ARTICLE

Simultaneous GLP-1 receptor activation and angiotensin receptor blockade increase natriuresis independent of altered arterial pressure in obese OLETF rats

Ruben Rodriguez[1] · Meagan Moreno[1] · Andrew Y. Lee[1] · Jose A. Godoy-Lugo[1] · Daisuke Nakano[2] ·
Akira Nishiyama[2] · David Parkes[3] · Mouhamed S. Awayda[4] · Rudy M. Ortiz[1]

Abstract

Obesity is associated with an inappropriately activated renin–angiotensin–aldosterone system, suppressed glucagon-like peptide-1 (GLP-1), increased renal Na^+ reabsorption, and hypertension. To assess the link between GLP-1 and angiotensin receptor type 1 (AT_1) signaling on obesity-associated impairment of urinary Na^+ excretion ($U_{Na}V$) and elevated arterial pressure, we measured mean arterial pressure (MAP) and heart rate by radiotelemetry and metabolic parameters for 40 days. We tested the hypothesis that stimulation of GLP-1 signaling provides added benefit to blockade of AT_1 by increasing $U_{Na}V$ and further reducing arterial pressure in the following groups: (1) untreated Long–Evans Tokushima Otsuka (LETO) rats ($n = 7$); (2) untreated Otsuka Long–Evans Tokushima Fatty (OLETF) rats ($n = 9$); (3) OLETF + ARB (ARB; 10 mg olmesartan/kg/day; $n = 9$); (4) OLETF + GLP-1 receptor agonist (EXE; 10 μg exenatide/kg/day; $n = 7$); and (5) OLETF + ARB + EXE (Combo; $n = 6$). On day 2, $U_{Na}V$ was 60% and 62% reduced in the EXE and Combo groups, respectively, compared with that in the OLETF rats. On day 40, $U_{Na}V$ was increased 69% in the Combo group compared with that in the OLETF group. On day 40, urinary angiotensinogen was 4.5-fold greater in the OLETF than in the LETO group and was 56%, 62%, and 58% lower in the ARB, EXE, and Combo groups, respectively, than in the OLETF group. From day 2 to the end of the study, MAP was lower in the ARB and Combo groups than in the OLETF rats. These results suggest that GLP-1 receptor activation may reduce intrarenal angiotensin II activity, and that simultaneous blockade of AT_1 increases $U_{Na}V$ in obesity; however, these beneficial effects do not translate to a further reduction in MAP.

Introduction

Obesity is a major health concern, with 35% of males and 40% of females in the United States currently classified as

✉ Ruben Rodriguez
rrodriguez3@ucmerced.edu

[1] Department of Molecular and Cellular Biology, University of California Merced, Merced, CA, USA

[2] Department of Pharmacology, Kagawa University Medical School, Kagawa, Japan

[3] DGP Scientific, Inc., Del Mar, CA, USA

[4] Department of Physiology and Biophysics, University of Buffalo, Buffalo, NY, USA

obese [1]. Obesity is associated with many adverse health outcomes such as metabolic syndrome, insulin resistance, attenuated glucagon-like peptide-1 (GLP-1) secretion, and hypertension [2–5]. Although many factors are associated with the development of hypertension in obesity, increased renal Na^+ reabsorption is among the primary factors [6, 7]. The mechanisms by which obesity increases renal Na^+ reabsorption are multifaceted and involve the physical compression of the kidneys by fat, increased sympathetic nervous system activity, and inappropriate activation of the renin–angiotensin–aldosterone system (RAAS) [8, 9]. Because of the many factors associated with the development of hypertension in obesity, treatment-resistant hypertension is more prevalent in obese individuals [10, 11]. Some treatment options make matters worse, because they can lead to the development or worsening of hyperglycemia [12, 13]. Angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs) are among the treatment options that do not lead to the development or

worsening of hyperglycemia. In contrast, ACEi and ARB decreased new-onset type 2 diabetes mellitus (T2DM) in individuals with cardiovascular risk factors or cardiovascular disease [14, 15]. Furthermore, in individuals without cardiovascular disease but with impaired fasting glucose or impaired glucose tolerance, treatment with an ACEi (ramipril) increased the regression to normoglycemia [16], suggesting that disrupting RAAS may prove beneficial for managing hypertension in those with metabolic derangements.

GLP-1 is an incretin hormone that is released into circulation during food intake and binds the GLP-1 receptor (GLP-1r) in the pancreas, where it stimulates insulin secretion in a glucose-dependent manner [17]. In addition to the pancreas, the GLP-1r is expressed in the lung, hypothalamus, hippocampus, cerebral cortex, gastrointestinal tract, heart, vasculature, and kidneys [18, 19], suggesting that GLP-1 may exert a variety of biological actions. To this end, GLP-1r agonists and dipeptidyl peptidase-4 inhibitors have been shown to increase urinary sodium excretion ($U_{Na}V$) and decrease blood pressure [20, 21], implicating GLP-1 signaling in the regulation of volume-dependent hypertension. The majority of studies that report a natriuretic and/or blood pressure-lowering effect examined the acute effects of these agents [22–27]. Furthermore, the few studies that assessed the chronic effects of GLP-1r agonists showed mixed results [28, 29]. Therefore, the objectives of this study were to evaluate (1) the potential added benefits of targeting two signaling pathways (GLP-1 and AT_1) that contribute to renal Na^+ handling and (2) if these benefits in renal Na^+ handling translate into amelioration of obesity-associated hypertension. To accomplish these objectives, we treated Otsuka Long–Evans Tokushima Fatty (OLETF) rats, a model of diet-induced obesity [30], with a GLP-1r agonist (exenatide) and an ARB (olmesartan). Moreover, we tested the hypothesis that stimulation of GLP-1 signaling provides added benefit to blockade of AT_1 by increasing renal Na^+ excretion and further reducing arterial pressure in obese OLETF rats.

Methods

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California, Merced.

Animals

Eight-week-old male, lean Long–Evans Tokushima Otsuka (LETO) and obese OLETF rats were studied (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan). Rats were divided into the following groups: (1) untreated LETO ($n = 7$) + vehicle (0.5% methylcellulose by oral gavage once daily); (2) untreated OLETF ($n = 9$) + vehicle; (3) OLETF + ARB (ARB; 10 mg olmesartan/kg by oral gavage once daily; $n = 9$); (4) OLETF + GLP-1r agonist (EXE; 10 µg exenatide/kg by osmotic mini-pumps for 42 days; $n = 7$); and (5) OLETF + ARB + Exe (Combo; $n = 6$). OLETF rats were assigned to groups, so that mean body mass (BM) at the onset of the study was within 5% of each other. Once assigned, a subset of animals from each group (LETO $n = 4$, OLETF $n = 6$, ARB $n = 4$, EXE $n = 5$, Combo $n = 5$) were surgically implanted with radio-telemeters (PA-C40; DSI, St. Paul, MN), as previously described [31, 32]. Animals were allowed to recover from the surgery for 7 days. Following the recovery period, all animals were transferred to metabolic cages and allowed to acclimate to the cages for 7 days. Treatments were started following the acclimation period. In the OLETF groups, mean arterial pressure (MAP) and heart rate were measured continuously for 30 s at 5 min intervals and are presented as the means for the 12 h light and 12 h dark cycles. In the LETO group, MAP and heart rate were measured continuously for 2 min once a day during the light cycle. Urine was collected at baseline and 2, 7, 13, 21, 28, and 40 days following the start of the treatments. At collection, urine volumes were recorded, samples were centrifuged ($3000 \times g \times 15$ min at 4 °C), and an aliquot transferred to a cryovial and immediately stored at -20 °C. All animals were housed in a specific pathogen-free facility under controlled temperature (23 °C) and humidity (55%) conditions on a 12 h light–dark cycle. All animals were given free access to water and standard laboratory chow (Teklad Diets, Madison, WI).

BM, water, and food intake

BM was measured daily to calculate the appropriate ARB dose. Water and food intake were measured for 24 h once a week.

