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The Effects of Exercise on Pressure Natriuresis

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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

THE EFFECTS OF EXERCISE ON PRESSURE NATRIURESIS

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
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Hypertension is a disease of high global burden and a leading risk factor for cardiovascular diseases and related deaths. The pressure natriuresis mechanism, which is an intrinsic response in the kidneys which increases urinary sodium excretion when renal perfusion pressure increases, plays a central role in the long-term regulation of mean arterial pressure. Studies have shown that a sustained impairment of the pressure natriuresis mechanism results in high blood pressure, making hypertension a disease of the kidneys.

Since exercise is recommended as a non-pharmacological treatment to manage hypertension, it is important to understand the means by which exercise is beneficial in hypertensive patients. Since pressure natriuresis is a key component in the regulation of blood pressure, understanding the relationship between exercise and pressure natriuresis is important. This study was designed to determine if voluntary exercise in young normotensive rats (from weaning to adulthood), altered pressure natriuresis.

Thirty-six (36) WKY, normotensive rats were set up into two (2) groups; sedentary (8 males & 8 females) and exercised (10 males & 10 females). Each rat had urinary sodium excretion measured at variable renal perfusion pressures (RPP) - baseline, lowered, and raised. Sodium excretion was measured and plotted against RPP to generate a pressure-natriuresis curve. Both male and female rats, (exercised and sedentary) had significant natriuresis responses to increases in RPP, but the response was greater in female rats than males. Exercise improved the pressure natriuresis relationship in both males and females, resulting in a steeper slope of the pressure natriuresis curve. This means that exercised rats had a greater increase in sodium excretion for any given increase in RPP. Our findings therefore demonstrated that exercise improves the pressure natriuresis relationship in both male and female rats, and this may partially explain the beneficial effects of exercise in hypertensive patients.

Introduction

Hypertension (also called high blood pressure) is the leading risk factor contributing to the global burden of cardiovascular disease (59). The American Heart Association, in 2018, estimated that 103 million Americans (31.5% of the population) have hypertension. In 2019, the World Health Organization released a global statistic on hypertension and stated that almost 1.3 billion (17%) of the world population is hypertensive and it is projected that more than 1.5 billion people will develop hypertension by 2025 (49).

According to the guidelines given in 2017 by the American College of Cardiology and the American Heart Association for the prevention, detection, evaluation and management of high blood pressure, hypertension is now defined as systolic blood pressure of greater than or equal to 130mmHg and diastolic blood pressure of greater than or equal to 80mmHg (104).

Hypertension directly poses a great public health challenge due to the associated systemic complications including stroke, blindness, kidney diseases, and heart diseases like myocardial infarction, angina, and heart failure, and contributes to about 12 million annual deaths worldwide. It is the major modifiable risk factor for developing cardiovascular and renal disease and there is no cut off for risk, which doubles with every 20 mmHg increase in systolic blood pressure (58). It is also important to note that hypertension is often a preventable and treatable disease (74).

Blood Pressure (BP) is the product of cardiac output (CO) and total peripheral resistance (TPR), and CO is the product of *heart rate* and *stroke volume*. Heart rate is the number of heart beats per minute while stroke volume is the volume of blood pumped from the left ventricle per beat. Peripheral vascular resistance is the resistance to flow through the blood vessels.

To maintain a normal BP, there must be a balance between the cardiac output and peripheral vascular resistance. Blood pressure increases with increased blood volume and cardiac output as well as increased blood viscosity and peripheral vascular resistance. Blood volume may be increased through excessive sodium intake, or from increased sodium and water retention from hormonal influences (aldosterone, antidiuretic hormone) or from chronic renal, cardiovascular or liver diseases. Any condition that increases heart rate, such as heart disease, thyroid disease, infections or side effect of certain medications, can also increase the cardiac output which increases blood pressure (8, 52).

Peripheral vascular resistance is influenced by the sympathetic nervous system (SNS), humoral factors, and local autoregulation. Increased vascular resistance may occur due to increased blood viscosity, endothelial dysfunction or rigidity of vessel walls from aging, atherosclerosis and other peripheral artery diseases through oxidative stress and vascular inflammation (17, 98). It is also elevated following increased sympathetic activity and in the presence of chemical substances such as endothelin, angiotensin II. The relationship between heart rate, stroke volume and peripheral resistance

explains why studies on hypertension have centered around cardiac or vascular theories (46). While blood pressure parameters are usually linked to the cardiovascular system, renal mechanisms have also been implicated in the long-term control of blood pressure regulation, especially the pressure natriuresis mechanism. The cardiovascular system depends on the kidneys for sodium and water balance in the body. It is thought that the pressure natriuresis system is the dominant method of controlling BP on a long-term basis and that it can compensate for other mechanisms that may cause hypertension such as, increased vascular resistance. This indicates that sustained impairment of pressure natriuresis will result in elevated blood pressure making hypertension is also a disease of the kidneys (96).

There is no known specific cause of hypertension, it is categorized as a multifactorial disease involving an interplay of genetic, environmental, vascular, neuronal and endocrine factors (70, 84).There is no known specific cause of hypertension, it is categorized as a multifactorial disease involving an interplay of genetic, environmental, vascular, neuronal and endocrine factors (70, 84).

Generally, the risks of developing hypertension are classified as modifiable and non-modifiable. Modifiable risks include physical inactivity, high salt intake, high saturated fat intake, smoking, alcohol consumption, and stress. Non-modifiable risks are those over which the individual has no control, and they include age, gender (more common in males), family history of hypertension, and race (more common in blacks) (104). Other risk factors

include presence of comorbidities like diabetes mellitus, chronic renal diseases, adrenal diseases, and thyroid diseases. (80).

The symptoms include, but are not limited to, headaches frequently noticed in the morning, eye ache or blurry vision, chest pain, palpitation, nosebleed, dizziness, and muscle tremors. However, many people are unaware of their condition as they tend to remain asymptomatic for a while and may only present when complications set in. Therefore, the disease is also referred to as a silent killer (34).

Treatments include lifestyle changes aimed at improving the modifiable risks, pharmacological and non-pharmacological (or surgical) options. The goal of treatment is not to aggressively reduce blood pressure as that could be detrimental, but to gradually lower it and work towards preventing complications and limiting adverse cardiovascular events and deaths (109).

About 15% of hypertensive patients fail to reach target blood pressure ranges, despite the use of up to three antihypertensive medications of different classes (76). It is also very difficult to accurately predict which type of antihypertensive medication will be most appropriate for each patient due to the multiple causative factors that may be involved.

As beneficial as the pharmacological approach is in the treatment of hypertension, the advantage of non-pharmacological prevention or management cannot be overemphasized (7). Exercise is often prescribed as a non-pharmacological treatment or prevention for hypertension. Exercising consistently for as little as 1 day per week (up to 30 minutes) has been shown

to be effective in reducing mortality among those with hypertension (7, 28). However, the physiology behind the beneficial effects of exercise in lowering blood pressure is not well understood.

Since the kidneys play a key role in blood pressure regulation, it is reasonable to postulate that exercise may affect kidney physiology. This study sought to determine if exercise alters kidney-salt regulation, elucidating a potential means by which exercise is beneficial to hypertensive patients.

Literature review

Hypertension

Epidemiology

The prevalence of hypertension increases with age and it is mainly related to vascular changes such as calcification and stiffness associated with ageing (77). It is found to be more common in men than women. The reason is believed to be the lack of endogenous estrogen which is important for normal endothelial functioning, and the presence of testosterone in males (4). Supporting the role of estrogen in hypertension is the higher prevalence of hypertension in postmenopausal women when compared to men of the same age group (13, 21, 27, 104).

A study conducted by Hu et al on hypertension in Southern China showed that the prevalence was slightly higher in males than females, and it increased with age in both genders. They also recorded more cases of hypertension

among obese patients, whether male or female. More postmenopausal women were found to be hypertensive then men in the same age group (45). It is thought to be due to the estrogen deficiency after menopause, which results in endothelial impairment (94). Similarly, a cross-sectional study conducted by Khanal et al in Nepal in which the ratio of male to female participants was 30:70 with an average age of 47 years, showed that the prevalence of hypertension was higher among men (up to 50% of recorded cases). About 60% of the hypertensive patients were males and females over the age of 60 years, further showing that hypertension increases with age. Obesity, smoking and alcohol consumption were other factors that were directly linked to hypertension among the participants (51).

To ascertain if differences in the prevalence of hypertension between genders occur earlier in life, a study conducted among young adults in the United States showed distinctions in the risk and prevalence of hypertension between genders as early as 20 years of age (21). As with other studies, they observed fewer cases of hypertension among premenopausal women. The researchers also proposed that these differences may be due to biological differences between the genders, or behavioral patterns such as sedentary lifestyle (higher in females), smoking (higher in males), and healthcare seeking attitudes (higher in females).

Several sex and age-related studies on hypertension have also been conducted on lab animals. A study by Saez et al examined in rats the effect of early onset disruption of renal development on the risk of developing

hypertension later in life. They induced nephron loss in the treatment group (consisting of males and females) by administering AT II receptor antagonist orally within 14 days of postnatal life. The rats were studied after a year, and they observed deterioration of renal structure and function in both sexes, but it was significantly higher in the male treated rats. This deterioration was characterized by reduced glomerular filtration rate, proteinuria, thickened glomerular basement membrane and progressively elevated blood pressure(85).

Classification

Hypertension can be classified into 2 main categories: essential and secondary.

Essential hypertension is also called primary or idiopathic. It is defined as elevated blood pressure with no identifiable or diagnosed underlying diseased condition. It is so called because it has a slow onset and is usually asymptomatic and long standing. The patients usually present with complications by the time it is diagnosed. It is the most common type of hypertension, accounting for about 95% of hypertensive cases worldwide (3, 38). The baseline abnormality in essential hypertension seems to be a primary dysfunction of the pressure natriuresis relationship (6). In some cases, it is linked to genetic (polygenic) factors, in which case it is called inheritable blood pressure. In other cases, it is related to modifiable (hypertensinogenic) factors like obesity/sedentary lifestyle, increased salt, and alcohol intake etc. Children’s risks of developing hypertension may be

increased due to non-modifiable factors that include prematurity, and low birth weight, and modifiable factors like secondary smoke exposure and poor sleep (10, 22). Most researchers believe that essential hypertension is due to a combination of genetic and environmental factors (48).

Secondary hypertension occurs due to an underlying disease condition and may have multiple etiologies. It accounts for less than 5% of hypertensive cases and it is associated with early onset development of hypertension. With genetic involvement, there is a monogenic mutation within the renal and adrenal systems, leading to a disruption of sodium regulation and blood pressure control (10, 82).

Children and adolescents are more prone to developing secondary hypertension (more than 50% of pediatric hypertension) and it has been linked mostly to renal diseases/tumors, cardiovascular and endocrine diseases (23, 73).

Blood pressure and autoregulatory mechanisms

For the cardiovascular system to function appropriately, the systemic arterial pressure must be maintained within normal limits. In each cardiac cycle, arterial blood pressure fluctuates between diastolic and systolic pressures and is controlled by autoregulatory mechanisms to keep it at homeostatic levels. The regulation is achieved by the interdependence of the 3 parameters that make up blood pressure: Heart rate (HR), ventricular stroke volume (SV) and total peripheral vascular resistance (TPVR) (2). These adjustments can either be short-term (seconds to minutes) or long-term (minutes to days). Short-term

regulation of arterial blood pressure is primarily by baroreceptor and chemoreceptor mechanisms, through the activity of the autonomic nervous system. Long-term regulation is of high clinical importance because of the prevalence of hypertension, and mainly involves the regulation of extracellular fluid volume (ECFV) by sodium regulatory mechanisms involving the kidney, including the widespread actions of angiotensin II and aldosterone (2). Blood volume is an important determinant of blood pressure as it affects the cardiac output. Blood volume itself is controlled by the ECFV, which is a function of the balance between sodium and water intake and elimination, mainly by the kidneys. Sodium is an osmotically active substance and will always be accompanied by water, whether it is being reabsorbed into the body or excreted into the urine. Thus, effective circulating volume is a function of body sodium regulation and is dependent on an interplay of hormones and Starling capillary forces (61, 89). An increase in body sodium and water causes an increase in body fluid volume and an increase in blood volume. Increased circulating blood volume results in increased peripheral venous pressure and venous return. Central venous pressure also becomes elevated following an increase in venous return, which results in increased end diastolic volume, stroke volume, cardiac output and blood pressure (61, 89, 97). Increased circulating volume also increases renal perfusion pressure (RPP) which inhibits renin secretion and the renin-angiotensin-aldosterone system (RAAS). Peritubular capillary hydrostatic pressure also increases leading to increased filtration in the renal medulla and reduced tubular reabsorption of salt and water. The resultant effect is increased sodium and water excretion which

reduces circulating volume, venous return, stroke volume and finally reduces the cardiac output and blood pressure to homeostatic levels (61, 89).

Reduced effective circulating volume (e.g. from dehydration or hemorrhage) has an opposite effect by reducing renal perfusion pressure which stimulates renin release to activate the RAAS pathway (*further details in sodium regulation section*). This causes increased reabsorption of sodium and water in the kidneys, due to the effects of aldosterone and antidiuretic hormone. Sodium retention causes an expansion of ECFV which in turn increases blood volume, stroke volume and cardiac output to raise the blood pressure back to the normal homeostatic level. There is also a reduction in capillary hydrostatic pressure in the renal medulla, which reduces filtration and increases sodium reabsorption causing increased blood volume and blood pressure (61, 89).

Sodium Regulation

The kidneys are paired bean-shaped retroperitoneal organs and are one of the most vital organs, central to homeostatic processes in the body. In addition to regulating blood pressure through sodium regulation, they are involved in toxin and waste (urine) excretion, reabsorption of electrolytes including Na^+, K^+, and Cl^-, regulation of plasma pH and osmolality, and hormone production (renin, erythropoietin and vitamin D) (25, 95).

The kidneys receive more than 20% of the CO through the renal artery, and this rich blood supply is essential for renal functions. The functional unit of the kidney is the nephron and there are about 3 million of these in both kidneys. Each nephron is made up of a renal corpuscle and a renal tubule. The renal

corpuscle consists of the glomerulus (a network of capillaries) enclosed by a glomerular or Bowman's capsule. The glomerulus, by pushing fluid through capillary pores, produces a filtrate that passes through the rest of the nephron to become urine. The renal tubule is made up of the proximal convoluted tubule (PCT), loop of Henle, distal convoluted tubule (DCT) and collecting duct. The PCT is important for selective reabsorption of water, sodium, glucose, and amino acids that entered the filtrate. The loop of Henle is a U-shaped tubule involved in concentrating the renal interstitium through a countercurrent mechanism involving salt and water. DCT reabsorbs Na^+ and Cl^- from the filtrate, and secretes K+, and these processes are regulated by the hormone aldosterone. It may also reabsorb less Na^+ in the presence of natriuretic substances like ANP (69).

