Insulin resistance in chronic kidney disease is ameliorated by spironolactone in rats and humans

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In this study, we examined the association between chronic kidney disease (CKD) and insulin resistance. In a patient cohort with nondiabetic stages 2–5 CKD, estimated glomerular filtration rate (eGFR) was negatively correlated and the plasma aldosterone concentration was independently associated with the homeostasis model assessment of insulin resistance. Treatment with the mineralocorticoid receptor blocker spironolactone ameliorated insulin resistance in patients, and impaired glucose tolerance was partially reversed in fifth/sixth nephrectomized rats. In these rats, insulin-induced signal transduction was attenuated, especially in the adipose tissue. In the adipose tissue of nephrectomized rats, nuclear mineralocorticoid receptor expression, expression of the mineralocorticoid receptor target molecule SGK-1, tissue aldosterone content, and expression of the aldosterone-producing enzyme CYP11B2 increased. Mineralocorticoid receptor activation in the adipose tissue was reversed by spironolactone. In the adipose tissue of nephrectomized rats, asymmetric dimethylarginine (ADMA; an uremic substance linking uremia and insulin resistance) increased, the expression of the ADMA-degrading enzymes DDAH1 and DDAH2 decreased, and the oxidative stress increased. All of these changes were reversed by spironolactone. In mature adipocytes, aldosterone downregulated both DDAH1 and DDAH2 expression, and ADMA inhibited the insulin-induced cellular signaling. Thus, activation of mineralocorticoid receptor and resultant ADMA accumulation in adipose tissue has, in part, a relevant role in the development of insulin resistance in CKD.

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Insulin resistance (IR) is defined as a clinical condition in which there is a reduced biological effect for any given blood concentration of insulin. The presence of IR has been reported in patients with chronic kidney disease (CKD).[1] IR in CKD patients is accompanied by hyperinsulinemia and glucose intolerance, as well as by derangement of insulin secretion.[2] Recent studies have described IR in CKD as a 'renal IR syndrome' that contributes to the comorbidity of cardiovascular disease, where the authors attributed this syndrome to a higher body mass index (BMI) and increased triglyceride concentrations.[3] Several factors have been proposed for the pathogenesis of IR in renal dysfunction, including uremic toxins.[4] These factors all inhibit insulin-stimulated glucose disposal in insulin target organs. It was revealed that excess concentrations of mineralocorticoid, aldosterone, or activation of its receptor mineralocorticoid receptor (MR) induce IR.[5,6] In some previous studies, plasma aldosterone concentrations have been shown to increase according to renal function deterioration.[7–9] Therefore, increased aldosterone concentrations might contribute to the development of IR in CKD.

Other studies have also revealed that one of the uremic toxins, the endogenous nitric oxide synthase (NOS) inhibitor asymmetric dimethylarginine (ADMA), is involved in the derangements of glucose metabolism in various pathological conditions. A recent study demonstrated that ADMA blocks insulin-induced glucose utilization in the adipocytes.[10,11] ADMA is degraded by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which is composed of the two isoforms DDAH1 and DDAH2,[12,13] each of which stems from different chromosomes and differs in several aspects. We previously demonstrated that, in Nx dog, DDAH2 expressions are downregulated in the kidney[14] and coronary endothelium,[15] which contributed to blunted vasodilative response. We also demonstrated recently that the DDAH2/ADMA pathway was involved in glucose-stimulated insulin secretion in pancreatic β-cells.[16] These data imply that tissue levels of DDAH/ADMA affect the local blood supply or cellular signaling, which is surmised to lead to systemic insulin-resistant state or glucose intolerance.[17]

In this study, we have identified plasma aldosterone concentration as an independent risk factor for IR in our

Table 1 | Characteristics of the study participants classified into five CKD stages

