Supplementary material

Supplementary methods and results for:

Impaired glucose and insulin homeostasis in moderate-severe CKD

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Supplementary methods: dose-ranging study

We performed an insulin dose-ranging study in order to determine an appropriate insulin infusion rate for the hyperinsulinemic-euglycemic clamp in our study population. The study included 5 participants with CKD (4 men, 1 women, median eGFR 38 mL/min/1.73m^2, eGFR range 20-43 mL/min/1.73m^2).

Each participant was admitted to the University of Washington Clinical Research Center after an overnight fast. Peripheral intravenous catheters were placed in each upper extremity, with one warmed ("arterialized") for blood sampling. Three fasting plasma samples were drawn 5 minutes apart.

Intravenous deuterated glucose (6,6-2D-glucose, Cambridge Pharmaceuticals, Cambridge, MA, 40 mg/mL in normal saline) was then administered as a priming dose (200 mg/m^2 per 100 mg/dL plasma glucose concentration infused over 5 minutes) followed by a continuous infusion (2 mg/min/m^2 maintained throughout the basal period and each stage of the hyperinsulinemic-euglycemic clamp). Basal plasma samples were drawn 150, 165, and 180 minutes after initiating the deuterated glucose infusion.

The low-dose hyperinsulinemic-euglycemic clamp was then initiated as a prime (80 mU/m^2/min for 5 minutes) followed by a constant rate (40 mU/m^2/min). A solution of 20% dextrose containing 4 mg/mL 6,6-2D-glucose was titrated to maintain blood glucose (measured every 5 minutes) near 90 mg/dL. Plasma samples were drawn 90, 105, and 120 minutes after initiating the low-dose clamp.

The medium-dose hyperinsulinemic-euglycemic clampwas then initiated as a prime (160 mU/m^2/min for 5 minutes) followed by a constant rate (80 mU/m^2/min). The dextrose plus 6,6-2D-glucose solution was titrated to continue to maintain blood glucose (measured every 5 minutes) near 90 mg/dL. Plasma samples were drawn 90, 105, and 120 minutes after initiating the medium-dose clamp.

Three of the five participants then underwent a high-dose hyperinsulinemic-euglycemic clamp, intended to determine the maximum rate of insulin-mediated glucose disposal. The high-dose hyperinsulinemic-euglycemic clampwas administered as a constant infusion of insulin at 400 mU/m^2/min (without prime). The dextrose plus 6,6-2D-glucose solution was titrated to continue to maintain blood glucose (measured every 5 minutes) near 90 mg/dL. Plasma samples were drawn 90, 105, and 120 minutes after initiating the high-dose clamp.

