	0.66 (0.6, 0.72)	< **0.001**
Tyrosine	0.65 (0.61, 0.69)	< **0.001**
Succinate	0.61 (0.55, 0.68)	< **0.001**
PGE	0.6 (0.53, 0.69)	< **0.001**
Citrulline	0.58 (0.53, 0.62)	< **0.001**
Leucine	0.56 (0.51, 0.61)	< **0.001**
iso-Leucine	0.55 (0.5, 0.6)	< **0.001**
Adenylosuccinate	0.53 (0.44, 0.65)	< **0.001**
Aspartic acid	0.51 (0.43, 0.6)	< **0.001**
Taurine	0.49 (0.41, 0.58)	< **0.001**
Malonic acid	0.38 (0.28, 0.51)	< **0.001**
Niacinamide	0.37 (0.29, 0.48)	< **0.001**
ADP	0.21 (0.14, 0.32)	< **0.001**
Linolenic acid	0.18 (0.15, 0.21)	< **0.001**

Supplemental table 2. Differences in plasma metabolic response post glucose challenge by CKD status. Result from regression analysis using SERFF normalized on sample type (fasting vs OGTT) by CKD status adjusted for age, sex, race, weight, and batch. Fold changes represent changes in metabolite levels with fasting levels after glucose load. (e.g. a fold change of 0.92 indicates a reduction of %8 in metabolite level. P-value for interaction represents the heterogeneity in fold change by disease status.

Metabolite name	Fold change between OGTT/fasting for Non-CKD (%95 Cl)	Fold change between OGTT/fasting for CKD (%95 Cl)	p-value for interaction
Succinate	0.48 (0.37, 0.62)	0.78 (0.71, 0.85)	< 0.001
Taurine	0.41 (0.28, 0.6)	0.75 (0.66, 0.85)	0.0032
Adenylosuccinate	0.41 (0.26, 0.65)	0.84 (0.72, 0.98)	0.0037
Hippuric acid	1.56 (0.67, 3.59)	5.68 (4.29, 7.54)	0.004
ADP	0.17 (0.07, 0.42)	0.63 (0.47, 0.86)	0.0063
Biotin	0.74 (0.59, 0.93)	1.02 (0.94, 1.1)	0.011
Niacinamide	0.35 (0.2, 0.6)	0.7 (0.58, 0.84)	0.019
Glycochenodeoxycholate	0.47 (0.16, 1.35)	1.69 (1.18, 2.42)	0.025
Inositol	0.92 (0.85, 0.99)	1 (0.98, 1.03)	0.026
Kynurenate	1.07 (0.82, 1.4)	0.78 (0.71, 0.85)	0.026
IMP	0.18 (0.07, 0.48)	0.55 (0.39, 0.76)	0.037
Uridine	0.69 (0.49, 0.96)	0.99 (0.89, 1.11)	0.043
Glucose	1.07 (0.88, 1.31)	1.34 (1.25, 1.43)	0.043
GDP	0.73 (0.59, 0.89)	0.9 (0.84, 0.97)	0.05
Melatonin	0.48 (0.28, 0.84)	0.83 (0.69, 1)	0.067
Glycocholate	0.7 (0.29, 1.66)	1.61 (1.2, 2.15)	0.073
2-Hydroxyglutarate	0.57 (0.43, 0.76)	0.73 (0.67, 0.8)	0.1
iso-Leucine	0.49 (0.4, 0.59)	0.58 (0.54, 0.61)	0.12
Urate	1.06 (1.02, 1.1)	1.03 (1.01, 1.04)	0.12
D-Leucic.Acid	0.68 (0.44, 1.04)	0.96 (0.83, 1.11)	0.13
Creatinine	0.87 (0.78, 0.97)	0.95 (0.91, 0.98)	0.15
Aspartic.Acid	0.63 (0.47, 0.83)	0.78 (0.71, 0.86)	0.15
1-Methyladenosine	0.89 (0.81, 0.99)	0.97 (0.94, 1.01)	0.15
Guanidinoacetate	0.67 (0.54, 0.82)	0.78 (0.73, 0.83)	0.15

Pentothenate	0.72 (0.54, 0.96)	0.9 (0.81, 0.99)	0.17
Glycerate	0.75 (0.5, 1.11)	0.99 (0.87, 1.14)	0.18
Aconitate	0.83 (0.69, 1)	0.95 (0.9, 1.02)	0.18
Pyridoxal-5-P	0.82 (0.56, 1.18)	1.07 (0.94, 1.21)	0.18
Trimethylamine-N-oxide.(TMAO)	0.41 (0.19, 0.87)	0.69 (0.54, 0.9)	0.2
F16BP/F26BP/G16BP	0.92 (0.84, 1.02)	0.99 (0.96, 1.02)	0.2
Citraconic.Acid	1.29 (0.78, 2.13)	0.92 (0.78, 1.09)	0.21
Erythrose	1.45 (0.89, 2.37)	1.04 (0.89, 1.23)	0.21
Glutamic.acid	0.59 (0.44, 0.79)	0.72 (0.65, 0.79)	0.22
Leucine	0.51 (0.42, 0.62)	0.57 (0.54, 0.61)	0.28
Glutaric.Acid/Oxaloacetate	0.87 (0.72, 1.05)	0.97 (0.91, 1.03)	0.28
Aminoisobutyrate	1.04 (0.83, 1.29)	0.92 (0.86, 0.99)	0.32
Proline	0.77 (0.66, 0.9)	0.84 (0.79, 0.88)	0.32
Hydroxyproline/Aminolevulinate	0.64 (0.42, 0.98)	0.79 (0.69, 0.92)	0.34
Pyruvate	1.08 (0.71, 1.64)	1.34 (1.16, 1.54)	0.34
Serine	0.75 (0.64, 0.89)	0.82 (0.77, 0.87)	0.37
Cystamine	1.09 (0.74, 1.6)	0.9 (0.79, 1.03)	0.38
L-Kynurenine	1.09 (0.9, 1.32)	0.99 (0.93, 1.06)	0.38
Arginine	0.72 (0.53, 0.99)	0.84 (0.75, 0.93)	0.39
Adipic.Acid	1.04 (0.75, 1.43)	0.89 (0.8, 0.99)	0.39
Valine	0.7 (0.61, 0.82)	0.75 (0.72, 0.79)	0.4
Carnitine	0.97 (0.84, 1.11)	1.02 (0.98, 1.07)	0.42
Phenylalanine	0.74 (0.66, 0.84)	0.78 (0.75, 0.81)	0.42
Asparagine	0.75 (0.67, 0.84)	0.79 (0.76, 0.82)	0.44
Glyceraldehyde	0.8 (0.59, 1.07)	0.9 (0.81, 1)	0.44
Chenodeoxycholate	0.94 (0.79, 1.12)	0.88 (0.82, 0.93)	0.45
Citrulline	0.63 (0.53, 0.74)	0.67 (0.63, 0.71)	0.48
Shikimic.Acid	1.17 (0.89, 1.55)	1.06 (0.96, 1.16)	0.49
Hypoxanthine	0.92 (0.7, 1.21)	1.01 (0.92, 1.11)	0.52
Linolenic.Acid	0.23 (0.17, 0.32)	0.26 (0.23, 0.29)	0.52
5-Hydroxytryptophan	1.06 (0.97, 1.15)	1.03 (1, 1.06)	0.54

GTP	0.95 (0.86, 1.05)	0.98 (0.95, 1.02)	0.54
Glycine	0.8 (0.67, 0.94)	0.84 (0.79, 0.89)	0.56
Oxypurinol	0.63 (0.44, 0.9)	0.7 (0.62, 0.79)	0.6
Xanthine	0.92 (0.71, 1.19)	0.99 (0.9, 1.08)	0.61
Cystathionine	0.9 (0.66, 1.23)	0.82 (0.74, 0.92)	0.61
Dimethylglycine	0.79 (0.58, 1.06)	0.85 (0.77, 0.95)	0.62
Glutamine	0.92 (0.77, 1.09)	0.88 (0.83, 0.93)	0.62
N-AcetylGlycine	0.71 (0.52, 0.96)	0.77 (0.69, 0.85)	0.62
Betaine	1.01 (0.84, 1.2)	0.96 (0.9, 1.02)	0.63
Tyrosine	0.68 (0.59, 0.78)	0.7 (0.67, 0.74)	0.63
Lysine	0.83 (0.72, 0.96)	0.86 (0.82, 0.9)	0.64
Oxalic.Acid	1.26 (0.91, 1.74)	1.16 (1.04, 1.29)	0.64
Methionine	0.67 (0.58, 0.78)	0.7 (0.66, 0.73)	0.65
Alanine	0.89 (0.72, 1.11)	0.94 (0.87, 1.01)	0.67
Threonine	0.78 (0.67, 0.91)	0.75 (0.72, 0.79)	0.69
Malonic Acid	0.95 (0.63, 1.43)	1.04 (0.9, 1.19)	0.69
Histidine	0.86 (0.76, 0.96)	0.84 (0.8, 0.87)	0.7
MethylSuccinate	0.76 (0.64, 0.89)	0.78 (0.74, 0.82)	0.73
Homovanilate	0.98 (0.74, 1.29)	0.93 (0.85, 1.02)	0.73
Kuraridinol	1.07 (0.84, 1.37)	1.02 (0.95, 1.1)	0.74
Creatine	1.14 (0.85, 1.53)	1.08 (0.98, 1.2)	0.75
Trimethylamine (TMA)	1.04 (0.9, 1.21)	1.06 (1.01, 1.12)	0.78
Propionate	0.83 (0.58, 1.19)	0.79 (0.7, 0.89)	0.78
Sorbitol	0.67 (0.55, 0.8)	0.68 (0.64, 0.73)	0.8
lactate	1.15 (0.82, 1.61)	1.2 (1.08, 1.35)	0.81
Allantoin	0.93 (0.76, 1.14)	0.95 (0.89, 1.02)	0.81
Choline	0.98 (0.78, 1.22)	0.95 (0.88, 1.03)	0.83
Fumaric.Acid/Maleic.Acid	0.95 (0.77, 1.19)	0.94 (0.87, 1.01)	0.89
PGE	0.75 (0.59, 0.96)	0.77 (0.71, 0.83)	0.89
Glycochenodeoxycholic.acid	0.83 (0.07, 9.83)	0.7 (0.34, 1.44)	0.9
Ornithine	0.77 (0.62, 0.97)	0.76 (0.71, 0.82)	0.92