Tissue collection

After the 6-week study period, animals were fasted for 12 h and tissues were collected the following morning. After measuring BM, animals were decapitated and trunk blood was collected into chilled vials containing 50 mM EDTA and a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Immediately following, the left kidney was removed, snap-frozen in liquid nitrogen, and stored at -80 °C until analyzed. Blood samples were centrifuged ($3000 \times g \times 15$ min at 4 °C) and plasma was transferred to cryovials and stored at -80 °C for later analyses.

Western blotting

A mid-transverse section of the kidney was used to extract proteins using RIPA buffer containing NP-40, EDTA, and protease and phosphatase inhibitor cocktail (Thermo, Waltham, MA). Tissue homogenate was centrifuged ($20,000 \times g \times 15$ min at $4\,^{\circ}$C), and the aqueous layer was transferred to a separate tube and stored at $80\,^{\circ}$C for later analyses. Total protein content was measured by Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein were separated on 6–15% Bis-Tris gels. Proteins were transferred using the Bio-Rad Trans-Blot SD semi-dry cell onto $0.45\,\mu$m polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA). Membranes were blocked with Odyssey blocking solution (LI-COR Biosciences, Lincoln, NE). After blocking, membranes were incubated overnight with the following primary antibodies against phosphorylated (p)-IGF-1 receptor-β (Tyr1135/1136)/(p)-insulin receptor (IR)-β (Tyr1150/1151), p-Akt (ser473), Akt (Cell Signaling, Danvers, MA), β-actin, IR-β (Santa Cruz Biotechnology, Santa Cruz, CA), γ-epithelial Na^+ channel (γENaC) [33], renal outer medullary K^+ channel (ROMK) (^{342}HNFGKTVEVETPHCAMCLYNEKDARARMKRG-YDNPNFVLSE VDETDDTQMA^{391}), and aquaporin 2 (AQP2) (^{254}RQSVELHSPQSLPRGSKA^{271}; Alomone Laboratories, Jerusalem, Israel) in Odyssey blocking solution $+0.2\%$ Tween 20 at $4\,^{\circ}$C. Membranes were washed, incubated for 1 h with specific secondary antibodies (IRDye: LI-COR Biosciences) in TBS-T $+5\%$ non-fat milk $+0.01\%$ SDS, re-washed, and scanned in an Odyssey infrared imager (LI-COR Biosciences). In addition to consistently loading the same amount of total protein per well, densitometry values were further normalized by correcting for the densitometry values of β-actin.

Biochemical analyses and homeostasis model assessment of insulin resistance

Fasting plasma glucose (FPG) and triglycerides (TGs) were measured using an Analox GM7 analyzer (Analox Instruments, London, UK). Fasting plasma non-esterified fatty acids (NEFA) (WAKO, Osaka, Japan), creatinine (Sigma-Aldrich), insulin (FPI), and leptin (EMD Millipore) were measured using commercially available kits. All samples were analyzed in duplicate and run in a single assay with intra-assay coefficients of variability of $<10\%$. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR $=$ (FPG $\times$ FPI)/2.430, with FPG in milligrams per deciliter and FPI in microunits per milliliter, as previously described [34].

Urine analyses

Plasma and urinary Na^+ and K^+ were measured using an EasyLyte analyzer (Medica, Bedford, MA). Urinary creatinine was measured using a commercially available colorimetric kit (Cayman Chemical, Ann Arbor, MI). Urinary aldosterone and corticosterone were extracted as previously described [35, 36] and measured using commercially available RIA kits (Siemens Healthcare Diagnostics, Los Angeles, CA, and MP Biomedicals, Solon, OH, respectively). Urinary angiotensinogen (Takara Bio USA, Mountain View, CA) was measured using a commercially available enzyme-linked immunosorbent assay kit. Urinary excretion values were calculated as the product of concentration and urine volume, and normalized by correcting for urinary creatinine excretion. Glomerular filtration rate (GFR) was estimated from samples taken on the last day of the study and calculated as follows: GFR $= (U_{cr} \times UV) \div P_{cr}$, where U_{cr} is urinary creatinine, UV is urine volume, and P_{cr} is plasma creatinine. Fractional excretions (FEs) for Na^+ and K^+ were calculated as follows: FE $= [(U_x \times P_{cr}) \div (P_x \times U_{cr})] \times 100$, where x is the electrolyte.

Statistical analyses

Means ($\pm$ SE) were calculated for all samples unless otherwise noted. One-way analysis of variance (ANOVA) followed by Tukey's honest significant difference or Games–Howell post hoc test were used to analyze differences between groups for protein expression and for end-of-study plasma and urinary analyses. For data measured repeatedly, we used a two-factor repeated-measures ANOVA with time as a within-subjects factor and group as a between-subjects factor. When significant interactions were observed, pair-wise comparisons were carried out using a Bonferroni correction. Relationships between dependent and independent variables were evaluated by linear regression analysis, and correlations were evaluated using Pearson's correlation coefficients. Means, regressions, and correlations were considered significant at $p < 0.05$ and analyses were performed with SPSS version 24 (IBM, Armonk, NY).

Results

Effects of GLP-1r activation alone and in combination with AT$_1$ blockade on MAP and heart rate

MAP and heart rate were measured daily by telemetry to accurately assess the effects of chronic GLP-1r agonism and AT$_1$ blockade on cardiovascular function.
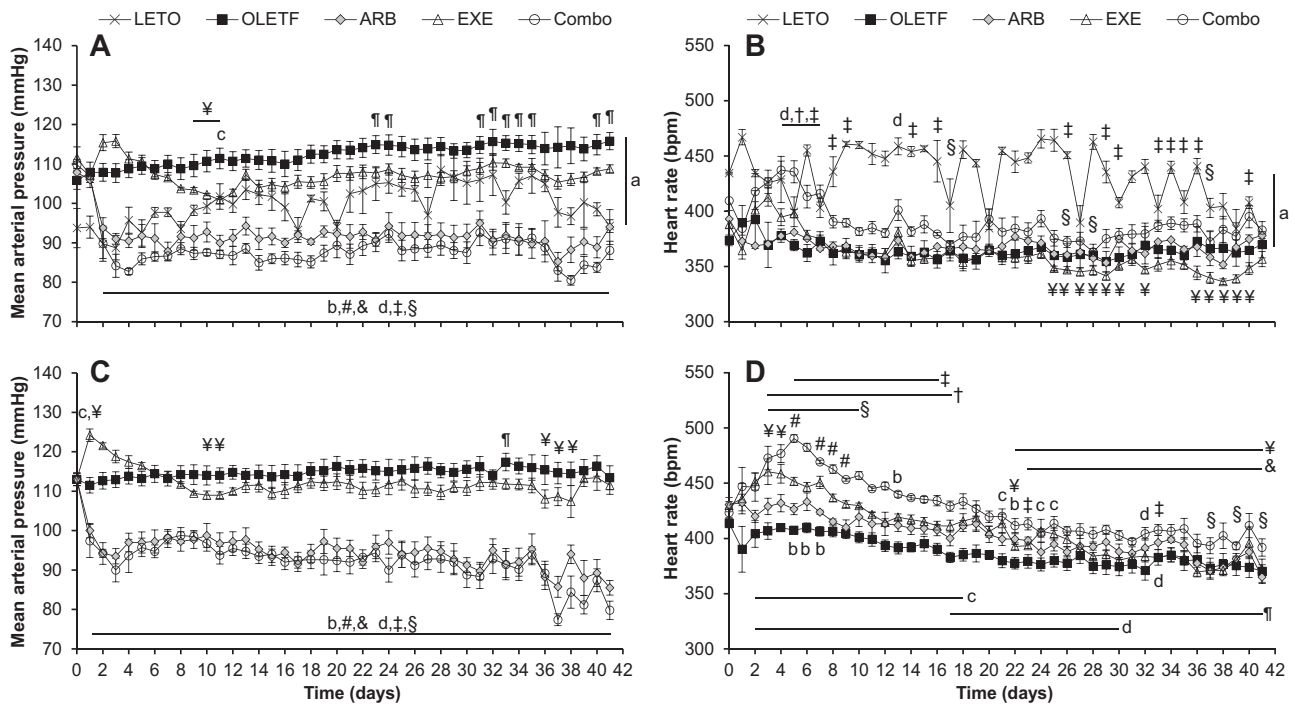
Fig. 1 Effects of exenatide alone and in combination with olmesartan on mean arterial pressure and heart rate. Mean (± SE) daily (a) arterial pressure and b heart rate during the c light cycle and d dark cycle, respectively, in the Long–Evans Tokushima Otsuka (LETO; $n = 4$), Otsuka Long–Evans Tokushima Fatty (OLETF; $n = 5$), OLETF + ARB (ARB; $n = 4$), OLETF + EXE (EXE; $n = 5$), and OLETF + ARB + EXE (Combo; $n = 5$) groups. a $p < 0.05$, LETO vs. OLETF; b $p < 0.05$, OLETF vs. ARB; c $p < 0.05$, OLETF vs. EXE; d $p < 0.05$, OLETF vs. Combo; #$p < 0.05$, ARB vs. EXE; †$p < 0.05$, ARB vs. Combo; ‡$p < 0.05$, EXE vs. Combo; ¶$p < 0.05$, vs. baseline for OLETF; &$p < 0.05$, vs. baseline for ARB; ¥$p < 0.05$ vs. baseline for EXE; §$p < 0.05$, vs. baseline for Combo