Regulation of sodium and body fluid volume is predominantly by the RAAS and pressure natriuresis mechanisms while sympathetic nervous control and natriuretic peptides make minor contributions (12).

Natriuretic peptides

Natriuretic peptides are endogenous hormones involved in natriuresis and diuresis. They are commonly grouped as cardiac or renal. The cardiac peptides are atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). ANP is mainly produced within the atria (47). BNP is mainly produced in the ventricles (88). ANP is usually secreted at a physiologically low concentration in response to atrial stretch. BNP is released with myocardial stretch. Both ANP and BNP can also be secreted in response to pressor

hormones such as epinephrine, phenylephrine, and angiotensin II (47, 65, 79). Both exhibit cardiac and renal protective properties and can mediate natriuresis, diuresis, increased glomerular filtration rate, reduction of renin-angiotensin activity, and increased cardiac output (65, 78, 79). However, they are quickly degraded from the system by an enzyme called neprilysin. BNP is reported to have a half life of about 20 minutes and ANP, about 7 minutes (16, 47, 99, 103).

The renal natriuretic peptide – urodilatin is structurally and functionally like ANP (1, 24). Urodilatin is secreted from the distal tubules of the kidneys in a circadian fashion and is influenced by increased dietary sodium intake or acute saline infusion (24, 30). Some studies have also shown that the synthesis of urodilatin is affected by cardiac sympathetic nerves (1, 33). Like the cardiac natriuretic peptides, urodilatin also has a very short half-life (11).

Sympathetic Nervous Control

The sympathetic nervous system (SNS) is involved in short- and long-term regulation of blood pressure. On a short-term basis, the sympathetic nerves are influenced by arterial baroreceptors which sense pressure changes in the arteries (60). A decrease in BP causes reduced baroreceptor firing which stimulates cardiac sympathetic drive and reduces vagal tone, resulting in increased firing from the sinoatrial node of the heart, and increased heart rate. Increased sympathetic activity also increases ventricular contractility which increases stroke volume and cardiac output. These combined effects restore BP to a normal level (60).

Long term regulation of BP by the SNS involves sodium regulation. It is thought that SNS nerves in the kidneys directly facilitate sodium and water reabsorption in the tubules, thus reduction in sympathetic outflow will result in increased sodium excretion and vice versa (61). Renin activity is also linked to sympathetic activity such that reduced sympathetic activity causes reduced renin secretion with reduced plasma angiotensin II formation. This leads to reduced sodium and water retention and increased salt and water excretion (12, 60, 61).

Renin-Angiotensin-Aldosterone System

The Renin-Angiotensin-Aldosterone system (RAAS) is an important hormonal system that mainly regulates BP by controlling water and electrolyte homeostasis and it significantly modulates pressure natriuresis (19, 35, 44).

As the name implies, there are 3 hormones involved in this system. Renin, a protease enzyme, is the rate-limiting enzyme and it is secreted by the juxtaglomerular cells of the kidneys. Synthesis of renin is directly influenced by increased sympathetic outflow via the beta-2 receptor, (as mentioned above in *sympathetic nervous control*), decreased renal perfusion pressure, reduced Na^+ delivery to the macula densa of the DCT, presence of prostaglandins, nitric oxide, adenosine and other mediators. (12, 61). Conversely, endothelin has been found to inhibit renin secretion (12).

Once renin is released, it acts on the renin substrate – angiotensinogen, a peptide hormone synthesized in the liver. Renin converts angiotensinogen to angiotensin I (AT I). AT I is then converted to Angiotensin II (AT II) by

angiotensin-converting enzyme (ACE), located on the lung endothelium (mainly) and kidney epithelial cells (12, 25, 61).

AT II is a potent vasoconstrictor which maintains or increases systemic blood pressure, as needed, by increasing peripheral vascular resistance. It also stimulates the release of aldosterone and antidiuretic hormone. These hormones stimulate sodium retention and thirst, respectively, to expand body fluid volume, CO and ultimately BP (25, 61). Both angiotensin and aldosterone have **anti-natriuretic effects**. AT II binds to 2 receptors; angiotensin II receptor 1 (AGTR1) and angiotensin II receptor 2 (AGTR2) (29, 90). The vasoconstrictive (anti-natriuretic) effect of AT II is mediated through AGTR1 while AGTR2 had been found to express an opposite effect (9, 12, 29). In a study conducted by Siragy et al on the function of AGTR2 in mice, they infused AGTR2 null mice with AT II for 1 week and found exaggerated anti-natriuretic effect characterized by sustained BP elevation and sodium retention. The wildtype mice however, had no changes in BP and urinary Na^+ (90). Padia et al further proposed from their study that AT II produces a **natriuretic effect** in the absence of AGTR1 and that to activate this effect, AGTR2 does not bind directly to AT II but to its metabolite – des-aspartyl1-AngII (71). This is similar to the findings from the study conducted by Siragy et al, suggesting that AGTR2 regulates BP by enhancing urinary sodium excretion (natriuresis) (9, 90).

Aldosterone significantly increases sodium reabsorption along with potassium secretion, mainly in the DCT and collecting duct of the nephrons, but also in

the gastrointestinal tract, sweat and salivary glands. This reabsorption/retention of sodium is important in maintaining effective circulating blood volume and normal blood pressure(61).

Pressure natriuresis (intrinsic renal control of arterial blood pressure)

Though not well understood by early researchers, hypertension was thought to be related to a dysfunction of the renal system. The role of renal sodium excretion in regulating arterial pressure remained rather vague, however, until the early 20th century when Guyton articulated the idea that long-term blood pressure regulation is complexly linked to renal excretory function (39, 40). A principal system for long-term control of arterial pressure is the pressure-natriuresis mechanism, by which increased renal perfusion pressure results in decreased sodium reabsorption and increased sodium excretion, with resultant changes in extracellular fluid volume (ECFV). Renal perfusion pressure directly controls sodium reabsorption in the proximal tubule. As renal perfusion pressure increases, renal peritubular capillary hydrostatic pressure (filtration) increases, with a resultant increase in renal interstitial hydrostatic pressure (RIHP). With increased filtration from the capillaries, more fluid exits into the renal interstitium thus, reducing sodium reabsorption and increasing urinary sodium excretion(20, 46). Nitric oxide has been shown to mediate and improve pressure natriuresis (37). Conversely, pressure natriuresis can become impaired in the presence of sympathetic overdrive, endothelial dysfunction, genetic mutations or renal abnormalities that increase sodium reabsorption at the distal tubule or antinatriuretic mediators like endothelin, AT

II, or aldosterone (40, 41, 46, 100). It follows that a sustained increase in blood pressure occurs if the pressure natriuresis response is impaired, making hypertension a disease of the kidney (15, 43, 46, 61).

Dahl and Heine studied the pressure natriuresis relationship in isolated kidneys of salt sensitive (SS) and salt resistant (SR) rats. Both groups were fed sodium restricted and high salt diets at different times in the study and they followed up with their blood pressure, urine volume and urinary sodium excretion. Both groups sustained normal blood pressure while on a salt restricted diet, while the SR rats on a high salt diet also remained normotensive throughout the study. On the other hand, blood pressure became progressively elevated in the SS rats fed a high salt diet. They found that the pressure natriuresis mechanism was preserved in both groups before the onset of hypertension but became depressed with sustained BP elevation (31).

The pressure-natriuresis relationship is most often studied as a linear relationship, which is a regression line of plots of renal perfusion pressure against urine sodium excretion. The slope of this curve indicates the sensitivity of the relationship (steeper means more sodium is excreted as RPP rises), and the Y intercept of parallel curves indicates a curve shift to the left (more sodium excreted at each RPP) or right (less sodium excreted at each RPP). Spontaneously hypertensive rats (SHR) are known to have abnormal pressure natriuresis, with a right shifted curve and a shallower slope (83).

Treatment of Hypertension

Treatment is generally individualized, based on the severity of hypertension, presence/absence of comorbidities, presence and/or risk of complications especially end organ damage. If a patient's BP is not well controlled after lifestyle modifications (reduced salt intake, frequent exercise, limited/no alcohol intake, increased fruits, and vegetable consumption), the clinician resorts to drug treatment and/or surgical management, as required. Blood pressure medications (antihypertensives) may also be the first management option if the patient presents with severe or complicated hypertension. Previously, monotherapy was adopted in the management of high blood pressure, but with increasing incidences of resistant hypertension and treatment failure, most patients are now placed on combination therapy.

Antihypertensive medications generally work by controlling cardiac output through modification of heart rate or stroke volume, or by regulating vascular resistance since BP is a product of cardiac output and peripheral resistance. Antihypertensives that reduce heart rate include β-blockers and calcium channel blockers (CCB). β-blockers inhibit β1 adrenergic receptors from binding to adrenaline, thus reducing heart rate, contractility, and cardiac output. Examples include Atenolol, Propranolol, Carvedilol. CCBs control BP by inhibiting calcium entry through calcium channels. This effect on cardiac muscles reduces the force of contraction and heart rate, while on the vascular smooth muscle, it prevents vasoconstriction. Ultimately, CCBs reduce cardiac output and cause a decrease in resistance. Examples of CCBs include

Nifedipine, Amlodipine, Nicardipine. Another class of antihypertensive called diuretics controls cardiac output by regulating blood volume. Diuretics inhibit sodium reabsorption and favor sodium excretion from the kidneys thus reducing circulating volume and stroke volume. They are the most common first line antihypertensives because sodium regulation is key to the regulation of blood volume and blood pressure and they have proven to be very effective over the years. Hydrochlorothiazide, Furosemide, Amiloride, Spironolactone, Indapamide are a few examples of diuretics.

The other antihypertensive class that regulates vascular resistance is known as vasodilators. They include CCBs, angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), renin inhibitors, α-blockers, and direct vasodilators. ACEIs work by preventing conversion of AT I to AT II and inhibiting the vasoconstrictive effect of AT II. Examples are Lisinopril, Captopril, and Enalapril. ARBs work like ACEIs but by blocking AT II from binding to AGTR1 to cause vasodilation. ARBs include Losartan, Telmisartan, and Valsartan. Renin inhibitors inhibit renin release and the only example licensed so far is Aliskiren. α-blockers inhibit α1 adrenergic receptors responsible for initiating smooth muscle contraction and examples include Prazosin and Doxazosin. Antihypertensives like hydralazine and minoxidil are known as direct vasodilators because they cause direct vasodilation on smooth muscles (3, 5, 19, 50, 64).

Surgical interventions are also available and vary based on the secondary causes. Angioplasty may be done to repair coarctation of the aorta,

adrenalectomy for diseased adrenal gland, nephrectomy for end stage renal disease or tumor, renal denervation and deep brain stimulation for resistant hypertension (66, 86, 91)

Exercise and hypertension

Physical activity is a major modifiable factor that reduces one's chances of developing hypertension (74). The beneficial effects of regular exercise for the promotion of health and prevention of diseases have been clearly shown. Regular exercise helps to **reduce** blood pressure, lower the **risk of heart disease, and** strengthen the heart**. Other** cardiovascular benefits of exercise **include** improvement of blood flow and oxygen to the body tissues, and **control of body weight**, with obesity also a risk factor for hypertension. Exercise also improves **sleep, mental health, and mood, and strengthens bones and muscles** (108)**.** A meta-analysis conducted by Wahid et al. to assess the relationship between physical activity and cardiovascular diseases showed that the risks of cardiovascular events declined with increasing physical activity. This suggests that even a minimal increase in physical activity results in significant cardiovascular benefit (101).

The burden of hypertension-related cardiovascular disease has become more worrisome as the incidence keeps increasing in children and adolescents. Although primary hypertension in childhood is commonly associated with obesity, it seems that other factors, such as dietary sodium intake and exercise, also influence BP levels (93). Several studies support that sympathetic nervous system imbalance, impairment of the physiological

mechanism of pressure natriuresis, hyperinsulinemia and early vascular changes are factors responsible for the early-onset development of hypertension. Although restriction of dietary sodium has been shown as a rational step in the prevention of hypertension in genetically predisposed individuals, interventional studies show that regular aerobic exercise can significantly reduce BP and reverse vascular changes in obese/hypertensive patients (93). T-cell and macrophage accumulation in the kidneys seen in cases of atherosclerosis and obesity are also known to impair the pressure natriuresis relationship. T-cells stimulate release of inflammatory cytokines which cause oxidative stress, renal injury and nephron loss resulting in renal sympathetic overdrive. Increased sympathetic activity in turn shifts the pressure natriuresis curve to the right (46). Obesity also disrupts pressure natriuresis through direct compression of the kidneys by renal fat, increased activation of RAAS, reduced natriuretic peptides and endothelial dysfunction (40, 42, 55).The burden of hypertension-related cardiovascular disease has become more worrisome as the incidence keeps increasing in children and adolescents. Although primary hypertension in childhood is commonly associated with obesity, it seems that other factors, such as dietary sodium intake and exercise, also influence BP levels (93).

Endothelial dysfunction has also been described to play a key role in the development of hypertension. The vascular endothelium secretes vasoactive substances including nitric oxide (NO), a potent vasodilator which maintains a basal dilated tone of the blood vessels and promotes natriuresis and diuresis

(67, 87, 92). NO has also been found to protect against plaque formation. Damage or dysfunction of the endothelium, therefore, reduces NO release which causes an increase in peripheral resistance, reduced blood flow, and elevated blood pressure [17]. Reduced NO has also been shown to shift the pressure natriuresis curve to the right (56).

Exercise has been associated with increased release of nitric oxide. Maiorana et al in a study on the relationship between exercise and nitric oxide, documented that individuals with previous endothelial dysfunction had improved dilation of blood vessels when they exercised regularly. The improvement manifested as a systemic reduction in total peripheral resistance rather than a local vasodilation of the exercised muscle or group of muscles (62). This suggests a mechanism of the relationship between exercise and lower blood pressure as there is increased blood flow to the renal interstitium which stimulates the pressure natriuresis mechanism.

Exercise has been shown to improve outcomes in hypertensive patients evidenced by reducing heart rate, improving blood flow and oxygen extraction. A study conducted on the effect of long-term exercise on the RAAS pathway showed that fractional sodium excretion improved in exercised hypertensive rats. This was attributed to reduced AGTR1 expression and increased urinary Na+ excretion in the exercised rats (14). Goessler et al also demonstrated that renin level is reduced with exercise training, thus reducing the levels of AT II and aldosterone (32). Finally, exercise improved urine sodium excretion in rats with congestive heart failure in a study conducted by Zheng et al (110).