G1P/G6P/F6P/F1P	0.95 (0.85, 1.06)	0.95 (0.91, 0.98)	0.94
Glucoronate	0.92 (0.7, 1.22)	0.92 (0.84, 1.01)	0.98
Cystine	0.98 (0.83, 1.14)	0.98 (0.93, 1.03)	0.99
Tryptophan	0.86 (0.73, 1.01)	0.86 (0.81, 0.91)	1

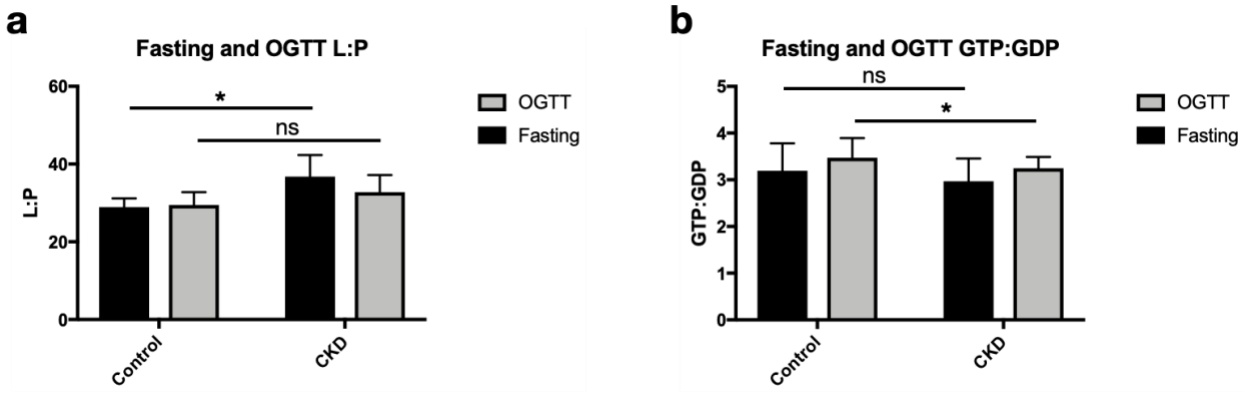
Supplemental table 3. Differences in fasting metabolites between CKD and controls (n=62). Percent differences are adjusted for age, sex, race, weight. Metabolites are listed according to decreasing percent difference.

Metabolite name	% Difference (95% CI)	p-value
IMP	**29 (11, 50)**	**1.17×10^{-3}**
Oxypurinol	27 (2, 59)	3.61×10^{-2}

Cystamine	22 (4, 42)	$1.30x10^{-2}$
Cystathionine	20 (7, 33)	$1.45x10^{-3}$
Kynurenate	**16 (5, 28)**	**$3.29x10^{-3}$**
Erythrose	15 (4, 26)	$6.17x10^{-3}$
D-Leucic caid	14 (-1, 31)	$6.21x10^{-2}$
Glucoronate	13 (2, 25)	$2.22x10^{-2}$
TMAO	10 (-5, 27)	$1.83x10^{-1}$
Allantoin	9 (4, 15)	$2.44x10^{-4}$
Creatinine	9 (3, 14)	$2.13x10^{-3}$
Propionate	7 (-1, 16)	$1.07x10^{-1}$
Ornithine	7 (1, 13)	$1.79x10^{-2}$
Citraconic acid	6 (-3, 16)	$2.25x10^{-1}$
L-Kynurenine	5 (-2, 12)	$1.60x10^{-1}$
Iso leucine	4 (-3, 12)	$2.24x10^{-1}$
Glycine	4 (-2, 11)	$1.52x10^{-1}$
Glycocholate	4 (-16, 29)	$7.21x10^{-1}$
Creatine	4 (-10, 20)	$6.04x10^{-1}$
Fumaric acid	4 (-1, 9)	$1.39x10^{-1}$
N-AcetylGlycine	4 (-8, 18)	$5.57x10^{-1}$
Lysine	4 (0, 8)	$7.48x10^{-2}$
Glutamic acid	3 (-5, 12)	$4.38x10^{-1}$
Trimethylamine	3 (-3, 9)	$2.88x10^{-1}$
Xanthine	3 (-2, 8)	$1.98x10^{-1}$
Carnitine	3 (-2, 9)	$2.97x10^{-1}$
1-Methyladenosine	3 (-1, 7)	$2.05x10^{-1}$
Cystine	3 (-3, 8)	$3.35x10^{-1}$
Pyridoxal-5-P	3 (-16, 25)	$8.07x10^{-1}$
Choline	2 (-4, 8)	$5.16x10^{-1}$
Asparagine	2 (-2, 6)	$3.67x10^{-1}$
Biotin	**2 (-4, 8)**	**$5.96x10^{-1}$**
Aminoisobutyrate	2 (-5, 8)	$6.51x10^{-1}$

Aconitate	2 (-3, 6)	$4.76x10^{-1}$
Oxaloacetate	2 (-3, 6)	$5.30x10^{-1}$
Valine	1 (-2, 6)	$4.67x10^{-1}$
Pentothenate	1 (-14, 19)	$8.58x10^{-1}$
Homovanilate	1 (-3, 6)	$5.43x10^{-1}$
Urate	1 (-2, 5)	$5.09x10^{-1}$
Leucine	1 (-3, 6)	$6.11x10^{-1}$
Shikimic ciad	1 (-10, 14)	$8.53x10^{-1}$
Betaine	1 (-5, 7)	7.46x10-1
Glycochenodeoxycholate	**1 (-20, 27)**	**$9.33x10^{-1}$**
Proline	1 (-5, 8)	$7.75x10^{-1}$
Histidine	1 (-3, 4)	$7.19x10^{-1}$
Threonine	1 (-5, 7)	$8.36x10^{-1}$
Uridine	0 (-7, 9)	$9.08x10^{-1}$
GDP	**0 (-3, 4)**	**$7.82x10^{-1}$**
Guanidinoacetate	0 (-5, 6)	$8.69x10^{-1}$
MethylSuccinate	0 (-4, 5)	$8.52x10^{-1}$
Glucose	**0 (-2, 2)**	**$7.59x10^{-1}$**
Glutamine	0 (-4, 4)	$9.88x10^{-1}$
Sorbitol	0 (-5, 4)	$9.45x10^{-1}$
Dimethylglycine	0 (-8, 8)	$9.57x10^{-1}$
lactate	0 (-5, 5)	$9.24x10^{-1}$
F16BP/F26BP/G16BP	0 (-2, 2)	$7.07x10^{-1}$
Chenodeoxycholate	0 (-4, 3)	$8.22x10^{-1}$
Adipic acid	-1 (-6, 4)	7.62x10-1
Citrulline	-1 (-8, 6)	$7.65x10^{-1}$
GTP	**-1 (-4, 2)**	**$4.48x10^{-1}$**
Alanine	-1 (-5, 3)	$5.83x10^{-1}$
Hyppuric acid	-1 (-18, 20)	$9.01x10^{-1}$
5-Hydroxytryptophan	-1 (-3, 1)	$2.41x10^{-1}$
Methionine	-2 (-7, 4)	$5.71x10^{-1}$

Hydroxyproline	-2 (-13, 12)	$8.03x10^{-1}$
2-Hydroxyglutarate	-2 (-9, 6)	$6.23x10^{-1}$
Inositol	**-2 (-4, 0)**	**$1.10x10^{-2}$**
Glycerate	-2 (-12, 9)	$7.04x10^{-1}$
Succinate	-2 (-7, 3)	$3.94x10^{-1}$
Phenylalanine	-2 (-5, 1)	$1.63x10^{-1}$
Serine	-2 (-6, 1)	$2.3x10^{-1}$
Tyrosine	-2 (-6, 2)	$2.48x10^{-1}$
Hypoxanthine	-3 (-7, 2)	$2.28x10^{-1}$
Oxalic acid	-4 (-9, 2)	$1.64x10^{-1}$
Glyceraldehyde	-4 (-11, 4)	$3.04x10^{-1}$
G1P/G6P/F6P/F1P	-4 (-7, -2)	$5.31x10^{-5}$
Arginine	-5 (-10, 0)	$4.09x10^{-2}$
Tryptophan	-6 (-10, -2)	$2.59x10^{-3}$
Taurine	**-6 (-12, 0)**	**$5.66x10^{-2}$**
PGE	-8 (-13, -4)	$5.61x10^{-4}$
Linolenic acid	-10 (-19, 1)	$6.74x10^{-2}$
Adenylosuccinate	**-10 (-17, -3)**	**$8.86x10^{-3}$**
Niacinamide	**-10 (-19, -1)**	**$3.95x10^{-2}$**
Aspartic acid	-11 (-19, -2)	$1.63x10^{-2}$
Pyruvate	-11 (-17, -5)	$6.73x10^{-4}$
Melatonin	-13 (-29, 6)	$1.68x10^{-1}$
ADP	**-19 (-31, -6)**	**$5.35x10^{-3}$**
Malonic acid	-34 (-45, -21)	$5.96x10^{-6}$



Supplemental figure 3.1 Distribution of L:P and GTP:GDP ratio in persons with CKD (n=41) and controls (n=21)**.** Panel A: L:P ratio at fasting and OGTT comparing CKD to controls. Panel B: GTP:GDP ratio at fasting and during OGTT comparing CKD to controls. Data points represent median and error bars represent interquartile range. Statistical analysis using ANOVA for multiple comparison testing ∗p-value<0.05, ∗∗p-value<0.01.

Supplemental table 4. The list of metabolites in each module from the WGCNA analysis.

