

2015

Hypothyroidism and the Development of Hypertension

Kaleb Short

Minnesota State University - Mankato

Follow this and additional works at: <http://cornerstone.lib.mnsu.edu/etds>

 Part of the [Cardiovascular Diseases Commons](#), [Endocrine System Diseases Commons](#), and the [Endocrinology, Diabetes, and Metabolism Commons](#)

Recommended Citation

Short, Kaleb, "Hypothyroidism and the Development of Hypertension" (2015). *All Theses, Dissertations, and Other Capstone Projects*. Paper 392.

This Thesis is brought to you for free and open access by the Theses, Dissertations, and Other Capstone Projects at Cornerstone: A Collection of Scholarly and Creative Works for Minnesota State University, Mankato. It has been accepted for inclusion in All Theses, Dissertations, and Other Capstone Projects by an authorized administrator of Cornerstone: A Collection of Scholarly and Creative Works for Minnesota State University, Mankato.

Hypothyroidism and the Development of Hypertension

By

Kaleb Fischer Short

A Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

In

Biology

Minnesota State University, Mankato

Mankato, Minnesota

April 2015

Date _____

Hypothyroidism and the Development of Hypertension

Kaleb Fischer Short

This thesis has been examined and approved by the following members of the student's committee.

Dr. David Sharlin (Advisor)

Dr. Penny Knoblich

Dr. Michael Bentley

Abstract

Hypothyroidism and the Development of Hypertension

Name: Kaleb Fischer Short

Degree: Master of Science in Biology

Institution: Minnesota State University, Mankato

Mankato, Minnesota, 2015.

Low thyroid hormone (TH) in adulthood is associated with increased risk of cardiovascular disease, including risk of hypertension. The Spontaneously Hypertensive Rat (SHR), exhibits alterations in thyroid function when compared to normotensive controls. Interestingly, inhibiting thyroid gland function before 4 weeks of age prevented hypertension in the SHR, indicating that TH is involved in the etiology of SHR hypertension. However, these studies utilized tail-cuff photoplethysmography (PPG), which is known to have stress-induced artifacts, and did not compare effects in the normotensive parent strain Wistar Kyoto (WKY) rat. Therefore, it is uncertain whether TH is responsible for an elevation in baseline blood pressure or whether hypertension in the SHR is caused by abnormal TH action in the cardiovascular system.

Considering this, the purpose of the present study was to investigate the effect of hypothyroidism on the SHR and its parent strain, WKY on blood pressure. To study the effect of thyroid hormone on blood pressure, we inhibited thyroid hormone production in WKY and SHR male rats with 1% KClO_4 and 0.05% MMI in drinking water from 4-14 weeks-of-age. Rats were allowed to recover from treatment weeks 14-17. Blood pressure was monitored weekly by tail-cuff PPG from 4-15 weeks and by implantable remote transmitters from 11-17 weeks. Pressure natriuresis was evaluated using flame photometry.

PPG data was consistent with previous literature that indicated hypothyroidism prevented hypertension in the SHR, but remote transmitter data indicated a comparable reduction in systolic (SAP) and mean arterial pressure (MAP) between strains. The observed difference in PPG can be explained by an exaggerated response of SAP to stress in SHRs which is normalized by hypothyroid treatment. Strain differences were observed in the effect of treatment on diastolic arterial pressure (DAP), pulse pressure (PP), heart rate, and pressure natriuresis. Endothelial dysfunction and increased arterial stiffness in the SHR provide potential explanations for differences in DAP, PP, and pressure natriuresis.

Acknowledgements

First, I would like to acknowledge my advisors, Dr. David Sharlin, and Dr. Penny Knoblich and my express my sincere gratitude for all of their patience and guidance in one of the most difficult undertakings of my life. Dr. Knoblich and her family have been great pillars of support and I would not have been able to accomplish this without their unconditional support and compassion. Dr. Sharlin's honest and earnest wisdom has guided me both in growing as a scientist and as an individual.

I also would like to thank Dr. Michael Bentley for being excellent committee member. He has extended knowledge and wisdom to me that have helped to shape and guide my professional and academic endeavors.

Last, and definitely not least, I would like to thank my family and friends who have been there to provide support in triumph and in failure. Graduate school would have been impossible without them.

Table of Contents

Abstract.....	3
Acknowledgements.....	5
Table of Contents.....	6
List of Figures.....	8
Introduction.....	10
Literature Review.....	12
Thyroid Hormone Secretion and Regulation	12
Arterial Pressure Regulation	13
Clinical Effects of Hypothyroidism on the Cardiovascular System	14
Effects of Thyroid Hormone on the Heart	15
Hemodynamic Effects of Thyroid Hormone.....	16
Thyroid Hormone in Nervous Control of the Cardiovascular System.....	18
Thyroid Function in the Rat Model of Hypertension.....	19
Materials and Methods.....	20
Rat Strains	20
Treatment	20
Water and Mass Measurement	21
Serum Collection and Measurement	21
Occlusion Tail-Cuff Photoplethysmography	22
Remote Monitoring of Blood Pressure.....	23
Stress Test	24
Na ⁺ Excretion	25
Data Analyses.....	26
Results.....	27
Serum T ₄	27
Weights.....	28
Systolic Arterial Pressure by Tail-Cuff Photoplethysmography.....	29
Pressure and Heart Rate by Remote Transmitter	30
Activity Differences	34

Stress Test	35
Pressure Natriuresis.....	36
Discussion.....	37
References.....	44

List of Figures

Figure 1. The effect of strain and treatment on serum T4

Figure 2. The effect of strain and treatment on weight.

Figure 3. The effect of strain and treatment on weight gain between weeks 14 and 17 in the recovery subset.

Figure 4. The effect of strain and treatment on systolic arterial pressure as measured by tail-cuff photoplethysmography.

Figure 5. The effect of strain and treatment on mean arterial pressure as measured by remote transmitter.

Figure 6. The effect of strain and treatment on systolic arterial pressure as measured by remote transmitter.

Figure 7. The effect of strain and treatment on diastolic arterial pressure as measured by remote transmitter.

Figure 8. The effect of strain and treatment on pulse pressure as measured by remote transmitter.

Figure 9. The effect of strain and treatment on heart rate as measured by remote transmitter.

Figure 10. The effect of strain and treatment on difference between heart rate at high activity and low activity at weeks 11-17 as measured by remote transmitter.

Figure 11. The effect of strain and treatment on difference between resting and stressed systolic arterial pressure (A) and pulse pressure (B) as measured by remote transmitter.

Figure 12. The effect of strain and treatment on Na⁺ excretion as measured by flame photometer.

Figure 13. The effect of strain and treatment on pressure response as measured by BIOPAC hardware.

Introduction

Low thyroid hormone in adulthood is associated with increased risk of cardiovascular disease (Rhee, Curhan, Alexander, Bhan, & Brunelli, 2013), including risk of hypertension (Saito, Ito, & Saruta, 1983; Streeten, Anderson, Howland, Chiang, & Smulyan, 1988). Interestingly, the Spontaneously Hypertensive Rat (SHR), a murine model of hypertension, exhibits abnormal thyroid function when compared to normotensive controls (Aoki, 1963a, 1963b; Fregly, 1975; Werner, Manger, Radichevich, Wolff, & von Estorff, 1975; Yamabe, 1970). Additionally, thyroid gland inhibition starting at 4 weeks of age has been found to prevent the onset of hypertension entirely in the SHR, indicating that thyroid hormone is involved in the development of hypertension (Aoki, 1963b; Rioux & Berkowitz, 1977).

Although these reports indicated involvement of thyroid hormone in the establishment of hypertension in the SHR, the effect of hypothyroidism on blood pressure has not been compared between the SHR and a normotensive strain. Therefore, it is uncertain whether thyroid hormone is responsible for a baseline elevation in blood pressure set point or whether hypertension in the SHR is caused by abnormal thyroid action in the cardiovascular system.

Given the abnormal thyroid function in the SHR and prevention of hypertension in the SHR by thyroidectomy in previous studies, it is hypothesized that: **hypertension in Spontaneously Hypertensive Rats is caused, in part, by abnormal thyroid hormone action in the cardiovascular system.**

In testing this hypothesis, we propose that the inhibition of thyroid gland activity will result in a greater overall decrease in mean arterial pressure in SHRs when compared to controls, than the decrease found in WKY rats given the same treatment.

Additionally, it is known that hypothyroidism induces reduced cardiac beta adrenergic receptor numbers, subsequently decreasing sensitivity to sympathetic stimulation (Bilezikian & Loeb, 1983; Rioux & Berkowitz, 1977). As tail-cuff photoplethysmography evokes a stress response in rats, it is unclear whether the decrease in systolic pressure in hypothyroid rats observed in prior studies is due to a basal reduction in systolic pressure or due to a reduction in the stress-induced sympathetic response. Prior research indicates that SHRs have higher sympathetic responsivity to restraint than normotensive rats (Chiueh & Kopin, 1978). Therefore, it is hypothesized that: **hypothyroid rats exhibit a reduced response of systolic arterial pressure to sympathetic stimulation. SHR hypothyroid rats will exhibit a greater reduction in systolic arterial pressure in response to sympathetic stimulation than WKY hypothyroid rats.**

In testing this hypothesis, we propose that the inhibition of thyroid gland activity will result in a smaller increase in systolic arterial pressure in response to acute stress when compared to euthyroid counterparts. SHR hypothyroid rats will exhibit a greater reduction in the systolic arterial pressure response to stress than WKY hypothyroid rats when compared to their euthyroid counterparts.

Lastly, hypothyroidism impairs nitric oxide synthesis (Virdis et al., 2009). Nitric oxide has a known effect on the pressure natriuresis response in rats (Guarasci & Kline,

1996). Since prior studies have reported that chronic hypothyroidism has no effect on the pressure natriuresis response in rats (Vargas, Atucha, Sabio, Quesada, & Garcia-Estan), it is hypothesized that: **developmentally hypothyroid rats will exhibit a comparable pressure natriuresis response to euthyroid rats.**

In testing this hypothesis, we propose that the inhibition of thyroid gland activity at weaning will result in an acute pressure natriuresis response comparable to that observed in euthyroid counterparts.

Literature Review

Thyroid Hormone Secretion and Regulation

Thyroid hormones, tetraiodothyronine (T_4 or thyroxine) and triiodothyronine (T_3), are hormones synthesized by the thyroid gland, a gland which resembles a bow tie in shape and size and is situated on the anterior side of the trachea. Iodine enters the follicular cells of the thyroid gland by active transport and is covalently bound to tyrosine residues within thyroglobulin, a glycoprotein synthesized within the follicular cells. This covalent bonding is referred to as iodination and forms either monoiodotyrosine (MIT) or diiodotyrosine (DIT). Two DIT molecules combine to form T_4 and the combination of one MIT and one DIT form T_3 . Ninety-five percent of thyroid hormone secreted by the thyroid gland is T_4 (Gard, 1998).