Parameter	Stage 1 (>90)	Stage 2 (60–89)	Stage 3 (30–59)	Stages 4 and 5 (>30)
n	19	89	78	14
Diseases (%)				
Glomerulonephritis	13 (68.4)	45 (50.1)	49 (62.8)	8 (57.1)
Nephrosclerosis	3 (15.7)	26 (29.2)	11 (14.1)	4 (28.6)
Polycystic kidney disease	0 (0)	5 (5.6)	7 (9.0)	1 (7.1)
Others	3 (15.7)	13 (14.6)	11 (14.1)	1 (7.1)
Age	53.4 ± 3.8	61.1 ± 1.8*	66.1 ± 1.7**	72.6 ± 1.7**
Gender (male/female)	11/8	49/40	42/36	7/7
BMI (kg/m^2)	23.6 ± 0.76	22.2 ± 0.52	23.6 ± 0.46	22.7 ± 1.0
Systolic BP (mm Hg)	128.9 ± 3.7	133.4 ± 1.6	135.5 ± 1.3	137.1 ± 1.8
Diastolic BP (mm Hg)	73.0 ± 2.2	76.1 ± 1.2	78.3 ± 1.4	76.1 ± 1.9
eGFR (ml/min per 1.73 m^2)	106.1 ± 3.2	72.3 ± 1.0**	45.7 ± 1.1**	17.6 ± 2.2**
Urinary protein (g/g creatinine)	0.072 ± 0.012	0.075 ± 0.009	0.151 ± 0.010**	0.627 ± 0.091**
Hb (g/dl)	13.1 ± 0.2	13.4 ± 0.2	13.3 ± 0.2	11.5 ± 0.5**
LDL-C (mg/dl)	121.1 ± 6.4	123.9 ± 4.3	112.9 ± 1.4	115.4 ± 11.5
TG (mg/dl)	95.6 ± 9.5	135.5 ± 10.4	131.9 ± 13.1	164.4 ± 29.9
Blood glucose (mg/dl)	101.1 ± 3.4	103.4 ± 2.1	106.7 ± 2.4	108.0 ± 2.7
IRI (mmol/l)	8.38 ± 0.79	9.98 ± 1.10	10.87 ± 1.70*	19.07 ± 4.60*
HOMA-IR	2.17 ± 0.21	2.63 ± 0.31	3.00 ± 0.54*	5.06 ± 1.25**
Aldosterone (pg/ml)	131.5 ± 9.1	130.8 ± 9.4	156.2 ± 10.7*	168.2 ± 8.9**
K (mEq/l)	4.14 ± 0.18	4.39 ± 0.15*	4.36 ± 0.15*	4.60 ± 0.22*
ARC (pg/ml)	5.77 ± 1.82	12.68 ± 2.08	16.24 ± 2.28	16.23 ± 5.22
ACTH (pg/ml)	23.0 ± 3.49	29.81 ± 2.56	34.82 ± 1.81	33.62 ± 5.70
Cortisol (pg/ml)	10.38 ± 0.79	11.4 ± 0.47	12.55 ± 0.35	11.40 ± 0.67

Abbreviations: ACTH, adrenocorticotropic hormone; ARC, active renin concentration; BMI, body mass index; BP, blood pressure; CKD, chronic kidney disease; eGFR, glomerular filtration rate; Hb, hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; IRI, immunoreactive insulin; K, potassium; LDL-C, low-density lipoprotein-cholesterol; TG, triglyceride.
*P < 0.05 vs. stage 1; **P < 0.01 vs. stage 1.

CKD cohort. By using Nx rats, we showed the coexistence of MR activation, ADMA accumulation, and impaired insulin signaling in the adipose tissue. We have provided evidence for a novel link between the aldosterone/MR pathway and the DDAH/ADMA pathway in the adipose tissue of CKD that induced the initiation of IR in CKD.

RESULTS

Human study

Characteristics of CKD patients in the study population. The baseline characteristics of the study participants are presented in Table 1. As the CKD stage advanced, fasting insulin concentrations (immunoreactive insulin, IRI) significantly increased without any changes in FBS concentration. Consistently, with the advances in the CKD stage, homeostasis model assessment of IR (HOMA-IR) values significantly increased. Plasma aldosterone concentrations also increased as the CKD stages advanced, although plasma cortisol, adrenocorticotropic hormone (ACTH), and active renin concentration were not significantly altered. The participants were taking various kinds of antihypertensives. However, the population taking each antihypertensive was not different between each stage of CKD patients (Table 2).

A linear regression analysis was performed between estimated glomerular filtration rate (eGFR) and the various indexes of glucose metabolisms. Both fasting serum insulin concentrations and HOMA-IR levels were correlated with eGFR levels (Figure 1).

Table 2 | Antihypertensive medications of CKD patients of each stage

	Stage 1	Stage 2	Stage 3	Stages 4 and 5
ARBs or ACE-Is	9/19 (47.3%)	41/89 (46.1%)	35/78 (44.9%)	6/14 (42.8%)
Ca channel blockers	9/19 (47.3%)	37/89 (41.6%)	36/78 (46.2%)	10/14 (71.4%)
Diuretics	2/19 (10.5%)	6/89 (6.7%)	4/78 (5.1%)	1/14 (7.1%)
β-Blockers	3/19 (15.8%)	12/89 (13.4%)	9/78 (11.5%)	2/14 (14.5%)

Abbreviations: ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CKD, chronic kidney disease.

Plasma aldosterone concentration is a determinant factor of IR in CKD. As renal dysfunction advances, plasma aldosterone concentrations increased and IR progressed (Table 1). In addition, aldosterone has been reported to inhibit insulin signaling and cause IR in vascular smooth muscle cells.[18] Therefore, we hypothesized that the increase in plasma aldosterone in CKD contributes to the development of IR. The fasting aldosterone concentrations had a significant correlation with fasting glucose concentrations (Figure 2a), fasting insulin concentrations (Figure 2b), and HOMA-IR levels (Figure 2c). Multiple regression analysis using the various anthropometric and biochemical parameters, as described in Materials and Methods, revealed that triglyceride, aldosterone, eGFR levels, and BMI levels were significant determinants of systemic IR in CKD (triglyceride; $\beta = 0.347$,