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RESEARCH ARTICLE

Ablation of TRPV1 Elevates Nocturnal Blood Pressure in Western Diet-fed Mice

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Abstract: ***Background*:** This study tested the hypothesis that genetically ablation of transient receptor potential vanilloid type 1 (TRPV1) exacerbates impairment of baroreflex in mice fed a western diet (WD) and leads to distinct diurnal and nocturnal blood pressure patterns.

***Methods*:** TRPV1 gene knockout (TRPV1$^{-/-}$) and wild-type (WT) mice were given a WD or normal diet (CON) for 4 months.

***Results*:** Capsaicin, a selective TRPV1 agonist, increased ipsilateral afferent renal nerve activity in WT but not TRPV1$^{-/-}$ mice. The sensitivity of renal sympathetic nerve activity and heart rate responses to baroreflex were reduced in TRPV1$^{-/-}$-CON and WT-WD and further decreased in TRPV1$^{-/-}$-WD compared to the WT-CON group. Urinary norepinephrine and serum insulin and leptin at day and night were increased in WT-WD and TRPV1$^{-/-}$-WD, with further elevation at night in TRPV1$^{-/-}$-WD. WD intake increased leptin, IL-6, and TNF-α in adipose tissue, and TNF-α antagonist III, R-7050, decreased leptin in TRPV1$^{-/-}$-WD. The urinary albumin level was higher in TRPV1$^{-/-}$-WD than WT-WD. Blood pressure was not different during daytime among all groups, but increased at night in the TRPV1$^{-/-}$-WD group compared with other groups.

***Conclusions*:** TRPV1 ablation leads to elevated nocturnal but not diurnal blood pressure, which is probably attributed to further enhancement of sympathetic drives at night.

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1. INTRODUCTION

Western diet (WD) intake leads to obesity which is linked to hypertension, diabetes, and renal dysfunction [1]. Recent studies have demonstrated that obesity is associated with increased sympathetic nerve activity (SNA) in the skeletal muscle and kidney [2, 3]. Increased efferent renal SNA (RSNA) in obesity is an important mechanism that triggers hypertension [4]; this concept is supported by the studies showing that bilateral renal denervation before weight gain prevented the development of obesity-related hypertension and attenuated sodium retention [5]. Arterial baroreflex (BR) is the primary feedback mechanism that regulates systemic blood pressure (BP) and cardiovascular homeostasis. Baroreflex sensitivity (BRS), which is attenuated whenever sympathetic activity is enhanced [6], is severely impaired in obese subjects [7]. Elevated SNA can occur in the absence of an elevated BP and is associated with a blunted BR control of heart rate (HR) [8]. Impairment of arterial BR control of efferent RSNA may be an important mechanism triggering cardiovascular dysfunction in obesity [9]. Chronic BR activation suppresses global sympathetic activity, and inhibition of RSNA has beneficial effects in obese hypertension [10]. Patients with impaired BR function have increased cardiovascular morbidity and mortality [11].

Function of sensory nerves, especially transient receptor potential vanilloid 1 (TRPV1)-positive nerves, is impaired in obesity, diabetes, and aging. Obesity decreased capsaicin-induced sensory neurotransmitter release [12]. TRPV1 is a non-selective cation channel, which is mainly expressed in primary sensory neurons and sensory C- and Aδ-fibers [13] and activated by noxious heat, acidic pH, capsaicin, and lipid metabolites [14, 15]. TRPV1 is expressed throughout the baroreceptive afferent pathway, which includes the nerve terminals innervating the ascending aorta, nodose ganglion neurons, and other afferent nerves. Moreover, TRPV1 ablation impaired the baroreflex control of efferent RSNA and HR, leading to dysregulation of BP [16].

TRPV1 plays an important role in regulating renal function. Ablation of TRPV1 exaggerated renal dysfunction and tissue damage in a mouse model of deoxycorticosterone acetate (DOCA)-salt hypertension [17]. Therefore, TRPV1

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may participate in the regulation of efferent RSNA and play a key role in the development of hypertension. TRPV1-positive sensory nerves are widely distributed in the kidney, especially in the renal pelvis [18]. TRPV1 activation leads to release of calcitonin gene-related peptide (CGRP) and substance P (SP), which can regulate SNA and BRS. CGRP facilitated BR-induced increases in HR and efferent RSNA [19], and SNA was significantly elevated in CGRP gene knockout mice [20]. Central SP increased BP, HR, and splanchnic nerve activity [21]. However, administration of SP or activation of TRPV1 with capsaicin in the unilateral renal pelvis led to increases in ipsilateral afferent renal nerve activity (ARNA) and elicited an inhibitory renorenal reflex, resulting in a contralateral natriuresis [22-24] and a long-lasting depression of efferent RSNA [25]. Increased ARNA would provide an important contribution to the maintenance of low efferent RSNA, which is essential in preventing renal sodium retention and in the regulation of arterial pressure [24, 25].

Although the results of previous studies indicated that TRPV1 is expressed in baroreceptive nerve endings and in renal pelvis, the role of TRPV1 in mediating the development of BRS impairment and hypertension in obesity is unknown. Thus, the purpose of this study was to test the hypothesis that activation of TRPV1 in the renal pelvis leads to an increase in ipsilateral ARNA and a decrease in contralateral RSNA, and ablation of TRPV1 leads to the increase in RSNA, which exacerbates impairment of BR and ARNA in mice fed a western diet (WD) and elevation of BP.