Module color	Metabolite name
black	Alanine
black	Pyruvate
black	lactate
black	Oxalic.Acid
blue	Glycine
blue	Aminoisobutyrate
blue	Choline
blue	Serine
blue	Asparagine
blue	Phenylalanine
blue	Arginine
blue	MethylSuccinate
blue	Citrulline
blue	G1P.G6P.F6P.F1P
blue	Prostaglandin E
brown	TMAO
brown	Dimethylglycine
brown	Proline
brown	Threonine
brown	Hydroxyproline
brown	Glutamine
brown	Propionate
brown	Hyppuric acid
brown	Linolenic acid
green	Malonic acid
green	D-Leucic acid
green	Urate
green	Inositol
green	Kynurenate
green	IMP
grey	Trimethylamine
grey	Creatinine

grey	Betaine
grey	Creatine
grey	Glutamic acid
grey	Carnitine
grey	13C-Arginine
grey	13C-Tyrosine
grey	L-Kynurenine
grey	5-Hydroxytryptophan
grey	Cystine
grey	Uridine
grey	1-Methyladenosine
grey	Glyceraldehyde
grey	C13-Lactate
grey	Glycerate
grey	N-AcetylGlycine
grey	Citraconic acid
grey	Hypoxanthine
grey	Adipic acid
grey	2-Hydroxyglutarate
grey	Oxypurinol
grey	Xanthine
grey	Allantoin
grey	Shikimic acid
grey	Aconitate
grey	Glucose
grey	13C-Glucose
grey	Glucoronate
grey	Pentothenate
grey	Cystathionine
grey	Melatonin
grey	Biotin
grey	Pyridoxal-5-P
grey	F16BP.F26BP.G16BP

grey	Chenodeoxycholate
grey	Glycochenodeoxycholate
grey	Glycocholate
grey	GTP
red	Fumaric acid
red	Glutaric acid
red	Homovanilate
red	Erythrose
turquoise	Valine
turquoise	Leucine
turquoise	Iso-Leucine
turquoise	Ornithine
turquoise	Lysine
turquoise	Methionine
turquoise	Cystamine
turquoise	Histidine
turquoise	Tyrosine
turquoise	Sorbitol
turquoise	Tryptophan
turquoise	Guanidinoacetate
yellow	Niacinamide
yellow	Taurine
yellow	Aspartic acid
yellow	Succinate
yellow	ADP
yellow	GDP
yellow	Adenylosuccinate

Chapter 4

Impaired incretin homeostasis in non-diabetic moderate-severe chronic kidney disease

4.1 Preface

This chapter is in press at the *Clinical Journal of American Society of Nephrology.*

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The article has been modified to satisfy the formatting requirements of this dissertation.

4.2 Abstract

Background: Incretins are regulators of insulin secretion and glucose homeostasis metabolized by dipeptidyl peptidase-4 (DPP-4). Moderate-severe CKD may modify incretin release, metabolism, or response.

Methods: We performed 2-hour oral glucose tolerance testing (OGTT) in 59 people with non-diabetic CKD (eGFR<60 ml/min per 1.73 m^2) and 39 matched controls. We measured total (tAUC) and incremental (iAUC) area under the curve of plasma total glucagon-like peptide-1 (GLP-1) and total glucose-dependent insulinotropic polypeptide (GIP). Fasting DPP-4 levels and activity were

measured. Linear regression was used to adjust for demographic, body composition, and lifestyle factors.

Results: Mean (SD) eGFR was 38 ±13 and 89 ±17ml/min per 1.73 m^2 in CKD and controls. GLP-1 iAUC and GIP iAUC were higher in CKD than controls with a mean of 1531 ±1452 versus 1364 ±1484 pMxmin, and 62370 ±33453 versus 42365 ±25061 pgxmin/ml, respectively. After adjustment, CKD was associated with 15271 pMxmin/ml greater GIP iAUC (95% CI 387, 30154) compared to controls. Adjustment for covariates attenuated associations of CKD with higher GLP-1 iAUC (adjusted difference, 122, 95% CI -619, 864). Plasma glucagon levels were higher at 30 minutes (mean difference, 1.6, 95% CI 0.3, 2.8 mg/dl) and 120 minutes (mean difference, 0.84, 95% CI 0.2, 1.5 mg/dl) in CKD compared to controls. There were no differences in insulin levels or plasma DPP-4 activity or levels between groups.

Conclusions: Overall, incretin response to oral glucose is preserved or augmented in moderate-severe CKD, without apparent differences in circulating DPP-4 concentration or activity. However, neither insulin secretion nor glucagon suppression are enhanced.

4.3 Introduction

Non-diabetic chronic kidney disease (CKD) is associated with metabolic dysregulation, including disrupted insulin and glucose homeostasis[1-3]. Factors contributing to CKD-associated glucometabolic complications include increased inflammation[4] and hyperglucagonemia[5]. An impaired response of incretin, a key regulator of insulin secretion and glucose homeostasis, could

be an important mechanism contributing to inadequate insulin secretion in CKD. However, understanding of how CKD impacts postprandial incretin secretion is limited.

Incretin hormones are secreted by the gut in response to nutrient intake and promote glucose-stimulated insulin secretion[6]. The two main incretin hormones are glucagon-like peptide-1(GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)[7] secreted by the enteroendocrine L and K cells, respectively [8,9]. GLP-1 and GIP account for up to 70% of postprandial insulin secretion (incretin effect) in healthy individuals[10]. Little is known about the independent effect of CKD on the secretion and response to incretins. However, the incretins have opposing effects on glucagon secretion with GLP-1 suppressing[12], and GIP stimulating glucagon secretion [13]. Whether and how GLP-1 and GIP in combination impact postprandial glucagon suppression in CKD remains unknown. Additionally, understanding the impact of CKD on dipeptidyl peptidase-4 (DPP-4), a ubiquitous enzyme inactivating incretin hormones, is lacking[14].

The current study investigates postprandial incretin hormone levels and their determinants using a standardized OGTT comparing non-diabetic patients with CKD and controls. We first describe the association of the presence and severity of kidney disease with circulating concentrations of incretin hormones in both fasted and postprandial states. We separately investigate the association of postprandial circulating incretin hormones with insulin, c-peptide, and glucagon levels during an OGTT by CKD status. We hypothesized non-diabetic CKD is associated with reduced incretin hormone release and impaired glucagon suppression contribute to glucometabolic complications underlying heightened cardiometabolic risk in CKD.

4.4 Methods

Study population and study design: The Study of Glucose and Insulin in Renal Disease (SUGAR) was a cross-sectional study of moderate-severe non-diabetic CKD. Subjects were recruited from nephrology and primary care clinics affiliated with the University of Washington and nearby institutions in Seattle, WA. From this population, a total of 98 participants were recruited for this study among which 59 had CKD (eGFR <60 ml/min per 1.73 m^2) and 39 were controls (eGFR >60 ml/min per 1.73 m^2) and spot urine albumin-to-creatinine ratios <30 mg/g), frequency matched on age, sex, and race. Eligibility was determined at the screening visit, at which eGFR was calculated from serum creatinine measured at a clinical laboratory. Exclusion criteria for both groups included age <18 years, a clinical diagnosis of diabetes, maintenance dialysis or fistula in place, history of kidney transplantation, use of medications known to reduce insulin sensitivity, fasting serum glucose ≥126 mg/dl, and hemoglobin<10 g/dl. A more detailed description of the study design, recruitment, and enrollment has been published previously[3,15].

CKD classification: Serum creatinine and cystatin C (Gentian) were measured in fasting serum using a Beckman DxC automated chemistry analyzer. Primary analyses used GFR estimated using the CKD-EPI creatinine-cystatin C equation (2012)[16] to follow precedent of the original eligibility criteria, categorizations, and analyses. Results were compared to race-neutral CKD-EPI creatinine-cystatin C equation (2021)[17].

Oral glucose tolerance test and hyperinsulinemic-euglycemic insulin clamp: A standard 75g OGTT was performed approximately one week after the hyperinsulinemic-euglycemic insulin clamp. Plasma glucose, insulin, total GLP-1, and total GIP concentrations were measured at –10, -5, 0, 30, 60, 90, and 120 minutes. We averaged -10 to 0 time points to generate baseline fasting

values. Plasma glucagon levels were measured at 0, 30, and 120 minutes. The postprandial incretin hormone responses were calculated as the area under the curves (AUC) using the trapezoid rule for the total duration of OGTT and evaluated both as total AUC (tAUC) and incremental AUC (iAUC), the latter only measuring the area above baseline level (incretin response). Glucose iAUC and 2-hour plasma glucose were calculated as a measure of glucose tolerance. Insulinogenic index was used to quantify the difference in plasma insulin divided by the difference in plasma glucose from baseline to 30 minutes of the OGTT. Clamp insulin sensitivity and Matsuda index were the primary and secondary measures of insulin sensitivity. Details of the clamp and OGTT procedures have been published previously[18].

Laboratory measures: Plasma samples were assayed for total GLP-1 and total GIP using multiplex electrochemiluminescence (Meso Scale Discovery, Rockville, MD, USA). Plasma glucagon was measured by ELISA (Mercodia). DPP-4 antigen concentration was determined by ELISA (eBioscience). Blood glucose concentrations were measured using the glucose hexokinase method (Roche Module P Chemistry autoanalyzer; Roche, Basel, Switzerland) and blood insulin concentrations were measured using 2-site immune-enzymometric assay (Tosoh 2000 Autoanalyzer). C-peptide concentrations were determined using a standard double-antibody radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). DPP-4 activity was assayed by incubating plasma with a colorimetric substrate, l-glycyl-l-prolyl p-nitroanilide, hydrochloride (Sigma), at 37°C. Inflammatory biomarkers were measured in fasting blood. CRP was measured with a Beckman Coulter[19] DxC chemistry analyzer. Serum TNF-α, IL-6, IFN- γ, and IL-1β were performed using commercial multiplex electroluminescence assays (Meso Scale Discovery, Rockville, MD, USA).

Covariates: Demographic and medical history of participants were self-reported. Cardiovascular disease (CVD) was defined as a physician diagnosis of myocardial infarction, stroke, resuscitated cardiac arrest, or heart failure or a history of coronary or cerebral revascularization. The Human Activity Profile (HAP) maximum activity score was used to quantify physical activity. Food intake was recorded using three days of prospective food diaries analyzed with Nutrition Data System for Research software. Body composition was measured by DXA (GE Lunar or Prodigy and iDXA).

Statistical analysis

Linear regression was used to test associations of CKD status with incretins (tAUC and iAUC), measures of insulin resistance, and inflammatory biomarkers adjusting biologically relevant confounders. Spearman correlation coefficient was used to evaluate univariable relationship between kidney function and incretin levels during the OGTT. The rate of acute incretin peripheral response was calculated using the difference of plasma incretin levels at baseline and 30 minutes post OGTT and over time. Analyses were conducted using R version 4.2.2[20]. Boxplots and scatterplots were made using GraphPad Prism version 10.0.0.

Study approval

The study was approved by the University of Washington Human Subjects Division (HSD). All participants provided written informed consent.

4.5 Results

Characteristics of the study participants.

