TITLE PAGE

Triiodothyronine reduces vascular dysfunction associated with hypertension by attenuating PKG/VASP signaling.

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Running Title: Triiodothyronine reduces hypertensive vascular dysfunction.

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LIST OF NON-STANDARD ABBREVIATIONS

Dahl SS rats	Dahl salt sensitive rats
ROS	Reactive oxygen species
PKG	Protein kinase G
VSMC	Vascular smooth muscle cells
VASP	Vasodilator-stimulated phosphoprotein
T3	Triiodothyronine

ABSTRACT/SIGNIFICANCE STATEMENT

Vascular dysfunction associated with hypertension comprises hypercontractility and impaired vasodilation. We have previously demonstrated that triiodothyronine (T3), the active form of thyroid hormone, has vasodilatory effects acting through rapid on-set mechanisms. In the present study, we examined whether T3 mitigates vascular dysfunction associated with hypertension. To test direct effects of T3 in hypertensive vessels, aortas from female Dahl salt-sensitive (Dahl SS) rats, fed a high-salt diet (8% NaCl, HS Group) and their age-matched controls, fed a standard low salt diet (0.3% NaCl, LS Group) for 16 weeks, were isolated and utilized in *ex vivo* vascular reactivity studies. We confirmed that the HS Group exhibited a higher systolic blood pressure in comparison to the control LS Group, and displayed aortic remodeling. Aortas from both groups were pre-treated with T3 (0.1 μM) for 30 minutes at 37°C in a 5% CO_2 incubator prior to functional vascular studies. T3 treatment significantly attenuated hypercontractility and improved impaired endothelium-dependent vasodilation in aortas from the HS group. These vascular improvements in response to T3 were accompanied by increased phosphorylation of vasodilator-stimulated phosphoprotein (VASP) at serine 239, a vasodilatory factor of the cGMP-dependent protein kinase (PKG)/VASP signaling pathway in vascular smooth muscle cells (VSMC). Moreover, increased production of reactive oxygen species (ROS) in aortas from the HS group were significantly reduced by T3, suggesting a potential anti-oxidant effect of T3 in the vasculature. These results demonstrate that T3 can mitigate hypertension-related vascular dysfunction, through the VASP signaling pathway and by reducing vascular ROS production. SIGNIFICANCE STATEMENT: This study demonstrates that T3

directly acts on vascular tone and has a beneficial effect in hypertension-induced vascular dysfunction. T3 augmented vasodilation and diminished vasoconstriction in blood vessels from hypertensive rats in association with activation of the PKG/VASP signaling pathway that activates vascular relaxation, and exerted an anti-oxidant effect. Collectively, these results show that T3 is a potential vaso-protective agent with rapid action on hypertension-related vascular dysfunction.

Keywords triiodothyronine・non-genomic effect・reactive oxygen species・endothelial dysfunction・VASP・hypertension

INTRODUCTION

Hypertension is a multifactorial disease characterized by an elevation of arterial blood pressure, which progressively increases the risk for cardiovascular disease (Mancia and Giannattasio, 2015). Reduction in vasodilation and increased vasoconstriction characterize vascular dysfunction in essential hypertension (Mordi et al., 2016). Thyroid hormones have also been identified as having an important role in maintaining vascular tone (Ojamaa et al., 1993; Ojamaa et al., 1996; Park et al., 1997; Khorshidi-Behzadi et al., 2013). An alteration of circulating levels of thyroid hormones can negatively affect homeostasis and vascular function (Khorshidi-Behzadi et al., 2013). In this regard, a strong association between thyroid hormone dysregulation and hypertension has been reported (Rapp, 1982). Using spontaneously hypertensive heart failure (SHHF) rats, our group reported that, although significant thyroid dysfunction is present in younger hypertensive animals, long-term treatment with a low dose of thyroid hormone, can attenuate cardiac dysfunction in SHHF rats (Kisso et al., 2008; Weltman et al., 2015). Among the experimental models of hypertension available, the Dahl salt sensitive (Dahl SS) rat strain is a genetic model of human hypertension that displays vascular dysfunction (Drenjancevic-Peric and Lombard, 2004; Teran et al., 2005) and rapid progression of hypertension in response to a high salt diet (Rapp, 1982; Sullivan, 1991).

However, the role of T3 in vascular dysfunction associated with hypertension in Dahl SS rats remains undetermined. T3 is known to cause rapid vascular relaxation and likely plays a crucial role in vascular homeostasis (Samuel et al., 2017). Our group has recently identified that T3 improves vascular function via a non-genomic mechanism

involving the protein kinase G/Vasodilator-stimulated phosphoprotein (PKG/VASP) signaling pathway (Samuel et al., 2017). In the current study, we aimed to determine whether T3 can improve vascular function in Dahl SS rats. The findings of this study provide evidence that T3 is a potent vasoactive factor that improves hypertensive vascular dysfunction.

