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Effects of Voluntary Exercise on Pressure-Induced Natriuresis in Hypertensive Female and Male Rats

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By

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

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Abstract

Hypertension (HT) is one of the major diseases associated with the circulatory system and remains a public health problem with a high rate of prevalence in the adult population in the USA. The pressure natriuresis mechanism which is Na+ excretion in respond to rise in blood pressure acts as a long-term regulator of arterial blood pressure. Recent studies have demonstrated that impairment of this long-term pressure natriuresis mechanism is involved in hypertension (HT) and many other renal disorders. Exercise is a nonpharmacological treatment to help control HT, and exercise has a beneficial effect on vascular health, endothelial functions, arterial stiffness, blood pressure, and VO_2 max (49). Since exercise is beneficial to hypertensive patients, it is important to understand the relationship between exercise and pressure natriuresis. The goal of this study was to determine if voluntary exercise in young spontaneously hypertensive female and male rats altered pressure natriuresis. Forty 4-week-old SHR (Twenty female and Twenty male) were randomly assigned into two treatment groups, exercise and sedentary. After 8 weeks, urine was collected during baseline, lowered, and raised renal perfusion pressures (RPPs). Urinary Na excretion was measured and pressure natriuresis curves (Na+ excretion vs RPP) were generated for each group. Exercise in female spontaneously hypertensive rats(SHRs) significantly improved the pressure natriuresis relationship, with the exercised female rats producing a greater increase in sodium excretion for any given increase in RPP. However, exercise in the males had no significant effect on the pressure natriuresis relationship. This study suggests that sex differences may play an important role in long-term pressure regulation and exercise performance in SHRs. Further understanding of the mechanisms behind this beneficial effect of exercise on pressure natriuresis may support the development of new therapeutics for hypertension.

Introduction

Hypertension and Prevalence

Hypertension is one of the most common disorders associated with the circulatory system and remains a major public health problem, with a high rate of prevalence in the adult population in the USA. Hypertension (HT) is defined as a chronic or temporary elevation of arterial blood pressure (59). Arterial pressure can be divided into systolic and diastolic pressure. Systolic pressure occurs as blood is pumped out of the heart through the aorta and pulmonary trunks. Diastolic pressure occurs as the heart rests between heartbeats, and these pressures are elevated in HT patients (59). Hypertension affects more than 1.2 billion individuals worldwide, and the 2017 medical guidelines showed that 81.9 million people depend on antihypertensive medication, while non-pharmacological interventions were recommended for only 9.4% of hypertensive patients (47). Patients with hypertension usually have several factors that can affect the results of research, including drug therapies, daily living activities, and level of stress, which makes it difficult to perform well-controlled long-term studies in human beings (77). Animal models with hypertension, such as rodents, are suitable models for study because researchers can control their diet, drugs and activity (77). The developmental period of hypertension is very short in spontaneously hypertensive rats (SHRs) when compared to humans. SHRs develop hypertension beginning at 7 weeks of age, with rapidly increasing BP with aging (77).So rat models are great model organism for study hypertension.

Health Impacts and Risk Factors

Hypertensive patients have an increased risk of stroke, heart failure, renal dysfunction, and other health-related problems (10, 54). Hypertension also increases the mortality rate of cardiovascular disease (47) and causes severe functional and structural damages to the brain, which can lead to vascular dementia (10). HT can occur due to hereditary and environmental factors. Environmental factors include too much salt intake, drinking too much alcohol, smoking, less potassium intake, stress and obesity, sedentary lifestyle, and many chronic conditions such as renal dysfunction (2, 40,44). Among environmental factors, high salt intake and high Na+ concentration in the blood is a major risk factor of getting HT. A high salt diet may induce changes in the vessel walls. High salt induce diet may also lead to alterations in collagen accumulation and increased vascular stiffness (30,68). High renal fibrosis is also observed in hypertensive subjects, however, it becomes less severe with daily exercise or aerobic training (20). Vascular structural changes, endothelial dysfunctions, renal dysfunctions, and sympathetic overstimulation (35) are major factors that lead to the pathophysiology of hypertension. Artery stiffness is also a significant risk factor in hypertension and other cardiovascular diseases (7). Other behavioral and environmental factors are also related to blood pressure. People who migrate from underdeveloped to developed areas often have increased blood pressure due to changes in nutrition, exercise activity, and stress (51).

Epidemiology - Sex Difference

Gender is one of the main risk factor and both men and women are prone to hypertension. Men are more prone to hypertension and other renal diseases than age-matched women (12,60,64). However, studies by Pinto et. al. demonstrated that the risk of hypertension increased with age for both sexes (60). Sex differences in hypertension are based on physiological factors due to aging, sex hormones, other disease conditions, and behavioral factors (4,67,60,12,51,45).

Aging is one of the main risk factors associate with hypertension. Compared to young individuals, elderly people have an increased risk of cardiovascular diseases (CVD) and renal inflammation due to physiological changes in their bodies. The systolic blood pressure (SBP) rises steadily, however diastolic blood pressure (DBP) increases to a maximum level and becomes plateaued or decreases with aging (60). The Framingham heart study reported that SBP was raised between the ages of 30-84 and DBP decreased between the ages of 60-84. (60,69).

Sex differences in hypertension are also associated with age. The rise in systolic pressure is steeper in women compared to age-matched men (60,17). A 25-year observational study in Sweden reported that (67 years) women had a higher average blood pressure than younger women, whether they were hypertensive or not. However, hypertension in men produced a greater increase in mortality risk compared to same-aged women (48). Young men are more prone to CVD and renal diseases than age-matched women (60,12,21). One study found that among 18-29-year-old white adults,1.5%of women versus 5% percent of men have hypertension (21). The death

rates were doubled among hypertensive middle-aged men compared with middle-aged women. Hypertension is more common in middle-aged men than women (4). The National Health and Nutrition Examination Surveys (NHANES III) have shown that normotensive and hypertensive young men have higher overall mean arterial pressure than women (13,56). Young women have fewer stiff arteries compared with same-aged men (68). Although an increase in artery stiffness occurs after menopause in women, rates always remain lower than in men (9,71,60,68). Higher levels of blood pressure in men compared to women increase the incidence of stroke, heart failure, and renal dysfunctions in men. Coronary artery diseases also develop in younger aged men compare to women (4). Moreover, high-density lipoprotein levels are lower in men (63,5) and this increases the risk of hypertension, coronary heart disease, stroke, heart failure, and renal disease**.** High-density lipoproteins lower the risk of hypertension and other CVD such as atherosclerosis.

The female sex hormone, estrogen, has a cardiac protective effect, and estrogen modulates vascular endothelial functions (33). A study by Beckhoff et. al. found that ovariectomized female SHR had an increased risk of hypertension (4,19). Reckelhoff et. al. demonstrated that testosterone injected into the spontaneously hypertensive rat results in a hypertensive shift in the pressure regulation (4,19).

Some forms of hypertension are more common in women. Hypertension occurs more often in type II diabetic women than men (60,17). ISH (Isolated systolic hypertension) is a form of hypertension that results in a diastolic blood pressure less than 80 mmHg, but a systolic blood pressure higher than 130 mmHg (60). The Framingham heart study found a higher prevalence of ISH in older women than in older men (60). This is due to

stiffer arteries with a wider radius or other physiological and behavioral factors. However, the higher total amount of activity and weekly vigorous activity had an inverse association with hypertension in women (5).

Lifestyle-related risk factors also vary between the sexes. According to a study by Bethany et. al. 35% of women and 29 % of men have a healthy weight (9). This might be due to a healthy diet and healthy living (9). The smoking percentage was increased in men compared to women. However, women have lower levels of physical activity compared to men (36% of women and 46% of men participate in regular physical activity) (9). Studies also reported that women have more awareness of hypertension and are more likely to report their normotensive or hypertensive BP than men. Women have more recent doctor visits compare to men. 46% of women have seen a medical professional recently compared to 30% of men (9). These factors can contribute to the sex difference in hypertension and other cardiovascular diseases. Although both men and women suffer from CVD later in their life, (5,72,21**)** there was a preventive effect of leisure-time physical activity on coronary artery disease, and hypertension, regardless of the degree of overweight or obesity (32,18,34).

Mean Atrial Pressure (MAP)

The blood pressure reading is usually marked as the systolic number over the diastolic number. Blood pressure in normotensive individuals is around 120mmHg over 80mmHg. A hypertensive patient will have high systolic blood pressure (SBP>140mmHg) and/or high diastolic blood pressure (DBP>90mmHg) (59). Mean arterial pressure (MAP) is the average arterial pressure over a single cardiac cycle

- systole, and diastole (22). MAP is influenced by cardiac output (CO) and vascular resistance (VR). Cardiac output is measured as the product of heart rate (HR) and stroke volume(SV)(22). Increased blood volume will increase the ventricular filling volume, (preload), the SV, and therefore increase CO and MAP (22) Systemic vascular resistance is primarily determined by the diameter of the blood arterioles. Decreasing the diameter of an arteriole increases vascular resistance because blood must pass through a smaller radius vessel. Short-term regulation of MAP is under baroreceptor reflex control, which uses the autonomic nervous system to affect both cardiac output and systemic vascular resistance to maintain MAP within the proper range (22). Long-term regulation is believed to be under the control of the renal system through blood volume regulation, which directly affects cardiac output (22).

