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Effect Modification of Body Mass Index and Kidney Function on Insulin Sensitivity Among Patients With Moderate CKD and Healthy Controls

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Introduction: Insulin resistance and obesity are prevalent in chronic kidney disease (CKD) patients. The interaction of body mass index (BMI) and kidney function across the continuum of estimated glomerular filtration rate (eGFR) is unknown.

Methods: In a cross-sectional study of 139 patients, 52 with CKD stages 3 and 4 and 87 patients with normal eGFR, we measured the insulin sensitivity index (ISI) using the hyperinsulinemic euglycemic clamp and homeostasis model assessment of insulin resistance (HOMA-IR). We investigated the interaction between eGFR and BMI in their association with ISI and HOMA-IR using linear models with robust standard errors.

Results: Median age was 56 (42, 66) years, 50.4% were female, and 36% were African American. Patients with low eGFR (~30 ml/min per 1.73 m²) had low ISI (2.3 mg/min per μ U/ml) regardless of BMI. Among patients with preserved eGFR (>90 ml/min per 1.73m²), BMI had a greater effect on ISI (6.3 mg/min per μ U/ml at a BMI of 20 kg/m² vs. 4.6 mg/min per μ U/ml at a BMI of 30 kg/m²) (*P* for interaction = 0.046). In models adjusted for demographics, and log transformed interleukin-6, high-sensitivity C-reactive protein, leptin, and adiponectin, a 1-SD (28 ml/min per 1.73 m²) lower eGFR was associated with a statistically significant 1.14-unit decrease in ISI (95% confidence interval = -1.80, -0.48) among nonobese patients. Among obese patients, the effect estimate was -0.25 (95% confidence interval = -0.88, 0.39). The association between BMI and HOMA-IR was stronger in patients with lower eGFR (*P* for interaction = 0.005).

Conclusion: Both eGFR and BMI are independently associated with insulin sensitivity, but the strength of the association between BMI and insulin sensitivity varies significantly across eGFR.

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nsulin resistance is common in patients with chronic kidney disease (CKD) and may represent a critical link between CKD and the increased cardiovascular disease (CVD) risk observed in this population. Prior literature suggests that in CKD, insulin resistance may be related primarily to a post-receptor binding defect in insulin action in the skeletal muscle¹; however, its metabolic correlates are multifactorial, such as decrease in clearance and dysregulation of adipocytokines, hyperinsulinemia, systemic inflammation, and increased oxidative stress.²⁻⁶ The co-occurrence of obesity in a significant proportion of CKD patients potentially exacerbates insulin resistance and compounds the risk of downstream cardio-metabolic effects including impaired glucose metabolism, endothelial dysfunction, and CVD. The potential interaction between the effects of impaired kidney function and obesity on insulin resistance may be related to the role played by the adipocytokines leptin and adiponectin, secreted by visceral adipose tissue and primarily cleared by the kidney. Leptin and adiponectin

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accumulate as kidney function declines^{7,8}; in maintenance hemodialysis patients, the leptin-to-adiponectin ratio has been shown to be the best correlate of insulin resistance validated against the gold standard hyperinsulinemic euglycemic clamp, when compared to more commonly used practical indices of insulin resistance such as the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulinsensitivity check index (QUICKI).⁹

The potential interaction between kidney function and measures of obesity—including adipocytokines as predictors of insulin sensitivity among nondiabetic persons with or without CKD has not been studied in detail. The aim of this study was to investigate the interaction between estimated glomerular filtration rate (eGFR) and surrogate measures of adiposity (body mass index and serum leptin) in their association with (i) peripheral insulin sensitivity measured via clampderived insulin sensitivity index (ISI), and (ii) hepatic insulin sensitivity measured using the HOMA-IR index, in a sample of nondiabetic subjects with preserved kidney function and CKD stages 3 and 4.