MATERIAL AND METHODS

Animal Model

Dahl SS rats were obtained from Harlan Laboratories (Indianapolis, IN). 8 week-old female Dahl SS rats were randomly assigned to a standard low salt diet (n=7) containing 0.3% NaCl (LS Group, Research Diets #D10101402, New Brunswick, NJ) or a high salt diet (n=7) containing 8% NaCl (HS group, Research Diets #D10101401, New Brunswick, NJ) for 16 weeks. Rats were housed in a temperature-controlled room with a 12 hours light/dark cycle, and were allowed food and water *ad libitium*. At the end of the experimental protocol, rats were anesthetized with an inhalation of 5% isoflurane in 100% oxygen. A thoracotomy was then performed and blood was obtained from the right ventricle and serum was collected for T3 analysis. Thoracic aortas were harvested for immediate analysis of vascular reactivity and other parameters under T3 treatment conditions. All experimental protocols were conducted in accordance with the Institutional Animal Care and Use Committee at New York Institute of Technology College of Osteopathic Medicine and with respect to the National Institute of Health (NIH, Publication N.85-23, revised 2010) Guidelines for the Care and Use of Laboratory Animals.

Blood Pressure Measurement

Blood pressure was measured in conscious restrained rats using CODA High Throughput Noninvasive Blood Pressure System and analyzed with CODA Version 4.1 software (Kent Scientific Corporation) according to manufacturer protocols. Rats were pre-trained/acclimated to the apparatus prior to pressure recordings. Twenty (20) measurements of blood pressure were averaged. Blood pressure was measured between 9am and 12pm.

Serum T3 measurements

Blood was collected from the right ventricle and left to clot at room temperature for 30 minutes and then centrifuged at 2800 rpm for 15 minutes. Serum was immediately aliquoted and stored at -80°C until assayed for total T3 (TT3), using an enzyme-linked immunosorbent assay (ELISA) kit (Monobind, Inc., Lake Forest, CA) according to the manufacturers' instructions.

Aortic Histomorphometric Analysis

Cryosections of aortas (5 μm) were mounted on slides and fixed in 10% formalin and stained with hematoxylin and eosin (Sigma Aldrich, St. Louis, MO, USA). High-resolution bright field and fluorescent light digital images were captured using an Olympus IX71 microscope equipped with a DP73 camera. Morphometric analysis including cross-sectional area and wall-to-lumen ratio of the aortas were obtained utilizing ImageJ

software (National Institutes of Health) according to established protocols (Gomez-Roso et al., 2009; Maia et al., 2014).

Vascular Reactivity

Isolated thoracic aortas were incubated in oxygenated Krebs buffer (130 mM NaCl, 14.9 mM $NaHCO_3$, 4.7 mM KCl, 1.18 mM KH_2PO_4, 1.17 mM $MgSO_4$-$7H_2O$, 1.56 mM $CaCl_2$-$2H_2O$, 0.026 mM EDTA, 5.5 mM glucose, pH 7.4), with the perivascular fat carefully removed. Aortas were cut into rings (2 mm in length) and cultured in Vascular Medium (ATCC, Manassas, VA), in an incubator at 37ºC supplied by 5% CO_2. Aortas were treated with 0.1 μM T3 (Sigma Aldrich, St. Louis, MO, USA) for 30 minutes. Concentration of T3 was chosen based on results from a previous study (Samuel et al., 2017). Stock solution of T3 was made by dissolving T3 in DMSO (ATCC, Manassas, VA) and further diluted in Krebs solution. A DMSO vehicle was used in controls. After treatment, aortic rings were mounted in a Multi-Wire Myograph System 620M (Danish Myo Technology, Aarhus, Denmark) for isometric tension recordings using a PowerLab 8/35 data acquisition system (ADInstruments Pty Ltd., Castle Hill, Australia). Aortic rings were equilibrated in Krebs buffer for 30 min, and in chambers perfused with 5% CO_2 in 95% O_2 at 37°C, as we have previously described (Carrillo-Sepulveda et al., 2013). In all experiments, aortic ring integrity was assessed by stimulation with 120 mM KCl (74.7 mM NaCl, 14.9 mM NaHCO3, 60 mM KCl, 1.18 mM, KH2PO4, 1.17 mM MgSO4-7H2O, 1.6 mM CaCl2-2H2O, 0.026 mM EDTA, 5.5 mM glucose). To test for the presence of endothelium, segments were contracted with 1 μM phelynephrine (PE) (Sigma Aldrich,

St. Louis, MO, USA) and once the vessels reached a stable maximum tension, the vessels were stimulated with 10 μM acetylcholine (ACh) (Sigma Aldrich, St. Louis, MO, USA) and relaxation was confirmed. Stock solution of PE and ACh were dissolved in distilled water. Aortic rings that achieved relaxation to ACh were considered to have a preserved endothelium. Cumulative concentration-response curves to ACh (1 nM to 10 μM) and PE (1 nM to 10 μM) were performed on intact aortic rings in the absence or presence of 0.1 μM T3. Endothelium-dependent relaxation was recorded for ACh after maximal pre-contraction with 1 μM PE.