2. MATERIALS AND METHODS

2.1. Animals and Diet

All experimental procedures were approved by the Animal Care and Use Committee of Michigan State University and conform to NIH guidelines. The male TRPV1 gene knockout ($TRPV1^{-/-}$) strain ($B6.129S4\text{-}TRPV1^{tm1Jul}$) and C57BL/6J wild-type (WT) strain (from Jackson Laboratory, Bar Harbor, Maine) were used. Three-week-old mice were divided to receive normal pellet diet (con; 8664, Harlan Teklad) or western diet (WD; 42% kcal from fat; 88137, Harlan Teklad) for 4 months, during which period mice had free access to tap water. Mice were maintained in a normal light/dark cycle.

2.2. Telemetry BP Assay

Mean arterial pressure (MAP) and HR were determined using a telemetry system (Data Sciences International, St. Paul, MN) according to the manufacturer's instruction. In brief, mice (14-week-old) were anesthetized with ketamine and xylazine (80 and 5 mg/kg i.p., respectively), then a transmitter catheter was implanted into the left carotid artery. MAP was measured at 7 days after surgery.

2.3. Intraperitoneal Glucose Tolerance Testing (IPGTT)

Experimental mice were fasted for 6-7 h, then glucose (2 g/kg body weight) was administered (i.p.) to the conscious mice. Tail vein blood was sampled for glucose determination using an Accu-Chek glucose meter (Roche Diagnostics) at 0, 30, 60, 90, and 120 min after glucose administration. The areas under the curves (AUC) for glucose concentrations were calculated according to the trapezoidal rule. Glucose tolerance was defined as AUC versus time curve calculated with the trapezoidal rule. Tail vein blood was also collected before IPGTT, and serum was immediately separated and stored at -80°C for insulin and leptin determination. Insulin and leptin levels were measured using a commercial kit (Crystal Chem Inc., Downers Grove, IL, USA).

2.4. Renal Pelvic Catheter [18, 26]

Anesthesia was induced with ketamine and xylazine (80 and 5 mg/kg, respectively, i.p.). Polyethylene catheters (PE10) were placed in the left jugular vein for drug infusion. Body temperature was maintained at 37 °C with an isothermal pad. Anesthesia was maintained with an intravenous infusion of ketamine and xylazine (40 and 2.5 mg/kg/h, respectively), which was initiated after 45 min of initial induction. Two MD-2000 microdialysis tubes (ID 0.18/OD 0.22 mm; BASi) were bonded together and placed inside the left ureter *via* a midline incision. One of the tubes, of which the tip extended 1 to 2 mm into the renal pelvis compared with the other, was used for drug perfusion, whereas the other was used for urine draining. The perfusion was performed at a rate of 10 μL/min, at which time the pelvis pressure did not change, and the drugs were perfused into the renal pelvis for 3 min. Mice were given 1 μM capsaicin into the renal pelvis.

2.5. Recording of ARNA and efferent RSNA [18, 26]

Renal nerves were isolated at the angle between the abdominal aorta and the renal artery *via* a left flank incision. The nerves were placed on the bipolar stainless-steel electrode to record multifiber nerve activity. The electrode was connected to a high-impedance probe (HIP-511, Grass Instruments). The signals were amplified x20,000, filtered with a high-frequency cutoff at 1,000Hz and a low-frequency cutoff at 100Hz by a Grass model P511 AC Amplifier and recorded by Gould 2400s recorder (Gould Instrument System, Valley View, Ohio, USA). After the renal nerve activity was verified using its pulse synchronous rhythmicity with the heartbeat, the nerves were sectioned and the efferent RSNA was recorded from a proximal renal nerve branch and ARNA from the distal cut end of a renal nerve. The electrode was fixed to the renal nerve with Kwik-Cast & Kwik-Sil (World Precision Instruments, Sarasota, Florida, USA). The effect of renal pelvis infused capsaicin on ipsilateral ARNA and on contralateral RSNA were evaluated in separate experiments. The experiment started after the placement of nerve electrodes and physiological stabilization for 60 min, MAP, HR and RSNA were recorded for 5 min. Capsaicin (1 μM) was perfused into the renal pelvis in 3-min periods, and the recovery value of renal nerve activity was recorded 10 min after the treatment. The postmortem renal nerve activity recorded as the background of renal nerve activity was subtracted from all of the values. Renal nerve activity was expressed in percentage of its baseline value.

2.6. Determination of Baroreflex Function [16]

The baroreflex function was evaluated by measuring the reflex changes in RSNA and HR in response to decreases

and increases in MAP induced by intravenous infusion of 50 μg/mL sodium nitroprusside (SNP, Hospira, Inc.) and 125 μg/mL phenylephrine (PE, Neo-synephrine, Hospira, Inc.), respectively. SNP and PE were administered *via* the jugular vein at an initial rate of 5 μL/min, increased by 5 μL/min every 30 s. The PE and SNP infusions were done separately with one drug administered after the BP response to the other drug had returned to baseline level, and the order of drugs was administered randomly. Infusions were stopped if MAP reached a minimum of 60 mmHg or a maximum of 140 mmHg. RSNA was defined as the recorded nerve activity after subtraction of the background noise. In each animal, absolute RSNA at the start of the experiment was defined as 100%. The postmortem renal nerve activity recorded as the background of renal nerve activity was subtracted from all of the values. Renal nerve activity was expressed in percentage of its baseline value. BR modulation of RSNA and HR was estimated by calculating (1) the percent change in integrated activity, and (2) the change in HR in relation to the change in mean BP induced by PE and SNP.