Renal Pressure Natriuresis

Arthur Guyton was the first to identify the role of the renal pressure mechanism in mean arterial pressure control (54). Pressure natriuresis is a major control mechanism for the regulation of body fluid volume and long-term arterial pressure or mean arterial pressure (54). The kidneys increase Na+ elimination when blood pressure is elevated to decrease blood volume, which decreases end-diastolic volume (EDV), stroke volume (SV), and CO (59). This eventually decreases MAP. Elevations of blood pressure at the kidney initiate decreased reabsorption of sodium and water by the renal tubules, and this results in a high Na+ concentration in the urine (natriuresis)(54). Dysfunction of this mechanism leads to numerous cardiovascular and renal diseases, including hypertension (54). The SHRs are a suitable model for examining changes in pressure

natriuresis because the SHR's renal pressure natriuresis mechanism is similar to that of humans, and SHR also develop hypertension as they age.

Hormone Regulation- RAAS

The main hormone system for the regulation of intravascular volume and systemic blood pressure is the renin-angiotensin-aldosterone system (RAAS)(35). This RAAS cascade influences the kidney, lungs, vasculature, and brain (37). This system affects vascular tone, sodium retention, oxidative stress, fibrosis, sympathetic tone, and inflammation (23). The RAAS is responsible for chronic alterations in blood pressure (37). Both kidneys secrete the enzyme renin, and this is the very first step of the RAAS cascade. Renin converts angiotensinogen to angiotensin I. Angiotensin I is quickly converted to angiotensin II by angiotensin-converting enzyme (ACE) located on the lung vascular endothelium (23,16). Angiotensin II (Ang II) binds to two major receptors, type 1 (AT1) and type 2 (AT2) receptors (58). AT1R enhances anti-natriuresis and raises BP, AT2R has the opposite effect of AT1R. AT2R enhances the natriuretic response and reduces blood pressure (15). RAAS activity is essential for long-term blood pressure regulation, same as the pressure natriuresis. However, RAAS is also responsible for elevated blood pressure in certain conditions. More importantly, RAAS blocking drugs have been available and commonly used in today's medical world. Overstimulation of RAAS can contribute to many CVDs and renal dysfunctions. Inhibition of ACE or renin concentrations can inhibit the RAAS cascade. The overall goal of inhibition of the RAA activity is to improve renal output and reduce cardiovascular mortality rates (23,16). The

RAAS cascade is affected by sex hormones, however, both sexes utilize the same antihypertensive drugs (73,24).

Ang II has major effects on the kidney, adrenal gland, arterioles, and brain (37). Angiotensin II (Ang II) causes increased blood pressure by raising vascular resistance through direct vascular constriction. In addition, Ang II also stimulates extracellular protein synthesis by boosting the signaling activity (38), which may lead to renal fibrosis. High renal fibrosis is one of the risk factors for elevated blood pressure (35). The RAAS stimulates the synthesis of pro-inflammatory molecules and pro-fibrotic agents that contribute to altering the function of some organs during certain disease conditions. In rodents, ANG II blocking agents protect against neurodegeneration and promote longevity (8). Angiotensin receptor blockers are used to decrease pre-hypertension (55) However decreased levels of Ang II also decrease arterial resistance, which lowers systolic and diastolic blood pressure (20).

Although the RAAS serves as a long-term blood pressure regulator, the RAAS can be activated or overstimulated due to several conditions that lead to the development of HT. Overexpression of plasma renin (PRA) and aldosterone (ALDO) is common in obese and hypertensive people, and these increase blood volume and vascular resistance. High levels of ALDO have been associated with increased aortic and arterial stiffness which increases the systolic and diastolic blood pressures (20). In obese individuals, elevated plasma renin, angiotensin, and aldosterone levels cause vasoconstriction, and this may lead to high blood pressure (20). The RAAS also is involved in the SHR’s pathophysiology of renovascular hypertension. Aldo causes an increase in sodium reabsorption and potassium excretion at the distal tubule and

collecting duct of the nephron (16). Aldosterone stimulates the insertion of the Na^+ channel/ basolateral Na-K ATPase protein (37). An increase in Na^+ in the body leads to an increase in osmolarity and an increase in blood and extracellular fluid. This will increase blood pressure due to the large blood volume (37). Ang II also acts on the brain which stimulates thirst and the release of anti-diuretic hormone (ADH). ADH acts to increases water reabsorption in the kidney (16,37).

Natriuretic Peptides

There are 3 major natriuretic peptides found in the human body. Atrial natriuretic peptide (ANP), brain natriuretic peptide (b-type) (BNP), and urodilatin (27,31,53,62).

These peptides play an important role in intravascular blood volume and vascular tone regulation (53). ANP is primarily stored in atrial granules and becomes a mature peptide inside the circulatory system. BNP is stored in atrial granules and ventricles, and it was first found in the brain tissue (61). The kidneys produce and secrete a compound similar to ANP, called urodilatin (31,61,62). Urodilatin, first discovered in urine, is related to the ANP family and is involved with the regulation of natriuresis (61). Fielder et al studied urodilatin concentrations in intact and cardiac denervated dogs. This study found that urodilatin is primarily involved with renal Na+ regulation and has a closer relation to Na+ excretion than circulating ANP (27,31, 39, 31). The study also indicated that natriuretic peptides attenuate sympathetic nerves and contribute to hormone regulation (39). Unlike other types of ANP, urodilatin does not circulate in the blood (27,31). Natriuretic peptides are involved in the communication that occurs between cardiovascular and metabolic diseases, such as between hypertension and diabetes

(39). Urodilatin is used as a diagnostic tool for CVD and heart failure (75). Natriuretic peptide-mediated therapeutics are an important treatment for patients suffering from metabolic diseases and CVDs. Administration of ANP and ANP antagonists has demonstrated that ANP has antihypertrophic and antifibrotic functions. In some in vitro studies, rodents lacking natriuretic receptor-A (NPR-A) developed cardiac hypertrophy and fibrosis (53). Nishikimi et al found that ANP and BNP have a cardiac protective function (53).

Hypertension Treatments- Pharmacological

Prescribed antihypertensive drugs are very common in the medical world. Treatments for HT in stage 1 and stage 2 are mainly based on antihypertensive drugs. Treatments are individualized for patients who are suffering from other CVD, renal disease, or diabetes (57). The treatments also depend on the stages of hypertension and age (57). According to the American Heart Society (AHS) and the American College of Cardiology (ACC), for stage 1 HT, the systolic range remained between 130-139, or the diastolic BP range remained between 80-89. Stage 2 HT patients have systolic BP over 140 and diastolic over 90 (57). Target blood pressure is changed if other diseases conditions are present (3). For example, in patients with chronic kidney disease and diabetes, the target blood pressure will be 130/80 (3).

Some common antihypertensive drugs are Thiazide -type diuretics, calcium channel blockers, ACE inhibitors and angiotensin 2 receptor blockers (ARBs). Thiazide diuretics, such as chlorthalidone and Indapamide, are a primary treatment for HT for all ages (3,26,25,57). Calcium channel blockers have been shown to reduce cardiovascular

mortality rates (57,76). These can be used as an alternative to diuretics (76). Some of the Ca++ channel blockers act as vasodilators (57,76,14). Ace inhibitors have shown effective cardiac protection for patients with kidney diseases and proteinuria. Ramipril is one of the most common ace inhibitors (76, 29). Beta-blockers are another antihypertensive drug. However, this drug has fewer protective effects in older patients (42).

Antihypertensive drug therapies are not always successful in patients with underlying disease conditions. Diabetic patients have a high risk of getting hypertension because of altered carbohydrate metabolism (46). ACE inhibitors, angiotensin (types 1(AT1)) receptor blockers (ARBs), and calcium channel blockers are not always used to treat hypertensive patients with diabetes because antihypertensive drug usage is associated with harmful side effects. Conditions such as hyperkalemia are increased in patients with renal abnormalities, heart failure, and diabetes (57,46). Many hypertensive patients with diabetes require combined antihypertensive drug therapy to lower blood pressure (46, 43). Researchers observed that most drugs improved endothelial dysfunction. However, some drugs also improve plasma adiponectin and leptin levels as well Adiponectin has cardioprotective functions (43). Leptin is directly associated with adipose tissue and causes several cardiovascular complications. Plasma leptin levels are mainly responsible for stimulating vascular inflammation, oxidative stress, and vascular smooth muscle hypertrophy (43). Leptin levels might responsible for diseases such as atherosclerosis, which is a major risk factor associate with hypertension. On the other hand, there were many complications associate with these drugs. Because of the excessive usage of anti-hypertensive drugs, side effects, and complexity of the drug

therapy, medical professionals have attempted to find alternative treatments (57,46). Minimal costs and side effects were associated with drug-free exercise training (50).