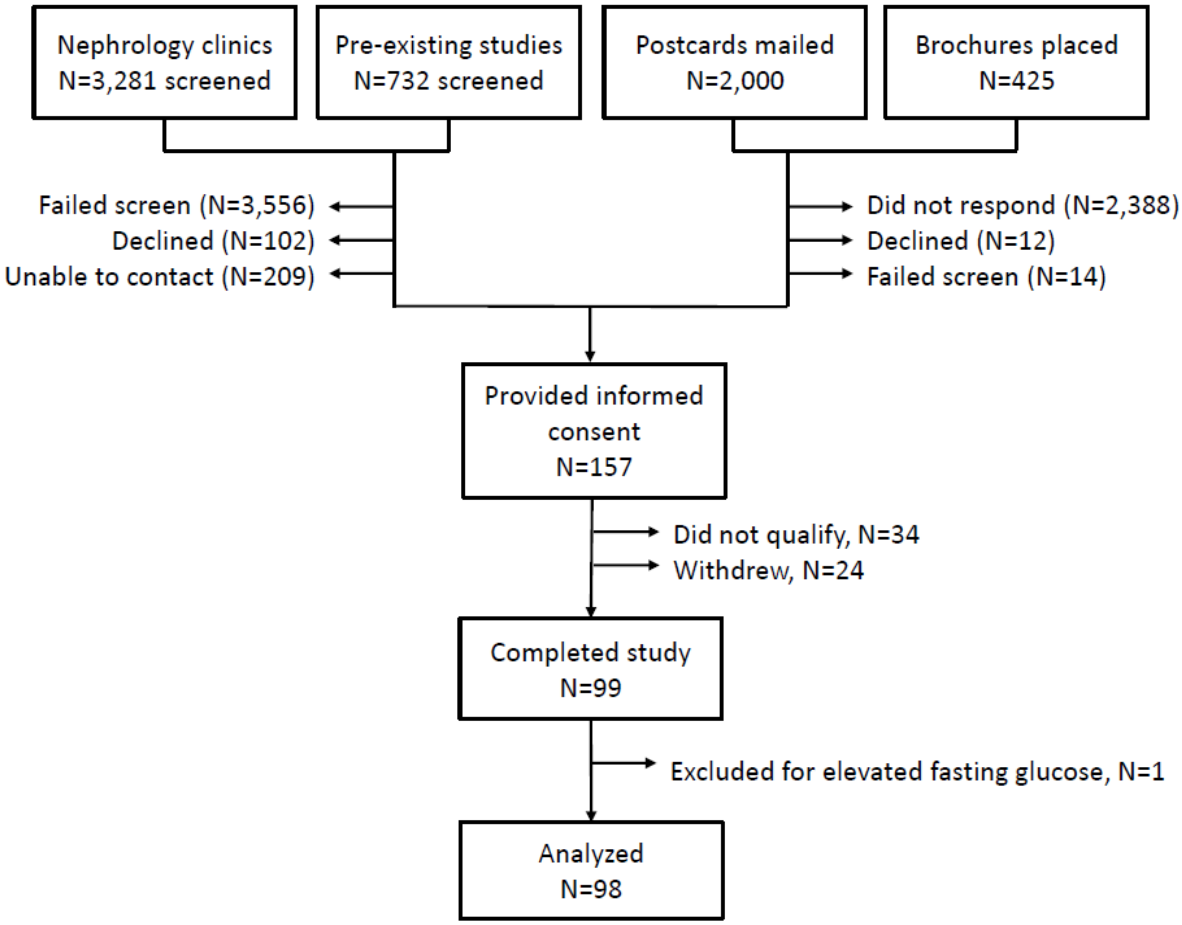
Plasma concentrations of insulin and glucose as well as the glucose concentration of the infusate were measured at the Northwest Lipid Research Laboratories, Seattle, WA. Glucose was measured using the glucose hexokinase method on a Roche Module P Chemistry autoanalyzer (Roche Diagnostics, Inc., Indianapolis, IN, inter-assay coefficient of variation [CV] 1.2-1.7%). Insulin was measured using a two site immune-enzymometric assay on the Tosoh 2000 auto-analyzer (CV 2.0-2.8%), calibrated to WHO IRP 66/304. The proportion of glucose present as 6,6-2D-glucose was determined at Vanderbilt University by gas chromatography–mass spectrometry using an Agilent (Santa Clara, CA) 7890C gas chromatograph interfaced with an Agilent 5975C mass selective detector as previously described (Mayerson AB, Hundal RS, Dufour S, Lebon V, Befroy D, Cline GW, Enocksson S, Inzucchi SE, Shulman GI, Petersen KF. The

effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. *Diabetes.* 2002 Mar; 51(3): 797–802.)

