ORIGINAL RESEARCH

Combined Salt and Caloric Restrictions: Potential Adverse Outcomes

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***Background*—**We hypothesized that caloric restriction (CR) and salt restriction (ResS) would have similar effects on reducing cardiovascular risk markers and that combining CR and ResS would be synergistic in modulating these markers.

***Methods and Results*—**To test our hypothesis, rats were randomized into 2 groups: ad libitum liberal salt diet (ad libitum/high-sodium, 1.6% sodium) or ResS diet (ad libitum/ResS, 0.03% sodium). CR was initiated in half of the rats in each group by reducing caloric intake to 60% while maintaining sodium intake constant (CR/high-sodium, 2.7% sodium or CR/ResS, 0.05% sodium) for 4 weeks. CR in rats on a high-sodium diet improved metabolic parameters, renal transforming growth factor-β and collagen-1α1 and increased plasma adiponectin and renal visfatin and NAD^+ protein levels. Although CR produced some beneficial cardiovascular effects (increased sodium excretion and reduced blood pressure), it also was associated with potentially adverse cardiovascular effects. Adrenal zona glomerulosa cell responsiveness and aldosterone levels and activation were inappropriately increased for the volume state of the rodent. Like CR on HS, CR on a ResS diet also produced relative increased zona glomerulosa responsiveness and an increased blood pressure with no improvement in metabolic parameters.

***Conclusions*—**These results suggest that combining CR and ResS may decrease the beneficial effects of each alone. Furthermore, CR, regardless of dietary salt intake, inappropriately activates aldosterone production. Thus, caution should be used in combining ResS and CR because the combination may lead to increased cardiovascular risk. **(*J Am Heart Assoc*. 2017;6:e005374. DOI: 10.1161/JAHA.116.005374.)**

Key Words: aldosterone • blood pressure • caloric restriction • high blood pressure • hypertension • insulin action • insulin resistance • mineralocorticoids • salt intake

Cardiovascular disease continues to be the number-1 cause for death, with worsening cardiometabolic factors being the primary mediators. Environmental factors, particularly diet, are substantial risk factors for the development of cardiometabolic abnormalities (eg, hypertension, insulin resistance, and dyslipidemia) and end-organ damage.[1] The 2 most frequently cited of these factors are salt and caloric intakes.[2-4] Based on the results of many observational studies, salt restriction (ResS) and caloric restriction (CR) are highly regarded to be effective nonpharmacologic interventions to reduce the risk of cardiovascular events.[5] Because of these results and supportive preclinical data,[6-8] government agencies and medical associations have strongly advocated both ResS and CR as national goals for the population in general.[9]

ResS activates the renin-angiotensin-aldosterone system (RAAS). This system plays a critical role in blood pressure (BP) regulation via its effects on both sodium and volume homeostasis and vascular reactivity and structure. However, there is abundant evidence showing that, in humans and rodents, chronic activation of the mineralocorticoid receptor and disordered angiotensin II (AngII) and/or aldosterone (Aldo) levels lead to increases in BP and cardiovascular and renal damage. Limited data suggest that CR reduces RAAS activity that would be beneficial.[10-12] However, the impact of ResS and CR on RAAS activation is uncertain.

Several recent studies suggest that sirtuins (silent information regulator 2 proteins [SIRT]), and specifically SIRT1, may be major mediators of the beneficial effects of CR. SIRTs are NAD-dependent histone deacetylases, which are reported to be involved in the cardioprotective effects of CR.[13-16] In addition, others have shown that SIRT1 mediates renal protective effects of CR.[17] Of interest, SIRT1 may also be involved in sodium homeostasis by modulating renal sodium

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Clinical Perspective

What Is New?

- Caloric restriction increases aldosterone secretion regardless of the level of sodium intake.
- The combination of caloric restricted and salt-restricted diets abolished the beneficial effects of each diet independently on reduction of blood pressure, improved insulin resistance, and decreased levels of adiponectin, visfatin, and nicotinamide adenine dinucleotide.
- Increases in blood pressure following caloric and salt restriction may, in part, be secondary to an enhanced adrenal zona glomerulosa cell response to secretagogues such as angiotensin II.

What Are the Clinical Implications?

- Caution should be exercised before advocating both significant caloric- and salt-restrictive diets, as their individual cardiovascular benefits may be reduced.
- In some individuals, caloric restriction may be associated with an increased risk of aldosterone-mediated cardiovascular and renal damage regardless of the level of sodium intake.

excretion[18-20] and has been shown to be increased in renal, cardiac, and fat tissue by a low-salt diet.[21]

However, there are no data to determine if the combination of CR and ResS has additive or synergistic effects in modifying potential cardiovascular and metabolic risk factors. Based on currently available published data, we hypothesized that CR and ResS would have similar effects on reducing cardiovascular and metabolic risk markers and that the combination of CR and ResS would be synergistic in modulating these markers. Thus, in rodents, we assessed the effects of 4 diets (liberal salt diet, CR/liberal salt diet, ResS diet, and CR/ResS diet) on our end points (BP, measures of RAAS activation, insulin resistance, and the levels of cardiovascular risk hormones) and potential mechanistic molecular biomarkers.

Materials and Methods

Animal Study

Male Wistar rats weighing 175 to 200 g were obtained from Charles River Laboratories, Inc (Wilmington, MA). All animals were housed in a room lighted 12 h/day at an ambient temperature of 22±1°C. Animals were allowed 4 days to acclimate after arrival and had free access to Laboratory Rodent Diet 5001 (LabDiet, St. Louis, MO) and tap water until the initiation of the experiment. To assess the influence of dietary sodium consumption, liberal salt (HS) and restricted salt (ResS) rodent chows (HS containing 1.6% Na^+ and 1.1% K^+, ResS containing 0.03% Na^+ and 1.1% K^+) were prepared as derivations from the Basal Diet 5755 (TestDiet, St. Louis, MO [Table 1]). Of importance in this study, mineral intake was maintained constant between CR and ad libitum (ie, non-CR) diets, particularly for sodium and potassium. Thus, the equivalent CR diets contained 2.7% Na^+ and 1.8% K^+ in CR/HS diets and 0.05% Na^+ and 1.8% K^+ in CR/ResS diets plus the appropriate enrichments in vitamins for each diet. The rats were divided into 2 groups 4 days before initiation of CR and fed with HS and ResS chow, respectively. During this period, 24-hour food consumption was measured daily to determine baseline food intake. After the acclimation period, each group was divided into 2 subgroups, ad libitum (ad libitum/HS or ad libitum/ResS) and CR (CR/HS or CR/ResS) and studied for 4 weeks. The ad libitum/HS and ad libitum/ResS groups had free access to the HS and the ResS chow, respectively, for the duration of the study. The CR/HS and CR/ResS groups received 60% of the food intake consumed by the animals during the baseline period when on the ad libitum/HS and the ad libitum/ResS diets, respectively. All rats were weighed weekly at the same time of day. In the last week of the intervention period, intraperitoneal glucose tolerance tests (ipGTT), BP measurements, and 24-hour urine collections were performed as described below. The studies were conducted in accordance with Harvard Medical School institutional guidelines for the humane treatment of animals.

Zona Glomerulosa Cell Stimulation

Adrenal glands were excised during euthanization, and zona glomerulosa (ZG) cells were isolated as previously reported.[22-24] Briefly, ZG cell suspensions were made by diluting pellets to obtain 1 to 2×10^5 cells/0.5 mL of modified Krebs-Ringer bicarbonate. Cells were then incubated in duplicate, in vehicle or in the presence of AngII (10^{-10} to 10^{-7} mol/L) for 1 hour at 37°C under 5% CO_2 and 95% O_2 atmosphere.

Intraperitoneal Glucose Tolerance Test

The procedure was performed as previously described by us.[25] Briefly, animals were fasted for 10 hours overnight before the ipGTT, and the next morning (day 21) body weights were recorded. Glucose solution (1.5 g/kg body weight) was infused intraperitoneally. Blood samples were obtained from the tail of conscious rats for determination of blood glucose levels before glucose administration and 15, 30, 60, 90, and 120 minutes after glucose administration. At the end of the ipGTT, rats were returned to cages and allowed access to food. Blood glucose levels were determined using a Freestyle glucometer (Abbott Laboratories, Abbott Park, IL).

Table 1. Diet Composition

		LS (LT072)		LS-CR (LT073)			HS (LT074)			HS-CR (LT075)			
Nutrition	Unit					In CR (%)			% of LS			In CR (%)	% of LS-CR
Protein	%		18		18	40		18	100		18	40	100
Arginine	%		0.71		0.71	40		0.71	100		0.71	40	100
Histidine	%		0.52		0.52	40		0.52	100		0.52	40	100
Isoleucine	%		0.97		0.97	40		0.97	100		0.97	40	100
Leucine	%		1.75		1.75	40		1.75	100		1.75	40	100
Lysine	%		1.47		1.47	40		1.47	100		1.47	40	100
Methionine	%		0.67		0.67	40		0.67	100		0.67	40	100
Cystine	%		0.07		0.07	40		0.07	100		0.07	40	100
Phenyalanine	%		0.97		0.97	40		0.97	100		0.97	40	100
Tyrosine	%		1.03		1.03	40		1.03	100		1.03	40	100
Threonine	%		0.78		0.78	40		0.78	100		0.78	40	100
Tryprophan	%		0.22		0.22	40		0.22	100		0.22	40	100
Valine	%		1.16		1.16	40		1.16	100		1.16	40	100
Alanine	%		0.56		0.56	40		0.56	100		0.56	40	100
Aspartic acid	%		1.31		1.31	40		1.31	100		1.31	40	100
Glutamic acid	%		4.14		4.14	40		4.14	100		4.14	40	100
Glycine	%		0.39		0.39	40		0.39	100		0.39	40	100
Proline	%		2.39		2.39	40		2.39	100		2.39	40	100
Serine	%		1.12		1.12	40		1.12	100		1.12	40	100
Taurine	%		0		0			0			0		
Fat (ether extract)	%		9.7		9.7	40		9.7	100		9.7	40	100
Fat (acid hydrolysis)	%		9.7		9.7	40		9.7	100		9.7	40	100
Cholesterol, ppm	%		46		46	40		46	100		46	40	100
Linoleic acid	%		3.22		3.22	40		3.22	100		3.22	40	100
Linolenic acid	%		0.06		0.06	40		0.06	100		0.06	40	100
Arachidonic acid	%		0.01		0.01	40		0.01	100		0.01	40	100
Omega-3 fatty acids	%		0.06		0.06	40		0.06	100		0.06	40	100
Total saturated fatty acids	%		2.63		2.63	40		2.63	100		2.63	40	100
Total monounsaturated fatty acids	%		3.2		3.2	40		3.2	100		3.2	40	100
Polyunsaturated fatty acids	%		3.3		3.3	40		3.3	100		3.3	40	100
Fiber (max)	%		3.9		4.6	29		3.9	100		4.6	29	100
Neutral detergent fiber	%		3.6		4.3	28		3.6	100		4.3	28	100
Acid detergent fiber	%		3.1		3.7	28		3.1	100		3.7	28	100
Nitrogen-free extract (by difference)	%		51.2		48	44		51.2	100		48	44	100
Starch	%		41.55		36.71	47		37.29	90		29.95	52	82
Glucose	%		48.79		43.94	46		44.53	91		37.19	50	85
Fructose	%		7.24		7.24	40		7.24	100		7.24	40	100
Sucrose	%		16.35		17.59	35		16.35	100		17.59	35	100
Lactose	%		0		0			0			0		

