Effect Of Hemp Seed Protein Isolate on Mesenteric Resistance Artery Isolated From The Spontaneously Hypertensive Rat

by

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ABSTRACT

Hypertensive vessels are characterized by attenuated endothelium-dependent vascular relaxation or endothelial dysfunction. Hemp seed protein isolate (HPI) is a novel protein that contains more than 80% of crude hemp protein and exhibits antihypertensive properties. In addition, HPI contains high levels of arginine, a pre-cursor for vasodilator nitric oxide (NO). However, it is unknown whether HPI can improve the vascular function of hypertensive vessels. Also, sex differences are observed in vascular function and BP regulation, with males at greater risk as opposed to age-matched pre-menopausal women.

The present study tested the hypothesis that direct treatment of HPI will lead to vascular relaxation of pre-constricted mesenteric resistance arteries. In addition, we also addressed sex differences in vascular response to HPI. To investigate the potential vasorelaxant activity, we tested HPI (0.1 to 100 µg/mL) on third-order mesenteric arteries using the pressure myography technique. We found that HPI relaxed mesenteric resistance arteries isolated from 16-week-old male and female Sprague Dawley (SD), spontaneously hypertensive rat (SHR), and Wistar-Kyoto (WKY) rats in a dose-dependent dependent manner. The vasorelaxant activity of HPI is probably due to the presence of amino acids such as arginine, cysteine, and serine. Also, male SD rats showed significantly lower %dilation compared to female SD rats. Whereas male WKY showed significantly higher HPI induced %dilation response compared to female WKY, female SHR, and male SHR. Sex differences observed in normotensive rats are probably due to bioactive components of HPI. Further studies are required to identify the mechanism involved in HPI-induced vasodilation and elucidate the sex difference observed.

In summary, we have shown for the first time that HPI is a vasodilator. Our study findings indicate that HPI may be used for improving the vascular function of hypertensive vessels.

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LIST OF ABBREVIATIONS

ABPM	ambulatory blood pressure monitoring
ACE	angiotensin converting enzyme
ANP	atrial natriuretic peptide
AOBP	automated office blood pressure
BP	blood pressure
BNP	brain natriuretic peptide
CNP	C-type natriuretic peptide
cGMP	cyclic GMP
DASH	Dietary Approaches to Stop Hypertension
DBP	diastolic blood pressure
DNP	dendroaspis type natriuretic peptide
DHF	defatted hemp flour
ECM	extracellular matrix
EDHF	endothelium derived hyperpolarizing factor
eNOS	endothelial nitric oxide synthase
HBPM	home blood pressure monitoring
HPH	hemp protein hydrolysate
HPI	hemp protein isolate
NO	nitric oxide
OBPM	office blood pressure measurement

PUFA	polyunsaturated fatty acid
RAAS	renin angiotensin aldosterone system
ROS	reactive oxygen species
SBP	systolic blood pressure
SHR	Spontaneously Hypertensive Rat
SD	Sprague Dawley
THC	tetrahydrocannabinol
VSMC	vascular smooth muscle cell
WHO	World Health Organization
WKY	Wistar Kyoto

CHAPTER I
INTRODUCTION AND LITERATURE REVIEW

1.1 Hypertension

1.1.1 Background

Hypertension is a medical condition characterized by persistently elevated arterial blood pressure (BP) during which systolic (SBP) and diastolic (DBP) blood pressures are greater than or equal to 140 mm Hg and 90 mm Hg, respectively.[1] BP threshold for hypertension diagnosis varies depending on the method employed (Table 1). For an accurate diagnosis of hypertension to be made, BP readings must be confirmed on two or more occasions in an office or clinical setting.[1] Other BP measurement techniques outside of clinical or office settings include home- and ambulatory methods which are explained in Table 2.

Hypertension is a major risk factor for various cardiovascular diseases, stroke, and kidney disease; its prevalence is observed to increase with age.[2] Approximately 90% of individuals with normal BP develop hypertension by the age of 55- 65 years.[3] Hypertension also leads to organ damage such as left ventricular hypertrophy, vascular dementia, peripheral artery disease, hypertensive retinopathy, and myocardial infarction.[4] Also, rate of hypertension is observed to be higher in men compared to age-matched premenopausal women.[5] Hypertensive individuals undergo treatments to achieve a BP level of <140/90 mm Hg.[3,6]

In Canada, cardiovascular complications arising from hypertension (heart failure and stroke) are the foremost cause of mortality and hospitalization.[3] Globally, 10.4 million deaths per year result from complications arising from elevated BP.[1] Also, there is a clear disparity in prevalence of hypertension among underdeveloped countries and developed countries such as Canada and USA, with the latter achieving better target control of BP.[7] The World Health Organization (WHO) aims to reduce the global burden of hypertension nearly to 33% by the next decade.[8]

Table 1:Blood pressure diagnosis in Canada (2020-2022 guidelines)[9]

Category	**SBP (mmHg)**	**DBP (mmHg)**	**BP measurement technique**
Optimum BP	<120	<80	
Hypertension	≥135	≥85	Automated office BP (AOBP) or home BP monitoring (HBPM)
	≥140	≥90	Office BP measurement (OBPM)
	≥130 (24-hour mean)	≥80 (24-hour mean)	Ambulatory BP monitoring (ABPM)
	≥135 (daytime mean)	≥85 (daytime mean)	ABPM
Hypertension (comorbid conditions)	≥130	≥80	OBPM
	≥135	≥85	HBPM

BP- blood pressure; SBP – systolic blood pressure; DBP- diastolic blood pressure

Table 2: Blood pressure measurement techniques[9,10]

BP measurement technique	Definition
AOBP	Conduct using an automated oscillometric device that records multiple BP readings. The patient may be accompanied or left unattended while the readings are taken.
OBPM	Perform using oscillometric or auscultatory devices such as mercury, aneroid or hybrid sphygmomanometers. BP is taken by the health care provider by cuffing the patient's upper arm.
HBPM	Patient's self- monitor BP using upper-arm cuff oscillometric device for at least 3-7 days with measurements taken in morning and bedtime.
ABPM	A fully automated upper-arm cuff oscillometric device must be worn by the patient which records BP every 15/30 min for 24 hours. This technique can identify various hypertension phenotypes.

AOBP – automated office blood pressure; OBPM – office blood pressure measurement; HBPM – home blood pressure monitoring; ABPM- ambulatory blood pressure monitoring

1.1.2 Classification

Essential hypertension

Essential or primary hypertension does not have a known specific cause or etiology and occurs due to the accumulation of several factors over time.[11] Primary hypertension accounts for the majority (approximately 95%) of hypertensive cases and is influenced by aging, genetics (family history), and environmental factors such lifestyle which includes factors like excess alcohol consumption, smoking, obesity, excess salt intake, and low dietary potassium and calcium intake.[11,12]

Secondary hypertension

Secondary hypertension affects less than 10% of the population.[13] It occurs due to specific underlying medical conditions or through usage of external substances such as prescription drugs, additive street drugs, herbal products, and food.[14] Examples of secondary causes include kidney diseases (renovascular disease, chronic kidney disease), endocrine disorders (hypo- and hyper-thyroidism), drugs (ergot alkaloids, nicotine), food (licorice), stress, and pregnancy.[13] Unlike primary hypertension which can only be controlled, secondary hypertension can be cured if the root cause is identified and addressed effectively.[14]

White coat hypertension

White coat or isolated clinic hypertension refers to elevated BP levels diagnosed inside a clinical or office setting in contrast to normal BP levels exhibited by the same patient in out-of-office diagnosis.[15] The latest guidelines provided by Hypertension Canada diagnose white coat hypertension as an office BP measurement ≥135/85 mm Hg but out-of-office value of less than 135/85 mm Hg.[9] However, the diagnostic threshold for white coat hypertension provided by the majority of other international guidelines (European Society of Cardiology, European Society of Hypertension, Japanese Society of Hypertension, China Hypertension League) is office BP ≥140/90 mm Hg, and out-of-office BP as <135/85 mm Hg.[15]

Masked hypertension

Masked hypertension refers to elevated BP levels diagnosed outside the clinic or office in contrast to normal BP levels inside clinic or office.[16] Hypertension Canada recommends repeated BP measurements using home blood pressure monitoring (HBPM) or ambulatory blood pressure monitoring (ABPM) techniques to confirm masked hypertension.[9]

Resistant hypertension

This phenotype of hypertension describes patients exhibiting resistance to anti-hypertensive drug treatment and requires concurrent usage of more than 3 antihypertensive drugs to achieve BP control.[17]

Isolated systolic hypertension

This type of hypertension is predominant in elderly population and is characterized by high SBP and normal DBP. [18] The prevalence of isolated systolic hypertension increases with aging (≥60 years) due to structural and mechanical changes in arterial wall, such as increased stiffness and reduced elasticity.[19]

Malignant hypertension

Malignant hypertension refers to patients exhibiting SBP and DBP greater than 180 mm Hg and 120 mm Hg, respectively.[20] This rare and severe form of hypertension emergency is characterized by damage to the retina, microvasculature, or organs such as kidney, brain, and heart.[21] Pathophysiological mechanisms that might lead to this life-threatening form of hypertension include immune system abnormalities, endothelium dysfunction, oxidative stress, inflammation, increased levels of angiotensin II, endothelin-1, aldosterone, and sympathetic activity.[22] Malignant form is observed in patients with existing long-term hypertension or due to renovascular causes.[22] Other reasons may include conditions such as idiopathic nephropathy, primary hyperaldosteronism, pheochromocytoma, or tumors.[22]

1.1.3 Pathophysiological mechanisms

Renin - angiotensin - aldosterone- system

The renin-angiotensin-aldosterone system (RAAS) plays a critical role in the elevation of arterial BP through effects on kidney fluid pressure balance and vasoconstriction.[23] Renin released from kidneys mediates conversion of angiotensinogen to angiotensin I, which then gets converted to angiotensin II by angiotensin-converting enzyme (ACE).[14] Angiotensin II exhibits vasoconstrictor effects and stimulates the release of aldosterone, hence leading to sodium and water reabsorption and increased BP.[14,24] Elevated BP is regulated by kidneys through decreased sodium reabsorption and increased sodium excretion, thus regulating BP and blood volume to normal.[25]

During hypertension, the excess of RAAS molecules - angiotensin II and aldosterone damage kidney and vessels resulting in end-organ damage, endothelial dysfunction, increased sodium reabsorption, and oxidative stress.[26,27] Angiotensin II plays role in vascular remodeling (structural and functional changes) through generating reactive oxygen species (ROS).[28] It stimulates and increases expression of NADPH oxidase, which thereby induces ROS production.[28] Similarly, aldosterone causes oxidative stress, vascular cell (endothelium and smooth muscle) inflammation and increased arterial stiffness.[29]

Role of genetic and environmental factors

Interaction between genetic and environmental factors influences the development of hypertension.[30] Genetic and environmental factors may account for 30% phenotype variability and specific genes (e.g., angiotensinogen gene variant 235T allele) may be responsible for control of SBP and DBP.[31,32] In addition, abnormalities in vascular cells during fetal development is linked to the pathogenesis of essential hypertension.[33] Hypertension may also be inherited from mother to offspring possibly due to tRNA mutations and mitochondrial DNA alteration.[34] Also, exposure to harmful environmental conditions such as air pollution may lead to early BP development in child.[35]

Environmental factors influencing changes in BP include alcohol intake, nutritional factors like salt and potassium intake, dietary patterns, obesity, sedentary lifestyle, and chronic stress.[36,37] A

four month supervised randomized clinical trial done in US on hypertensive subjects (n=150) showed that lifestyle modifications involving exercise, restricted sodium and calorie intake, dietary approaches to stop hypertension (DASH) diet resulted in significant control over BP.[38]

Role of natriuretic peptides

Natriuretic peptides are hormones such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and dendroaspis-type natriuretic peptide (DNP).[39] These are essential for maintaining cardiovascular homeostasis, extracellular fluid, electrolyte balance, and BP.[39] ANP and BNP are mainly produced in the heart upon stretching of the atrial and ventricular wall, whereas CNP is generated by endothelial cells.[39] ANP and BNP maintain sodium and water balance and reduce BP through mechanisms such as vasodilation and increased renal excretion.[40,41] Deficiency of these peptides is linked to hypertension, obesity, and type 2 diabetes.[26]

Role of the sympathetic nervous system

Chronic activation of the sympathetic nervous system also plays a role in the etiology of hypertension.[42] The role of the sympathetic nervous system in short-term regulation of BP is well established, however precise mechanisms leading to hypertension requires further research.[43] Stimulation of sympathetic nervous system results in increased cardiac output (heart rate and stroke volume) and RAAS activation.[44] Catecholamines, nor-epinephrine (NE) and epinephrine are released, stimulating the conversion of pro-renin to renin which causes an increase in BP through RAAS system.[45] In addition, sympathetic activity is also affected by age and sex, with older females (>60 age) showing increased activity compared to males.[44] Sympathetic activity progresses as hypertension advances in essential and resistant hypertensive individuals.[42,44] In addition, chronically elevated sympathetic nerve activity in hypertensive patients is linked to left ventricular hypertrophy and other pathophysiological outcomes such as cardiac arrhythmias, atherosclerosis, and tachycardia.[46]