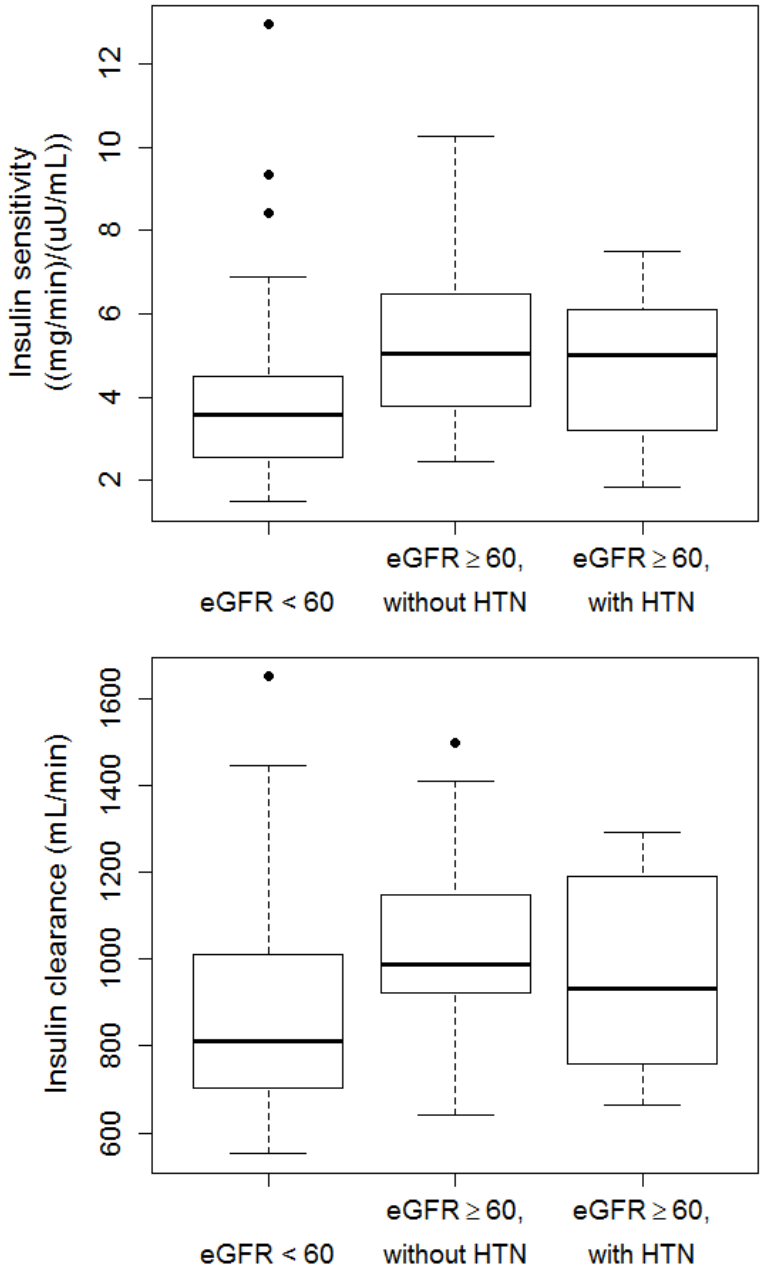
Isotopic steady state concentrations were achieved during the final 30 minutes of each stage of the clamp. The rates of glucose appearance (Ra) and disappearance (Rd) were calculated based on steady-state equations modified to include the use of a labeled dextrose infusion (Wolfe RR (1992) Radioactive and Stable Isotope Tracers in Biomedicine. Wiley-Liss, New York). Endogenous glucose production was defined as endogenous Ra of glucose at each stage of the clamp.