Light cycle

Baseline MAP was greater in OLETF rats than in LETO rats, remaining so for the remainder of the study (Fig. 1a). From day 2 to the end of the study, MAP was decreased in the ARB and Combo groups compared with that in the OLETF group (Fig. 1a). Exenatide treatment transiently decreased MAP, reaching its nadir on day 11, after which time, MAP returned to and hovered around its baseline levels (Fig. 1a). Baseline heart rate was greater in LETO rats, remaining so for the duration of the study (Fig. 1b). From day 4 to day 7, heart rate increased in the Combo group compared with that in the OLETF group; after that, heart rate hovered around baseline levels (Fig. 1b). On day 25, heart rate decreased below baseline levels in the EXE group and remained lower for the remainder of the study (Fig. 1b).

Dark cycle

From day 1 to the end of the study, MAP was decreased in the ARB and Combo groups compared with that in the OLETF group (Fig. 1c). Exenatide treatment initially increased MAP, peaking on day 1, then gradually decreasing to OLETF levels, where it remained for the rest of the study (Fig. 1c). From day 2, heart rate was increased in the EXE and Combo groups, peaking on day 3 in the EXE group and day 5 in the Combo group, then gradually decreasing to the levels observed in the OLETF rats by day 18 in the EXE group and day 30 in the Combo group (Fig. 1d). Collectively, these results suggest that the beneficial effects of GLP-1r activation on MAP may be more prominent during the light cycle and that the addition of an AT$_1$ inhibitor does not lead to added benefits.

GLP-1r activation in combination with AT$_1$ blockade decreases BM but does not improve the biochemical parameters of metabolic syndrome

BM, food intake, FPG, FPI, leptin, TG, and NEFA were measured, and HOMA-IR was calculated to assess whether GLP-1r activation alone or in combination with AT$_1$ blockade would protect against the development of metabolic syndrome and insulin resistance. From baseline, BM was greater in OLETF rats than in LETO rats (Fig. 2a). On day 2, BM was lower in the EXE and Combo groups than in the OLETF group, remaining lower in the Combo group for the remainder of the study (Fig. 2a). From baseline, food
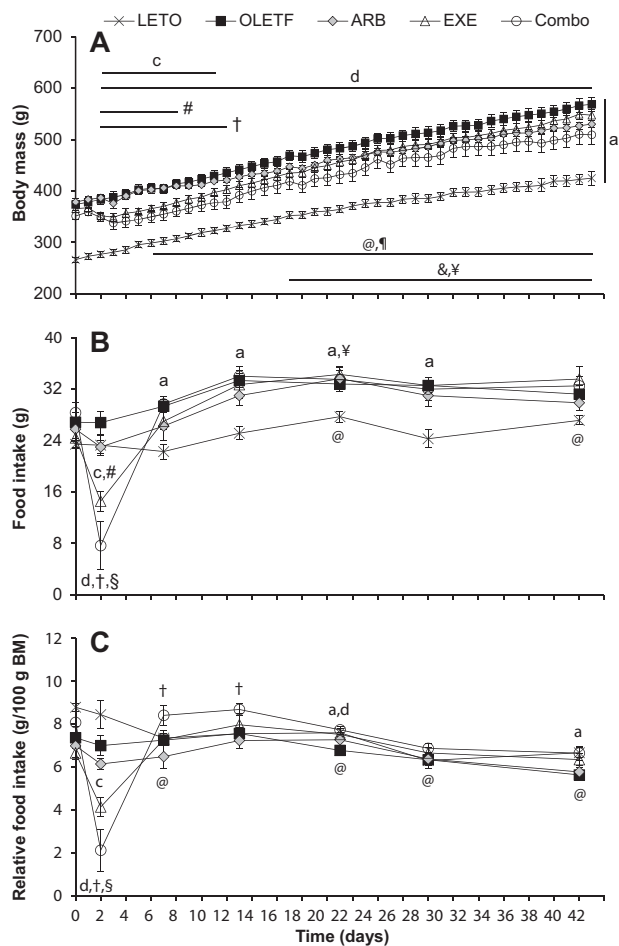
Fig. 2 Exenatide in conjunction with olmesartan treatment decreased body mass. Mean (± SE) (**a**) daily body mass, and weekly (**b**) absolute and **c** relative food intake in the Long–Evans Tokushima Otsuka (LETO; $n = 7$), Otsuka Long–Evans Tokushima Fatty (OLETF; $n = 9$), OLETF + ARB (ARB; $n = 9$), OLETF + EXE (EXE; $n = 7$), and OLETF + ARB + EXE (Combo; $n = 6$) groups. **a** $p < 0.05$, LETO vs. OLETF; **c** $p < 0.05$, OLETF vs. EXE; **d** $p < 0.05$, OLETF vs. Combo; #$p < 0.05$, ARB vs. EXE; †$p < 0.05$, ARB vs. Combo; @$p < 0.05$, vs. baseline for LETO; ¶$p < 0.05$, vs. baseline for OLETF; &$p < 0.05$, vs. baseline for ARB; ¥$p < 0.05$ vs. baseline for EXE; §$p < 0.05$, vs. baseline for Combo

intake was greater in the OLETF rats than in the LETO rats (Fig. 2b). On day 2, food intake was 46% and 71% lower in the EXE and Combo, respectively, compared with that in the OLETF group (Fig. 2b). From day 7, food intake was not different between OLETF rats and the intake of any of the treatment groups (Fig. 2b). On day 2, relative food intake was 41% and 70% lower in the EXE and Combo groups, respectively, compared with that in the OLETF group (Fig. 2c). On day 21, relative food intake was 14% greater in the Combo group than in the OLETF group; notwithstanding, there was no difference in relative food intake between the OLETF group and the treatment groups for the remainder of the study (Fig. 2c). Mean FPG and FPI

were 32% and 128% higher, respectively, in the OLETF group compared with those in the LETO group, and FPG was 9% lower in the ARB group compared with that in the OLETF group. Neither exenatide nor combination treatment had a significant effect on these parameters (Table 1). Mean fasting plasma TG and leptin were increased 133% and 3.6-fold, respectively, in the OLETF group compared with those in the LETO group, but none of the treatments had a significant effect on TG (Table 1). Mean fasting plasma NEFA did not differ in any of the groups (Table 1). HOMA-IR was 2.4-fold higher in the OLETF group than in the LETO group, but none of the treatments had a significant effect on HOMA-IR (Table 1). These data suggest that the decrease in BM in the Combo group did not translate into profound or sustained improvements in the biochemical parameters of metabolic syndrome or insulin resistance. In addition, these data suggest that the arterial pressure-reducing effects of the treatments are not secondary to BM reduction, as the improvements in MAP did not match the changes in BM.