The study included a total of 98 participants, 59 had CKD and 39 were healthy controls. Among CKD participants the mean (± SD) age was 63.6 ± 13.9 years, 51% were female, and 22% self-

reported as Black participants. Mean (range) eGFR was 37.6 (9.5 to 59.5 ml/min per 1.73 m^2) compared to 88.8 (61 to 117 ml/min per 1.73 m^2) among controls (Table 1). Compared with controls, participants with CKD were more likely to have cardiovascular disease, to be smokers, be less physically active, have higher body weight, fat mass, and plasma inflammatory markers, and have lower daily calorie intake (Table 1).

Table 1. Characteristics of participants in the Study of Glucose and Insulin in Renal Disease.

Characteristics	Controls	CKD
Number	39	59
Demographics		
Age, mean (SD)	61.0 (12.4)	63.6 (13.9)
Female, No. (%)	17 (44)	30 (51)
Race, No. (%)		
Asian/Pacific Islander	1 (3)	5 (8)
Black	4 (10)	13 (22)
White	34 (87)	41 (69)
Medical history and lifestyle, No. (%)		
History of CVD	2 (5)	19 (32)
Currently smoking	3 (8)	10 (17)
Physical activity, HAP score	83.5 (8.7)	76.8 (9.5)
Calorie intake, kcal/day	2047.9 (556.4)	1758.2 (540.8)
Fat intake, gram/day	81.8 (32.1)	70.6 (26.4)
Carbohydrate intake, gram/day	243.1 (78.3)	209.2 (79.0)
Protein intake, gram/day	79.6 (24.5)	71.0 (26.1)
Medication use		
Any antihypertensive medication	13 (33)	53 (90)
RAS antagonists	8 (21)	38 (64)

Diuretics	2 (5)	27 (46)
β-Blockers	3 (8)	23 (39)
Calcium-channel blockers	3 (8)	27 (46)
Physical characteristics, Mean (SD)		
BMI (kg/m^2)	27.5 (6.3)	30.2 (6.0)
Body weight (kg)	82.1 (20.6)	88.1 (19.8)
Fat-free mass (kg)	56.1 (13.1)	53.7 (11.7)
Fat mass (kg)	27.1 (14.0)	31.9 (11.6)
Laboratory data		
Serum creatinine (mg/dl), median (IQR)	0.9 (0.7 to 1.0)	1.7 (1.5 to 2.1)
Serum Cystatin-C (mg/L), median (IQR)	0.9 (0.7 to 1.0)	1.6 (1.4 to 2.0)
eGFR (mL/min/1.73 m^2), CKD-EPI 2012, mean (SD)	88.8 (17.1)	37.6 (12.5)
eGFR (mL/min/1.73 m^2), CKD-EPI 2021, mean (SD)	91.1 (18.3)	38.4 (12.3)
Urine albumin excretion rate (mg/24 hours), median (IQR)	5.7 (3.5 to 8.5)	39.2 (14.2 to 225.1)
CRP (mg/dL), median (IQR)	0.1 (0.06 to 0.3)	0.3 (0.1 to 0.7)
IL-6 (pg/mL), median (IQR)	0.9 (0.6 to 1.4)	1.5 (0.9 to 2.1)
TNF-α (pg/mL), median (IQR)	1.6 (1.3 to 1.9)	2.7 (2.1 to 3.0)

Chronic kidney disease was defined as estimated glomerular filtration rate <60 ml/min per 1.73 m^2; controls as ≥60 ml/min per 1.73 m^2. Data are means (SDs) for continuous variables, N (percentages) for categorical variables, and medians (interquartile ranges). Abbreviations: SD, standard deviation; IQR, interquartile range; CVD, cardiovascular disease; HAP, human activity profile; RAS, renin-angiotensin system, eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha.

CKD was associated with greater fasting plasma incretin levels and varied incretin response during an OGTT.

In the overall cohort, eGFR was inversely correlated with only total GLP-1 levels (tAUC), but not GLP-1 response (iAUC) (Figure 1A and 1C). In comparison, eGFR was inversely correlated with

both total GIP and GIP response in the overall cohort (Figure 1B and 1D). CKD was associated with a higher fasting GLP-1 levels with a mean of 16.2 ± 11.6 compared to 8.5 ± 3.3pM among controls (*P*<0.01) (Table 2, Supplemental Table 1). GLP-1 tAUC measured during the OGTT was higher in participants with CKD versus controls (Table 2, Figure 2A). After adjustment CKD was associated with a 1100 pM x min higher GLP-1 tAUC (95% CI of 119 to 2080; *P*=0.03) (Table 3). In contrast, we found no significant difference in GLP-1 response (GLP-1 iAUC) compared to controls (Tables 2 and Table 3).

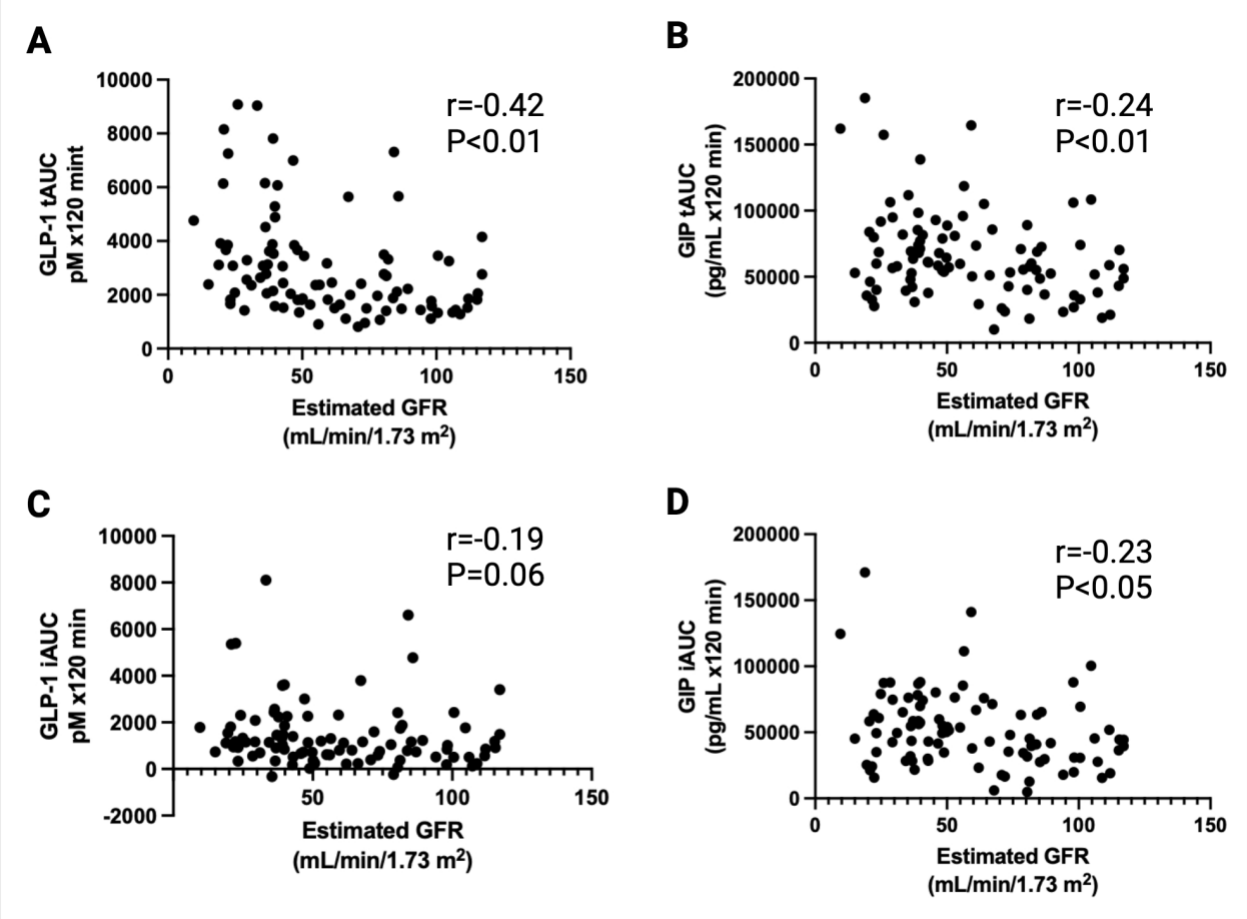

Figure 4.1 Association of estimated GFR with plasma incretin levels during OGTT. eGFR<30 (n=17), eGFR 30-45 (n=22), eGFR 45-60 (n=19), and eGFR>60 (n=39). CKD-EPI creatinine-cystatin equation (2012) was used to estimate GFR. Spearman correlation coefficients were used to estimate the univariate relationship between incretin response and kidney function.

Fasting GIP level was higher among the CKD group with a mean of 134.5 ± 104.1 versus 97 ± 112.6 pg/ml in controls (P<0.01) (Table 2, Supplemental Table 1), but the estimated mean difference was not significant after adjusting for potential confounders (Supplemental Table 1). In contrast, both total postprandial GIP level and GIP response were elevated in CKD compared to controls (Table 2 and Figure 2B). Adjusting for potential confounders attenuated the estimated association by 24% to an estimated mean difference of 15271 pg x min/ml higher GIP iAUC (95% CI of 387 to 30154; P=0.04) in CKD compared to controls (Table 3). These differences in incretin levels were observed in the absence of differences in fasting plasma DPP-4 antigen levels and DPP-4 activity between among CKD and controls (Figure 3A and 3B).

Table 2. Fasting and OGTT glucose homeostasis and physiological measurements by CKD status.