Exercise and Cardiovascular Physiology

Health professionals, ACC, and AHS also recommend lifestyle modifications such as daily exercise, for elevated blood pressure (57, 3,40). The American College of Sports Medicine (ACSM) found that at least 30 minutes of dynamic aerobic endurance exercise and resistance exercise per day helped to reduce elevated blood pressure in humans (1,40). Aerobic and regular physical activity reduces the risk of getting hypertension and other cardiovascular diseases. Studies also found that eight weeks of resistance or endurance training similarly reduce pre-hypertensive blood pressure and improved endothelial function in pre-hypertensive patients (6). There are different exercise training strategies and different types of exercise that help to reduce arterial pressure. Human studies have shown that exercise reduces systolic, diastolic, and mean arterial pressure (28,40). Exercise training sessions enhance endothelial function and decrease systolic and diastolic blood pressure by approximately 8-17 mmHg and 6-13 mmHg, respectively (1,6). Resistance exercise leads to a reduction of resting SBP and DBP which reduces systemic and pulmonary blood pressure (6).

In addition to lowering blood pressure, these studies found that aerobic training decreased central pulse wave velocity (PWV) and plasma aldosterone (Aldo) levels. Aerobic training in obese hypertensives has decreased plasma Aldo level and it was significantly correlated with PWV reduction(20). Increased Plasma Aldo level can stimulate RAAs and increase Na reabsorption which results in hypertension. PWV is

used to measure an individual's arterial stiffness. Increase plasma aldosterone concentrations lead to greater PWV in hypertensive adults. An increase in blood pressure causes the vascular tone to increase. Exercise increases sympathetic activity, which results in increased cardiac output and an increased blood flow to the lungs (49). A recent study investigated the effect of resistance and aerobic exercise on central blood pressure and myocardial oxygen use in hypertensive and pre-hypertensive patients. The study found that exercise training reduces peripheral arterial stiffness and myocardial oxygen demand (7). Physical activity can increase blood flow to the kidneys, reduce urinary proteins, reduce renal inflammation, and inhibit fibrogenesis of the kidney (20,35). Exercise reduces cardiovascular risk factors by decreasing blood pressure, improving lipid values, and reducing oxidative stress (35).

It is clear that exercise training is beneficial to hypertensive patients, however, the exact physiological mechanism by which exercise is beneficial is unclear. Some studies supported that the effects of exercise training are due to reduced sympathetic activity and improved autonomic functions (77). Other studies suggest that exercise training is associated with neuronal plasticity in the brain area that regulates blood pressure (77). Moreover, exercise training reduces renal fibrosis in SHR and helps to prevent kidney damage (35). These studies found differences in the pathophysiology of kidney sections in SHR-sedentary and SHR-exercised groups, showing that renal fibrosis was significantly different between the SHR exercise and sedentary groups (35).

It is crucial for the understanding of hypertension to investigate the physiological mechanisms underlying this disease and the mechanisms by which exercise can mitigate these effects. Exercise training can reduce visceral fat and improve Na+

elimination by altering renal function. Exercise training increased blood flow to the renal interstitial which stimulates the pressure natriuresis mechanism. Furthermore, elevations of blood pressure at the kidney also initiate decreased reabsorption of sodium in renal tubules, and high Na+ concentration in the urine (54). Exercise also alters kidney filtration by improving capillary hydrostatic pressure and increasing fluid exit into the renal interstitium. This results in greater Na+ excretion. Moreover, physical activity improves kidney functions, glomerular filtration capacity and contributes to improve Na+ excretion in normotensive and hypertensive subjects. Most studies on exercise and hypertension focus on aerobic exercise such as walking, running, swimming, and cycling (28).

This Study

Regular exercise has been reported to have benefits for the SHRs such as decreased blood pressure, reduced oxidative stress, reduced renal inflammation, and reduced fibrogenesis. However, the mechanisms for the decrease in blood pressure are not clear. A previous study in this lab found that voluntary exercise improved pressure natriuresis in a normotensive rat (WKY). Therefore, this study was designed to investigate the effects of voluntary exercise on pressure natriuresis in a hypertensive rat, the spontaneously hypertensive rat (SHR) (35). I hypothesized that exercise training will improve the pressure-natriuresis relationship (increase urinary sodium excretion with increases in renal perfusion pressure) in exercised female and male SHR rats. Exercised rats who allowed to exercise for 8-9 weeks will excrete more sodium for any increase in blood pressure than sedentary rats (not allowed to exercise).

Methods

Exercise Protocol:

Twenty female SHR and twenty male SHR from our colony were weaned at 4 weeks of age and randomly assigned to the sedentary or exercise group. Exercised rats (10 male and 10 female) were housed individually with a 12-inch diameter stainless steel exercise wheel, with an attached wheel counter, to monitor wheel revolutions to determine the voluntary running distance. Sedentary rats (10 male and 10 female rats) were housed individually without running wheels. Due to endemic respiratory disease in the rat colony, all study rats were treated with oral antibiotics (trimethoprim-sulfa @ 50 mg/kg/day Trimethoprim and 250 mg/kg/day sulfa) to prevent lung diseases. Antibiotics were mixed with water for 10 days after assigned into groups. SHR-ex female and SHR-ex male rats were allowed to exercise for at least 8 weeks before the acute study. The number of wheel revolutions was recorded twice weekly and converted to a weekly running distance in kilometers.

Surgical Setup:

Rats were weighed, then placed in an anesthetic chamber, and anesthetized with isoflurane gas (3%) in oxygen. Once asleep, rats were dosed with Inactin (100mg/kg) and allowed 10-15 minutes for the Inactin to take effect. Rats were monitored under anesthesia to avoid breathing difficulties, cardiac depression, or insufficient anesthesia. The rat's ventral neck and abdomen, and medial region of each thigh were shaved using an electric clipper. Rats were then placed on a heating pad and a rectal

temperature probe was inserted. Rectal temperature was monitored and kept at 36ºC by adjusting the heating pad and using a heat lamp (on and off) when necessary. An incision was made in the ventral surface of the neck and a polyethylene tube was inserted into the trachea (PE 240) to assist with respiration. A saline and heparin (0.3 ml heparin-1000U/ml in 40ml saline solution) filled catheter (PE 50 attached to an adapter and a 3 way-stopcock) was inserted and tied into the isolated carotid artery. A pressure transducer in the catheter was connected to a computer for the measurement of blood pressure. A saline filled catheter was inserted into the isolated jugular vein and connected to an infusion pump, which infused saline (20ml/kg/hour) to maintain body fluid balance. Next, an incision was made on the medial surface of the right thigh. The femoral artery was isolated, a heparin-saline filled catheter inserted (PE 20), tied in place, and a pressure transducer in the catheter was connected to the computer for measurement of renal perfusion pressure (RPP) during the low-pressure periods.

A ventral midline incision was made in the abdomen and the intestines were moved to one side and wrapped in plastic wrap to keep them moist. The mesenteric and celiac arteries were isolated using gentle blunt dissection with cotton swabs and forceps, and a suture was looped around each artery as it exits the aorta. The suture ends were pulled through a small piece of tubing (PE 190). Tightening the suture ends closed off these arteries to raise the renal perfusion pressure (RPP). The aorta was isolated cranial to the renal arteries and caudal to the renal arteries, for later manipulation of RPP via aortic constriction.

The bladder was isolated, a small cut was made in the cranial edge, and a lipped urinary catheter (PE 240) was inserted and tied in place for urine collection. The screw

on the clamp was tightened or loosened to constrict or dilate the forceps to adjust RPP in a finely controlled manner. Next, the exposed tissue was covered with saline-moistened gauze, and the rat was allowed to equilibrate (rest) for 45 minutes. BP recordings were collected briefly, every 10-15 minutes during this period to monitor catheter viability and blood pressure. Starting surgery time, starting and ending saline infusion time, and saline infusion volume were recorded to ensure similar conditions for all rats.

Data collection

After equilibration, blood pressure and heart rate were monitored continuously for the remainder of the study, via the carotid artery and femoral artery catheters, using computer-connected data collection hardware (Biopac). For every 15 minutes, urine was aspirated from the bladder catheter and bladder using a tuberculin 1ml (TB) syringe with an attached PE 50 tube. Urine was placed into a TB syringe, measured, and covered with parafilm. Samples were stored at 10 degrees C until analysis by flame photometer. All urine collected between collection periods, when RPP was being adjusted, was discarded. To determine the baseline values of blood pressure and urine sodium excretion, two fifteen-minute recordings with corresponding urine samples were taken. Baseline blood pressure was averaged for the two periods. Then, the aortic clamp (forceps) was tightened (cranial to the renal arteries) until the blood pressure at the kidneys (RPP) was 20 mmHg below the baseline mean, as measured by the femoral catheter. Urine was collected over two additional 15-minute periods (low RPP). Next, the aortic clamp was released and the sutures around the celiac and mesenteric

arteries were tightened to increase blood pressure above the baseline mean by 30 mmHg, measured via the carotid catheter. The aortic clamp was moved to a location caudal to the renal arteries. The tension on the mesenteric suture and/or the aortic clamp was adjusted as needed to maintain the desired elevation in RPP. Urine was collected during three additional 15-minute periods (A third period was added due to a longer adjustment time in urinary Na+ excretion observed when RPP was raised, than when lowered. At the end of the acute study, a blood sample was drawn from the lower aorta using a TB syringe, and hematocrit was measured to check for appropriate hydration. Anesthetized rats were killed by the creation of a pneumothorax. The kidneys were removed and weighed.