Obesity linked to elevated RAAS components but not corticosterone

Urinary aldosterone excretion ($U_{aldo}V$), urinary corticosterone excretion (U_BV), and urinary angiotensinogen excretion ($U_{Agt}V$) were measured to assess the contribution of RAAS and corticosterone to anti-natriuresis in obesity, and to determine whether GLP-1r activation alone or in combination with AT_1 blockade would ameliorate this effect. From baseline, $U_{aldo}V$ was greater in OLETF rats than in LETO rats (Fig. 3a). On day 2, $U_{aldo}V$ was 124% higher in the EXE group than in OLETF rats and was 37% lower in the Combo group than in the OLETF group, remaining lower for the rest of the study (Fig. 3a). On day 7, $U_{aldo}V$ was 61% less in the EXE group compared with that in the OLETF (Fig. 3a). From the onset to the end of the study, there was no difference in U_BV between LETO and OLETF rats or between OLETF rats and the treatment groups (Fig. 3b). BM was positively correlated with $U_{aldo}V$ [$U_{aldo}V = 0.131$ $BM - 19.047$ ($R = 0.621$; $P = 0.0001$)], but not with U_BV [$U_BV = -0.141$ $BM + 409.91$ ($R = 0.078$; $P = 0.375$)]. On day 40, $U_{Agt}V$ was 4.5-fold greater in OLETF rats than in LETO rats and was 56%, 62%, and 58% lower in the ARB, EXE, and Combo groups, respectively, compared with that in the OLETF group (Fig. 4). Collectively, these data suggest that obesity is associated with an inappropriate elevation in RAAS components but not corticosterone levels in OLETF rats, and that combination treatment reversed these effects.
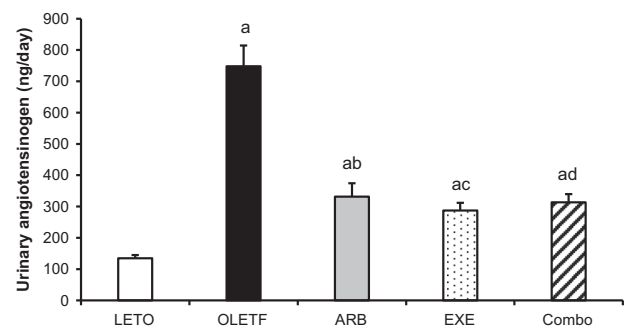
Table 1 Plasma parameters associated with the metabolic syndrome

	LETO ($n = 7$)	OLETF ($n = 9$)	ARB ($n = 9$)	EXE ($n = 7$)	Combo ($n = 6$)
Glucose (mmol/L)	5.6 ± 0.2	$7.4 \pm 0.2^*$	$6.7 \pm 0.1^{*\#}$	$7.1 \pm 0.2^*$	$7.0 \pm 0.1^*$
Insulin (mmol/L)	130 ± 18	$296 \pm 31^*$	229 ± 28	$325 \pm 45^*$	$300 \pm 27^*$
HOMA-IR (relative units)	0.8 ± 0.1	$2.7 \pm 0.4^*$	1.6 ± 0.2	$2.5 \pm 0.4^*$	2.3 ± 0.2
Triglycerides (mmol/L)	0.6 ± 0.1	$1.4 \pm 0.1^*$	1.0 ± 0.1	$1.5 \pm 0.2^*$	1.1 ± 0.1
NEFA (mmol/L)	0.54 ± 0.06	0.55 ± 0.04	0.62 ± 0.04	0.53 ± 0.05	0.51 ± 0.06
Leptin (µg/L)	2.8 ± 0.3	$13.0 \pm 2.5^*$	6.6 ± 0.6	$10.2 \pm 1.5^*$	7.2 ± 1.2

$^*p < 0.05$, vs. LETO

$^\#p < 0.05$ vs. OLETF. NEFA, non-esterified fatty acids
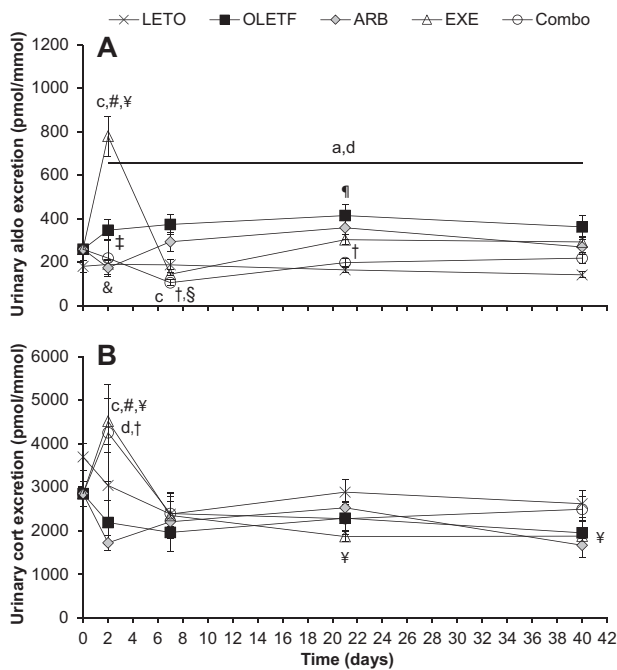
Fig. 3 Exenatide in conjunction with olmesartan treatment decreased urinary aldosterone excretion. Mean ($\pm$ SE) urinary (**a**) aldosterone and **b** corticosterone excretion in the Long–Evans Tokushima Otsuka (LETO; $n = 7$), Otsuka Long–Evans Tokushima Fatty (OLETF; $n = 9$; $n = 5$ for B), OLETF + ARB (ARB; $n = 9$; $n = 4$ for B), OLETF + EXE (EXE; $n = 7$), and OLETF + ARB + EXE (Combo; $n = 6$) groups. **a** $p < 0.05$, LETO vs. OLETF; **c** $p < 0.05$, OLETF vs. EXE; **d** $p < 0.05$, OLETF vs. Combo; #$p < 0.05$, ARB vs. EXE; †$p < 0.05$, ARB vs. Combo; ‡$p < 0.05$, EXE vs. Combo; $p < 0.05$, vs. baseline for OLETF; &$p < 0.05$, vs. baseline for ARB; ¥$p < 0.05$ vs. baseline for EXE; §$p < 0.05$, vs. baseline for Combo

Fig. 4 Effects of exenatide alone and in combination with olmesartan on urinary angiotensinogen excretion. Mean ($\pm$ SE) urinary angiotensinogen excretion in the Long–Evans Tokushima Otsuka (LETO; $n = 7$), Otsuka Long–Evans Tokushima Fatty (OLETF; $n = 6$), OLETF + ARB (ARB; $n = 6$), OLETF + EXE (EXE; $n = 7$), and OLETF + ARB + EXE (Combo; $n = 6$) groups. **a** $p < 0.05$, LETO vs. OLETF; **b** $p < 0.05$, OLETF vs. ARB; **c** $p < 0.05$, OLETF vs. EXE; **d** $p < 0.05$, OLETF vs. Combo

Effects of GLP-1r activation alone and in combination with AT$_1$ blockade on renal electrolyte handling