Immunoblotting

Protein expression was analyzed by Western blotting. Total protein was extracted from the aortas of HS and LS rats after treatment with 0.1 μM T3 for 30 minutes. Equivalent amounts of protein (40 μg) were loaded and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to PVDF membranes (Thermo Fisher Scientific Inc., Rockfold, IL, USA) as previously described (Samuel et al., 2017). Membranes were blocked for 1 hour with 5% non-fat milk solution in Tris-buffered saline with 0.1% Tween-20 (TBST), then incubated overnight at 4°C with the following specific primary antibodies: PKG (1:1000, catalog no.3248, Cell Signaling Techonology, Danvers, MA), total and phosphorylated VASP (1:1000, catalog no. 3132, catalog no. 3114, Cell Signaling Techonology, Danvers, MA) followed by anti-IgG-HRP secondary antibodies (1:10000, catalog no. 7074, catalog no. 7076, Cell Signaling Technology, Danvers, MA). Membranes were stripped and re-probed with an internal loading control, β-actin (1:10000, catalog no. 4967, Cell Signaling, Danvers, MA). Protein bands were detected using Enhanced Chemiluminescence (ECL) Ultra reagents (Lumigen Inc.,

Southfield, MI). PKG data are presented as percent (%) relative to the LS group after normalization to β-actin. Phospho-VASP measurements were normalized to total VASP levels and expressed as percent (%) relative to the LS group.

Measurement of reactive oxygen species (ROS)

Global reactive oxygen species (ROS) were detected in aortic tissues using 25 μM fluorescent probe dihydroethidium (DHE, Sigma Aldrich, St. Louis, MO, USA) as previously described (Carrillo-Sepulveda et al., 2015). Aortic rings were obtained from HS and LS groups. Briefly, the aortic rings were pre-treated with 0.1 μM T3 for 30 min, embedded in Tissue Tek OCT compound (Sakura Finetek, Terrance, CA), snap-frozen, and stored at -80°C. Cryosections of 5 μm thickness were cut and placed on Superfrost Plus slides (Menzel-Gläzer, Germany). 50 μL of DHE solution was applied to each tissue section and then covered with a coverslip. The slides were incubated in a light-protected humidified chamber at 37°C for 30 min before imaging. Fluorescence of DHE was detected using an Olympus IX71 fluorescence microscope fitted with a DP73 camera. Quantitative analysis was performed to detect changes in fluorescence using Image J software (National Institutes of Health). Background fluorescence was subtracted for each image prior to quantification. The results are expressed as a percentage (%) fluorescence in HS and HS+T3 aortic rings in comparison to the LS tissue.

Statistical Analysis

Results are expressed as means ± SEM. All data were analyzed using GraphPad Prism version 7 (GraphPad Software, San Diego, CA). Statistical significance among groups was determined using one-way ANOVA followed by Bonferroni post hoc tests. Student *t* test was used when appropriate. $P<0.05$ was considered statistically significant.

RESULTS

Characterization of Dahl SS rats

Female Dahl SS rats fed a high salt diet for 16 weeks (HS group) exhibited increased systolic and diastolic blood pressure in comparison to rats fed a low salt diet for the same period of time (LS group), as shown in the Fig.1 A and Fig.1B, respectively. Concentrations of total T3 in serum of LS and HS rats at the end of the study were not significantly different (1.732 ± 0.150 and 1.834 ± 0.335 ng/ml, respectively). In addition to hypertension, the HS group also exhibited marked aortic remodeling, as evident in the histological sections of thoracic aorta in Figure 2A. Increased cross-sectional area (Fig. 2B) and greater wall-to-lumen ratio were observed in the HS group compared with the LS group, confirming aortic remodeling (Fig. 2C).

T3 mitigates hypertensive vascular dysfunction

Vascular dysfunction in hypertensive Dahl SS rats has been well described (Luscher et al., 1987). To determine whether hypertensive vascular dysfunction is mitigated by short-term treatment with T3, aortas from the HS and LS groups were isolated and utilized for vascular reactivity studies. Impaired endothelium-dependent relaxation recorded in aortas from the HS group was attenuated with short-term T3 treatment (Fig.

3A), with the maximal effect generated by the agonist (EMax) of 46.85 ± 2.90% vs 63.41 ± 4.9 %, which is HS vs HS+T3 group, respectively (Fig.3B). In contrast, T3 did not change relaxation response in aortas from the LS group under the same conditions (Fig. 3C, D). Aortas from the HS group showed increased contractility in response to PE that was significantly reduced with short-term T3 treatment (Fig. 4A-B). T3 treatment did not alter contractile response in aortas from the LS group (Fig. 4C-D). Moreover, *in vivo* experiments showed that rats from HS group treated with T3 (5μg/Kg/day) in drinking water for 4 weeks (Supplemental Methods) did not display changes in water intake (Supplemental Figure 1) or blood pressure (Supplemental Figure 2).