Numerous professional organizations recommend exercise as an initial lifestyle therapy to prevent, treat, and control hypertension. Exercise is now prescribed in terms of frequency, duration, and intensity. The popular recommendation is moderately intense aerobic exercise (such as walking, running, cycling, swimming), lasting for an average of 30 minutes per session and at least 3-4 days per week (104). According to the latest physical activity guidelines for Americans, adults are encouraged to engage in at least 2.5 hours of moderately intense aerobic exercise per week or at least 75 minutes of vigorous exercise per week (28, 75). A review article published by Patel et al, however, shows that aerobic exercise is effective for cardiac remodeling while both aerobic and anerobic exercises can improve high density lipoprotein and reduce low density lipoproteins and triglycerides (72). Findings from an aerobic exercise study conducted on older patients with resistant hypertension also showed up to 6mmHg reduction in systolic blood pressure (18).

Background

Despite numerous studies on hypertension, more research is necessary for improved understanding and management of this disease. Currently, the mechanisms by which exercise lowers blood pressure or prevents hypertension are not fully understood. In addition, the mechanism by which exercise in children and adolescents, reduces the risk of later hypertension development, is unclear. Furthermore, effects of exercise on urine sodium

excretion have been investigated in rats with congestive heart failure or hypertension, but the effect on the pressure natriuresis relationship in normal rats has not been reported. This study examined the effects of exercise in young, growing rats on the pressure natriuresis relationship, once they reach adulthood.

Problem statement

Since exercise is recommended as a non-pharmacological approach to managing hypertension, and much more as an important factor for preventing hypertension, understanding the mechanism of this effect is critical. Pressure natriuresis is known to be altered in hypertension and is believed to contribute significantly to the rise in mean arterial pressure. Therefore, this research investigated in normotensive male and female rats, the relationship between exercise during growth and development, and pressure natriuresis, to observe effects of exercise in the absence of underlying disease.

Hypotheses

1. Exercise in rats during growth and development will improve the pressure natriuresis relationship as adult rats.
2. Exercise will have a greater effect on pressure natriuresis in females than males.

Previous work

Rats are an acceptable model for the study of hypertension because they have similar cardiovascular systems and mechanisms of blood pressure control as found in humans (57).

Previous studies conducted in this laboratory on SHR (Spontaneously Hypertensive Rats) and WKY (Wistar-Kyoto Normotensive rats) showed that exercised rats excreted more urinary sodium when blood pressure was raised, suggesting that exercise might modify how the kidney responds to blood pressure. The surgical manipulation was, however, not tightly regulated, hence, there was significant variation in the renal perfusion pressures across the study groups. Furthermore, the study did not investigate the sodium excretion in response to a lowered blood pressure. The current study carefully regulated the increase in blood pressure, and added a decrease in blood pressure, measured sodium excretion, and the change in sodium excretion, at each level to produce a pressure-natriuresis curve for each group.

Materials and Methods

Forty normotensive male and female Wistar Kyoto (WKY) rats were weaned at 4 weeks, then randomly assigned to the exercise or sedentary (control) group. Rats were housed in standard plastic cages with corn cob bedding, given standard rat chow and water ad libitum, and kept at 22^{o} C with a 12:12 light cycle.

Control/sedentary group*:* 8 male (8 Sed M) and 8 female (Sed F) rats were housed individually with no exercise wheel for 8-10 weeks.

Exercise group: 10 male (EX M) and 10 female (Ex F) rats were housed individually in a cage with an exercise wheel for 8 to 10 weeks. The exercise wheel was connected to a counter which monitored the number of wheel revolutions. This counter was read, recorded, and reset twice weekly.

Antibiotics: Early in the study, several rats developed respiratory distress during anesthesia and died. Necropsy revealed pneumonia, commonly due to an opportunistic bacterium, *Pneumocystis (carinii or wakefieldiae).* To prevent the pneumonia, all rats in both the control and exercise groups were subsequently placed on antibiotics for the first 10 days of the study. Trimethoprim/Sulfamethoxazole was added to their drinking water to deliver a dose of 50mg trimethoprim/250mg sulfamethoxazole /kg per day. The average rat weight at set up period was about 200g (0.2 kg) and each drank about 50mls of water per day. Based on the weight and the quantity of water consumed per day, each treated rat received a daily dose of 60mg (1.25mls) of antibiotics in 50mls of drinking water.

Surgical Setup

At the end of the sedentary or exercise period, the rats were ready for surgery. Each rat (13-15 weeks of age) was anaesthetized with 3% isoflurane in oxygen, and once asleep, dosed with intraperitoneal Inactin, at a dose of

100mg/kg. Inactin became unavailable for part of the study, therefore, eleven rats (5 sedentary males, 1 exercised female, 5 sedentary females) were dosed with a different combination of intraperitoneal anesthetics – Tilzolan and Xylazine. After several adjustments, the most effective dose was determined to be 20mg/kg and 0.5mg/kg for Tilzolan and Xylazine, respectively, with 25% of the initial dose given intraperitoneally as needed to maintain anesthetic depth. The other 75% is given deep into the thigh muscle. If a repeat dose was required, the rat was given 20% of the initial intramuscular dose, as required.

Each rat was shaved on the ventral surface from the neck to the caudal abdomen, and along the inner thighs. The rat was then placed on a heating pad, in dorsal recumbency. A rectal temperature probe was inserted to monitor the rat's temperature, and the heating pad adjusted to keep temperature at 37^{o}C.

Next, a tracheal tube (PE 240) was inserted directly into the trachea to aid respiration as rats breathe poorly when anaesthetized. The carotid and femoral arteries were isolated and catheterized with heparin/saline (0.3ml heparin/50 ml saline) filled PE 50 and PE 20 tubing, respectively. Tubing was connected to stopcocks and both arterial catheters were connected to pressure transducers which interfaced with a computer using physiological software (BIOPAC). The jugular vein was also catheterized with saline filled tubing (PE 50) attached to a stopcock for the infusion of saline at a rate of 20ml/kg/hr.

The abdomen was opened, a thermometer probe placed within the abdomen to determine abdominal temperature more accurately, and the temperature was kept between 35ºC – 36ºC. (The open abdominal cavity resulted in an artificially low temperature reading from the rectal probe.) The celiac and mesenteric arteries were isolated near their origin, and a thread placed around each artery and pulled through plastic tubing (PE 160) for later tightening to raise renal perfusion pressure. The aorta was isolated above and below the renal arteries and the tips of a forceps passed through an adjustable bulldog clamp were placed with one prong either side of the aorta above the renal arteries. The apex of the bladder was incised and a tube (PE 240) with a lip flamed on one side placed in the lumen and tied in place with suture.

Exposed abdominal organs were covered with wet saline sponges and wrapped in plastic, then the rat was allowed to stabilize for a 45-minute equilibration period. Tuberculin syringes with the tips cut off, were labeled as urine tubes to collect and record urine volumes. After the equilibration period, the computer recording continued throughout the rest of the acute study. A total of seven, 15-minute, urine collection periods were utilized – two at baseline, two at the lower blood pressure (20 mmHg below baseline), and three at a higher blood pressure (30 mmHg above baseline).

After the two baseline periods, the bulldog clamp was slowly tightened to constrict the forceps on the aorta (above the renal artery) until the femoral BP was 20mmHg below the animal's baseline BP. Two 15-minute urine samples

were collected and recorded. This gave the manipulated **low value** BP. To raise blood pressure, the string ends protruding through the tubes on the celiac and mesenteric arteries were tightened and clamped to raise blood pressure by trapping additional blood in the aorta. In addition, the forceps was moved to the aorta, below the renal arteries, and tightened until blood pressure reached 30mmHg above the animal's baseline BP. This produced the **high value**. Three 15-minute urine samples were collected.

The combination anesthesia (Tilzolan/Xylazine) raised the baseline mean arterial pressure in the rats approximately 30 mmHg above that of the Inactin treated rats. To maintain consistency between these two anesthetics, the "baseline value" was used as the "high value". The renal perfusion pressure was lowered by closing the forceps above the renal arteries until pressure reached the mean Inactin baseline value of approximately 100 mmHg, and this was used as the baseline value. The low value was then set 20 mmHg lower (80mmHg) and was attained by further tightening the forceps on the aorta above the renal artery.

Urine preparation and sodium analysis

Urine volumes were measured to the nearest 0.01ml, covered with parafilm, and stored at 10 ^{o}C until analysis by flame photometer. During analysis, each urine sample less than 0.15 ml was diluted to a volume of 0.30 ml with DDI water to ensure an adequate sample size. Any sample greater than 0.15 ml was diluted to twice the original volume so the sodium concentration did not exceed that recommended by the operations manual of the flame photometer.

Following the directions for the flame photometer, each of the samples was further diluted to 1:200 and analyzed in duplicate. Total sodium excretion in each 15-minute period was calculated by multiplying the average sodium concentration in each sample by the urine volume for that period. The concentration of sodium was adjusted for weight (sodium excretion per kilogram body weight per 15 minutes) and changes in urine volume and sodium excretion from baseline were also calculated.

Data Analyses

18 male and 18 female WKY rats were studied and randomly assigned to exercise or sedentary treatment groups (8 sedentary males (Sed M), 8 sedentary females (Sed F), 10 exercised males (EX M) and 10 exercised females (Ex F)). Two rats from the initial sedentary male group, and two from the sedentary female group were discarded, due to the erratic outcomes from changing anesthetic drugs - the baseline urinary sodium excretion was extremely high (up to four times the highest baseline urinary sodium excretion), hence the non-uniform sample size between the control and treatment groups.

The renal perfusion pressures (RPP) were measured using the BIOPAC software, and the data was transferred to an Excel spread sheet. The means and standard errors of the RPP for each collection period were calculated.

Sigma Plot 12.5 program was used for statistical analysis. A one-way ANOVA was used to compare body weights, ages at the acute study, acute study duration, saline infusion rate, and kidney weights. A two-way repeated measure ANOVA was used to compare RPP, urine volumes, and urine sodium excretions, and change in each value from baseline, within each group, and between groups. Results of each manipulation were averaged, and the average data analyzed using a two-way repeated measure ANOVA. In a similar manner, change from baseline data was averaged for each manipulation and analyzed. Holm-Sidak post hoc test was carried out for multiple comparison of periods and groups.

A pressure natriuresis curve was created for each group by plotting urine sodium excretion against RPP for each rat and generating a linear regression line. Slopes and Y intercepts were compared between groups using Sigma Plot software.

Statistical significance was accepted when $p<0.05$.

Results

General data

No differences were found between any rat groups in age, length of time of acute study, saline flow rate, hematocrit, kidney weight or body weight, except the male rat kidney and body weights which were greater than the female rats ($p < 0.05$) (Table 1).

Exercise

Male and female rats ran similar weekly distances throughout the duration of the exercise weeks, except for week 4, during which the female rats ran a greater mean distance than the males (Figure 1).

Renal Perfusion Pressure (RPP)

a) *RPP data*

For all groups, the lowered and raised RPP values were significantly different ($p<0.001$) than baseline, and high values were different than low values. The EX M group had significantly lower ($p < 0.05$) baseline values (99±4 vs 107±3, and 100±3 vs 106±3 for baseline 1 and baseline 2 respectively) and lower values for both low periods (77±3 vs 87±3 for both low periods) than the Sed M group (Figure 2). No differences were found in RPP between the exercised and sedentary females.

b) *Mean of the RPP for each manipulation*

The average of the RPP values for each manipulation was plotted for all subgroups. The mean baseline and low values remained significantly lower ($p=0.021$, $p=0.039$ respectively) in Ex M than Sed M (Figure 3). No differences were found between the exercise and sedentary females.

c) *Mean RPP changes from baseline (∆RPP) for each manipulation*

For this analysis, the change in RPP (∆RPP) from baseline to the low or to the high values were averaged for each manipulation in all groups and plotted against their respective manipulations. No significant difference in mean ∆RPP was observed between Ex M and Sed M values. There was also no significant difference between Ex F and Sed F mean ∆RPP values across all period manipulations (Figure 4).

Urine Volume

Urine volumes in both exercise groups increased significantly over both baseline and low RPP values during all high RPP periods ($p < 0.05$). In the sedentary groups, urine volume was significantly increased over the low RPP periods during all high periods but was only significantly greater than baseline during high 3 in the males, and high 2 and 3 in the females ($p < 0.05$). Exercised males had a significantly higher urine volume than the sedentary males during the second raised RPP period (high 2) ($p = 0.036$). No sex difference in urine volumes was found between the male and female sedentary groups, or the exercised groups (Figure 5).

Urine sodium (Na+) excretion

a) *Na+ excretion*

Sodium excretion was significantly increased ($p <0.05$) above both baseline and low values when RPP was raised in the exercised male and female rats (Ex M and Ex F) (Figure 6). However, in the sedentary groups, sodium excretion was significantly elevated ($p < 0.05$) above

baseline and low values only during the third period of raised RPP (high-3) (Figure 6).

Exercised males had a significantly greater ($p < 0.05$) urine sodium excretion than sedentary males during the second and third periods of raised RPP (high-2 and high-3). No between group differences in Na+ excretion were found in the female rats, and no sex differences were observed between the exercised males and females, or the sedentary males and females.

b) *Means of Na+ excretion for each manipulation*

The average Na+ excretion was calculated for each manipulation. Urine Na+ excretion was significantly greater in the high RPP periods of exercised males and, sedentary and exercised females when compared to baseline and low values ($p < 0.05$). In Sed M, the Na+ excretion was not significantly altered by any manipulation. Comparison of Na+ excretion between the groups showed that the exercised males had a greater sodium excretion during elevated RPP (high) than the sedentary males (Figure 7).

c) *Means sodium excretion changes from baseline (∆ Na+ excretion) for each manipulation*

The mean ∆Na+ excretion was calculated for each manipulation and is shown in Figure 8. The change in sodium excretion during the high RPP was significantly different than the change during the low RPP in

sedentary females, and exercised males and females ($p < 0.05$). The change in sodium excretion during high RPP was significantly greater in the Ex M compared to that of Sed M ($p=0.0041$) (Figure 8) but no significant differences were found between the female groups.

Pressure natriuresis curves

Exercise had a significant effect on the pressure natriuresis curve in both male and female rats. The slope of the curve was greater in the exercised male rats than in the sedentary male rats (2.07 vs 0.79, $p = 0.01$) and in the exercised female rats than the sedentary female rats (3.49 vs 1.81, $p=0.04$) (Figures 9 and 10). A sex difference was observed in the pressure natriuresis curves, with sedentary female rats having a lower y-intercept than the sedentary males (-132 vs –53; $p=0.039$), and the exercised females having a greater slope than the exercised males ($p=0.041$) (Figure 11).

Discussion

There were no significant differences in general data between the groups. The exercised and sedentary rats of the same sex had similar body and kidney weights, however male rats had higher body weights and kidney weights than females. Hematocrit and rate of saline infusion was also not significantly different between the groups which indicates similar level of hydration.