MATERIALS AND METHODS

Design and Study Sample

The Insulin Resistance in Chronic Kidney Disease (IRCKD) study was a cross-sectional clinic-based investigation that enrolled 140 subjects: 52 patients with CKD stages 3 and 4 and 88 controls (eGFR > 60ml/min per 1.73 m^2). Patients with CKD were recruited from the Nashville Veterans Affairs (VA) medical center. Controls were recruited using Researchmatch.org, Vanderbilt University Medical Center e-mail distribution lists, and flyers in the outpatient clinics. All study participants were nondiabetic at enrollment and had a body mass index (BMI) $> 18 \text{ kg/m}^2$. Diabetes status was ascertained by medical history, chart review, antidiabetic medication use, glycated hemoglobin, or fasting glucose. In the CKD group, we included patients ≥ 18 years of age with eGFR <60 ml/min per 1.73 m² without proteinuria >5 g/24 h, who had blood pressure <160/100 mm Hg, with no change in their blood pressure medications 1 month prior to enrollment. Controls were 50 to 80 years of age and prehypertensive (systolic blood pressures 120–139 mm Hg and/or diastolic blood pressure 80-89 mm Hg) who had an eGFR ≥ 60 ml/min per 1.73 m² with no proteinuria. Controls on antihypertensive therapy were excluded. Given our focus on investigating patterns of insulin sensitivity among obese and normal-weight patients along the kidney function continuum, we excluded patients with BMI <18 kg/m² (considered to be underweight).

In both groups, we excluded pregnant or breastfeeding women, patients on any insulin sensitizer or medication for treatment of metabolic syndrome, patients with decompensated heart failure, or those who had had an acute CVD event in the last 6 months. We also excluded patients who had received systemic glucocorticoids/immunomodulators within 1 month of enrollment or had active/severe inflammatory diseases.

The study was approved by the Vanderbilt University Medical Center and VA institutional review boards, and informed consent was obtained from all participants.

Study Procedures

Blood Samples

Samples used to measure glucose were processed using GM9 glucose analyzer (Analox Instruments, Atlanta, GA). Samples for insulin, adiponectin, and leptin were drawn into ethylenediamine tetraacetic acid (EDTA) tubes, centrifuged within 10 to 15 minutes at 3000 RPM, and stored at -80° . Insulin and leptin samples were analyzed at Vanderbilt's Hormonal Lab Core. High-molecular-weight adiponectin and interleukin 6 (IL-6) were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) and high-sensitivity C-reactive protein (hsCRP) was measured at the Vanderbilt Clinical laboratory by immunoturbidimetric method.

Hyperinsulinemic Euglycemic Clamp

The clamp was performed to assess whole-body glucose disposal in all study participants following an 8-hour overnight fast using the glucose clamp technique originally described by DeFronzo et al.¹⁰ Briefly, 2 peripheral i.v. catheters were placed in the upper extremities, and fasting blood samples were drawn. An i.v. infusion of human regular insulin (50 units/50 ml of 0.9% normal saline solution) was initiated at a constant rate of 80 mU/m² per min for CKD patients and 40 mU/ m^2 per min for controls to achieve hyperinsulinemia, to suppress hepatic glucose production, and to increase glucose disposal in skeletal muscle. Because of increased resistance to insulin action in CKD patients compared to controls, a higher dose of insulin was required to completely suppress hepatic glucose generation. Concurrently, an intravenous infusion of 20% dextrose was administered at a variable rate in order to "clamp" blood glucose concentrations in the euglycemic range (90-105 mg/dl), and a glucose analyzer was used to measure blood glucose every 5 minutes. An infusion of potassium phosphate was also given to prevent hypokalemia resulting from hyperinsulinemia. Within 150 minutes of initiation of the insulin infusion, a steady state of euglycemia, defined as <10% variation in blood glucose readings for \geq 30 minutes at a constant infusion of 20% dextrose, was achieved.

Assuming that hepatic glucose production was suppressed by the hyperinsulinemic state and that no net change in blood glucose concentrations under steadystate clamp conditions were observed, we estimated the glucose disposal rate (M), which is equal to the average glucose infusion rate during steady state. The estimated M value was normalized to body weight and used to calculate the clamp-derived ISI.

Study Outcomes

The main dependent variables for this study were muscle and hepatic insulin sensitivity measured using clamp-derived ISI and HOMA-IR, respectively. The gold standard for assessing peripheral insulin sensitivity is the hyperinsulinemic euglycemic clamp, which measures the whole-body insulin sensitivity *in vivo* as it directly measures glucose disposal after suppression of hepatic glucose output under steady-state conditions.¹⁰ The HOMA-IR is the product of fasting plasma insulin and glucose, which is an index of hepatic insulin resistance¹¹

ISI = M/(G × ΔI),¹² where M is the glucose disposal rate in mg/kg/min and is calculated as M = (dextrose infusion rate at steady state in ml/h × 184) / (weight in kg × 60); G is the mean steady state glucose concentration in mg/dl; and ΔI is the difference in plasma insulin concentrations at the beginning and the end of the steady state period meausred in $\mu U/ml$.