Urinary Na$^+$ excretion (U$_{Na}$V), urinary K$^+$ excretion (U$_K$V), U$_{Na}$V/Na$^+$ intake, U$_K$V/K$^+$ intake, Na$^+$ balance, K$^+$ balance, and urinary Na$^+$/K$^+$ ratio were calculated to assess the effects of GLP-1r activation and AT$_1$ blockade on renal

function and electrolyte balance. From baseline to the end of the study, there were no differences in U$_{Na}$V, U$_K$V, U$_{Na}$V/Na$^+$ intake, U$_K$V/K$^+$ intake, Na$^+$ balance, K$^+$ balance, or urinary Na$^+$/K$^+$ ratio between LETO and OLETF rats (Fig. 5a-c and Figure. S1 and 2a, b). On day 2, U$_{Na}$V, U$_{Na}$V/Na$^+$ intake, and the urinary Na$^+$/K$^+$ ratio were 60%, 40%, and 61% lower, respectively, in the EXE group compared with those in the OLETF group, and U$_{Na}$V and the urinary Na$^+$/K$^+$ ratio were 62% and 53% lower, respectively, in the Combo group compared with that in the OLETF group (Fig. 5a, c and Figure S1A). In contrast, on day 2, U$_K$V/K$^+$ intake was 132% greater in the EXE group and 167% greater in the Combo group compared with that in the OLETF group (Figure S1A). On day 7, U$_K$V was 24% lower in the EXE group than in the OLETF group (Fig. 5b). On day 40, U$_{Na}$V, U$_K$V, and the urinary Na$^+$/K$^+$ ratio were 69%, 35%, and 32% greater, respectively, in the Combo group than in the OLETF group (Fig. 5a-c); however, these effects disappeared when U$_{Na}$V and U$_K$V were corrected for Na$^+$ and K$^+$ intake (Figure S1A & B). Nevertheless, on day 40, Na$^+$ balance was 122% lower in the Combo group than in the OLETF (Figure S2A). The
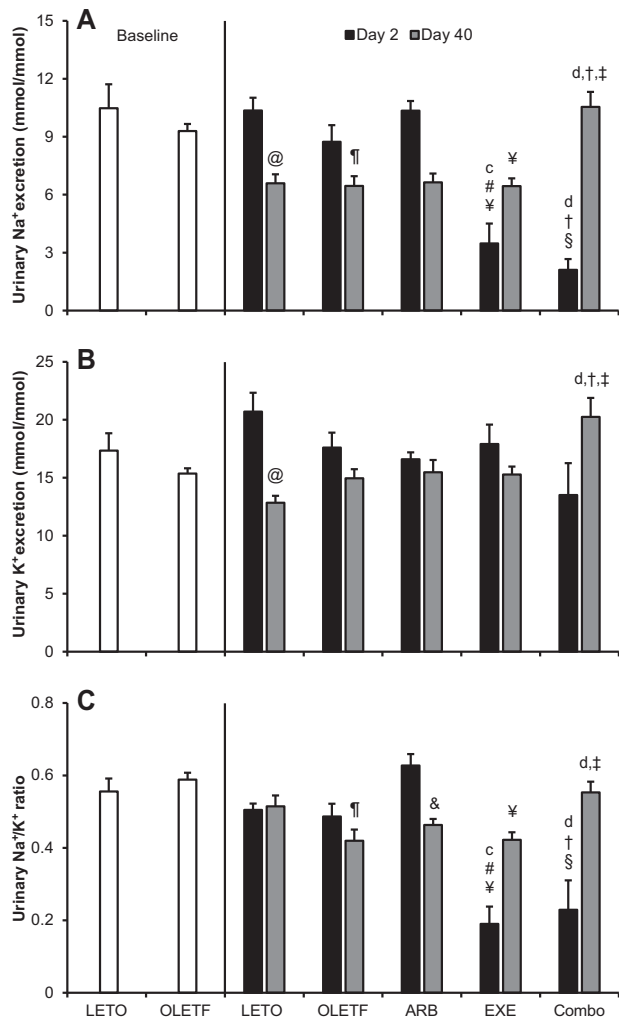
Fig. 5 Effects of exenatide alone and in combination with olmesartan on urinary Na^+ and K^+ excretion. Mean ($\pm$ SE) urinary (**a**) Na^+ ($U_{Na}V$), **b** K^+ (U_KV) excretion, and urinary (**c**) Na^+/K^+ ratio in the Long–Evans Tokushima Otsuka (LETO; $n = 7$), Otsuka Long–Evans Tokushima Fatty (OLETF; $n = 9$), OLETF + ARB (ARB; $n = 9$), OLETF + EXE (EXE; $n = 7$), and OLETF + ARB + EXE (Combo; $n = 6$) groups. **c** $p < 0.05$, OLETF vs. EXE; **d** $p < 0.05$, OLETF vs. Combo; #$p < 0.05$, ARB vs. EXE; †$p < 0.05$, ARB vs. Combo; ‡$p < 0.05$, EXE vs. Combo; @$p < 0.05$, vs. baseline for LETO; ¶$p < 0.05$, vs. baseline for OLETF; &$p < 0.05$, vs. baseline for ARB; ¥$p < 0.05$ vs. baseline for EXE; §$p < 0.05$, vs. baseline for Combo
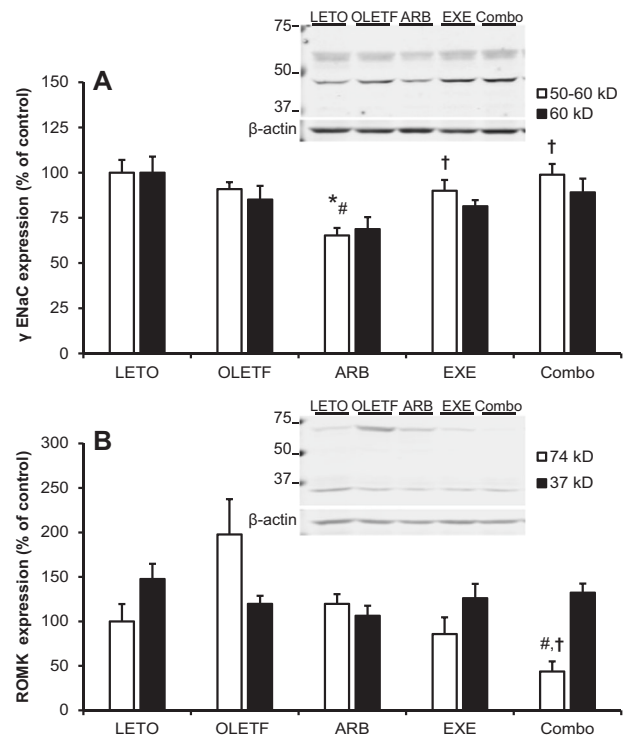

Fig. 6 Exenatide in conjunction with olmesartan treatment decreased renal ROMK protein expression. Mean ($\pm$ SE) kidney (**a**) γENaC and **b** ROMK (% change from LETO), and the representative western blotting bands in the Long–Evans Tokushima Otsuka (LETO; $n = 5$), Otsuka Long–Evans Tokushima Fatty (OLETF; $n = 8$), OLETF + ARB (ARB; $n = 8$), OLETF + EXE (EXE; $n = 6$), and OLETF + ARB + EXE (Combo; $n = 5$) groups. *$p < 0.05$ vs. LETO; #$p < 0.05$ vs. OLETF; †$p < 0.05$ vs. ARB

Effects of GLP-1r activation alone and in combination with AT$_1$ blockade on Na$^+$ and K$^+$ channels in the kidney

Protein expression of γENaC, ROMK, and AQP2 was measured to assess whether the hyperinsulinemia and increased $U_{aldo}V$ in OLETF rats altered the protein expression of Na^+ and K^+ channels in the kidney. In addition, the phosphorylation of IR and AKT were measured to assess the link between changes in renal electrolyte channels and insulin signaling. Cleaved and active γENaC migrated as a doublet band at ~60 kDa with an additional cleavage product migrating to ~50 kDa, as previously shown [33]. Mean protein expression of cleaved and active γENaC was not different among the groups. However, the mean protein expression of the 50–60 kDa multiband was decreased 28% in the ARB group compared with that in the OLETF group, and combination treatment prevented this effect (Fig. 6a). Mean protein expression of glycosylated ROMK was increased 98% in the OLETF group compared to that in the LETO group ($P = 0.052$) and was decreased 78% in the Combo compared with that in the OLETF group

treatments had no detectable effect on plasma Na^+ or K^+ (Table S1). Furthermore, the effects on electrolyte excretion were independent of changes in absolute or relative water intake, urine volume, urinary creatinine excretion (Figure S3), estimated GFR, FE_{Na}^+, and FE_K^+ (Table S1). Collectively, these data demonstrate that prolonged GLP-1r activation in conjunction with AT$_1$ blockade increase $U_{Na}V$ and U_KV in response to increased Na^+ and K^+ intake; however, the increase in urinary Na^+/K^+ ratio suggests that tubular K^+ secretion may be blunted in this group.