Continued

Table 1. Continued

		LS (LT072)		LS-CR (LT073)			HS (LT074)			HS-CR (LT075)			
Nutrition	Unit					In CR (%)			% of LS			In CR (%)	% of LS-CR
Total digestible nutrients	%		32.9		34.1	38		32.9	100		34.1	38	100
Energy	kcal/g		3.63		3.51	42.0		3.63			3.51	42.0	
		kcal	%	kcal	%		kcal	%		kcal	%		
Protein		0.719	19.8	0.719	20.5	40	0.719	19.8	100	0.719	20.5	40	100
Fat (ether extract)		0.869	23.9	0.869	24.8	38	0.869	23.9	100	0.869	24.8	40	100
Carbohydrates		2.047	56.3	1.92	54.7	42	2.047	56.3	100	1.92	54.7	44	100
Minerals	%		7.3		9.3	24		7.3	100		9.3	24	100
Calcium	%		0.6		1	0		0.6	100		1	0	100
Phosphorus	%		0.53		0.78	12		0.53	100		0.78	12	100
Phosphorus (available)	%		0.53		0.78	12		0.53	100		0.78	12	100
Potassium	%		1.1		1.8	2		1.1	100		1.8	2	100
Magnesium	%		0.09		0.13	13		0.09	100		0.13	13	100
Sulfur	%		0.12		0.18	10		0.12	100		0.18	10	100
Sodium	%		0.03		0.05	0		1.6	5333		2.7	−1	5400
Chlorine	%		0.54		0.84	7		2.97	550		4.93	0	587
Fluorine	ppm		4.8		8	0		4.8	100		8	0	100
Iron	ppm		62		100	3		62	100		100	3	100
Zinc	ppm		26		40	8		26	100		40	8	100
Manganese	ppm		63		105	0		63	100		105	0	100
Copper	ppm		23		38	1		23	100		38	1	100
Cobalt	ppm		3.11		5.19	0		3.11	100		5.19	0	100
Iodine	ppm		0.55		0.92	0		0.55	100		0.92	0	100
Chromium	ppm		2.92		4.88	0		2.92	100		4.88	0	100
Selenium	ppm		0.07		0.07	40		0.07	100		0.07	40	100
Vitamins													
Carotene	ppm		0		0			0			0		
Vitamin A	IU/g		21		35	0		21	100		35	0	100
Vitamin D_3	IU/g		2.1		3.5	0		2.1	100		3.5	0	100
Vitamin E	IU/g		48		80	0		48	100		80	0	100
Vitamin K	ppm		10		16.7	0		10	100		16.7	0	100
Thiamine hydrochloride	ppm		20		33	1		20	100		33	1	100
Riboflavin	ppm		20		32.8	2		20	100		32.8	2	100
Niacin	ppm		87		144	1		57	66		144	−52	100
Pantothenic acid	ppm		54		90	0		54	100		90	0	100
Folic acid	ppm		4		6.6	1		4	100		6.6	1	100
Pyridoxine	ppm		15.92		26.41	0		15.92	100		26.41	0	100
Biotin	ppm		0.4		0.6	10		0.4	100		0.6	10	100
Vitamin B_{12}	μg/kg		23		36	6		23	100		36	6	100
Choline chloride	ppm		1351		1351	40		1351	100		1351	40	100

CR indicates caloric restriction; HS, high sodium; LS, low sodium.

Heart Rate and BP Measurements

Heart rate and BP were measured in conscious rats on day 24 using tail-cuff plethysmography (CODA noninvasive BP system, Kent Scientific, Litchfield, CT) as previously described.[26] Rats were warmed to 30°C for about 10 minutes and allowed to rest quietly before BP measurement. BP was measured in the afternoon in a quiet room, and the rats were kept calm and handled by the same person. No sedation was used. Rats were acclimatized to the tail-cuff BP measurement procedure for 1 week before the final measurements. We have previously demonstrated a very strong correlation between tail-cuff and telemetry BP measurements using this approach.[26]

Urine Analyses

On day 25, rats were individually housed in metabolic cages, and 24-hour urine samples were collected. Urinary volume (UV) was assessed, and samples were stored at −80°C for subsequent determination of urinary protein, urinary sodium excretion (UNa^+), and urinary creatinine. Urinary protein was determined by Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL). UNa^+ was measured using the Stanbio Sodium kit (Stanbio Laboratory, Boerne, TX), and urinary creatinine was measured by Creatinine Liquicolor (Stanbio Laboratory). Urinary protein-to-creatinine ratio was calculated as urinary protein concentration (mg/mL)/urinary creatinine concentration (mg/dL).

Tissue Collection and Blood Sampling at Euthanasia

On the last day of the study (day 28), rats were euthanized under inhalatory anesthesia with isoflurane. Blood samples were drawn from the abdominal aorta. The left ventricle and kidneys were rapidly excised, weighed, quickly frozen in liquid nitrogen, and stored at −80°C until processing. Blood was collected in purple-top and yellow-top BD Microtainer tubes (EDTA plasma and serum, respectively). The plasma and serum were separated by centrifugation and stored at −80°C.

Measurement of Blood Hormones and Kidney NAD^+ Concentration

Serum insulin was measured by Rat Insulin ELISA (ALPCO Diagnostics, Salem, NH). Homeostasis model assessment insulin resistance (HOMA-IR) was calculated by the following formula: HOMA-IR=fasting blood glucose (mmol/L)×fasting insulin (μU/mL)/22.4. Serum Aldo and plasma corticosterone levels were determined using a solid-phase radioimmunoassay (Diagnostic Products, Los Angeles, CA) and corticosterone ^3H radioimmunoassay kit (MP Biomedicals, LLC, Solon, OH). Plasma renin activity (PRA) was measured by radioimmunoassay (DiaSorin, Stillwater, MN). Plasma adiponectin level was measured by ELISA (Rat Adiponectin, EMD Millipore, Darmstadt, Germany). Kidney NAD^+ concentration was determined by NAD^+/NADH Quantification Colorimetric Kit (BioVision, Milpitas, CA).

Western Blotting

Kidney tissues were homogenized in ice-cold RIPA buffer (Boston BioProducts, Inc, Ashland, MA) with protease/phosphatase inhibitors (Thermo Scientific Inc, Rockford, IL) as previously described.[27] Protein extracts (20 μg) were mixed in 6× SDS sample buffer (Boston BioProducts, Ashland, MA) with 0.6 mol/L dithiothreitol, heated at 99°C for 5 minutes, and size fractionated by electrophoresis on 10% SDS-polyacrylamide gels. Proteins were electrophoretically transferred to Hybond enhanced chemiluminescence nitrocellulose membranes (Amersham Biosciences Corp, Piscataway, NJ). The membranes were blocked in 2% nonfat dried milk in TBS-T (Boston BioProducts) for 1 hour at room temperature on an orbital shaker and then overnight with rabbit anti-WNK4 antibody (1:2000, EMD Millipore, 07-2270, Darmstadt, Germany) or rabbit anti-Visfatin antibody (1:2000, Abcam Inc, ab109210, Cambridge, MA) at 4°C. After 3 washes in TBS-T, the membrane was incubated for 2 hours at 4°C with horseradish peroxidase–conjugated second antibody (1:5000, goat antirabbit IgG horseradish peroxidase conjugate, sc-2004, Santa Cruz Biotechnology, Inc, Dallas, TX) and analyzed using an enhanced chemiluminescence method (Denville Scientific, Inc, Metuchen, NJ). The signals were quantified by ImageJ software. The blots were subsequently reprobed for β-actin (1:50 000, Monoclonal Anti-β-Actin-Peroxidase clone AC-15, Sigma-Aldrich Co LLC, St. Louis, MO), and the results were normalized to β-actin to correct for loading.

Real-Time Polymerase Chain Reaction Analysis

Quantitative real-time polymerase chain reaction was performed as previously described.[27] Briefly, total mRNA was extracted using the RNeasy mini kit (Qiagen, Redwood City, CA), and the complimentary DNA was synthesized by First Strand cDNA Synthesis Kit (GE Healthcare UK Limited, Little Chalfont, Buckinghamshire, UK) with random hexamer primers. The ABI PRISM 7000 Sequence Detection System real-time quantitative polymerase chain reaction (Applied Biosystems, Foster City, CA) was used to perform the real-time polymerase chain reaction using TaqMan Gene Expression Assays for rat mineralocorticoid receptor, serum and glucocorticoid-regulated kinase 1, epithelial sodium channel-α, protein kinase with no lysine 4 (WNK4), and sodium

chloride cotransporter (NCC) (Applied Biosystems). Reactions were analyzed with the ABI software using the standard curve method. Target gene expression was normalized to 18S rRNA levels.