Within groups, all raised and lowered RPP values were significantly different from baseline values. The finding was similar when means of each

manipulation were compared to baseline, indicating that the induced changes in RPP were adequate. Between group comparisons showed that Ex M had significantly lower baseline and lowered RPP manipulation than the Sed M but found no difference between the female groups. The blood pressure lowering effect of exercise has been previously described and may have been responsible for this difference between the male groups, but this is unclear when dealing with anesthetized rats (102). The lack of difference in baseline RPP between the female groups may have been due to a sex difference in the effects of exercise, or simply the effects of general anesthetic.

The change in RPP from baseline for both the low and high manipulations was similar between the groups, indicating that the manipulated change in values were of similar magnitudes. This assures a consistent RPP change in determining the associated urine sodium excretion and avoids the potential complications of a non-linear pressure natriuresis relationship that may be evident only at greater distances from baseline.

Urine volumes and sodium excretion changed as expected, with changes in RPP resulting in parallel changes in urine volume and sodium excretion. It is well established that diuresis accompanies increases in RPP and natriuresis (36). Urine volumes were similar between groups, with one exception during high-2, when exercised males had a greater volume than sedentary males. There were no significant differences between Na+ excretion at the baseline RPP or at the lowered RPP across all groups. It is important to note that both male and female rats, exercised and sedentary, had measurable pressure

natriuresis responses, excreting more sodium into the urine as RPP was raised. The effect was also amplified in the exercised groups as high RPP manipulations resulted in significantly greater urinary Na+ excretion compared to the sedentary groups, even though the change in RPP from baseline was similar. This greater sodium excretion during the high RPP manipulations was significant only in the males, with the exercised male rats excreting significantly more sodium than the sedentary. The exercised females had a higher mean Na+ excretion than the sedentary females when RPP was raised, but this was not significant, likely affected by the large standard error. There was also no statistical sex difference in urine sodium excretion between males and females of the same treatment group.

Due to the large variation in baseline sodium excretion, change from baseline was calculated for all rats for each manipulation. Exercised male rats had a greater increase in urine sodium excretion when RPP was raised, than the sedentary males. Although the exercised female rats had a larger mean increase in sodium excretion during the high RPP manipulations, this was not significant, again, likely influenced by the large standard error.

A pressure natriuresis curve was created for each group and compared between the groups for statistical differences. The steeper slopes found in both male and female exercised rats when compared to the respective sedentary groups, indicates an improved sensitivity of the kidney to changes in RPP. Exercise resulted in a greater increase in urine sodium excretion for any given increase in RPP. Sex differences in the pressure-natriuresis

relationship were found as well, with the female rats showing a more sensitive renal sodium response to RPP changes than the male rats of the same treatment group. Although the slope for the sedentary groups was not statistically different, the slope difference resulted in a more negative y-intercept in the female, than in the male rats. The greater slope in the exercised females when compared to the exercised males indicates a greater increase in urine sodium excretion for any increase in RPP. Thus, it appears that female rats have a stronger pressure-natriuresis response.

The changes in the kidney induced by exercise are not well defined but some theories have however been proposed. Exercise improves endothelial function including greater nitric oxide (NO) production. This could result in a greater medullary blood flow and capillary hydrostatic pressure, resulting in a greater interstitial pressure that inhibits sodium reabsorption (56). Regular physical activity has been shown to improve shear stress, which in turn increases NOS activity. NO induces vascular remodeling and improves blood flow (26). Exercise also resulted in improved urine sodium excretion and increased sympathoinhibition, in response to blood volume expansion in rats with heart failure in the study by Zheng et al. The authors suggested that exercise may have improved the weak natriuresis effect in the rats with cardiac failure through 2 mechanisms. There is improved neuronal release of NO with prolonged exercise, which may be responsible for increased inhibition of renal sympathetic activity. They also explained that plasma level of AT II which intensifies sympathetic nervous stimulation, is reduced with

exercise training (110). Waldman et al also demonstrated that exercise improved sympathoinhibition and reduced AT II hypertensive rats to levels similar to those of normotensive rats (102).

Studies have shown that female sex hormones, especially estrogen, have protective cardiac and renal properties (53, 105, 106). A study conducted by Ji et al showed that estrogen inhibits oxidative stress by suppressing reactive oxygen species (ROS) (68, 107). Estrogen also regulates RAAS by reducing renin levels and angiotensin converting enzyme (ACE) activity, thus inhibiting sympathetic activity and preventing sodium retention (68, 107). Conversely, testosterone is associated with increased secretion of renin and angiotensinogen (54, 81). Nitric oxide is also increased in females due to upregulation of nitric oxide synthase (NOS) activity by estrogen (63, 105). Thus, the stronger pressure natriuresis relationship observed in females was due to the sex hormone difference and not necessarily the effect of exercise.

Since sympathetic overdrive, RAAS activation and endothelial dysfunction are factors shown to impair pressure natriuresis, **exercise** may modulate these factors resulting in **improved pressure natriuresis.**

Conclusion

This study sought to demonstrate that effects of exercise on blood pressure may be due to an effect on the pressure natriuresis relationship of the kidneys. The findings clearly demonstrate that exercise improves the

pressure natriuresis relationship in both male and female rats, supporting our first hypothesis. However, we did not see a greater effect in female rats than males (second hypothesis) but did find that the pressure natriuresis relationship in female rats is more sensitive than that of males. Since sympathetic overdrive, activation of RAAS and endothelial dysfunction are factors that have been shown to impair pressure natriuresis, this study therefore supports that exercise may modulate those factors resulting in inhibition of sympathetic activity and RAAS as well as increased secretion of NO.

Future Research

We have an ongoing study on the effects of exercise on pressure natriuresis in hypertensive rats and we would also like to study the exact mechanisms by which exercise improve pressure natriuresis.

Tables and Figures

Table 1: General data from rats

Group	Age (wks)	Acute Study Total Time (hr)	Flow Rate (ml/kg/hr)	Hematocrit (%)	Kidney Weight (g)	Body Weight (kg)
Sedentary Males	15.6 ± 0.6	5.72 ± 0.37	20.76 ± 1.24	58.5 ± 1.88	2.85 ± 0.10*	0.397 ± 0.011*
Exercised Males	15.2 ± 0.6	6.01 ± 0.35	19.17 ± 0.49	58.6 ± 1.2	2.98 ± 0.14*	0.384 ± 0.013*
Sedentary Females	17.6 ± 1.3	5.65 ± 0.20	21.36 ± 0.27	49.4 ± 3.3	1.99 ± 0.10	0.254 ± 0.011
Exercised Females	15.0 ± 0.8	5.34 ± 0.20	20.91 ± 0.48	52.1 ± 3.3	1.98 ± 0.08	0.259 ± 0.008

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

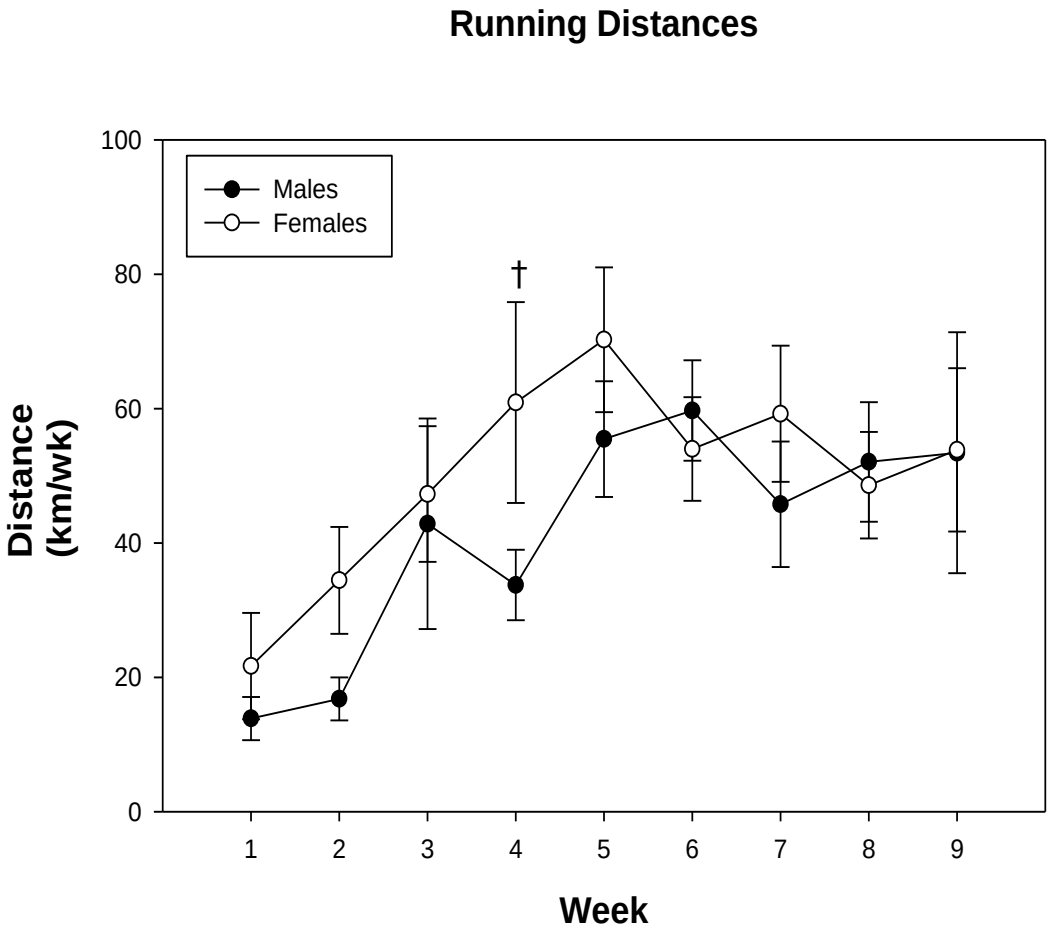

Figure 1: Mean weekly running distances in male and female rats. † $p < 0.05$ when compared to the male rats during the same week.

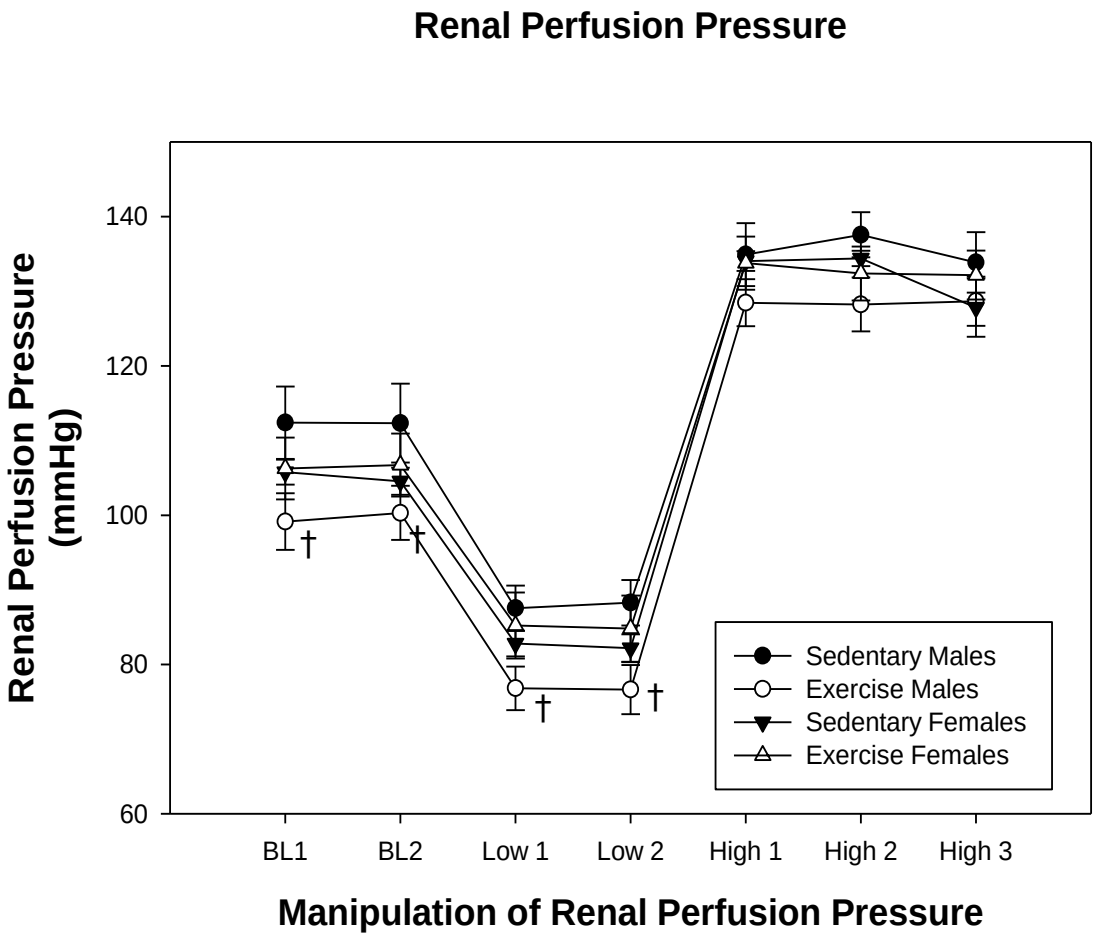

Figure 2: Renal perfusion pressure (RPP) during baseline (BL), lowered (low), and raised (high) manipulations in sedentary and exercised male and female rats. † $p < 0.05$ when compared to the same period in the sedentary rats of the same sex.

