THE EFFECT OF ACUTE PHOSPHATE INTAKE ON MUSCLE METABOREFLEX ACTIVATION AND VASCULAR FUNCTION

by

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ABSTRACT

Emerging evidence suggests that long term high consumption of inorganic phosphate (Pi) is associated with an increased risk of cardiovascular disease. Importantly, with the growing abundance of processed foods, dietary intake of Pi in the United States has doubled the daily-recommended allowance. Recently, Mizuno *et al*. demonstrated that chronic high dietary Pi consumption results in significantly exaggerated increases in mean arterial pressure (MAP) in response to exercise and muscle metaboreflex activation in rodents. Whether acute high Pi influences MAP responses in humans remains unknown. Notably, acute high Pi consumption has been shown to impair vascular function in healthy men, but a recent study does not support this finding. Therefore, the goal of this research was to investigate whether acute high Pi consumption affects exercise and muscle metaboreflex activation, and whether vascular function is also affected. To investigate, subjects performed static handgrip (HG) at 35% maximal voluntary contraction, followed by post-exercise ischemia (PEI) to isolate the muscle metaboreflex. Vascular function was assessed via brachial artery flow-mediated dilation (FMD), and arterial stiffness was assessed using pulse wave velocity (PWV). Pi group (N= 13; 23 ± 1 yrs; mean ± SEM) ingested a monosodium phosphate drink containing 2,000 mg of phosphorus and 1,520 mg of sodium, and a control group (N=5; 23 ± 2 yrs) ingested a drink containing 1,520 mg

of sodium, to match the sodium amount in the Pi drink. After 60 minutes, measures were repeated and blood was drawn at 60 and 120 min post. Serum phosphate was significantly elevated at 60 min (baseline, 3.2 ± 0.2 mg/dL; 60 min, 4.5 ± 0.2 mg/dL; $p<0.01$) and 120 min (4.9 ± 0.3 mg/dL; $p<0.01$) post- Pi, but did not change with control ($p=0.51$). Metaboreflex-induced increases in MAP were not different following Pi ($P=0.192$) or Control ($P=0.472$). In contrast, macrovascular function only was significantly attenuated after Pi drink, (pre, 5.1± 0.5%; post, 3.2 ± 0.5%; $p<0.01$), with no changes with Control drink. For microvascular function, there were no differences pre vs. post with the Pi *(P* =0.721) or Control drink (*P*=0.302) and there were no differences in arterial stiffness following Pi drink ($P=0.163$) or Control drink ($P=0.394$). In summary, acute high Pi consumption did not affect the pressor response during exercise or muscle metaboreflex activation, or arterial stiffness, whereas macrovascular function was impaired. These data suggest a preserved pressor response, but peripheral macrovasculature is vulnerable to acute high Pi in young healthy men.

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Review of the Literature

Introduction

Phosphate (Pi) is an essential micronutrient required for critical biological reactions in all living organisms, and is present in most foods as both organic and inorganic forms [1]. Specifically, inorganic phosphate is a common additive used in a variety of processed foods, and its presence is immense in the American diet, with the growing consumption of foods containing phosphate additives [2,3]. Importantly, chronic high consumption of Pi is becoming a public health concern, as excess Pi intake is associated with an increased risk of cardiovascular disease (CVD), even in the general population [4]. CVD is the number one cause of death in the United States, with over 630,000 deaths annually. Despite advancing medical treatments, alternative treatment and prevention methods to reduce CVD risk factors include dietary interventions, such as avoiding foods containing Pi additives [5].

It has been reported in animal models that chronic Pi consumption increases blood pressure at rest [6], and during exercise [7]; however the exact mechanisms of Pi-induced hypertension remains unknown, and there have been no studies which have extended these findings from rodents to humans. Additionally, endothelial dysfunction has recently been studied as a possible contributor to the pathogenesis of CVD, however the effect of Pi on the vasculature in healthy adults have been equivocal [8-10]. Therefore, the purpose of this study was to determine the influence of acute high Pi consumption on the pressor response during isometric handgrip exercise and isolated muscle metaboreflex activation during post exercise ischemia, in young, healthy humans. Additionally, we determined whether macrovascular function, assessed by brachial artery flow-mediated dilation (FMD) and microvascular function, assessed by the reactive hyperemia response, would

be attenuated following acute high Pi consumption. Lastly, as another measure of central and systemic vascular function, we determined whether acute high Pi would affect arterial stiffness in young, healthy individuals.

Part I. Phosphate and Cardiovascular Disease

Phosphate

Phosphate (Pi) is an essential micronutrient required for critical biological reactions of the human body, such as cellular metabolism, energy storage, and formation of cell membranes [1]. During absorption, Phosphorus is bound with oxygen, where it exists as phosphate, and is strictly regulated through the intestine-bone-renal axis. Additionally, Pi makes up 0.7 to 1.1 % of the adult body; where 85% is complexed with calcium as hydroxyapatite in the bone, and the remaining 15% is distributed through fluids and soft tissue [11].