HOMA-IR = fasting serum insulin (μ U/ml) × fasting plasma glucose (mg/dl)/405.^{5,11,13,14}

Covariates

The main independent variables for this study were eGFR, BMI (weight [kg]/height $[m]^2$), and serum leptin. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.¹⁵ Participants were classified as being obese based on the World Health Organization cut-points as BMI \geq 30 kg/m², and CKD was defined as eGFR <60 ml/min per 1.73 m². Covariates included demographics, inflammatory markers (IL-6 and hsCRP) and adipocytokines (adiponectin and leptin).

Statistical Analysis

Interaction Between eGFR and Continuous BMI as Determinants of ISI and HOMA-IR

A multivariable linear regression model was fitted with ISI as the dependent variable; eGFR and BMI as main independent variables (modeled linearly), and including an eGFR \times BMI interaction term. Estimates of mean ISI were obtained for the range of values of eGFR and BMI for which we had the most data (2.5th to 97.5th percentile). Model-based estimates of mean ISI were plotted against both eGFR and BMI using contour plots to display potential interaction patterns. This

analysis was repeated using log leptin in place of BMI as a measure of functional fat. All the above analyses were repeated for HOMA-IR as the dependent variable.

Linear Relationship Between eGFR and Measures of Insulin Sensitivity Across BMI Categories

To investigate potential effect modification of the association of eGFR with ISI by obesity, a linear model with ISI as the dependent variable was fitted with the following parameters: eGFR, BMI category (obese vs. nonobese) and an eGFR \times BMI category interaction term (model 1). Linear contrasts were used to obtain the mean difference (β) in ISI per SD change in eGFR among obese and nonobese participants. This analysis was repeated using the HOMA-IR index as the dependent variable. Models were subsequently adjusted for plasma levels of inflammatory markers (IL-6 and hsCRP) and adipocytokines (leptin and adiponectin) in model 2. These variables (IL-6, hsCRP, adiponectin, and leptin) were log-transformed and modeled using restricted cubic splines with 4 knots. Knots were placed at quantiles of covariate distributions, equally spaced in sample size.¹⁶ Confounding effects were examined by further adjustment for age, sex, and race in model 3. Analyses restricted to healthy controls were also performed.

In fully stratified analyses, adjusted mean differences in HOMA-IR scores were computed across 4 categories (CKD/obese, CKD/nonobese, non-CKD/obese, nonobese/non-CKD) using linear models, with the nonobese/non-CKD group as the referent category.

All models were fitted using robust standard errors to relax homoscedasticity assumptions. A 2-sided 5% significance level was used for statistical inferences, and all analyses were performed using Stata version 15.1 (StataCorp LLC, College Station, TX). There were 6 missing values for hsCRP, 2 for serum adiponectin, and 1 for serum IL-6. Complete case analyses were performed.

RESULTS

Baseline Characteristics of the Study Sample

Baseline characteristics of the 139 participants (52 CKD patients and 87 controls) included in these analyses are presented in Table 1. The median age was 56 years (interquartile range [IQR] = 42, 66); 49.6% were male and 36% were African American. Median (IQR) eGFR among CKD cases and controls was 46.1 (40.4, 52.6) and 94.3 (84.9, 107.0) ml/min per 1.73 m², respectively. Compared to controls, CKD patients were older, were more likely to be obese, and had higher systolic blood pressure. CKD patients also had higher median fasting glucose, fasting insulin, HOMA-IR, and serum leptin, but had lower adiponectin, M values, and ISI compared to controls (Table 1).