T_4 binds to thyroid hormone receptors with less affinity than T_3 , and is often thought of as a precursor to T_3 . The deiodinase enzymes function to remove iodine atoms from iodothyronines as a mechanism of activation or deactivation within target tissues. Type 2 deiodinase (D2), removes an iodine atom from the outer ring of T_4 to create T_3

(activation). Type 3 deiodinase (D3) can remove an iodine atom from the inner ring of T_4 to create reverse T_3 (rT_3) (inactivation) or remove an iodine atom from the inner ring of T_3 to create T_2 (inactivation). Type 1 deiodinase (D1) is found most commonly in the liver and kidney and can remove an iodine atom from either ring of thyroid hormones to make T_2 , rT_3 , or T_3 (Bates, St Germain, & Galton, 1999).

Circulating thyroid hormone in the blood is regulated via the hypothalamic-pituitary-thyroid (HPT) axis. Thyrotropin releasing hormone (TRH) is produced by neurons of the paraventricular nucleus of the hypothalamus. TRH stimulates the release of thyroid stimulating hormone (TSH) production and release by the anterior pituitary. TSH stimulates the thyroid gland to increase synthesizing activity of thyroid hormones. Thyroid hormones act on the pituitary gland and hypothalamus to inhibit the release of TSH and TRH, respectively, via a classical negative feedback loop (Gard, 1998).

Thyroid hormone receptors mediate the majority of biological processes regulated by thyroid hormones. There are two extant thyroid receptor genes, $TR\alpha$ and $TR\beta$. The T_3 receptor product of $TR\alpha$ is $TR\alpha 1$ and is expressed primarily in the heart, brain, and skeletal muscle. The T_3 receptor products of $TR\beta$ are $TR\beta 1$, which is systemically expressed, $TR\beta 2$, which is primarily expressed in the retina, brain, and inner ear, and $TR\beta 3$, which is primarily expressed in the kidney, liver, and lung (Cheng, Leonard, & Davis, 2010).

Arterial Pressure Regulation

Mean arterial pressure (MAP) is the average pressure in the arteries over one cardiac cycle, which includes systole (ventricular contraction), and diastole, (ventricular

relaxation/filling). Diastolic arterial pressure (DAP) is the minimum pressure in the arteries in a cardiac cycle. Systolic Arterial Pressure (SAP) is the maximum pressure in the arteries in a cardiac cycle. Pulse Pressure (PP) is the difference between SAP and DAP.

MAP is a function of total peripheral resistance (TPR), the amount of resistance that opposes blood flow in the systemic circulation, and cardiac output (CO), the amount of blood pumped by the heart each minute (L/min). Cardiac output is the product of stroke volume (SV), the amount of blood pumped from the ventricles of the heart in each cardiac cycle (L), and heart rate (beats per minute (bpm)). Acutely, when a decrease in MAP occurs, it stimulates the arterial baroreceptors, and the central nervous systems enacts an increase in sympathetic stimulation. This increase in sympathetic stimulation functions to increase heart rate, increase total peripheral resistance, and increase stroke volume. Long-term, MAP is increased by the kidneys through increasing fluid and ion reabsorption and decreasing fluid and ion excretion, resulting in an increase in blood volume (Mohrman & Heller, 1997). The larger blood volume increases stroke volume, and subsequently MAP.

Clinical Effects of Hypothyroidism on the Cardiovascular System

In the hypertensive population, there is a 30-50% increased prevalence of hypothyroidism compared to the general population (Fletcher & Weetman, 1998; Streeten et al., 1988). A review of published data indicates that hypertension occurs in about 22% of hypothyroid patients compared to 3-18% of the general population (Danzi & Klein, 2003). Clinical cardiovascular observations in hypothyroidism include bradycardia, narrowed pulse pressure, and diastolic hypertension (I. Klein & Ojamaa,

2001). Additionally, subclinical hypothyroidism has been reported as a risk factor for atherosclerosis and myocardial infarction (Hak et al., 2000). A recent study found that subclinical hypothyroidism and hypothyroidism were associated with a higher rate of all-cause mortality in patients with congestive heart failure (Rhee et al., 2013). In most cases, the effects of hypothyroidism on blood pressure are reversible with thyroid hormone replacement (Fletcher & Weetman, 1998; Saito & Saruta, 1994).

Effects of Thyroid Hormone on the Heart

Thyroid hormone excess has been found to increase cardiac contractility in humans (Amidi, Leon, DeGroot, Kroetz, & Leonard, 1968), rats (Korecky, Zak, Schwartz, & Aschenbrenner, 1987), and cats (Buccino, Spann, Pool, Sonnenblick, & Braunwald, 1967). The expression and subsequent production of phospholamban in cardiomyocytes is negatively transcriptionally regulated, in part, by T_3 (Kiss, Jakab, Kranias, & Edes, 1994). Phospholamban regulates the active transport of Ca^{2+} into the sarcoplasmic reticulum by the Ca^{2+} -ATPase pump (SERCA2). Conditional SERCA2 knockout mice have severely impaired diastolic and systolic function (Louch et al., 2010). Mice lacking phospholamban are observed to have increased cardiac contractility and when treated with thyroid hormones, there was no additional increase in contractility. This suggests that the relationship between cardiac contractility and thyroid hormones is primarily mediated through phospholamban activity (Kiss et al., 1998). Overall, this suggests T_3 has an important influence on systolic contraction and diastolic relaxation and provides a mechanistic explanation of clinical observations of reduced stroke volume and narrowed pulse pressure in hypothyroid patients.

Hypothyroidism reduces beta adrenergic receptors by 30-40% and increases alpha-1 adrenergic receptors in the rat heart (Bilezikian & Loeb, 1983). This finding would be consistent with clinical observations of bradycardia. Interestingly, it has been found that changes in heart rate, contractility and blood pressure with changes in thyroid hormone levels occur independently of the beta adrenergic receptor activity, indicating the complex nature of these effects. (Bachman et al.).

Thyroid hormone positively regulates the alpha myosin heavy chain and negatively regulates the beta myosin heavy chain in the heart (I. Klein & Danzi, 2007). Thyroid receptor beta and alpha 1 are involved in the negative regulation of the beta myosin heavy chain as well as the induction of the myosin heavy chain (Mansen, Yu, Forrest, Larsson, & Vennstrom, 2001). Shifts from alpha to beta myosin in transgenic mice have been found to reduce contractile function, increase systolic and diastolic ventricular volumes (Krenz & Robbins, 2004). Together these findings outline changes in myosin chain ratios associated with hypothyroidism as a potential mechanism of diastolic dysfunction observed in hypothyroidism.

Hemodynamic Effects of Thyroid Hormone

T₃ decreases vascular resistance acutely by dilating the arterioles (Park et al., 1997). This is a direct relaxational effect of T₃ on vascular smooth muscle cells (Ojamaa, Klemperer, & Klein, 1996). Clinically, T₃ has been administered to promote and facilitate increases in cardiac output and vasodilation in the pathological failing heart and normal heart in instances of advanced congestive heart failure (Hamilton et al., 1998) and in coronary bypass patients (Klemperer et al., 1995). This relaxational effect can, in part, be attributed to thyroid hormone involvement in the synthesis of nitric oxide, a vasodilator.

In hyperthyroidism, nitric oxide synthesis is enhanced and in hypothyroidism, nitric oxide synthesis is impaired through thyroid hormone regulation of nitric oxide synthase (Virdis et al., 2009).

Chronic and acute inhibition of nitric oxide synthase is reported to blunt the response of sodium excretion to increases in blood pressure, or pressure natriuresis (Guarasci & Kline, 1996). Since nitric oxide synthase inhibition is associated with decreased pressure natriuresis and nitric oxide synthase is positively regulated by thyroid hormone, augmented pressure natriuresis in hyperthyroidism and blunted pressure natriuresis in hypothyroidism would be anticipated. However, hyperthyroidism blunts the pressure natriuresis response in rats, but chronic hypothyroidism is not associated with an alteration of the pressure natriuresis response (Vargas et al., 1994). This suggests that alternative mechanisms regulating pressure natriuresis compensate (or in the case of chronic hyperthyroidism, overcompensate) for chronic changes nitric oxide synthase activity associated with altered thyroid states.

Additionally, hypothyroidism has been observed to decrease sensitivity of rat aortic smooth muscle to alpha and beta adrenergic agonists (Rahmani, Cheema, Sen, Peoples, & Riley, 1987; Rioux & Berkowitz, 1977). As the dominant effect of adrenergic receptors in vascular smooth muscle is to constrict, the decrease in adrenergic sensitivity resulting from hypothyroidism should have a vasodilatory effect and decrease resistance. However, increased arterial stiffness and a related increase in systemic vascular resistance and a subsequent increase in afterload are observed in hypothyroidism (Obuobie et al., 2002). Increased afterload and systemic vascular resistance should result in an increase in blood pressure.

In hyperthyroidism, the decrease in systemic vascular resistance leads to a fall in effective arterial filling volume, causing an increase in renin release and activation of the renin-angiotensin system (Resnick & Laragh, 1982). An activation of the renin-angiotensin system stimulates renal sodium absorption and a subsequent increase in plasma volume (I. L. Klein, G.S., 2000). Additionally, thyroid hormone stimulates erythropoiesis (Dainiak, Hoffman, Maffei, & Forget, 1978). In hyperthyroidism, the combination of an increase in plasma volume and erythropoiesis results in an overall increase in blood volume and preload. The opposite is true in a hypothyroid state and the net effect of decreased preload and blood volume is a direct decrease in cardiac output (I. Klein & Danzi, 2007). The combined direct effects of changes in preload, contractility, heart rate and blood volume result in a 50-300 percent increase in cardiac output in hyperthyroid individuals, compared to euthyroid counterparts and a 30-50 percent decrease in cardiac output in hypothyroid individuals compared to euthyroid counterparts (I. Klein & Danzi, 2007).

Thyroid Hormone in Nervous Control of the Cardiovascular System

In addition to local tissue effects of thyroid hormone in the cardiovascular system, recent research implicates thyroid hormone involvement in central nervous control of cardiac function (Fliers, Klieverik, & Kalsbeek, 2010). Injection of thyroid hormone into the hypothalamus of rats inhibits the AMPK pathway and inhibits sympathetic nervous system activity (Lopez et al., 2010), though studies of chronic inhibition of thyroid activity indicate decreased response to sympathetic stimulation (Bilezikian & Loeb), indicating that chronic vs. acute changes in thyroid state and local thyroid levels determine the ultimate effect on sympathetic activity and responsiveness to sympathetic

stimulation. Recently, a group of thyroid hormone sensitive neurons in the anterior hypothalamus involved in cardiovascular regulation have been observed in decreased numbers in mice with defective TR α 1 receptors. These mice were also observed to have a permanent increase in blood pressure even after the administration of T₃; indicating that developmental thyroid signaling is critical in the establishment of cardiovascular mechanisms involved in the regulation of blood pressure (Mittag et al., 2013).

Thyroid Function in the Rat Model of Hypertension

The Spontaneously Hypertensive Rat (SHR), a model of essential hypertension, has altered thyroid function. A study of thyroid function in SHRs found plasma and pituitary TSH were significantly elevated and plasma T₄ was significantly lower in the SHR when compared to normotensive WKY rats. Plasma T₃, however, was found to be normal. The monoiodotyrosine/diiodotyrosine ratio (MIT/DIT ratio) was found to be elevated in the SHR, suggesting an abnormality in thyroidal synthesis of thyroid hormones. Consistent with this idea, abnormalities in hydrolytic enzymes and thyroglobulin were reported in SHRs (Kojima, Kubota, Sato, Yamada, & Harada, 1976). Several studies report elevated thyroid secretion rates, though the same studies yield equivocal results on iodine uptake and the rate of thyroidal radioactivity release (Fregly, 1975; Yamabe, 1970).