Data Analysis

The data were analyzed using 2-way ANOVA (HS and ResS) and presented as means±SE. The Fisher least significant difference test was used for comparison of multiple means by GraphPad Prism 6 (GraphPad Software Inc, La Jolla, CA). Differences were considered statistically significant if $P<0.05$. All studies were accomplished with the individual performing the study blinded as to the intervention group from which the tissues were obtained. For the ZG dose-response curves to AngII, data were analyzed using mixed-model regression with polynomial components and autoregressive covariance to compare diets while accommodating doses and replicates within samples. SAS version 9.4 (SAS Institute, Cary, NC) was used for these analyses.

Results

Body Weights, Sodium and Potassium Intake, and Tissue Weights

The rats on normal caloric intake gained weight over the 4-week study period with no statistically significant differences between the HS and ResS groups. The weight gain in the CR groups was reduced, resulting in a 23% weight difference between the CR and the ad libitum groups at the end of the study (Table 2). Food intake of the 2 CR groups was well controlled and about 40% less than each ad libitum group. However, the sodium intake was not statistically different in ad libitum and CR groups (Table 2). Kidney size was reduced in both ResS and CR groups, as compared with the ad libitum/HS group, whereas left ventricular weight was reduced only by CR (Table 2). No statistically significant reduction in left ventricular and kidney weight was detected in animals with the combined regimen (ResS and CR), and the tissue weights in this group were similar to those of the CR/HS group (Table 2).

Hemodynamic and Urine Parameters

BP was significantly reduced to similar levels by either ResS or CR (Figure 1A and 1B). Interestingly, the BP-lowering effect exerted by ResS or CR disappeared in the CR/ResS group. No statistically significant differences were detected in pulse pressure or heart rate in response to either ResS or CR (data not shown). Despite similar water and Na^+ intake in the ad libitum/HS and CR/HS groups (Table 2), UV and UNa^+ were significantly increased in the CR/HS. Both UV and UNa^+ were clearly reduced by ResS (Figure 2A and 2B). The combination of CR with ResS showed no statistically

Table 2. Physiological Parameters in Rats With or Without CR at the End of Study

	Ad Libitum/HS	Ad Libitum/ResS	CR/HS	CR/ResS
Body weight, g	386±9	396±10	282±5*	298±8*,†
Average food intake, g/d	26.8±0.6	25.6±0.8	16.0±0.4*	15.5±0.5*,†
Average Na^+ intake, mEq/d	18.62±0.41	0.03±0.00*	18.80±0.44	0.03±0.00*
Average K^+ intake, mEq/d	7.5±0.2	7.3±0.2	7.4±0.2	7.1±0.2
Average water intake, g/d	80.1±3.4	50.4±3.1*	85.1±3.4	48.3±2.1*
LV weight/TL, g/mm	21.2±0.4	19.3±1.1	15.6±0.5*	15.4±1.1*,†
Kidney weight/TL, g/mm	39.9±2.3	33.2±1.9*	28.7±1.7*	23.6±0.3*,†
PRA, ng/(mL·h)	7.6±1.4	11.7±0.9*	6.1±1.0	11.8±1.5*
Aldosterone, ng/dL	6.3±1.0	20.4±1.3*	12.1±1.3*	19.1±2.7*
Aldosterone/PRA ratio	1.0±0.2	1.8±0.3	2.6±0.5*	1.8±0.2
Corticosterone, ng/mL	540±83	543±57	829±66*	532±83
Fasting glucose, mg/dL	117±3	124±6	102±3*	112±7
Fasting insulin, μU/mL	6.3±1.2	14.4±3.5*	4.3±1.7	9.6±3.6
HOMA-IR	2.1±0.4	5.1±1.4*	1.2±0.5	3.1±1.3

Data are mean±SEM (n=12 rats/group for body weight and food, Na^+, K^+, water intake; n=9 rats/group for aldosterone, PRA, corticosterone; n=6 rats/group for tissue weights; n=6 rats/group for fasting glucose, fasting insulin, and HOMA-IR). CR indicates caloric restriction; HOMA-IR, homeostatic model assessment of insulin resistance; HS, high sodium; LV, left ventricular; PRA, plasma renin activity; ResS, sodium restriction; TL, tibia length.

*$P<0.05$ vs ad libitum/HS.

†$P<0.05$ vs ad libitum/ResS by Fisher least significant difference test.

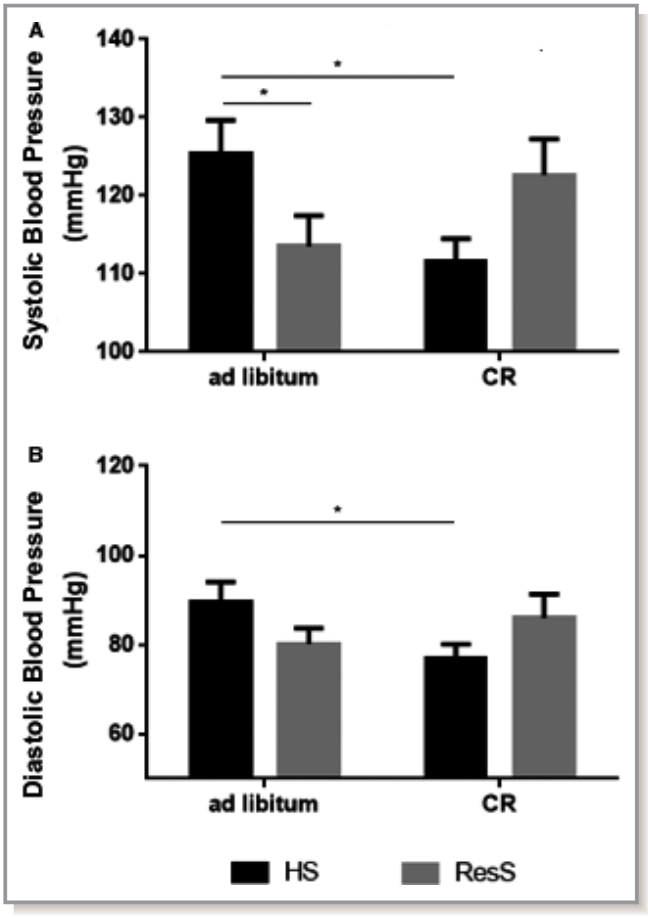

Figure 1. Hemodynamic parameters in rats. Rats were divided into 4 groups as defined in Methods: ad libitum/HS (containing 1.6% Na^+, 1.1 K^+ and no caloric restriction); ad libitum/ResS (containing 0.03% Na^+, 1.1% K^+ and no caloric restriction); caloric restricted to 60% on a high-salt diet (CR/HS) and caloric restricted to 60% on a restricted-salt diet (CR/ResS). Systolic blood pressures (A) and diastolic blood pressures (B) were measured by tail-cuff method on day 24 of each diet. Data are shown as mean±SEM (n=12 rats/group). *P<0.05 by Fisher least significant difference test after 2-way ANOVA analyses. If * is not indicated, the comparisons were not statistically significant. CR indicates caloric restriction; HS, high sodium; ResS, sodium restriction.

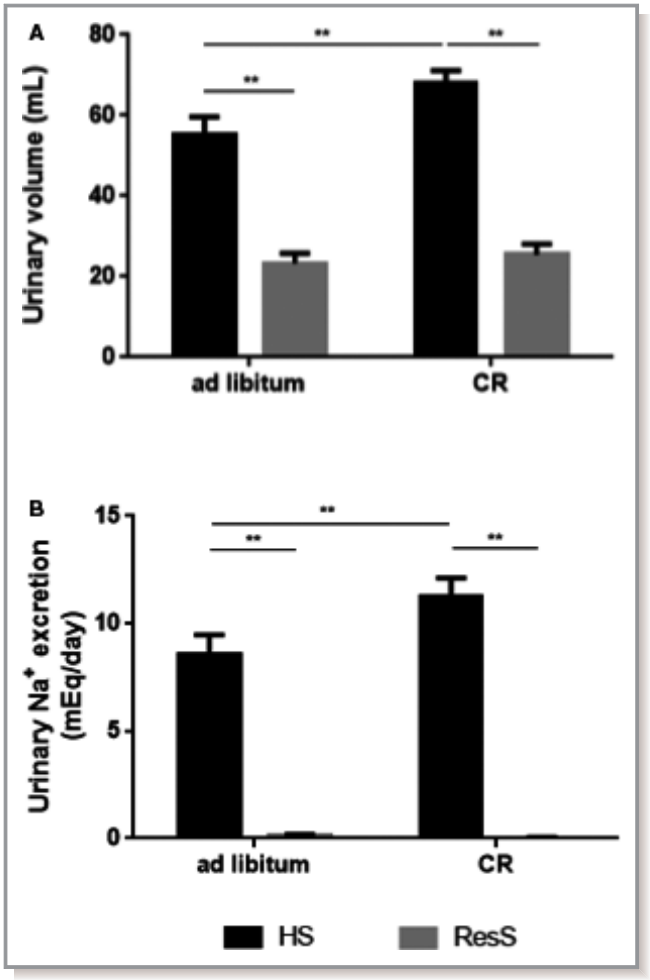

Figure 2. Urinary volume and sodium excretion in rats. Rats were placed individually in metabolic cages, and 24-hour urines were collected on day 25. Urinary volume (A) and sodium concentration were measured, and 24-hour urinary sodium excretion (B) was calculated. Data are shown as mean±SEM (n=12 rats/group). **P<0.01 by Fisher least significant difference test after 2-way ANOVA analyses. If * is not indicated, the comparisons were not statistically significant. CR indicates caloric restriction; HS, high sodium; ResS, sodium restriction.

significant effects on UV and UNa^+; UV and UNa^+ were similar in the CR/ResS and the ad libitum/ResS groups (Figure 2A and 2B).