T3 activates PKG/VASP signaling in the aortas of HS treated rats

Based on our previous study showing that T3 promotes vascular relaxation via a PKG/VASP signaling pathway in cultured vascular smooth muscle cells (VSMC) (Samuel et al., 2017), we examined whether improvement of function in hypertensive aortas caused by short-term treatment with T3 is associated with activation of PKG/VASP signaling. Immunoblot analysis revealed that expression of PKG in aortas from the HS group was significantly reduced in comparison to the LS group (Fig. 5). Moreover, levels of phosphorylated VASP, a downstream target of PKG and a marker of vasorelaxation, were decreased in aortas from the HS group as compared to the LS group (Fig. 6). Short-term T3 treatment significantly increased VASP phosphorylation in aortas from HS group (Fig.6). Together, these results indicate that T3 is able to activate the PKG/VASP signaling pathway in hypertensive vessels.

Effect of T3 on vascular oxidative stress

It has been well-established that oxidative stress is involved in the pathogenesis of hypertensive vascular dysfunction (Touyz and Schiffrin, 2004). To further investigate whether T3 exerts an antioxidant effect in hypertensive vessels, we measured oxidative stress by detecting aortic ROS levels by using DHE staining. The increased production of ROS found in aortas from the HS group was significantly reduced with short-term T3 treatment (Fig. 7).

DISCUSSION

The main findings of the current study are that short-term T3 treatment 1) attenuated vascular dysfunction by increasing vasodilation and diminishing vasoconstriction; 2) activated the vascular PKG/VASP signaling pathway; and 3) reduced oxidative stress in aortas from hypertensive Dahl SS rats. Collectively, these data imply that T3 exerts direct vascular effects and supports T3 as a potential vaso-protective agent for hypertension-related vascular dysfunction.

A series of studies has demonstrated that administration of T3 at pharmacological concentration causes vascular relaxation (Liu et al., 2014; Samuel et al., 2017). For several years, T3-induced vascular relaxation was attributed to endothelium-dependent mechanisms (Lozano-Cuenca et al., 2016; Razvi et al., 2018), while the VSMC-related mechanisms mediating the vasodilatory effect of T3 remained understudied. In this regard, early work by Ojamaa et al. demonstrated that T3 can directly exert its vasodilatory effect through VSMC mechanisms (Ojamaa et al., 1993; Ojamaa et al., 1996). Consistent with that finding, our group has recently shown that T3

elicits rapid vascular relaxation via activation of PKG/VASP signaling in VSMC (Samuel et al., 2017). Despite growing evidence for participation of both endothelial and VSMC on T3-induced vascular relaxation (Cai et al., 2015), additional studies are needed to fully understand the crosstalk between endothelium-dependent and -independent mechanisms involved in the vascular effects of T3 in different pathophysiological contexts.

An association between thyroid hormone dysregulation and hypertension has been reported in clinical and basic experimental studies (Rapp, 1982; Polat Canbolat et al., 2017; Gu et al., 2018). We hypothesized that short-term T3 administration would increase vasodilation and decrease vasoconstriction in hypertensive Dahl SS rats. Results of the present study show that T3 reduces vascular hypercontractility in aortas from hypertensive Dahl SS rats. This rapid-onset effect of T3 on vasomotor response has been previously observed by us and other groups (Hiroi et al., 2006; Samuel et al., 2017; Llevenes et al., 2018). PKG plays an important role in maintenance of vascular smooth muscle phenotype and vascular relaxation (Choi et al., 2018). Activation of PKG in VSMC can lead to phosphorylation of VASP at serine 239, resulting in vascular relaxation (Holt et al., 2016). In the present study, we found decreased protein expression of PKG in aortas from Dahl SS rats, which were accompanied by reduced levels of phosphorylated VASP, indicating downregulation of the PKG/VASP signaling pathway in hypertensive blood vessels. Importantly, T3 significantly increased levels of phosphorylated VASP in aortas from Dahl SS rats, and this effect was associated with reduced vascular hypercontractility. These findings are in accordance with our previous study (Samuel et al., 2017), in which we reported that T3 rapidly potentiates vascular

relaxation via increased phosphorylation of VASP, which corresponds to a non-genomic, non-canonical effect of T3 (Bassett et al., 2003; Flamant et al., 2017). These new findings firmly establish that T3 is able to induce significant post-translational modification of VASP in blood vessels to cause rapid changes in vascular reactivity and support an important vasoactive role.