Table 1. Baseline characteristics of st	study participants
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	Overall	CKD ^c patients	Controls
Characteristic ^b	n = 139	n = 52	n = 87
Age, yr	56 (42, 66) ^a	66 (59, 70)	49 (36, 60)
Male, n (%)	69 (49.6)	36 (69.2)	33 (37.9)
African American (n (%)	50 (36.0)	15 (28.9)	35 (40.2)
BMI (kg/m ²)	28.7 (25.3, 33.5)	30.3 (26.7, 34.9)	27.9 (24.6, 32.7)
BMI ≥30 kg/m², n (%)	63 (45.3)	28 (53.9)	35 (40.2)
SBP, mm Hg	129 (121, 136)	134 (126, 146)	127 (120, 134)
eGFR, ml/min per 1.73 m ²	81.3 (50.7, 101.2)	46.1 (40.7, 52.6)	94.3 (84.9, 107.0)
Proteinuria, mg/dl	11.0 (4.0–36.0)	11.0 (4.0–36.0)	0 (0)
Urine protein-to-creatinine ratio ^d	0.10 (0.03, 0.33)	0.10 (0.03, 0.33)	0 (0)
Fasting plasma glucose, mg/dl	101 (94, 107)	106 (100, 115)	98 (92, 104)
Fasting insulin, µU/mI	10.6 (7.7, 19.3)	17.6 (11.6, 26.1)	8.8 (5.9, 14.3)
HOMA-IR index	2.7 (1.8, 4.9)	4.5 (3.1, 6.9)	2.1 (1.5, 3.5)
Glucose disposal rate, mg/kg per min	6.4 (4.7, 9.8)	5.1 (3.6, 6.4)	7.7 (5.3, 10.8)
ISI, mg/min per μU per mI)	3.7 (2.6, 5.9)	2.8 (2.0, 3.5)	4.22 (3.1, 6.7)
Leptin (ng/ml)	28.7 (16.4, 52.4)	31.6 (17.0, 51.4)	26.2 (15.9, 52.7)
Adiponectin (µg/ml)	15.9 (8.7, 31.4)	14.6 (7.6, 23.2)	17.8 (8.7, 35.5)
HMW adiponectin (µg/ml)	1.5 (0.7, 3.0)	1.2 (0.7, 2.1)	1.8 (0.7, 3.3)

BMI, body mass index; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HMW, high molecular weight; HOMA-IR, homeostasis assessment model of insulin resistance; ISI, insulin sensitivity index; SBP, systolic blood pressure.

^aData for continuous variables are presented as median (interquartile range).

^bMost between-group comparisons were statistically significant (P = 0.005 for SBP, P = 0.02 for BMI, and P < 0.001 for the variables) except for race, leptin, and adiponectin. ^cCKD = eGFR <60 ml/min per 1.73 m².

^dUrine protein and creatinine levels were measured in spot urine samples.

Relationship Among eGFR, BMI, and ISI

Log ISI was positively correlated with eGFR (r = 0.39) and inversely correlated with BMI (r = -0.30) and log leptin (r = -0.42). The contour plot in Figure 1a shows the association of ISI with both eGFR and BMI. Patients who had concurrently the lowest eGFR and highest BMI values had the lowest mean ISI (2.3 mg/min per μ U per ml; depicted in the upper-left portion of the plot), whereas study participants with both the highest eGFR and lowest BMI values had the highest mean ISI (7.4 mg/min per µU per ml; depicted in the lower-right portion of the plot). The accentuated curvature of the contour lines is suggestive of interaction between BMI and eGFR in the association with ISI. In Figure 1a, patients with low eGFR (in particular, the lower margin of the CKD stage 3 range, ~ 30 ml/min per 1.73 m²) had low ISI (2.9 mg/min per µU per ml, and hence insulin resistant) even with BMI within the normal range $(20-24.9 \text{ kg/m}^2)$. At higher eGFR, there was a greater change in ISI per unit change in BMI. For example, at an eGFR of 90 ml/min per 1.73 m², the mean ISI was 6.3 at a BMI of 20 kg/m² and only 4.6 at a BMI of 30 kg/m², a difference of 1.7 units. The P value for interaction was 0.046, indicating differences in the association between BMI and ISI at lower versus higher eGFR. Similar interaction patterns were observed in interaction analyses using log leptin in place of BMI (Supplementary Figure S1). At a low eGFR of 30 ml/min per 1.73 m², mean ISI remained low at 3.0 mg/min per μ U per ml, with increasing values of log leptin from 1.4 to 2.9. At a higher eGFR of 90 ml/min per 1.73 m², a

similar increase in log leptin (from 1.4 to 2.9) was associated with a significant drop in mean ISI from 7.8 to 5.4 mg/min per μ U per ml. The *P* value for interaction was 0.01, indicating significant differences in the association between log leptin and ISI at lower versus higher eGFR.