Role of inflammation

The inflammatory response is mediated by innate immune cells, and this complex process involves interaction between extracellular matrix, inflammatory mediators, and immune cells.[47] The level of inflammation is marked by the presence of C-reactive proteins which increase as inflammation progresses.[48] High C-reactive protein level in serum is considered a risk factor for atherosclerosis

development, as it stimulates the release of various adhesion molecules from the endothelium.[49] The ATTICA cross-sectional study in Greece revealed that pre-hypertensive subjects (653 men and 535 women) exhibited significantly greater inflammatory markers such as tumor necrosis factor-α, C-reactive protein levels compared to normotensives.[50] Hypertension may develop due to chronic inflammation in combination with oxidative stress.[51] Phagocytes such as neutrophils produce ROS to protect from pathogens but accumulation of ROS leads to vascular damage due to oxidative stress.[51]

1.1.4 Anti-hypertensive treatment

Hypertension is treated either through a non-pharmacological approach, such as lifestyle modifications, or through pharmacological treatment or both.[52]

Recommended lifestyle changes are weight reduction by restricting calorie intake or by exercise, controlled alcohol consumption, dietary changes such as low sodium intake, potassium supplements, and diet rich in fruits, vegetables, and low-fat dairy. [53] Lifestyle changes are recommended for all patients with established hypertension as a first-line treatment approach.[1] However, in most cases, a combination of lifestyle modification with use of anti-hypertensive drugs is necessary to achieve sufficient control of BP.[1]

Hypertension is treated using drugs belonging to classes such as diuretics (thiazide or similar), ACE inhibitors, angiotensin receptor blockers, and long-acting calcium channel blockers.[54] To achieve optimal BP control, combination drug therapy (two or more drugs) has proved to be more efficient than single-drug treatment.[55] Combination drug therapy combines low dosage anti-hypertensive drugs exerting different mechanisms of action to achieve better efficacy and lower side effects.[56] Some of the side effects from these drugs include dizziness, headache, ankle swelling, cough, rash, anxiety, and diarrhea.[57] A cohort study on North American older adults (>65 years) prescribed with antihypertensive medication reported side effects such as keratinocyte carcinoma and melanoma as a result of prolonged elevated thiazide diuretic usage.[58]

Apart from these potential side effects, the cost of medication is a deep concern. Affordability of the drugs is an obstacle to many older patients, and Canada remains the only country in the world with a universal health care system lacking an affordable universal prescription drug coverage.[59]

Hence, it is necessary to pursue cost-effective natural alternative remedies for hypertension management.

1.1.5 Dietary approaches to treat hypertension

Health Canada defines functional food as "similar in appearance to, or conventional food consumed as part of usual diet and shows physiological benefits".[60]

Hypertension risk can be reduced through diet by incorporating functional foods such as nuts, legumes, dark chocolate, fish oil, whole grains, soy protein, fruits, and vegetables.[61] These are rich in bioactive compounds such as omega-3 fatty acid, fiber, flavonoids, and polyphenols which exhibit cardioprotective effects.[61] Hypertension Canada recommends adoption of diet patterns such as DASH diet, reduced salt and alcohol intake, exercise, and weight loss to manage hypertension and improve overall cardiac health.[9]

Sodium

To reduce hypertensive risk and other cardiovascular diseases, WHO recommends consuming less than 2 g/day of sodium (i.e. 5 g of salt) in adults.[62] Moderate salt intake of 3 g per day is ideal to reduce the risk of hypertension, stroke, and cardiac disorders.[63] This was concluded from a meta-analysis of 34 trials on 3230 individuals with salt intake ranging between 2.4 g/day to 6 g/day.[63] Results from this study showed that average BP (SBP/DBP) was significantly reduced by 5.39/2.82 mm Hg in hypertensive patients, and by 2.42/1.00 mm Hg in normotensive individuals .[63]

DASH

The DASH diet incorporates fruits, vegetables, low-fat dairy or fat-free alternatives, whole grain, poultry, fish, legumes, and nuts.[64] The diet encourages reduced consumption of food containing saturated fat, total fat, refined grain (e.g. white bread, pasta), red meat, sweets and sugar-rich snacks.[64] In addition, this dietary pattern provides various nutrients such as magnesium, potassium, calcium, protein, fiber, and vitamins.[64] Numerous studies demonstrated a positive correlation between DASH diet and reduced BP. A trial conducted with 459 adults, aged 22 years or older, who adhered to the DASH diet for 8 weeks, showed that SBP level lowered by 11.4 mm Hg and DBP by 5.5 mm Hg among hypertensive patients and by 3.5 mm Hg and 2.1 mm Hg in non-hypertensive individuals.[65] The DASH diet (3 weeks) also lowered BP and improved endothelial

function in 15 obese hypertensive individuals.[66] Also, combination of the DASH diet and sodium restriction for 30 days reduced BP significantly in 412 participants.[67]

1.1.6 Dietary protein in hypertension

Consumption of peptides derived from both plant and animal protein source have reduced BP in hypertensive patients.[68–71] The source of protein is not a major factor for efficacy, but rather amino acid composition and amount of protein consumed are important determinants in hypertension treatment.[72] Some of the plausible mechanism through which proteins reduce BP includes natriuresis, regulation of insulin sensitivity, vasodilation, and inhibition of enzymes such as ACE and renin.[73,74]

The International Study of Salt and Blood Pressure (INTERSALT), which included 10020 adults worldwide aged 20-59 years, investigated the relationship between dietary protein and BP by assessing nitrogen, urea, and sulfate level in urine.[75] High protein consumption (>30%) was inversely associated with BP.[75] Another cross-sectional study, International Population Study on Macronutrients and BP (INTERMAP) with more than 4000 participants aged 40-59 years from Japan, China, United States, and United Kingdom also showed an inverse relationship between protein intake and BP.[76]

The Optimal Macronutrient Intake Trial to Prevent Heart Disease (OmniHeart) explored effects of three types of diets (i.e., carbohydrate, protein, and unsaturated fat) in adults whose SBP and DBP ranged between 120-159 mm Hg and 80-99 mm Hg, respectively.[77] Results indicated significantly reduced mean BP (SBP/DBP) among hypertensive participants consuming protein-rich diets (-3.5 mm Hg / -2.4 mm Hg) and unsaturated fat diets (-2.9 mm Hg / -1.9 mm Hg) in comparison to carbohydrate diets.[77] The authors recommended modifying the DASH diet by replacing carbohydrates (partially) and incorporating more proteins (preferably plant proteins) or monounsaturated fat for improving overall cardiac health.[77]

One of the most widely studied plant proteins is soy protein which has documented health benefits such as BP control, mitigation of inflammation, hypoglycemic effects, cholesterol-lowering, and weight loss.[78] Antihypertensive activity exhibited by soy protein is attributed to the high content of amino acids such as arginine and the polyphenol isoflavone.[78] Examples of other protein sources with antihypertensive benefits include whey, pea, chia seed, fish, walnut, mushroom, and bitter melon.[79–82] Hemp seed protein has emerged as a nutrient-rich plant protein with an essential amino

acid profile similar to soy but with better digestibility.[83] In addition hemp protein has several health benefits which will be discussed in later sections.

1.1.7 Effect of dietary protein on vascular function

The most widely studied mechanism of action on antihypertensive effects exhibited by dietary food protein or peptides is through renin or ACE inhibition.[45] Although RAAS components are important molecular targets for hypertension control, other mechanisms targeting endothelial function have also been reported which will be discussed in this section.

Shobako et al. studied the effects of rice bran-derived peptide, Leu-Arg-Ala (LRA) on mesenteric artery isolated from male SHR.[84] Results indicated significant increase in vascular relaxation in response to increasing concentrations of LRA (0.01 μM – 10 μM).[84] Following removal of endothelium from mesenteric artery, LRA-induced vasorelaxation was nullified which suggests that dilation of vessels is endothelium-dependent.[84] In addition, LRA-induced vasorelaxation was inhibited after treatment with the nitric oxide synthase inhibitor, L-NAME.[84] Thus, LRA-induced vasorelaxation was proposed to be NO-endothelium mediated.[84]

Another study examined the rapeseed derived protein, rapakinin (Arg-Ile-Tyr) in mesenteric artery isolated from male SHR and WKY rats.[85] A significant dose-dependant (3 μM – 100 μM) increase in vascular relaxation was seen in both SHR and WKY rats.[85] Results showed rapakinin mediates vascular relaxation via endothelium-dependant relaxing factor – prostaglandin (PG).[85] Further tests revealed vasorelaxation was mediated through PGI_2 IP and cholecystokinin (CCK) – CCK_1 receptors.[85]

Other food-derived antihypertensive peptides which improved endothelial function and induced vasorelaxation include milk – casein-derived peptides, valyl prolyl proline, and isoleucyl prolyl proline.[86] In addition 4-week supplementation of dietary protein mixture (pea, soy, milk, egg) in obese individuals under fasting showed improved endothelial function after analyzing biomarkers of endothelial dysfunction (e.g. soluble intercellular adhesion molecule 1, vascular cell adhesion molecule 1, soluble endothelial selectin and von Willebrand factor) in these individuals.[87]Also, ground hemp seed diet improved vascular endothelial function in isolated thoracic arteries isolated from obese male zucker rats (a rat model characterised by vascular dysfunction).[88] This study showed greater % relaxation to acetylcholine in rats fed with hemp seed diet, but no significant response in rats fed with hemp seed oil diet.[88]

1.2 Vascular effects of hypertension

Increased vascular resistance to blood flow caused by narrowing and stiffening of arteries is a trademark of essential hypertension.[33,89] This is due to alterations in the structure, mechanics, and function of resistance arteries.[33]

Resistance arteries are blood vessels comprising small arteries and arterioles with a lumen size less than 400 μm and 100 μm respectively.[90] As blood flows across the arterial bed, the largest BP drop (70%) is observed across microvasculature.[89] Abnormalities such as lumen narrowing in these arteries generate significantly increased peripheral resistance to blood flow.[91] The relationship between blood pressure and peripheral resistance is explained by the following equation.[92]

mean blood pressure = cardiac output x peripheral resistance

1.2.1 Structural and mechanical alterations

Resistance arteries undergo structural alterations during hypertension - namely eutrophic and hypertrophic remodeling characterized by changes in lumen diameter and media cross-section area.[90] Eutrophic remodeling is characterized by decreased diameter and increased media-lumen ratio, and is reportedly a greater predictor of cardiovascular diseases.[93,94] Hypertrophic vascular remodeling is characterized by an increase in vessel wall thickness; thus exhibiting increased wall to lumen ratio and media cross-sectional area.[95]

Essential hypertension involves increased peripheral vascular resistance, as small arteries/arterioles undergo remodelling which leads to a significant rise in resistance that is explained by Poiseuille's law.[95]

According to the Hagen-Poiseuille equation,

$$flow = \frac{\delta P \pi r^4}{8\eta L},$$

Also,

$$Blood\ flow = \frac{\delta P}{resistance},$$

Thus, re-arranging the equation,

$$Resistance = \frac{8\eta L}{\pi r^4}$$

r = radius of the vessel

L = length of the vascular segment

δP = pressure difference

η = viscosity of blood

Thus, resistance is affected by three parameters – vessel radius, length, and viscosity. Out of these, radius has a significant impact on resistance and blood flow, as resistance is inversely proportional to the fourth power of the radius. Hence, a small reduction in diameter generates a four-fold increase in resistance, which in turn increases BP.