Oxidative stress is another key component playing a role in the pathogenesis of hypertensive vascular dysfunction (Touyz, 2004). Damage from oxidative stress has been shown to cause impaired vasoconstriction and vasodilatation (Rodrigo et al., 2003; Paravicini and Touyz, 2006). Consistent with these previous findings, we also found that vascular dysfunction in hypertensive Dahl SS rats is accompanied by augmented vascular oxidative stress, which was markedly reduced by short-term T3 treatment. These data support the hypothesis that reduction of vascular ROS levels and subsequent increase in nitric oxide (NO) bioavailability caused by T3 may be a mechanism contributing, at least in part, to reduced vasoconstriction as well as increased endothelium-dependent vasodilation observed in aortas from hypertensive Dahl SS rats directly treated with T3.

This antioxidant effect of T3 is in accordance with others studies that have suggested T3 as a potential antioxidant agent. For example, thyroid hormones have free radical scavenging capacity and are able to reduce low-density lipoprotein oxidation *in vitro* (Oziol et al., 2001). Moreover, considering that oxidative stress also plays a key role in renal damage in Dahl SS rats (De Miguel et al., 2011), further studies investigating the effects of T3 treatment in kidneys of hypertensive animals may expand

the benefits of T3 to renal microvasculature. Moreover, future studies will be designed to examine the mechanisms by which T3 reduces vascular oxidative stress.

While we cannot account in the present study for T3 causing endothelium-independent vasodilation in hypertensive blood vessels, our data implies that T3 activates endothelial cells (acetylcholine curve data, Fig. 3), resulting in NO release, which stimulates cyclic GMP (cGMP) production and activates PKG that consequently phosphorylates VASP (western blot data, Fig. 6), causing smooth muscle cell relaxation. Moreover, T3 significantly reduced vascular oxidative stress, implying an increase in vascular NO bioavailability due to reduced ROS levels. Therefore, it is likely that the antioxidant effect of T3 also contributes to it vasodilator effect.

In conclusion, our data demonstrate that T3 not only plays a role in promoting vasodilation but also decreases vasoconstriction in hypertensive vasculature, most likely via a combination of endothelial and VSMC-related mechanisms.

The therapeutic utilization of T3 in treating cardiovascular disorders has been under continued debate (Gerdes and Iervasi, 2010). Our findings offer further evidence that T3 exerts a protective effect in the cardiovascular system. Improved understanding of crosstalk between endothelial and VSMC-related mechanisms by which T3 regulates vascular function may open new avenues of research and treatment options for vascular dysfunction found in cardiovascular disease. The present study was not intended to examine T3 as a potential agent reducing blood pressure, but specifically examined the short-term direct effects of T3 in blood vessels from hypertensive rats. In fact, *in vivo* treatment with T3 did not show effect in blood pressure in the Dahl salt-sensitive rat model, which was expected since the regulation of blood pressure in this

model is mostly attributed to renin angiotensin system and renal injury mechanisms. Rapid vascular effects of T3 without affecting blood pressure have also been observed in human studies. Hamilton et al. showed that short-term intravenous administration of T3 in humans caused peripheral vasodilatory effects and reduced systemic vascular resistance without causing changes in blood pressure (Hamilton et al., 1998).

Although serum T3 levels were normal, results imply impaired thyroid hormone signaling in vessels from Dahl SS rats. Our group has previously reported that low cardiac tissue T3 levels were related to reduced cardiac function in diabetic rats despite normal serum thyroid hormone levels (Weltman et al., 2014). Our data suggest that thyroid hormone bioavailability may also be altered in vascular tissue despite normal serum hormone levels, and merits further investigation.

Despite advances in anti-hypertensive drug therapy in recent decades showing significantly prolonged lifespan of hypertensive patients, some patients do not benefit from these medications (Mozaffarian et al., 2015). This indicates that additional vasoactive agents are needed to attenuate vascular complications that negatively impact hypertensive individuals. Our data do not postulate T3 as a treatment for high blood pressure, but raise the interesting possibility that T3 can directly ameliorate vascular dysfunction via both endothelial and VSMC-related mechanisms, which represents a critical preclinical step in the development of hypertension.

In conclusion, our results indicate that T3 mitigates vascular dysfunction in hypertensive Dahl SS rats via an antioxidant effect and activation of the PKG/VASP signaling pathway, increasing our knowledge of the mechanisms of non-genomic effects of T3 in the vascular system.

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Disclosures

Conflict of interest: None.

AUTHORSHIP CONTRIBUTIONS

Participated in research design: Carrillo-Sepulveda, Maracheril, Gerdes

Conducted experiments: Carrillo-Sepulveda, Panackal, Maracheril, Maddie, Patel, Ojamaa

Performed data analysis: Carrillo-Sepulveda, Panackal, Maddie, Ojamaa, Savinova, Gerdes

Wrote or contributed to the writing of the manuscript: Carrillo-Sepulveda, Panackal, Maracheril, Maddie, Ojamaa, Savinova, Gerdes

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LEGENDS FOR FIGURES

Figure 1. High salt diet increased systolic and diastolic blood pressure. Systolic (A) and diastolic (B) blood pressure in Dahl SS rats fed a high salt diet (HS Group) and low salt diet (LS Group) for 16 weeks measured by tail-cuff method. Data are expressed as means ± SEM; n=7 per group. *P<0.05 compared with LS group.