PRA and Aldo Levels

Consistent with previously published results, PRA and serum Aldo were increased by ResS, confirming that the systemic RAAS was activated to maintain body electrolytes and volume (Table 2). Interestingly, the Aldo/PRA ratio in the CR/HS was significantly higher than those in the ad libitum/HS because Aldo levels were higher and PRAs were lower in the CR/HS group (Table 1), suggesting that CR enhances the ZG response to AngII. However, this effect was not observed on the ResS diet, as PRA, Aldo, and the Aldo/PRA ratio did not differ significantly between the CR/ResS and the ad libitum/ResS groups (Table 2).

Aldosterone Production by Ex Vivo Zona Glomerulosa Cells

To characterize directly the effects of the diets on Aldo production, we then turned to an isolated ZG preparation. Aldo levels, basally and in response to AngII, were significantly increased following both HS and ResS and CR (Figure 3). These data support the hypothesis that CR may be associated with an increased risk of Aldo-mediated cardiovascular and renal damage regardless of the level of sodium intake. Thus,

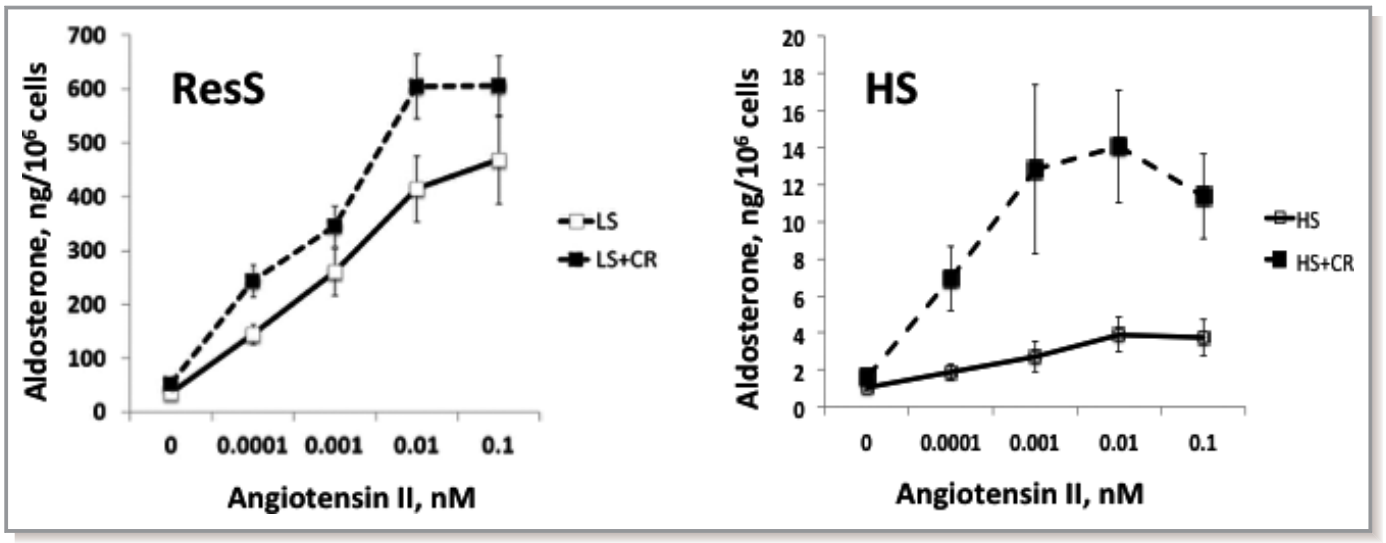

Figure 3. Angiotensin II-stimulated aldosterone production by ex vivo rat zona glomerulosa cells. CR increased angiotensin II-stimulated aldosterone production in ResS and HS. Ex vivo zona glomerulosa cells were harvested from rats following treatment with the 4 diets as shown in Methods and stimulated with increasing concentrations of angiotensin II for 1 hour at 37°C. Data are shown as mean±SEM (n=3 separate studies, replicated 4 times except for AngII 10^{-10} and 10^{-9} mol/L doses [2 replicates]; HS: P=0.027; ResS: P=0.0068). The data were analyzed using mixed-model regression with polynomial components and autoregressive covariance to compare diets while accommodating doses and replicates within samples. SAS version 9.4 (SAS Institute, Cary, NC) was used for these analyses. AL indicates aldosterone; AngII, angiotensin II; CR, caloric restriction; HS, high sodium; LS, low sodium; ResS, sodium restriction.

the increase in BP with CR and ResS may, in part, be secondary to the markedly enhanced ZG cell response to AngII.

Renal Sodium-Handling Factors

Serum Aldo, mineralocorticoid receptor, and epithelial sodium channel-α gene expressions were significantly upregulated by ResS (Figure 4), as anticipated to maintain normal sodium/volume homeostasis. Surprisingly, mineralocorticoid receptor, serum and glucocorticoid-regulated kinase 1, and epithelial sodium channel-α gene expressions also were increased by CR (Figure 4) under liberal salt intake conditions. WNK4 and NCC are additional factors involved in the renal responses to changes in sodium intake.[28-30] Although there was a significant change in WNK4 gene expression at the end of the 4-week study, WNK4 protein levels were significantly reduced in the ResS and CR groups as compared with the HS group. This decrease in WNK4 protein was not observed when animals received the combined CR/ResS diet (Figure 5A and 5C). As anticipated, NCC gene expression was significantly upregulated with ResS; however, it was also increased in the CR/HS group when sodium intake was not reduced (Figure 5B). These results suggest that CR produces changes in the kidney similar to those occurring with ResS, with both CR and ResS leading to enhanced renal sodium reabsorption. Interestingly, the effects of combining CR and ResS were not additive, as the effects of UV and UNa^+ during CR/ResS were not significantly different from those of ad libitum/ResS (Figure 2A and 2B).

The Effect of ResS or CR on Markers of Kidney Injury

Urinary protein-to-creatinine ratio, a surrogate marker for kidney injury, was significantly increased by ResS (Figure 6A), raising the possibility that ResS increases renal injury. However, the profibrotic factors transforming growth factor-β1 and collagen type 1α1 chain were not significantly changed in the kidney (Figure 6B and 6C). In contrast, CR markedly reduced both urinary protein-to-creatinine ratio and collagen type 1α1 gene expression (Figure 6A and 6C), suggesting that CR under liberal salt intake conditions protects the kidney against injury and as such may suppresses kidney fibrosis.

Glucose Tolerance and Insulin Sensitivity

CR has been reported to upregulate insulin sensitivity in normal animals.[31] In agreement with these reports, both fasting glucose and the AUC of blood glucose during the ipGTT were significantly reduced in the CR/HS group. However, these effects were dampened when sodium restriction was added to CR (Table 2). Indeed, the blood glucose levels at some time points during the ipGTT (15 and 90 minutes) were significantly higher in CR/ResS than in CR-HS (Figure 7A). In addition, the AUC was significantly lower only in the CR/HS condition as compared with HS (Figure 7B). These results indicate that the beneficial effect of CR on glucose homeostasis is masked when it is combined with ResS.

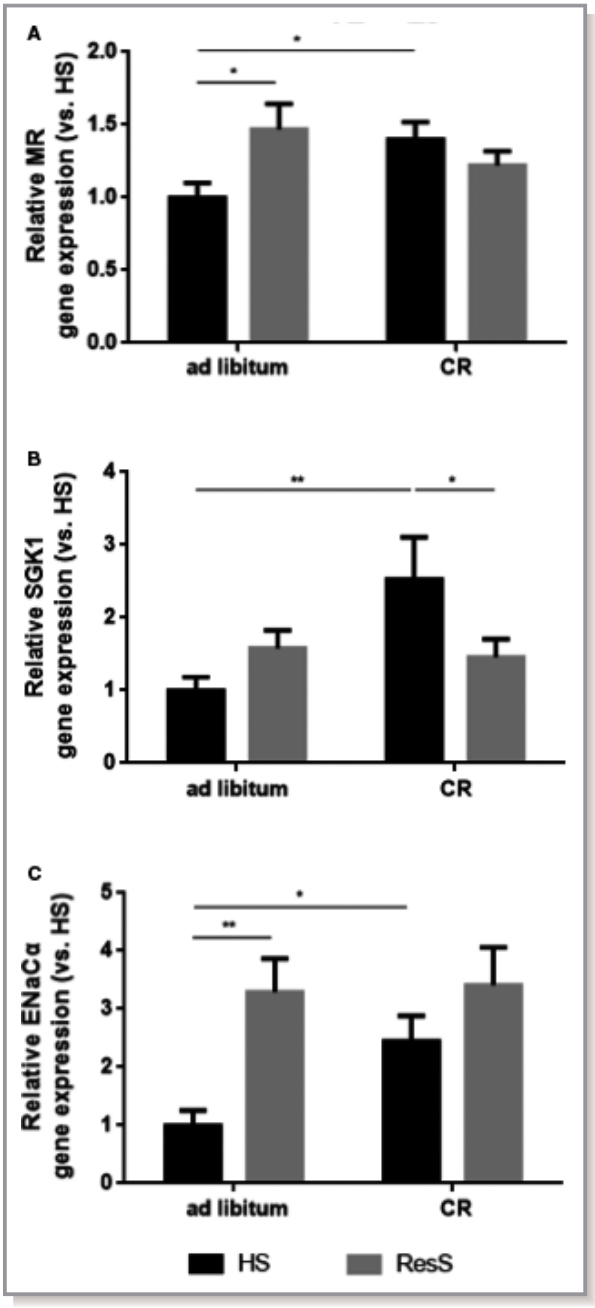

Figure 4. Gene expression of MR, SGK1, and ENaCα in kidney. Gene expressions of mineralocorticoid receptor (MR) (A), serum and glucocorticoid-regulated kinase 1 (SGK1) (B), and epithelial sodium channel-α (ENaCα) (C) in kidney were evaluated by real-time polymerase chain reaction. Each gene's expression level was normalized by 18S rRNA expression level. Data are shown as mean±SEM (n=12 rats/group). *P<0.05, **P<0.01 by Fisher least significant difference test after 2-way ANOVA analyses. If * is not indicated, the comparisons were not statistically significant. CR indicates caloric restriction; HS, high sodium; ResS, sodium restriction.