Adult offspring of gestationally hypothyroid normotensive rats are reported to have elevated systolic, diastolic, and mean arterial pressure when compared with euthyroid counterparts (Santos et al., 2012). Given the finding that SHRs are hypothyroxinemic in comparison to normotensive strains, hypertension in the SHR may be partially attributable to gestational hypothyroidism.

Most interestingly, radioactive thyroid ablation and thyroparathyroidectomy of 4 week old rats have been found to prevent the onset of hypertension in SHRs. Moreover, thyroid hormone replacement in these animals was found to trigger a complete restoration of hypertension in both studies. (Aoki, 1963b; Rioux & Berkowitz, 1977). However, Rioux and Berkowitz found that thyroidectomy at 10 weeks of age did not significantly reduce already present hypertension, though it prevented further development of hypertension. Notably, despite the maintenance of established hypertension in rats thyroidectomized at 10 weeks, heart rate was severely reduced. This suggests that thyroid hormone is required for the development of elevated blood pressure, but removal of thyroid hormone does not function to reverse an established elevation in blood pressure. It is unclear whether thyroid hormone is generally responsible for an increased baseline in blood pressure in all rats, or whether a specific abnormality in thyroid hormone-mediated mechanisms in the SHR is responsible for the development of hypertension, as the effect of thyroidectomy on blood pressure has not been compared between SHRs and a normotensive strain.

Materials and Methods

Rat Strains

Spontaneously Hypertensive Rats (SHR) and Wistar Kyoto (WKY) Rats from MNSU's in house breeding program were used. They are descendants of rats supplied by both Charles River Laboratories and Taconic.

Treatment

At four weeks of age, male rats were weaned and divided into two test groups, euthyroid (control group) and hypothyroid treatment. Control rats were administered tap water from MNSU's animal care facility. Hypothyroid rats were administered 0.05% methimazole and 1.0% perchlorate in drinking water from MNSU's animal care facility. All rats were fed the standard LabDiet 5001 Rodent Diet. At postnatal week 14, a subset of hypothyroid rats were removed from treatment and monitored to week 17 (WKY Hypothyroid, SHR Hypothyroid (n=4 for each group)). A subset of control rats continued to participate in the study through week 17 (WKY Control, SHR Control (n=4)). This subset of rats will be referred to as the "recovery subset".

Water and Mass Measurement

To determine that there was not a strain bias in water consumption between treatment groups, water consumption was measured every two days for the first two weeks of treatment. Mass was measured using a scale (US-BenchTop-PRO 200g + 0.1g balance). Water consumption was measured by cage with each cage housing two rats. Individual rats were weighted every two days and water consumption for each two day period was divided by the total weight of the two rats. (WKY Control (cages=5), WKY Hypothyroid (cages=5), SHR Control (cages=4), SHR Hypothyroid (cages=4)).

Weekly mass was measured using a US-BenchTop-PRO 200g + 0.1g balance (WKY Control (n=17), WKY Hypothyroid (n=17), SHR Control (n=14), SHR Hypothyroid (n=15)) from week 4 to week 14. Mass was also measured at week 17, the end of the recovery period, in the recovery subset of animals.

Serum Collection and Measurement

To confirm thyroid insufficiency in treatment rats, blood was collected at postnatal week 15 (WKY Control, WKY Hypothyroid, SHR Control, SHR Hypothyroid (n=6/group)).

To confirm thyroid hormone recovery, blood was collected at postnatal week 17 in the recovery subset (WKY Control, WKY Hypothyroid, SHR Control, SHR Hypothyroid (n=4/group)). The rats were first anesthetized in an anesthetic chamber using isoflurane (3% in oxygen) followed by right ventricular puncture using a 22g needle and 10 mL syringe to collect blood. Blood was placed into a serum separator test tube, allowed to coagulate for 20 minutes, and then centrifuged at 1100-2000 RCF for 10 minutes. Serum was placed into microcentrifuge tubes and frozen at -18 degrees Celcius until assay.

Serum T₄ was measured using Diagnostic Automation competitive enzyme-linked immunosorbant assay (ELISA) test kits. 25 µL of 0, 0.05, 1, 2, 5, 10, 15, and 25 µg/dL standards and serum samples were pipetted into coated wells. 100 µL of working conjugate reagent was pipetted into each well and mixed thoroughly for 30 seconds, then incubated at room temperature for 60 minutes. Following incubation, well contents were removed and rinsed 5 times with distilled water. 100 µL of TMB reagent was pipetted into each well and contents were gently mixed for 5 seconds, then incubated in the dark for 20 minutes. 100 µL of stop solution was pipetted into each well and wells were gently mixed for 30 seconds. Absorbance was read at 450 nm with a Thermo Scientific Multiskan Spectrum Microplate Photometer. Standard values were used to generate a standard curve and concentration values were interpolated for serum samples using the standard curve.

Occlusion Tail-Cuff Photoplethysmography

Starting at postnatal week 4, systolic arterial pressure (SAP) was recorded weekly in a subgroup of rats via tail-cuff photoplethysmography (WKY Control (n=10), WKY Hypothyroid (n=9), SHR Control (n=8), SHR Hypothyroid (n=10)). Rats were carefully placed in clear plastic restraining chambers to limit movement during data collection. Rats were placed under a heat lamp to facilitate clear pulse detection, and IITC Life Science tail-cuff photoplethysmography sensors were placed on the rats' tails. BioPac software was used to interface between the IITC plethysmography units and a computer, which recorded pulse and pressure. Once a pulse was detected, the tail cuff was inflated to 300 mmHg, or until a pulse wave was no longer detected. The tail cuff was then slowly deflated until the pulse was again detected. The blood pressure at the reappearance of the pulse wave is equal to systolic arterial pressure. This process was repeated for a total of six pressure readings. Rats were then returned to their housing. The last three clear systolic pressures were averaged for each rat to determine the weekly SAP value. This procedure was continued to postnatal week 15.

Remote Monitoring of Blood Pressure

At postnatal week 10, a subgroup of rats was implanted with remote monitoring blood pressure transmitters to more accurately measure unstressed blood pressure. Rats greater than 150 grams at the time of implant were implanted with Data Sciences International TA11PA-C40 PhysioTel transmitters (WKY Control (n=8), WKY Hypothyroid (n=6), SHR Control (n=6)). Due to growth retardation associated with hypothyroid treatment, some rats were less than 150 grams at the time of implant, and therefore were implanted with much smaller Data Sciences International TA11PA-C10 PhysioTel transmitters (WKY Hypothyroid (n=1), SHR Hypothyroid (n=7)).

For implantation, rats were placed in an anesthetic chamber and anesthetized with isoflurane (3% in oxygen). Rats were then removed from the chamber, a mask was placed over the nose and mouth, and isoflurane anesthesia was maintained at an appropriate depth. Sterile techniques were followed for all surgical procedures. The inner surface of the thigh was shaved and scrubbed with chlorhexidine, a disinfectant scrub. The rat was placed on a heating pad, temperature was recorded rectally, and rectal temperature was maintained at 37 degrees Celsius by adjusting the heating pad control. An inguinal incision was made, the femoral artery was gently isolated, and the catheter from the remote monitoring device was inserted into a branch of the femoral artery and advanced into the descending aorta. The C40 transmitter was placed subcutaneously on the rat's side. The C10 transmitter was placed subcutaneously on the ventral surface of the abdomen. The catheter was tied in place with 4-0 silk, and the skin closed with 4-0 nylon. The rat received an injection of Rimadyl (carprofen) (10 mg/kg) to control post-op pain. The rat was allowed to recover for at least 5 days before data collection began.

Data was collected from postnatal week 11 to postnatal week 14 (WKY Control (n=8), WKY Hypothyroid (n=7), SHR Control (n=6), SHR Hypothyroid (n=7)). The transmitters sent a radio signal to a receiver attached to a computer. Blood pressure was monitored in 10 second segments, once an hour, for 48 successive hours out of each week. Batteries were be shut off during the days the rats were not monitored to conserve battery life. Batteries were turned off or on by waiving a magnet over the surface of the rat.

Stress Test

As a measure of sympathetic response, the recovery subset of rats (WKY Control, WKY Hypothyroid, SHR Control, SHR Hypothyroid (n=4 for each group)) was subjected to a stress test at weeks 14 and 17. Heart rate and pressure were sampled continuously via remote transmitter for 50 seconds in undisturbed rats for a baseline recording. The rat was then placed in a restrainer and the proximal tail was held while heart rate and blood pressure were sampled continuously for 50 seconds. The rat was then returned to its housing. Stress response was determined as a measure of change from baseline average to stressed average in heart rate and pressure.

Na⁺ Excretion

Pressure natriuresis was measured acutely in a subgroup of rats (WKY Control (n=6), WKY Hypothyroid (n=6), SHR Control (n=6), SHR Hypothyroid (n=5)). The rat was anesthetized using isoflurane anesthetic gas (3% in oxygen) and administered Inactin (thiobutabarbital) (100 mg/kg) intraperitoneally once anesthetized. The ventral abdomen and neck were shaved. The rat was placed on a heating pad, temperature was recorded rectally, and rectal temperature was maintained at 37 degrees Celsius by adjusting the heating pad control. An incision was made on the ventral neck and a breathing tube (PE 240) was inserted into the trachea, a cannula (PE 50) was inserted into the jugular vein for saline infusion (12 mL/kg/hr) to maintain fluid balance, and a cannula (PE 50) was inserted into the carotid artery and connected to a blood pressure transducer which was connected to BIOPAC hardware which interfaced with a computer for recording blood pressure and heart rate. The abdomen was opened, and ligation sutures were pre-placed around the mesenteric and celiac arteries, and the lower aorta, caudal to the renal (kidney)

arteries. For urine collection, a tube (PE 240) was placed into the bladder lumen and stabilized with a purse string suture.

After a 30-minute period of equilibration, blood pressure was recorded continuously on the computer. Urine was gently aspirated from the bladder tube and bladder at the end of each period, measured, and stored at 3 degrees Celsius until analysis. A baseline fifteen-minute urine sample was collected from all rats in both groups. Following the baseline period, the artery ligation sutures were tied off, which caused a rise in blood pressure. Urine and blood pressure readings were collected for three additional fifteen-minute periods. Urine samples were measured and diluted to 0.3 mL. 1:100 dilutions of samples were prepared with a Jenway Series 7 Diluter. Sodium in urine samples was measured using a Jenway PFP7 Flame Photometer, and pressure natriuresis was compared within each strain between control and hypothyroid groups.

Data Analyses

Repeated measures two-way ANOVAs followed by a Bonferroni post hoc tests were used to determine if there was a significant effect of treatment (hypothyroid or control) or strain (SHR or WKY) on mean arterial pressure, diastolic pressure, systolic pressure, pulse pressure and heart rate. Two separate repeated measures two-way ANOVAs were used for data for weeks 11-14 of treatment and the recovery subset through week 17.