Figure 2. Aortic remodeling in Dahl SS rats. Representative thoracic aorta cross-sections (A) stained with hematoxylin & eosin. Medial cross-sectional area (B) was increased in the HS group. Wall-to-lumen ratio (C) was increased in the HS group. Data are expressed as means ± SEM; n=8-12 per group. *P<0.05 compared with LS group.

Figure 3. T3 improved relaxation response in hypertensive aortas. Cumulative concentration-curve and maximum response (Emax) to ACh in aortas from Dahl SS rats fed a high salt diet (HS Group) (A and B) and low salt diet (LS Group) (C and D). Aortas from both groups were pre-treated with 0.1 μM T3 for 30 minutes (open squares and circles). Data are expressed as means ± SEM; n=7 per group. **P<0.05 compared with HS group.

Figure 4. T3 attenuated augmented contractile response in hypertensive aortas. Cumulative concentration-curve and maximum response (Emax) to PE in aortas from

Dahl SS rats fed a high salt diet (HS Group) (A and B) and low salt diet (LS Group) (C and D). Aortas from both groups were pre-treated with 0.1 μM T3 for 30 minutes (open squares and circles). Data are expressed as means ± SEM; n=7 per group. **P<0.05 compared with HS group.

Figure 5. Decreased aortic PKG expression in Dahl SS rats fed a high salt diet. Levels of PKG were assessed in aortas from HS and LS group using western blot. Top panel: representative immunoblots. Bottom panel: quantification of intensity of bands. Data are expressed as means ± SEM, n=7 per group, *P<0.05 vs. LS group.

Figure 6. T3 increased phosphorylation of VASP in hypertensive aortas. Aortas were treated with 0.1 μM T3 for 30 minutes. After treatment, levels of phosphorylated VASP in the serine 239 were assessed using western blot. Top panel: representative immunoblots. Bottom panel: quantification of intensity of bands. Data are expressed as means ± SEM, n=7 per group, *P<0.05 vs. LS group, **P<0.05 vs. HS group.

Figure 7. T3 decreased oxidative stress in hypertensive aortas. Aortas from experimental groups were either treated or not treated with 0.1 μM T3 for 30 min followed by detection of oxidative stress using DHE probe. Top panel: representative photomicrographs depicting DHE (*red fluorescence*) in aortas. Bottom panel: quantification of fluorescent intensity in each aorta cross-section. Data are expressed as means ± SEM, n=7 per group, *P<0.05 vs. LS group, **P<0.05 vs. HS group. Scale bar represents 100μm.