A study by Rizzoni et.al. showed increased media to lumen ratio and smaller internal diameter in subcutaneous small arteries taken from 59 essential hypertensive patients.[96] Vascular smooth muscle cells (VSMC) vis-a-vis increased collagen and fibronectin are involved in the remodeling of resistance arteries.[97] As the arterial wall is exposed to chronic tensile strain, it responds with VSMC hyperplasia and changes in extracellular matrix (ECM) composition.[98] Restructuring of these small arteries may also be due to factors such as chronic vasoconstriction, endothelial dysfunction, apoptosis leading to inward growth, angiotensin II and endothelin-dependent inflammation.[99] Small arterial remodeling may be initially considered adaptive but may become maladaptive, leading to end-organ damage and cardiac complications.[99,100]

In addition to remodeling, hypertensive vessels show mechanical changes such as increased arterial stiffness, reduced compliance, and distensibility.[101] Overproduction of collagen, fibronectin, and proteoglycans, as well as decreased elastin levels, contribute to the stiffening and thickening of the ECM and vascular wall.[98]

1.2.2 Functional alterations

The vascular endothelium is the innermost lining of the blood vessel that plays a major role in vascular constriction and relaxation.[102] It secretes vasoactive substances which are essential in maintaining vascular tone.[103] These include vasodilators such as NO, endothelium-derived hyperpolarizing factor (EDHF), prostacyclin, and constrictors such as thromboxane and endothelin-1.[104] NO plays a key role in vasodilation and normally helps to maintain blood pressure

and reduce peripheral resistance.[105] L-arginine is converted by the enzyme NO synthase, to synthesize NO, which then diffuses into adjacent vascular smooth muscle cells and leads to relaxation of vessels.[106]

Impaired endothelium-dependant relaxation was first reported in arteries isolated from hypertensive rats (SHR).[107,108] Also, hypertensive vessels showed impaired vasorelaxation response to agonists such as acetylcholine.[108] Therefore, endothelial dysfunction during hypertension due to decreased NO level causes impaired vascular relaxation, thereby increasing peripheral resistance.[105,109] Factors contributing to impaired vascular relaxation include aging and increased production of ROS resulting in NO inhibition.[110]

1.3 Hemp

Industrial hemp (*Cannabis sativa* L.) is a versatile crop cultivated with ≤ 0.3% w/w of tetrahydrocannabinol (THC) present on the leaves and flowering heads.[111] In Canada, production of industrial hemp is authorized under license issued by Health Canada.[111] The crop is known for oilseed and fiber and is useful for textile, paper, canvas, rope, cosmetics, nutrition, industrial and medicinal purposes.[112] The stem of hemp plant is a rich source of cellulose and woody fibers with antibacterial properties that are useful in surgical device, automotive, and construction industries.[113] Hemp cultivation was initially illegal in Canada due to ẟ-9 THC content, but presently hemp varieties with low THC content are grown as an important agricultural commodity.[114] Industrial hemp and marijuana, both belong to plant genus *Cannabis* but differ in psychoactive component THC, as industrial hemp (*C. sativa sativa)* is high in cannabidiol (CBD), an antipsychotic agent, and low in THC (<0.3%) content whereas marijuana (*C. sativa indica)* is high in THC and low in CBD.[115,116] In addition to being a versatile crop with multiple uses, cultivation of industrial hemp is sustainable and environment - friendly since it can be grown under various climatic conditions without chemicals such as herbicides.[117] It is a fast-growing plant with high yield and the entire plant is used for various purposes, thus generating zero waste.[117] Manitoba is one of the top 3 provinces in Canada with over 92 licenses issued for industrial hemp cultivation.[118] In addition, Canada's first and largest hemp seed processing units are located in Manitoba namely- Hemp Oil Canada and Manitoba Harvest.[119] With growing interest in hemp seed products, the Canadian hemp industry has the potential to be worth 1 billion dollars by 2030.[120]

1.3.1 Industrial hemp seed

In Canada, demand for hemp grain products such as hemp oil, seeds, and protein powder is the driving force behind cultivation of industrial hemp crop. [119]

Hemp seed has been used as a source of food for over 3000 years, and hemp seed and hempseed oil have served as food and traditional medicine in China and across Asia.[121] Interestingly, reduced incidence of cardiovascular risk and increased longevity among the people of Bama Yao have been attributed to their use of hemp seed oil.[122] In recent years, hemp seed and its constituents are incorporated in food products such as flours, flavoring sauces, hemp energy bars, protein powders, salad dressing, dietary supplements as well as in cosmetics, and skin care purposes.[123]

The major constituent of hemp seed is oil or fat, followed by proteins, carbohydrates, fiber, vitamins, and minerals.[114] Bioactive constituents present in hemp seed that are responsible for nutritional benefits include amino acids, fatty acids, lignanamides, and polyphenols.[124]

Hemp seed oil content is approximately 25-35% and it differs between various cultivars; the oil fraction also varies based on environmental conditions.[125] Hemp seed oil contains more than 70% of polyunsaturated fatty acids (PUFA) and approximately 17% of monounsaturated fatty acids.[126] Linoleic acid and α-linolenic acid are the most prominent fatty acids present, accounting for approximately 54% and 10% of PUFA respectively.[126] Incorporating these essential fatty acids that are present in optimal ratio of 3:1 (linoleic acid: linolenic acid) into the diet serves as an excellent source of nutrition.[127] These are required by the human body for various biochemical pathways and get metabolized into eicosanoids and prostaglandins.[127] The PUFA improves metabolism, inhibits tumor growth, decreases BP, and has anti-inflammatory properties.[127] Apart from these fatty acids, hemp seed oil also contains tocopherols, β-caryophyllene and methyl salicylate which has cholesterol lowering properties, as well as anti-viral, anti-inflammatory, antioxidant and cytoprotective properties.[127]

The hemp seed carbohydrate fraction ranges between 20-30%.[123] A major portion of carbohydrate is insoluble dietary fiber that is found in the hull of the seed.[128] Consumption of these dietary fibers reduce the risk of type-2 diabetes by reducing appetite and obesity.[129] The carbohydrate fraction also contains soluble fiber (3g/100g) and starch in small amounts.[128] Besides carbohydrates, hemp seeds also contain a rich array of vitamins and minerals such as vitamins E, B1, and B2, and

phosphorous, potassium, calcium, magnesium, sodium, and iron.[121] Among these, phosphorous is the most abundant macronutrient in hemp seed (1.16 g per100 g).[121] Apart from these components, hemp seed also contains antinutritional compounds like phytic acid, tannins, cyanogenic glycosides, and saponins which are also present among other legumes, pulses, and oil seeds.[125]

Phenylpropionamide (lignanamide) isolated from hemp seed exhibits neuroprotective anti-inflammatory action and improved cognition in mice, and potentially may be used in Alzheimer's disease and other neurodegenerative disorders.[124] A randomized controlled trial showed hemp seed oil (30 ml) consumption daily for 8 weeks improved skin conditions of 15 female and 1 male eczema patients.[130] Other positive effects exhibited by hemp seed consumption in human and animal studies include cardiac protection post-ischemia, reduced cholesterol, reduced damage to the arterial wall, promotion of blood circulation, in rheumatoid arthritis, and pain relief.[131–133]

1.3.2 Industrial hemp seed protein

Generally, the protein content present in whole hemp seed ranges between 20-25% but this value varies depending on cultivars.[134] Proteins are usually isolated from hemp seed cake or meal (hemp seed flour) which is a residue or by-product obtained after hemp oil extraction.[135] HPI is the purified form obtained from hemp seed containing more than 80% protein.[136] The protein fractions present include storage proteins - edestin and albumin that are rich in essential amino acids and exhibit good digestibility.[137] Hemp seed proteins contain sulphur group proteins (methionine-cystine) as well as amino acids such as arginine, aspartic acid, and glutamic acid.[138] Arginine is important for NO biosynthesis that is associated with antihypertensive effects, as well as improved immune and cognitive function.[139] Similarly, aspartic acid is essential for neuronal function and glutamic acid is a neurotransmitter.[140,141] Levels of arginine, methionine, and cysteine is higher in HPI compared to soy protein isolate.[142] The presence of sulphur amino acids adds to nutritional quality since many other protein sources such as pea and legumes lack them.[136]

HPI is hypoallergenic; allergenic peptides such as thaumatin-like protein and lipid transfer protein present in hemp are reduced during the production of HPI; thus mild allergenic activity makes HPI a suitable ingredient for food preparation.[143] *in vitro* digestibility studies have indicated that HPI is easily digested by pepsin and trypsin in comparison to soy protein isolate.[144] High digestibility was further confirmed by a recent study that analyzed in vitro digestion of HPI using a gastrointestinal digestion static model.[145] Results showed only a few peptides survived the

digestion process.[145] *in vivo* digestibility study reported that protein digestibility- corrected amino acid score for hemp seed proteins are in the same range as lentils, pinto beans (pulse proteins) and higher than whole wheat or cereal grain products.[135]

Also, most of the essential amino acids present in HPI meet the standards set by Food and Agriculture Organization /World Health Organization (FAO/WHO) for infants (2-5 years old) and children (10-12 years old).[142] In addition, Wang et al. reported that the ratio of essential amino acid to total amino acid (E/T) is greater in HPI compared to soy protein isolate.[138] The nutritional quality of a protein is determined by amino acids present, bioavailability, and digestibility.[125] Hemp protein meets all these requirements and can be used for human consumption.[125]

Hemp seed protein has several health benefits. A study conducted on 10 healthy volunteers (3 men, 7 women) given meals containing 30g hemp protein showed significantly increased levels of metabolites such as glucagon-like peptides, ferulic acid, and salicylic acid in the plasma of these participants.[146] Presence of these metabolites is beneficial in terms of reducing inflammation and treating type-2 diabetes.[146] Also, treatment with peptides isolated from hemp protein has shown anti-cancer activity by reducing proliferation and inducing cell death of liver cancer cells.[147] Another recent study showed hemp seed proteins reduced ROS levels in endothelial cells and inflammatory cytokine production, which are biomarkers of endothelial dysfunction.[147] Hemp seed protein also improves cardiovascular health which will be discussed below.

1.3.3 Antihypertensive effects of hemp seed protein

Girgih et al., studied the antihypertensive effects of hemp seed protein hydrolysates (HPH) [obtained from pepsin/pancreatic digestion of HPI] and peptides fractions [obtained from ultrafiltration of HPH] on 20-week-old male SHR.[148] Oral administration of HPH significantly reduced SBP by -20 mm Hg within 2 hours, -30 mm Hg after 8 hours.[148] In addition, *in vitro* tests showed HPH inhibited ACE and renin enzymes. [148] Girgih and colleagues also identified bioactive short-chain peptides present in HPH exhibiting antioxidant and antihypertensive activity.[149] These peptides: Trp-Val-Tyr-Tyr and Pro-Ser-Leu-Pro-Ala (PSLPA) were orally administered to 30-week-old male SHR showed significant reduction in SBP levels.[149] Additionally, another study found 4 new synthetic peptides (GVLY, LGV, IEE, and RVR) derived from hemp seed protein with ACE inhibitory effects.[150] The highest ACE inhibition was observed with GVLY with an IC50 value of 16 ± 1.5 µM (IC50 is inhibitory concentration needed to bring 50% ACE inhibition)

followed by LGV and RVR whereas IEE was inactive as it inhibited ACE only by 20.5%.[150] These bioactive peptides have the potential to be used as functional food but there is a lack of human intervention trials using these hemp-derived peptides. So far, there is only one registered clinical trial that will be exploring benefits of dietary HPI and effect of these dietary protein with added bioactive peptides for consumption in humans.[151]

Girgih et al., also explored the antihypertensive effects of HPI among young and adult SHR rats.[152] 8-week-old SHR rats fed with a 1% HPI diet for 8 weeks exhibited significant reduction in SBP, plasma ACE, and plasma renin level.[152] Similarly, 20-week-old SHR rats fed with 1% HPI diet for a period of 4 weeks exhibited reduced SBP, plasma ACE, and renin levels.[152] The study showed HPI has both preventive and treatment effects on hypertension and can be used as a potential anti-hypertensive functional food.[152]

CHAPTER II

2.1 Rationale

Prevention and treatment of hypertension by incorporating healthy diet is a subject of growing interest. There is ongoing research to identify novel food sources useful in treatment of chronic diseases. Hemp seeds are a great source of protein and other nutrients. Globally, within the last decade, there is growing consumer demand for hemp seed products such as food (hemp milk, oil, protein powders), nutritional supplements, and hemp-derived personal care products (soaps, gels, cosmetics).[153]

Hemp seed proteins show potential antioxidant and antihypertensive properties, thus benefiting overall cardiovascular health.[154] Research has shown the ability of HPI-containing diets in reducing elevated BP in male SHR rats.[152] Previously reported mechanisms underlying the antihypertensive effects of HPI focused on renin and ACE inhibition.[152] Although RAAS components are important molecular targets for hypertension control, other mechanisms such as direct vasorelaxant activity of food-derived proteins may be involved. There is a need to expand research beyond the usual ACE and renin inhibition and identify potential mechanisms through which HPI exhibits anti-hypertensive effects. Also, no previous study has investigated the effect of HPI on vascular function.

Generally, the incidence of hypertension is observed to be higher among men compared to age-matched premenopausal women.[5] Even among normotensive subjects, plasma renin is found to be higher in men compared to women.[155] Sex differences in BP are also observed among male and female SHR.[156] In addition, previous studies have shown endothelial dysfunction is higher in male SHR compared to female SHR and sexual dimorphism is observed in vascular function, with male mesenteric vessels showing greater response to Ang II.[157,158] In addition, research has not examined if there are any sex differences in response to HPI diet.

HPI has high levels of arginine, a major precursor for NO synthesis. NO is a potent vasodilator that plays a major role in regulating hypertension. Also, the bioavailability of NO is greater in females (healthy and hypertensive) compared to males.[5,159] This study aims to find if HPI has a direct effect on vascular function or arterial dilation of SHR rats and compare % dilation response to controls (WKY). We also aim to address if there are any sex differences in vascular response to HPI.

2.2 Hypothesis

We hypothesize that direct treatment of HPI on arteries will lead to vasorelaxation. Since there is sex differences in vascular function,[160] we hypothesize that female rats will show greater vasodilation compared to age-matched male rats.