Measurements	**Controls (n=39)**	**CKD (n=59)**	**P-value**
Fasting measurements, Mean (SD)			
Fasting glucose, mg/dL	98.4 (9.2)	100.7 (8.6)	0.19
Fasting glucagon, pmol/L	5.7 (3.7)	6.8 (4.5)	0.20
Fasting insulin, μU/mL	6.9 (4.6)	10.3 (7.0)	**<0.01**
GLP-1, pM	8.5 (3.3)	16.2 (11.6)	**<0.01**
GIP, pg/ml	96.9 (112.6)	134.5 (104.1)	**<0.01**
C-peptide, ng/mL	2.1 (1.0)	3.8 (1.8)	**<0.01**
Free fatty acid, mEq/L	0.5 (0.1)	0.5 (0.2)	0.73
OGTT measurements, Mean (SD)			
Insulinogenic index, (μU /ml)/(mg/dL)	1.0 (1.2)	1.1 (0.9)	0.64
Glucose tAUC, (mg x 120min)/mL	19220 (3705)	19712 (3189)	0.48
Glucose iAUC, (mg x 120min)/mL	7402 (3127)	7583 (2884)	0.55
2-hour glucose, mg/dL	149.1 (44.5)	151.6 (35.4)	0.75

Insulin iAUC, (120min x μU)/mL	6108 (4748)	7975 (5405)	0.08
30 min glucagon, pmol/L	3.5 (2.0)	5.1 (3.6)	**0.01**
2-hour glucagon, pmol/L	1.3 (0.9)	2.2 (1.7)	**<0.01**
2-hour GLP-1, pM	14.8 (11.6)	20 (11.7)	**0.04**
2-hour GIP, pg/mL	442 (313)	622 (365)	**0.01**
GLP-1 iAUC, pM x 120min	1364 (1484)	1531 (1452)	0.58
GLP-1 tAUC, pM x 120min	2384 (1546)	3486 (1996)	**<0.01**
GIP iAUC, pg/mL x 120min	42365 (25061)	62370 (33453)	**<0.01**
GIP tAUC, pg/mL x 120min	53994 (28191)	78510 (38924)	**<0.01**
2-hour C-peptide, ng/mL	10.1 (4.0)	15.7 (7.8)	**<0.01**
C-peptide iAUC, ng/mL x120min	724 (329)	913 (443)	**<0.01**
2-hour free fatty acid, mEq/L	0.04 (0.04)	0.07 (0.06)	**<0.01**
Hyperinsulinemic-euglycemic clamp			
Insulin sensitivity, (mg/min)/(μU /mL)	5.0 (2.0)	3.9 (2.0)	**0.03**
2-hour GLP-1, pM	4.2 (1.7)	10.3 (9.1)	**<0.01**
2-hour GIP, pg/mL	65.9 (50.2)	98.9 (82.8)	**0.01**
2-hour glucagon, pmol/L	1.6 (1.6)	2.4 (2.6)	0.13

Cells represent means (SDs).

The rate of acute GIP increase in the first 30 minutes of OGTT was greater in CKD compared to controls. The mean rate of increase in GIP within the first 30 minutes of the OGTT was 249 ± 111 vs 177 ± 101 pg/ml/min in CKD and controls, respectively. CKD patients had an estimated mean 167pg/ml/min greater rate of increase in GIP (95% CI of 50 to 284; P<0.01) compared to controls after adjustment for potential confounders (Supplemental Table 2). Further adjustment for fasting plasma GIP levels did not meaningfully impact estimates of association. In contrast, the CKD patients did not differ meaningfully or significantly in their mean rate of increase in GLP-1 (Supplemental Table 2).

Table 3. Association of CKD with measures of GLP-1 and GIP during 2-hour OGTT. Mean differences represent the differences associated with CKD (vs controls) with 95% confidence intervals and P-values. Covariates were added one at a time to the base model which included age, sex, and race. The fully adjusted model is adjusted for age, sex, race, fat-free mass, fat mass, physical activity, calorie intake, smoking status, and CVD. GLP-1 and GIP were measured during OGTT.

Covariate adjustment	**GLP-1 AUC**				**GIP AUC**			
	GLP-1 iAUC		**GLP-1 tAUC**		**GIP iAUC**		**GIP tAUC**	
	Difference (95% CI), pM x min	***P***	**Difference (95% CI), pM x min**	***P***	**Difference (95% CI), (pg x min)/mL**	***P***	**Difference (95% CI), (pg x min)/mL**	***P***
None (unadjusted)	166 (-435 to 769)	0.58	1102 (350 to 1854)	**<0.01**	20005 (7517 to 32493)	**<0.01**	24516 (10116 to 38916)	**<0.01**
Age, sex, and race	92 (-504 to 690)	0.76	1192 (406 to 1978)	**<0.01**	18971 (5923 to 32018)	**<0.01**	21613 (6885 to 36340)	**<0.01**
Weight	162 (-447 to 771)	0.60	1224 (417 to 2031)	**<0.01**	19629 (6244 to 33014)	**<0.01**	21908 (6783 to 37032)	**<0.01**
Fat mass	216 (-401 to 833)	0.49	1223 (400 to 2045)	**<0.01**	19715 (5800 to 33630)	**<0.01**	21349 (5670 to 37029)	**<0.01**
Fat-free mass	144 (-515 to 803)	0.66	1095 (217 to 1972)	**0.01**	21408 (6540 to 36277)	**<0.01**	23465 (6720 to 40210)	**<0.01**
Physical activity	96 (-570 to 761)	0.77	1019 (135 to 1903)	**0.02**	19725 (4849 to 34600)	**<0.01**	21558 (4809 to 38308)	**0.01**
Calorie intake	93 (-628 to 814)	0.79	1022 (62 to 1981)	**0.04**	14485 (-2.7 to 28972)	0.05	15510 (-583 to 31603)	0.06
Smoking status	92 (-634 to 818)	0.80	1018 (53 to 1983)	**0.04**	14490 (-100 to 29080)	0.05	15528 (-677 to 31733)	0.06
Fully adjusted model	122 (-619 to 864)	0.74	1100 (119 to 2080)	**0.03**	15271 (387 to 30154)	**0.04**	16974 (515 to 33432)	**0.04**

GIP response, but not GLP-1 response was associated with insulinotropic effects during OGTT.

Total postprandial insulin levels during the OGTT did not significantly differ between CKD and controls, whereas C-peptide levels were more consistently greater at each time point in CKD

during the OGTT (Figure 2C and 2D). No significant differences were observed in insulin response (insulin iAUC) and insulinogenic index between CKD and controls (Table 2). Similarly, we found no meaningful difference by CKD status in glucose tolerance measured by glucose iAUC (Table 2, Figure 2E). The correlation of GLP-1 and GIP responses with insulin and C-peptide response, and glucose iAUC were generally weaker in patients with CKD compared to controls (Supplemental Figure 1A-1F).

Plasma glucagon levels were elevated in CKD compared to controls in response to OGTT.

Fasting plasma glucagon levels were not significantly different between CKD and controls (Table 2, Supplemental Table 1, Figure 2F). Plasma glucagon levels were higher at 30 minutes and 120 minutes in CKD compared to controls (Table 2, Figure 2F). The percent change in glucagon levels from baseline to 30 minutes post OGTT was attenuated in CKD with a median [IQR] of -27% [-11 to -46] versus -38% [-19 to -57] among controls. The percent change from baseline was also modestly attenuated at 2 hours post OGTT among CKD with median [IQR] of -70% [-57 to -80] compared to -78% [-60 to -88] in controls.

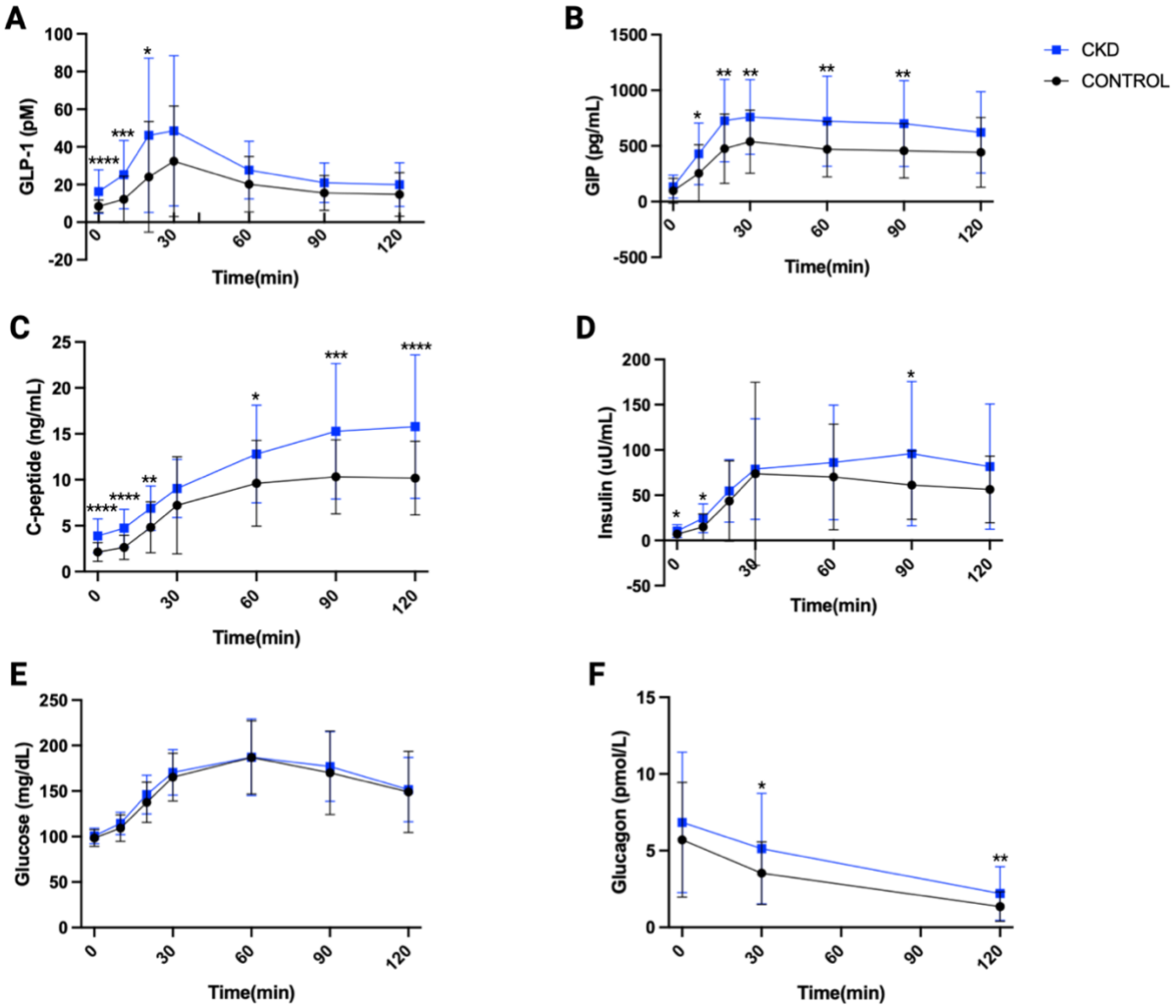

Figure 4.2 Changes in plasma glucose, glucagon, and pro-insulin factors in response to OGTT comparing CKD and controls. Data points and error bars are means and SD, respectively. Unpaired *t* test corrected by multiple hypothesis testing (Bonferroni) was used to evaluate differences between CKD and controls at each timepoint. "****"= $P<0.0001$, "***" = $P<0.001$, "**" = $P<0.01$, "*" = $P<0.05$.