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Urine Analysis

Parafilm-sealed urine samples were placed in a refrigerator until later analysis. Urine samples less than 0.20 ml were diluted to 0.30 ml using double deionized (DDI) water to ensure adequate volume for analysis. Those volumes of 0.20 ml or greater were diluted to a volume twice the original to be certain the sodium concentration did not exceed the recommended upper limit of the flame photometer. To meet the requirements of the flame photometer, each diluted original urine sample was diluted a second time, 1:200 with DDI water, using an automated diluter. Two of these dilutions were made for each urine sample, assuring sodium measurement in duplicate for each urine collection period. Following the standard operating procedure, the flame photometer was calibrated using a standard 140 mmol/L Na+ solution, diluted 1:200 with DDI water.

Deionized water was aspirated to set the zero. Once the Na+ ion concentration was determined, the two samples for each period were averaged, unless the two differed by more than 5%. In this case, the original urine was reanalyzed. Overall, there were 14 urine samples analyzed for each rat (a duplicate per each collection period). The sodium excretion was calculated for each rat and period by multiplying the sodium concentration by the urine volume after the first dilution. Group averages were calculated for each urine collection period.

Data Analysis

The urine sodium content was measured using a flame photometer. Total sodium excretion was calculated for each of the samples by multiplying sodium concentration by the urine volume. For each rat group, urine sodium excretions ug/kg body weight/15 minutes were plotted against the mean arterial pressure. A pressure natriuresis curve was created by inserting a best-fit regression line for each group. Differences between groups were determined by parallel line comparison or comparison of the y-intercepts. Heart rate and RPP data were measured using the Biopac software, and group means were calculated, for each 15 minutes period. Wheel revolutions were converted into km and weekly means for males and females were plotted against weeks. Running distances, RPP, initial urine volumes, and urine sodium excretion were compared using a two-way ANOVA with repeated measures. We have used equality variance test and normality test to identify the equality in the variance. Results are express as a mean +/- standard error. (Outliers were identified by values two standard deviations from the

mean, confirmed using a Grubbs test, and removed from the data). Two sedentary males, one exercise male, and one sedentary female were removed

Results

General data

Table 1 contains the general data for sedentary and exercised SHRs. Hypertensive sedentary and exercise rats of the same sex did not differ in age, length of time of the acute study, saline flow rate, hematocrit percentage, kidney weights or body weights. Male SHR kidney weights and body weights were greater than the female rats (One-way Anova, $p < 0.05$)(Table 1).

Running Distance

Both male and female rats increased running distances from week 1 to week 4, however, the female rats ran an overall greater mean distance than the males (Figure 1). Female rats ran greater distances than the male rats during individual weeks 4 and 6 (Anova, $p< 0.05$)(figure 1).

Renal Perfusion Pressure

Baseline, lowered and raised RPP values were similar among the groups, (Figure 2) (Male SHR, ANOVA, df=18, Fstat=1.523, P value=0.233),(Female SHR, ANOVA, df=17, F stat=0.0213, P value=0.886). The change in RPP (ΔRPP) from baseline, for all

raised and lowered RPP values, was also not different among the groups (Figure 3). (ANOVA, df=35, Fstat=0.187, P value=0.904.)

Urine Volume

In the sedentary groups and the exercise males, urine volume was increased over the baseline periods during all high periods. In the exercise female group, only high 2 and high 3 urine volumes were greater than baseline values. There was no difference between treatment groups of the same sex (Figure 4).(ANOVA, df=35, Fstat=1.866, P value=0.153).

Urine Na excretion

Exercised males increased urine sodium excretion over baseline during all high periods. Sedentary males had a greater urinary Na excretion than baseline in the high 2 and high3 periods. In the exercise females, urine Na excretion was increased in all high periods when compared to baseline values. Sodium excretion in the sedentary females increased over baseline in only the high 2 and high 3 periods. There was no difference in Na+ excretion among the four groups, and no differences were observed between treatment groups of same sex (Figure 5).(Male SHR, ANOVA, df=18, Fstat=1.280, P value=0.273), (Female SHR, ANOVA, df=17, F stat=0.957, P value=0.342).

Pressure Natriuresis Curves

Pressure Natriuresis -Male: Exercise had no significant effect on the pressure natriuresis curve in male rats. There was no statistical difference between the slope, or the Y-Intercept of the pressure natriuresis curve of the exercise males when compared to sedentary males (Figure 6).(Parallel line Analysis, F stat=0.046, P value=0.8306) Test for equality of Intercepts, F stat=2.5155, P value=0.1150).

Pressure Natriuresis-Female: Exercise had a significant effect on the pressure natriuresis curve in female rats. The slope of the curve was greater in the exercised female rats than in the sedentary female rats (Figure 7).(Parallel line Analysis, F stat =10.3305, P=0.0017).

Pressure Natriuresis-All Groups

A sex difference was observed in the pressure natriuresis curves when like treated males and female rats were compared. Parallel line comparison and y intercept methods used to compare slope differences The slope of sedentary males was significantly steeper than the sedentary females, and the Y-intercept of exercise males was significantly more negative than that of the exercise females (Figure 8). (Exercise males and exercise female - Parallel line analysis, F stat=1.0202, P value=0.3142, Equality of Intercept , F stat=11.5769, P value=0.0009). (Sedentary male and sedentary female - Parallel line analysis, F stat=12.2415, P value=0.0006).

Discussion

The major finding of this study was the difference in the pressure natriuresis curve of exercised female rats when compared to sedentary females. Exercise females had an improved pressure natriuresis response, indicated by the steeper slope of the curve (Figure 7). This results in a greater increase in urine Na+ excretion for any given increase in RPP. However, exercise males had no significant pressure natriuresis changes. The general data of this investigation, summarized in table 1, show no differences between the groups, except that the males were heavier, and had greater kidney weights than the female rats. Similar hematocrit percentages and saline infusion rates indicate similar levels of hydration among the groups, and thus cannot explain the lack of exercise effect in the male rats.

There were no differences between renal perfusion pressure (RPP), or change in RPP from baseline, during any manipulation periods in sedentary and exercised rats (Figures 2 and 3), indicating the RPP values and manipulations do not explain the change in the pressure natriuresis slope. Urine volumes and raw sodium excretion amounts were elevated above baseline values in all rats when RPP was raised, confirming that a pressure natriuresis response was stimulated. Urine volume and sodium excretion was not different within the same sex, however, when RPP was raised, males had larger urine volume than females undergoing the same treatment, perhaps due to the larger size and blood volume. The effect of exercise was only evident when urinary sodium excretion was plotted against RPP, indicating the relationship between sodium excretion and RPP was altered.

Between sex, differences were noted when pressure natriuresis curves were compared between like treatment groups. Sedentary males had a steeper slope than females, and exercised males had a more negative Y-intercept than exercised females. These results suggest a more sensitive pressure natriuresis relationship in the male SHR than in the female. It is unclear if the differences in pressure natriuresis in the sedentary males versus the females played a role in the lack of effect of exercise in the male SHR. Another potential explanation for a lack of exercise effect in the males is the lower running distances of the male rats, particularly noticeable during the earlier weeks when hypertension would be developing. The enhanced exercise performance of females compared with males, along with the difference in exercise effects, suggests that sex /gender difference in exercise performance may influence long-term pressure regulation. Previous studies in this laboratory showed that WKY females also had enhanced running performance compared to male WKY rats, but the difference in running distances was smaller. Other studies have also shown that female rats had enhanced running performance over male rats (45, 41). A sex difference in the effect of hypertension on renal functions/glomerular filtration rates, or endothelial function could modify the effects of exercise. Researchers have determined sexually dimorphic characteristics to voluntary cage-wheel exercise in rats. Some cardiac morphological differences such as cardiac mass was significantly greater in female rats compared to males (45). The same study showed that females had a significantly greater increase in heart mass for every hour of activity than the male rats (45).

Prior studies in Dr. Knoblich's laboratory on normotensive WKY rats found that exercise improved pressure natriuresis in both males and females, and lowered baseline blood

pressure in anesthetized male, but not female WKY rats (Effects of voluntary exercise on pressure natriuresis in WKY rats). In the WKY, exercise improved the slope of the pressure natriuresis curve in both female and male rats when compared to sedentary groups. Some sex differences were found when comparing treatments between the sexes in WKY rats. Sedentary females had a more negative Y-intercept than sedentary males, and exercised females had a steeper slope than exercised males. When compared to the WKY rats, the sex differences in the pressure-natriuresis curves of the SHR in the current study were reversed, with the sedentary males showing a greater slope than females, and exercise males having a more negative Y-intercept than females. The difference in exercise effects in the SHR, when compared to the WKY, may be the result of physiological alterations due to hypertension.

The mechanism by which exercise improved pressure natriuresis is unclear. Running and swimming appear to improve endothelial function (74,6). After 8 weeks of training, the dilation of the brachial artery when blood flow increases (flow-mediated dilation or FMD) was changed. However, there was no difference in the response between males and females (6). NO release from the endothelial cell is one of the main causes of FMD. Endothelial dysfunction is often found in hypertensive patients. Endothelial dysfunction can cause abnormalities in vessel walls including renal arteries, and this dysfunction is linked to many renal diseases including renal fibrosis, which is prevalent in hypertensive individuals (74,6). The production of nitric oxide by healthy endothelium is believed to be a key factor in the pressure natriuresis response. Continuous development of renal medullary oxidative stress causes kidney dysfunctions (54). NO may play a major role in Na+ excretion and in modulating the pressure natriuresis response in hypertensive rats,

which have lower levels of NO (54). One study found that continuous NO production was required to prevent the development of cardiovascular diseases like hypertension because NO can stimulate renal blood flow. Inhibition of NO production results in reduced renal medullary blood flow (54). NO acts as a signal to reduce tubular sodium reabsorption which increases Na+ excretion in normotensive rats. The authors concluded that reduced renal blood flow influences sodium excretion in hypertensive rats (54). These findings suggest that NO plays a major role in maintaining pressure natriuresis, and improvements in endothelial function with exercise could increase NO synthesis and pressure natriuresis.