2.7. Adipose Tissue Histopathological Analysis and Incubation

Hematoxylin and eosin (HE) staining of adipose tissue was routinely performed. Epididymal fat pads were dissected into proximal and distal segments for incubation. The adipose tissues were sectioned in cold Ringer bicarbonate (KRB, pH 7.4, in mM): 120 NaCl, 4.75 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 5.5 glucose, 20 HEPES; with 1% bovine serum albumin, penicillin (100 U/ml), and streptomycin (100 μg/ml). Each treatment group was run in duplicate and consisted of one proximal and one distal piece of adipose tissue. The samples obtained from the same mouse were incubated in the presence or in the absence of TNF-α antagonist III, R-7050 (10 μM). Thus, drug-treated tissue was always compared with untreated tissue from the same animal. Adipose samples were balanced for 60 min, then incubated in fresh KRB in 95% air and 5% CO_2 for 3 h at 37°C with drug treatment. The incubation media were collected and saved at −80°C for leptin, IL-6, TNF-α levels measurement by ELISA.

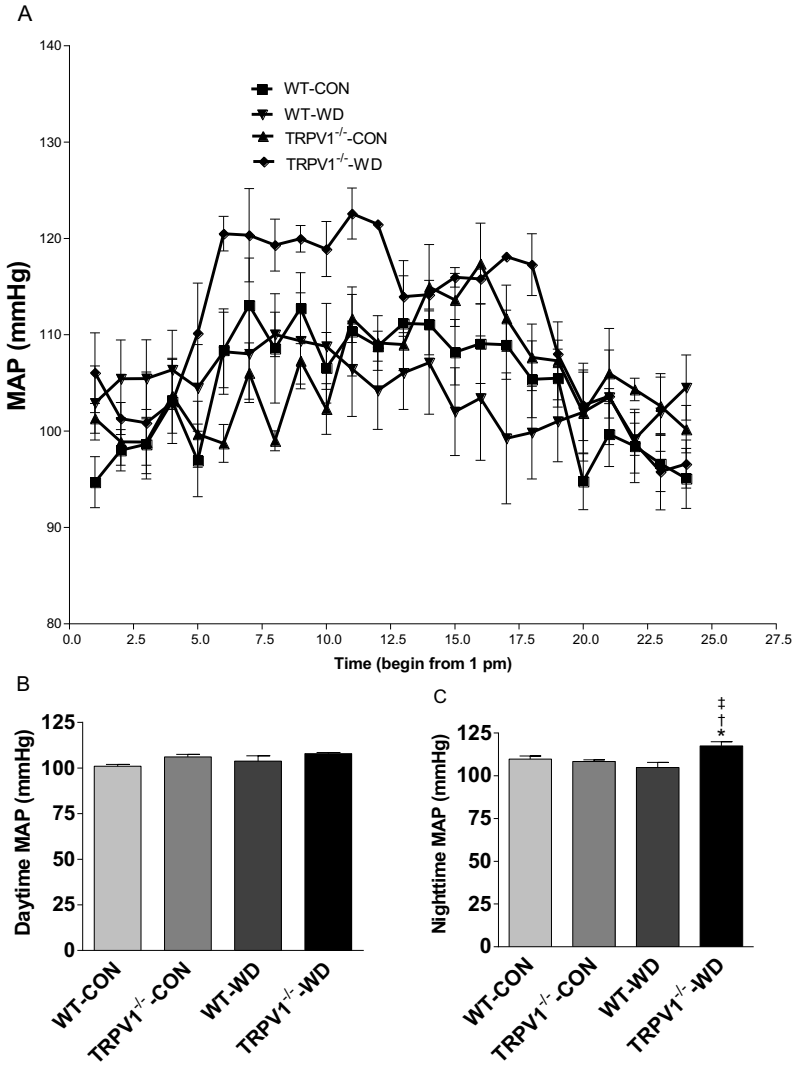


Fig. (1). Panel (**A**) shows the long-term telemetric recording of blood pressure in wild-type (WT) and transient receptor potential vanilloid 1 (TRPV1) gene knockout ($TRPV1^{-/-}$) mice with normal diet (CON) and western diet (WD). Bar graphs show mean arterial pressure (MAP) during daytime (from 6 am to 6 pm) (**B**) and nighttime (from 6pm to 6 am) (**C**) in WT and $TRPV1^{-/-}$ mice, with CON or WD fed. Values are mean ± SEM; n=5; *P<0.05 *vs.* WT-CON; †P<0.05 *vs.* $TRPV1^{-/-}$-CON; ‡P<0.05 *vs.* WT-WD.

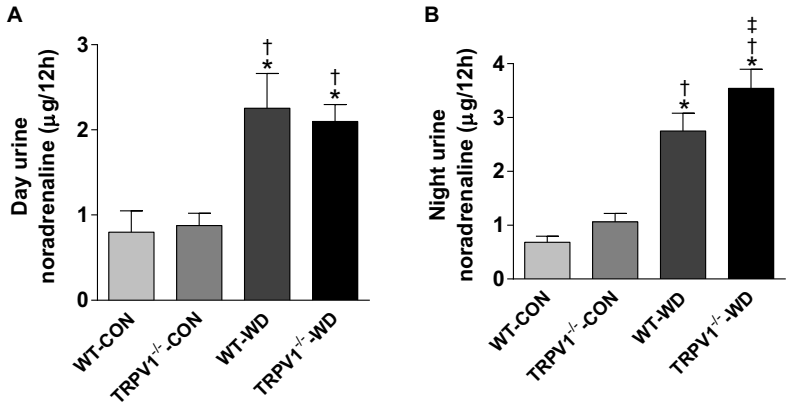


Fig. (2). Effect of WD on urinary norepinephrine at daytime (**A**) and nighttime (**B**) in WT and TRPV1$^{-/-}$ mice. Values are mean ± SEM; n=6; *P<0.05 *vs.* WT-CON; †P<0.05 *vs.* TRPV1$^{-/-}$-CON; ‡P<0.05 *vs.* WT-WD.