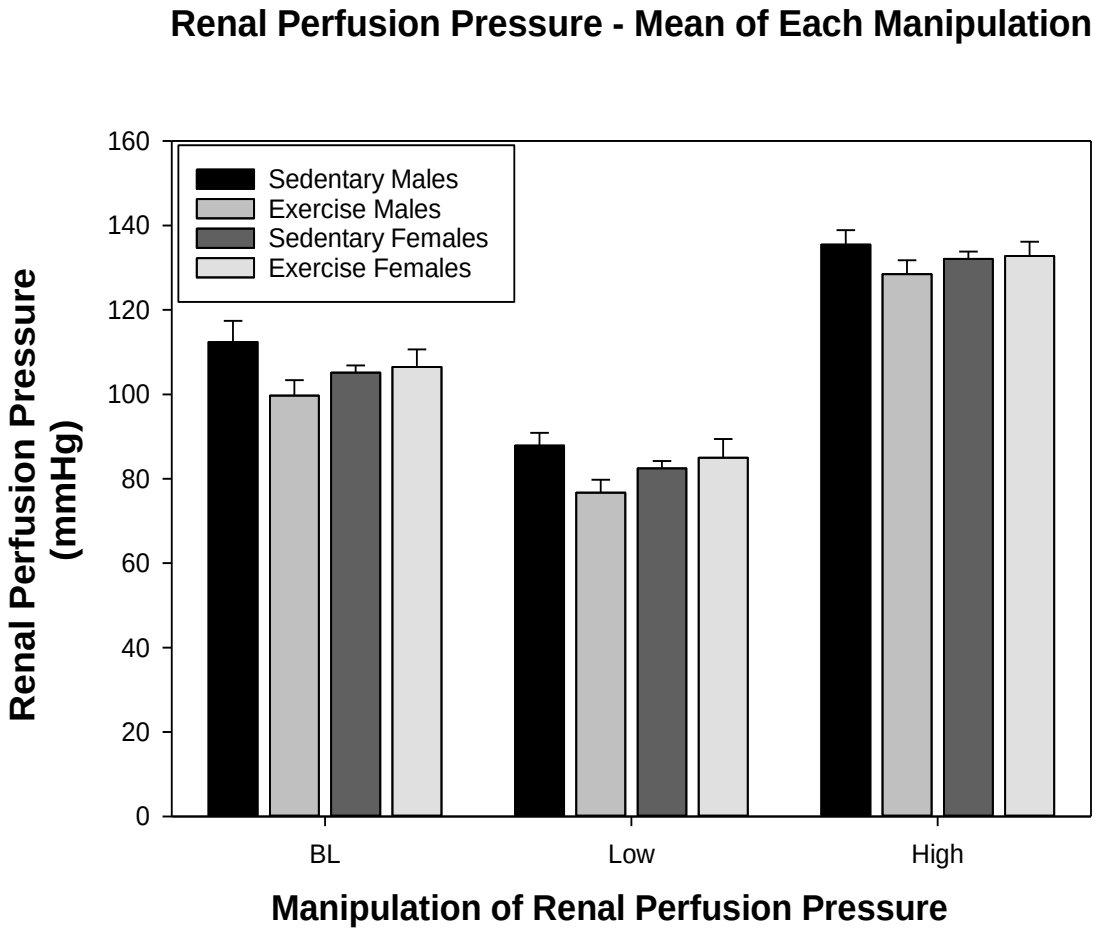

Figure 3: Mean renal perfusion pressure during baseline (BL) lowered (low) and raised (high) manipulations in sedentary and exercised male and female rats. † $p < 0.05$ when compared to the same period in the sedentary rats of the same sex.

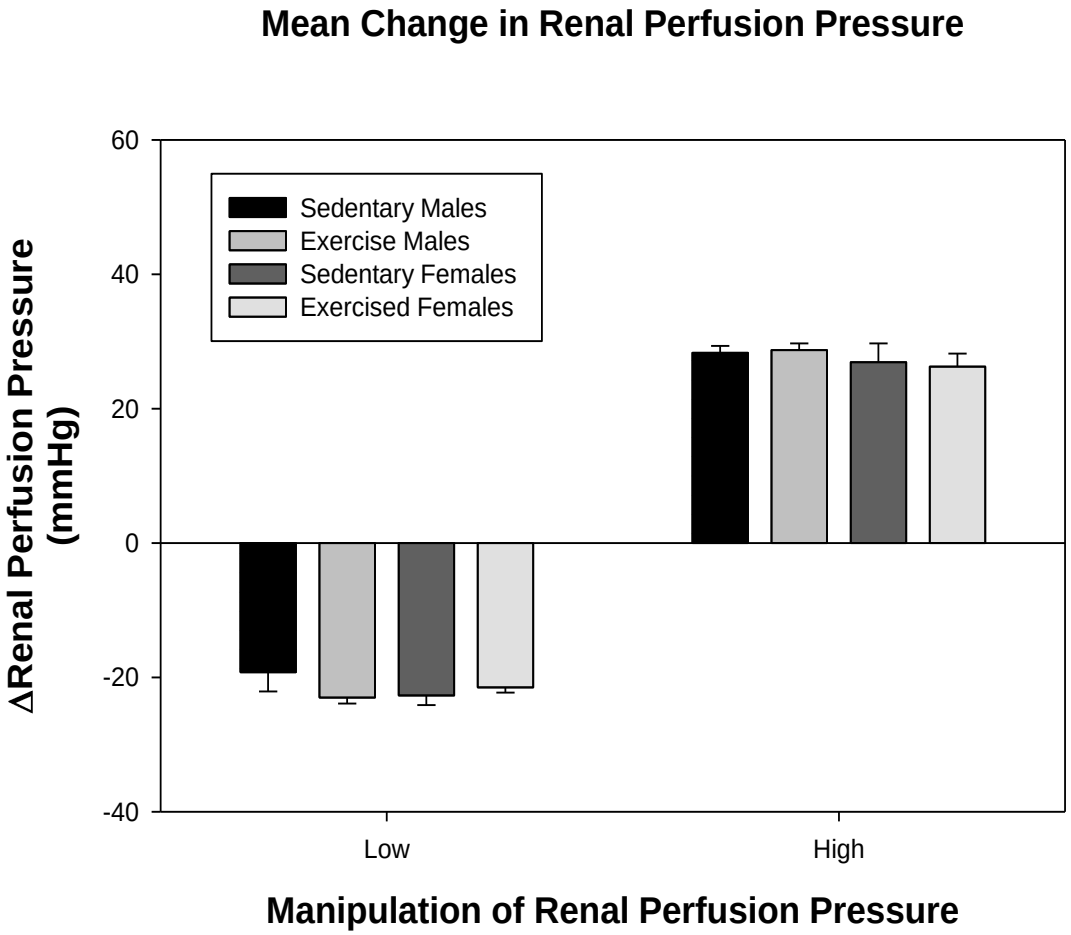

Figure 4: Mean change from baseline in renal perfusion pressure during lowered (low) and raised (high) manipulations in sedentary and exercised male and female rats.

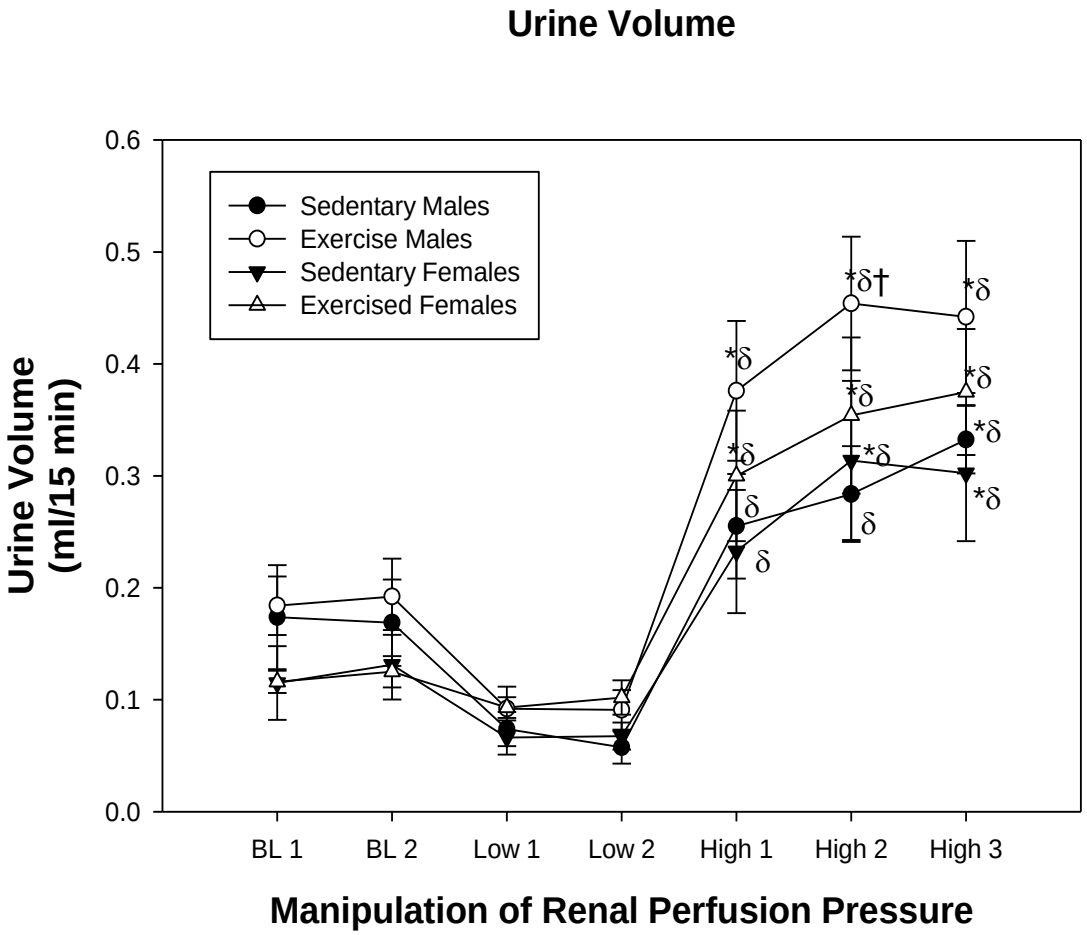

Figure 5: Urine volume during baseline (BL), lowered (low), and raised (high) manipulations of renal perfusion pressure in sedentary and exercised male and female rats.

*$p < 0.05$ when compared to either baseline value. δ $p < 0.05$ when compared to either low value. † $p < 0.05$ when compared to the same period in the sedentary rats of the same sex.

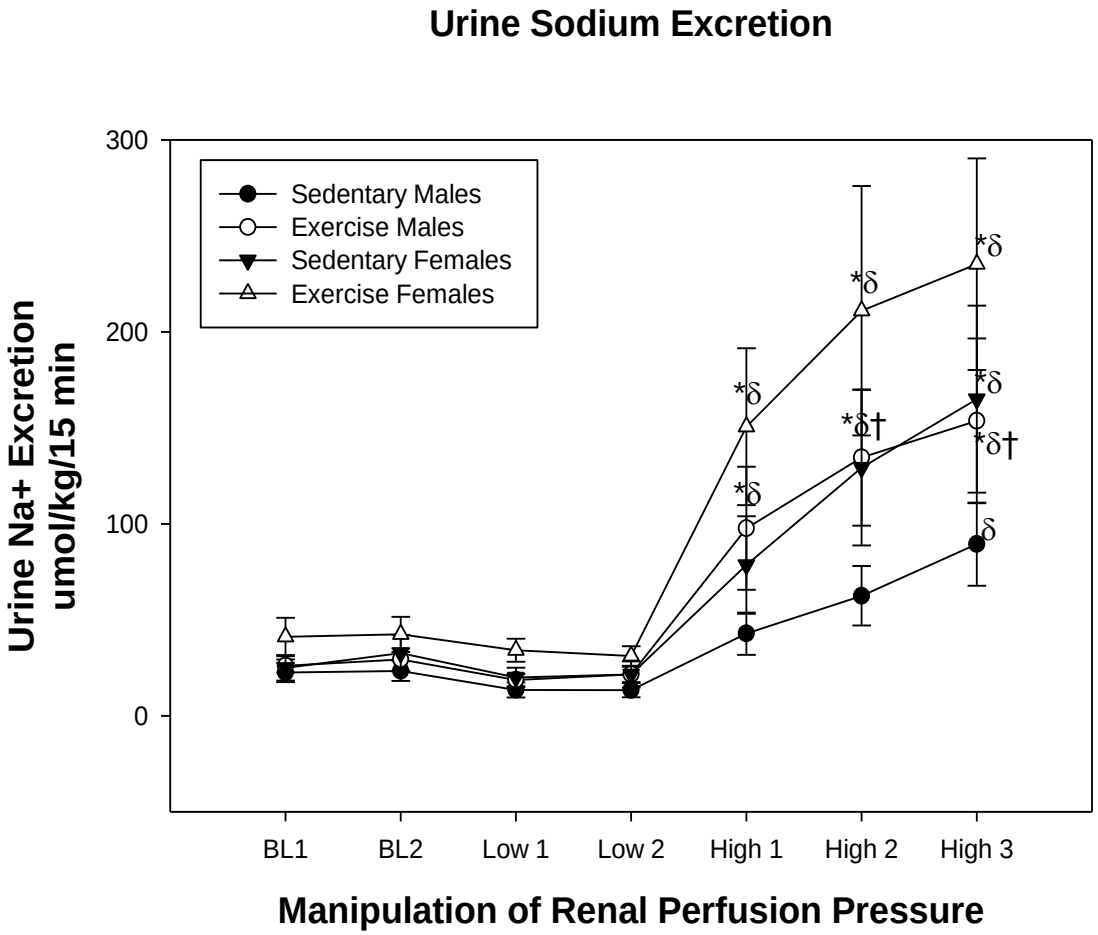

Figure 6: Urine sodium excretion during baseline (BL), lowered (low), and raised (high) manipulations of renal perfusion pressure in sedentary and exercised male and female rats.

*$p < 0.05$ when compared to either baseline value. δ $p < 0.05$ when compared to either low value. † $p < 0.05$ when compared to the same period in the sedentary rats of the same sex.

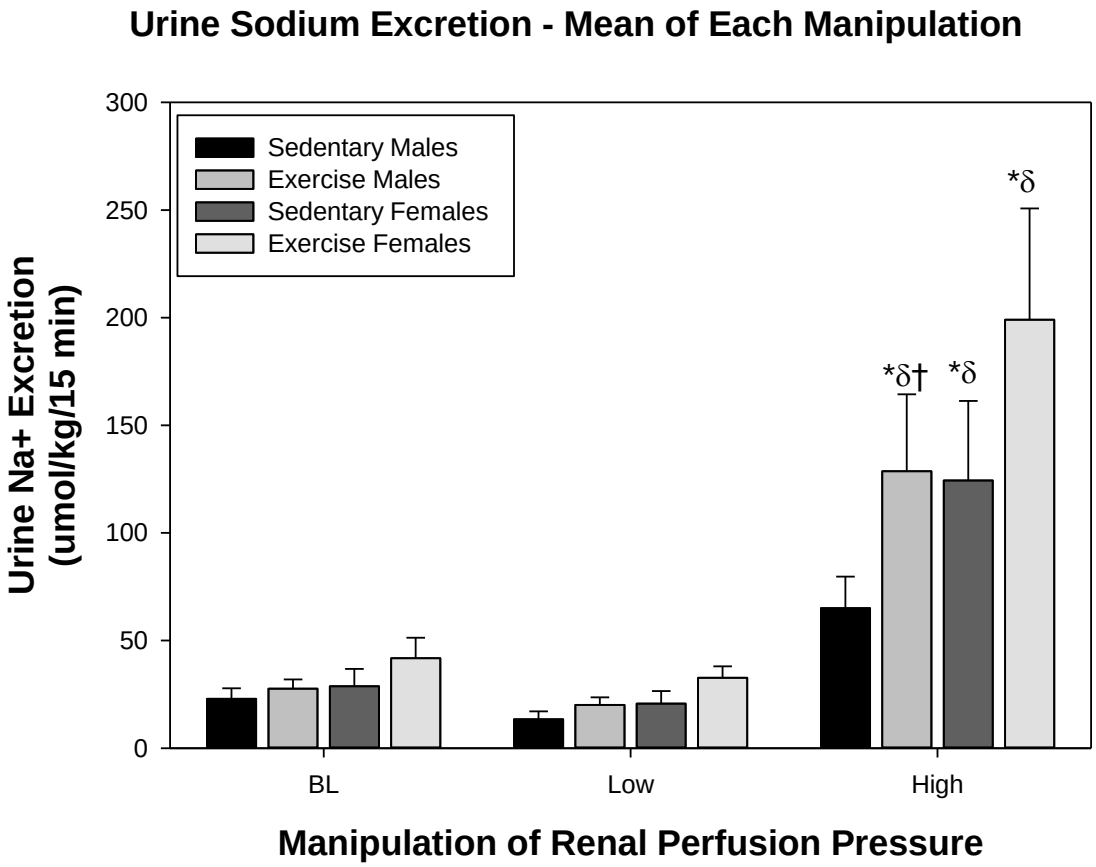

Figure 7: Mean urinary sodium excretion during baseline (BL) lowered (low) and raised (high) manipulations in sedentary and exercised male and female rats.