Exercise can have beneficial effects on other aspects of kidney function. Glomerular blood flow regulation and glomerular filtration rate/ capacity are the main factors associated with body fluid regulation, and these are negatively impacted by hypertension (11,74). Juxtamedullary nephrons of normotensive rats tend to remain healthy, however, in SHR, the juxtamedullary complex is complicated and has an increased risk of pathological lesions such as fibrinoid necrosis, fibro sclerosis, and glomerulosclerosis (11,74). Moreover, filtration is not normal in the cortical nephrons of the SHR (74). In contrast to normotensive rats, the renal medulla can be damaged under hypertensive conditions. Experiments in animal models have shown the benefits of exercise for kidney disease associated with CVD. Renal inflammation kidney diseases always follow cardiovascular diseases. Glomerulosclerosis was reduced in swim exercised rats when compared to sedentary rats. Glomerular filtration rates were increased in female and male exercised groups compared to sedentary groups (74). Another study found that the systolic blood pressure and renal fibrosis in hypertensive

rats improved after exercise training (36). Moreover, exercise groups had a decrease in inflammatory protein levels, fibrotic-related protein levels, and connecting tissue growth factors (36, 74). These factors are a major part of renal diseases which can affect the kidney filtration capacity and glomerular filtration rate in hypertensive rats. Like our SHR kidney weights, there was no difference in kidney weights between groups in a study conduct by C. Huang. However, this study histologically analyzed the kidneys and found SHR had enlarged areas of fibrosis when compared with WKY groups. SHR-exercised rats had a significant decrease in renal fibrosis (36).

Sex differences in the effects of hypertension produce different outcomes in males and females. Hypertensive males experience more oxidative stress than hypertensive females, and males also experience abnormal pressure natriuresis due to high oxidative stress (70). Researchers examined the influence of sex hormones on renal oxidative stress in hypertension because oxidative stress due to reactive oxygen species plays an important role in renal pressure natriuresis. Reactive oxygen species such as hydrogen peroxide can result in abnormal Na+ handling, and Na+ excretion in hypertensive individuals by reducing renal blood flow (70). Research conducted by J Sullivan et al found that male SHRs have increased urinary H2O2 excretion compared to females (70). Estrogen suppresses reactive oxygen species, and testosterone stimulates reactive oxygen species, leading to sexual dimorphism in hypertensive rats (70).

Young women have estrogen receptors in many tissues and cells, including vessels, muscles, endothelial cells, and myocardial cells, while premenopausal women have fewer estrogen receptors in the same tissues (66). Some population-based studies demonstrated that postmenopausal women have a high risk of getting cardiovascular

diseases. The authors suggest that postmenopausal women who carry ESR1 (estrogen receptor) have an increased risk of myocardial infarction and other CVD (66). Exercise and estrogen also maintain normal myosin patterns in slow and fast muscle (41). Female sex hormones have a strong effect on cardiovascular functions and musculoskeletal functions (41).

Hypertensive patients often have elevated levels of vasoconstrictive agents, such as vasopressin, and angiotensin II (54). Vasoconstrictor hormones such as angiotensin, increase Na+ reabsorption in hypertensive rats. Furthermore, many studies involve the renin-angiotensin-aldosterone system (RAAS) in hypertensive rats because the RAAS is the other long-term regulator of blood pressure that is involved with hormones. Female sex hormones and estrogen receptors in endothelial cells can regulate this RAAS activity. A study by Nickenig et al demonstrated that Ang II caused significantly stronger vasoconstriction in ovariectomized rats, supporting the idea that there is a strong correlation between sex hormones and the RAAS (52). Female sex hormones have protective cardiovascular effects and estrogen therapies have been used to prevent CVD. Estrogen therapy reduces the risk of coronary heart disease because it can reduce coronary artery angiotensin (angiotensin-converting enzyme) activity (71). Estrogen therapy also increases cardiac atrial natriuretic peptide (ANP) in circulating blood which acts as a vasodilator and promotes natriuresis (65).

The male sex hormone testosterone also contributes to sex differences in SHRs. Studies by Jane et al showed that testosterone increases the risk of hypertension in male SHR via the RAAS (64). Plasma renin concentration is high in males compared to females (67). Renin converts angiotensinogen to angiotensin I, which subsequently gets

converted to angiotensin II. Ovariectomized female rodents treated with testosterone had similar serum testosterone levels and blood pressure levels compared to males (64).

Conclusion

Our hypothesis was partially correct. Our results support the idea that exercise, as a non-pharmacological treatment, improves the pressure natriuresis response in female hypertensive rats. In males, the natriuresis response was not improved, possibly due to sex differences in the effects of exercise, other physiological factors, or differences in running distances. It is unclear if greater running distances early in hypertension development would result in similar effects in the males. Because hypertension results in endothelial dysfunction and progressive renal damage, it is not surprising that some of the benefits of exercise on hypertension may be within the kidneys. The exact mechanism by which pressure natriuresis was altered in the exercised rats is unclear, but improved endothelial function and nitric oxide production, resulting in less renal fibrosis, and improved renal function, remains a possibility. Identifying the specific mechanisms could support the development of therapeutic strategies for hypertension that focus on pressure natriuresis.

Future Studies

The exact mechanism and mediators behind the alterations in pressure natriuresis should be determined. Further examination of gender differences associated with pressure natriuresis, and the effects of sex hormones combined with exercise on pressure natriuresis in female and male SHR and WKY rats would also be beneficial.

Table and Figures

Table 1: General data from sedentary and exercise SHRs. Age at the time of the study, acute study total time, saline infusion rate (flow rate), hematocrit, kidney weight, and body weight are shown as means ± SE. SHR = spontaneously hypertensive rats

Group	Age (wks)	Acute study (hr)	Flow rate (ml/kg/hr)	Hematocrit (%)	Kidney weight (g)	Body weight (kg)
Sedentary Male	16.43 ± 0.90	6.46 ± 0.32	19.05 ± 1.15	67.20 ± 2.59	2.60 ± 0.08*	0.32 ± 0.011*
Exercise Male	17.65 ± 0.74	5.99 ± 0.23	18.64 ± 0.83	62.10 ± 2.89	2.90 ± 0.05*	0.36 ± 0.011*
Sedentary Female	16.91 ± 1.01	5.41 ± 0.25	25.96 ± 1.34	62.67 ± 1.14	1.59 ± 0.03	0.21 ± 0.004
Exercise Female	16.67 ± 0.46	6.12 ± 0.28	22.06 ± 1.06	62.00 ± 3.21	1.85 ± 0.04	0.23 ± 0.004