Greater inflammation was associated with greater incretin levels and incretin response in CKD.

In the overall cohort, plasma TNF-α levels were significantly associated with GIP response, and CRP levels were significantly associated with GLP-1 response (Supplemental Table 3). In the CKD subgroup, greater CRP was also associated with greater GLP-1 response (Supplemental Table 3). Among patients with CKD each 1 mg/dL greater plasma CRP was associated with 0.58 greater pM GLP-1 response (95% CI of 0.37 to 0.8; $P<0.01$) in CKD (Supplemental Table 3).

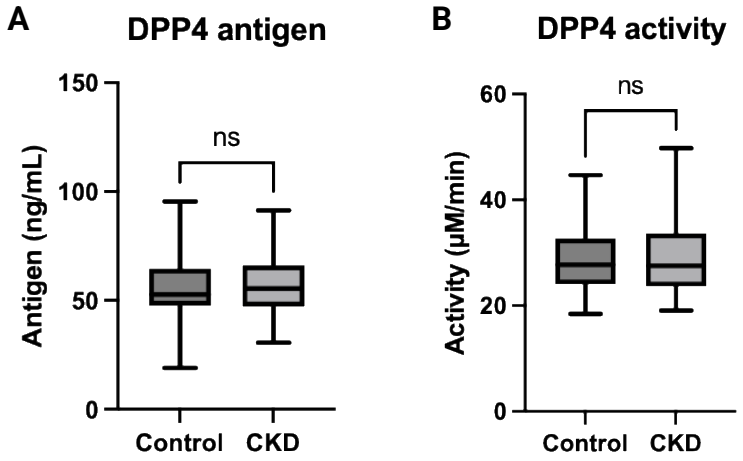

Figure 4.3 Comparison of fasting plasma DPP-4 antigen and activity levels among CKD (n=43) and controls (n=34). Box plots represent median and IQR and the whiskers represent minimum and maximum values. Unpaired *t* test was used to determine the difference between the two groups.

Sensitivity analyses using the CKD-EPI creatinine-cystatin C 2021 equation yielded similar outcomes.

The eGFR was similar among CKD and controls compared to the 2012 equation (Table 1). Results using the 2021 GFR equation were similar to the 2012 equation (Supplemental Table 4 and Supplemental Figure 2).

4.6 Discussion

Our findings demonstrate the presence and severity of non-diabetic moderate-severe CKD is associated with greater plasma levels of incretins during fasting and in response to an OGTT. The elevated incretin levels during fasting and postprandial conditions were observed in the absence of any significant difference in fasting glucagon levels, or DPP-4 levels. Acute GIP release and GIP response (iAUC) during the OGTT were higher in CKD versus controls. The correlation of

incretin levels with OGTT stimulated insulin or c-peptide was attenuated in those with CKD compared with controls. Concomitantly, CKD was associated with elevated postprandial plasma glucagon levels and impaired glucagon suppression post OGTT. In CKD, inflammation was associated with elevated incretin response. Overall, our findings show non-diabetic moderate-severe CKD is associated with greater postprandial incretin levels and an augmented GIP response during OGTT do not translate into meaningful improvements in insulin, glucose, or glucagon homeostasis.

Elevated fasting and post-prandial plasma incretin levels in CKD was independent of differences in circulating fasting DPP-4 levels and activity, suggesting these differences are unlikely due to reduced incretin degradation. Our findings are consistent with other studies in patients with non-diabetic end-stage renal disease (ESRD). One prior study showed greater GLP-1 levels in response to a high-calorie mixed meal in non-diabetic end-stage renal disease (ESRD) subjects compared to healthy controls[21] while another small study of nine non-diabetic hemodialysis patients and 10 healthy controls found elevated fasting and postprandial total GIP response during a standardized meal[22]. The influence of the uremic milieu on potential alternative incretin degradation pathways is unknown.

In our study, CKD was associated with a greater rate of GIP increase in the first 30 minutes of OGTT compared to controls (Supplemental Table 2) independent of differences in fasting levels of GIP implying these differences may be independent of reduced clearance of GIP. Controversy exists regarding the role of renal clearance on incretin response. A prior small case-control study in a select group of patients with more modest kidney disease (mean creatinine clearance 46 ml/min) suggested similar metabolic clearance rates and plasma half-life of intact GLP-1 and intact GIP but prolonged metabolite half-lives with intravenous GLP-1 and GIP infusion in CKD

compared to controls[23]. This prior study was limited by the lack of urinary measurements to assess clearance and measurement of lean mass influencing the volume of distribution. Another study in patients with ESRD treated with dialysis showed no difference in incretin response compared with controls suggesting a preserved ability to degrade and eliminate active GLP-1 and GIP and their metabolites in ESRD[24]. Our study is the first demonstrating unaltered DPP-4 levels and activities in non-diabetic CKD supporting these prior observations in ESRD.

Disruption of postprandial incretin hormone response (iAUC) in CKD appeared to influence downstream insulin, c-peptide, and glucagon homeostasis during the OGTT. In healthy adults, GIP is considered more strongly insulinotropic than GLP-1[25]. Consistent with these findings we found a stronger positive correlation between GIP response and insulin/C-peptide compared to GLP-1. Furthermore, CKD was associated with a weaker correlation between GIP response and insulin/C-peptide compared to controls. In comparison, we found no meaningful correlation of GLP-1 with insulinotropic response. Our findings expand on prior studies suggesting non-diabetic patients with CKD demonstrate a blunted insulinotropic effect of incretins akin to patients with type 2 diabetes and normal kidney function [26,27]. However, CKD patients appeared to have numerically greater baseline-corrected insulin response (insulin iAUC) reflecting reduced insulin clearance[3] and a similar acute insulin response estimated by the insulinogenic index compared to controls (Table 2). This may suggest altered glucose homeostasis in CKD patients may be attributed to inadequate augmentation of the insulin response by incretin hormones (especially GLP-1) or resistance to insulin's actions on peripheral tissues. Our findings are consistent with results from a randomized double-blind study also showed non-diabetic ESRD patients exhibit reduced incretin-stimulated insulin secretion despite adequate insulin response during IV glucose stimulation[28]. Mechanistic studies of CKD in 5/6th nephrectomized mice showed impaired β-cell insulin secretion in response

to glucose[29], but none have investigated β-cell resistance to GIP-induced insulin secretion. Thus, it is important to evaluate the incretin response to carbohydrate consumption in non-diabetic CKD, especially in the β-cells of the endocrine pancreas where GLP-1 and GIP receptors are abundantly expressed[30].

The attenuated suppression of glucagon during the OGTT in non-diabetic moderate-severe CKD suggests potential disruption of alpha cell response to incretins in CKD. Despite declines in glucagon levels during the OGTT in both CKD and controls, postprandial glucagon levels remained significantly higher in the CKD group compared to controls. These findings are in line with other studies of patients with type 2 diabetes and non-diabetic patients with ESRD[5,24,31-33]. It suggests an altered counterregulatory balance between GIP induction and GLP-1 suppression of alpha cell glucagon production in CKD during OGTT-induced hyperglycemia. Sustained and elevated postprandial glucagon levels could have adverse impacts on glycemic control and amino acid catabolism contributing to muscle wasting in patients with CKD[34-36].

Inflammation may contribute to heightened incretin response to OGTT. The association of inflammatory biomarkers, including CRP and IL-6 with GLP-1 levels has been reported in other observational studies[37-39]. Interestingly, the contrary has been observed with long-term incretin-based therapies, significantly decreasing circulating proinflammatory cytokines[40-42]. Mechanistic studies are needed to investigate the link between systemic inflammation and incretin levels in CKD and if lifestyle or pharmacologic therapies reducing inflammation and catabolism simultaneously improve incretin effects.

Our study had notable strengths and limitations. First, we recruited a well-characterized group of non-diabetic CKD participants across the spectrum of moderate-severe CKD including measures of body composition and lifestyle factors. Second, we used an OGTT to comprehensively measure

gut-derived incretin hormones, glucagon, insulin, and glucose. Third, we employed a rigorous analysis method adjusting for a wide range of clinically relevant confounders. Our study was not without limitations. First, our assays measured total GLP-1 and GIP levels in the plasma, so the proportion of active from the total GLP-1 and GIP and their renal clearance was not directly measured. Second, serial blood sample collections during OGTT were acquired without the addition of a DPP-4 inhibitor which may have impacted the levels of glucagon, GLP-1 and GIP. However, both the plasma fasting DPP-4 antigen levels and its activity were similar in CKD and controls. Finally, despite normal fasting glucose levels, both controls and CKD patients included individuals with impaired glucose tolerance (IGT). However, the inclusion of individuals with IGT in our control group may suggest observed estimated differences in incretin levels and responses between CKD and controls are conservative.

In conclusion, non-diabetic CKD is associated with disruption of incretin homeostasis and evidence of attenuated incretin effects on insulin, C-peptide, and glucagon secretion. These changes may contribute to the metabolic dysregulation associated with kidney disease and reveal a potential role for incretin-mimetics to counter attenuated incretin effects. Indeed, a recent pharmacokinetic study of combination GLP-1 and GIP in the form of single-dose tirzepatide, a dual GLP-1 and GIP receptor agonist, showed similar drug clearance and tolerability in healthy controls compared to patients across all stages of CKD, including ESRD[43]. Studies are needed to investigate the differential efficacy of GLP-1 and GIP single and dual agonist on insulin, glucose and glucagon homeostasis and links to outcomes in non-diabetic CKD.