*p < 0.05 when compared to baseline value. δ p < 0.05 when compared to low value. † p < 0.05 when compared to the same period in the sedentary rats of the same sex.

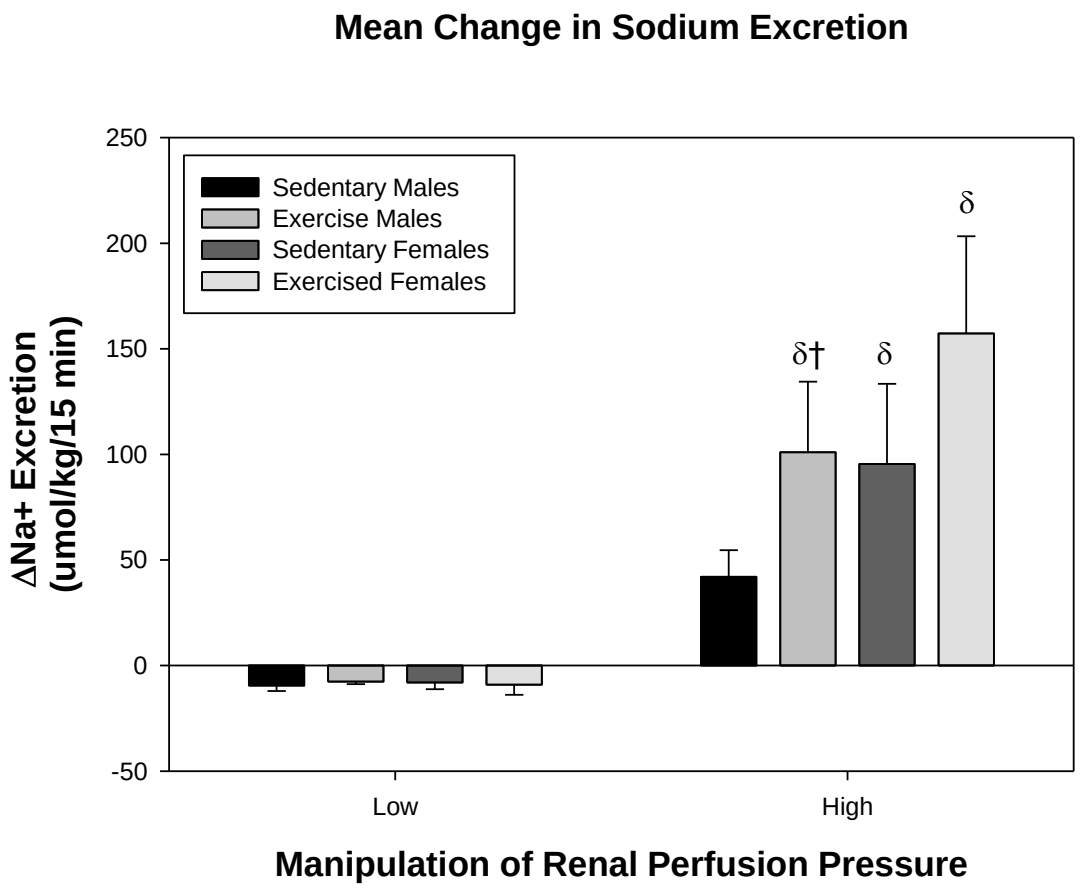

Figure 8: Mean change from baseline in urine sodium excretion during lowered (low) and raised (high) manipulations in sedentary and exercised male and female rats.

δ $p < 0.05$ when compared to low value. † $p < 0.05$ when compared to the same period in the sedentary rats of the same sex.

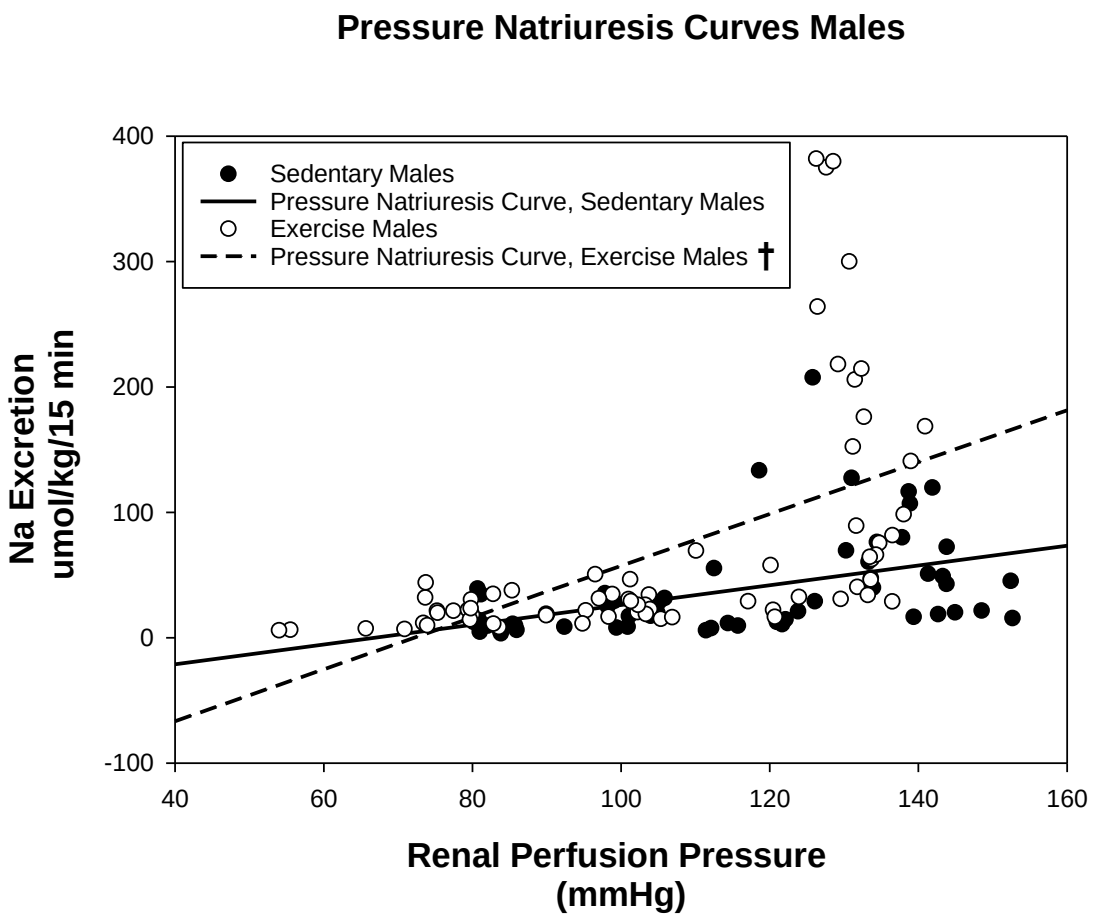

Figure 9: Pressure natriuresis curves in sedentary and exercised male rats.

† $p < 0.05$ when the slope is compared to that of the sedentary rats.

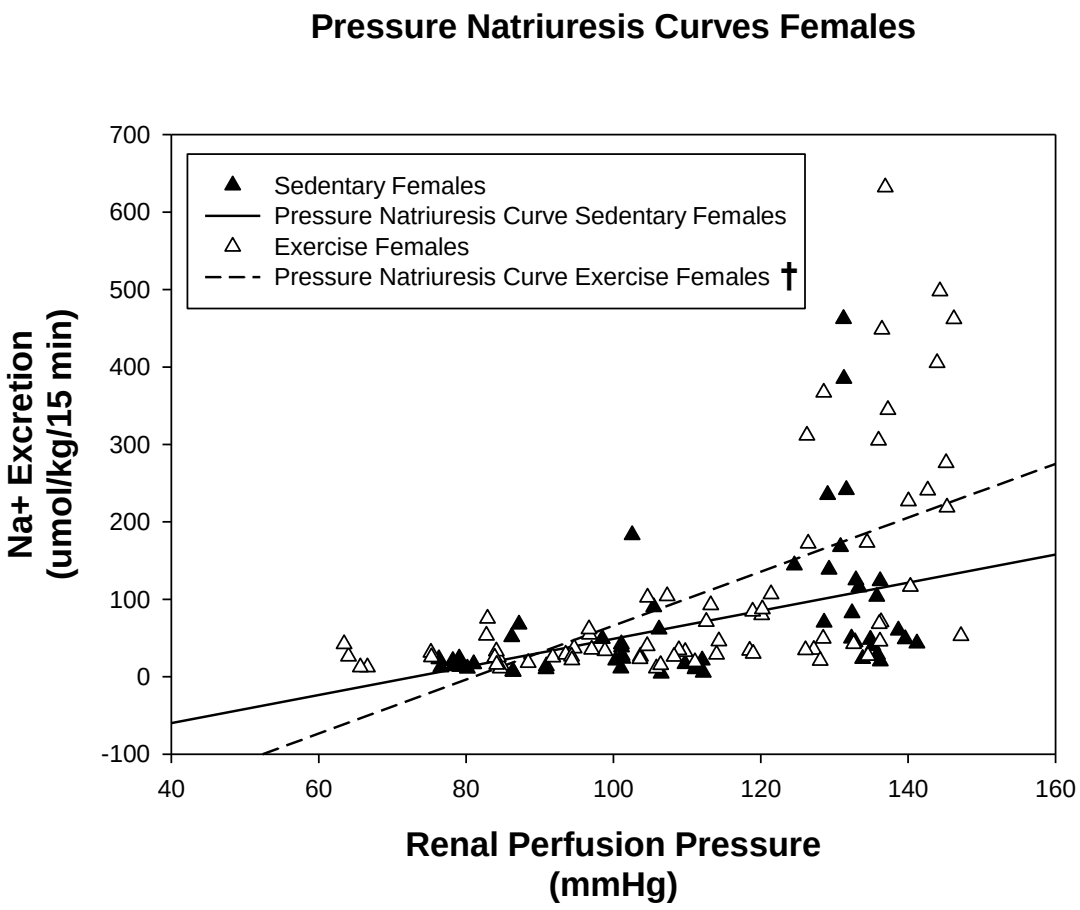

Figure 10: Pressure natriuresis curves in sedentary and exercised female rats. **†** $p < 0.05$ when the slope is compared to that of the sedentary rats.

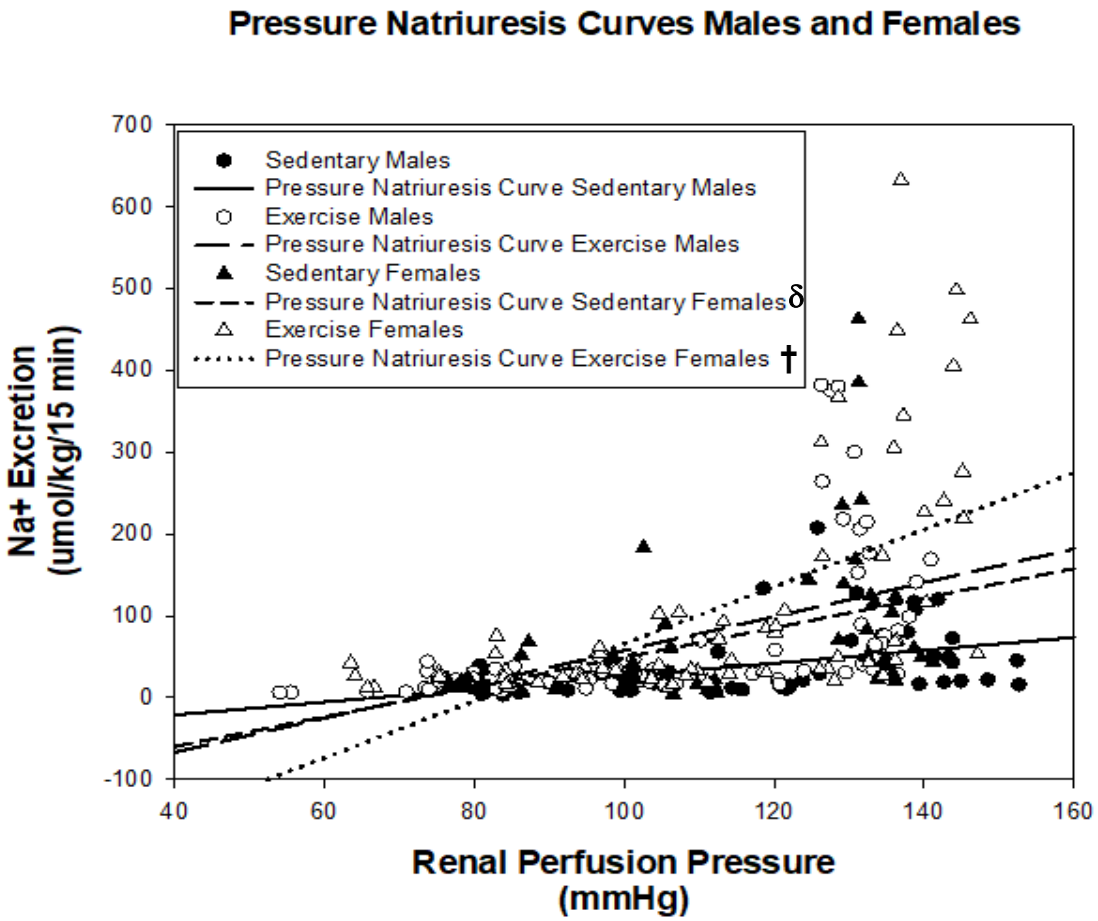

Figure 11: Pressure natriuresis curves in sedentary and exercised, male and female rats. † $p < 0.05$ when the slope of the female rats is compared to that of males of the same treatment. δ $p < 0.05$ when the y-intercept of the female rats is compared to that of males of the same treatment.