Figure 1b shows differential slopes for the linear relationship between ISI and eGFR across BMI categories. The regression slope for the association between eGFR and ISI was greater in nonobese compared to obese subjects. A 1-SD (28 ml/min per 1.73 m²) lower eGFR was associated with a significant 1.33-unit lower (95% confidence interval [CI] = -1.97, -0.70) ISI among nonobese participants and only a 0.51-unit lower (95% CI = -0.98, -0.05) ISI among obese participants (P for interaction = 0.04). Among nonobese subjects, after adjustment for log IL-6, log hsCRP, log leptin, and log adiponectin, lower eGFR remained associated with a significantly lower ISI ($\beta = -1.36$; 95% CI = -2.02, -0.70) (Table 2). In obese participants, there was a significant attenuation of the regression slope between eGFR and ISI ($\beta = -0.40$; 95% CI = -0.83, 0.04), which was no longer significant. In models additionally adjusted for demographics (age, sex, and race), similar patterns were observed for nonobese ($\beta = -1.14$; 95% CI = -1.80, -0.48) and obese ($\beta = -0.25$; 95% CI = -0.88, 0.39) patients. We found similar patterns in analysis restricted to control subjects. After full adjustment for demographics (age, sex, and race), log IL-6, log hsCRP, log leptin, and log adiponectin, a 1-SD lower eGFR remained associated



Figure 1. (a) Interaction between estimated glomerular filtration rate (eGFR) and body mass index (BMI) for the association with clamp-derived insulin sensitivity index (ISI). Patients with low eGFR (in particular, the lower margin of the chronic kidney disease [CKD] stage 3 range, 30 ml/ min per 1.73 m²) had lower ISI even with BMI within the normal range. At higher eGFR, there was a greater change in ISI per unit change in BMI. *P* for interaction = 0.046, indicating differences in the association between BMI and ISI at lower versus higher eGFR. (b) Linear relationship between clamp-derived ISI and eGFR across BMI categories. A 1-SD lower eGFR was associated with a greater decrease in ISI among nonobese compared to obese participants. (c) Linear relationship between clamp-derived ISI and BMI across eGFR categories. A 1-SD higher BMI was associated with a greater decrease in ISI among participants with eGFR ≥ 60 compared to those with eGFR <60.

with significant а 2.06-unit lower (95%) CI = -3.96, -0.15) ISI in nonobese subjects but only a nonsignificant 0.80-unit lower (95% CI = -2.01, 0.41) ISI among obese subjects. Figure 1c shows a steeper regression slope for the association of 1-SD higher BMI with ISI among participants with eGFR ≥ 60 $(\beta = -0.97; 95\% \text{ CI} = -1.55, -0.38)$ compared to patients with eGFR <60 (β =-0.43;95% CI = -0.82, -0.04).

 Table 2.
 Association of of lower eGFR with insulin sensitivity index in obese and nonobese subjects in sequentially adjusted models

	Nonobese		Obese	
Model	βª	95% CI	βª	95% CI
Model 1	-1.33	(-1.97, -0.70)	-0.51	(-0.98, -0.05)
Model 2	-1.36	(-2.02, -0.70)	-0.40	(-0.83, 0.04)
Model 3	-1.14	(-1.80, -0.48)	-0.25	(-0.88, 0.39)

Cl, confidence interval; eGFR, estimated glomerular filtration rate.

^aTabulated values (β) represent the decrease in mean insulin sensitivity index (in mg/ min per μU per ml units) per standard deviation (28 ml/min per 1.73 m²) lower eGFR among obese (body mass index ≥30 kg/m²) and nonobese subjects. Model 1 comprises eGFR, body mass index (binary variable: <30 kg/m² or ≥30 kg/m²), and the eGFR × body mass index interaction term. Model 2 includes model 1 variables, log high-sensitivity C-reactive protein, log interleukin-6, log leptin, and log adiponectin. Model 3 includes model 2 variables and demographics (age, sex, and race). Log HOMA-IR was inversely correlated with eGFR (r = -0.49) and positively correlated with BMI (r = 0.52) and log leptin (r = 0.46). Figure 2a shows the association of HOMA-IR with eGFR and BMI. The

Relationship Among eGFR, BMI, and HOMA-IR

association of HOMA-IR with eGFR and BMI. The HOMA-IR scores were lower for persons with higher eGFR compared to lower eGFR, at any BMI value. For example, at a BMI of 30 kg/m², the HOMA-IR was 2.1 for persons with an eGFR of 120 ml/min per 1.73 m² compared to 4.8 for persons with an eGFR of 30 ml/ min per 1.73 m². Hence, persons with higher eGFR (120 ml/min per 1.73 m²) were significantly insulin sensitive (low HOMA-IR) even with BMI in the obese range. More importantly, the change in HOMA-IR per unit change in BMI was greater at lower eGFR compared to higher eGFR (as evidenced by the greater gradient of contours at lower vs. higher eGFR). Among persons with higher eGFR (120 ml/min per 1.73 m²), HOMA-IR scores remained low at 2.1 with increasing BMI values from 20 to 30 kg/m². At a lower eGFR of 30 ml/min per 1.73 m², a similar gradient in BMI (20 to 30 kg/m²) was associated with a significant increase in mean HOMA-IR from 2.1 to