2.3 Objective

To determine the effects of HPI on mesenteric resistance arteries *ex-vivo* isolated from an animal model.

1. Determine the effects of HPI on vascular function (endothelium-dependent and -independent relaxation) of mesenteric resistance arteries isolated from male and female SD rats.
2. Test the treatment effects of HPI on vascular function in male and female SHR (vs normotensive control).

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

Natural hemp seeds (2.27 kg) were purchased from Manitoba Harvest Hemp Foods (Winnipeg, Manitoba). Other analytical grade reagents for hemp preparation – acetone (ACS grade), sodium hydroxide pellets were procured from Fisher Scientific Canada, and concentrated HCL was from Sigma Aldrich.

Reagents for pressure myography – sodium chloride (NaCl), potassium chloride (KCl), magnesium sulfate ($MgSO_4$), monopotassium phosphate ($KHPO_3$), calcium chloride dihydrate ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), glucose, nor-epinephrine (NE), acetylcholine (Ach), and sodium nitroprusside (SNP) were purchased from Sigma Aldrich. Sodium EDTA was purchased from Omnipur. Pierce - BCA Assay Kit was obtained from Thermo Scientific.

3.2 Animals

SD, SHR and WKY rats were purchased from Charles River (Saint-Constant, Canada) at 14 weeks of age. Each group had 10 male and 10 female rats. These animals were housed in the R.O. Burrell Animal Facility under a daily light/dark cycle of 12-hour light/12-hour dark. Animals were housed at 20-23^{0}C and 30-70% humidity. The animals were fed *ad libitum* with a standard diet (Prolab RMH 3000) and water upon arrival. They were acclimatized for 2 weeks and sacrificed at 16-weeks of age.

3.3 Blood pressure and body weight

Body weight measurements were taken on the day of sacrifice. Rats were trained for BP measurement for the 7 days prior to sacrifice. BP was measured at the end of the acclimatization period (15–16-week age) using tail-cuff plethysmography (CODA non-invasive system, Kent Scientific). It consisted of 5 acclimation and 15 data cycles and an average of 7 accepted cycles were taken for measurement.

3.4 Methods

3.4.1 Hemp protein isolate preparation

Hemp seeds were ground in a laboratory blender to obtain hemp seed flour. The ground mixture was defatted using acetone at 1:10 (w/v) at room temperature for 1 hour. The mixture was allowed to settle followed by decantation of acetone and a second consecutive extraction of the residue. The residual flour was air-dried in the fume hood overnight and the resultant dried defatted hemp flour (DHF) was stored at -20^{0}C.

Production of HPI from DHF was done according to the method from Tang et.al, with few modifications.[142] DHF was dispersed in distilled water (1:20 w/v), with the pH of the solution adjusted to 10 using 2 M NaOH and stirred for 2 hours using a magnetic stirrer at room temperature. The mixture was centrifuged for 30 min, 3700g at 4^{0}C. The supernatant was collected, filtered, and adjusted to pH 4.2 using 2 M HCl to precipitate proteins. The mixture was again centrifuged for 30 min 3700g at 4^{0}C. The supernatant was removed, and the protein precipitate was washed with distilled water and centrifuged for another 30min at 4^{0}C. The precipitate was collected, adjusted to pH 7 using 2 M NaOH, and freeze-dried to obtain HPI powder. The resultant HPI powder was stored at -20^{0}C.

HPI at semi-log doses of 0.1, 0.3, 1, 3, 10, 30, and 100 µg/mL was tested on the isolated mesenteric arteries by pressure myography. A dose-response relationship between varying concentrations of HPI on vasodilatory function was assessed. The dose was selected based on a previous study that used 50 and 100 µg/mL of HPI on human monocytes.[161]

3.4.2 HPI solution preparation

Protein dispersion (5.0%, w/v) was prepared by dissolving HPI powder in distilled water. The dispersion was stirred for 1 h at room temperature using a magnetic stirrer. The solution was then centrifuged at 4000 rpm for 23 min at 20^{0}C and the protein concentration of the supernatant was measured with Pierce BCA Protein Assay Kit. The resultant HPI stock solution was stored at -20^{0}C.

HPI solutions (0.1, 0.3, 1, 3, 10, 30, 100 μg/ml) were prepared from the stock solution on the day of the experiment by dissolving X μg/ml of stock in Y ml Krebs-NE solution at pH 7.4. X and Y were calculated using the formula:

$$C_1V_1 = C_2V_2$$

3.4.3 Amino acid content determination

HPI samples were hydrolyzed using methods from the Association Of Official Analytical Chemists (AOAC) and ISO (the International Organization for Standardization).[162,163] Following hydrolysis, amino acids were derived by dissolving the sample in Waters AccQ-tag reagent. Identification and quantification of the derived amino acids were determined by Shimadzu Nexera X2 Ultra-High-Performance Liquid Chromatograph (UPLC) system.

3.4.4 Proximate analysis

Moisture content, dry matter, crude protein, crude fiber, fat, and ash content present in HPI were analyzed by Central Testing Laboratory Ltd (Winnipeg, Manitoba).

3.4.5 Pressure myography

Isolation of vessels

At 16-weeks age, rats were euthanized using isoflurane anesthesia. Heparin (0.1 mL/100 g body weight) was injected via the saphenous vein. The chest cavity was opened, and hearts were excised. Following this, the mesenteric arterial bed was removed and placed in an ice-cold Krebs solution. The composition of 1x Krebs include (mmol/L): 118.9 NaCl, 4.7 KCl, 1.18 $MgSO_4*7H_2O$, 1.18 KH_2PO_4, 2.5 $CaCl_2*2H_2O$, 24.9 $NaHCO_3$, 5.5 glucose and 0.026 Na*EDTA. The arterial bed was secured onto a dissection dish filled with Krebs solution. Third-order mesenteric arterial segment free of connective and adipose tissue were isolated (Figure 1). Mesenteric resistance arteries were selected for the purpose of this study since these arterioles undergo remodelling (increased wall to lumen ratio) during hypertension.[164] In addition, mesenteric arteries are less branched compared to other arteries (renal, femoral, and coronary) thus making it easier to study using the pressure myography technique.[165]

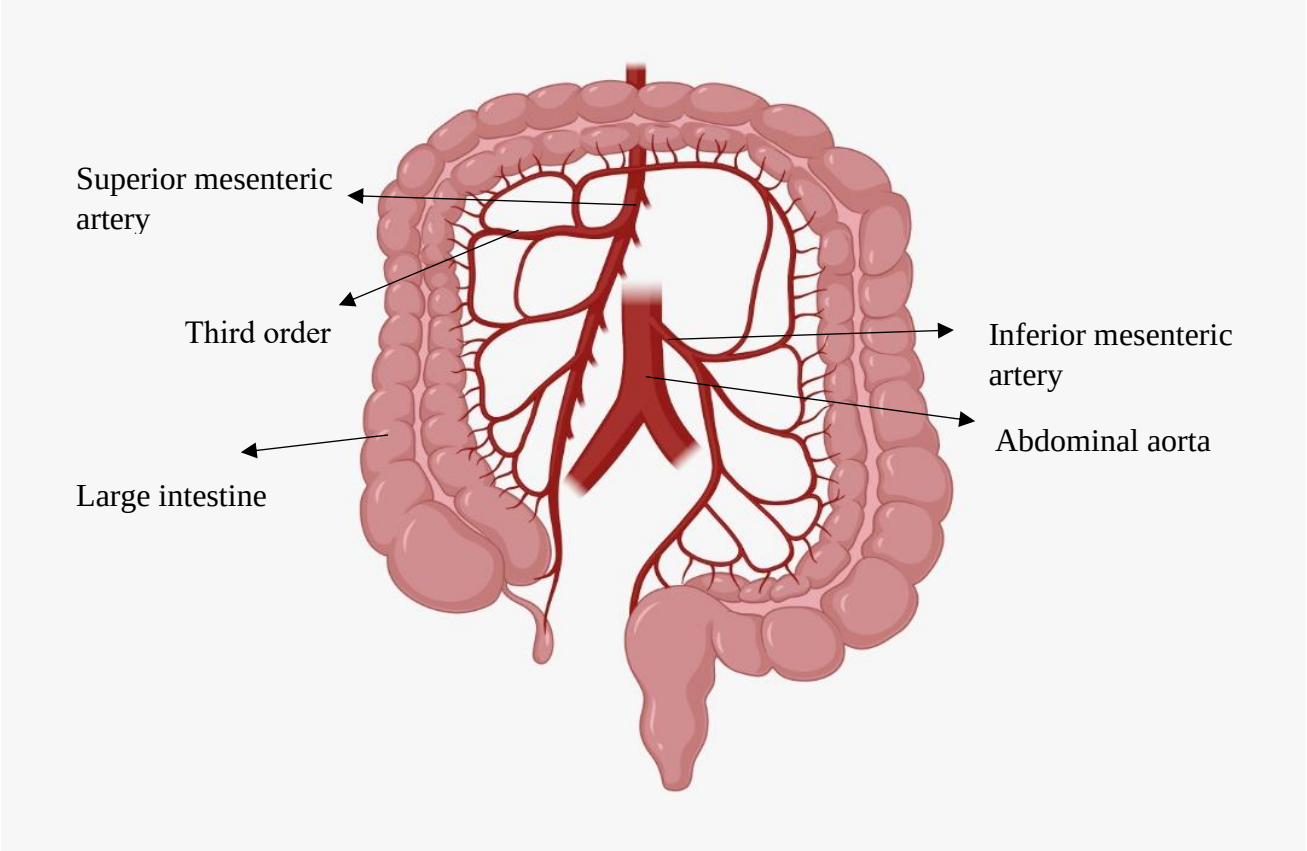

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Figure 1. Mesenteric arterial bed. The third-order arterial segment was isolated for all experiments.

The arterial segment was mounted onto a pressure myograph chamber (Living Systems Instrumentation) filled with Krebs solution. One end of the artery was placed onto a glass cannula that is connected to a pressure regulating device – peristaltic pump and pressure servo control (Living systems instrumentation Burlington, Vermont). The other end of the vessel was placed on the opposing glass cannula. Both ends of the vessel were secured using nylon ties and adjusted to be parallel without stretch.

The vessels were equilibrated for 45-60 minutes at 45 mm Hg, 37^0C using Krebs solution that was bubbled with a gas mixture (5% carbon dioxide, 21% oxygen, and balance nitrogen) which ensures adequate oxygenation. Following equilibration, 125 mM KCl was used to check the viability of the vessels. Constriction of the vessels >50% is required to confirm vessel viability. After confirmation of viability, vessels were equilibrated again with Krebs solution for 45-60 min.

Functional properties

Following equilibration, vessels were pre-constricted using 10^{-5}M NE. Vessels were then treated, in the continued presence of NE, with increasing concentrations of HPI (0.1, 0.3, 1, 3, 10, 30, and 100 µg/mL). The lumen diameter, as well as left wall and right wall thickness was taken following each concentration using a video dimension analyzer (Living systems instrumentation, Burlington, Vermont).

Vessels were once again equilibrated for 45 – 60 min, followed by treatment with NE (pre-constriction). The arterial segment was exposed, again in the presence of NE, with consecutive Ach doses (10^{-9} M – 10^{-4} M). Following treatment with Ach, the vessels were equilibrated, pre-constricted with NE, and treated with increasing concentrations of SNP (10^{-9} M – 10^{-4} M). Vascular dimensions were acquired as indicated above following each treatment. The change in arterial diameter was calculated for each concentration using the following equation:

$$\textbf{Percentage dilation} = [(D_x - D_i) / (D_{eq} - D_i)] * 100$$

D_x = arterial diameter at each dose of agonist (HPI/Ach/SNP)

D_i = initial diameter at 10^{-5}M NE (pre-constriction)

D_{eq}= baseline diameter following equilibration

3.4.6 Statistical analysis

Statistical analysis was performed using Graph Pad Prism Software version 9.2.0. All results are expressed as mean ± SEM. $p < 0.05$ was considered statistically significant. Values that were +/- 3 standard deviations from the mean were removed as outliers.

For the SD rat study, an unpaired t-test was used to determine the difference between male and females for body weight and BP. Two-way analysis of variance (ANOVA) was used to determine the effect of sex and dose of HPI, Ach, and SNP on % dilation of blood vessels. Post hoc tests to determine differences among groups were corrected for multiple comparisons using Tukeys.

For the SHR vs WKY study: To determine the effect of genotype and sex on BP and body weight, data were analyzed by two-way ANOVA. The effect of genotype, sex, dose, and their interactions on the functional properties of the blood vessels were analyzed by three-way ANOVA. Post hoc tests used to determine differences between groups were corrected for multiple comparisons using Tukeys.