(Fig. 6b). The mean protein expression of non-glycosylated ROMK (Fig. 6b) and AQP2 (Figure S4) was not different among the groups. The mean protein expression of p-IR or the p-IR: IR ratio did not change in any of the groups (Figure S5A & C). The mean protein expression of IR was increased 46% in the Combo compared to that in the OLETF group (Figure S5B). The mean protein expression of p-AKT and AKT, and the p-AKT: AKT ratio did not differ in any of the groups (Figure S6A, B & C). Collectively, these results suggest that (1) the increase in $U_{Na}V$ in the Combo group is not due to decreased cleaved and active γENaC protein expression or improvements in renal insulin signaling and (2) enhanced K^+ retention may be due to decreased glycosylated ROMK protein expression.

Discussion

Obesity accounts for 78% of the risk for essential hypertension in men and 65% in women [4]. Many factors are associated with the development of hypertension in obesity, with increased renal Na^+ reabsorption being among the principal factors [6, 7]. The mechanisms by which obesity increases renal Na^+ reabsorption are multifactorial and involve inappropriate activation of the RAAS, physical compression of the kidneys by fat, and increased sympathetic nervous system activity [8, 9]. In addition to the aforementioned mechanisms, obesity may also increase renal Na^+ reabsorption by decreasing GLP-1 secretion [5]. GLP-1 decreases sodium reabsorption in the proximal tubule by inhibiting NHE3 activity [37, 38]. In addition, studies have shown that acute GLP-1r activation decreases angiotensin II (Ang II) levels in healthy and T2DM subjects; [24, 39] however, whether this effect contributes to the natriuretic and blood pressure-lowering effect of GLP-1r agonists is not known. Therefore, this study aimed to evaluate (1) the potential added benefits of targeting two signaling pathways (GLP-1 and AT_1) that are known to contribute to impaired renal Na^+ handling, and (2) if these benefits in renal Na^+ handling translated into amelioration of obesity-associated hypertension. To this end, the present study demonstrated that chronic simultaneous targeting of GLP-1r activation and AT_1 blockade increased $U_{Na}V$, and that this benefit did not translate to further reductions in MAP compared to ARB treatment in obese OLETF rats.

In addition to their blood glucose lowering effects [40], GLP-1r agonists decrease blood pressure by decreasing renal Na^+ reabsorption and by improving endothelial function [41]. In the present study, GLP-1r activation decreased $U_{Na}V$ on day 2, which most likely resulted from the acute anorexic effects of GLP-1r activation [42]. Although this anorexic effect reduced BM in the EXE and Combo groups, the reduction in BM was lost in the EXE

group after 10 days of treatment, but persisted in the Combo group. This decrease in BM unlikely contributed to the increased $U_{Na}V$ observed at the end of the study, as this effect did not match the time course of the decrease in BM. Although $U_{Na}V$ increased on day 40 in the Combo group, this effect disappeared when $U_{Na}V$ was corrected for dietary Na^+ intake, suggesting that combination treatment protected against Na^+ retention, which is supported by a reduction in Na^+ balance on day 40 in the Combo group compared with that in the OLETF group. This finding is noteworthy, because increased Na^+ intake without the appropriate increased $U_{Na}V$ can disrupt the normal circadian rhythm of blood pressure [43]. Furthermore, although $U_{aldo}V$ remained low in the Combo group for the duration of the study, cleaved and active γENaC protein expression was not suppressed in the Combo group, suggesting that aldosterone may not regulate activation of γENaC [33]. Given these results, the natriuresis observed in the Combo group may be the result of decreased NHE3 activity independent of aldosterone. This potential exists because obesity-associated insulin resistance (similar to the OLETF phenotype) decreases GLP-1r activation [5], increases plasma insulin levels [44], and intrarenal Ang II [45], all of which are factors that increase NHE3 activity, leading to increased Na^+ reabsorption [46–48]. In the present study, renal IR phosphorylation (activation) was not profoundly different among the groups, suggesting that insulin signaling likely did not contribute to the observed effects. Furthermore, blockade of AT_1 and activation of GLP-1r (via exenatide) independently did not have profound effects on $U_{Na}V$, despite having decreased $U_{Agt}V$ (an index of intrarenal Ang II activity) [49], suggesting that intrarenal Ang II and/or AT_1 activation likely did not alter NHE3 activity. However, $U_{Na}V$ was increased in the Combo group, suggesting that AT_1 blockade and GLP-1r activation synergistically altered NHE3 activity to contribute to the natriuresis.

Along with increased $U_{Na}V$, U_KV was increased in the Combo group compared to that in the OLETF group. Nevertheless, the urinary $Na^+:K^+$ ratio was also increased in this group, suggesting that tubular K^+ secretion may have been blunted. The decrease in protein expression of glycosylated ROMK in this group may contribute to the blunted secretion of tubular K^+. Collectively, these results suggest that AT_1 blockade in conjunction with GLP-1r activation increases $U_{Na}V$ and decreases U_KV and that the natriuretic effect may be aldosterone independent. Nevertheless, the decrease in U_KV may be aldosterone dependent through downregulation of the protein expression of glycosylated ROMK.

Obesity inappropriately activates RAAS, increasing circulating levels of angiotensinogen, renin, angiotensin-converting enzyme, and aldosterone [50]. In the present study, we evaluated $U_{Agt}V$ as an index of intrarenal Ang II

activity [49] and $U_{aldo}V$ to better define the effects of GLP-1r activation and AT_1 inhibition on aldosterone, as there are circadian variations of plasma aldosterone [51]. To this end, obese OLETF rats were characterized by greater $U_{Agt}V$ and $U_{aldo}V$ compared with LETO rats. Notwithstanding, chronic GLP-1r activation or AT_1 blockade reduced $U_{Agt}V$ and their combination had no additional benefits, suggesting a common mediator responsible for this beneficial effect. In support of this view, GLP-1r activation reduces plasma Ang II levels in patients with T2DM [24] and protects against Ang II-induced hypertension in C57BLK/6J mice [52]. Although GLP-1r activation reduced $U_{Agt}V$, it acutely increased $U_{aldo}V$, which may be secondary to the acute anorexic effects of GLP-1r activation [42]. On day 7, $U_{aldo}V$ was lower in the EXE group than in the OLETF group and returned to baseline levels soon thereafter. Furthermore, Combo treatment decreased BM and the obesity-associated elevation in $U_{aldo}V$ for the duration of the study. These results suggest that GLP-1r activation may inhibit inappropriate RAAS activation in obese insulin-resistant conditions.

In the present study, GLP-1r activation acutely increased MAP and heart rate during the dark cycle, which may have been accomplished by increasing sympathetic outflow [53, 54]. However, the effect on MAP was reversed by ARB co-treatment, suggesting that sympathetic stimulation of RAAS may mediate this effect. Nonetheless, the increase in heart rate was not reversed by ARB co-treatment; on the contrary, heart rate increased higher and for a longer duration in the Combo group, suggesting that alternative mechanisms may be responsible for this effect but are beyond the scope of this study. After the initial increase in MAP following exenatide treatment, MAP slowly decreased during the light cycle until it reached its nadir at day 11, remaining at baseline levels for the reminder of the study. Simultaneous targeting of GLP-1r activation and AT_1 blockade did not have additive effects on MAP, which is indicative of the strict regulation of arterial pressure at some minimum threshold regardless of metabolic condition. Interestingly, chronic GLP-1r activation decreased MAP only during the light cycle and this effect was independent of increased $U_{Na}V$, suggesting that GLP-1r activation may transiently regulate MAP independent of its postprandial effects and enhanced $U_{Na}V$. In support of this view, exenatide treatment improved endothelial function after an overnight fast, and this effect was reversed by the addition of a GLP-1r antagonist in patients with impaired glucose tolerance or recently diagnosed type 2 diabetes [55]. Taken together, these results suggest that in insulin-resistant conditions, chronic GLP-1r activation may decrease arterial pressure by improving endothelial function independent of increased natriuresis.