* $p < 0.05$ when compared to the female rats of the same treatment

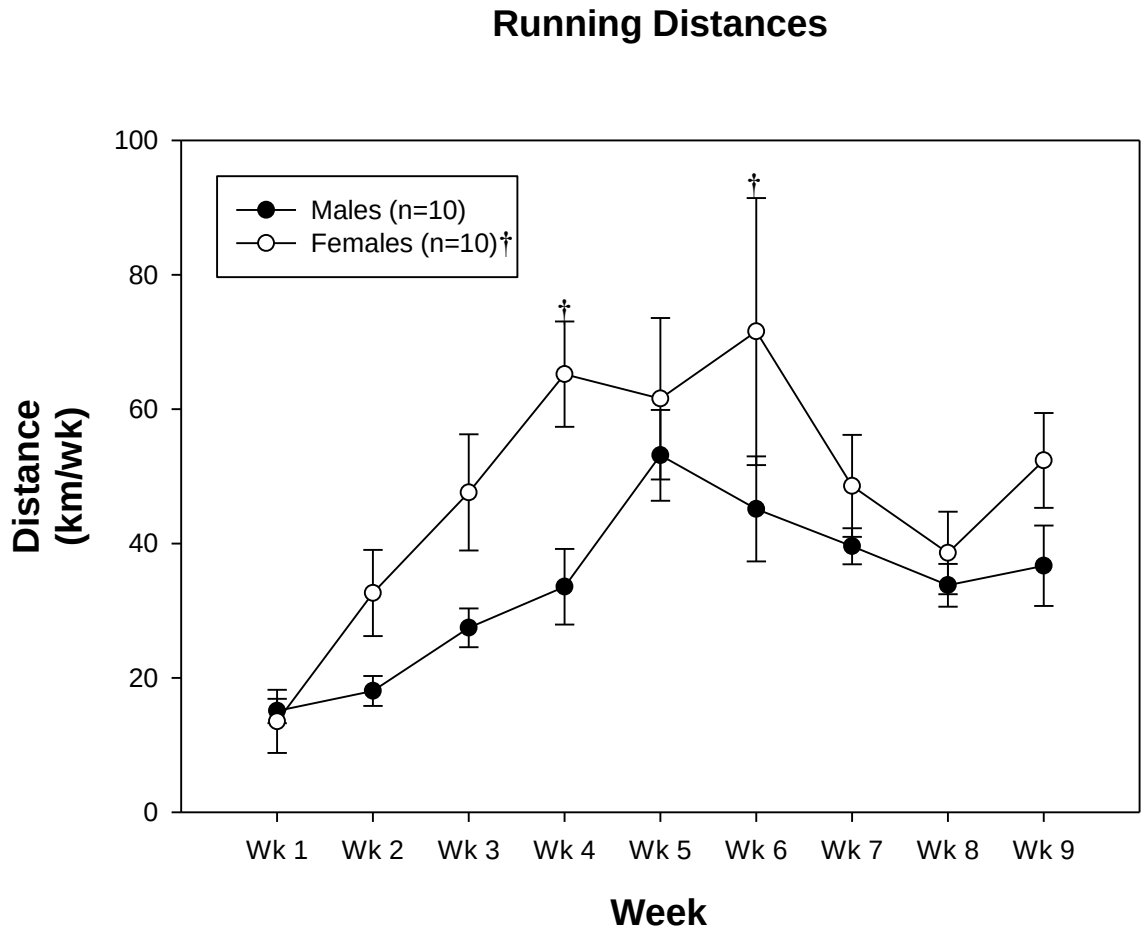

Figure 1: Weekly running distances in male and female SHR. Values are mean ± SE. † $p < 0.05$ when compared to the male rats.

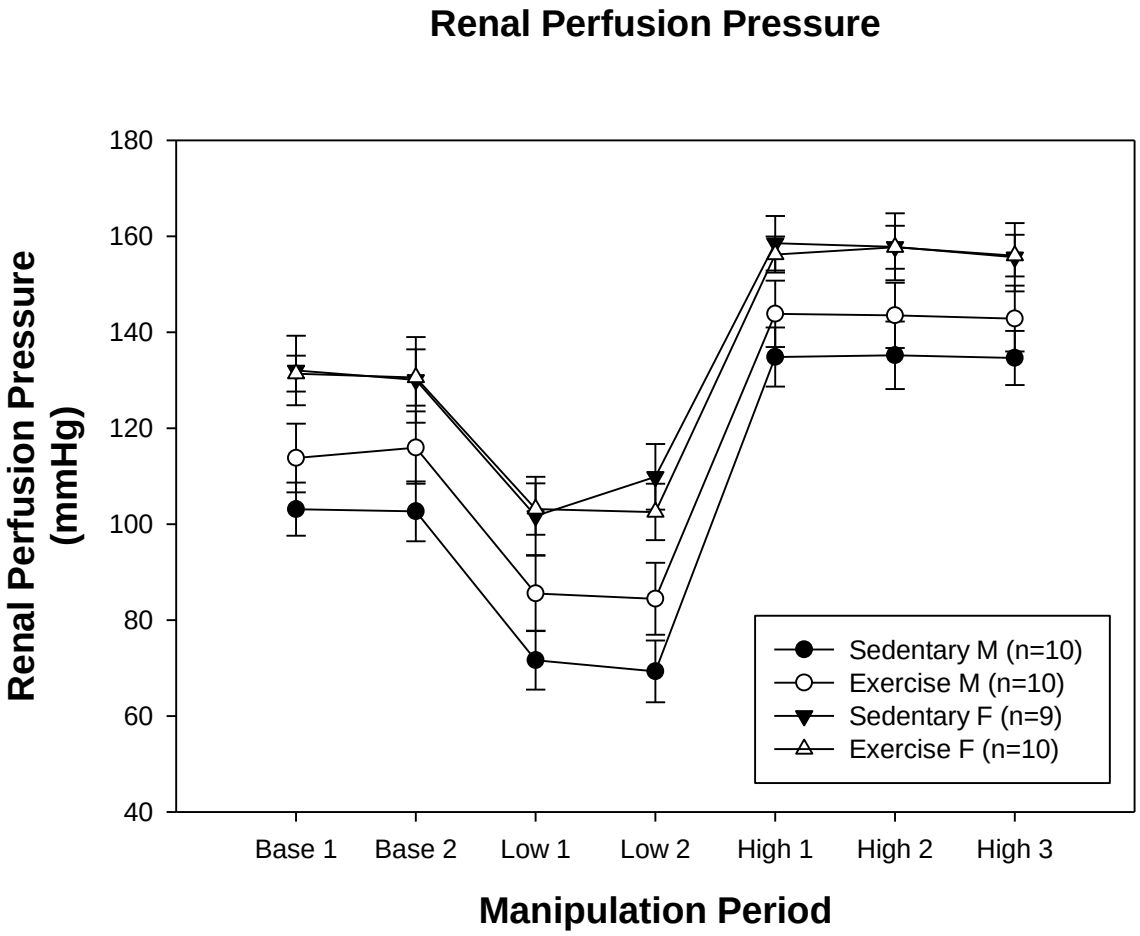

Figure 2: Renal perfusion pressure (RPP) during baseline (BL), lowered (low), and raised (high) manipulations in sedentary and exercised male and females SHR. Values are mean ± SE.

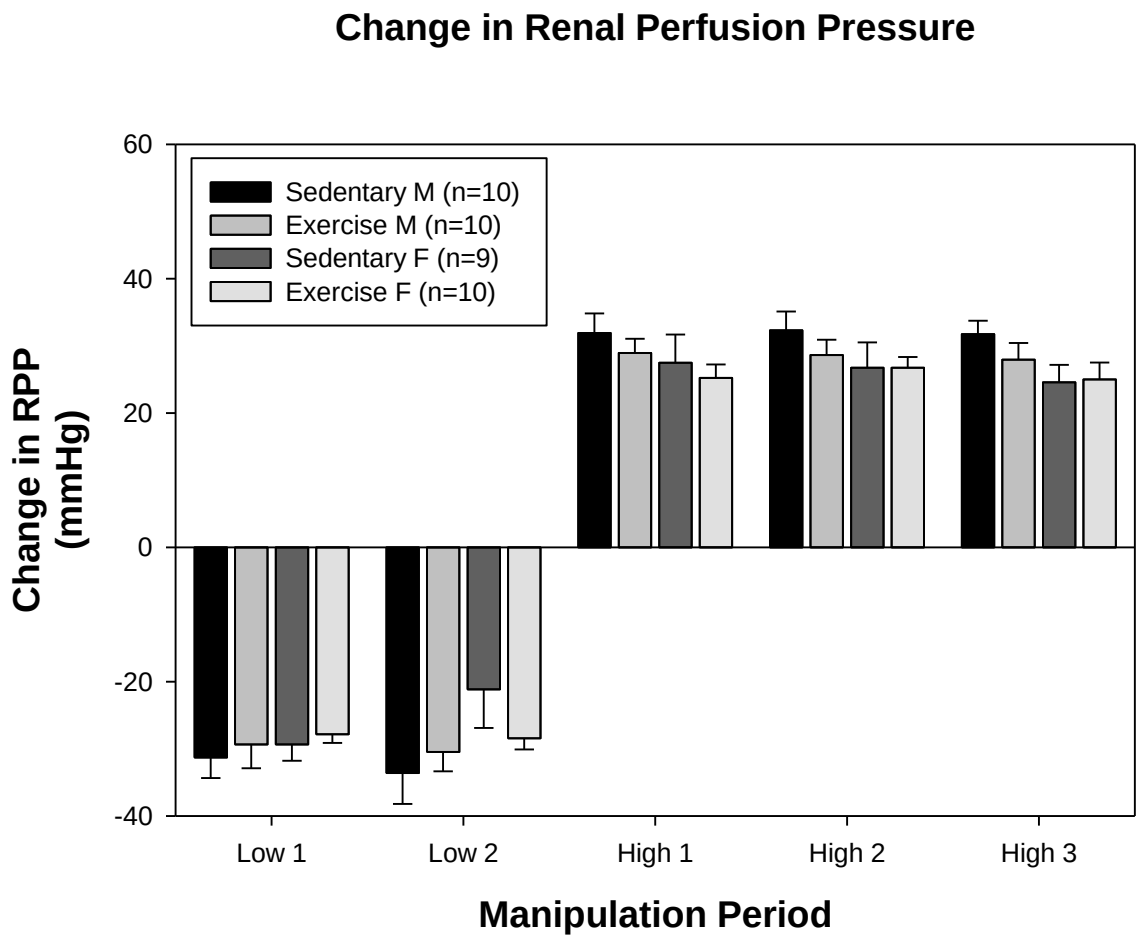

Figure 3: Change from baseline in renal perfusion pressure during lowered (low) and raised (high) manipulations in sedentary and exercised male and female SHR. Values are mean ± SE.