Weekly blood pressure measurements were normalized by calculating the difference between the actual pressure, and the group mean. A repeated measures two-way ANOVA, followed by a Bonferroni post hoc test was used to determine if there was a significantly difference between strains in hypothyroid effects on mean arterial

pressure, diastolic pressure, systolic pressure, pulse pressure and heart rate. Weeks 11-14 of treatment, and the recovery subset through week 17 were analyzed separately.

A one-way ANOVA followed by a Bonferroni post hoc test was used to determine if there was a significant effect of strain or treatment on serum T₄ at weeks 14 and 17.

A two-way ANOVA followed by a Bonferroni post hoc test was also used to determine if there was a significant effect of strain or treatment on urine volume excretion or Na⁺ excretion when blood pressure was raised.

Tertiles of activity were determined for each strain/treatment group. Mean diastolic arterial, systolic arterial, mean arterial, and pulse pressures and heart rate were determined for high, low, and medium tertiles. For each animal, low means were subtracted from high means to determine and compare differences in pressure and heart rate base on activity. Repeated measures two-way ANOVAs followed by a Bonferroni post hoc tests were used to determine if there was a significant effect of treatment (hypothyroid or control) or strain (SHR or WKY) on differences between high and low activity values for mean arterial pressure, diastolic pressure, systolic pressure, pulse pressure and heart rate. Data for weeks 11-14 of treatment and the recovery subset through week 17 were analyzed separately.

Results

Serum T₄

Figure 1 summarizes mean serum T₄ for animals sacrificed at postnatal week 14 and postnatal week 17. Serum T₄ was significantly lower in WKY hypothyroid rats and

SHR hypothyroid rats compared to their respective controls at week 14 as well as significantly lower T₄ in SHR controls than in WKY controls at week 14. No significant differences were detected between any groups in the recovery subset sacrificed at week 17.

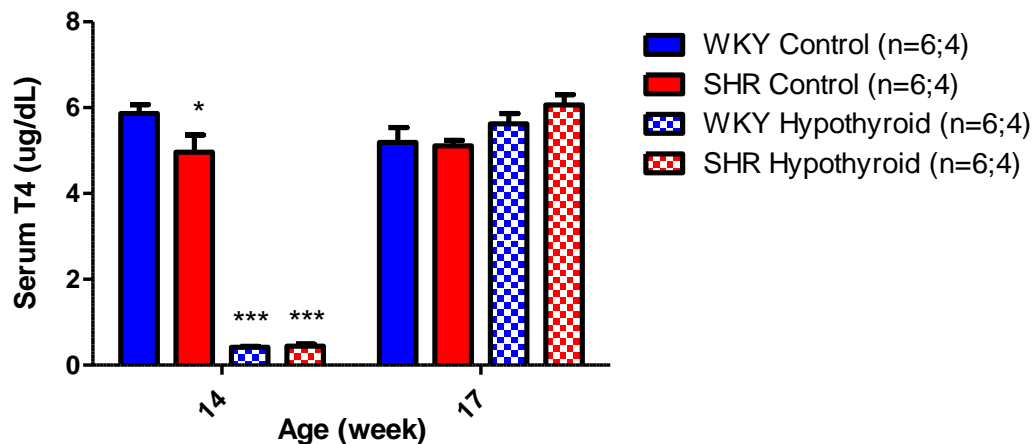


Figure 1. The effect of strain and treatment on serum T₄ as measured by ELISA. Bars represent mean serum T₄ ± standard error at postnatal weeks 14 and 17. *p<0.05 between SHR controls and WKY controls ***p<0.001 when hypothyroid rats are compared to control rats of the same strain. (n=week 14;week 17)

Weights

Figure 2 summarizes mean weekly weights at postnatal week 4 through 14. WKY hypothyroid rats and SHR hypothyroid rats had significantly lower weights compared to their respective controls from week 6 through week 14.

Figure 3 summarizes mean weight gain between week 14 and 17 in the recovery subset. SHR control rats experienced a significantly lower weight gain compared to WKY hypothyroid rats. No significant differences were detected between any other groups.

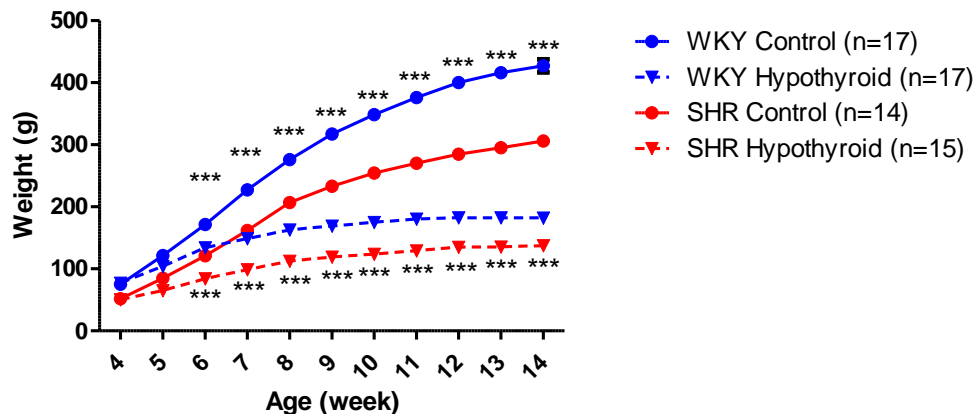


Figure 2. The effect of strain and treatment on weight. Bars represent mean weight \pm standard error at postnatal weeks 4-14. *** $p < 0.001$ when hypothyroid rats are compared to control rats of the same strain.

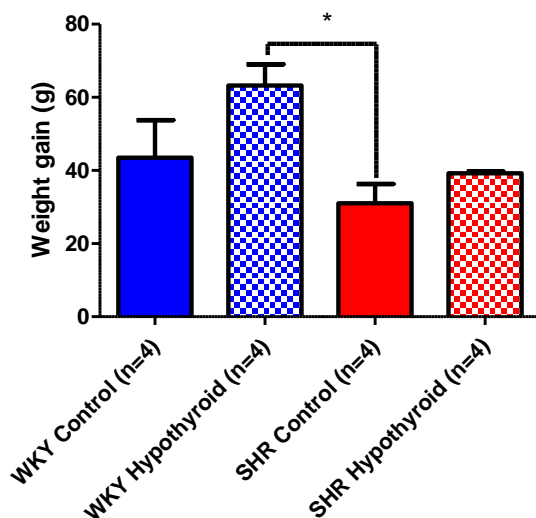


Figure 3. The effect of strain and treatment on weight gain between weeks 14 and 17 in the recovery subset. Bars represent mean weight gain \pm standard error. * $p < 0.05$ between SHR control and WKY hypothyroid rats.

Systolic Arterial Pressure by Tail-Cuff Photoplethysmography

Figure 4 summarizes mean weekly systolic arterial pressures at postnatal week 4 through 15 as measured by tail-cuff photoplethysmography. SHR hypothyroid rats had significantly lower systolic arterial pressures compared to SHR controls weeks 6-15.

WKY hypothyroid rats had significantly lower systolic arterial pressures compared to WKY controls at weeks 5, 7, 11-13. WKY treatment groups exhibited a significantly lower difference in systolic arterial pressure than that between SHR treatment groups at weeks 4, 8, 9, 11, and 13-15.

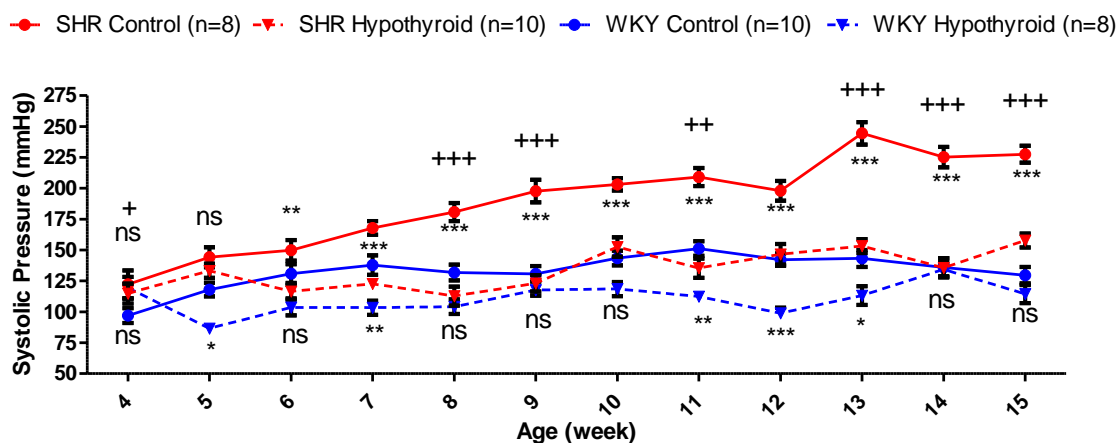


Figure 4. The effect of strain and treatment on systolic arterial pressure as measured by tail-cuff photoplethysmography. Bars represent mean systolic arterial pressure \pm standard error at postnatal weeks 4-15. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when hypothyroid rats are compared to control rats of the same strain. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$ when the magnitude of difference between control and hypothyroid rats is compared between SHR and WKY strains.

Pressure and Heart Rate by Remote Transmitter

Figure 5 summarizes mean weekly mean arterial pressures at postnatal week 11 through 17 as measured by remote transmitter. Both the SHR and WKY hypothyroid rats had significantly lower mean arterial pressures compared to their respective controls weeks 11-14. SHR hypothyroid rats in the recovery subset had significantly lower mean arterial pressures compared to SHR controls weeks 15-17. WKY hypothyroid rats in the recovery subset had significantly lower mean arterial pressures compared to WKY controls at weeks 15-17. In the hypothyroid SHR, MAP decreased significantly during recovery, when compared to week 14. No significant differences were found between strains in mean arterial pressure changes in response during treatment or recovery.

Figure 6 summarizes mean weekly systolic arterial pressures at postnatal week 11 through 17 as measured by remote transmitter. Both the SHR and WKY hypothyroid rats had significantly lower systolic arterial pressures compared to their respective controls weeks 11-14. Both the WKY and SHR hypothyroid rats in the recovery subset had significantly lower systolic arterial pressures compared to their respective controls weeks 15-17. No significant differences were found between strains in systolic arterial pressure response during treatment or recovery.

Figure 7 summarizes mean weekly diastolic arterial pressures at postnatal week 11 through 17 as measured by remote transmitter. Diastolic pressure was not significantly different in SHR hypothyroid rats compared to SHR controls weeks 11-14, but WKY hypothyroid rats had significantly lower diastolic arterial pressures compared to WKY controls at weeks 11-14. SHR hypothyroid rats in the recovery subset had significantly lower diastolic arterial pressures compared to SHR controls weeks 16-17, but diastolic pressure, although remaining low, was not significantly different in WKY hypothyroid rats in the recovery subset compared to WKY controls at weeks 15-17. When compared to SHR, WKY rats exhibited a significantly greater change in diastolic arterial pressure in response to treatment weeks 11-14. In hypothyroid SHR, diastolic pressure decreased significantly during recovery, when compared to week 14. No significant differences were found between strains in the change in diastolic arterial pressure in response to treatment withdrawal weeks 15-17.