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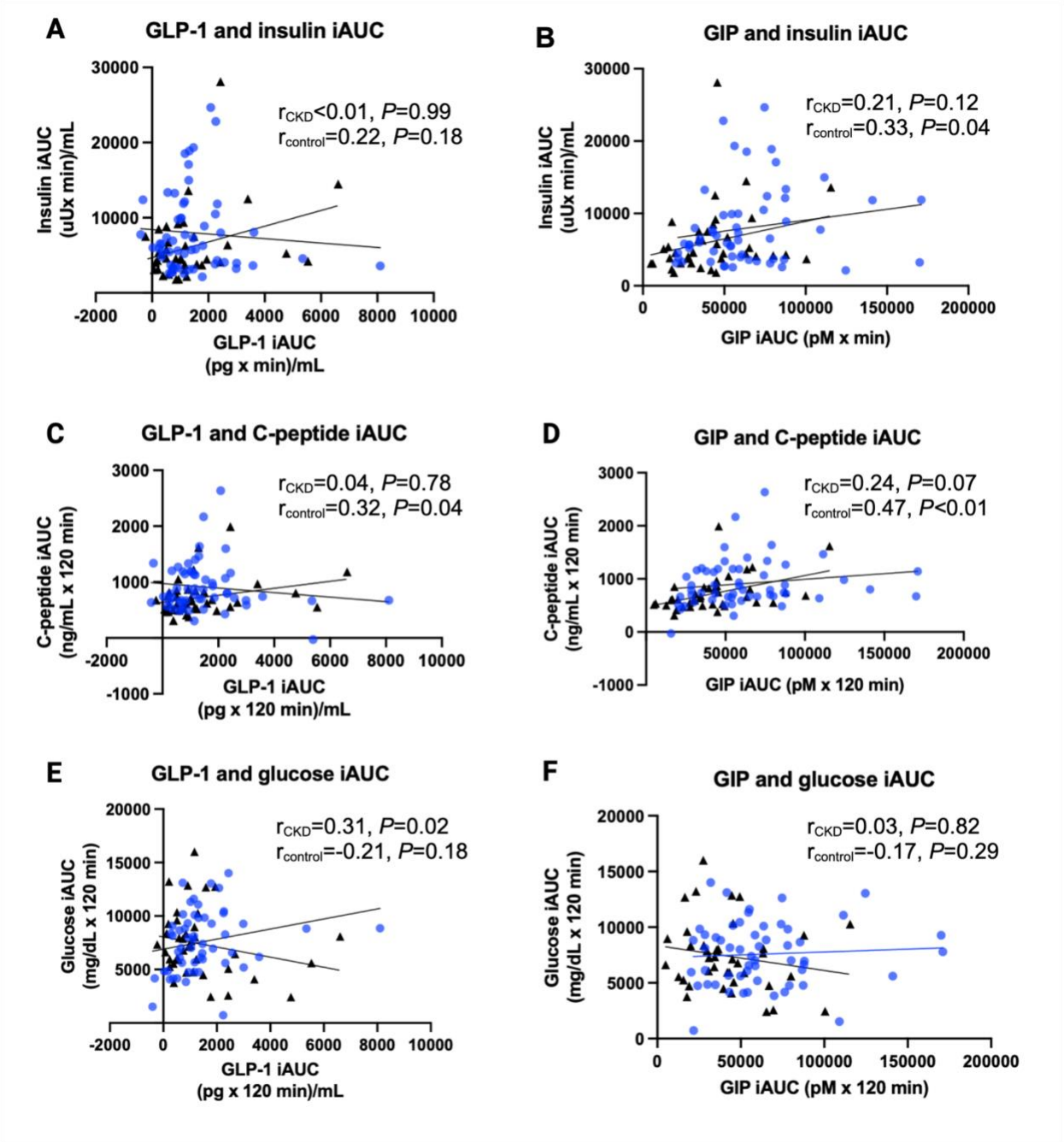
4.10 Supplemental Materials

Supplemental Figure 4.1 The correlation between incretin response with insulin, C-peptide, and glucose iAUCs in CKD and controls during OGTT. Spearman correlation coefficient was used to estimate the univariate relationship between incretin response and insulin secretion. Black triangles represent controls and blue circle represent CKD.



Supplemental Table 1. Association of CKD with fasting GLP-1, GIP, and glucagon measurements. Mean differences represent the differences associated with CKD (vs controls) with 95% confidence intervals and P-values. Covariates were added one at a time to the base model which included age, sex, and race. The fully adjusted model is adjusted for age, sex, race, fat-free mass, fat mass, physical activity, calorie intake, smoking status, and CVD.

Covariate adjustment	**Fasting GLP-1**		**Fasting GIP**		**Fasting glucagon**	
	Difference (95% CI), pM	***P***	**Difference (95% CI), pg/mL**	***P***	**Difference (95% CI), pmol/L**	***P***
None (unadjusted)	8.8 (5.1 to 12)	<0.01	52 (18 to 85)	<0.01	1.1 (-0.6 to 2.9)	0.20
Age, sex, and race	10.1 (6.2 to 14)	<0.01	36 (4.3 to 68)	0.03	1.7 (0.03 to 3.4)	0.04
Weight	9.9 (5.8 to 14)	<0.01	35 (2.2 to 67)	0.04	1.6 (-0.1 to 3.3)	0.06
Fat mass	9.4 (5.2 to 13)	<0.01	31 (-2.6 to 65)	0.07	1.5 (-0.2 to 3.3)	0.09
Fat-free mass	8.7 (4.3 to 13)	<0.01	30 (-6.5 to 66)	0.11	1.3 (-0.6 to 3.2)	0.17
Physical activity	8.6 (4.1 to 13)	<0.01	29.5 (-7.3 to 66)	0.11	1.1 (0.8 to 3.1)	0.24
Calorie intake	8.5 (3.7 to 13)	<0.01	24 (-13 to 61)	0.20	0.9 (-1.1 to 2.9)	0.36
Smoking status	8.5 (3.7 to 13)	<0.01	25.2 (-12 to 62)	0.18	0.8 (-1.1 to 2.8)	0.40
Fully adjusted model	8.8 (3.9 to 14)	<0.01	30 (-7.7 to 67)	0.11	1.0 (-0.9 to 3.0)	0.30

Supplemental Table 2. Estimated differences in the rate of acute incretin peripheral response between CKD and controls. Mean differences represent the differences associated with CKD (vs controls) with 95% confidence intervals and *P*-values. Covariates were added one at a time to the base model which included age, sex, weight, and smoking status.

Covariate adjustment	**Acute GLP-1 response**		**Acute GIP response**	
	Difference (95% CI), pM/min	***P***	**Difference (95% CI), pg/mL/min**	***P***
None (unadjusted)	7.5 (-6.4 to 21.3)	0.29	174 (61 to 287)	<0.01
Age, sex, weight, smoking status	7.5 (-6.4 to 21.3)	0.29	172 (58 to 285)	<0.01
Fat mass, fat-free mass, calorie intake	10.6 (-4.1 to 25.3)	0.16	167 (50 to 284)	<0.01
Physical activity	10.7 (-4.0 to 25.3)	0.16	167 (50 to 284)	<0.01

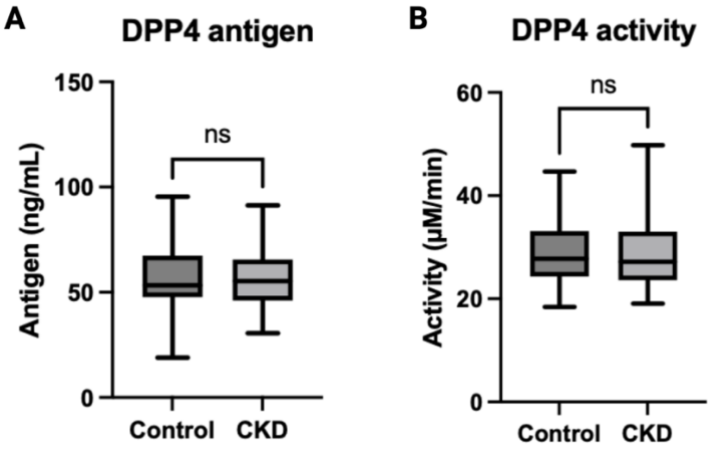
Supplemental Table 3. Association of inflammatory biomarkers with incretin response during OGTT in the CKD and controls. Cells represent association coefficients with 95% confidence intervals and *P*-values. The coefficients represent the estimated change in incretin response with one unit increase in the corresponding inflammatory biomarker. Association coefficients were obtained using linear regression adjusting for adjusted for age, sex, race, fat-mass, fat-free mass, calorie intake, physical activity, and smoking status. * Significant association of inflammatory biomarker with GLP-1 or GIP response in the overall cohort. "**" = *P*<0.01, "*" = *P*<0.05.

Inflammatory biomarker	**GLP-1 iAUC**				**GIP iAUC**			
	Overall	**Control**	**CKD**	***P* for interaction**	**Overall**	**Control**	**CKD**	***P* for interaction**
	b (95% CI)	**b (95% CI)**	**b (95% CI)**		**b (95% CI)**	**b (95% CI)**	**b (95% CI)**	
C-reactive protein ** (mg/dL)	0.29 (-0.1 to 0.5)	0.04 (-0.26 to 0.35)	0.58 (0.38 to 0.78)**	**<0.01**	-0.06 (-4.4 to 4.3)	0.83 (-4.7 to 6.4)	0.5 (-6.3 to 7.2)	0.88
IL-6 (pg/mL)	-0.08 (-0.4 to 0.2)	-0.08 (-0.40 to 0.23)	-86 (-336 to 165)	0.32	5.7 (-0.1 to 11.5)	6.1 (1.0 to 11)	2155 (-4074 to 8384)	0.28
IFN-γ (pg/mL)	1.0 (-6.1 to 8.3)	170 (-61 to 401)	4.1 (-2.8 to 11)	0.42	36 (-109 to 181)	1770 (-2514 to 6054)	34 (-141 to 209)	0.29
IL-1β (pg/mL)	-0.03 (-0.33 to 0.27)	-0.17 (-0.5 to 0.16)	410 (-4594 to 5415)	0.73	-2.8 (-9.0 to 3.3)	-1.5 (-7.4 to 4.4)	43070 (-80512 to 166653)	0.34
TNF-α* (pg/mL)	186 (-204 to 575)	283 (-651 to 1216)	441 (-136 to 1020)	0.88	8139 (370 to 15907)*	-4659 (-21511 to 12191)	12223 (-2042 to 26489)	0.11

Supplemental Table 4. Association of CKD with measures of GLP-1 and GIP response during 2-hour OGTT using the CKD-EPI Creatinine-Cystatin C Equation (2021). Mean differences represent the differences associated with CKD (vs controls) with 95% confidence intervals and P-values. Covariates were added one at a time to the base model which included age, sex, and race. The fully adjusted model is adjusted for age, sex, race, fat-free mass, fat mass, physical activity, calorie intake, smoking status, and CVD.