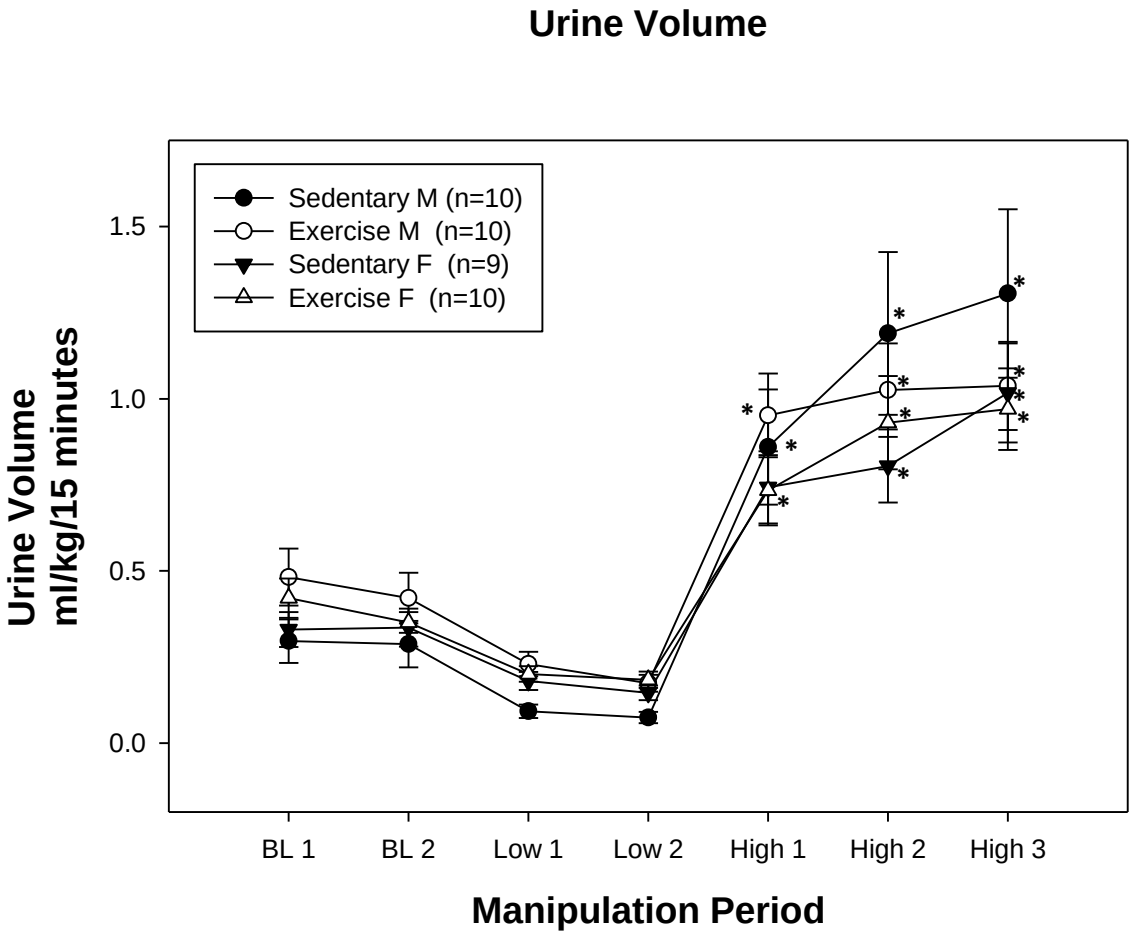

Figure 4: Mean urine volume during baseline (BL), lowered (low), and raised (high) manipulations of renal perfusion pressure in sedentary and exercised male and female SHR. Values are mean ± SE. *P < 0.05 when compared to baseline

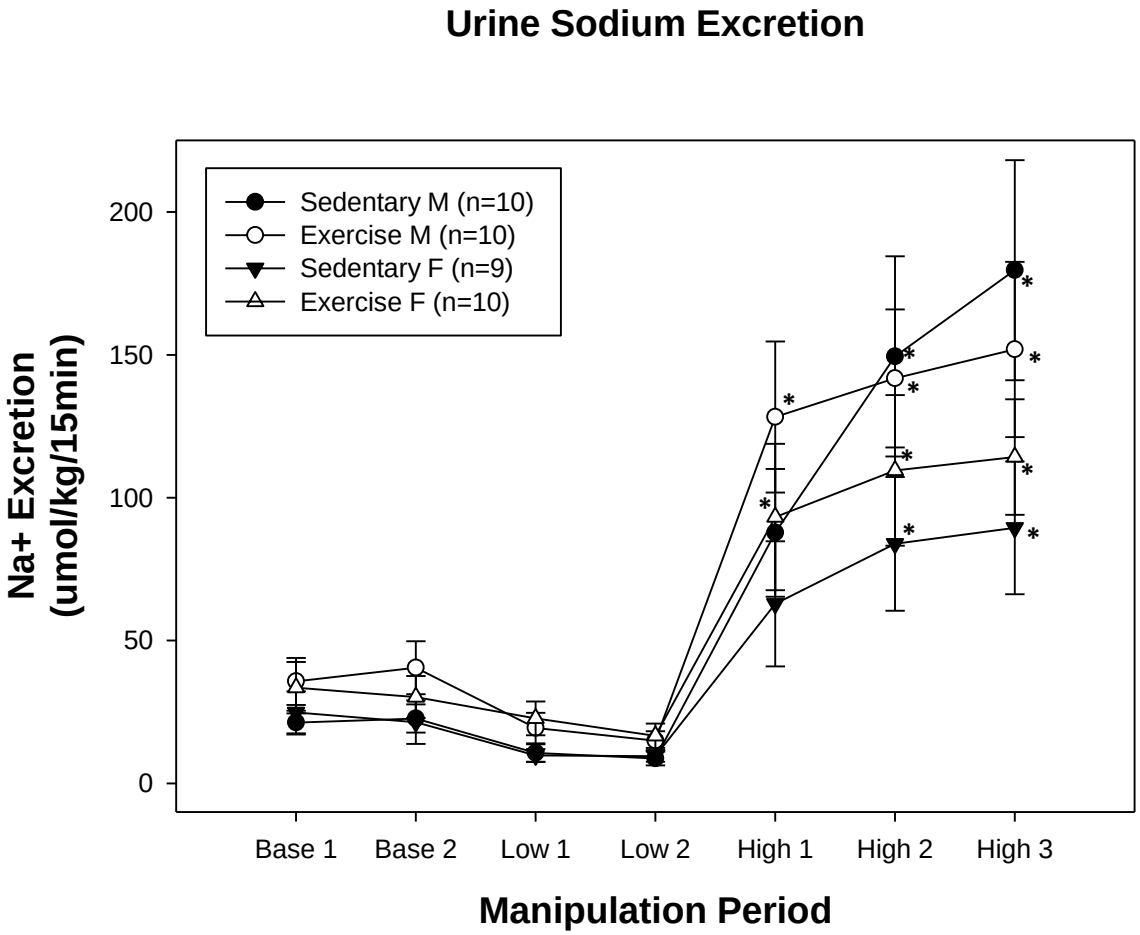

Figure 5: Urine sodium excretion during baseline (BL), lowered (low), and raised (high) manipulations of renal perfusion pressure in sedentary and exercised male and female SHR. Values are mean ± SE. *P < 0.05 when compared to baseline.