Supplementary Figures

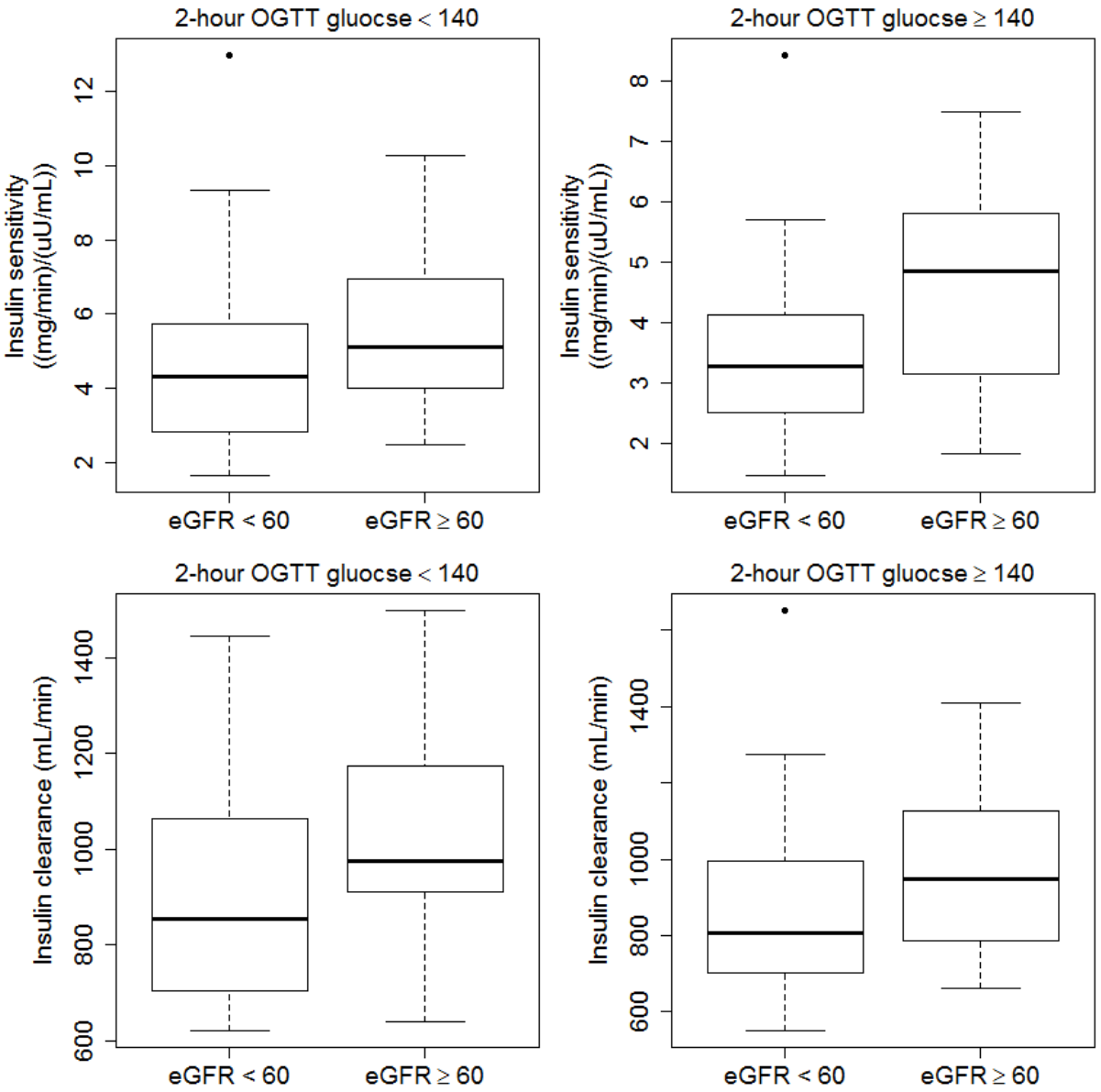
Supplementary Figure S1. Flow diagram of participant enrollment and analysis.