2.8. Plasma and Urine Analysis

At the end of the 16-wk treatment, mice were placed in mouse metabolic cages for 24 h urine collection. Urinary albumin was measured with an enzyme-linked immunosorbent assay kit (Exocell, Philadelphia, PA, USA). Urinary 12(s)-HETE was measured with a 12(s)-HETE EIA kit (Enzo Inc, Farmingdale, NY, USA). Plasma creatinine and urea concentrations were assayed with kits (Biovision, Milpitas, California). Endogenous creatinine clearance is a sensitive and accurate method in assessing glomerular filtration rate (GFR). Plasma and urine creatinine is determined using assay kit (Biovision) and calculation of creatinine clearance: GFR=U[Cr]∗[Volume]/ P[Cr]∗[Time] [27].

2.9. Statistical Analysis

All values are expressed as mean ± SEM. Differences among groups were compared by one-way ANOVA followed by Tukey-Kramer multiple comparison test. Differences between two groups were analyzed by *t* test. The results were considered statistically significant at P<0.05.

3. RESULTS

3.1. WD Intake Elevated Nocturnal BP in TRPV1$^{-/-}$ Mice

A 16-week WD feeding resulted in a significant increase in body weight in both strains (WT: 44.2 ± 5.8 g and TRPV$^{-/-}$: 46.4 ± 3.1 g) compared with mice fed standard chow (WT: 28.6 ± 3.4 g and TRPV$^{-/-}$: 30.0 ± 1.6 g). WT and TRPV1$^{-/-}$ mice did not show differences in body weight when fed the same diet. MAP detected by telemetry was not different at the daytime between all groups but increased at the nighttime in TRPV1$^{-/-}$-WD compared with WT-CON, TRPV1$^{-/-}$-CON, and WT-WD (P<0.05, Fig. **1**).

3.2. WD Intake Increased RSNA, Decreased Ipsilateral ARNA and Contralateral RSNA Response to Capsaicin Stimulation

Compared with the CON group, urine NE was significantly increased in WD groups, a further increase was observed in the TRPV1$^{-/-}$-WD group at the nighttime but not in daytime (P<0.05, Fig. **2**). These results suggested that WD-induced MAP elevation in TRPV1$^{-/-}$ mice at night may be related to increased RSNA activity at the nighttime. To examine the effect of WD intake on ARNA, and whether ARNA is involved in the increased RSNA in WD fed mice, capsaicin, a TRPV1 agonist, was perfused into the renal pelvis which can stimulate ARNA in WT mice but not in TRPV1$^{-/-}$ mice. Ipsilateral ARNA was increased by capsaicin perfusion, which was suppressed by WD intake (WT-CON: 216.8 ± 18.1%, WT-WD: 142.2 ± 18.1%, P<0.05, Fig. **3**) in WT mice. Ipsilateral ARNA was not altered when capsaicin was perfused into the renal pelvis in TRPV1$^{-/-}$ mice. In other experimental mice groups, renal pelvis perfused capsaicin induced contralateral RSNA suppression with less suppression in WT-WD mice (P<0.05, Fig. **3**). There is no relevant change in BP, HR, and respiratory rate upon pelvis perfusion of capsaicin (data not shown). These results suggest that WD intake decreased ipsilateral ARNA and suppressed contralateral RSNA in WT mice, which may be related to increase RSNA activity in WD fed mice.

3.3. WD Intake Decreased BRS with A Further Decrease in TRPV1$^{-/-}$ Mice

To further clarify whether BRS is changed in WD fed mice in both strains. Baseline MAP and HR of anaesthetized mice did not differ among WT-CON (95 ± 9 mmHg, 417± 33 bpm), WT-WD (99 ± 8 mmHg, 429 ± 43 bpm), TRPV1$^{-/-}$-CON (99 ± 7 mmHg, 422 ± 30 bpm), and TRPV1$^{-/-}$-WD (95 ± 9 mmHg, 428 ± 37 bpm) groups. BRS was evaluated by the average index, *i.e.* related changes in HR and RSNA induced by PE or SNP. BRS was reduced to a similar degree in TRPV1$^{-/-}$-CON and WT-WD and further decreased in TRPV1$^{-/-}$-WD (Fig. **4**, P<0.05). These results suggest that WD intake and TRPV1 ablation together may lead to lower BRS and higher RSNA activity and increase in BP at night in TRPV1$^{-/-}$-WD mice.

3.4. WD Intake Impaired Glucose Tolerance, Increased Serum Insulin and Leptin Levels, with Further Increased Leptin Level at Night in TRPV1$^{-/-}$-WD Mice

Fasting blood glucose, insulin, and leptin concentrations were higher in mice fed WD compared with CON in both strains, with further increased leptin level at night in TRPV1$^{-/-}$-WD mice (Figs. **5** and **6**, P<0.05). There was no difference in the fasting blood glucose levels and serum insulin levels