Figure 2. (a) Interaction between estimated glomerular filtration rate (eGFR) and body mass index (BMI) for the association with the homeostasis assessment model of insulin resistance (HOMA-IR). The HOMA-IR score was lower for persons with higher eGFR compared to lower eGFR regardless of BMI. Also, persons with high eGFR (e.g., 120 ml/min per 1.73 m²) had low HOMA-IR even with BMI in the obese range. The change in HOMA-IR per unit change in BMI was greater at lower eGFR. *P* for interaction = 0.005, indicating significant differences in the association between BMI and HOMA-IR at lower versus higher eGFR. (b) Linear relationship between HOMA-IR and glomerular filtration rate (GFR) across BMI categories. Each 1-SD lower eGFR was associated with a greater increase in HOMA-IR among obese compared to nonobese participants. (c) Linear relationship between HOMA-IR and BMI across eGFR categories. A 1-SD higher BMI was associated with a greater increase in HOMA-IR among participants with eGFR <60 compared to those with eGFR \geq 60.

4.8. The *P* value for interaction was 0.005, indicating significant differences in the association between BMI and HOMA-IR at lower versus higher eGFR. Similar interaction patterns were observed in interaction analyses using log leptin instead of BMI (Supplemental Figure S2) for the association with HOMA-IR.

Figure 2b indicates differences in regression slopes for the linear relationship between HOMA-IR and eGFR across BMI categories (*P* for interaction = 0.09). After full adjustment for demographics, log IL-6, log hsCRP, log leptin, and log adiponectin, a 1-SD (28 ml/min per 1.73 m²) lower eGFR was associated with a greater increase in HOMA-IR score among obese ($\beta = 1.06$; 95% CI = 0.36, 1.76) compared to nonobese participants (β = 0.50; 95% CI = -0.07, 1.08). Figure 2c shows a greater effect of higher BMI on HOMA-IR among participants with eGFR <60 ($\beta = 1.69$; 95% CI = 0.98, 2.39) compared to patients with eGFR ≥ 60 ($\beta = 0.96$; 95% CI = 0.46, 1.47).

Table 3 shows adjusted mean differences in HOMA-IR scores for participants classified as CKD/nonobese, obese/non-CKD, and CKD/obese participants compared to the referent non-CKD/nonobese group. In model 1, the mean difference ($\beta = 4.39$) in HOMA-IR scores for the CKD/obese group was greater than the sum of the mean differences for the CKD/nonobese ($\beta = 1.91$) and the non-CKD/obese groups ($\beta = 1.78$), suggesting significant synergistic interaction (*P* for interaction < 0.001) between CKD and obesity with respect to higher mean HOMA-IR scores. After full adjustment for demographics, inflammatory markers, and adipocytokines, CKD patients (both obese and nonobese) still had higher HOMA-IR scores compared to the referent non-CKD/ nonobese group, whereas obese participants with preserved eGFR no longer had significantly lower HOMA-IR scores.

DISCUSSION

We investigated the the association of eGFR and measures of adiposity (BMI and serum leptin) with muscle and hepatic insulin sensitivity in a nondiabetic population comprising patients with CKD stage 3 and 4 and

		Model 1	Model 2	Model 3	
Subject group	n	β ^α (95% CI) ^b	β (95% Cl)	β (95% CI)	
Non-CKD, nonobese ^c	52	Ref.	Ref.	Ref.	
CKD, nonobese	24	1.95 (1.03, 2.87)	1.73 (0.98, 2.48)	1.21 (0.21, 2.20)	
Non-CKD, obese	35	1.81 (0.93, 2.69)	0.63 (-0.31, 1.57)	0.39 (-0.54, 1.31)	
CKD, obese	28	4.64 (3.42, 5.87)	3.02 (1.72, 4.32)	2.30 (0.90, 3.70)	

BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; HOMA-IR, homeostasis model assessment of insulin resistance; Ref., referent.

Tabulated values are mean differences (β) in HOMA-IR index between CKD and/or obese subjects compared to the referent non-CKD/nonobese group.

^bRobust 95% confidence intervals were computed using the Huber–White Sandwich estimator.