Interestingly, HOMA-IR as well as fasting insulin were significantly higher in ad libitum/ResS versus ad libitum/HS (Table 1), suggesting that ResS itself may impair the insulin response and partly cancel the potential beneficial effect of CR. However, other metabolic parameters were improved with CR regardless of salt intake. For example, postprandial triglyceride levels were reduced by CR in both HS and ResS (HS 223.8±22.8; HS+CR 149.4±21.1; ResS 281.4±37.9; ResS+CR 134.1±19.4 [mg/dL, mean±SEM, P<0.025]).

Adiponectin, Kidney Visfatin Expression, and Kidney NAD^+ Level

Increased adiponectin, visfatin, and NAD^+ levels are associated with improvement of insulin sensitivity and renal protection. These 3 factors were all increased by CR but not by ResS. However, as was shown for other beneficial effects of CR, when the rats also ingested a ResS diet, improvement in these factors was blunted (Figure 8A through 8D).

Discussion

In this study we report on the novel effects of CR on Aldo production. Aldo responses to stimulation with AngII in ex vivo ZG cells were greater in rats following CR regardless of the level of sodium intake. This response was significantly amplified when rats were on a ResS diet. On the liberal salt diet, CR reduced BP, an effect that was abolished when ResS was added to CR, potentially secondary to the enhanced ZG cell response to AngII. In agreement with the literature, CR improved insulin sensitivity, reduced markers of kidney injury, and increased renal protective factors, eg, adiponectin and visfatin. Surprisingly, combining ResS with CR reversed most of the beneficial effects of CR, resulting in increased insulin resistance and decreased levels of adiponectin, visfatin, and NAD^+. These results suggest that combining ResS with CR does not result in additive beneficial effects. Indeed, many of the beneficial effects of CR appear to be reversed in the presence of ResS intake. Thus, our initial hypothesis that the combination of CR with ResS would elicit additive benefits was rejected, and our findings raise a note of caution concerning the combination of these 2 individually beneficial interventions in clinical settings.

There is a large body of literature documenting that both CR and ResS can substantially reduce BP in humans. However, there are limited studies assessing the effect of both together. For example, in 1990 the Hypertension Prevention Trial reported on changes in BP in subjects with high-normal BP randomized to a control treatment group with no dietary counseling or counseling for CR, ResS, or CR+ResS.[32] Their initial measurements after 6 months of treatment were similar to those found in the present rat study. All groups were associated with reduced BP, unfortunately even the control group. CR and ResS produced nearly

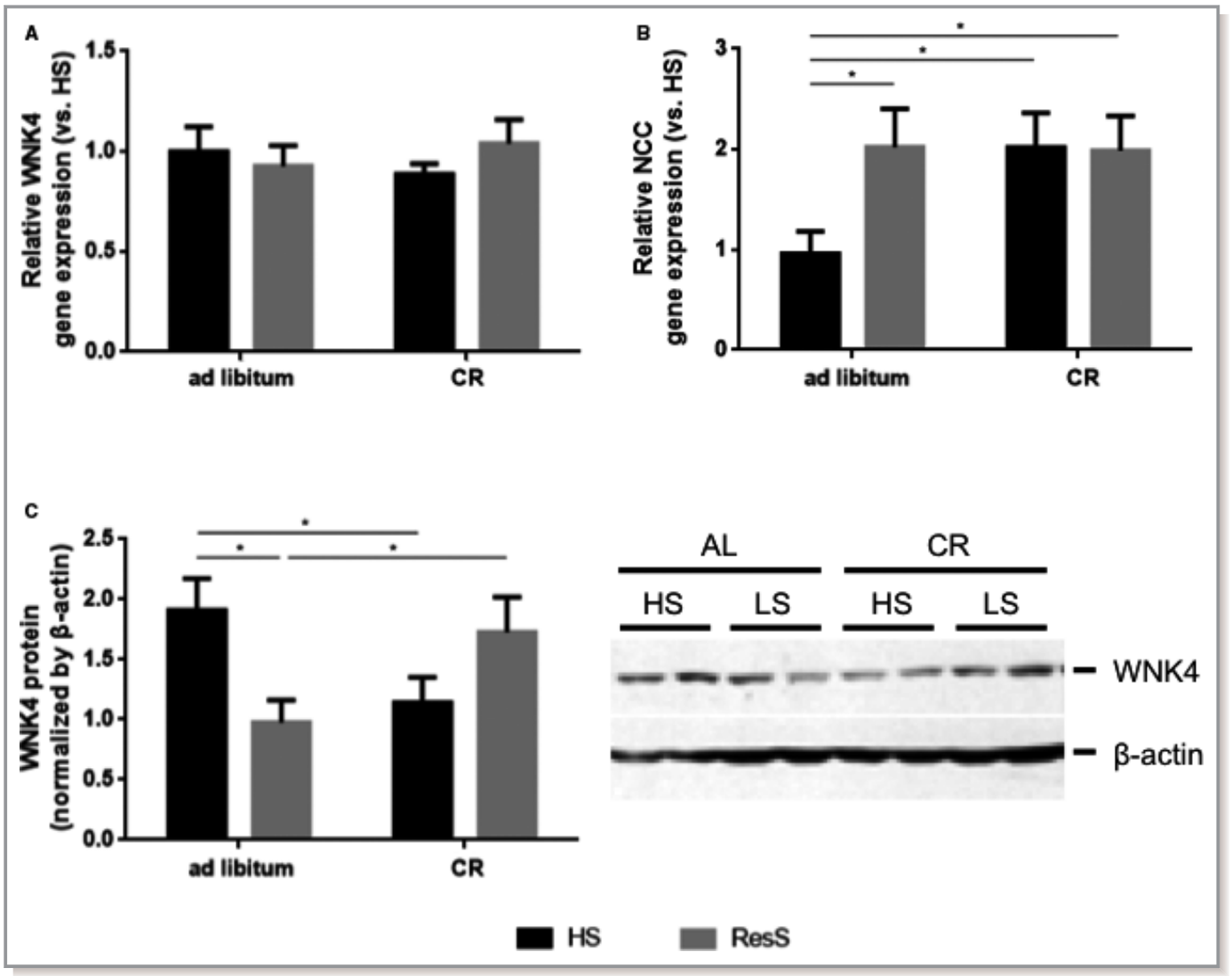

Figure 5. Gene and protein expressions of WNK4 and NCC in kidney. Gene expressions of protein kinase with no lysine 4 (WNK4) (A) and sodium chloride cotransporter (NCC) (B) in kidney were evaluated by real-time polymerase chain reaction. Each gene expression level was normalized by 18S rRNA expression level. Kidney WNK4 protein expression (C) was analyzed by western blotting, and protein expression level was normalized by β-actin protein expression. Data are shown as mean±SEM (n=12 rats/group). *P<0.05 by Fisher least significant difference test after 2-way ANOVA analyses. If * is not indicated, the comparisons were not statistically significant. CR indicates caloric restriction; HS, high sodium; ResS, sodium restriction.

identical systolic BP reductions, similar to what was observed in our study. As in our study, the BP response to the combination of CR and ResS was blunted: compared with each of the diet treatments alone, only about half of the reduction occurred. However, there are limitations to this study because the diets were not provided, and CR alone also was associated with sodium reduction—2 concerns not present in our study.

Reduction of salt intake is recognized as an effective nonpharmacological intervention to reduce BP and cardiovascular risk.[2,3,8,28] Although BP was reduced in the present study, ResS also impaired insulin sensitivity (Table 2) and increased proteinuria (Figure 6). Lima et al reported similar results in a chronic ResS study (42 weeks) using aged rats.[33] Moreover, a low-salt diet increases insulin resistance in humans.[34] In addition, ResS impairs the endothelial effect of insulin[35] and also enhances cardiac fibrosis in a rat model of heart failure.[36] These results suggest that ResS may not always provide cardiometabolic benefits. Insulin resistance due to chronic ResS is reversed by α- and β-blockade and by L-arginine,[37] indicating that the sympathetic nervous system and the L-arginine–nitric oxide pathway are involved in the insulin resistance induced by chronic ResS. In addition, there is evidence that RAAS activation is involved in insulin resistance in both skeletal muscle and adipose tissue.[38,39] Thus, the increase in RAAS that occurs with ResS,[40-42] could

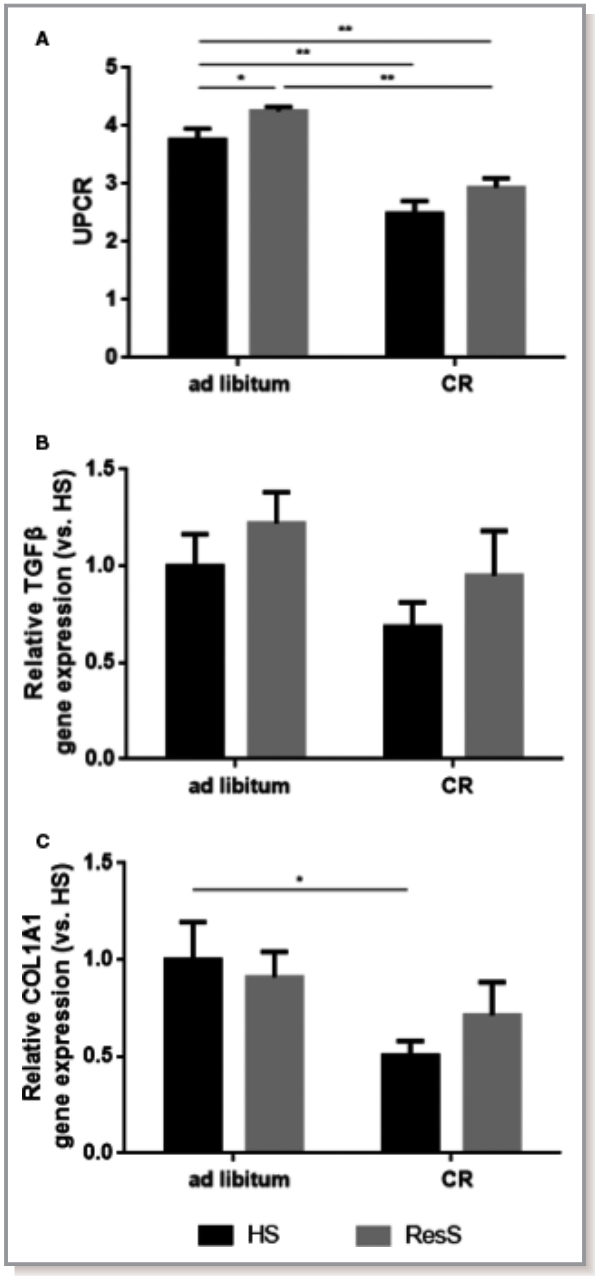

Figure 6. Urinary protein excretion and gene expression of TGFβ and COL1A1 in kidney. The effect of ResS or CR on urinary protein-to-creatinine ratio (UPCR) (A), kidney transforming growth factor-β1 (TGFβ) (B), and collagen type 1α1 chain (COL1A1) (C) gene expression were evaluated by real-time polymerase chain reaction. Each gene expression was normalized by 18S rRNA expression level. Data are shown as mean±SEM (n=12 rats/group). *P<0.05, **P<0.01 by Fisher least significant difference test after 2-way ANOVA analyses. If * is not indicated, the comparisons were not statistically significant. CR indicates caloric restriction; HS, high sodium; ResS, sodium restriction.

be another mediator of insulin resistance with ResS. However, more precise measurements (glucose insulin clamp studies) are needed to confirm the observations in this study.