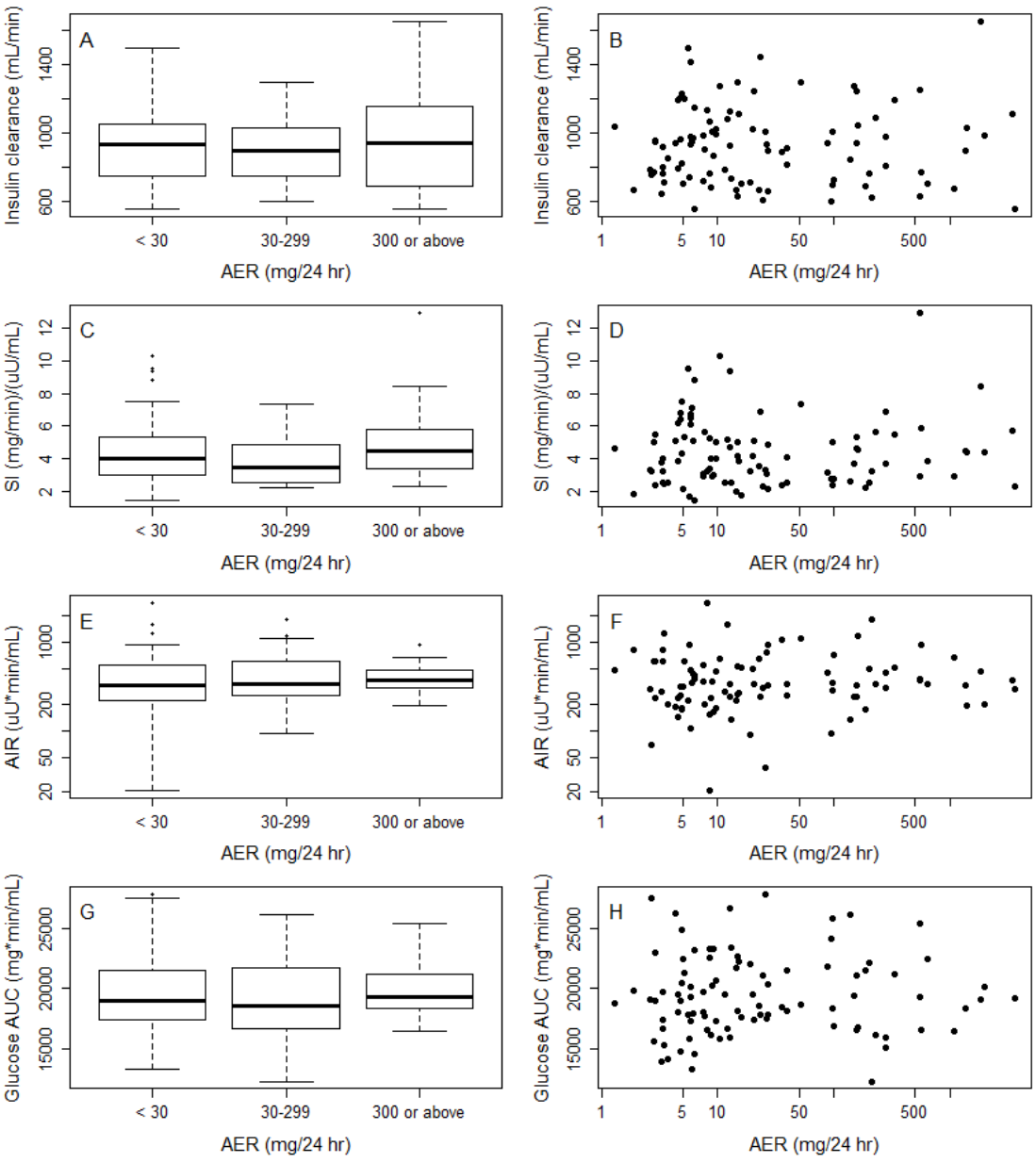
Supplementary Figure S2. Associations of chronic kidney disease with insulin sensitivity and insulin clearance, stratifying control subjects by hypertension status. Distributions of insulin sensitivity (measured by hyperinsulinemic-euglycemic clamp) and insulin clearance (measured by hyperinsulinemic-euglycemic clamp) are summarized using box plots and scatterplots. Box plots display median with 5th, 25th, 75th, and 95th percentiles, with participants outside the 5th – 95th percentiles noted as data points. Abbreviations: eGFR = estimated glomerular filtration rate; HTN = hypertension.