CHAPTER IV
RESULTS

4.1 Proximate composition of HPI

The proximate components present in HPI obtained from Central Testing Laboratory (Winnipeg) are shown in Table 3. On average, HPI contains approximately 0.96% moisture and 99% dry matter. In the current study, average crude protein content and fat content is 82.79% and 16.31% respectively. The fibre content is less than 1% since much of the fibre is present in the seed hull and we used shelled hemp seeds. Dehulled hemp seeds yield hemp hearts that are rich in oil and protein content.[135] Ash content is approximately 6.4% and the total digestible nutrients present in HPI is approximately 84%

Table 3. Proximate composition of HPI

Parameter	**HPI**
Moisture (%)	0.96 ± 0.891
Dry Matter (%)	99.04 ± 0.891
Crude Protein (%)	82.79 ± 0.141
Crude Fibre (%)	0.385 ± 0.007
Fat (%)	16.31 ± 0.155
Ash (%)	6.465 ± 0.64
Calcium (%)	0.075 ± 0.007
Phosphorus (%)	0.63 ± 0.028
Magnesium (%)	0.02 ± 0.000
Potassium (%)	0.06 ± 0.014
Sodium (%)	0.61 ± 0.000
Copper (mg/kg)	14.82 ± 1.739
Iron (mg/kg)	88.165 ± 0.771
Manganese (mg/kg)	37.07 ± 1.174
Zinc (mg/kg)	79.17 ± 3.309
Total digestible nutrients (%)	84.19 ± 0.240

Each value is presented as mean ± standard deviation of duplicate measurements

4.2 Amino acid composition

The amino acid profile of HPI, expressed as crude content (g/100g) and percentage of their protein content is shown in Table 4. Glutamic acid and arginine accounts for 30% of the total amino acids present in HPI. Overall, amino acid composition of HPI obtained in our present study is similar with the previous studies.[138,166]

Table 4. The amino acid composition of HPI

	Crude content (g/100g)	**% Amino acid**
His	1.238	1.958
Ser	3.211	5.079
Arg	8.855	14.008
Gly	2.481	3.926
Asp	6.942	10.982
Glu	11.173	17.675
Thr	2.291	3.625
Ala	2.587	4.092
Pro	2.387	3.776
Cys	0.669	1.058
Lys	2.205	3.488
Tyr	2.493	3.944
Met	1.542	2.440
Val	3.420	5.411
Ile	2.770	4.382
Leu	4.576	7.240
Phe	3.403	5.384
Trp	0.962	1.521

His- Histidine; Ser – serine; Arg – arginine; Gly- glycine; Asp- aspartic acid; Glu- glutamic acid; Thr -threonine; Ala- alanine; Pro- proline; Lys- lysine; Cys- cysteine; Tyr- tyrosine; Met- methionine; Val- valine; Ile -isoleucine; Leu- leucine; Phe- phenylalanine; Trp- tryptophan

4.3 Body weight and BP – SD

At 16 weeks of age, male SD rats weighed significantly higher compared to female SD rats (456.9 ± 7.69 g vs 299.0 ± 7.88 g) ($p<0.05$; Figure 2 A). There was no significant difference in SBP or DBP between the sexes. SBP and DBP in male SD rats were 122.7 ± 7.79 mm Hg and 85.75 ± 7.04 mm Hg, respectively. Whereas in female SD rats, SBP and DBP were 116.6 ± 4.93 and 77.66 ± 3.73, respectively (Figure 2 B and C).

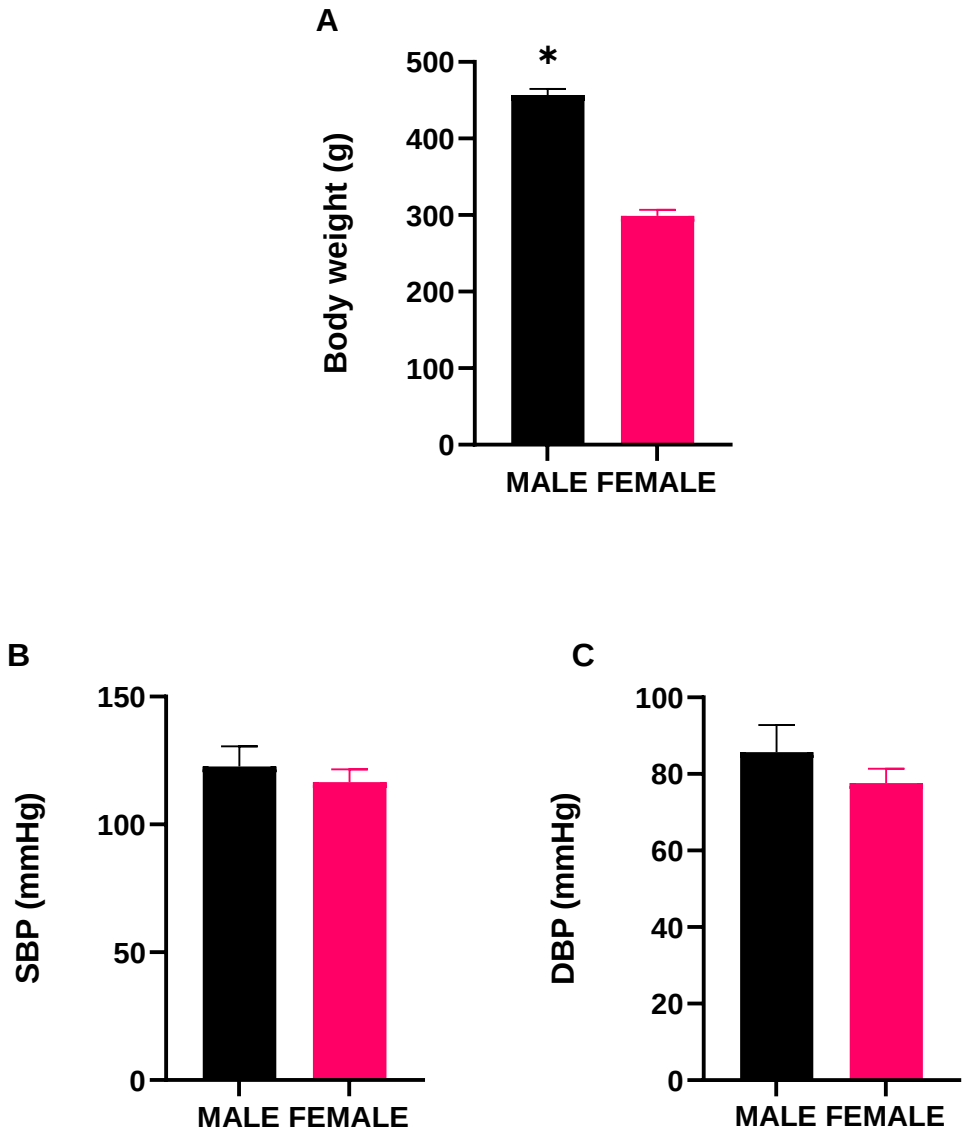

Figure 2. Body weight and BP of male and female SD rats

Data expressed as mean ± SEM. n=8 (Male), n=9 (Female). * Indicates statistically significantly different ($p<0.05$). SD – Sprague Dawley rats; SBP- systolic blood pressure; DBP- diastolic blood pressure.

4.4 Functional response in SD rat mesenteric resistance arteries – HPI

Figure 3 shows the relaxation response to HPI doses on NE induced pre-constricted mesenteric resistance arteries isolated from 16-week-old male and female SD rats. There was not a significant interaction, but a significant effect of dose and sex on % dilation. Tukey's multiple comparison test revealed that % dilation at 0.1 µg/ml concentration was 42.9 – 50.8 % significantly lower compared to 10, 30 and 100 µg/ml. Dilation response at 10, 30 and 100 µg/ml doses were not different from each other. At the lowest concentration (0.1 µg/ml), male and female rats showed % dilation response of 8.12 ± 1.73 % and 17.31 ± 4.56 % respectively. Overall, male SD rats had a significantly ($p<0.05$) lower % dilation in response to HPI compared to female SD rats.

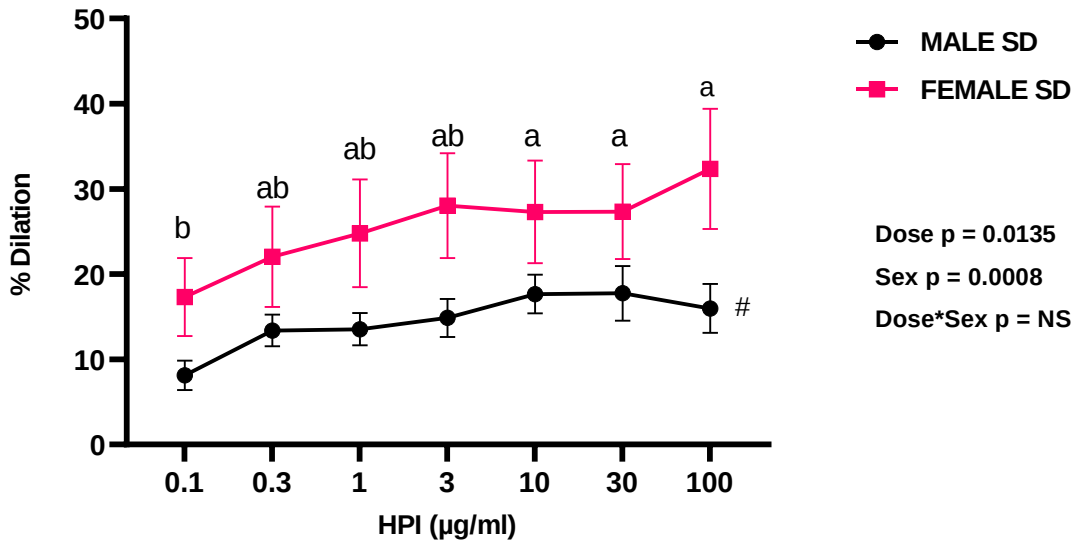

Figure 3. Vasorelaxation induced by HPI on mesenteric resistance artery isolated from male and female SD rats. Data expressed as mean ± SEM. Log-transformed data for normality but non transformed data are presented. Doses with different letters are significantly different. # indicates % dilation response is significantly different between male and female SD rats. $p<0.05$. n=8 (Male), n=9 (Female). HPI- hemp protein isolate, SD – Sprague Dawley.

4.5 Functional response in SD rat mesenteric resistance arteries – Ach

Endothelium-dependant relaxation was investigated in male and female SD rats by examining the % dilation response to Ach (Figure 4). Lowest % dilation of 10.56 ± 2.32 % was observed at 10^{-9} M and the highest % dilation of 83.81 ± 3.28 % was seen at 10^{-4} M concentration. Our results showed a 70-88% lower % dilation at 10^{-9} M vs 10^{-7} M, 10^{-6} M, 10^{-5} M, and 10^{-4} M ($p<0.05$). Also, the % dilation observed at 10^{-8} M and 10^{-7} M was 53-83% lower compared to 10^{-6} M, 10^{-5} M, and 10^{-4} M ($p<0.05$). Overall, Ach relaxed the pre-constricted vessels in a concentration dependant manner. No significant differences were observed between the sexes.

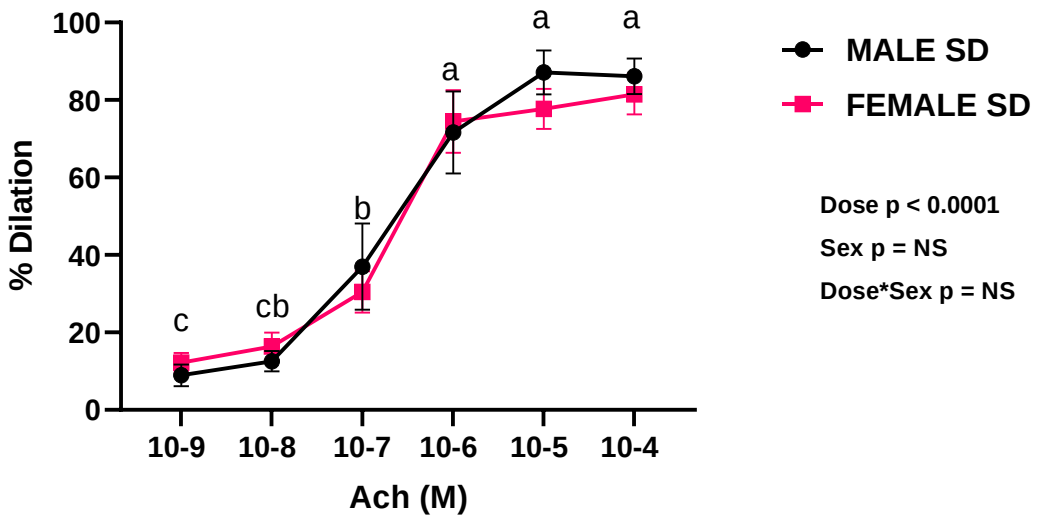

Figure 4. Vasorelaxation induced by Ach on mesenteric resistance artery isolated from male and female SD rats. Data expressed as mean ± SEM. Doses with different letters are significantly different. $p<0.05$. n=8 (Male), n=7 (Female). Ach- acetylcholine, SD – Sprague Dawley

4.6 Functional response in SD rat mesenteric resistance arteries – SNP

Endothelium-independent vasodilation was investigated by analyzing % dilation response to increasing doses of SNP (from 10^{-9} M up to 10^{-4} M) in mesenteric resistance arteries pre-constricted with 10^{-5} M NE (Figure 5). No significant % dilation difference was observed between male and female SD rats. There was a statistically significant effect of SNP dose on % dilation. At 10^{-9} M, % dilation was 10.89 ± 1.52 %, which was 56-65% lower compared to the % dilation at 10^{-5} M and 10^{-4} M, (24.97 ± 6.63 % and 30.73 ± 3.8 % respectively, $p<0.05$).