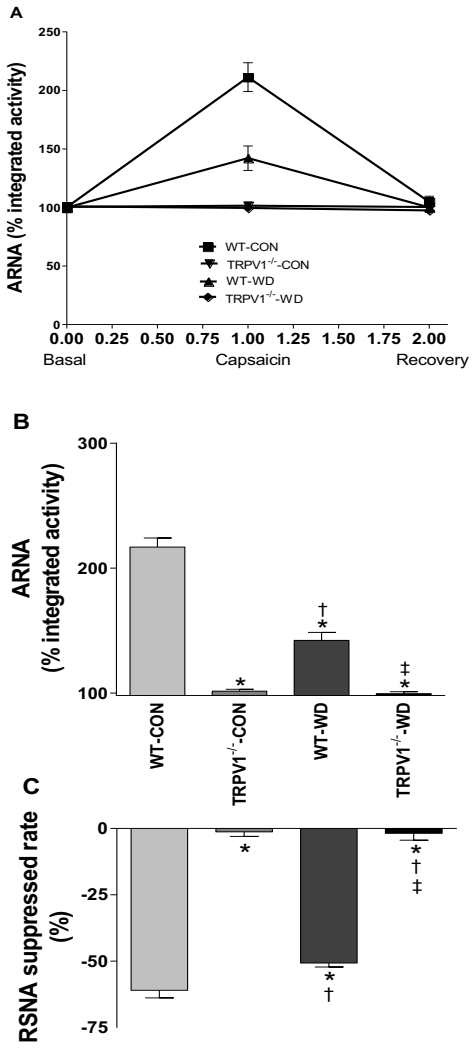


Fig. (3). Effects of capsaicin, a specific TRPV1 agonist, on ipsilateral afferent renal nerve activity (ARNA) or contralateral renal sympathetic nerve activity (RSNA). (**A**) Capsaicin (10^{-6} M) perfused into the left renal pelvis in WT and TRPV1$^{-/-}$ fed with CON and WD. Quantification of ARNA (**B**) and RSNA (**C**). Values are mean ± SEM; n=5-8; *P<0.05 *vs.* WT-CON; †P<0.05 *vs.* TRPV1$^{-/-}$-CON; ‡P<0.05 *vs.* WT-WD.

between mice fed CON and WD. Glucose tolerance were significantly higher in both strains with WD intake (Fig. **5**, P<0.05), showing AUC of glucose tolerance test was a significant increase in both strains with WD intake versus CON intake mice (Fig. **5**, P<0.05). Moreover, glucose tolerance was more impaired in TRPV1$^{-/-}$ mice than WT mice in both WD intake or CON intake (Fig. **5**, P<0.05).

3.5. WD Intake Increased Inflammatory Cells in Adipose Tissue with Increased Leptin, IL-6 and TNF-a Released from the Adipose Tissue

Leptin from adipose tissue can reflect inflammation and status of lipid metabolism. To further clarify whether inflammation is increased in adipose tissue with WD fed mice, adipose tissue HE staining was routinely performed**,** which showed that WD intake qualitatively increased adipose cell sizes with the infiltration of immune cells in adipose tissue in

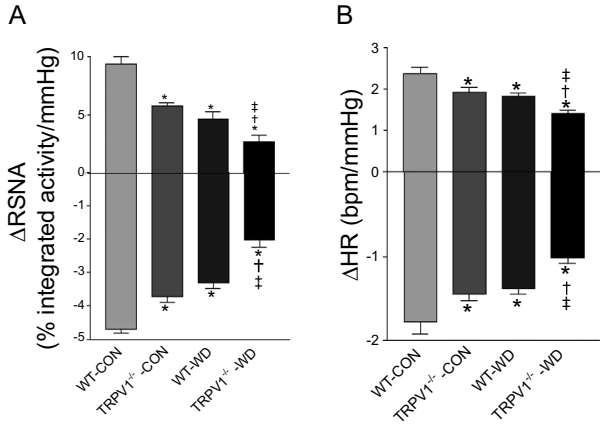
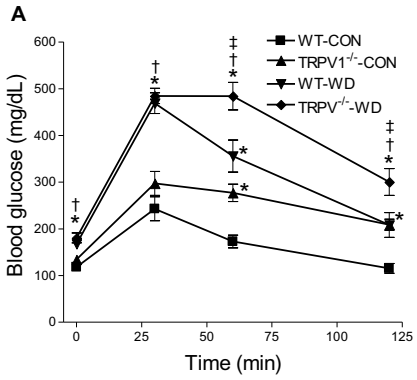


Fig. (4). Effects of WD on the baroreflex function. Reflex changes in RSNA (**A**) and heart rate (HR) (**B**) in response to blood pressure changes induced by IV injection of phenylephrine (PE, 125 μg/mL) or sodium nitroprusside (SNP, 50 μg/mL). Values are mean±SEM; n=5-8; *P<0.05 *vs.* WT-CON; †P<0.05 *vs.* TRPV1$^{-/-}$-CON; ‡P<0.05 *vs.* WT-WD.



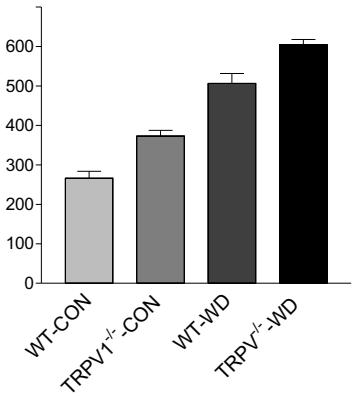


Fig. (5). Effects of WD on the fasting glucose level and glucose tolerance test. (**A**) Fasting glucose levels and glucose tolerance test were measured in WT and TRPV1$^{-/-}$ mice before and after i.p. glucose (2g/kg body weight). (**B**) Mean area under the curve (AUC) of glucose was calculated. Values are mean±SEM; n=5; *P<0.05 *vs.* WT-CON; †P<0.05 *vs.* TRPV1$^{-/-}$-CON; ‡P<0.05 *vs.* WT-WD.

both strains (Fig. **7**). This is consistent with higher leptin, and IL-6 concentrations in adipose tissues in mice fed WD