The mean H0MA-IR index in the referent category (non-CKD, nonobese) was 1.98. CKD: estimated GFR <60 ml/min per 1.73 m². Obese: body mass index \ge 30 kg/m².

In model 1, mean differences (β) are unadjusted. In model 2, mean differences (β) are adjusted for log high-sensitivity C-reactive protein, log interleukin-6, log leptin, and log adiponectin. Model 3 includes model 2 variables and demographics (age, sex, and race).

persons with preserved eGFR. Our findings suggest that eGFR and BMI are both independently associated with insulin sensitivity, but that the strength of the association between BMI and insulin sensitivity varies significantly across eGFR levels and insulin sensitivity phenotype.

Skeletal muscle insulin sensitivity or clamp-derived ISI was largely reduced at low eGFR and less strongly associated with BMI (or leptin) at low eGFR. Meanwhile, the association of BMI with hepatic insulin sensitivity (HOMA-IR) was more pronounced at lower eGFR levels, with progressively higher BMI levels correlating with significantly higher hepatic insulin resistance (elevated HOMA-IR). Hence, obese patients with CKD had markedly pronounced hepatic insulin resistance (elevated HOMA-IR) beyond what was expected from the independent contribution of reduced eGFR and high BMI, highlighting a synergistic interaction between obesity and CKD.

Patients with CKD are at increased risk for cardiovascular (CV) mortality, which is not entirely explained by traditional CV risk factors.¹⁷ Insulin resistance is associated with increased CVD risk even in the absence of diabetes¹⁸ and is considered a "nontraditional" CV risk factor in CKD¹⁹ through its effects on inflammation, endothelial function, and oxidative stress.²⁰⁻²³ Current evidence suggests that insulin resistance might also accelerate the progression of kidney disease.^{24,25} Furthermore, our group²⁶ previously reported that insulin resistance might have a significant role in protein-energy wasting in CKD, especially in maintenance hemodialysis patients.²⁷ Characterizing the unmechanisms is key for derlying developing interventions that can improve insulin resistance and its consequences in CKD.

Insulin resistance is present even at earlier stages of CKD,²⁸ and its prevalence increases with further decline in kidney function.²⁹ Although the pathophysiology of insulin resistance in uremia has been recognized and explored over decades, evidence related to the effects of moderate CKD and adiposity in insulin

resitance remains unsettled. Fliser et al. reported that no correlation was observed between eGFR and insulin sensitivity measured by the i.v. glucose tolerance test.²⁸ Studies have suggested that BMI was the primary determinant of insulin resistance across the CKD spectrum in nondiabetic patients with CKD stage 3 and 4, measured by the hyperinsulinemic euglycemic clamp³⁰ and HOMA-IR.⁵ In contrast, similar to our results, Kobayashi et al. reported a correlation between kidney function and insulin sensitivity measured by clamp in nondiabetic patients with varying stages of CKD.²⁴ In addition, data from the Uppsala Longitudinal Study of Adult Men study, which included a cohort of elderly men with baseline eGFR >50 ml/min per 1.73 m², demonstrated a positive correlation between eGFR and insulin sensitivity measured by the clamp technique.³¹

Our current findings introduce the notion that reduced kidney function interacts with BMI in the context of peripheral insulin sensitivity. At lower eGFR levels, kidney disease seems to be the driver of peripheral insulin resistance, whereas at higher eGFR, BMI may be the primary regulator of peripheral insulin resistance independent of eGFR. The pathophysiology of low eGFR-associated peripheral insulin resistance is complex and likely multifactorial. Potential factors involved include decreased clearance of insulin and adipocytokines, chronic inflammation, metabolic acidosis, increased oxidative stress, uremic toxins, vitamin D deficiency, uncontrolled secondary hyperparathyroidism, and anemia.³² We suggest that adipocytokine dysregulation may be the most crucial factor promoting insulin resistance and the interaction patterns observed in this study.

Our study further suggests distinct patterns for ISI and HOMA-IR. Although higher BMI is associated with reduced ISI at all eGFR levels, the magnitude of this association is lower as eGFR declines. At an eGFR of \sim 30 ml/min per 1.73 m², subjects exhibit marked peripheral insulin resistance regardless of their BMI. In contrast, the correlation of BMI with hepatic insulin

resistance, measured by HOMA-IR, is greater at low eGFR levels. This divergence in the results between peripheral and hepatic insulin resistance is consistent with the literature regarding the existence of peripheral insulin resistance in CKD.