References

1. **Abassi ZA**, **Golomb E**, **Klein H**, **Keiser HR**. Urodilatin: A Natriuretic Peptide of Renal Origin. *Cardiovasc Drug Rev* 10: 199–210, 1992.

2. **Ackermann U**. Regulation of arterial blood pressure. *Surg* 22: 120a-120f, 2004.

3. **Alexander M**, **Madhur M**, **Harrison D**. Hypertension: Practice Essentials, Background, Pathophysiology [Online]. *Medscape Cardiol.* https://emedicine.medscape.com/article/241381-overview [13 Jul. 2020].

4. **August P**. Hypertension in Men. *J Clin Endocrinol Metab* 84: 3451–3454, 1999.

5. **Bethesda**. Alpha 1 Adrenergic Receptor Antagonists [Online]. National Institute of Diabetes and Digestive and Kidney Diseases. http://www.ncbi.nlm.nih.gov/pubmed/31644028 [25 Jul. 2020].

6. **Bolívar JJ**, **Cappuccio F**. Essential Hypertension: An Approach to Its Etiology and Neurogenic Pathophysiology. *Int J Hypertens* 2013: 11, 2013.

7. **Brown RE**, **Riddell MC**, **Macpherson AK**, **Canning KL**, **Kuk JL**. The Joint Association of Physical Activity, Blood-Pressure Control, and Pharmacologic Treatment of Hypertension for All-Cause Mortality Risk. *Am J Hypertens* 26: 1005–1010, 2013.

8. **Bruss ZS**, **Raja A**. Physiology, Stroke Volume [Online]. StatPearls

Publishing. http://www.ncbi.nlm.nih.gov/pubmed/31613466 [5 Aug. 2020].

9. **Carey RM**, **Padia SH**. Role of angiotensin AT $_2$ receptors in natriuresis: Intrarenal mechanisms and therapeutic potential. *Clin Exp Pharmacol Physiol* 40: 527–534, 2013.

10. **Carretero OA**, **Oparil S**. Essential Hypertension. *Circulation* 101: 329–335, 2000.

11. **Carstens J**, **Grønbæk H**, **Larsen HK**, **Pedersen EB**, **Vilstrup H**. Effects of urodilatin on natriuresis in cirrhosis patients with sodium retention. *BMC Gastroenterol* 7: 1, 2007.

12. **Castrop H**, **Höcherl K**, **Kurtz A**, **Schweda F**, **Todorov V**, **Wagner C**. Physiology of kidney renin. *Physiol. Rev.* 90 American Physiological Society Bethesda, MD: 607–673, 2010.

13. **Choi HM**, **Kim HC**, **Kang DR**. Sex differences in hypertension prevalence and control: Analysis of the 2010-2014 Korea national health and nutrition examination survey. *PLoS One* 12, 2017.

14. **Ciampone S**, **Borges R**, **De Lima IP**, **Mesquita FF**, **Cambiucci EC**, **Gontijo JAR**. Long-term exercise attenuates blood pressure responsiveness and modulates kidney angiotensin II signalling and urinary sodium excretion in SHR. *JRAAS - J. Renin-Angiotensin-Aldosterone Syst.* (2011). doi: 10.1177/1470320311408750.

15. **Crowley SD**, **Coffman TM**. The inextricable role of the kidney in

hypertension. *J. Clin. Invest.* 124 American Society for Clinical Investigation: 2341–2347, 2014.

16. **Daniels LB**, **Maisel AS**. Natriuretic Peptides. (2007). doi: 10.1016/j.jacc.2007.09.021.

17. **Dharmashankar K**, **Widlansky ME**. Vascular endothelial function and hypertension: Insights and directions. *Curr. Hypertens. Rep.* 12 NIH Public Access: 448–455, 2010.

18. **Dimeo F**, **Pagonas N**, **Seibert F**, **Arndt R**, **Zidek W**, **Westhoff TH**. Aerobic exercise reduces blood pressure in resistant hypertension. *Hypertension* 60: 653–658, 2012.

19. **Drawz P**, **Ghazi L**. Advances in understanding the renin-angiotensin-aldosterone system (RAAS) in blood pressure control and recent pivotal trials of RAAS blockade in heart failure and diabetic nephropathy. *F1000Research* 6 Faculty of 1000 Ltd: 2017.

20. **Evans RG**, **Majid DS**, **Eppel GA**. MECHANISMS MEDIATING PRESSURE NATRIURESIS: WHAT WE KNOW and WHAT WE NEED TO FIND OUT. *Clin Exp Pharmacol Physiol* 32: 400–409, 2005.

21. **Everett B**, **Zajacova A**. Gender differences in hypertension and hypertension awareness among young adults. *Biodemography Soc Biol* 61: 1–17, 2015.

22. **Ewald DR**, **Haldeman LA**. Risk Factors in Adolescent Hypertension. *Glob Pediatr Heal* 3: 2333794X1562515, 2016.

23. **Feig DI**, **Johnson RJ**. Hyperuricemia in childhood primary hypertension. *Hypertension* 42: 247–252, 2003.

24. **Forssmann W-G**, **Meyer M**, **Forssmann K**. The renal urodilatin system: clinical implications [Online]. www.elsevier.com/locate/cardiores [9 Jul. 2020].

25. **Fountain JH**, **Lappin SL**. Physiology, Renin Angiotensin System [Online]. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/pubmed/29261862 [10 Jul. 2020].

26. **Francescomarino S Di**, **Sciartilli A**, **Valerio V Di**, **Baldassarre A Di**, **Gallina S**. The effect of physical exercise on endothelial function. *Sport. Med.* 39 Springer: 797–812, 2009.

27. **Fryar CD**, **Ostchega Y**, **Hales CM**, **Zhang G**, **Kruszon-Moran D**. Key findings Data from the National Health and Nutrition Examination Survey [Online]. https://www.cdc.gov/nchs/data/databriefs/db289_table.pdf#2. [11 Jun. 2020].

28. **Füzéki E**, **Banzer W**. Physical activity recommendations for health and beyond in currently inactive populations. *Int. J. Environ. Res. Public Health* 15 MDPI AG: 2018.

29. **Gasparo M de**, **Catt K J**, **Inagami T**, **Wright J W**, **Unger T**. International union of pharmacology. XXIII. The angiotensin II receptors - PubMed [Online]. *Pharmacol Rev* 52: 415–472, 2000.

https://pubmed.ncbi.nlm.nih.gov/10977869/ [26 Jul. 2020].

30. **Gerzer R**, **Drummer C**. Is the Renal Natriuretic Peptide Urodilatin Involved in the Regulation of Natriuresis? *J Cardiovasc Pharmacol* 22: S86–S87, 1993.

31. **Girardin E**, **Caverzasio J**, **Iwai J**, **Bonjour JP**, **Muller AF**, **Grandchamp A**. Pressure natriuresis in isolated kidneys from hypertension-prone and hypertension-resistant rats (Dahl rats). *Kidney Int* 18: 10–19, 1980.

32. **Goessler K**, **Polito M**, **Cornelissen VA**. Effect of exercise training on the renin-angiotensin-aldosterone system in healthy individuals: A systematic review and meta-analysis. *Hypertens. Res.* 39 Japanese Society of Hypertension: 119–126, 2016.

33. **Goetz K**, **Drummer C**, **Long Zhu J**, **Leadley R**, **Fiedler F**, **Gerzer R**, **Goetz K**, **Zhu J**, **Leadley R**, **Drummer C**, **Fiedler F**, **Gerzer R**. *Evidence that Urodilatin, Rather Than ANP, Regulates Renal Sodium Excretion*. [date unknown].

34. **Goodhart AK**. Hypertension from the patient's perspective. *Br. J. Gen. Pract.* 66 Royal College of General Practitioners: 570, 2016.

35. **Granger JP**. Pressure natriuresis. Role of renal interstitial hydrostatic pressure. *Hypertension* 19: I9–I9, 1992.

36. **Granger JP**, **Hall JE**. Role of the Kidney in Hypertension. In: *Comprehensive Hypertension*. Elsevier Inc., 2007, p. 241–263.

37. **Guarasci GR**, **Kline RL**. Pressure natriuresis following acute and chronic inhibition of nitric oxide synthase in rats. *Am J Physiol - Regul Integr Comp Physiol* 270, 1996.

38. **Gupta-Malhotra M**, **Banker A**, **Shete S**, **Sharukh Hashmi S**, **Tyson JE**, **Barratt MS**, **Hecht JT**, **Milewicz DM**, **Boerwinkle E**. Essential Hypertension vs. Secondary Hypertension Among Children. *Am J Hypertens* 28: 73, 2015.

39. **Guyton AG**. Physiologic regulation of arterial pressure. *Am J Cardiol* 8: 401–407, 1961.

40. **Hall JE**. The kidney, hypertension, and obesity. In: *Hypertension*. 2003.

41. **Hall JE**, **do Carmo JM**, **da Silva AA**, **Wang Z**, **Hall ME**. Role of the kidney in hypertension. In: *Hypertension*. Future Medicine Ltd., 2013, p. 67–83.

42. **Hall JE**, **Do Carmo JM**, **Da Silva AA**, **Wang Z**, **Hall ME**. Obesity-Induced Hypertension: Interaction of Neurohumoral and Renal Mechanisms. *Circ Res* 116: 991–1006, 2015.

43. **Hall JE**, **Coleman TG**, **Guyton AC**. The Renin-Angiotensin System Normal Physiology and Changes in Older Hypertensives. *J Am Geriatr Soc* 37: 801–813, 1989.

44. **Hall JE**, **Guyton AC**, **Mizelle HL**. Role of the renin-angiotensin system in control of sodium excretion and arterial pressure [Online]. *Acta Physiol Scand* : 48–62, 1990. https://pubmed.ncbi.nlm.nih.gov/2220409/

[17 Jul. 2020].

45. **Hu L**, **Huang X**, **You C**, **Li J**, **Hong K**, **Li P**, **Wu Y**, **Wu Q**, **Bao H**, **Cheng X**. Prevalence and risk factors of prehypertension and hypertension in Southern China. *PLoS One* 12: 170238, 2017.

46. **Ivy JR**, **Bailey MA**. Pressure natriuresis and the renal control of arterial blood pressure. *J Physiol* 592: 3955–67, 2014.

47. **Jordan J**, **Birkenfeld AL**, **Melander O**, **Moro C**. Natriuretic peptides in cardiovascular and metabolic crosstalk implications for hypertension management. *Hypertension* 72 Lippincott Williams and Wilkins: 270–276, 2018.

48. **Jordan J**, **Kurschat C**, **Reuter H**. Arterial hypertension-diagnosis and treatment. *Dtsch Arztebl Int* 115: 557–558, 2018.

49. **Kearney PM**, **Whelton M**, **Reynolds K**, **Muntner P**, **Whelton PK**, **He J**. Global burden of hypertension: analysis of worldwide data. *Lancet* 365: 217–223, 2005.

50. **Khalil H**, **Zeltser R**. Antihypertensive Medications [Online]. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/pubmed/32119466 [25 Jul. 2020].

51. **Khanal MK**, **Dhungana RR**, **Bhandari P**, **Gurung Y**, **Paudel KN**. Prevalence, associated factors, awareness, treatment, and control of hypertension: Findings from a cross sectional study conducted as a part of a community based intervention trial in Surkhet, Mid-western region

of Nepal. *PLoS One* 12, 2017.

52. **King J**, **Lowery DR**. Physiology, Cardiac Output [Online]. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/pubmed/29262215 [5 Aug. 2020].

53. **Knowlton AA**, **Lee AR**. Estrogen and the cardiovascular system. *Pharmacol Ther* 135: 54–70, 2012.

54. **Komukai K**, **Mochizuki S**, **Yoshimura M**. Gender and the renin-angiotensin-aldosterone system. *Fundam Clin Pharmacol* 24: 687–698, 2010.

55. **Kotsis V**, **Stabouli S**, **Papakatsika S**, **Rizos Z**, **Parati G**. Mechanisms of obesity-induced hypertension. *Hypertens Res* 33: 386–393, 2010.

56. **Lee JU**. Nitric oxide in the kidney: Its physiological role and pathophysiological implications. *Electrolyte Blood Press.* 6 Korean Society of Electrolyte and Blood Pressure Research: 27–34, 2008.

57. **Leong X-F**, **Ng C-Y**, **Jaarin K**. Animal Models in Cardiovascular Research: Hypertension and Atherosclerosis. *Biomed Res Int* 2015: 1–11, 2015.

58. **Lewington S**, **Clarke R**, **Qizilbash N**, **Peto R**, **Collins R**, **Prospective Studies Collaboration**. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. [Online]. *Lancet (London, England)* 360: 1903–13, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12493255 [19

Oct. 2018].