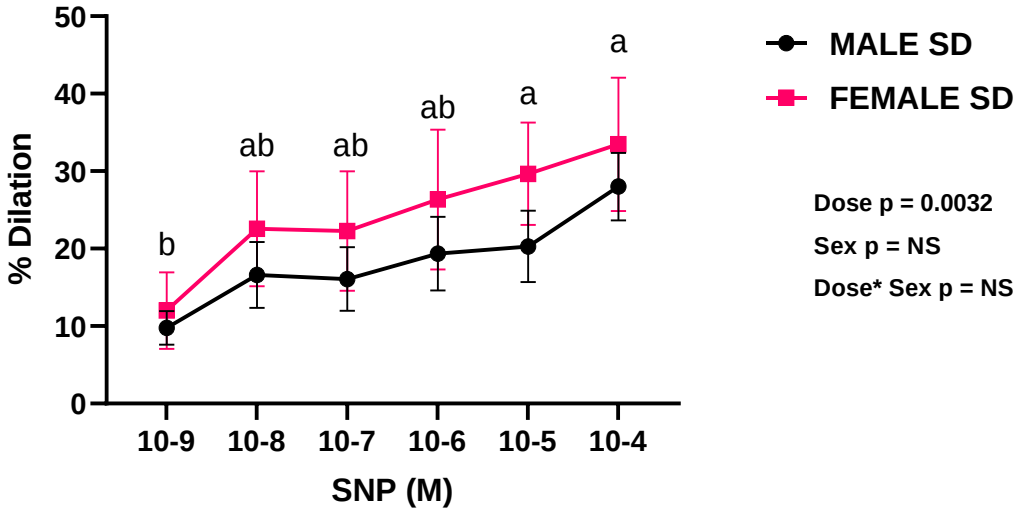

Figure 5. Vasorelaxation induced by SNP on mesenteric resistance artery isolated from male and female SD rats. Data expressed as mean ± SEM. Log-transformed data for normality but non-transformed data are presented. Doses with different letters are significantly different. $p<0.05$. n = 8 (Male), n=8 (Female). SNP- sodium nitroprusside, SD – Sprague Dawley

4.7 Body weight – SHR vs WKY

At 16-weeks of age, male rats (SHR and control) weighed significantly higher (58% greater) compared to female rats ($p<0.05$) (Figure 6). There was no effect of genotype on body weight.

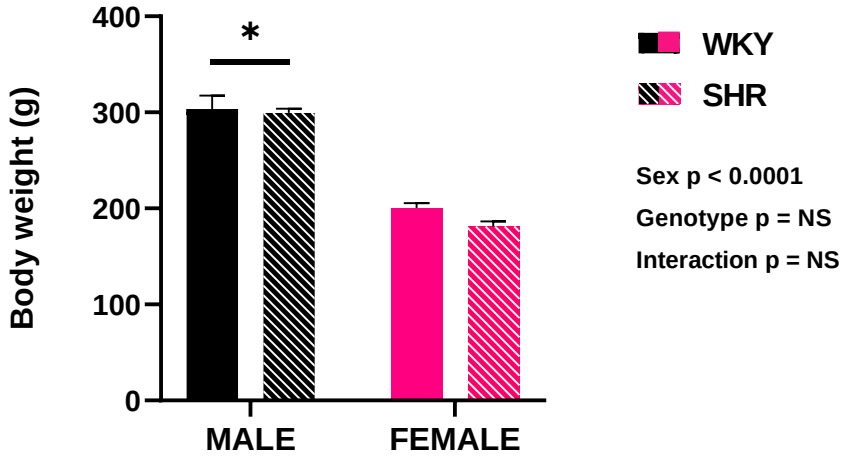

Figure 6. Body weight of male and female, SHR and WKY rats

Data expressed as mean ± SEM. * Indicates significantly different. ($p<0.05$). Solid color represents WKY rats, and stripes represent SHR rats. n=9 (Male SHR), n=9 (Female SHR), n=6 (Male WKY), n=6 (Female WKY). SHR- spontaneously hypertensive rat; WKY – Wistar - Kyoto rat.

4.8 BP measurement – SHR vs WKY

Male rats had 10% higher SBP compared to female rats ($p<0.05$; Figure 7 A) Also, SBP was 35% higher in SHR rats compared to WKY ($p<0.05$) (Figure 7 B). In comparison, no significant difference in DBP was observed between male and female rats (Figure 7 C). However, SHR showed 50% higher DBP compared to the control rats ($p<0.05$; Figure 7 D).

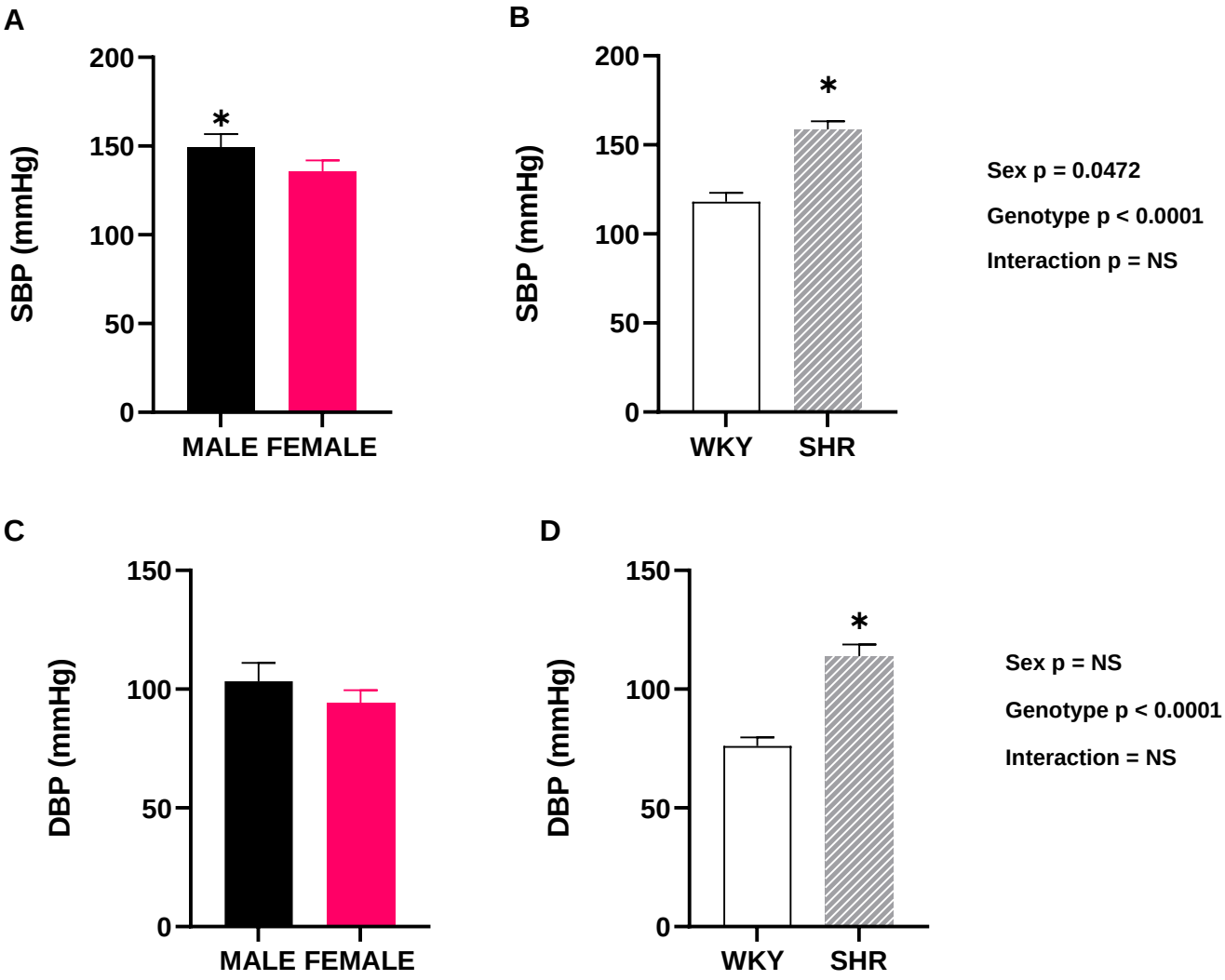

Figure 7. BP measured from male and female, SHR and WKY rats

(A) Effect of sex on SBP. (B) Effect of genotype (SHR vs WKY) on SBP. (C) Effect of sex on DBP. (D) Effect of genotype on DBP. Data expressed as mean ± SEM. n=18 (SHR), n=12 (WKY), n=15 (Male), n=15 (Female). * Indicates significantly different. ($p<0.05$). SHR- spontaneously hypertensive rats, WKY- Wistar-Kyoto rats.

4.9 Functional response in hypertensive mesenteric resistance arteries – HPI

To test whether HPI induced vasodilation in hypertensive vessels and in their normotensive controls, we used mesenteric resistance arteries isolated from 16-week-old SHR and WKY rats. HPI was added in increasing doses (starting from 0.1 μg/ml to 100 μg/ml) to pre-constricted third-order mesenteric vessels. Also, to examine whether sex affected HPI vasodilation response, we used male and female hypertensive rats. Our results showed that there was a significant interaction between genotype and sex on % dilation response (Figure 8 A). Male WKY rats showed 45-53% higher % dilation compared to all other groups ($p<0.05$). HPI dilated vessels (both hypertensive and controls) in concentration dependant manner. HPI dose of 0.1 μg/ml showed the lowest vasodilation (8.65 ± 1.02 %) and was significantly different from 1, 3, 10, 30, and 100 μg/ml (Figure 8 B). The 1, 3, 10, 30 and 100 μg/ml doses resulted in vasodilation ranging from 15.76 ± 1.57 % to 20.69 ± 1.83 % but were not significantly different from each other.

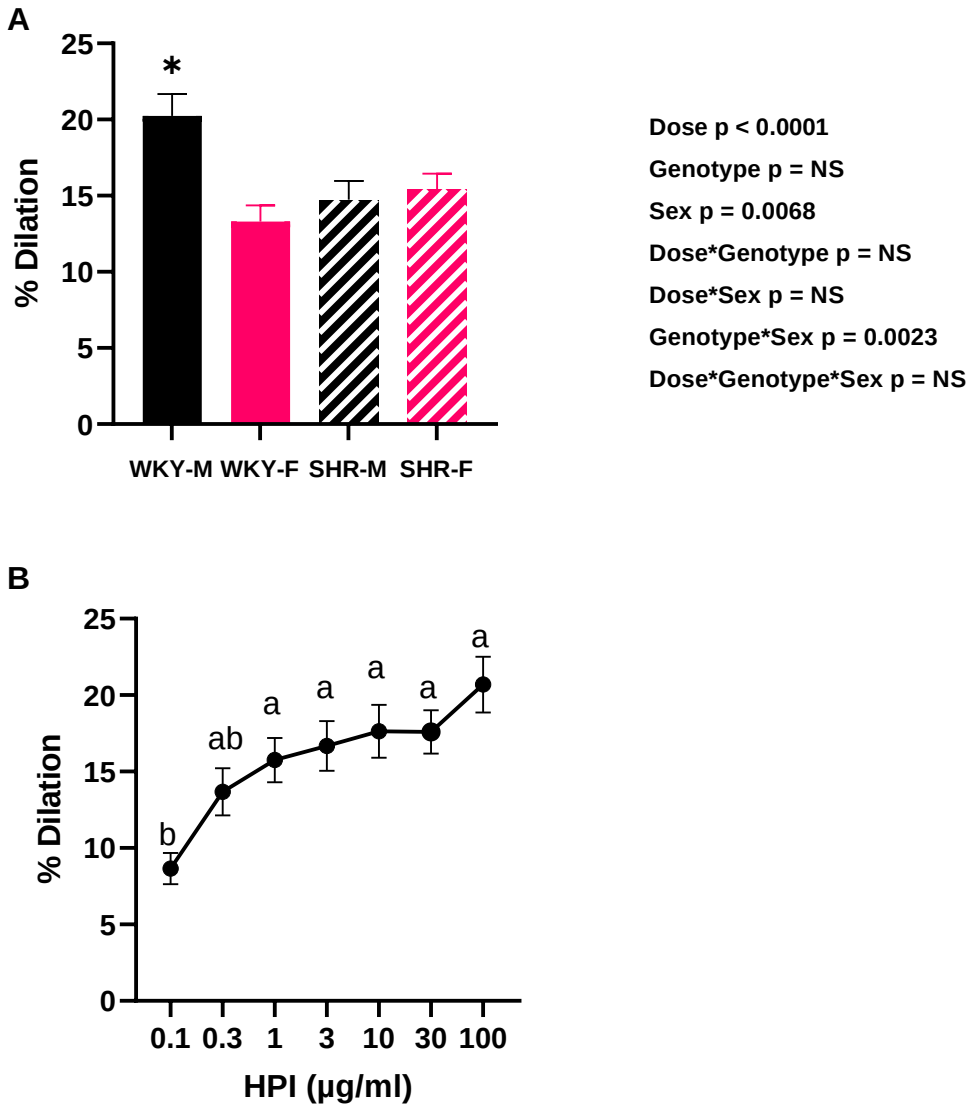

Figure 8. Vasorelaxation induced by HPI on mesenteric resistance arteries isolated from male and female SHR and their controls. (A) Effect of genotype*sex interaction on % dilation. n=42 (Male WKY); n=35 (Female WKY); n=54 (Male SHR), n=62 (Female SHR). * Indicates significantly different. ($p<0.05$). (B) Effect of HPI dose on % dilation. n = 26-28 per dose. Means with different letters are significantly different from each other. Data expressed as mean ± SEM. HPI- hemp protein isolate; SHR- spontaneously hypertensive rats, WKY- Wistar-Kyoto rats, NS – not significant.