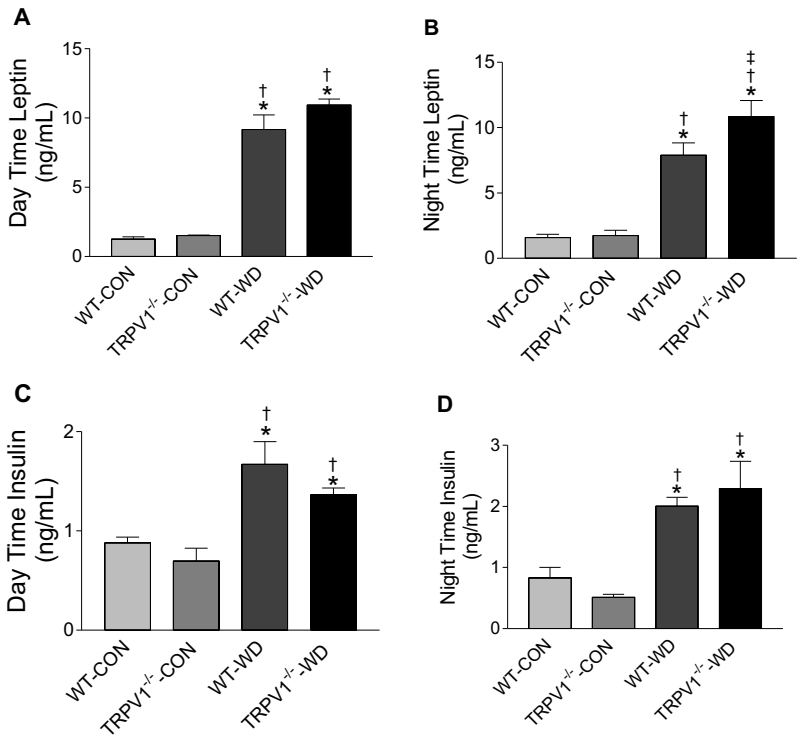


Fig. (6). Effects of WD on the fasting insulin and leptin level. Daytime collected the serum at 2pm, and nighttime collected the serum at 12am. Values are mean±SEM; n=5; *P<0.05 *vs.* WT-CON; †P<0.05 *vs.* TRPV1$^{-/-}$-CON; ‡P<0.05 *vs.* WT-WD.

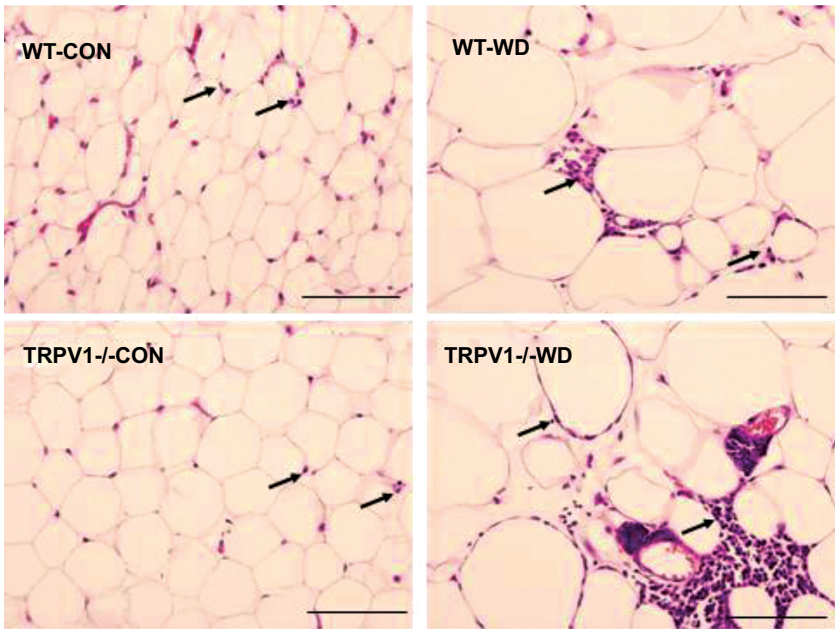


Fig. (7). Adipose tissue hematoxylin and eosin (HE) staining. H&E-stained sections are shown from the epididymal adipose tissue of WT or TRPV1$^{-/-}$ mice with CON or WD fed mice. The arrows indicate the infiltration of immune cells in adipose tissue. Scale bar=100μm.

compared with fed CON in both strains, with further elevated levels in TRPV1$^{-/-}$-WD (Fig. **8**, P<0.05). Moreover, adipose tissues TNF-α level is higher in TRPV1$^{-/-}$ mice fed WD compared with WT mice fed WD and fed CON in both strains (Fig. **8**, P<0.05). The adipose tissues obtained from the same animal were incubated in the TNF-α antagonist III, R-7050 (1 μM). The leptin level had no difference among the four groups with the presence of theTNF-α antagonist (Fig. **8**, P<0.05).

3.6. WD Intake Increased Urinary 12(s)-HETE Level, and Impaired Renal Function

Our previous study showed that 12-HETE enhanced ARNA and substance P/calcitonin gene-related peptide release but suppressed renin activity and these effects were abolished when TRPV1 was blocked. To further clarify whether 12(S)-HETE is increased with WD intake, we measured the urinary 12(S)-HETE level. Urinary 12(S)-