Figure 8 summarizes mean weekly pulse pressures at postnatal week 11 through 17 as measured by remote transmitter. Both the WKY and SHR hypothyroid rats had significantly lower pulse pressures compared to their respective controls weeks 11-14.

SHR hypothyroid rats in the recovery subset had significantly lower pulse pressures compared to SHR controls at week 15. WKY hypothyroid rats in the recovery subset had significantly lower pulse pressures compared to WKY controls at week 17. SHRs exhibited a significantly greater response of pulse pressure to treatment compared to WKY rats weeks 11-14. The recovery subset of SHRs exhibited a significantly greater change in pulse pressure in response to treatment compared to the recovery subset of WKY rats at week 15. In the hypothyroid SHR, pulse pressure increased significantly during recovery when compared to week 15.

Figure 9 summarizes mean weekly heart rate at postnatal week 11 through 17 as measured by remote transmitter. Both the WKY and SHR hypothyroid rats had significantly lower heart rates compared to their respective controls weeks 11-14. WKY rats exhibited a significantly greater change in heart rate in response to treatment compared to SHRs weeks 12-14. No significant differences were found between strains in response of heart rate to treatment withdrawal weeks 15-17.

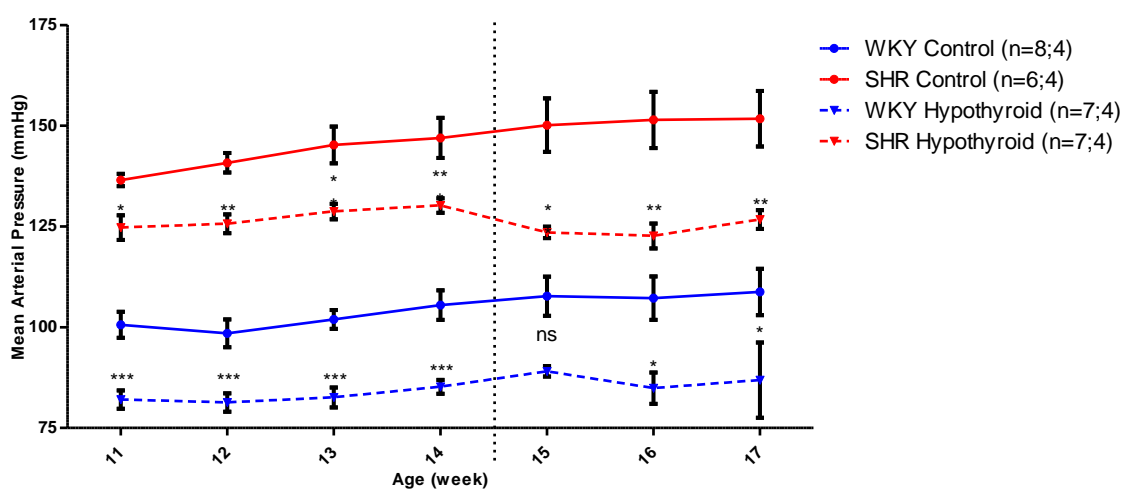


Figure 5. The effect of strain and treatment on mean arterial pressure as measured by remote transmitter. Bars represent mean, mean arterial pressure \pm standard error at postnatal weeks 11-17. * $p < 0.05$, ** $p < 0.01$,

*** $p < 0.001$ when hypothyroid rats are compared to control rats of the same strain. (n=week 11-14; week 15-17)

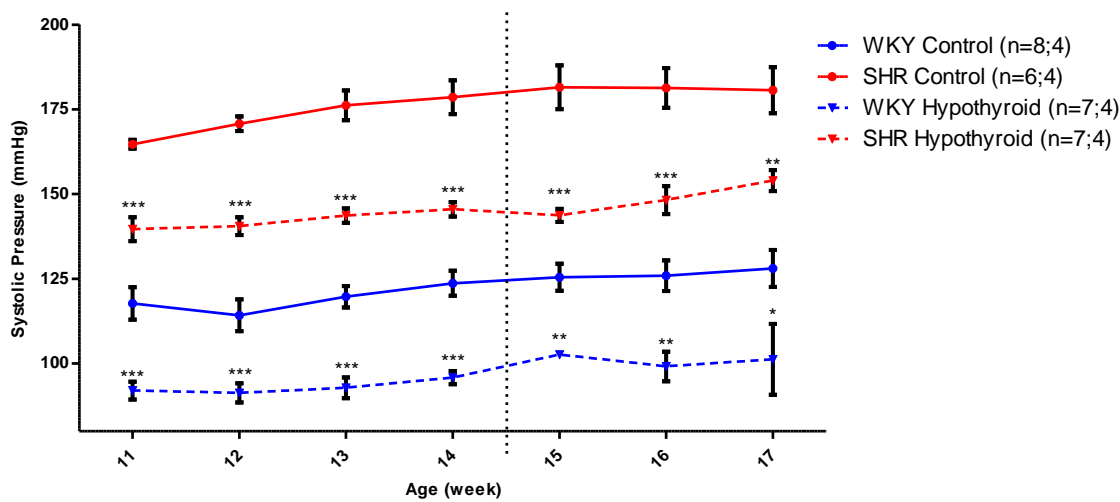


Figure 6. The effect of strain and treatment on systolic arterial pressure as measured by remote transmitter. Bars represent mean systolic arterial pressure \pm standard error at postnatal weeks 11-17. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when hypothyroid rats are compared to control rats of the same strain. (n=week 11-14; week 15-17)

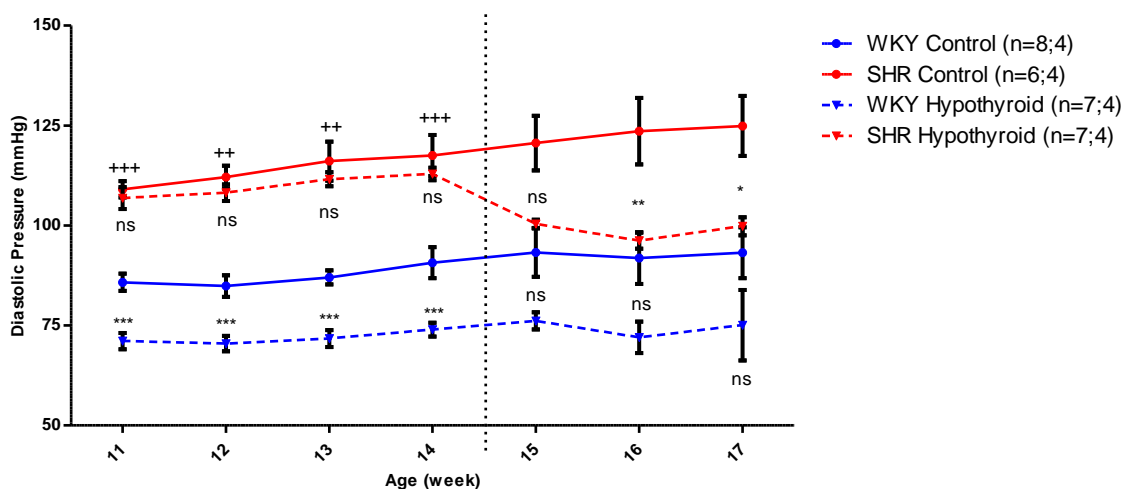


Figure 7. The effect of strain and treatment on diastolic arterial pressure as measured by remote transmitter. Bars represent mean diastolic arterial pressure \pm standard error at postnatal weeks 11-17. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when hypothyroid rats are compared to control rats of the same strain. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$ when the magnitude of difference between control and hypothyroid rats is compared between SHR and WKY strains. (n=week 11-14; week 15-17)

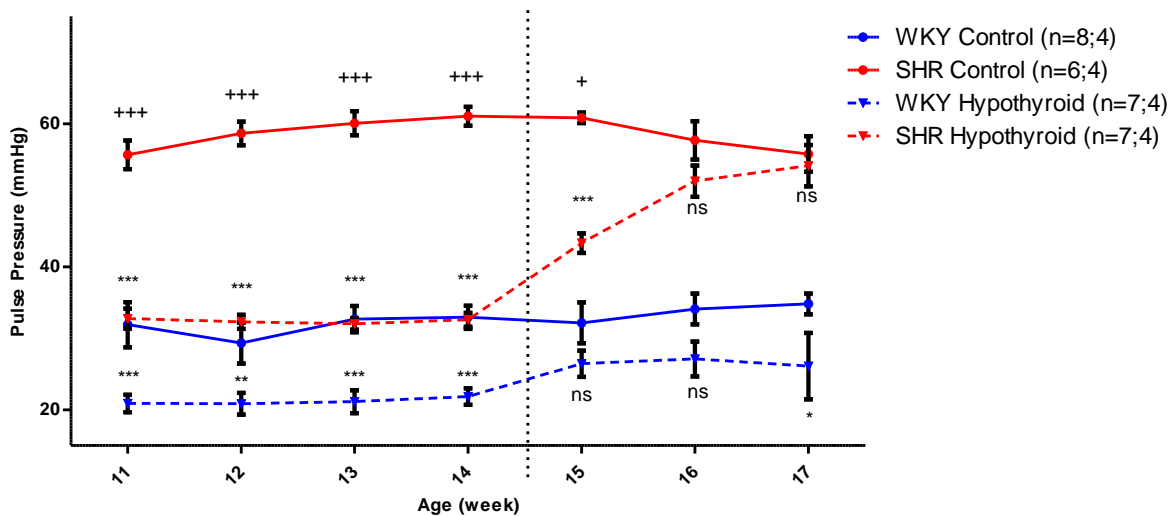


Figure 8. The effect of strain and treatment on pulse pressure as measured by remote transmitter. Bars represent pulse pressure \pm standard error at postnatal weeks 11-17. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when hypothyroid rats are compared to control rats of the same strain. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$ when the magnitude of difference between control and hypothyroid rats is compared between SHR and WKY strains. (n=week 11-14; week 15-17)

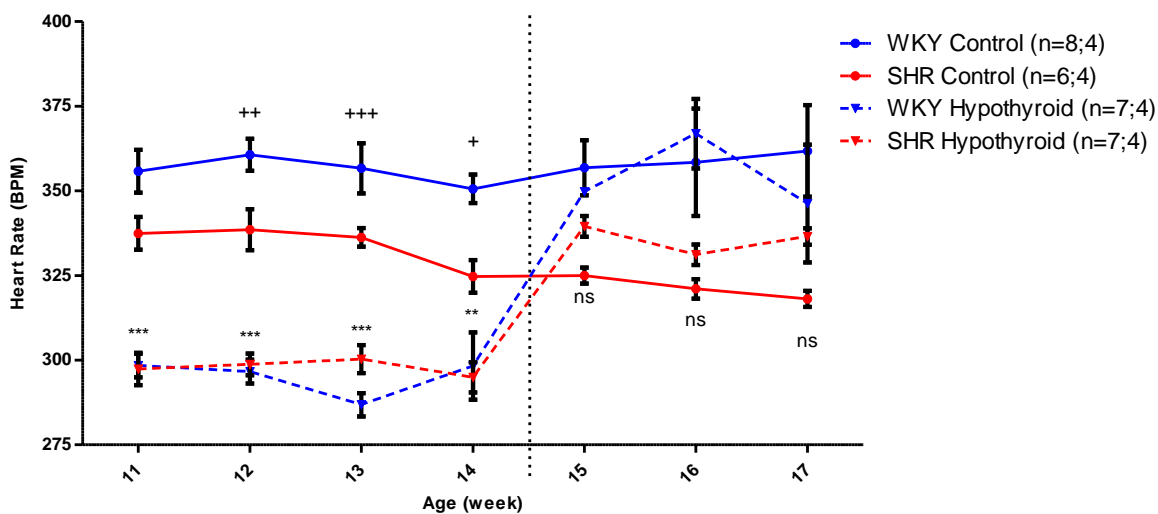


Figure 9. The effect of strain and treatment on heart rate as measured by remote transmitter. Bars represent mean heart rate \pm standard error at postnatal weeks 11-17. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when hypothyroid rats are compared to control rats of the same strain. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$ when the magnitude of difference between control and hypothyroid rats is compared between SHR and WKY strains. (n=week 11-14; week 15-17)

Activity Differences

Figure 10 summaries mean difference between heart rate at high and low tertiles of activity as measured by remote transmitter. Hypothyroid SHRs and hypothyroid WKYs had a significantly blunted response of heart rate to increased activity compared to their respective controls weeks 11-14. No significant differences in heart rate response to increased activity were found between any groups at weeks 15-17.