Supplementary Figure S3. Associations of chronic kidney disease with insulin sensitivity and insulin clearance, stratified by plasma glucose contentration two hours after oral glucose administration. Distributions of insulin sensitivity (measured by hyperinsulinemic-euglycemic clamp) and insulin clearance (measured by hyperinsulinemic-euglycemic clamp) are summarized using box plots and scatterplots. Box plots display median with 5th, 25th, 75th, and 95th percentiles, with participants outside the 5th – 95th percentiles noted as data points. Abbreviations: eGFR = estimated glomerular filtration rate; OGTT = oral glucose tolerance test (with glucose results in mg/dL).



Supplementary Table S4. Associations of albuminuria with measures of glucose and insulin homeostasis. Distributions of insulin sensitivity (measured by hyperinsulinemic-euglycemic clamp), insulin clearance (measured by hyperinsulinemic-euglycemic clamp), insulin secretion (acute insulin response to intravenous glucose), and glucose tolerance (glucose area under the curve during the oral glucose tolerance test) are summarized using box plots and scatterplots. Box plots display median with 5th, 25th, 75th, and 95th percentiles, with participants outside the 5th – 95th percentiles noted as data points.



Supplementary Table S1. Clinical conditions to which CKD was attributed among the 59 participants with estimated GFR <60 mL/min/1.73m^2.

Clinical condition	**N**	**N with kidney biopsy**
Hypertension	23	0
Primary glomerular diseases		
Focal and segmental glomerulosclerosis	4	3
Membranous glomerulopathy	1	1
C1q nephropathy	1	1
Polycystic kidney disease	6	0
Renovascular disease	3	0
Other		
Nonsteroidal anti-inflammatory drugs	1	0
Reflux nephropathy	1	1
Renal agenesis	1	0
Tubulointerstitial nephritis with uveitis	1	0
Unknown	17	0

Supplementary Table 2. Correlations of continuous covariates with measures of glucose and insulin homeostasis in SUGAR.

	Insulin sensitivity (mg/min)/(µU/mL)	Insulin clearance (mL/min)	Insulin secretion (µU*min/mL)	Glucose tolerance (µU*min/mL)
Age	-0.15	-0.02	-0.16	0.21
Physical activity	0.25	0.14	-0.08	-0.16
Dietary intake				
Energy	0.19	0.33	-0.12	0.06
Fat	0.19	0.30	-0.08	0.03
Carbohydrates	0.04	0.14	-0.07	0.01
Protein	0.12	0.24	-0.11	0.11
Glycemic Index	-0.26	-0.28	0.21	-0.06
Height	0.19	0.51	0.04	-0.05
Weight	-0.07	0.33	0.25	0.07
Fat free mass	0.06	0.46	0.18	-0.01
Fat mass	-0.20	0.01	0.27	0.07
Systolic blood pressure	-0.11	-0.07	0.10	0.12
Diastolic blood pressure	-0.07	0.02	0.20	0.02
Estimated GFR	0.11	0.18	-0.04	-0.12
Urine albumin excretion	0.05	0.03	0.04	0.03
Hemoglobin	0.17	0.24	-0.27	0.23
Parathyroid hormone	0.08	0.02	0.15	0.05

Insulin sensitivity and clearance were measured during the hyperinsulinemic-euglycemic clamp. Insulin secretion (AIR) was measured as the incremental plasma insulin area under the curve during minutes 2-10 of the intravenous glucose tolerance test. Glucose tolerance was measured as glucose area under the curve during the oral glucose tolerance test. Urine albumin excretion was log-transformed. Entries are Pearson correlation coefficients.

Supplementary Table 3. Average values for measurements of glucose and insulin homeostasis for categorical variables in SUGAR.