59. **Lim SS**, **Vos T**, **Flaxman AD**, **Danaei G**, **Shibuya K**, **Adair-Rohani H**, **Amann M**, **Anderson HR**, **Andrews KG**, **Aryee M**, **Atkinson C**, **Bacchus LJ**, **Bahalim AN**, **Balakrishnan K**, **Balmes J**, **Barker-Collo S**, **Baxter A**, **Bell ML**, **Blore JD**, **Blyth F**, **Bonner C**, **Borges G**, **Bourne R**, **Boussinesq M**, **Brauer M**, **Brooks P**, **Bruce NG**, **Brunekreef B**, **Bryan-Hancock C**, **Bucello C**, **Buchbinder R**, **Bull F**, **Burnett RT**, **Byers TE**, **Calabria B**, **Carapetis J**, **Carnahan E**, **Chafe Z**, **Charlson F**, **Chen H**, **Chen JS**, **Cheng AT-A**, **Child JC**, **Cohen A**, **Colson KE**, **Cowie BC**, **Darby S**, **Darling S**, **Davis A**, **Degenhardt L**, **Dentener F**, **Des Jarlais DC**, **Devries K**, **Dherani M**, **Ding EL**, **Dorsey ER**, **Driscoll T**, **Edmond K**, **Ali SE**, **Engell RE**, **Erwin PJ**, **Fahimi S**, **Falder G**, **Farzadfar F**, **Ferrari A**, **Finucane MM**, **Flaxman S**, **Fowkes FGR**, **Freedman G**, **Freeman MK**, **Gakidou E**, **Ghosh S**, **Giovannucci E**, **Gmel G**, **Graham K**, **Grainger R**, **Grant B**, **Gunnell D**, **Gutierrez HR**, **Hall W**, **Hoek HW**, **Hogan A**, **Hosgood HD**, **Hoy D**, **Hu H**, **Hubbell BJ**, **Hutchings SJ**, **Ibeanusi SE**, **Jacklyn GL**, **Jasrasaria R**, **Jonas JB**, **Kan H**, **Kanis JA**, **Kassebaum N**, **Kawakami N**, **Khang Y-H**, **Khatibzadeh S**, **Khoo J-P**, **Kok C**, **Laden F**, **Lalloo R**, **Lan Q**, **Lathlean T**, **Leasher JL**, **Leigh J**, **Li Y**, **Lin JK**, **Lipshultz SE**, **London S**, **Lozano R**, **Lu Y**, **Mak J**, **Malekzadeh R**, **Mallinger L**, **Marcenes W**, **March L**, **Marks R**, **Martin R**, **McGale P**, **McGrath J**, **Mehta S**, **Mensah GA**, **Merriman TR**, **Micha R**, **Michaud C**, **Mishra V**, **Mohd**

Hanafiah K, **Mokdad AA**, **Morawska L**, **Mozaffarian D**, **Murphy T**, **Naghavi M**, **Neal B**, **Nelson PK**, **Nolla JM**, **Norman R**, **Olives C**, **Omer SB**, **Orchard J**, **Osborne R**, **Ostro B**, **Page A**, **Pandey KD**, **Parry CDH**, **Passmore E**, **Patra J**, **Pearce N**, **Pelizzari PM**, **Petzold M**, **Phillips MR**, **Pope D**, **Pope CA**, **Powles J**, **Rao M**, **Razavi H**, **Rehfuess EA**, **Rehm JT**, **Ritz B**, **Rivara FP**, **Roberts T**, **Robinson C**, **Rodriguez-Portales JA**, **Romieu I**, **Room R**, **Rosenfeld LC**, **Roy A**, **Rushton L**, **Salomon JA**, **Sampson U**, **Sanchez-Riera L**, **Sanman E**, **Sapkota A**, **Seedat S**, **Shi P**, **Shield K**, **Shivakoti R**, **Singh GM**, **Sleet DA**, **Smith E**, **Smith KR**, **Stapelberg NJC**, **Steenland K**, **Stöckl H**, **Stovner LJ**, **Straif K**, **Straney L**, **Thurston GD**, **Tran JH**, **Van Dingenen R**, **van Donkelaar A**, **Veerman JL**, **Vijayakumar L**, **Weintraub R**, **Weissman MM**, **White RA**, **Whiteford H**, **Wiersma ST**, **Wilkinson JD**, **Williams HC**, **Williams W**, **Wilson N**, **Woolf AD**, **Yip P**, **Zielinski JM**, **Lopez AD**, **Murray CJL**, **Ezzati M**, **AlMazroa MA**, **Memish ZA**. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet (London, England)* 380: 2224–60, 2012.

60. **Lohmeier TE**. The Sympathetic Nervous System and Long-Term Blood Pressure Regulation [Online]. https://academic.oup.com/ajh/article-abstract/14/S3/147S/205220 [10 Jul. 2020].

61. **Lote CJ**. *Principles of Renal Physiology*. 5th ed. New York: Springer-

Verlag New York, 2012.

62. **Maiorana A**, **O???Driscoll G**, **Taylor R**, **Green D**. Exercise and the Nitric Oxide Vasodilator System. *Sport Med* 33: 1013–1035, 2003.

63. **Neugarten J**, **Ding Q**, **Friedman A**, **Lei J**, **Silbiger S**. Sex hormones and renal nitric oxide synthases. *J Am Soc Nephrol* 8: 1240–1246, 1997.

64. **Nguyen Q**, **Dominguez J**, **Nguyen L**, **Gullapalli N**. Hypertension management: An update [Online]. *Am Heal Drug Benefits* 3: 47–55, 2010. www.AHDBonline.com [25 Jul. 2020].

65. **Nishikimi T**, **Maeda N**, **Matsuoka H**. The role of natriuretic peptides in cardioprotection. *Cardiovasc. Res.* 69: 318–328, 2006.

66. **O'Callaghan EL**, **McBryde FD**, **Burchell AE**, **Ratcliffe LEK**, **Nicolae L**, **Gillbe I**, **Carr D**, **Hart EC**, **Nightingale AK**, **Patel NK**, **Paton JFR**. Deep Brain Stimulation for the Treatment of Resistant Hypertension. *Curr. Hypertens. Rep.* 16 Current Medicine Group LLC 1: 1–10, 2014.

67. **O'Connor PM**, **Cowley AW**. Modulation of pressure-natriuresis by renal medullary reactive oxygen species and nitric oxide. *Curr. Hypertens. Rep.* 12 NIH Public Access: 86–92, 2010.

68. **O'Hagan TS**, **Wharton W**, **Kehoe PG**. Interactions between oestrogen and the renin angiotensin system - Potential mechanisms for gender differences in Alzheimer's disease [Online]. *Am. J. Neurodegener. Dis.* 1 E-Century Publishing Corporation: 266–279, 2012. www.AJND.us/ [6

Aug. 2020].

69. **Ogobuiro I**, **Tuma F**. Physiology, Renal [Online]. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/pubmed/30855923 [24 Jul. 2020].

70. **Oparil S**, **Amin Zaman ; M**, **Calhoun DA**. Pathogenesis of Hypertension Clinical Principles Physiologic Principles [Online]. www.annals.org [5 Aug. 2020].

71. **Padia SH**, **Howell NL**, **Siragy HM**, **Carey RM**. Renal angiotensin type 2 receptors mediate natriuresis via angiotensin III in the angiotensin II type 1 receptor-blocked rat. In: *Hypertension*. Hypertension, p. 537–544.

72. **Patel H**, **Alkhawam H**, **Madanieh R**, **Shah N**, **Kosmas CE**, **Vittorio TJ**. Aerobic vs anaerobic exercise training effects on the cardiovascular system . *World J Cardiol* 9: 134, 2017.

73. **Patel N**, **Walker N**. Clinical assessment of hypertension in children. *Clin Hypertens* 22, 2016.

74. **Pescatello LS**, **MacDonald H V**, **Lamberti L**, **Johnson BT**. Exercise for Hypertension: A Prescription Update Integrating Existing Recommendations with Emerging Research. *Curr Hypertens Rep* 17: 87, 2015.

75. **Piercy KL**, **Troiano RP**. Physical Activity Guidelines for Americans From the US Department of Health and Human Services. *Circ Cardiovasc Qual Outcomes* 11: e005263, 2018.

76. **Pimenta E**, **Calhoun DA**. Treatment of resistant hypertension. *J Hypertens* 28: 2194–2195, 2010.

77. **Pinto E**. Blood pressure and ageing. *Postgrad. Med. J.* 83 BMJ Publishing Group: 109–114, 2007.

78. **Potter LR**. Natriuretic peptide metabolism, clearance and degradation. *FEBS J.* 278: 1808–1817, 2011.

79. **Potter LR**, **Yoder AR**, **Flora DR**, **Antos LK**, **Dickey DM**. Natriuretic peptides: Their structures, receptors, physiologic functions and therapeutic applications. *Handb. Exp. Pharmacol.* 191: 341–366, 2009.

80. **Puar THK**, **Mok Y**, **Debajyoti R**, **Khoo J**, **How CH**, **Ng AKH**. Secondary hypertension in adults. *Singapore Med J* 57: 228–232, 2016.

81. **Quan A**, **Chakravarty S**, **Chen JK**, **Chen JC**, **Loleh S**, **Saini N**, **Harris RC**, **Capdevila J**, **Quigley R**. Androgens augment proximal tubule transport. *Am J Physiol - Ren Physiol* 287: 452–459, 2004.

82. **Raina R**, **Krishnappa V**, **Das A**, **Amin H**, **Radhakrishnan Y**, **Nair NR**, **Kusumi K**. Overview of monogenic or Mendelian forms of hypertension. *Front. Pediatr.* 7 Frontiers Media S.A.: 263, 2019.

83. **Roman RJ**, **Cowley AW**. Abnormal pressure-diuresis-natriuresis response in spontaneously hypertensive rats. *Am J Physiol - Ren Fluid Electrolyte Physiol* 248: F199–F205, 1985.

84. **Ruppert V**, **Maisch B**. Genetics of Human Hypertension. *Herz* 28 Herz:

655–662, 2003.

85. **Saez F**, **Reverte V**, **Paliege A**, **Moreno JM**, **Llinás MT**, **Bachmann S**, **Salazar FJ**. Sex-dependent hypertension and renal changes in aged rats with altered renal development. *Am J Physiol Physiol* 307: F461–F470, 2014.

86. **Saha SP**, **Ziada KM**, **Whayne TF**. Surgical, interventional, and device innovations in the management of hypertension. *Int. J. Angiol.* 24 Thieme Medical Publishers, Inc.: 1–10, 2015.

87. **Sandoo A**, **van Zanten JJCSV**, **Metsios GS**, **Carroll D**, **Kitas GD**. The endothelium and its role in regulating vascular tone. *Open Cardiovasc Med J* 4: 302–12, 2010.

88. **Semenov AG**, **Tamm NN**, **Seferian KR**, **Postnikov AB**, **Karpova NS**, **Serebryanaya D V**, **Koshkina E V**, **Krasnoselsky MI**, **Katrukha AG**. Processing of pro-B-type natriuretic peptide: furin and corin as candidate convertases. *Clin Chem* 56: 1166–76, 2010.

89. **Sharma R**, **Sharma S**. Physiology, Blood Volume [Online]. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/pubmed/30252333 [17 Jul. 2020].

90. **Siragy HM**, **Inagami T**, **Ichiki T**, **Carey RM**. Sustained hypersensitivity to angiotensin II and its mechanism in mice lacking the subtype-2 (AT2) angiotensin receptor. *Proc Natl Acad Sci U S A* 96: 6506–6510, 1999.

91. **Smithwick RH**. Surgical treatment of hypertension. *Am J Med* 4: 744–

759, 1948.

92. **Spieker LE**, **Flammer AJ**, **Lüscher TF**. The vascular endothelium in hypertension. [Online]. *Handb Exp Pharmacol* : 249–83, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16999229 [12 Dec. 2018].

93. **Stabouli S**, **Papakatsika S**, **Kotsis V**. The role of obesity, salt and exercise on blood pressure in children and adolescents. *Expert Rev Cardiovasc Ther* (2011). doi: 10.1586/erc.11.63.

94. **Staessen JA**, **Celis H**, **Fagard R**. The epidemiology of the association between hypertension and menopause. In: *Journal of Human Hypertension*. Nature Publishing Group, p. 587–592.

95. **Suzuki M**. Physical exercise and renal function. *J Phys Fit Sport Med* 4: 17–29, 2015.

96. **Taddei S**, **Bruno RM**, **Masi S**, **Solini A**, **Camm AJ**, **Lüscher TF**, **Maurer G**, **Serruys PW**. Epidemiology and pathophysiology of hypertension Chapter: Epidemiology and pathophysiology of hypertension Author(s): ESC CardioMed (3 edn) Epidemiology and pathophysiology of hypertension. doi: 10.1093/med/9780198784906.001.0001.

97. **Tansey EA**, **Montgomery LEA**, **Quinn JG**, **Roe SM**, **Johnson CD**. Understanding basic vein physiology and venous blood pressure through simple physical assessments. *Adv Physiol Educ* 43: 423–429, 2019.

98. **Trammel JE**, **Sapra A**. Physiology, Systemic Vascular Resistance [Online]. In: *StatPearls*. StatPearls Publishing https://www.ncbi.nlm.nih.gov/books/NBK556075 [5 Aug. 2020].

99. **Tsai SH**, **Lin YY**, **Chu SJ**, **Hsu CW**, **Cheng SM**. Interpretation and use of natriuretic peptides in non-congestive heart failure settings. *Yonsei Med. J.* 51 Yonsei University College of Medicine: 151–163, 2010.

100. **Wadei HM**, **Textor SC**. The role of the kidney in regulating arterial blood pressure. *Nat Rev Nephrol* 8: 602–609, 2012.

101. **Wahid A**, **Manek N**, **Nichols M**, **Kelly P**, **Foster C**, **Webster P**, **Kaur A**, **Friedemann Smith C**, **Wilkins E**, **Rayner M**, **Roberts N**, **Scarborough P**. Quantifying the Association Between Physical Activity and Cardiovascular Disease and Diabetes: A Systematic Review and Meta-Analysis. *J Am Heart Assoc* 5, 2016.

102. **Waldman BM**, **Augustyniak RA**, **Chen H**, **Rossi NF**. Effects of voluntary exercise on blood pressure, angiotensin II, aldosterone, and renal function in two-kidney, one-clip hypertensive rats. *Integr Blood Press Control* 10: 41–51, 2017.

103. **Weber M**, **Mitrovic V**, **Hamm C**. B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide - Diagnostic role in stable coronary artery disease [Online]. In: *Experimental and Clinical Cardiology*. Pulsus Group, p. 99–101. /pmc/articles/PMC2274852/?report=abstract [24 Jul. 2020].

104. **Whelton PK**, **Carey RM**, **Aronow WS**, **Casey DE**, **Collins KJ**, **Dennison Himmelfarb C**, **DePalma SM**, **Gidding S**, **Jamerson KA**, **Jones DW**, **MacLaughlin EJ**, **Muntner P**, **Ovbiagele B**, **Smith SC**, **Spencer CC**, **Stafford RS**, **Taler SJ**, **Thomas RJ**, **Williams KA**, **Williamson JD**, **Wright JT**. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. *J Am Coll Cardiol* 71: e127–e248, 2018.

105. **Xiao S**, **Gillespie DG**, **Baylis C**, **Jackson EK**, **Dubey RK**. Effects of estradiol and its metabolites on glomerular endothelial nitric oxide synthesis and mesangial cell growth. *Hypertension* 37: 645–650, 2001.

106. **Xue B**, **Johnson AK**, **Hay M**. Sex differences in angiotensin II- and aldosterone-induced hypertension: The central protective effects of estrogen. *Am J Physiol - Regul Integr Comp Physiol* 305: R459, 2013.

107. **Xue B**, **Zhang Z**, **Beltz TG**, **Guo F**, **Hay M**, **Johnson AK**. Estrogen regulation of the brain renin-angiotensin system in protection against angiotensin II-induced sensitization of hypertension. *Am J Physiol - Hear Circ Physiol* 307: H191, 2014.

108. **Yang YJ**. An Overview of Current Physical Activity Recommendations in Primary Care. *Korean J Fam Med* 40: 135–142, 2019.

109. **Zanchetti A**. Treatment goals in hypertension. *Am J Med* 76: 1–3, 1984.

110. **Zheng H**, **Li Y-F**, **Zucker IH**, **Patel KP**. Exercise training improves renal excretory responses to acute volume expansion in rats with heart failure. *Am J Physiol Physiol* 291: F1148–F1156, 2006.