SIRT proteins are reported to be involved in the cardioprotective effects of CR.[13-16] Seven homologues, SIRT1-7, have been identified in mammals, and SIRT1, the most extensively studied family member, plays a major role in mediating the beneficial effects of CR. In mammals visfatin is the rate-limiting enzyme in the NAD^+ biosynthesis pathway.[43] Because NAD^+ is an indispensable cofactor for SIRT1,[44] visfatin is recognized to be a critical upstream regulator of SIRT1.[44] In fact, visfatin was recently reported to be necessary for CR-induced beneficial effects on oxidative stress, mitochondrial biogenesis, and metabolic adaptation.[45] Consistent with these results, our study shows that visfatin protein and NAD^+ levels were increased in the kidneys of CR animals (Figure 8). In addition, CR reduced BP and proteinuria with an increase in sodium excretion even when a liberal sodium intake was maintained during CR. These results suggest that CR could lower BP by enhancing excretion of Na^+, potentially mediated by SIRT1, which may be involved in sodium homeostasis by modulating renal sodium excretion.[18-20] Interestingly, Aldo levels, epithelial sodium channel-α, and NCC expressions in the kidney (major regulators of sodium excretion) were increased during CR even with a liberal sodium intake and reached similar levels to those observed in rats maintained on a normal calorie/sodium-restricted diet (Table 2, Figures 4 and 5). These results suggest that in the presence of a liberal salt intake, CR induces changes in volume-modulating factors similar to those observed with salt restriction. These findings imply that when rodents are caloric restricted, they "sense" that they are also sodium/volume restricted because CR enhances sodium excretion (Figure 2) and increases Aldo levels and production (Table 1, Figure 3). That they are not actually volume depleted and may in fact be overly volume repleted is indicated by the observed suppression of PRA compared with a liberal salt diet without caloric restriction. These results suggest that factors associated with CR are directly modulating Aldo production and downstream messengers.

Adiponectin is a well-known adipokine that is upregulated by CR. Exogenously infused adiponectin improves insulin sensitivity and reduces kidney injury in diabetic mice.[46] Several studies in humans showed that low levels of adiponectin were associated with high BP,[47-50] but the mechanism is not fully understood. In endothelial cells, adiponectin activates the AMP kinase pathway, thereby stimulating nitric oxide synthesis and bioavailability.[51] Thus, this protective effect on endothelial function may partially explain the link between adiponectin and BP control. In our study adiponectin levels were increased by CR with a liberal salt diet (Figure 8), suggesting that adiponectin is involved in mediating some of the CR-induced beneficial effects, specifically in the presence of a liberal salt diet.

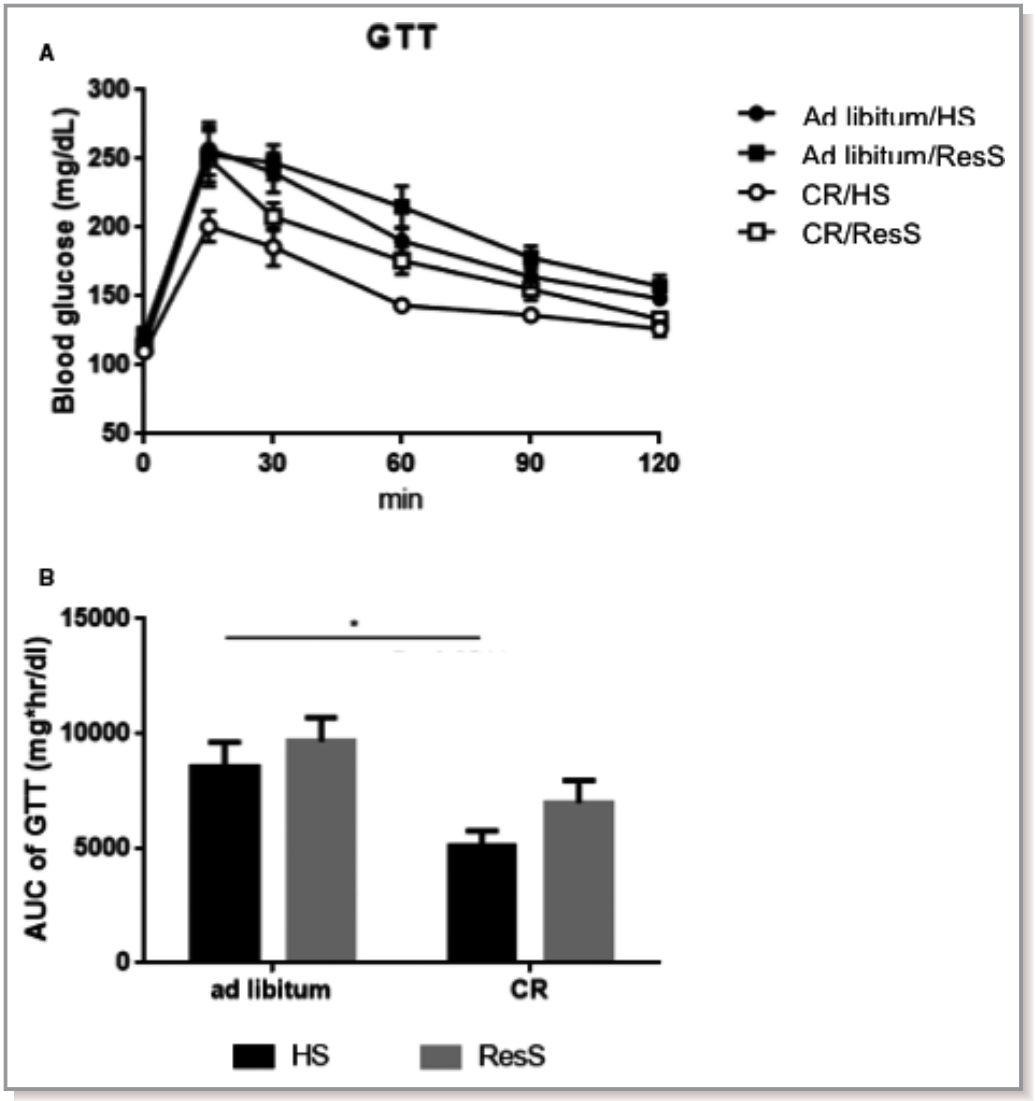

Figure 7. Glucose tolerance test in rats. Glucose solution (1.5 g/kg body weight) was infused intraperitoneally in conscious rats after 10 hours overnight fasting on day 21 of study, and blood glucoses (A) were measured at 0, 15, 30, 90, 120 minutes after glucose challenge. The areas under the curve (AUC) of blood glucose (B) were calculated. Data are shown as mean±SEM (n=12 rats/group). *P<0.05 by Fisher least significant difference test after 2-way ANOVA analyses. If * is not indicated, the comparisons were not statistically significant. AUC indicates area under the concentration-time curve; CR, caloric restriction; GTT, glucose tolerance test; HS, high sodium; ResS, sodium restriction.

We had not anticipated that the combination of ResS with CR would cancel out each other's beneficial effects on BP, insulin sensitivity, proteinuria, and cardioprotective factors such as adiponectin, visfatin protein, and NAD^+ (Figures 1, 7, and 8). Why did this occur? One possible explanation may be related to our finding of increased Aldo production by ZG cells following CR. Limited reports are available on CR, Aldo levels, and RAAS activity.[10-12] Early studies by Sowers et al showed that in obese adult humans, CR for up to 12 weeks was associated with reduced weight, PRA, and Aldo levels.[10] Ho et al reported similar findings in 25 otherwise healthy obese subjects.[11] However, the combined effects of ResS and CR are not clear.[12] It has been proposed that CR leads to reduced sodium intake, which mediates the BP-lowering effects, thus confounding studies of CR. In our study sodium and potassium were maintained constant between CR and ad libitum (ie, non-CR) diets. Another possible explanation may relate to the activation of RAAS, which has been reported to suppress SIRT1 activation by CR in skeletal muscle or kidney.[52,53] The RAAS activation by ResS could suppress CR-induced upregulation of cardiac protective factors and cancel the beneficial effects of CR. In fact, visfatin and NAD^+