4.10 Functional response in hypertensive mesenteric resistance arteries – Ach

Figure 9 shows vasorelaxation responses induced by Ach in mesenteric resistance arteries pre-constricted with 10^{-5} M NE. Vessels isolated from WKY rats showed significantly greater dilation response compared to SHR (54.79 ± 4.09 % vs 46.39 ± 2.97 %) ($p<0.05$; Figure 9 A). Also, sexes responded differently to Ach dose as shown in Figure 9 B. Ach (10^{-9} M to 10^{-4} M) induced vasorelaxation in concentration dependant manner. At the lowest concentration (10^{-9} M), male and female vessels dilated by 14.43 ± 3.38 % and 8.59 ± 1.25 % respectively. At the highest concentration (10^{-4} M), male and female vessels dilated by 63.10 ± 6.97 % and 77.79 ± 3.17 %, respectively. In male rats, vasorelaxation at concentrations 10^{-9} M and 10^{-8} M was significantly lower compared to response at 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M. Whereas in females, vessels dilated significantly lower at concentrations of 10^{-9}, 10^{-8} and 10^{-7} M compared to 10^{-6}, 10^{-5}, and 10^{-4} M.

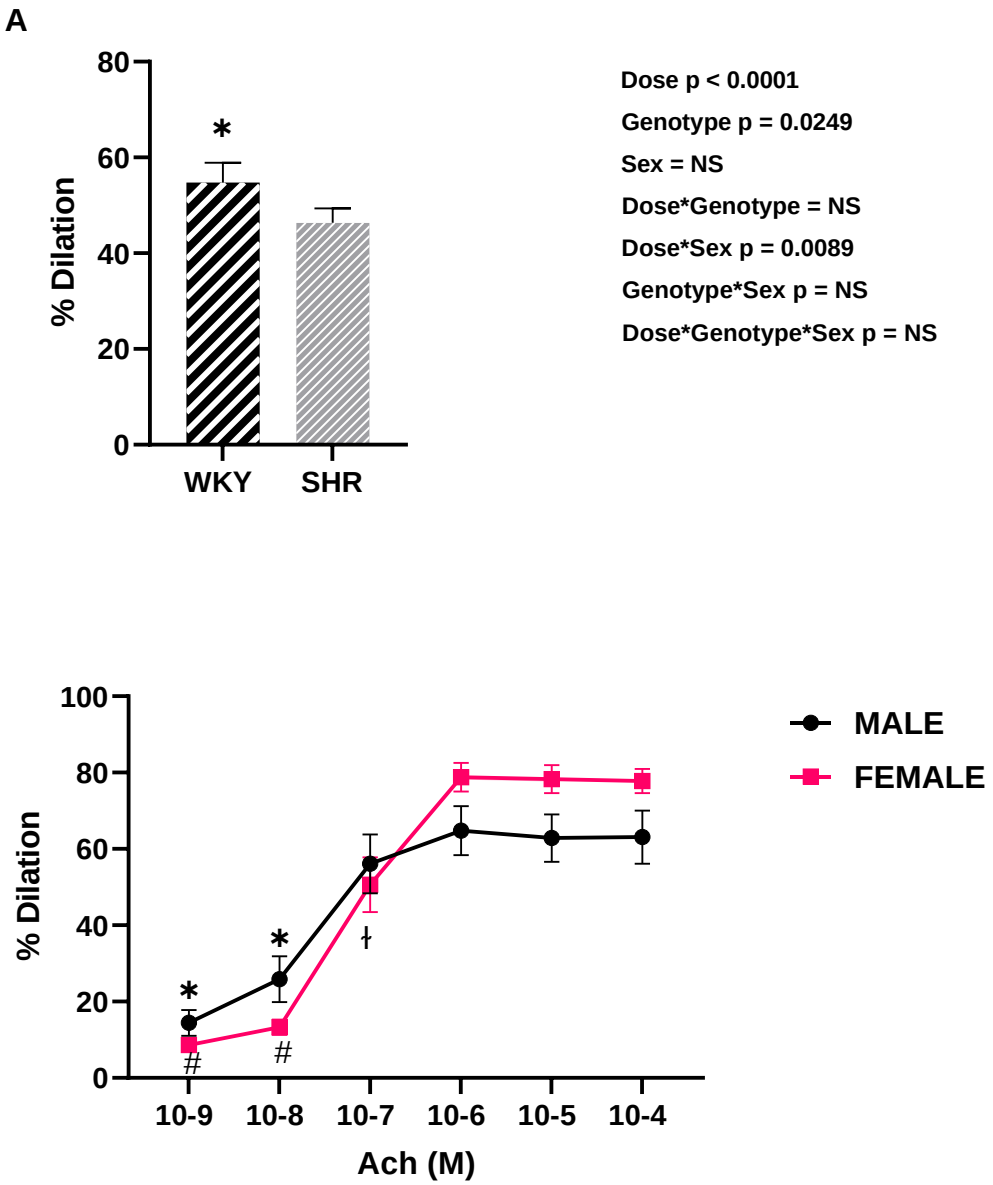

Figure 9. Vasorelaxation induced by Ach on mesenteric resistance arteries isolated from male and female SHR and their controls. (A) Effect of genotype on % dilation. n=70 WKY; n =105 SHR. * Indicates significantly different ($p<0.05$). (B) Effect of dose*sex interaction on % dilation. * Indicates within males significantly different from means without symbol; #, ł indicates within females significantly different from means without matching symbol. Data expressed as mean ± SEM. $p<0.05$. n=13-15 per dose (Male); n=14-15 per dose (Female). Ach- acetylcholine; SHR- spontaneously hypertensive rats, WKY- Wistar-Kyoto rats, NS – not significant.

4.11 Functional response in hypertensive mesenteric resistance arteries – SNP

Figure 10 shows endothelium-independent vasodilation induced by cumulative doses of SNP on pre-constricted third-order mesenteric arteries isolated from 16-week-old male and female SHR rats and their controls. SNP induced relaxation in a dose-dependant manner (Figure 10 A). SNP at 10^{-9} M showed significantly lower vasodilation response compared to 10^{-5} M and 10^{-4} M ($p< 0.05$) (Figure 10 A). Maximal dilation response of 36.44 ± 4.68 % was observed at 10^{-4} M SNP but was not significantly different from the 10^{-5} M dose. In addition, WKY rats showed significantly higher % dilation compared to SHR rats (51.31 ± 4.46 % vs 18.11 ± 1.53 %) ($p<0.05$; Figure 10 B). Vasodilation in response to SNP was not significantly different in females and males (33.25 ± 3.26 % vs 22.83 ± 2.47 %) (Figure 10 C).

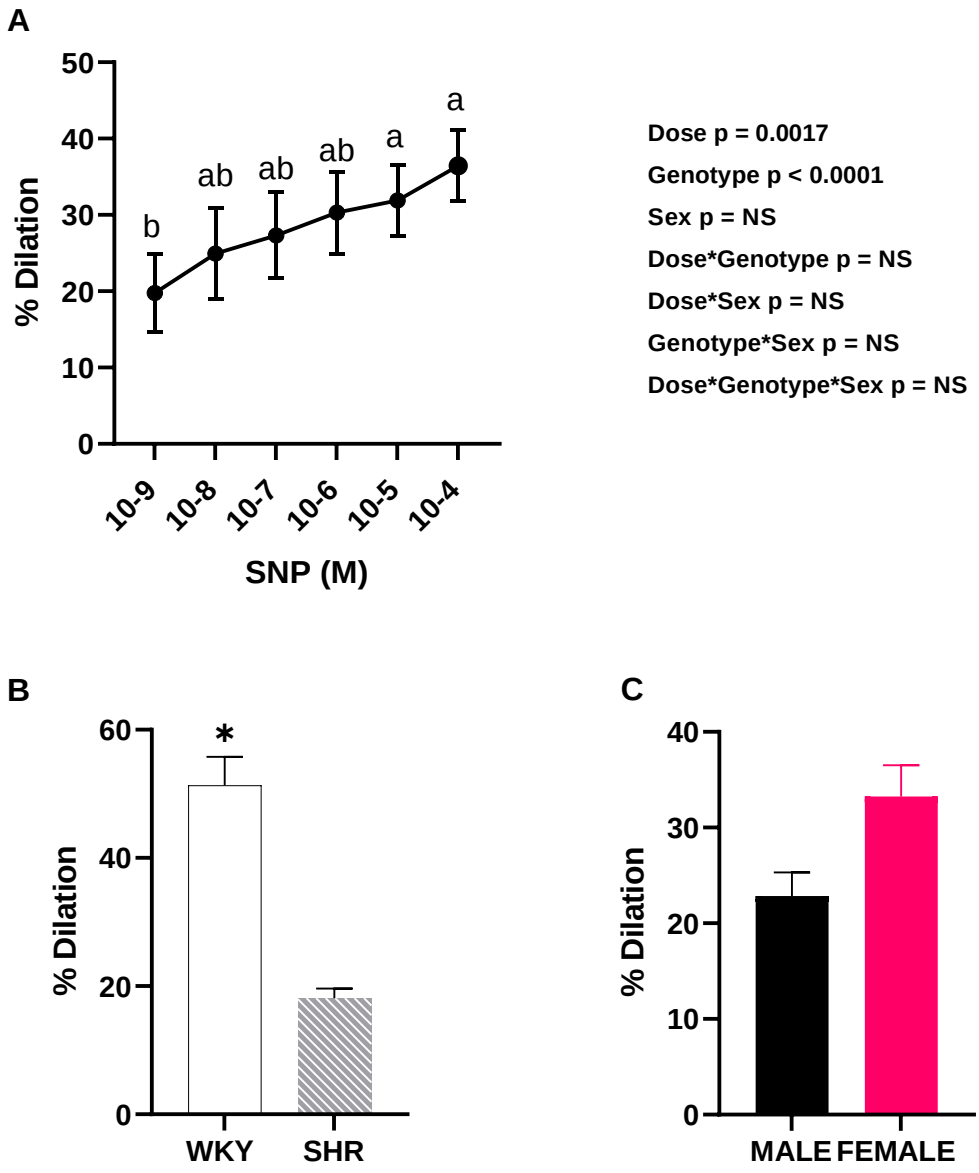

Figure 10. Vasorelaxation induced by SNP on mesenteric resistance arteries isolated from male and female SHR rats and their controls. (A) Effect of dose on % dilation. n = 24 – 26 per dose. Different letters are significantly different ($p<0.05$). (B) Effect of genotype on % dilation. n = 48WKY; n=104 SHR. * Indicates significantly different ($p<0.05$). (C) Effect of sex on % dilation. n=68 (Male), n=84 (Female). Data expressed as mean ± SEM. SNP- sodium nitroprusside; SHR- spontaneously hypertensive rats, WKY- Wistar-Kyoto rats, NS – not significant.

CHAPTER V
DISCUSSION

Discussion

Previous studies have indicated that hypertension was attenuated through HPI diet in SHR.[152] Presently, there is one ongoing double blind, randomized clinical trial investigating the effect of HPI consumption in human hypertensive subjects.[151] Since HPI may play a role in the hypertension management, we aimed to determine if HPI exerts direct effect on vascular function. The main finding of our study is that HPI dilates pre-constricted mesenteric vessels isolated from both normotensive and hypertensive animals. We also observed sex differences in HPI induced dilation response. To my knowledge, this is the first study to report vasodilatory effect of HPI.