No significant differences were detected between any groups in mean difference between high and low activity for parameters other than heart rate.

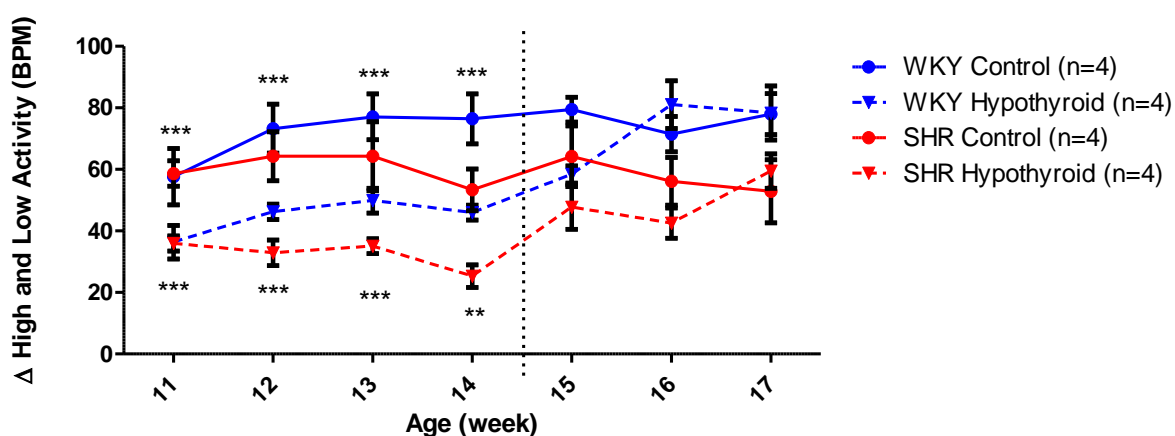


Figure 10. The effect of strain and treatment on difference between heart rate at high activity and low activity at weeks 11-17 as measured by remote transmitter. Bars represent mean difference between high activity and low activity \pm standard error at postnatal weeks 11- 17. ** $p < 0.01$, *** $p < 0.001$ when hypothyroid rats are compared to control rats of the same strain.

Stress Test

Figure 11A summarizes mean differences between resting and stressed systolic arterial pressure as measured by remote transmitter. SHR controls had a significantly greater increase in systolic arterial pressure with stress than WKY controls at weeks 14 and 17, and a significantly greater increase in systolic arterial pressure with stress than SHR hypothyroid rats at week 14. During recovery at week 17, SHR hypothyroid rats had

a significantly greater increase in systolic arterial pressure with stress than WKY hypothyroid rats.

Figure 11B summarizes mean difference between resting and stressed pulse pressure as measured by remote transmitter. SHR controls had a significantly greater increase in pulse pressure with stress than WKY controls at weeks 14 and 17, and a significantly greater increase in pulse pressure with stress than SHR hypothyroid rats at week 14.

No differences were found between any groups in the effects of stress on heart rate, mean arterial pressure, or diastolic pressure.

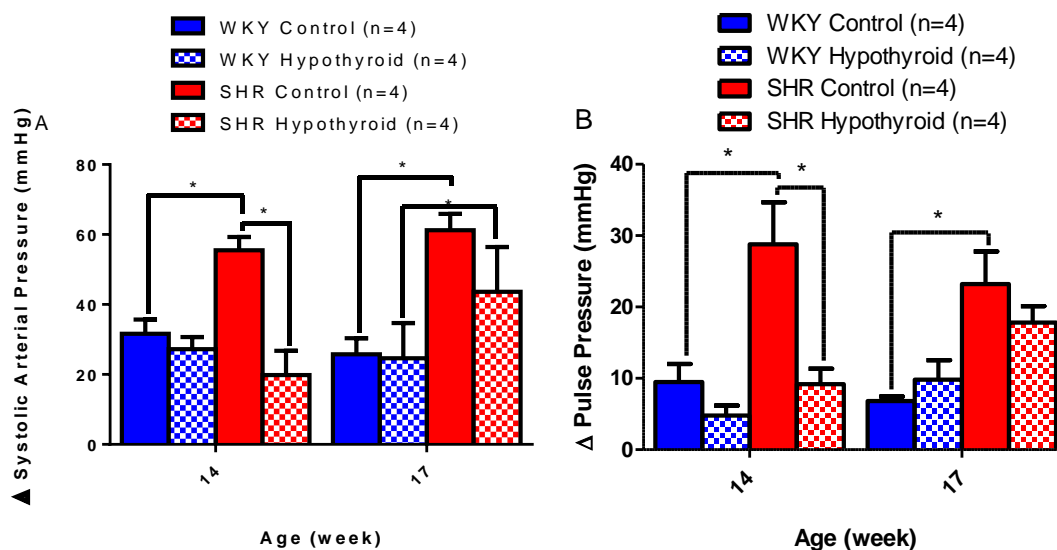


Figure 11. The effect of strain and treatment on difference between resting and stressed systolic arterial pressure (A) and pulse pressure (B) as measured by remote transmitter. Bars represent mean systolic arterial pressure (A) and pulse pressure (B) \pm standard error at postnatal weeks 14 and 17. * $p < 0.05$

Pressure Natriuresis

Figure 12 summarizes mean Na^+ excretion at four 15 minute periods at postnatal week 15 as measured by flame photometer. WKY hypothyroid rats had significantly

higher pressure natriuresis compared to WKY controls periods 2-4, and sodium excretion was greater than baseline values during period 4.

No significant differences were found between any groups in urine volume excretion.

Figure 13 summarizes mean arterial pressure at eight 7.5 minute intervals at postnatal week 15 as measured by BIOPAC hardware. No significant differences in mean arterial pressure were found between WKY hypothyroid and SHR hypothyroid rats and their respective controls.

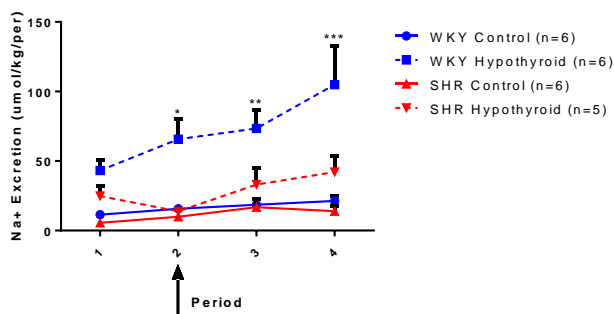


Figure 12. The effect of strain and treatment on Na⁺ excretion as measured by flame photometer. Bars represent mean Na⁺ excretion \pm standard error at each 15 minute period at postnatal week 14. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when WKY hypothyroid rats are compared to WKY control rats. Arrow represents ligation of celiac and mesenteric arteries and abdominal aorta caudal to the kidneys.

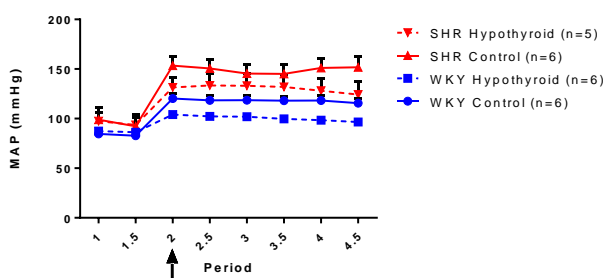


Figure 13. The effect of strain and treatment on pressure response as measured by BIOPAC hardware. Bars represent mean, mean arterial pressure \pm standard error at 7.5 minute intervals at postnatal week 14. Arrow represents ligation of celiac and mesenteric arteries and abdominal aorta caudal to the kidneys.

Discussion

Induction of hypothyroidism in experimental animals with methimazole and perchlorate treatment was confirmed by measuring a reduction in total serum T₄. Furthermore, treated animals exhibited severe growth retardation, an effect consistent with overt developmental hypothyroidism. When treatment was withdrawn, serum T₄ was normalized and weight was gained at a rate that was similar to their respective controls, suggesting of a return to euthyroid status.

Consistent with previous results (Aoki, 1963b; Rioux & Berkowitz, 1977), tail-cuff photoplethysmography indicated that hypothyroidism induced at weaning prevented the development of hypertension in SHR. However, in WKY rats, the effect of hypothyroidism on systolic blood pressure was minimal. Conversely, remote transmitter data showed a comparable reduction of systolic arterial pressure and mean arterial pressure in hypothyroid SHRs and hypothyroid WKY rats when compared to their respective controls. The stress test data provide a potential explanation for the discrepancy between tail-cuff photoplethysmography and remote transmitter data. SHR control rats exhibited a much greater increase in systolic arterial pressure under stressed conditions than WKY rats, but this exaggerated response was absent in the hypothyroid SHRs. Thus, during tail cuff photoplethysmography, the stressed systolic arterial pressure in the control SHRs was much higher than that of the hypothyroid SHRs, which did not exhibit an exaggerated stress response, resulting in an exaggerated difference in the systolic arterial pressures between SHR controls and SHR hypothyroid animals.

Potential explanations of the reduction of pressure in response to stress observed in hypothyroid SHRs are decreased sensitivity to adrenergic stimulation, lower blood

volume, and reduced maximum cardiac output of the heart due to morphological changes associated with hypothyroidism. Prior research indicates that the effects of thyroid hormone on the heart, in stress-free environment, are independent of the beta adrenergic receptors (Bachman et al.). Additionally, the present study found that pressure changes associated with changes in activity were normal, indicative of a normal response despite a reduction in baseline pressures. These observations support the idea that the reduction in stress-induced response of systolic blood pressure is due to morphological changes resulting in a reduction in cardiac output. However, it cannot be ruled-out that alterations in adrenergic signaling occur in the hypothyroid state; resulting in a lower the maximum sympathetic response to a stressful environment.