Covariate adjustment	**GLP-1 exposure**				**GIP exposure**			
	GLP-1 iAUC		**GLP-1 tAUC**		**GIP iAUC**		**GIP tAUC**	
	Difference (95% CI), pM x min	***P***	**Difference (95% CI), pM x min**	***P***	**Difference (95% CI), (pg x min)/mL**	***P***	**Difference (95% CI), (pg x min)/mL**	***P***
None (unadjusted)	233 (-362 to 828)	0.44	1187 (449 to 1925)	**<0.01**	17900 (5424 to 30377)	**<0.01**	23054 (8722 to 37387)	**<0.01**
Age, sex, and race	224 (-364 to 814)	0.45	1326 (559 to 2094)	**<0.01**	17244 (4234 to 30254)	**<0.01**	20281 (5633 to 34930)	**<0.01**
Weight	268 (-325 to 861)	0.37	1336 (558 to 2115)	**<0.01**	17432 (4244 to 30621)	**0.01**	20232 (5379 to 35086)	**<0.01**
Fat mass	325 (-274 to 925)	0.28	1341 (549 to 2132)	**<0.01**	17452 (3760 to 31144)	**0.01**	19676 (4303 to 35048)	**0.01**
Fat-free mass	272 (-366 ro 910)	0.40	1234 (393 to 2075)	**<0.01**	18719 (4144 to 33294)	**0.01**	21406 (5054 to 37757)	**0.01**
Physical activity	226 (-419 to 871)	0.49	1163 (314 to 2011)	**<0.01**	17007 (2422 to 31592)	**0.02**	19487 (3124 to 35849)	**0.02**
Calorie intake	235 (-462 to 932)	0.50	1077 (259 to 2095)	**0.01**	12043 (-2090 to 26177)	0.09	13949 (-1673 to 29613)	0.08
Smoking status	235 (-466 to 937)	0.50	1178 (256 to 2101)	**0.01**	12043 (-2188 to 26275)	0.09	13966 (-1784 to 28718)	0.08
Fully adjusted model	284 (-439 to 1007)	0.43	1304 (360 to 2248)	**<0.01**	13099 (-1566 to 27764)	**0.08**	15931 (-217 to 32079)	0.05

Supplemental Figure 4.2 Comparison of fasting plasma DPP4 antigen and activity levels among CKD (n=41) and controls (n=36) using the CKD-EPI Creatinine-Cystatin C Equation (2021). Box plots represent median and IQR and the whiskers represent minimum and maximum values. Unpaired t-Test was used to determine the difference between the two groups.



Chapter 5

Conclusions & Future Work

5.1 Abstract

In this dissertation, the focus has been on patient-oriented research in persons with CKD, employing metabolic imaging and multi-omics approaches to unravel its pathophysiology and its impact on metabolism, muscle function, and physical performance. The investigation comprehensively delved into metabolic, physiological, and cardiometabolic disruptions associated with CKD. Chapter 1 provided a thorough overview of CKD and its related comorbidities, highlighting metabolic and physiological complexities. Chapter 2 explored the effects of NR and CoQ10 supplementation on systemic markers of mitochondrial function and physical endurance in sedentary CKD patients. Chapters 3 and 4 focused on metabolic and physiological aberrations in non-diabetic moderate-severe CKD. We identified altered metabolic pathways associated with insulin response disruptions and assessed postprandial incretin hormone levels and their determinants through standardized oral glucose tolerance testing. The dissertation concludes in Chapter 5 with a summary of findings, concluding remarks, and prospects for future research endeavors.

5.2 Summary of Work

Chapter 1 is a brief review of CKD health complexities and associated comorbidities and its detrimental effects on metabolism, muscle function, and overall health. It provides an overview of hormonal, inflammatory, and nutritional factors as mediators in development of metabolic and hemodynamic dysfunction, cardiovascular disease, and patient outcomes. These factors include

elevated chronic inflammation (IL-6, TNF-α, C-reactive protein), insulin resistance, dyslipidemia, and hypertension. In addition, nutritional and lifestyle factors such as reduced protein intake and impaired amino acid metabolism are main contributors of skeletal muscle dysfunction and exercise intolerance in CKD. Finally, we emphasized the role of mitochondria function in the development of CKD and CKD-associated comorbidities and highlighting it as a therapeutic target.

Chapter 2 investigates the impact of two pharmacological agents known to target mitochondrial function on physical endurance measures and systemic markers of mitochondrial metabolism in a sedentary CKD cohort. To this end, we conducted a randomized, placebo-controlled, crossover trial to test the impact of CoQ10 and NR on physical endurance capacity, work efficiency, and metabolic/lipid profiles in 25 patients with CKD. While we did not observe meaningful improvements in physical endurance outcomes post NR or CoQ10, short term CoQ10 and NR supplementation resulted in biologically plausible changes in mitochondrial metabolism and the plasma lipid profile. CoQ10 treatment led to improved β oxidation resulting in a systemic increase in plasma free fatty acids and a decrease in complex triglycerides while NR supplementation altered levels of TCA cycle intermediates and resulted in a broad decrease of plasma lipid species, including lipotoxic subclasses of sphingolipids. The observed impacts on the metabolic profile suggest early beneficial changes in systemic mitochondrial metabolism and lipid profile that argue for future trials with a longer treatment duration.

In **chapters 3** and **4**, we focus on better understanding of metabolic and physiological derangements associated with non-diabetic CKD. In **chapter 3**, I investigated the plasma metabolic response to a 75g oral glucose load to identify specific metabolites and metabolic pathways underlying impaired insulin resistance in a non-diabetic CKD population. We performed targeted plasma metabolic profiling comparing 41 nondiabetic patients with moderate-to-severe

CKD to 20 healthy controls during an oral glucose challenge. Our goal was to identify specific metabolic alterations in nondiabetic CKD to better understand the metabolic and biological basis for glucose intolerance and insulin resistance in CKD. We showed that patients with CKD demonstrate an attenuated plasma metabolome response to OGTT compared to controls independent of alterations in the fasting state. We observed an attenuated plasma response to OGTT particularly in vitamin B family members, mitochondrial energy metabolism, and purine metabolism. Together, our findings suggest CKD is associated with a suppressed anabolic response to glucose challenge consistent with prior findings of impaired anabolic response to insulin in this population.

Chapter 4 builds on the findings from chapter 3 by assessing incretin, glucagon, and insulin homeostasis in response to a 75g oral glucose load in the same cohort. Our goal was to understand how the presence and severity of nondiabetic kidney disease influences incretin homeostasis in addition to factors contributing to these differences. We investigated the response to standardized oral glucose tolerance testing in a well-characterized cohort of 59 patients with non-diabetic moderate-severe CKD and control participants. We reported substantial disruption of incretin and glucagon homeostasis in patients with CKD independent of biologically relevant confounders. Overall, our study suggests that the alteration in the incretin system and glucagon dynamics are contributing factors to metabolic disturbances in CKD.

5.3 Conclusions

The findings in the dissertation add to the collective understanding of metabolic and physiological derangements associated with CKD and sheds light in the link between metabolic alterations and

physiological disturbances in CKD. The disruption in incretin homeostasis and blunted incretin response changes may contribute to the metabolic dysregulation associated with kidney disease and reveal a potential role for incretin-mimetics to address the attenuated incretin effects observed in our study. These findings highlight the need for attention to the differential response of incretin-mimetics by CKD status to inform their clinical application addressing the metabolic complications associated with CKD.

Additionally, we examined the feasibility and efficacy of pharmacological interventions such as NR and CoQ10 that target mitochondrial metabolism for their potential effects on clinically relevant outcomes in CKD such as physical performance, mitochondrial metabolism, and cardiometabolic health in a sedentary CKD population. We also explored the impact of NR and CoQ10 in dyslipidemia management in CKD and found favorable changes in plasma lipid profile with both NR and CoQ10. Our work added robust clinical evidence to the body of preclinical evidence supporting the efficacy of NR and CoQ10 for beneficial impacts of NR and CoQ10 on metabolic health and factors linked with patient outcome.

5.4 Future Work

5.4.1 Exercise intervention in CKD

The role of exercise interventions (aerobic and resistance training) is largely unexplored in CKD. Patients with CKD have lower physical function, and impaired physical performance, contributing to a high prevalence of frailty and mobility disability, and an increased risk for mortality. This is partly due to the impact of uremic milieu on mitochondrial function contributing to skeletal muscle dysfunction and exercise intolerance. In chapter 1, we explored potential pharmacological

interventions (NR and CoQ10) that directly impact mitochondrial function. Another approach to improve mitochondrial function is aerobic and strength training. Strategic exercise interventions could improve multitude of CKD complications including metabolic and skeletal muscle function, endocrine, inflammation, and oxidative stress. To this end, we recently completed a 12-week randomized home-based exercise trial combining aerobic and strength training in sedentary individuals with CKD. The participants were randomized to usual care (no exercise) or exercise intervention. The goal of the study is to assess changes in skeletal muscle mitochondrial function, metabolism, and physical function. The primary outcome of the study is assessment of in vivo mitochondrial function using a novel non-invasive phosphorous magnetic resonance spectroscopy (^{31}P MRS). The secondary outcomes include changes in physical performance outcomes, and measures of ex vivo mitochondrial function in the skeletal muscle and immune cells. Completing this work would allow us to assess the impact and efficacy of pharmacological (NR and CoQ10) and lifestyle (Exercise and health education) intervention on metabolism and physical functioning in a vulnerable CKD population.

5.4.2 Further assessment of NR and CoQ10 in CKD

In a complementary study of the finding from chapter 1, we are performing a comprehensive assessment of NR and CoQ10 supplementation in a range of cardiometabolic health biomarkers. To complement our metabolomics and lipidomics findings, we have performed RNA-sequencing to investigate changes in the transcriptome post NR and CoQ10 supplementation. This will allow us to further assess changes in mitochondria-associated gene expressions thought to be mechanistically linked to metabolic disturbances in CKD. Additionally, we have measured plasma

markers of oxidative stress (F2-isoprostanes) and a panel of inflammatory cytokines. Together, these findings will provide a comprehensive assessment of biologically and clinically relevant changes with NR and CoQ10 in cardiometabolic health biomarkers in persons with moderate to severe CKD