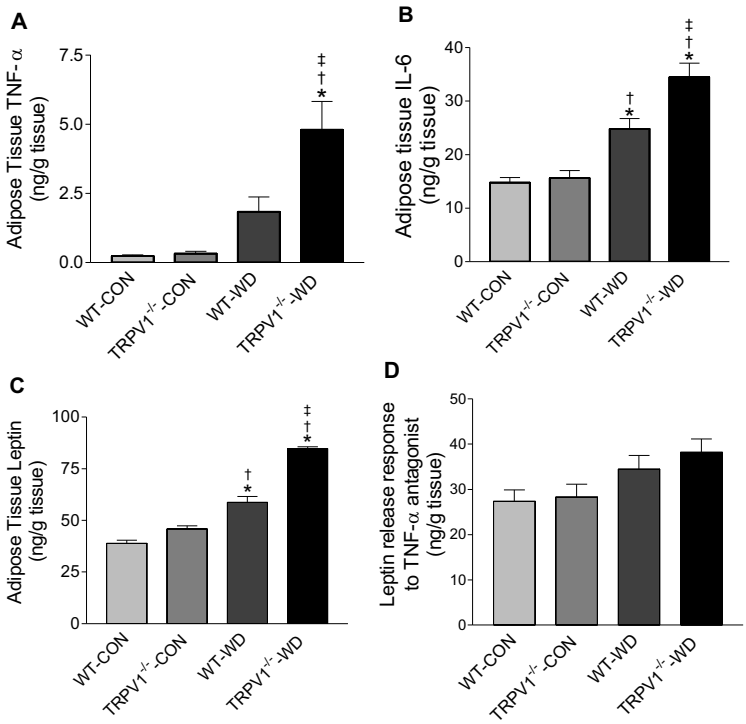


Fig. (8). Effects of Western diet (WD) on TNF-α (**A**), IL-6 (**B**), and leptin (**C**) from the adipose tissue. TNF-α antagonist III, R-7050 (10^{-6} M) decrease the leptin (**D**) released from the adipose tissue. Values are mean±SEM; n=5-8; *P<0.05 *vs.* WT-CON; †P<0.05 *vs.* TRPV1$^{-/-}$-CON; ‡P<0.05 *vs.* WT-WD.

Table 1. Urinary 12(s)-HETE level and impaired renal function.

	WT-CON	TRPV1$^{-/-}$-CON	WT-WD	TRPV1$^{-/-}$-WD
12(S)-HETE (pg/24h)	11.9±1.1	11.6±1.3	13.6±0.4*†	13.5±0.5*†
Plasma urea (mM)	6.1±0.8	6.4±0.8	6.7±0.6	7.6±1.1*
Plasma creatinine (mM)	0.23±0.05	0.25±0.04	0.32±0.05*†	0.33±0.02*†
Urinary albumin (μg/24h)	7.1±1.3	7.6±1.4	15.2±2.9*†	22.1±3.5*†‡
GFR (μL/min)	0.22±0.05	0.21±0.02	0.25±0.07	0.21±0.03

*P<0.05 *vs.* WT-CON; †P<0.05 *vs.* TRPV1$^{-/-}$-CON; ‡P<0.05 *vs.* WT-WD.

HETE concentrations were higher in mice with WD intake compared with CON intake in both strains (Table **1**, P<0.05), and there was no difference between mice in CON intake or WT intake. We further examined the role of WD intake on renal functions. Plasma creatinine levels and urinary albumin levels were increased in mice with WD intake compared with CON intake in both strains, with further elevated urinary albumin levels in TRPV1$^{-/-}$-WD compared with WT-WD (Table **1**, P<0.05). Moreover, plasma urea level is higher in TRPV1$^{-/-}$-WD compared with WT-WD and CON intake in both strains (Table **1**, P<0.05). However, there was no change in GFR among all groups (Table **1**).

4. DISCUSSION

Our present study shows that WD intake causes obesity, showing higher fasting glucose, insulin, leptin levels and increased SNA. TRPV1 ablation further increased the leptin level and SNA at night and exacerbated the WD intake-induced impairment of BR control on RSNA and HR and induced hypertension at night time.

BRS represents an index of arterial baroreflex function. In the present study, compared with the control value, the BR regulated RSNA and HR modulation was clearly impaired in TRPV1$^{-/-}$ mice. Therefore, it can be concluded that TRPV1 ablation is a factor responsible for BRS inhibition. TRPV1 is widely distributed along the entire baroreceptive afferent pathway [16], including ascending aorta and aortic arch [16], the nodose ganglion neurons [28, 29], and visceral afferent fibers [30-32]. TRPV1 function impaired on baroreceptive nerve endings can cause dysfunction in BP regulation [16]. TRPV1 ablation lead to impairment in sensory nerve function, imbalance in autonomic nervous system, and decrease BRS [33].

Intact arterial baroreceptors are necessary for maintaining arterial BR function. Baroreceptor impairment produced by WD intake may be caused by (1) a direct impairment in baroreceptor function by the hyperglycemia, hyperleptinemia, and hyperinsulinemia [34-37]; (2) an impairment in TRPV1 function or expression [38]; or (3) a reduction in arterial distensibility [39-41]. The reduction in arterial distensibility in WD intake may have resulted from aortic wall hypertrophy, increase in collagen content, inflammation, hyperleptinemia and hyperlipidemia [39, 42-44]. As our data show, further increase in inflammation, leptin, and SNA levels when WD intake and TRPV1 ablation are combined may finally be responsible for the fact that in these conditions the BR is further impaired and blood pressure is increased. Hour-by-hour analysis of MAP with telemetry suggests that TRPV1 ablation with WD intake results in pressure rising during the dark cycle and falling during the light cycle, which is consistent with the increased SNA and leptin levels in TRPV1$^{-/-}$-WD at nighttime.