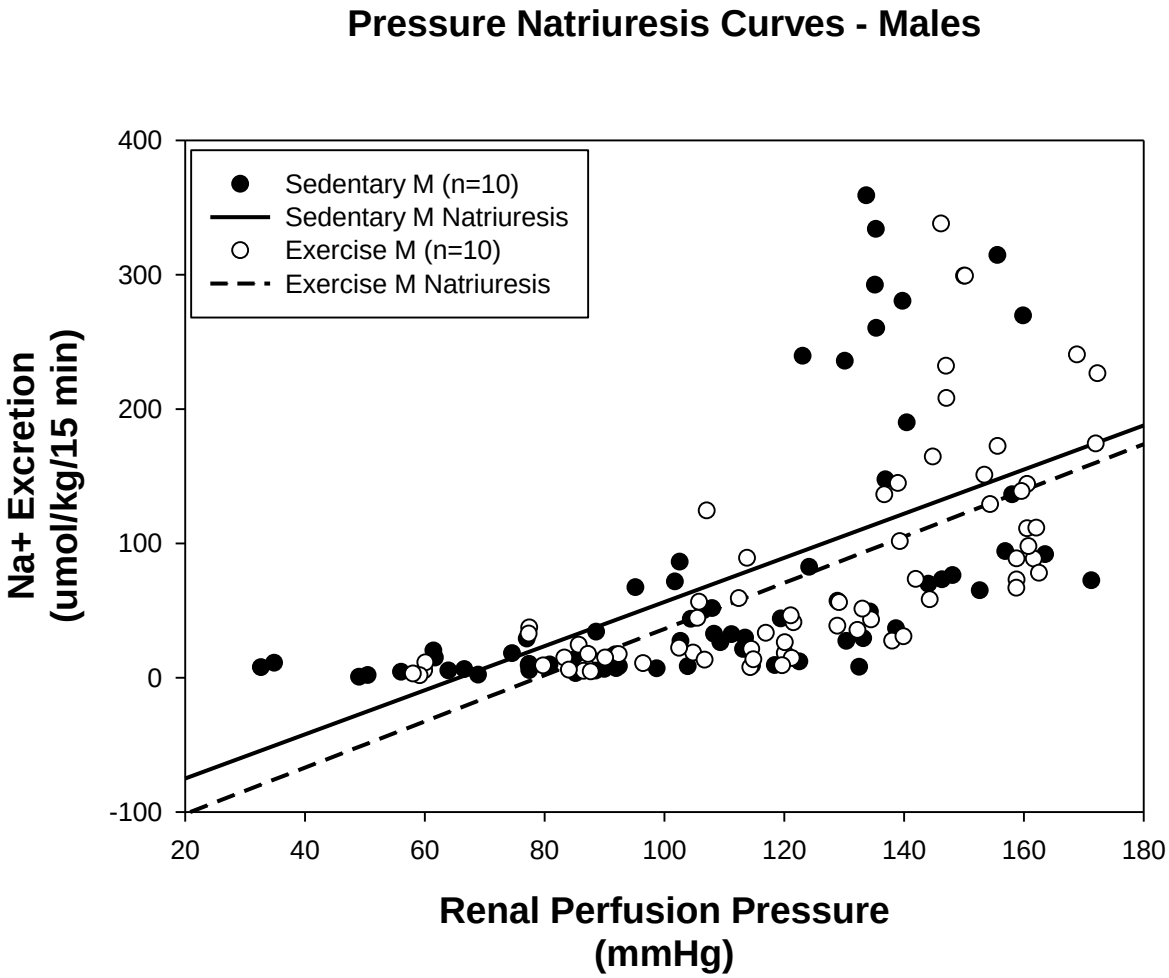

Figure 6: Pressure natriuresis curves in sedentary and exercised male SHR. Neither the slope or the Y-intercept was different between the groups.

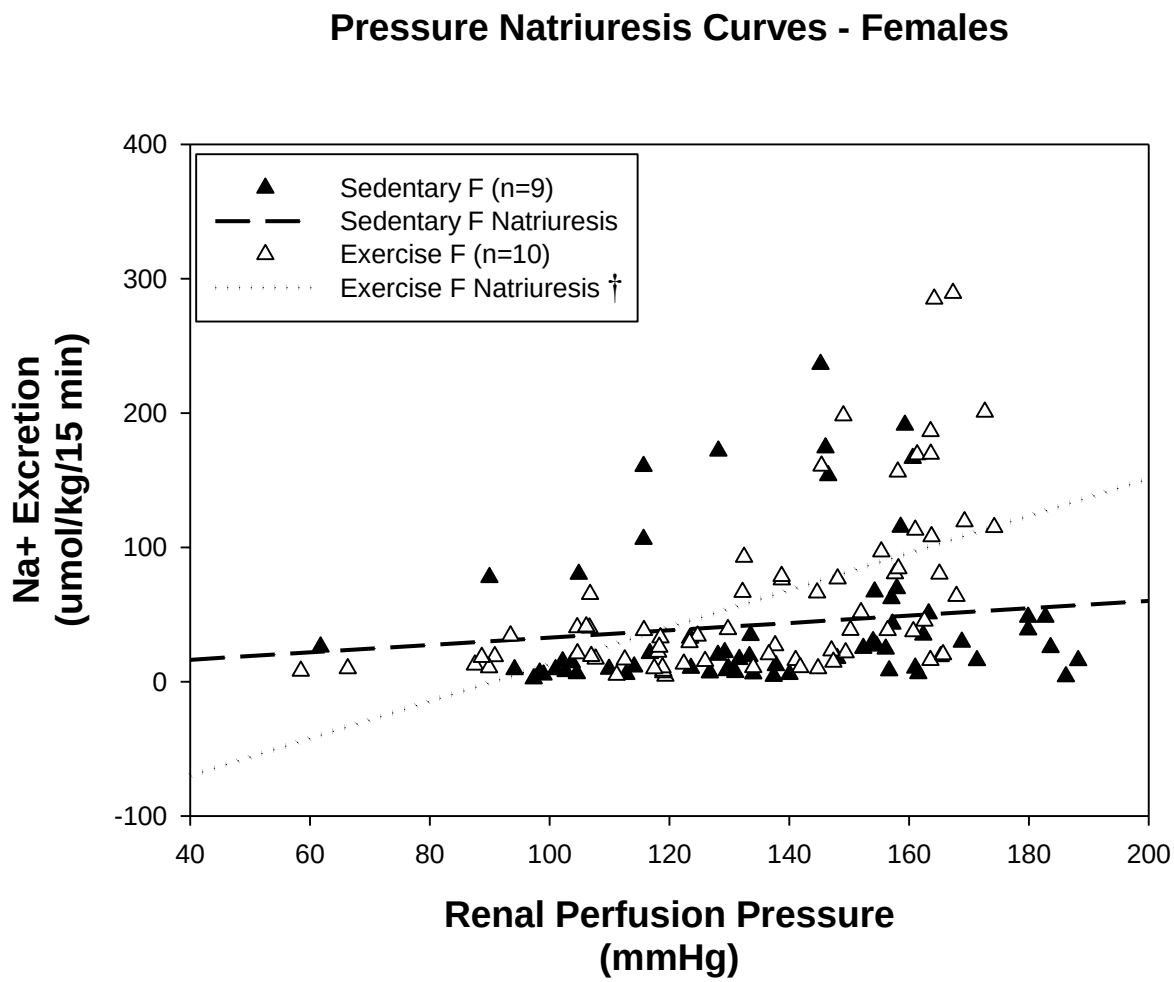

Figure 7: Pressure natriuresis curves in sedentary and exercised female SHR. † $P < 0.05$ when compared to the slope of the natriuresis curve of the sedentary rats.

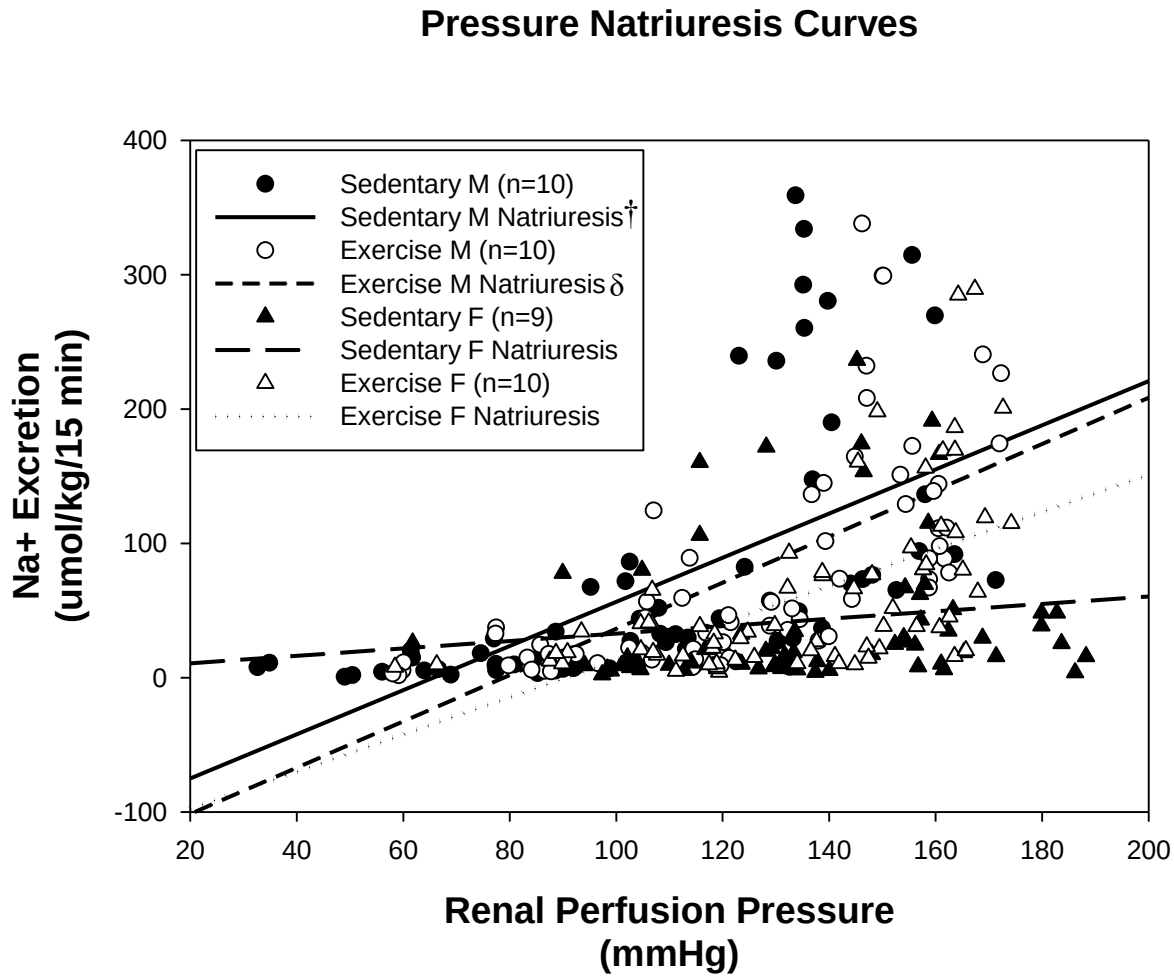

Figure 8: Pressure natriuresis curves in sedentary and exercised, male and female SHR.

† $P < 0.05$ when the slope is compared to that of the female rats of the same treatment.

δ $P < 0.05$ when the Y-intercept is compared to the female rats of the same treatment.

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