During treatment, diastolic arterial pressure was reduced in hypothyroid WKY animals compared to WKY controls, but hypothyroid SHRs did not display significantly lower diastolic arterial pressure compared to control SHRs. However, when treatment was withdrawn, the diastolic arterial pressures of the SHR hypothyroid rats dropped and were significantly lower than those of the SHR controls. WKY hypothyroid rats were not observed to have significantly reduced diastolic arterial pressure compared to their controls during the recovery period, but this is may be a limitation of n, as the mean and systolic arterial pressures remained reduced compared to controls during this time period.

If the reductions in mean arterial and systolic arterial pressure in the hypothyroid SHRs were due to a reduction in resistance or blood volume, we would anticipate a corresponding reduction in diastolic pressure. However, the SHR hypothyroid rats did not experience a reduction in diastolic pressure indicating that the reductions in mean arterial and systolic arterial pressure are most likely due to the reduction in stroke volume (Danzi

& Klein, 2003; Polikar, Burger, Scherrer, & Nicod, 1993) and reductions in contractility and ejection speed observed in hypothyroidism.

A potential explanation of the difference in effect of hypothyroid treatment on diastolic arterial pressure between strains could be endothelial dysfunction (Kung & Luscher) observed in SHRs. Evidence suggests that SHRs have altered endothelial function (Mirsky, Pfeffer, Pfeffer, & Braunwald). Interestingly, thyroid hormone signaling is involved in the synthesis of nitric oxide (Viridis et al.). Considering this, an impaired ability of the endothelium to release vasodilatory paracrine agents may indicate that the SHR is especially dependent on thyroid mediated nitric oxide synthesis.

Sustained reductions in systolic, diastolic, and mean arterial pressures in hypothyroid animals persisted, even after three weeks of treatment withdrawal, which is contrary to previous studies (Rioux & Berkowitz) and is indicative of lasting morphological changes induced by hypothyroidism at weaning. Studies of gestational hypothyroidism have reported permanent increases in blood pressure despite return to euthyroid status and have outlined changes in the autonomic nervous system's control of blood pressure as a cause (Mittag, Davis, Vujovic, Arner, & Vennstrom; Santos et al.). It is possible that treatment at weaning also induced changes in autonomic nervous system control of blood pressure. A decrease in blood pressure rather than increase may be due to a difference in timing of hypothyroidism.

The reductions in pulse pressure and heart rate in hypothyroid SHRs and hypothyroid WKY animals compared to their respective controls are consistent with clinical observations of reduced heart rate and pulse pressure in hypothyroid human patients. However, the reduction in pulse pressure observed clinically is typically due to

diastolic hypertension, not systolic hypotension, which was observed in hypothyroid SHR the present study. As prior clinical studies observed adult onset hypothyroidism and subclinical hypothyroidism, the difference in timing of the onset of hypothyroidism in the present study is a potential explanation for these differences. The observed differences in effect of hypothyroid treatment on pulse pressure between strains could be due to increased arterial stiffness (Safar, Chamiot-Clerc, Dagher, & Renaud) and left ventricular dysfunction (Mirsky et al.) observed in SHRs. Increased arterial stiffness in SHRs compared to WKYs provides an explanation for the high pulse pressure observed in control SHRs. Left ventricular dysfunction has been observed in hypothyroidism (Biondi et al.) and may be due to alterations in myosin chain expression that have been observed in hypothyroidism (I. Klein & Danzi). Considering that diastolic function is impaired in euthyroid SHR, SHRs may be more vulnerable to alterations in myosin chain expression.

While hypothyroid SHRs exhibited a smaller reduction in heart rate compared to WKYs, the heart rates of the two hypothyroid groups did not significantly differ. This difference in reduction in heart rate is most likely due to the lower baseline heart rate of the SHR compared to that of the WKY (van den Buuse).

A strain specific increase in pressure natriuresis was observed in WKY hypothyroid rats compared to WKY controls, but in hypothyroid SHRs, a non-significant increase in pressure natriuresis was measured. If these acute observations are representative of chronic kidney behavior, WKY hypothyroid animals may experience a reduction in blood volume compared to controls. This is consistent with the reduced blood volume observed in hypothyroidism in humans (Danzi & Klein, 2003) and may

provide another potential explanation for the strain specific reduction in diastolic arterial pressure under treatment in WKY rats.

Additionally, pressure natriuresis is related to endothelial function and endothelial function is impaired in the SHR (Mirsky et al.). Nitric oxide has known involvement in pressure natriuresis (Guarasci & Kline) and thyroid hormone signaling is involved in its synthesis (Viridis et al.). Since nitric oxide synthase inhibition is associated with decreased pressure natriuresis and nitric oxide synthase is positively regulated by thyroid hormone, augmented pressure natriuresis in hyperthyroidism and blunted pressure natriuresis in hypothyroidism would be anticipated. However, hyperthyroidism blunts the pressure natriuresis response in rats, but chronic hypothyroidism is not associated with an alteration of the pressure natriuresis response (Vargas et al., 1994) and the present study found augmented pressure natriuresis in hypothyroid WKYs compared to the controls . This suggests that alternative mechanisms regulating pressure natriuresis compensate for chronic reduction of nitric oxide synthase activity associated with hypothyroidism. Thyroid hormone's effect on the synthesis of nitric oxide may be of greater importance in the SHR, due to the underlying endothelial dysfunction, and they may lack the natriuretic compensatory mechanism that appears to be present in the WKY. This may be a potential explanation as to why hypothyroid SHRs did not experience a significant elevation in pressure natriuresis when compared to their controls. This finding also emphasizes that hypothyroidism likely induces a multifaceted effect on the cardiovascular system.

Although there is clear evidence that thyroid hormone has direct effects on the heart, vasculature and autonomic nervous control of blood pressure, hypothyroidism is a systemic condition and it cannot be ruled out that the observed effects are the result of an

indirect effect. For example, induction of hypothyroidism through thyroid inhibition increases TSH and TRH by removing negative feedback inhibition as well as disrupting other hormone pathways (Brent, 2012; Burstein, Draznin, Johnson, & Schalch; Miell et al., 1993). It is therefore possible that some effects observed in the present study are due to effects related to treatment and are not directly associated with thyroid hormone action. However, given that prior literature identifies direct and indirect action of thyroid hormone on the cardiovascular system, it is a reasonable assumption that the observations of the present study are due to direct and indirect effects of reduced thyroid hormone.

Weight gain is associated with an increase in blood pressure (Masuo, Mikami, Ogihara, & Tuck) and it is possible that effects seen in the present study were related to growth retardation induced by hypothyroidism. However, the recovery subset of rats in the present study did not experience a significant increase in systolic or mean arterial pressure, despite returning to euthyroid status and gaining weight. This observation suggests that growth retardation was not the cause of the reductions in systolic and mean arterial pressure experienced by hypothyroid animals.

In revisiting our hypotheses, we reach several conclusions. First, inhibition of thyroid gland activity resulted in a decrease in mean arterial pressure in SHR when compared to controls that was equal to the decrease found in WKY rats given the same treatment. This finding indicates abnormal thyroid hormone action following weaning is not a major factor in the development of hypertension in the SHR. However, differences in response to treatment in diastolic arterial pressure and pulse pressure between the SHR and WKY rats are indicative of strain differences of thyroid hormone action in cardiovascular system.

Second, inhibition of thyroid gland activity in SHR_s resulted in a smaller increase in systolic arterial pressure in response to acute stress when compared to euthyroid SHR_s, but no significant differences in stress response were observed between hypothyroid and control WKY_s. This indicates that hypothyroid treatment reduced the effect of sympathetic stimulation on systolic arterial pressure in SHR_s, but not in WKY rats.

Lastly, hypothyroid treatment resulted in significantly greater pressure natriuresis in WKY rats when compared to WKY euthyroid controls. However, no significant differences in increases in pressure natriuresis were found between control SHR_s and hypothyroid SHR_s. This indicates that sustained hypothyroidism beginning at weaning increases the pressure natriuresis response in WKY rats, but not SHR.

References

- Amidi, M., Leon, D. F., DeGroot, W. J., Kroetz, F. W., & Leonard, J. J. (1968). Effect of the thyroid state on myocardial contractility and ventricular ejection rate in man. *Circulation*, *38*(2), 229-239.
- Aoki, K. (1963a). Experimental Studies on the Relationship between Endocrine Organs and Hypertension in Spontaneously Hypertensive Rats. I. Effects of Hypophysectomy, Adrenalectomy, Thyroidectomy, Nephrectomy and Sympathectomy on Blood Pressure. *Jpn Heart J*, *4*, 443-461.
- Aoki, K. (1963b). Experimental Studies on the Relationship between Endocrine Organs and Hypertension in Spontaneously Hypertensive Rats. II. Effects of Various Hormones on Blood Pressure. *Jpn Heart J*, *56*, 561-576.
- Bachman, E. S., Hampton, T. G., Dhillon, H., Amende, I., Wang, J., Morgan, J. P., & Hollenberg, A. N. (2004). The metabolic and cardiovascular effects of hyperthyroidism are largely independent of beta-adrenergic stimulation. *Endocrinology*, *145*(6), 2767-2774. doi: 10.1210/en.2003-1670
- Bates, J. M., St Germain, D. L., & Galton, V. A. (1999). Expression profiles of the three iodothyronine deiodinases, D1, D2, and D3, in the developing rat. *Endocrinology*, *140*(2), 844-851. doi: 10.1210/endo.140.2.6537
- Bilezikian, J. P., & Loeb, J. N. (1983). The influence of hyperthyroidism and hypothyroidism on alpha- and beta-adrenergic receptor systems and adrenergic responsiveness. *Endocr Rev*, *4*(4), 378-388. doi: 10.1210/edrv-4-4-378