Phosphate Bioavailability

Because Pi exists in all living organisms, it is found in most foods as both organic and inorganic forms [12]. Organic Pi is found naturally in almost all fruits and vegetables, however, total Pi content values from these sources tend to be relatively low. Whole grains, nuts, and legumes are higher in Pi, but since the human digestive system lack the enzyme that releases Pi from phytic acid found in plants (phytase), the Pi bioavailability is low, as only 20-40% of Pi is absorbed in the body [12]. On the contrary, inorganic Pi such as food additives, are commonly used as preservatives, flavor enhancers, color stabilizers, and emulsifiers, and are presented in the most readily absorbable state, with up to a 90% absorption rate. Importantly, for practical purposes, it can be assumed that

diets high in inorganic Pi only, will be the focus for this topic, since the Pi bioavailability found in naturally occurring foods is relatively low.

Sources of Inorganic Phosphate Additives

Due to the wide diversity of uses, Pi additives in the food industry are immense, with more than 100 million pounds of Pi additives used annually in the United States alone [13]. Pi additives, including those combined with sodium, are commonly used in several milk and dairy products, processed meats, seafood, and beverages. Dark colas and sodas in particular are the beverages that contain the highest amounts of Pi additives (in the form of phosphoric acid), whereas the meat and baking industries produce the highest Pi containing foods. Like sodium, Pi has become nearly indispensable in food manufacturing, substantially enhancing the Pi content of processed foods [13]. Importantly, since Pi food additives are regarded as generally safe for consumption, there are little to no restrictions by the food industry, which can present a major public health barrier for individuals trying to reduce excess Pi intake [12].

Recommended vs. Actual Estimated Intake of Phosphate

The daily recommended allowance for Pi is 700 mg/day for adults [11]. Importantly, over the last 20 years, with the growing abundance of processed foods, dietary intake of Pi in the United States has doubled the daily-recommended allowance, with a mean daily intake of 1495 mg (fifth percentile = 874 mg; fiftieth percentile = 1,445 mg; ninety-fifth percentile = 2,282 mg) [11]. The prevalence of these additives will continue to rise as food manufacturers find ways to improve convenience and increase

shelf life through the addition of Pi ingredients [2]. Moreover, it difficult to measure Pi, as quantified values are not listed on food labels, or clearly defined in national nutrition databases [14]. In this regard, the daily Pi consumption of an individual can be underestimated by more than 20% [12].

Cardiovascular Disease

Cardiovascular disease (CVD) is a general term for a classification of chronic diseases and conditions that involve the heart and blood vessels. CVD is the number one cause of death in the industrialized world, with over 630,000 deaths every year in the United States alone [15]. According to the National Center for Health Statistics of the Centers for Disease Control and Prevention, over 28.4 million adults in the United States have been diagnosed with CVD [15], which is roughly 11.7% of the population. The most common type of CVD is coronary heart disease (CHD), a disease which is characterized by hardened and narrow coronary arteries due to the build-up of plaque on the arterial walls (also called atherosclerosis). Other CVD include myocardial ischemia, peripheral vascular disease, heart failure, and stroke, and total more than $200 billion in treatment costs annually. Importantly, as the number one cause of death, it is a serious public health concern that requires several prevention interventions at both individual and community levels. According to the American Heart Association, major risk factors include both genetic and environmental factors; however modifiable lifestyle factors, such as poor diet, lack of exercise, smoking, obesity, and excessive alcohol use greatly increase this risk, as half of the United States population has at least one of these risk factors [15, 16]. Poor dietary lifestyle choices are one of the most influential risk factors for CVD, as the Western

diet is composed of mostly foods with high sugar, sodium, saturated fat, and cholesterol content, which all contribute to high blood pressure and cholesterol levels; both key factors that lead to CVD. It is important to educate those at risk, as CVD risk can be significantly reduced with modified lifestyle changes.

Phosphate and Cardiovascular Disease

Emerging evidence suggests that long term high consumption of Pi is associated with an increased risk of CVD and all-cause mortality [13, 17]. Moreover, even relatively small elevations in serum phosphate in the high normal range (2.5–4.5 mg/dL) have been correlated with endothelial dysfunction [8, 9], a condition that precedes atherosclerosis [18]. Dhingra *et al. [19]* evaluated 3,368 participants from the Framingham Offspring Study who were free of clinical CVD and had normal kidney function. After a mean duration of follow-up of 16 years, it was reported that serum phosphate levels greater than 3.5 mg/dL were associated with an estimated 55% greater risk of incident CVD compared to levels less or equal than 2.8 mg/dL. Similar large-scale studies have confirmed these findings [17, 19-21], however there are very limited clinical studies that have reported the direct influence of Pi intake and CVD [8, 9].