Our results also suggest that inflammation and adipocytokine dysregulation potentially play a key role in peripheral insulin resistance in obese patients, which is consistent with the current literature. Accumulating evidence has provided a direct link between inflammation and obesity-induced insulin resistance over the last decades. Adipose tissue-derived pro-inflammatory cytokines such as tumor necrosis factor $-\alpha$ (TNF- α) were found to play a role in obesity-linked insulin resistance.³³ Inflammatory cytokines (TNF-a, IL-6) and nuclear factor $-\kappa B$ (NF-kB) are known to induce insulin resistance by increased serine phosphorylation of insulin receptor substrate 1 (IRS-1).³⁴⁻³⁶ Chronic kidney disease induces systemic inflammation through adipose tissue dysfunction, which, when combined with decreased clearance of adipocytokines, exacerbates insensitivity to the metabolic actions of insulin.

Current evidence regarding hepatic insulin action in CKD remains unclear. Earlier studies by DeFronzo et al.²⁹ and Friedman et al.³⁷ documented that altered insulin action in CKD is primarily due to a dysfunction in glucose uptake into the skeletal muscle. However, Rubenfeld and Garber³⁸ reported increased glucose production in CKD patients mainly from increased gluconeogenesis measured using radiolabeled alanine, suggesting both peripheral and hepatic insulin resistance. In the current study, we report that HOMA-IR scores are greatest at low eGFR levels. Studies have shown that reduced eGFR leads to hyperinsulinemia (due to decreased clearance), which is associated with decreased expression of GLUT4, impaired insulin signaling, and peripheral insulin resistance. Instead of a decreased endogenous insulin production to regain homeostasis, a maladaptive response occurs that is characterized by increased insulin production to overcome insulin resistance, which self-perpetuates insulin resistance. Hence, in the context of concurrently reduced eGFR, decreased insulin and adipocytokine clearance, and hyperinsulinemia, progressively higher BMI or increased adiposity correlates with worsening insulin resistance. This may explain in part why the synergistic interaction of concurrently low eGFR and high BMI correlates with much higher HOMA-IR scores than expected from the singular contrubution of either low eGFR or high BMI, as seen in Table 3.

Limitations of this study include the cross-sectional design, which raises the possibility of reverse causality and limits causal inferences between CKD and insulin resistance. In addition, our study had a relatively small sample size with insufficient control matching. Data from larger prospective studies would strengthen the potential for causal inferences. Also, our study population consists mainly of patients with CKD stage 3, which limits the generalizability to patients with more advanced CKD. Another limitation is the use of BMI as a surrogate of adiposity in the CKD population. The fact that BMI does not incorporate the protein energy wasting in patients with CKD might lead to misclassification of patients with sarcopenic obesity as normal, when their body fat percentage would in fact classify them as obese. This is commonly referred as the "obesity paradox."³⁹ Strengths of our study include the use of the gold standard hyperinsulinemic euglycemic clamp to measure peripheral insulin sensitivity and enrolling participants with a sufficiently wide range of eGFR to investigate the association with insulin sensitivity across BMI levels.

In conclusion, although kidney disease and adiposity are both independent factors contributing to insulin resistance in CKD, the contribution of BMI to insulin resistance depends on the stage of kidney disease. Lower eGFR is associated with impaired insulin action in peripheral tissues, regardless of adiposity. This association of eGFR with insulin sensitivity seems to be mediated primarily by inflammation and adipocytokine dysregulation among obese subjects but not in nonobese patients. Low eGFR and obesity are associated with pronounced insulin resistance in the liver, highlighting a synergistic interaction between kidney function and obesity in hepatic insulin resistance. Additional studies are needed to explore the underlying mechanisms of insulin resistance in CKD.

DISCLOSURE

All the authors declare no competing interests.

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AUTHOR CONTRIBUTIONS

EAA: data curation, software, formal analysis, writing, original draft preparation. MS: investigation, data curation, writing, original draft preparation. AA: investigation. EDS: conceptualization, methodology, supervision. TAI: conceptualization, methodology, writing, reviewing and editing, supervision. AMH: conceptualization, methodology, writing, reviewing and editing, supervision. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Interaction between estimated glomerular filtration rate (eGFR) and log leptin for the association with clamp-derived insulin sensitivity index (ISI).

Figure S2. Interaction between estimated glomerular filtration rate (eGFR) and log leptin for the association with the homeostasis assessment model of insulin resistance (HOMA-IR).

STROBE Statement

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