Arterial baroreceptors not only play a role in short term regulation of blood pressure, but also in long-term regulation of blood pressure regulation under salt intake. Howe *et al*. showed that increasing dietary salt intake resulted in hypertension in sinoaortic-denervated but not in baroreceptor intact rats [45]. Osborn and Hornfeldt showed that high salt diet increased arterial pressure in sinoaortic-denervated but not sham rats [46]. Capsaicin-sensitive sensory nerve degeneration causes an increase in BP with salt intake and enhancement of RSNA. Sensory nerve TRPV1 activation inhibits the SNA to maintain blood pressure within normal range [47]. Our present study has shown that the sensitivity of BRS was reduced to the same degree in TRPV1$^{-/-}$-CON and WT-WD and further decreased in TRPV1$^{-/-}$-WD. In the recent study, it has been shown that baroreflex activation can chronically suppress the SNA and decrease arterial pressure. BR activation reduced arterial pressure in hypertension [48] and a pronounced fall in plasma norepinephrine (NE) concentrations prolonged BR activation led to substantial reductions in MAP by suppressing the SNA [49, 50]. Aged and type II diabetes impaired BR led to increased RSNA and hypertension [51-53]. TRPV1 ablation with WD intake further decrease BRS may be related to further increase SNA and increase BP.

Our study shows that WD intake decreased ARNA, and the afferent renal denervation impairs BR control of efferent RSNA [54]. Our previous studies have shown that the activation of the TRPV1 expressed in the renal pelvis leads to an increase in ipsilateral ARNA and contralateral renal excretory function [22, 23]. Kopp *et al*. reported renal sensory nerves activation was decreased in streptozotocin-treated rats and obese Zucker diabetic fatty rats, and the decreased responsiveness of the renal sensory nerves in STZ rats contribute to induce hypertension. Decreased renal sensory nerve activity would lead to increase renal sympathetic nerve activity, which plays an important role in the development of hypertension [55, 56]. Increased RSNA may influence the rennin angiotensin aldosterone system and play a role in long-term changes in BP. Bilateral renal denervation, which can decrease the RSNA, prevented the onset or reduced the magnitude of the hypertension in a large number of different experimental animal models of hypertension and in resistant hypertension [57-59]. In present study, we showed that WD intake impaired sensory nerve function leading to decrease capsaicin-induced ipsilateral ARNA and contralateral RSNA inhibition. The systemic SNA was elevated with WD intake, showing urinary NE level increased in WD intake mice, with further elevated NE levels in TRPV1$^{-/-}$-WD.

TRPV1 stimulation can cause a sympathoexcitatory reflex or a sympathoinhibitory reflex depending on the tissue type. TRPV1 channels appear preferentially on sensory neurons in dorsal root ganglia and sensory nerve terminals of unmyelinated C-fibers or thinly myelinated Aδ-fibers that innervate a number of organs/tissues [28-31, 60, 61]. In the lung, capsaicin injected into the pulmonary circulation activates C fibers that play a role in evoking a pulmonary chemoreflex [62, 63], which is characterized by a triad of apnea, bradycardia, and hypotension. In the heart, epicardial application of capsaicin stimulates cardiac TRPV1 receptors, evoking a sympathoexcitatory reflex [31]. In the kidney, activation of the TRPV1 channels by capsaicin and endogenous capsaicin activator resulting in increases in SP release, ARNA, and a contralateral natriuresis [18, 61, 64, 65]. The renal pelvis and pelvis-ureteric junction are heavily innervated by TRPV1-positive sensory nerves located between the layers of smooth muscles and epithelia [65, 66]. TRPV1 may be activated by metabolic by-products and inflammatory mediators themselves, such as metabolic product of arachidonic acid (AA) and 12/15-lipoxygenase, which may be upregulated in the mice with WD intake. Our previous studies have shown that 12-Hydroperoxyeicosatetraenoic acid (12(s)-HPETE) and 12-HETE may activate of TRPV1, enhance ARNA and SP/CGRP release but suppress renin activity [61]. We measured the urinary 12 (S)-HETE levels, a 12/15-lipoxygenase metabolic product, which are increased in WD intake in both strains and may counteract ARNA decrease in WT-WD but not in TRPV1$^{-/-}$-WD. However, suppression of ARNA is observed in WT-WD, which may be related to WD-induced activation of the RAAS [54, 67].

CONCLUSION

Our data show that TRPV1 ablation with WD intake elevated nocturnal but not diurnal BP, which is consistent with the increased SNA and leptin levels in TRPV1$^{-/-}$-WD at nighttime. Leptin appears to have the most robust evidence linking it to the development of obesity-related hypertension [68], which can increase RSNA [69, 70]. Insulin has been found to increase leptin secretion [71, 72]. However, the plasma insulin level has not been found different between TRPV1$^{-/-}$-WD and WT-WD at the daytime and the nighttime. WD intake increased adipose cell sizes with a large number of immune cell infiltration in adipose tissue in both strains, with further elevated levels in TRPV1$^{-/-}$-WD. Kirchgessner found that TNF-α increased leptin 5.5-fold from 3T3-L1 adipocytes within 6 h [73]. TNF-α acutely activates leptin expression and anti-inflammatory agents can abrogate TNF-α induced hyperleptinemia [74]. Our data show that adipose tissue TNF-α levels are higher in TRPV1$^{-/-}$ mice with WD intake compared to WT-WD and with CON intake in both strains. TNF-α antagonist III, R-7050 (10 μM) decreased leptin releasing in both strains. The data indicate that TRPV1 ablation increased inflammation in adipose tissue may be related to higher leptin release from TRPV1$^{-/-}$-WD mice.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experimental procedures were approved by the Animal Care and Use Committee of Michigan State University, USA.

HUMAN AND ANIMAL RIGHTS

No humans were used in the study. The research was conducted in accordance with the ethical standards. All care and use of laboratory animals were followed and conform to NIH guidelines.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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