	Insulin sensitivity (mg/min)/(μU/mL)	Insulin clearance (mL/min)	Insulin secretion (μU*min/mL)	Glucose tolerance (μU*min/mL)
Sex				
Female	4.2 (2.2)	830 (173)	359 (2.0)	19233 (3637)
Male	4.5 (1.9)	1012 (238)	334 (2.3)	19758 (3188)
Race				
White	4.5 (1.9)	956 (225)	307 (2.0)	19616 (3383)
Black	4.2 (2.7)	865 (215)	589 (2.6)	18932 (3901)
Asian/Pacific Islander	2.9 (0.6)	708 (155)	338 (1.5)	19886 (2097)
Cardiovascular disease				
No	4.5 (2.2)	927 (222)	363 (2.1)	19202 (3492)
Yes	3.9 (1.4)	915 (252)	291 (2.3)	20689 (2785)
Current smoking				
No	4.2 (1.8)	907 (209)	355 (2.2)	19324 (3389)
Yes	5.8 (3.1)	1039 (310)	290 (2.0)	20714 (3333)
Any antihypertensive medication				
No	5.1 (2.2)	1020 (196)	352 (2.2)	18575 (3439)
Yes	4.0 (1.9)	878 (228)	343 (2.2)	19981 (3305)

Insulin sensitivity and clearance were measured during the hyperinsulinemic-euglycemic clamp. Insulin secretion was measured as the incremental plasma insulin area under the curve during minutes 2-10 of the intravenous glucose tolerance test. Glucose tolerance was measured as glucose area under the curve during the oral glucose tolerance test. Entries are mean (SD), except for AIR, which presents geometric mean (SD).

Supplemental Table 4. Associations of antihypertensive medication use with measures of glucose and insulin homeostasis in SUGAR.

	N	Insulin sensitivity (mg/min)/(μU/mL)	Insulin clearance (mL/min)	Insulin secretion (μU*min/mL)	Glucose tolerance (μU*min/mL)
RAAS inhibitors	46	-0.41 (-1.31, 0.48)	-48 (-133, 37)	12 (-21, 59)	433 (-1164, 2030)
Beta-blockers	26	-0.92 (-1.82, -0.01)	-89 (-210, 32)	-13 (-40, 26)	307 (-1098, 1713)
Calcium-channel blockers	30	0.22 (-0.75, 1.18)	-66 (-173, 40)	-33 (-52, -7)	1483 (-265, 3232)
Diuretics	29	-0.17 (-1.11, 0.76)	24 (-81, 128)	6 (-26, 53)	272 (-1402, 1946)

Insulin sensitivity and clearance were measured during the hyperinsulinemic-euglycemic clamp. Insulin secretion was measured as the incremental plasma insulin area under the curve during minutes 2-10 of the intravenous glucose tolerance test. Glucose tolerance was measured as glucose area under the curve during the oral glucose tolerance test.

Results are from linear regression models including yes/no variables for each class of antihypertensive medication, plus variables for age, sex, race, and CKD status.

For insulin sensitivity, insulin clearance, and glucose AUC, estimates (95% CI) are average differences in outcome comparing those on antihypertensive medication and those not, adjusted for age, sex, race, CKD status, and other antihypertensive classes.

For insulin secretion, estimates (95% CI) are average percent differences comparing those on antihypertensive medication and those not, adjusted for age, sex, race, CKD status, other antihypertensive classes, and log-insulin sensitivity.

Supplemental Table 5. Associations of CKD with insulin sensitivity and insulin clearance, excluding one participant with outlying values of estimated GFR and insulin sensitivity.