In summary, the present study demonstrates that enhancing GLP-1 and blocking AT_1 signaling produce synergistic effects on renal Na^+ and K^+ regulation, resulting in increased $U_{Na}V$ and decreased U_KV. Furthermore, GLP-1r activation alone reduced $U_{Agt}V$, and this effect was not enhanced further by AT_1 blockade. Nonetheless, co-treatment decreased $U_{aldo}V$ for the duration of the study. Despite these beneficial effects, simultaneously targeting GLP-1r activation and AT_1 blockade did not translate to additive reductions in MAP. Interestingly, these effects were independent of improvements in hyperglycemia and hyperlipidemia in OLETF rats, suggesting that the obesity-associated impairments in renal electrolyte regulation are reconcilable during the early events contributing to the development of T2DM and not profoundly impacted by elevated glucose and TGs at this stage. Nonetheless, further studies are warranted in humans to confirm these findings.

Compliance with ethical standards

References

1. Flegal KM, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in obesity among adults in the united states, 2005 to 2014. JAMA. 2016;315:2284–91.
2. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006;444:840–6.
3. Després J-P, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006;444:881–7.
4. Garrison RJ, Kannel WB, Stokes J, Castelli WP. Incidence and precursors of hypertension in young adults: The Framingham Offspring Study. Prev Med. 1987;16:235–51.
5. Færch K, Torekov SS, Vistisen D, Johansen NB, Witte DR, Jonsson A, Pedersen O, Hansen T, Lauritzen T, Sandbæk A, Holst J, Jørgensen ME. Glp-1 response to oral glucose is reduced in prediabetes, screen-detected type 2 diabetes, and obesity and influenced by sex: The Addition-pro Study. Diabetes. 2015;64:2513–25.
6. Fujiwara K, Hayashi K, Matsuda H, Kubota E. Altered pressure-natriuresis in obese zucker rats. Hypertension. 1999;33:1470–5.
7. Hall JE, Brands MW, Dixon WN, Smith MJ. Obesity-induced hypertension. Ren Funct Syst hemodynamics Hypertens. 1993;22:292–9.
8. Hall JE. The kidney, hypertension, and obesity. Hypertension. 2003;41:625–33.
9. Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. Circ Res. 2015;116:991–1006.
10. Bramlage P, Pittrow D, Wittchen H, Kirch W, Boehler S, Lehnert H, Hoefler M, Unger T, Sharma A. Hypertension in overweight

and obese primary care patients is highly prevalent and poorly controlled. Am J Hypertens. 2004;17:904–10.

11. Lloyd-Jones DM, Evans JC, Larson MG, O'Donnell CJ, Roccella EJ, Levy D. Differential control of systolic and diastolic blood pressure: factors associated with lack of blood pressure control in the community. Hypertension. 2000;36:594–9.

12. Gress TW, Nieto JF, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. N Engl J Med. 2000;342:905–12.

13. Eriksson JW, Jansson P-A, Carlberg B, Hägg A, Kurland L, Svensson MK, Ahlström H, Ström C, Lönn L, Öjbrandt K, Johansson L, Lind L. Hydrochlorothiazide, but not candesartan, aggravates insulin resistance and causes visceral and hepatic fat accumulation the mechanisms for the diabetes preventing effect of candesartan (medica) study. Hypertension. 2008;52:1030–7.

14. Tocci G, Paneni F, Palano F, Sciarretta S, Ferrucci A, Kurtz T, Mancia G, Volpe M. Angiotensin-converting enzyme inhibitors, angiotensin ii receptor blockers and diabetes: a meta-analysis of placebo-controlled clinical trials. Am J Hypertens. 2011;24:582–90.

15. McMurray JJ, Holman RR, Haffner SM, Bethel MA, Holzhauer B, Hua TA, Belenkov Y, Boolell M, Buse JB, Buckley BM, Chacra AR, Chiang F-TT, Charbonnel B, Chow C-CC, Davies MJ, Deedwania P, Diem P, Einhorn D, Fonseca V, Fulcher GR, Gaciong Z, Gaztambide S, Giles T, Horton E, Ilkova H, Jenssen T, Kahn SE, Krum H, Laakso M, Leiter LA, Levitt NS, Mareev V, Martinez F, Masson C, Mazzone T, Meaney E, Nesto R, Pan C, Prager R, Raptis SA, Rutten GE, Sandstroem H, Schaper F, Scheen A, Schmitz O, Sinay I, Soska V, Stender S, Tamás G, Tognoni G, Tuomilehto J, Villamil AS, Vozár J, Califf RM. Effect of valsartan on the incidence of diabetes and cardiovascular events. N Engl J Med. 2010;362:1477–90.

16. Bosch J, Yusuf S, Gerstein HC, Pogue J, Sheridan P, Dagenais G, Diaz R, Avezum A, Lanas F, Probstfield J, Fodor G, Holman RR. Effect of ramipril on the incidence of diabetes. N Engl J Med. 2006;355:1551–62.

17. Meloni AR, DeYoung MB, Lowe C, Parkes DG. Glp-1 receptor activated insulin secretion from pancreatic β-cells: mechanism and glucose dependence. Diabetes, Obes Metab. 2013;15:15–27.

18. Dunphy JL, Taylor RG, Fuller PJ. Tissue distribution of rat glucagon receptor and glp-1 receptor gene expression. Mol Cell Endocrinol. 1998;141:179–86.

19. Richards P, Parker HE, Adriaenssens AE, Hodgson JM, Cork SC, Trapp S, Gribble FM, Reimann F. Identification and characterization of glp-1 receptor–expressing cells using a new transgenic mouse model. Diabetes. 2014;63:1224–33.

20. Sufiun A, Rafiq K, Fujisawa Y, Rahman A, Mori H, Nakano D, Kobori H, Ohmori K, Masaki T, Kohno M, Nishiyama A. Effect of dipeptidyl peptidase-4 inhibition on circadian blood pressure during the development of salt-dependent hypertension in rats. Hypertens Res. 2015;38:237–43.

21. Rieg T, Gerasimova M, Murray F, Masuda T, Tang T, Rose M, Drucker DJ, Vallon V. Natriuretic effect by exendin-4, but not the dpp-4 inhibitor alogliptin, is mediated via the glp-1 receptor and preserved in obese type 2 diabetic mice. Am J Physiol Ren Physiol. 2012;303:F963–71.

22. Muskiet MHA, Tonneijck L, Smits MM, Kramer MHH, Diamant M, Joles JA, van Raalte DH. Acute renal haemodynamic effects of glucagon-like peptide-1 receptor agonist exenatide in healthy overweight men. Diabetes, Obes Metab. 2016;18:178–85.

23. Tonneijck L, Smits MM, Muskiet MH, Hoekstra T, Kramer MHH, Danser AH, Diamant M, Joles JA, van Raalte DHH. Acute renal effects of the glp-1 receptor agonist exenatide in overweight type 2 diabetes patients: a randomised, double-blind, placebo-controlled trial. Diabetologia. 2016;59:1412–21.

24. Skov J, Pedersen M, Holst JJ, Madsen B, Goetze JP, Rittig S, Jonassen T, Frøkiaer J, Dejgaard A, Christiansen JS. Short-term

25. Moroi M, Kubota T. Diuretic and natriuretic effects of dipeptidyl peptidase-4 inhibitor teneligliptin: the contribution of glucagon-like peptide-1. J Cardiovasc Pharmacol. 2015;66:159–64.