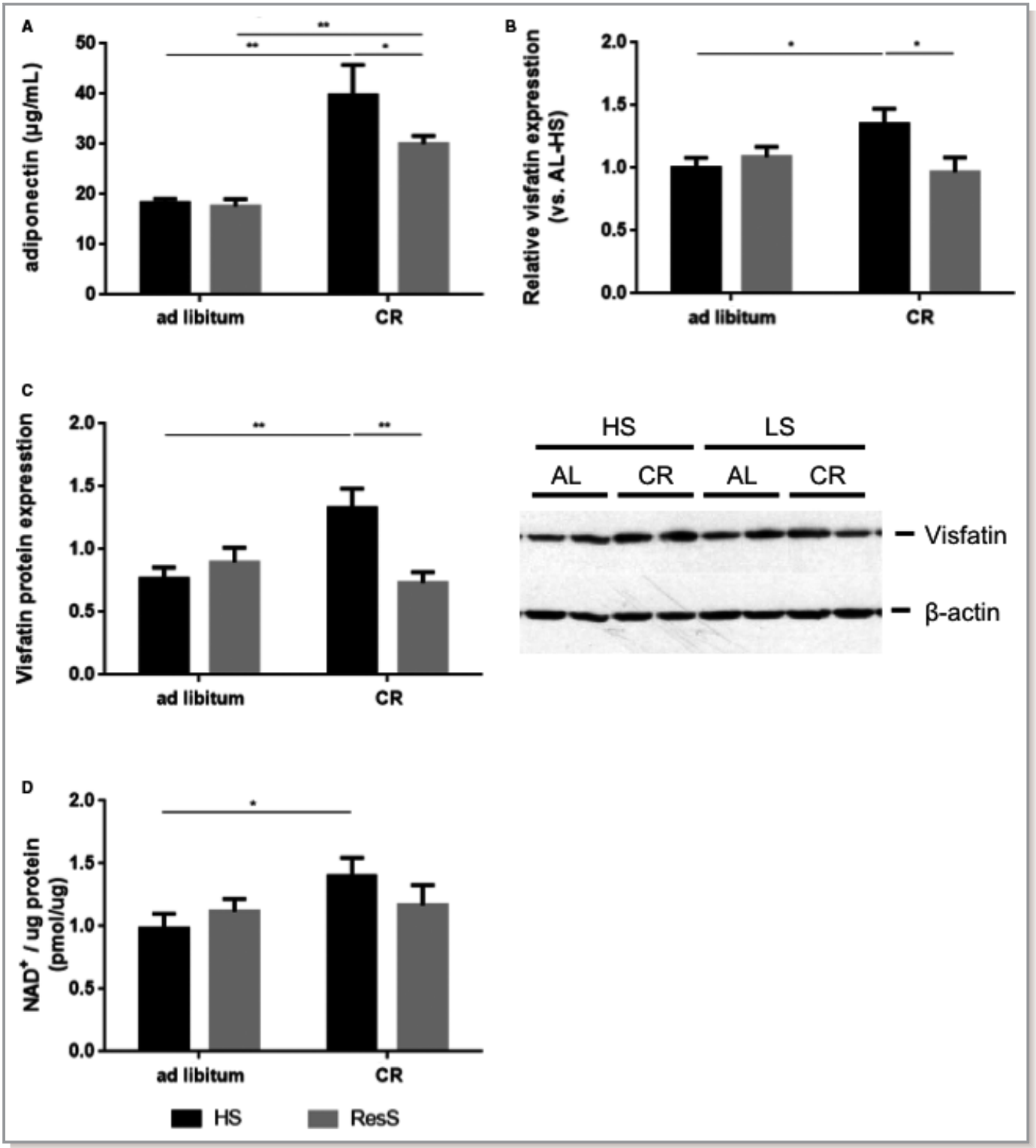

Figure 8. Serum adiponectin, gene and protein expression of visfatin and NAD^+ levels in kidney. Plasma adiponectin level (A) was measured by using the Rat Adiponectin ELISA kit. Kidney visfatin gene expression (B) was evaluated by real-time polymerase chain reaction, and gene expression level was normalized by 18S rRNA expression level. Kidney visfatin protein expression (C) was analyzed by Western blotting, and protein expression levels were normalized by β-actin protein expression. Kidney NAD^+ concentration (D) was determined by NAD^+/NADH Quantification Colorimetric Kit (BioVision, Milpitas, CA). Data are shown as mean±SEM (n=12 rats/group). **P*<0.05, ***P*<0.01 by Fisher least significant difference test after 2-way ANOVA analyses. If * is not indicated, the comparisons were not statistically significant. AL indicates aldosterone; CR, caloric restriction; HS, high sodium; LS, low sodium; ResS, sodium restriction.

levels were upregulated by CR but reduced when combined with ResS (Figure 8), suggesting that SIRT1 activity may be decreased in the CR/ResS. Upregulated adiponectin also was suppressed by the combination (Figure 8).

There are several potential limitations of the present study. First, CR likely induced stress in these rats, given their increased corticosterone levels. The level of stress was not controlled in our experimental design. However, the increased corticosterone levels were observed only in the presence of a liberal salt intake. Second, salt intake may have been different in the CR versus nonrestricted rats as was observed in humans[32]; however, this is unlikely because the 24-hour sodium excretions were identical, and the feeding frequency was staggered to minimize even a timing difference in salt intake. Third, other nutrients may have been different in the CR versus non-CR rats, which could have been further exaggerated in the sodium-restricted CR rats and thus contribute to the observed phenotypes. We think this is unlikely because the compositions of all diets were identical except for the decrease in fat and carbohydrates in the CR diets and sodium in the ResS diets (see Table 1 for diet compositions). Fourth, the difference in Aldo levels could be secondary to modulation of other Aldo regulatory factors. However, the confirmation from the ex vivo studies makes this possibility unlikely. Fifth, our studies were performed on healthy male rats; therefore, extrapolation to diseased rodents or humans should be done with caution and only following specific additional studies. In fact, dietary sodium restriction increases serum adiponectin in *db/db* mice,[54] but no change was observed in the current study. In addition, high sodium intakes have been associated with increased glucocorticoid production, insulin resistance, and metabolic syndrome in patients with hypertension and/or metabolic syndrome,[55] although no change in glucocorticoid levels and rather improved insulin sensitivity were observed on a liberal salt diet in our study of healthy rodents. Finally, where we did not identify a statistically significant difference does not mean that the comparators are equivalent, as in some cases the lack of a significant difference could have been limited by the sample size.

In conclusion, both CR and ResS induce similar beneficial cardiometabolic changes in rats. However, the beneficial effect of each is reduced when both treatments are applied concomitantly, suggesting that clinically their combination should be advocated with caution until more data are available. These unanticipated results may, in part, be secondary to CR-mediated altered Aldo production.

Acknowledgments

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Disclosures

None.

References

1. Kannel WB. Fifty years of Framingham Study contributions to understanding hypertension. *J Hum Hypertens*. 2000;14:83–90.
2. Mozaffarian D, Fahimi S, Singh GM, Micha R, Khatibzadeh S, Engell RE, Lim S, Danaei G, Ezzati M, Powles J. Global sodium consumption and death from cardiovascular causes. *N Engl J Med*. 2014;371:624–634.
3. O'Donnell M, Mente A, Rangarajan S, McQueen MJ, Wang X, Liu L, Yan H, Lee SF, Mony P, Devanath A, Rosengren A, Lopez-Jaramillo P, Diaz R, Avezum A, Lanas F, Yusoff K, Iqbal R, Ilow R, Mohammadifard N, Gulec S, Yusufali AH, Kruger L, Yusuf R, Chifamba J, Kabali C, Dagenais G, Lear SA, Teo K, Yusuf S. Urinary sodium and potassium excretion, mortality, and cardiovascular events. *N Engl J Med*. 2014;371:612–623.
4. Nguyen NT, Magno CP, Lane KT, Hinojosa MW, Lane JS. Association of hypertension, diabetes, dyslipidemia, and metabolic syndrome with obesity: findings from the National Health and Nutrition Examination Survey, 1999 to 2004. *J Am Coll Surg*. 2008;207:928–934.
5. Bales CW, Kraus WE. Caloric restriction: implications for human cardiometabolic health. *J Cardiopulm Rehabil Prev*. 2013;33:201–208.
6. Takatsu M, Nakashima C, Takahashi K, Murase T, Hattori T, Ito H, Murohara T, Nagata K. Calorie restriction attenuates cardiac remodeling and diastolic dysfunction in a rat model of metabolic syndrome. *Hypertension*. 2013;62:957–965.
7. Benstein JA, Feiner HD, Parker M, Dworkin LD. Superiority of salt restriction over diuretics in reducing renal hypertrophy and injury in uninephrectomized SHR. *Am J Physiol*. 1990;258:F1675–F1681.
8. Hattori T, Murase T, Takatsu M, Nagasawa K, Matsuura N, Watanabe S, Murohara T, Nagata K. Dietary salt restriction improves cardiac and adipose tissue pathology independently of obesity in a rat model of metabolic syndrome. *J Am Heart Assoc*. 2014;3:e001312. DOI: 10.1161/JAHA.114.001312.
9. Appel LJ, Frohlich ED, Hall JE, Pearson TA, Sacco RL, Seals DR, Sacks FM, Smith SC Jr, Vafiadis DK, Van Horn LV. The importance of population-wide sodium reduction as a means to prevent cardiovascular disease and stroke: a call to action from the American Heart Association. *Circulation*. 2011;123:1138–1143.
10. Sowers JR, Whitfield LA, Beck FWJ, Catania RA, Tuck ML, Dornfeld L, Maxwell M. Role of enhanced sympathetic nervous system activity and reduced Na+, K+-dependent adenosine triphosphatase activity in maintenance of elevated blood pressure in obesity: effects of weight loss. *Clin Sci (Lond)*. 1982;63:121s–124s.
11. Ho JT, Keogh JB, Bornstein SR, Ehrhart-Bornstein M, Lewis JG, Clifton PM, Torpy DJ. Moderate weight loss reduces renin and aldosterone but does not influence basal or stimulated pituitary-adrenal axis function. *Horm Metab Res*. 2007;39:694–699.
12. Prineas RJ. Clinical interaction of salt and weight change on blood pressure level. *Hypertension*. 1991;17:I143–I149.
13. Bordone L, Guarente L. Calorie restriction, SIRT1 and metabolism: understanding longevity. *Nat Rev Mol Cell Biol*. 2005;6:298–305.
14. Sundaresan NR, Pillai VB, Gupta MP. Emerging roles of SIRT1 deacetylase in regulating cardiomyocyte survival and hypertrophy. *J Mol Cell Cardiol*. 2011;51:614–618.
15. Chalkiadaki A, Guarente L. Sirtuins mediate mammalian metabolic responses to nutrient availability. *Nat Rev Endocrinol*. 2012;8:287–296.
16. Wang Y. Molecular links between caloric restriction and Sir2/SIRT1 activation. *Diabetes Metab J*. 2014;38:321–329.