Vascular effect of HPI on SD rats

First, we explored the effects of HPI on mesenteric resistance arteries isolated from male and female SD rats. In our study we demonstrated that HPI dilated pre-constricted mesenteric resistance arteries isolated from male and female SD rats. In addition, our results showed significant sex differences in HPI induced vasodilation in SD rats, with male SD rats showing significantly lower % dilation compared to female SD rats. Male and female SD rats showed a maximum dilation of 17.75 ± 3.22 % and 32.36 ± 7.05%, respectively. Research shows female SD rats has higher endothelial NO synthase (eNOS) protein expression compared to males.[167] In addition, the vasodilatory pathway of the RAS system is enhanced in female SD rats due to greater expression of receptor AT_2R which has vasodilatory action.[168] Sexual dimorphism is observed in endothelial cell morphology, receptor and protein expression in male and female SD rats.[169] These might be the plausible reasons for the greater % dilation shown in response to HPI by female SD rats compared to male SD.

Improved endothelial function in male and female arteries due to food proteins have been reported in several studies. Previous studies explored the vasodilatory effects of egg white hydrolysates at doses 0.1 μM to 0.1 mM on thoracic aortic rings isolated from 13-week-old male SD rats.[170] These peptides produced a maximal % dilation of approximately 40.5% at 0.1 mM concentration.[170] Whey protein consumption improved flow-mediated dilation in mild hypertensive men and women,[171] and consumption of soy protein isolate in postmenopausal women (aged 47-70) showed improved endothelial function or flow-mediated dilation due to the antioxidant effect of phytoestrogens.[172] Hemp seeds do not contain phytoestrogens but rather vasodilatory activity may be due to its high arginine content or presence of other bioactive vasorelaxant components. Sex

differences in terms of HPI response is a novel discovery and this is the first study to report it. Male and female arteries showed a dose dependant increase in vasodilation in response to HPI. Although we observed sex differences via HPI-induced dilation in SD rats, we did not observe any sex differences in Ach and SNP induced vasodilation. We can speculate that there might be sex-dependant variability in vascular response to bioactive compounds present in HPI. For example, gastro intestinal breakdown of certain bioactive peptides such as lacto-ghrestatin were found to be degraded in different regions in men and women.[173] Our present study showed vasodilatory response of HPI, but the mechanism is unknown. Also, further research is required to understand the mechanism behind the sex differences observed in SD rats in response to HPI.

Vascular effect of Ach and SNP on SD rats

Furchgott and Zawadzki (1980) first reported the imperative role of endothelial cells in arterial dilation by Ach via muscarinic receptors.[174] Later, it was found that Ach is an agonist that triggers endothelium-dependant vessel dilation by stimulating release of NO and other vasodilators, whereas SNP is a NO donor that causes endothelium – independent vasodilation.[175] Ach is one of the widely used agonists used to study endothelium-dependant dilation. Whereas SNP is a prodrug that releases NO, which directly acts on VSMC triggering a biochemical cascade that results in relaxation of the vessel.[176,177] This donated NO activates soluble guanylate cyclase which subsequently increases cyclic GMP levels to induce vascular smooth muscle relaxation.[178] In our present study, male and female SD rats vessels showed concentration - dependant dilation varying between 10.56 ± 1.64 % to 83.81 ± 2.32 % in response to Ach (10^{-9} M to 10^{-4} M). Ach induced vasodilatory response observed in SD rats in our present study is similar to previous studies.[179,180] SNP induced dilation varied between 10.89 ± 1.57 % and 30.73 ± 3.85 % for the same concentrations. Vasodilatory response to Ach and SNP indicates that our vessel endothelium and smooth muscle cells are intact and were not damaged during the experiment. Furthermore, the absence of sex differences in response to Ach and SNP suggest that HPI related sex differences are not due to changes in vascular reactivity to NO but probably due to bioactive properties of HPI alone.

Vascular effect of HPI on SHR rats and their controls

SHR

SHR is the most widely used genetic animal model for essential hypertension research. This strain was first developed by Okamoto and Aoki in 1963 by mating a male hypertensive Wistar strain rat (SBP 150-175 mm Hg) with a female Wistar rat that had slightly above average BP (SBP 130-140 mm Hg).[181] They successfully created SHR animal model that fully developed hypertension within fifteen weeks of age.[181] Unlike SHR rats which undergoes BP changes with age, WKY rats show stable BP throughout their life-span and thus is the most commonly used control strain for SHR.[181,182] Also, SHR shows greater total peripheral resistance and structural changes compared to age-matched WKY controls.[183] In particular, SHR mesenteric resistance vessels show impaired endothelium-dependant relaxation.[184] In addition, the SHR animal model shows characteristics similar to those found in human essential hypertension such as genetic etiology, increase in BP with age, cardiovascular complications (left ventricular hypertrophy, congestive heart failure etc.) arising due to hypertension.[185]

As expected, in our present study, BP was significantly higher in the SHR group compared to their age-matched normotensive controls, WKY. Also, male animals showed significantly higher SBP, and body weight compared to female groups. In humans as well, males show higher BP compared to age matched women irrespective of race or ethnicity. Sex differences in BP is attributed to higher circulating Ang 1-7 receptors in females that help to reduce BP.[186,187]

Effect of HPI in SHR

Vasorelaxation activity by HPI in the isolated mesenteric artery of SD rats prompted our investigation on hypertensive arteries isolated from SHR. This is the first study to report the vasodilatory effects of HPI on hypertensive arteries. We observed a dose-dependant increase in vasodilation in both SHR and WKY animals. HPI-induced dilation on pre-constricted mesenteric resistance arteries was lower in SHR compared to male WKY rats. Also, WKY rats showed significantly higher endothelium-dependant (Ach) and -independent dilation (SNP) compared to SHR. This indicates that vasodilatory capacity of SHR rats is attenuated. Overall, HPI induced dilation in a concentration-dependant manner, with male WKY rats showing the highest % dilation response compared to all other animal groups. However, further studies are required to identify mechanism through which HPI dilates arteries.

Impaired vasodilatory response to Ach can be considered as an indicator for endothelial dysfunction. Essential hypertensive patients show impaired vasodilation to Ach compared to normotensive subjects.[188] Peripheral vasculature undergoes changes during hypertension, thus Ach-induced dilation or endothelium dependant dilation is impaired in SHR rats.[189] Previous studies have also reported that Ach-induced dilation is significantly higher in mesenteric resistance arteries isolated from WKY compared to SHR.[190] Similarly, Ach induced vasorelaxation is reportedly higher in WKY compared to other hypertensive rat strains such as stroke-prone SHR due to endothelial dysfunction in the latter.[191]

There is a genetic component to impaired vasorelaxation as well. Taddei et al found that normotensive offspring of hypertensive subjects showed impaired dose-dependant vasodilation to Ach in comparison to offspring from control because of defective L-arginine-NO signaling.[192] In contrast, vasodilation to SNP produced similar results between offspring of hypertensive patients and normotensive subjects.[192]

In our present study, mesenteric vessels isolated from WKY rats showed significantly higher Ach induced dilation compared to SHR animals. Thus, indicating impaired endothelium – dependant vascular function in hypertensive vessels. During cardiovascular diseases, endothelium-independent vasodilation is impaired due to structural and functional alterations in arteries and VSMC.[193] Similarly, in our present study, endothelium-independent dilation induced by SNP was significantly impaired in SHR. Thus, we can speculate that attenuated vasodilatory response to HPI in SHR rats is probably due to impaired endothelium or VSMC of the hypertensive vessels.

Sex difference:

Male and female SHR showed minimal dilation of 7.25% and 9.30% respectively at lowest HPI dose of 0.1μg/ml. There was no significant HPI induced % dilation difference between male and female SHR. Although SHR animals showed vasodilatory response, it was significantly lower than male WKY. Also, there was no significant difference between male and female % dilation response to SNP. However, in response to Ach, there was a statistically significant interaction between sex and dose, therefore male and female animals responded significantly different to Ach doses. Previous research has shown that endothelium-dependant and independent dilation is greater in females compared to males.[194,195] However, some studies indicated that % relaxation to Ach in third order mesenteric arteries was similar or not significantly different between groups i.e male

SHR, female SHR, male WKY, and female WKY at 12-13 weeks of age.[157] Research has shown sexual dimorphism in NO production, with females or pre-menopausal women exhibiting greater NO biosynthesis compared with men.[196] Sex difference is also attributed to difference in receptors. Female WKY and SHR show greater expression of vasodilatory receptors (β_1 and β_3).[197] Ang II mediates vasoconstriction through activation of Type 1 receptor and male SHR shows greater Ang II type 1 receptor (AT_1) mRNA levels in kidney, aorta and mesenteric vessels.[158] In our present study, sex difference induced by HPI in WKY group might be due to changes in vascular reactivity to HPI.

NO production mechanisms in vasculature:

L-arginine is the precursor or substrate, metabolised with the help of NOS to produce NO in the vascular endothelium.[198] All isomers of enzyme NO synthase (neuronal NOS, inducible NOS, eNOS) hydroxylates and oxidizes L-arginine to generate NO.[199] The human body obtains arginine either through diet or endogenously through cellular protein turnover and de-novo synthesis of arginine precursor compounds.[200] In our present study, HPI contains 14 % arginine, which can bind with eNOS, produce NO and may partly contribute to the vasorelaxation of the pre-constricted arteries.

Vasorelaxation generally occurs with the help of endothelium derived relaxing factors such as NO, PGI_2 and EDHF through different biochemical pathways. Vascular endothelial cells release these vasoactive substances in response to stimulus such as hormones (circulating and local), shear stress, platelet products or any vasoactive agonists.[201] However, several experimental models of hypertension demonstrated that NOS dependant pathway via cGMP becomes primary vasorelaxant pathway in hypertensive small mesenteric arteries.[202]

Plausible mechanisms through which HPI causes vasorelaxation:

<u>Vasorelaxant amino acids</u>

Apart from arginine, HPI also contains other amino acids with vasorelaxation property. L-serine promoted dose-dependent vasodilation in mesenteric resistance arteries with intact endothelium through activation of calcium-activated potassium channel (K_{Ca}). [203,204] HPI contains 5% serine which might contribute to vasorelaxation observed in our study. Apart from serine, HPI also contains cysteine which can inhibit ROS production and protect endothelial cells from oxidative

stress related injury.[205,206] Cysteine is also considered a precursor to hydrogen sulfide (H_2S), which is an EDHF with vasodilatory property.[207] Production of H_2S is predominantly catalysed by enzymes cystathionine-β-synthase and cystathionine-γ-lyase (CSE), expressed in VSMC and endothelial cells.[208–210] Vasorelaxation is significantly impaired in mutated murine mesenteric resistance arteries lacking these enzymes.[211] Although cysteine levels (1%) present in HPI are quite lower in comparison to serine and arginine, it might also contribute to vasodilation observed.

Other pathways

Other pathway might include vasodilation induced by the omega-3 PUFA present in HPI. In the present study, fat component accounts for 16% of total HPI. PUFA are known to improve vascular function either by increasing NO availability through eNOS activation or direct vasorelaxation by generating epoxides.[212] Epoxides activates K_{Ca} channel which results in hyperpolarisation and relaxation of vessels.[212]

Also, ground hemp seed diet improved functioning of the impaired potassium channels (K_{ATP} and BK_{Ca}) in isolated thoracic aorta of male obese Zucker rats.[88] These potassium channels expressed in VSMC and endothelial cells help in endothelial function by facilitating release of vasoactive substances.[213] In addition, these channels might help in BP regulation under hypertensive conditions.[213] The study showed hemp seed diet improved vasodilatory response but not with hemp seed oil diet. Perhaps we can hypothesize that HPI improves functioning of potassium channels, and this might also be another plausible mechanism behind vasodilation shown by HPI.

Future directions

Our current study showed HPI dilated mesenteric vasculature containing viable endothelium. In future experiments, HPI should be tested on endothelium-removed vasculature to characterize the mechanism behind vasodilation. If HPI dilates endothelium denuded vessel, it indicates vasorelaxation mechanism involves both endothelium-dependent and independent pathways. Also, studies should identify the receptors and relaxing factors involved (e.g., NO, prostacyclin or EDHF). HPI should be tested on vessels treated with NO inhibitor such as L-NAME to see if dilation is mediated with NO or with the help of other endothelium derived relaxing mediators. If vessel dilates in the presence of L-NAME, this might indicate HPI relaxes vessels through other vasodilatory mediators such as prostaglandins or EDHF. To identify, if vasorelaxation is mediated

via prostaglandins, COX inhibitor – indomethacin should be used. If vasorelaxation is blocked by indothemacin, this indicates HPI relaxation is mediated via prostaglandins. In addition, future studies should identify the exact bioactive components present in HPI responsible for the dilatory property.

Conclusion

The exact mechanisms responsible for vasodilation induced by HPI remains to be determined. The high content of arginine in HPI or perhaps other bioactive compounds (PUFA, vasorelaxant amino acids) may be responsible for vasorelaxation. However, further tests are required to identify exact components causing vasodilation. HPI has several cardiovascular health benefits such as ACE and renin inhibition, and vasodilatory properties. From this present study, we can conclude that HPI apart from their nutritional role can modulate vascular function.

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