- Biondi, B., Fazio, S., Palmieri, E. A., Carella, C., Panza, N., Cittadini, A., . . . Sacca, L. (1999). Left ventricular diastolic dysfunction in patients with subclinical hypothyroidism. *J Clin Endocrinol Metab*, *84*(6), 2064-2067. doi: 10.1210/jcem.84.6.5733
- Brent, G. A. (2012). Mechanisms of thyroid hormone action. *J Clin Invest*, *122*(9), 3035-3043. doi: 10.1172/JCI60047
- Buccino, R. A., Spann, J. F., Jr., Pool, P. E., Sonnenblick, E. H., & Braunwald, E. (1967). Influence of the thyroid state on the intrinsic contractile properties and energy stores of the myocardium. *J Clin Invest*, *46*(10), 1669-1682. doi: 10.1172/JCI105658
- Burstein, P. J., Draznin, B., Johnson, C. J., & Schalch, D. S. (1979). The effect of hypothyroidism on growth, serum growth hormone, the growth hormone-dependent somatomedin, insulin-like growth factor, and its carrier protein in rats. *Endocrinology*, *104*(4), 1107-1111. doi: 10.1210/endo-104-4-1107
- Cheng, S. Y., Leonard, J. L., & Davis, P. J. (2010). Molecular aspects of thyroid hormone actions. *Endocr Rev*, *31*(2), 139-170. doi: 10.1210/er.2009-0007
- Chiueh, C. C., & Kopin, I. J. (1978). Hyperresponsivity of spontaneously hypertensive rat to indirect measurement of blood pressure. *Am J Physiol*, *234*(6), H690-695.
- Dainiak, N., Hoffman, R., Maffei, L. A., & Forget, B. G. (1978). Potentiation of human erythropoiesis in vitro by thyroid hormone. *Nature*, *272*(5650), 260-262.
- Danzi, S., & Klein, I. (2003). Thyroid hormone and blood pressure regulation. *Curr Hypertens Rep*, *5*(6), 513-520.
- Fletcher, A. K., & Weetman, A. P. (1998). Hypertension and hypothyroidism. *J Hum Hypertens*, *12*(2), 79-82.
- Fliers, E., Klieverik, L. P., & Kalsbeek, A. (2010). Novel neural pathways for metabolic effects of thyroid hormone. *Trends Endocrinol Metab*, *21*(4), 230-236. doi: 10.1016/j.tem.2009.11.008
- Fregly, M. J. (1975). Thyroid activity of spontaneous hypertensive rats. *Proc Soc Exp Biol Med*, *149*(1), 124-132.
- Gard, P. R. (1998). *Human endocrinology*. London ; Bristol, PA: Taylor & Francis.
- Guarasci, G. R., & Kline, R. L. (1996). Pressure natriuresis following acute and chronic inhibition of nitric oxide synthase in rats. *Am J Physiol*, *270*(2 Pt 2), R469-478.
- Hak, A. E., Pols, H. A., Visser, T. J., Drexhage, H. A., Hofman, A., & Witteman, J. C. (2000). Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam Study. *Ann Intern Med*, *132*(4), 270-278.
- Hamilton, M. A., Stevenson, L. W., Fonarow, G. C., Steimle, A., Goldhaber, J. I., Child, J. S., . . . Hage, A. (1998). Safety and hemodynamic effects of intravenous triiodothyronine in advanced congestive heart failure. *Am J Cardiol*, *81*(4), 443-447.
- Kiss, E., Brittsan, A. G., Edes, I., Grupp, I. L., Grupp, G., & Kranias, E. G. (1998). Thyroid hormone-induced alterations in phospholamban-deficient mouse hearts. *Circ Res*, *83*(6), 608-613.
- Kiss, E., Jakab, G., Kranias, E. G., & Edes, I. (1994). Thyroid hormone-induced alterations in phospholamban protein expression. Regulatory effects on

- sarcoplasmic reticulum Ca²⁺ transport and myocardial relaxation. *Circ Res*, 75(2), 245-251.
- Klein, I., & Danzi, S. (2007). Thyroid disease and the heart. *Circulation*, 116(15), 1725-1735. doi: 10.1161/CIRCULATIONAHA.106.678326
- Klein, I., & Ojamaa, K. (2001). Thyroid hormone and the cardiovascular system. *N Engl J Med*, 344(7), 501-509. doi: 10.1056/NEJM200102153440707
- Klein, I. L., G.S. (2000). The cardiovascular system in thyrotoxicosis. In U. R. Braverman LE (Ed.), *Werner & Ingbar's the thyroid: a fundamental and clinical text* (pp. 596-604). Philadelphia: Lippincott Williams & Wilkins.
- Klemperer, J. D., Klein, I., Gomez, M., Helm, R. E., Ojamaa, K., Thomas, S. J., . . . Krieger, K. (1995). Thyroid hormone treatment after coronary-artery bypass surgery. *N Engl J Med*, 333(23), 1522-1527. doi: 10.1056/NEJM199512073332302
- Kojima, A., Kubota, T., Sato, A., Yamada, T., & Harada, A. (1976). Abnormal thyroid function in spontaneously hypertensive rats. *Endocrinology*, 98(5), 1109-1115. doi: 10.1210/endo-98-5-1109
- Korecky, B., Zak, R., Schwartz, K., & Aschenbrenner, V. (1987). Role of thyroid hormone in regulation of isomyosin composition, contractility, and size of heterotopically isografted rat heart. *Circ Res*, 60(6), 824-830.
- Krenz, M., & Robbins, J. (2004). Impact of beta-myosin heavy chain expression on cardiac function during stress. *J Am Coll Cardiol*, 44(12), 2390-2397. doi: 10.1016/j.jacc.2004.09.044
- Kung, C. F., & Luscher, T. F. (1995). Different mechanisms of endothelial dysfunction with aging and hypertension in rat aorta. *Hypertension*, 25(2), 194-200.
- Lopez, M., Varela, L., Vazquez, M. J., Rodriguez-Cuenca, S., Gonzalez, C. R., Velagapudi, V. R., . . . Vidal-Puig, A. (2010). Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nat Med*, 16(9), 1001-1008. doi: 10.1038/nm.2207
- Louch, W. E., Hougen, K., Mork, H. K., Swift, F., Aronsen, J. M., Sjaastad, I., . . . Sejersted, O. M. (2010). Sodium accumulation promotes diastolic dysfunction in end-stage heart failure following Serca2 knockout. *J Physiol*, 588(Pt 3), 465-478. doi: 10.1113/jphysiol.2009.183517
- Mansen, A., Yu, F., Forrest, D., Larsson, L., & Vennstrom, B. (2001). TRs have common and isoform-specific functions in regulation of the cardiac myosin heavy chain genes. *Mol Endocrinol*, 15(12), 2106-2114. doi: 10.1210/mend.15.12.0735
- Masuo, K., Mikami, H., Ogihara, T., & Tuck, M. L. (2000). Weight gain-induced blood pressure elevation. *Hypertension*, 35(5), 1135-1140.
- Miell, J. P., Taylor, A. M., Zini, M., Maheshwari, H. G., Ross, R. J., & Valcavi, R. (1993). Effects of hypothyroidism and hyperthyroidism on insulin-like growth factors (IGFs) and growth hormone- and IGF-binding proteins. *J Clin Endocrinol Metab*, 76(4), 950-955. doi: 10.1210/jcem.76.4.7682563
- Mirsky, I., Pfeffer, J. M., Pfeffer, M. A., & Braunwald, E. (1983). The contractile state as the major determinant in the evolution of left ventricular dysfunction in the spontaneously hypertensive rat. *Circ Res*, 53(6), 767-778.
- Mittag, J., Davis, B., Vujovic, M., Arner, A., & Vennstrom, B. (2010). Adaptations of the autonomous nervous system controlling heart rate are impaired by a mutant

- thyroid hormone receptor-alpha1. *Endocrinology*, 151(5), 2388-2395. doi: 10.1210/en.2009-1201
- Mittag, J., Lyons, D. J., Sallstrom, J., Vujovic, M., Dudazy-Gralla, S., Warner, A., . . . Vennstrom, B. (2013). Thyroid hormone is required for hypothalamic neurons regulating cardiovascular functions. *J Clin Invest*, 123(1), 509-516. doi: 10.1172/JCI65252
- Mohrman, D. E., & Heller, L. J. (1997). *Cardiovascular physiology* (4th ed.). New York: McGraw-Hill, Health Professions Division.
- Obuobie, K., Smith, J., Evans, L. M., John, R., Davies, J. S., & Lazarus, J. H. (2002). Increased central arterial stiffness in hypothyroidism. *J Clin Endocrinol Metab*, 87(10), 4662-4666. doi: 10.1210/jc.2002-020493
- Ojamaa, K., Klemperer, J. D., & Klein, I. (1996). Acute effects of thyroid hormone on vascular smooth muscle. *Thyroid*, 6(5), 505-512.
- Park, K. W., Dai, H. B., Ojamaa, K., Lowenstein, E., Klein, I., & Sellke, F. W. (1997). The direct vasomotor effect of thyroid hormones on rat skeletal muscle resistance arteries. *Anesth Analg*, 85(4), 734-738.
- Polikar, R., Burger, A. G., Scherrer, U., & Nicod, P. (1993). The thyroid and the heart. *Circulation*, 87(5), 1435-1441.
- Rahmani, M. A., Cheema, I. R., Sen, S., Peoples, B., & Riley, S. R. (1987). The effect of hyperthyroidism and hypothyroidism on alpha 1- and alpha 2-adrenergic responsiveness in rat aortic smooth muscle. *Artery*, 14(6), 362-383.
- Resnick, L. M., & Laragh, J. H. (1982). PLasma renin activity in syndromes of thyroid hormone excess and deficiency. *Life Sci*, 30(7-8), 585-586.
- Rhee, C. M., Curhan, G. C., Alexander, E. K., Bhan, I., & Brunelli, S. M. (2013). Subclinical hypothyroidism and survival: the effects of heart failure and race. *J Clin Endocrinol Metab*, 98(6), 2326-2336. doi: 10.1210/jc.2013-1039
- Rioux, F., & Berkowitz, B. A. (1977). Role of the thyroid gland in the development and maintenance of spontaneous hypertension in rats. *Circ Res*, 40(3), 306-312.
- Safar, M., Chamiot-Clerc, P., Dagher, G., & Renaud, J. F. (2001). Pulse pressure, endothelium function, and arterial stiffness in spontaneously hypertensive rats. *Hypertension*, 38(6), 1416-1421.
- Saito, I., Ito, K., & Saruta, T. (1983). Hypothyroidism as a cause of hypertension. *Hypertension*, 5(1), 112-115.
- Saito, I., & Saruta, T. (1994). Hypertension in thyroid disorders. *Endocrinol Metab Clin North Am*, 23(2), 379-386.
- Santos, S. O., Loureiro, S. M., Alves, I. G., Jesus, C. S., Santos, P. R., Santos, M. R., . . . Badaue-Passos, D., Jr. (2012). Experimental gestational hypothyroidism evokes hypertension in adult offspring rats. *Auton Neurosci*, 170(1-2), 36-41. doi: 10.1016/j.autneu.2012.07.004
- Streeten, D. H., Anderson, G. H., Jr., Howland, T., Chiang, R., & Smulyan, H. (1988). Effects of thyroid function on blood pressure. Recognition of hypothyroid hypertension. *Hypertension*, 11(1), 78-83.
- van den Buuse, M. (1994). Circadian rhythms of blood pressure, heart rate, and locomotor activity in spontaneously hypertensive rats as measured with radio-telemetry. *Physiol Behav*, 55(4), 783-787.

- Vargas, F., Atucha, N. M., Sabio, J. M., Quesada, T., & Garcia-Estan, J. (1994). Pressure-diuresis-natriuresis response in hyperthyroid and hypothyroid rats. *Clin Sci (Lond)*, *87*(3), 323-328.
- Virdis, A., Colucci, R., Fornai, M., Polini, A., Daghini, E., Duranti, E., . . . Monzani, F. (2009). Inducible nitric oxide synthase is involved in endothelial dysfunction of mesenteric small arteries from hypothyroid rats. *Endocrinology*, *150*(2), 1033-1042. doi: 10.1210/en.2008-1112
- Werner, S. C., Manger, W. M., Radichevich, I., Wolff, M., & von Estorff, I. (1975). Excessive thyrotropin concentrations in the circulation of the spontaneously hypertensive rat. *Proc Soc Exp Biol Med*, *148*(4), 1013-1017.
- Yamabe, H. (1970). Studies on the thyroid in spontaneously hypertensive rats. I. Thyroid activity. *Jpn Circ J*, *34*(4), 233-244.