17. Kume S, Uzu T, Horiike K, Chin-Kanasaki M, Isshiki K, Araki S, Sugimoto T, Haneda M, Kashiwagi A, Koya D. Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. *J Clin Invest*. 2010;120:1043–1055.
18. Hao CM, Haase VH. Sirtuins and their relevance to the kidney. *J Am Soc Nephrol*. 2010;21:1620–1627.
19. Zhang D, Li S, Cruz P, Kone BC. Sirtuin 1 functionally and physically interacts with disruptor of telomeric silencing-1 to regulate α-ENaC transcription in collecting duct. *J Biol Chem*. 2009;284:20917–20926.
20. Kone BC. Epigenetics and the control of the collecting duct epithelial sodium channel. *Semin Nephrol*. 2013;33:383–391.
21. Yang SY, Lin SL, Chen YM, Wu VC, Yang WS, Wu KD. A low-salt diet increases the expression of renal sirtuin 1 through activation of the ghrelin receptor in rats. *Sci Rep*. 2016;6:32787.
22. Braley LM, Menachery AI, Yao T, Mortensen RM, Williams GH. Effect of progesterone on aldosterone secretion in rats. *Endocrinology*. 1996;137:4773–4778.
23. Chong C, Hamid A, Yao T, Garza A, Pojoga L, Adler GK, Romero JR, Williams GH. Regulation of aldosterone secretion by mineralocorticoid receptor-mediated signaling. *J Endocrinol*. 2017;232:525–534.
24. Braley LM, Menachery A, Williams GH. Angiotensin II's role in mediating angiotensin I- and tetradecapeptide-induced steroidogenesis by rat glomerulosa cells. *Endocrinology*. 1981;109:960–965.
25. Chuengsamarn S, Garza AE, Krug AW, Romero JR, Adler GK, Williams GH, Pojoga LH. Direct renin inhibition modulates insulin resistance in caveolin-1-deficient mice. *Metabolism*. 2013;62:275–281.
26. Pojoga LH, Romero JR, Yao TM, Loutraris P, Ricchiuti V, Coutinho P, Guo C, Lapointe N, Stone JR, Adler GK, Williams GH. Caveolin-1 ablation reduces the adverse cardiovascular effects of N-ω-nitro-L-arginine methyl ester and angiotensin II. *Endocrinology*. 2010;151:1236–1246.
27. Pojoga LH, Williams JS, Yao TM, Kumar A, Raffetto JD, do Nascimento GR, Reslan OM, Adler GK, Williams GH, Shi Y, Khalil RA. Histone demethylase LSD1 deficiency during high-salt diet is associated with enhanced vascular contraction, altered NO-cGMP relaxation pathway, and hypertension. *Am J Physiol Heart Circ Physiol*. 2011;301:H1862–H1871.
28. Lai L, Feng X, Liu D, Chen J, Zhang Y, Niu B, Gu Y, Cai H. Dietary salt modulates the sodium chloride cotransporter expression likely through an aldosterone-mediated WNK4-ERK1/2 signaling pathway. *Pflugers Arch*. 2012;463:477–485.
29. Zhou B, Wang D, Feng X, Zhang Y, Wang Y, Zhuang J, Zhang X, Chen G, Delpire E, Gu D, Cai H. WNK4 inhibits NCC protein expression through MAPK ERK1/2 signaling pathway. *Am J Physiol Renal Physiol*. 2012;302:F533–F539.
30. McCormick JA, Ellison DH. The WNKs: atypical protein kinases with pleiotropic actions. *Physiol Rev*. 2011;91:177–219.
31. Jiang HY, Koike T, Li P, Wang ZH, Kawata Y, Oshida Y. Combined effects of short-term calorie restriction and exercise on insulin action in normal rats. *Horm Metab Res*. 2010;42:950–954.
32. The Hypertension Prevention Trial: three-year effects of dietary changes on blood pressure. Hypertension Prevention Trial Research Group. *Arch Intern Med*. 1990;150:153–162.
33. Lima NK, Lima FB, dos Santos EA, Okamoto MM, Sumida DH, Hell NS, Furukawa LN, Heimann JC. Effect of lifelong high- or low-salt intake on blood pressure, left ventricular mass and plasma insulin in Wistar rats. *Am J Med Sci*. 2006;331:309–314.
34. Garg R, Williams GH, Hurwitz S, Brown NJ, Hopkins PN, Adler GK. Low-salt diet increases insulin resistance in healthy subjects. *Metabolism*. 2011;60:965–968.
35. Vecchione C, Morisco C, Fratta L, Argenziano L, Trimarco B, Lembo G. Dietary sodium restriction impairs endothelial effect of insulin. *Hypertension*. 1998;31:1261–1265.
36. Mori T, Kurumazuka D, Matsumoto C, Shirakawa H, Kimura S, Kitada K, Kobayashi K, Matsuda H, Hayashi T, Kitaura Y, Matsumura Y. Dietary salt restriction activates mineralocorticoid receptor signaling in volume-overloaded heart failure. *Eur J Pharmacol*. 2009;623:84–88.
37. Ruivo GF, Leandro SM, do Nascimento CA, Catanozi S, Rocha JC, Furukawa LN, Dolnikoff MS, Quintao EC, Heimann JC. Insulin resistance due to chronic salt restriction is corrected by α and β blockade and by L-arginine. *Physiol Behav*. 2006;88:364–370.
38. Feraco A, Armani A, Mammi C, Fabbri A, Rosano GM, Caprio M. Role of mineralocorticoid receptor and renin-angiotensin-aldosterone system in adipocyte dysfunction and obesity. *J Steroid Biochem Mol Biol*. 2013;137:99–106.
39. Lastra G, Dhuper S, Johnson MS, Sowers JR. Salt, aldosterone, and insulin resistance: impact on the cardiovascular system. *Nat Rev Cardiol*. 2010;7:577–584.
40. Guyton AC. Blood pressure control—special role of the kidneys and body fluids. *Science*. 1991;252:1813–1816.
41. Hall JE. Control of sodium excretion by angiotensin II: intrarenal mechanisms and blood pressure regulation. *Am J Physiol*. 1986;250:R960–R972.
42. Hurwitz S, Fisher ND, Ferri C, Hopkins PN, Williams GH, Hollenberg NK. Controlled analysis of blood pressure sensitivity to sodium intake: interactions with hypertension type. *J Hypertens*. 2003;21:951–959.
43. Rongvaux A, Shea RJ, Mulks MH, Gigot D, Urbain J, Leo O, Andris F. Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. *Eur J Immunol*. 2002;32:3225–3234.
44. Canto C, Auwerx J. Targeting sirtuin 1 to improve metabolism: all you need is NAD(+). *Pharmacol Rev*. 2012;64:166–187.
45. Song J, Ke SF, Zhou CC, Zhang SL, Guan YF, Xu TY, Sheng CQ, Wang P, Miao CY. Nicotinamide phosphoribosyltransferase is required for the calorie restriction-mediated improvements in oxidative stress, mitochondrial biogenesis, and metabolic adaptation. *J Gerontol A Biol Sci Med Sci*. 2014;69:44–57.
46. Guo X, Zhou G, Guo M, Cheung AK, Huang Y, Beddhu S. Adiponectin retards the progression of diabetic nephropathy in *db/db* mice by counteracting angiotensin II. *Physiol Rep*. 2014;2:e00230.
47. Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, Matsuzawa Y. Decreased plasma adiponectin concentration in patients with essential hypertension. *Am J Hypertens*. 2003;16:72–75.
48. Huang KC, Chen CL, Chuang LM, Ho SR, Tai TY, Yang WS. Plasma adiponectin levels and blood pressures in nondiabetic adolescent females. *J Clin Endocrinol Metab*. 2003;88:4130–4134.
49. Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, Fu Y, Motone M, Yamamoto K, Matsuo A, Ohashi K, Kihara S, Funahashi T, Rakugi H, Matsuzawa Y, Ogihara T. Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension*. 2004;43:1318–1323.
50. Kim DH, Kim C, Ding EL, Townsend MK, Lipsitz LA. Adiponectin levels and the risk of hypertension: a systematic review and meta-analysis. *Hypertension*. 2013;62:27–32.
51. Wang ZV, Scherer PE. Adiponectin, cardiovascular function, and hypertension. *Hypertension*. 2008;51:8–14.
52. Kackstein K, Teren A, Matsumoto Y, Mangner N, Mobius-Winkler S, Linke A, Schuler G, Punkt K, Adams V. Impact of angiotensin II on skeletal muscle metabolism and function in mice: contribution of IGF-1, Sirtuin-1 and PGC-1α. *Acta Histochem*. 2013;115:363–370.
53. Fan YY, Kohno M, Hitomi H, Kitada K, Fujisawa Y, Yatabe J, Yatabe M, Felder RA, Ohsaki H, Rafiq K, Sherajee SJ, Noma T, Nishiyama A, Nakano D. Aldosterone/mineralocorticoid receptor stimulation induces cellular senescence in the kidney. *Endocrinology*. 2011;152:680–688.
54. Baudrand R, Lian CG, Lian BQ, Ricchiuti V, Yao TM, Li J, Williams GH, Adler GK. Long-term dietary sodium restriction increases adiponectin expression and ameliorates the proinflammatory adipokine profile in obesity. *Nutr Metab Cardiovasc Dis*. 2014;24:34–41.
55. Baudrand R, Campino C, Carvajal CA, Olivieri O, Guidi G, Faccini G, Vohringer PA, Cerda J, Owen G, Kalergis AM, Fardella CE. High sodium intake is associated with increased glucocorticoid production, insulin resistance and metabolic syndrome. *Clin Endocrinol (Oxf)*. 2014;80:677–684.

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