26. Zhou X, Huang C-h, Lao J, Pocai A, Forrest G, Price O, Roy S, Kelley DE, Sullivan KA, Forrest MJ. Acute hemodynamic and renal effects of glucagon-like peptide 1 analog and dipeptidyl peptidase-4 inhibitor in rats. Cardiovasc Diabetol. 2015;14:1–10.

27. Kim M, Platt MJ, Shibasaki T, Quaggin SE, Backx PH, Seino S, Simpson JA, Drucker DJ. Glp-1 receptor activation and epac2 link atrial natriuretic peptide secretion to control of blood pressure. Nat Med. 2013;19:567–75.

28. Zavattaro M, Caputo M, Samà M, Mele C, Chasseur L, Marzullo P, Pagano L, Mauri M, Ponziani M, Aimaretti G, Prodam F. One-year treatment with liraglutide improved renal function in patients with type 2 diabetes: a pilot prospective study. Endocrine. 2015;50:620–6.

29. Scholten BJ, Lajer M, Goetze JP, Persson F, Rossing P. Time course and mechanisms of the anti-hypertensive and renal effects of liraglutide treatment. Diabet Med. 2015;32:343–52.

30. Moran TH, Bi S. Hyperphagia and obesity in oletf rats lacking cck-1 receptors. Philos Trans R Soc Lond B Biol Sci. 2006;361:1211–8.

31. Ortiz RM, Graciano ML, Mullins JJ, Mitchell KD. Aldosterone receptor antagonism alleviates proteinuria, but not malignant hypertension, in cyp1a1-ren2 transgenic rats. Am J Physiol Ren Physiol. 2007;293:F1584–591.

32. Ortiz RM, Graciano ML, Seth D, Awayda MS, Navar LG. Aldosterone receptor antagonism exacerbates intrarenal angiotensin ii augmentation in ang ii-dependent hypertension. Am J Physiol Ren Physiol. 2007;293:F139–47.

33. Awayda MS, Awayda KL, Pochynyuk O, Bugaj V, Stockand JD, Ortiz RM. Acute cholesterol-induced anti-natriuretic effects: role of epithelial na+ channel activity, protein levels, and processing. J Biol Chem. 2011;286:1683–95.

34. Cacho J, Sevillano J, de Castro J, Herrera E, Ramos MP. Validation of simple indexes to assess insulin sensitivity during pregnancy in wistar and sprague-dawley rats. Am J Physiol Endocrinol Metab. 2008;295:E1269–76.

35. Ortiz RM, Wang TJ, Wade CE. Urea and osmotic excretion in rats exposed to chronic centrifugation. Aviat Space Environ Med. 1999;70:1089–95.

36. Ortiz RM, Wang T, Wade CE. Influence of centrifugation and hindlimb suspension on testosterone and corticosterone. Aviat Space Environ Med. 1999;70:499–504.

37. Crajoinas RO, Oricchio FT, Pessoa TD, Pacheco BPM, Lessa LMA, Malnic G, Girardi ACC. Mechanisms mediating the diuretic and natriuretic actions of the incretin hormone glucagon-like peptide-1. Am J Physiol Ren Physiol. 2011;301:F355–63.

38. Carraro-Lacroix LR, Malnic G, Girardi ACC. Regulation of na+/h+ exchanger nhe3 by glucagon-like peptide 1 receptor agonist exendin-4 in renal proximal tubule cells. Am J Physiol Ren Physiol. 2009;297:F1647–55.

39. Skov J, Dejgaard A, Frøkiær J, Holst J, Jonassen T, Rittig S, Christiansen J. Glucagon-like peptide-1 (glp-1): effect on kidney hemodynamics and renin-angiotensin-aldosterone system in healthy men. J Clin Endocrinol Metab. 2013;98:E664–71.

40. Holst J, Vilsbøll T, Deacon CF. The incretin system and its role in type 2 diabetes mellitus. Mol Cell Endocrinol. 2009;297:127–36.

41. Goud A, Zhong J, Peters M, Brook RD, Rajagopalan S. Glp-1 agonists and blood pressure: a review of the evidence. Curr Hypertens Rep. 2016;18:16.

42. Barrera JG, D'Alessio DA, Drucker DJ, Woods SC, Seeley RJ. Differences in the central anorectic effects of glucagon-like peptide-1 and exendin-4 in rats. Diabetes. 2009;58:2820–7.

43. Takeshige Y, Fujisawa Y, Rahman A, Kittikulsuth W, Nakano D, Mori H, Masaki T, Ohmori K, Kohno M, Ogata H, Nishiyama A. A sodium-glucose co-transporter 2 inhibitor empagliflozin prevents abnormality of circadian rhythm of blood pressure in salt-treated obese rats. Hypertens Res. 2016;39:415–22.

44. Rodriguez R, Viscarra JA, Minas JN, Nakano D, Nishiyama A, Ortiz RM. Angiotensin receptor blockade increases pancreatic insulin secretion and decreases glucose intolerance during glucose supplementation in a model of metabolic syndrome. Endocrinology. 2012;153:1684–95.

45. Nishiyama A, Nakagawa T, Kobori H, Nagai Y, Okada N, Konishi Y, Morikawa T, Okumura M, Meda I, Kiyomoto H, Hosomi N, Mori T, Ito S, Imanishi M. Strict angiotensin blockade prevents the augmentation of intrarenal angiotensin ii and podocyte abnormalities in type 2 diabetic rats with microalbuminuria. J Hypertens. 2008;26:1849–59.

46. Fuster DG, Bobulescu IA, Zhang J, Wade J, Moe OW. Characterization of the regulation of renal na+/h+ exchanger nhe3 by insulin. Am J Physiol Ren Physiol. 2007;292:F577–85.

47. Li XC, Shull GE, Miguel-Qin E, Zhuo JL. Role of the na+/h+ exchanger 3 in angiotensin ii-induced hypertension. Physiol Genom. 2015;47:479–87.

48. Farah LX, Valentini V, Pessoa TD, Malnic G, McDonough AA, Girardi ACC. The physiological role of glucagon-like peptide-1 in the regulation of renal function. Am J Physiol Ren Physiol. 2015;310:F123–7.

49. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. Pharmacol Rev. 2007;59:251–87.

50. Engeli S, Böhnke J, Gorzelniak K, Janke J, Schling P, Bader M, Luft FC, Sharma AM. Weight loss and the renin-angiotensin-aldosterone system. Hypertension. 2005;45:356–62.

51. Lamarre-Cliche M, Jd Champlain, Lacourcière Y, Poirier L, Karas M, Larochelle P. . Effects of circadian rhythms, posture, and medication on renin-aldosterone interrelations in essential hypertensives*. Am J Hypertens. 2005;18:56–64.

52. Hirata K, Kume S, Araki S-i, Sakaguchi M, Chin-Kanasaki M, Isshiki K, Sugimoto T, Nishiyama A, Koya D, Haneda M, Kashiwagi A, Uzu T. Exendin-4 has an anti-hypertensive effect in salt-sensitive mice model. Biochem Biophys Res Commun. 2009;380:44–9.

53. Barragán JM, Rodríguez RE, Eng J, Blázquez E. Interactions of exendin-(9–39) with the effects of glucagon-like peptide-1-(7–36) amide and of exendin-4 on arterial blood pressure and heart rate in rats. Regul Pept. 1996;67:63–68.

54. Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, Elmquist JK. Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. J Clin Invest. 2002;110:43–52.

55. Koska J, Sands M, Burciu C, D'Souza KM, Raravikar K, Liu J, Truran S, Franco DA, Schwartz EA, Schwenke DC, D'Alessio D, Migrino RQ, Reaven PD. Exenatide protects against glucose- and lipid-induced endothelial dysfunction: evidence for direct vasodilation effect of glp-1 receptor agonists in humans. Diabetes. 2015;64:2624–35.