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FIGURES

Figure 1

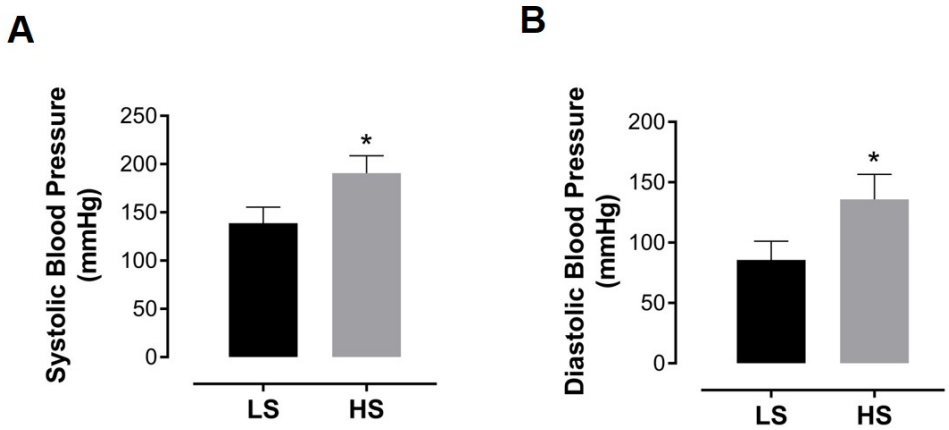

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Figure 2

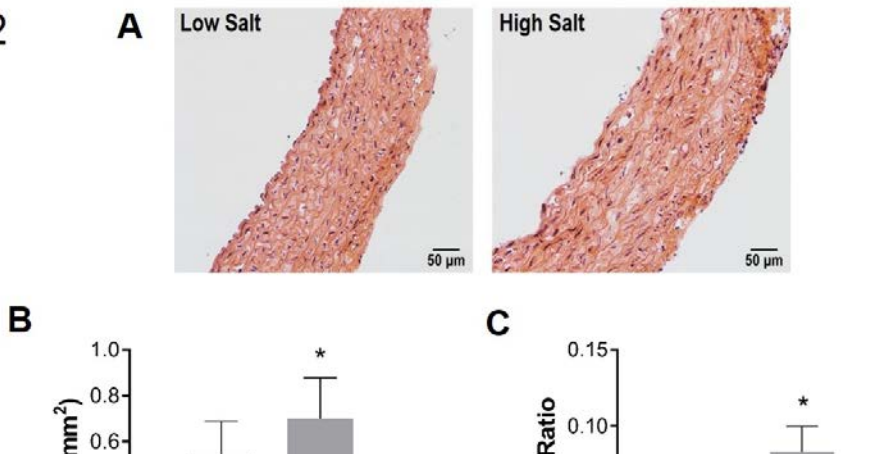

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Figure 3

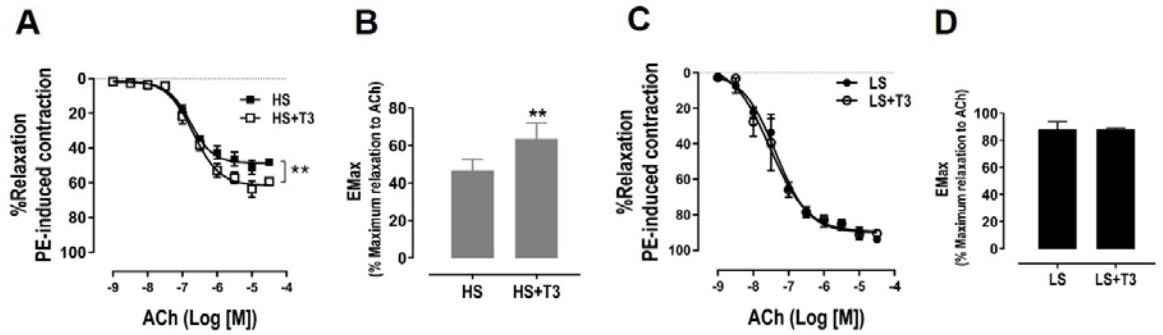

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Figure 4

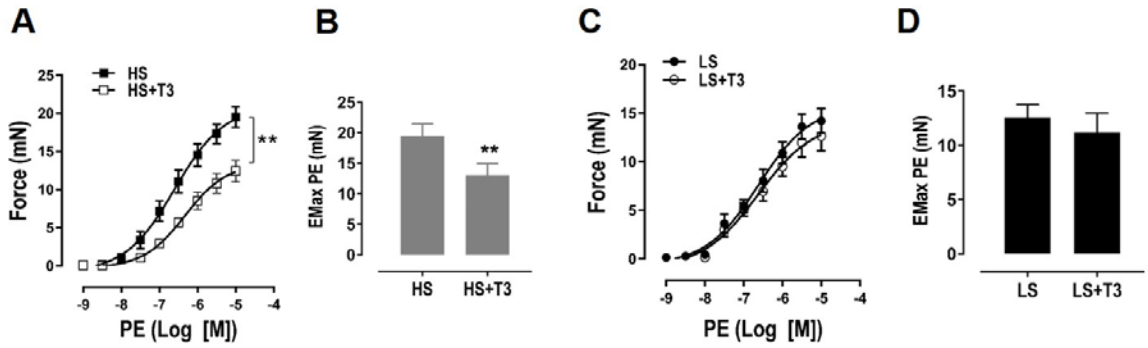

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Figure 5

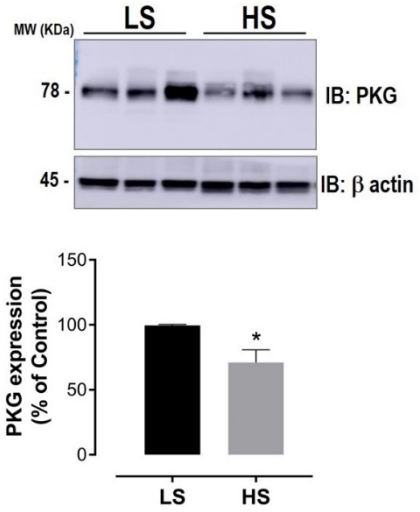

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Figure 6

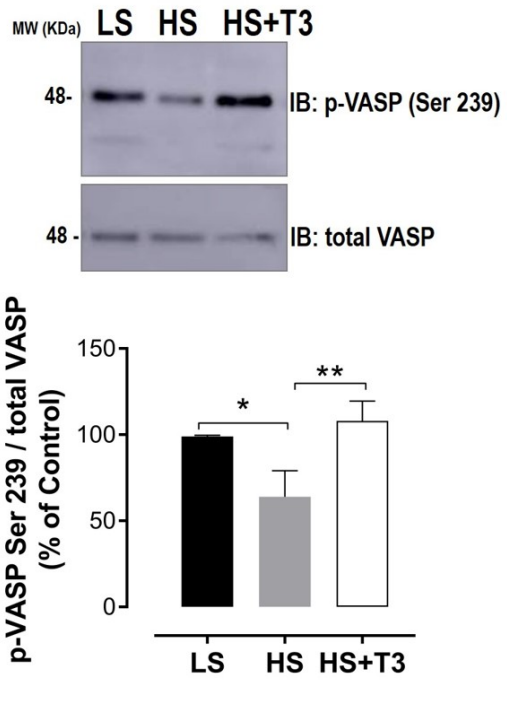

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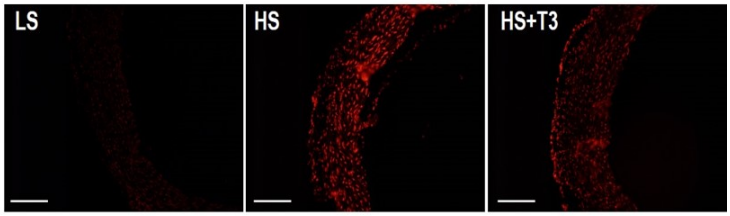
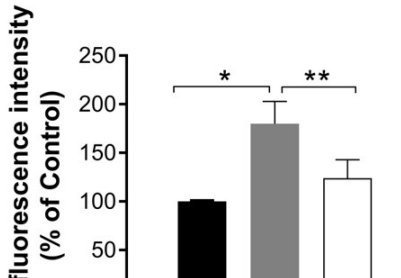
Figure 7





Triiodothyronine reduces vascular dysfunction associated with hypertension by attenuating PKG/VASP signaling.

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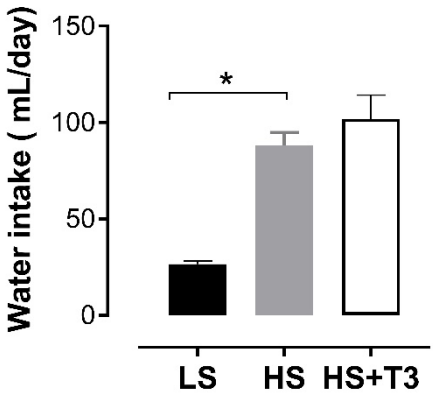
Supplemental Methods

Animals and Study Design

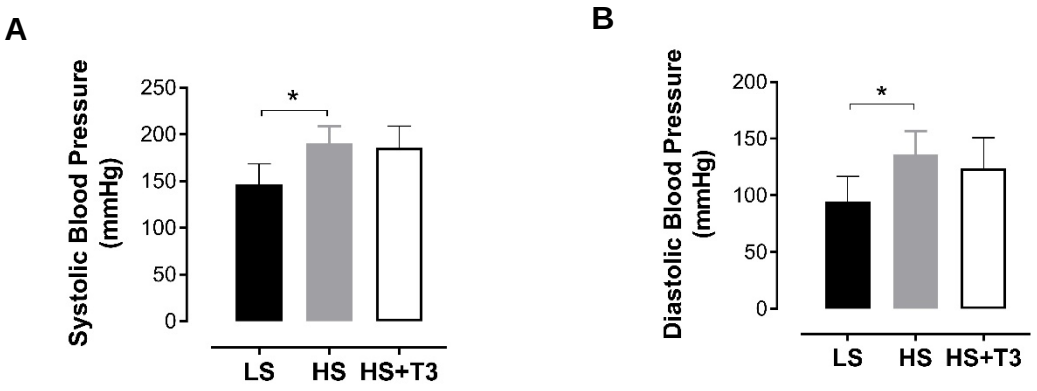
Female Dahl SS rats assigned to a high salt diet (n=7-8/per group) containing 8% NaCl (HS group, Research Diets #D10101401, New Brunswick, NJ) were randomly allocated in two experimental sub-groups at 11 weeks of age. One group received regular drinking water (HS group) and another T3 supplemented water (5μg/Kg/day) for four weeks. The efficacy of T3 supplementation at the selected dose and delivery method was previously established by our group (Weltman et al., 2013; Weltman et al., 2015; Zhang et al., 2018). Water intake was measured at baseline for HS diet (8 weeks of age), and bi-weekly thereafter initiation of HS protocol.

Supplemental Figure 1



Supplemental Figure 1. T3 treatment did not alter water intake in the Dahl SS rats fed a high salt diet (HS Group). HS Group were treated with T3 (5μg/Kg/day) for four weeks; water intake was measured three weeks after initiation of T3 treatment. Data are expressed as means ± SEM; n=4 in the low salt group (LS group), n=8 in the HS group, and n=7 in the HS+T3 group. *P<0.0001 compared with LS group.

Supplemental Figure 2



Supplemental Figure 2. T3 treatment did not decrease blood pressure in Dahl SS rats fed a high salt diet (HS Group). HS Group was treated with T3 (5μg/Kg/day) for four weeks and systolic (A) and diastolic (B) blood pressure was measured by tail-cuff method. Data are expressed as means ± SEM; n=9 per group. *P<0.005 compared with the low salt group (LS group).

Supplemental References

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