	Insulin sensitivity (mg/min)/(μU/mL)		Insulin clearance (mL/min)	
Covariate adjustments	**Difference (95% CI)**	**p-value**	**Difference (95% CI)**	**p-value**
None (unadjusted)	-1.3 (-2, -0.5)	0.0009	-129 (-215, -42)	0.004
Age/race/sex	**-1 (-1.7, -0.3)**	**0.008**	**-91 (-170, -11)**	**0.03**
+ Height & weight	-0.8 (-1.5, -0.1)	0.02	-102 (-178, -25)	0.009
+ Fat mass	-0.8 (-1.5, -0.1)	0.02	-103 (-183, -22)	0.01
+ Fat-free mass	-1 (-1.7, -0.3)	0.008	-85 (-162, -7)	0.03
+ Physical activity	-1 (-1.7, -0.2)	0.01	-106 (-194, -18)	0.02
+ Macronutrient intake	-1 (-1.7, -0.2)	0.01	-90 (-168, -13)	0.02
+ Glycemic index	-0.9 (-1.6, -0.2)	0.02	-82 (-160, -4)	0.04
+ Smoking	-1.1 (-1.8, -0.4)	0.003	-100 (-175, -25)	0.009
+ Cardiovascular disease	-1 (-1.7, -0.2)	0.01	-98 (-181, -16)	0.02
Fully adjusted model	-0.82 (-1.51, -0.13)	0.02	-92 (-169, -16)	0.02

Cell contents are the difference associated with CKD (versus control subjects), with 95% confidence interval and p-value. Individual covariates (paired for height and weight) were added to one at a time to the base model including age, sex, and race. The fully adjusted model adjusts for age, sex, race, fat mass, fat-free mass, physical activity, glycemic index, and smoking.

Insulin clearance and sensitivity were measured during the hyperinsulinemic-euglycemic clamp.

Supplemental Table 6. Associations of CKD with Matsuda index, fasting insulin concentration, and insulinogenic index.

	Matsuda index		Fasting insulin (µU/mL)		Insulinogenic index (µL/mL)/(mg/dL)	
Covariate adjustments	**Difference (95% CI)**	**p-value**	**Difference (95% CI)**	**p-value**	**% Difference (95% CI)**	**p-value**
None (unadjusted)	-2.4 (-4.1, -0.6)	0.007	2.8 (0.7, 4.9)	0.009	20 (-12, 64)	0.25
Age/race/sex	**-2.2 (-3.8, -0.6)**	**0.008**	**3 (0.8, 5.2)**	**0.006**	**5 (-24, 45)**	**0.77**
+ Height & weight	-1.3 (-2.6, 0)	0.06	1.5 (-0.2, 3.1)	0.08	-4 (-30, 32)	0.81
+ Fat mass	-1.3 (-2.6, 0)	0.06	1.5 (-0.2, 3.2)	0.08	-4 (-30, 31)	0.78
+ Fat-free mass	-2.3 (-3.9, -0.7)	0.004	3.3 (1.3, 5.3)	0.001	9 (-19, 48)	0.56
+ Physical activity	-2 (-3.6, -0.4)	0.01	2.7 (0.6, 4.7)	0.01	5 (-25, 45)	0.79
+ Macronutrient intake	-2.3 (-4.1, -0.6)	0.009	3.3 (1.1, 5.4)	0.003	5 (-23, 43)	0.75
+ Glycemic index	-2 (-3.6, -0.5)	0.01	2.7 (0.5, 4.8)	0.01	4 (-25, 44)	0.80
+ Smoking	-2.4 (-4.1, -0.7)	0.005	3.2 (1, 5.3)	0.004	12 (-19, 54)	0.49
+ CV disease	-2.1 (-3.7, -0.5)	0.01	3 (0.6, 5.3)	0.01	5 (-24, 45)	0.77
Fully adjusted model	**-1.5 (-2.9, -0.2)**	**0.02**	**2.1 (0.5, 3.6)**	**0.009**	**7 (-22, 46)**	**0.67**

Cell contents are the difference associated with CKD (versus control subjects), with 95% confidence interval and p-value. Individual covariates (paired for height and weight) were added to one at a time to the base model including age, sex, and race. The fully adjusted model adjusts for age, sex, race, fat mass, fat-free mass, physical activity, glycemic index, and smoking. CV = cardiovascular.