Part II. Blood Pressure Responses to Exercise and Phosphate

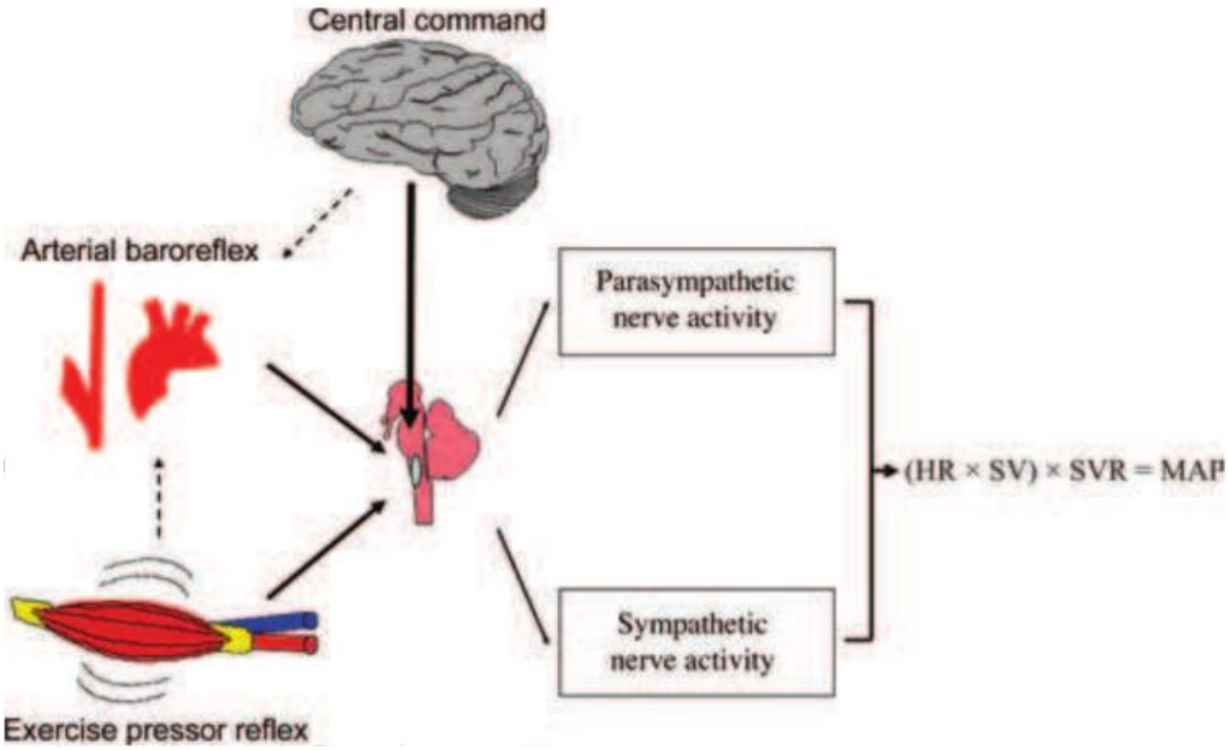
Blood Pressure Responses to Exercise

It is well known that cardiovascular responses to exercise is rapidly matched to its intensity or its perceived effort [22-24]. During exercise, working skeletal muscle require an increase in oxygen, therefore an increase in blood flow is required to meet this demand. Higher blood flow is attained through changes in cardiac output and vascular resistance, which directly influences an increase in blood pressure [25]. Mean arterial pressure (MAP), heart rate (HR), and respiration rate all increase according to exercise demand, and continue until blood flow perfusion to the working muscle is matched to its metabolic needs [28]. The sensory and effector neural mechanisms that precisely regulate these cardiovascular and hemodynamic changes during exercise are central command, the exercise pressor reflex, and the baroreflex [24, 26-28].

Autonomic Nervous System

The autonomic nervous system (ANS) is a system consisting of central (CNS) and peripheral neural control that contain two major components; the sympathetic and parasympathetic. Anatomically, the ANS includes the hypothalamus, the brainstem and the spinal cord, with nerves emerging from the CNS, and branching out to every part of the body [29]. Afferent neurons (sensory neurons) provide feedback and efferent (motor neurons) carry out actions from the CNS (Figure 1), which enables the ANS to regulate and coordinate several bodily functions such as MAP, HR, respiratory rate, arousal, and digestion [29].

The sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) work together antagonistically to modulate vital functions in the body. The SNS is associated with arousal and energy generation, and plays an important role in the regulation of cardiovascular function during rest and exercise [30, 31]. Importantly, the SNS innervates the vasculature, controlling peripheral vasoconstriction, and regulating blood pressure. Additionally, it also directly innervates the heart and elevates HR, contractility and MAP, thus decreasing parasympathetic activity [27]. The PNS is associated with rest and digestion, and innervates the pacemaker cells in the sinoatrial node of the heart, which reduces HR and predominates in the resting state. [29]. Cardiovascular adjustments during exercise, mediated by both the SNS and the PNS, have been studied extensively in both human and animal models [23,33-36]. In addition, control of the ANS is also dependent on other regulatory neural mechanisms including central command, the exercise pressor reflex, and the arterial baroreflex.



Williamson, 2006

Figure 1. A diagram sourced from [24] showing how neural signals from central command in the brain, the exercise pressor reflex, and the baroreceptors, all modulate sympathetic and parasympathetic activity during exercise. Changes in heart rate, stroke volume, and vascular resistance influence mean arterial pressure, which is matched appropriately according to exercise demand.

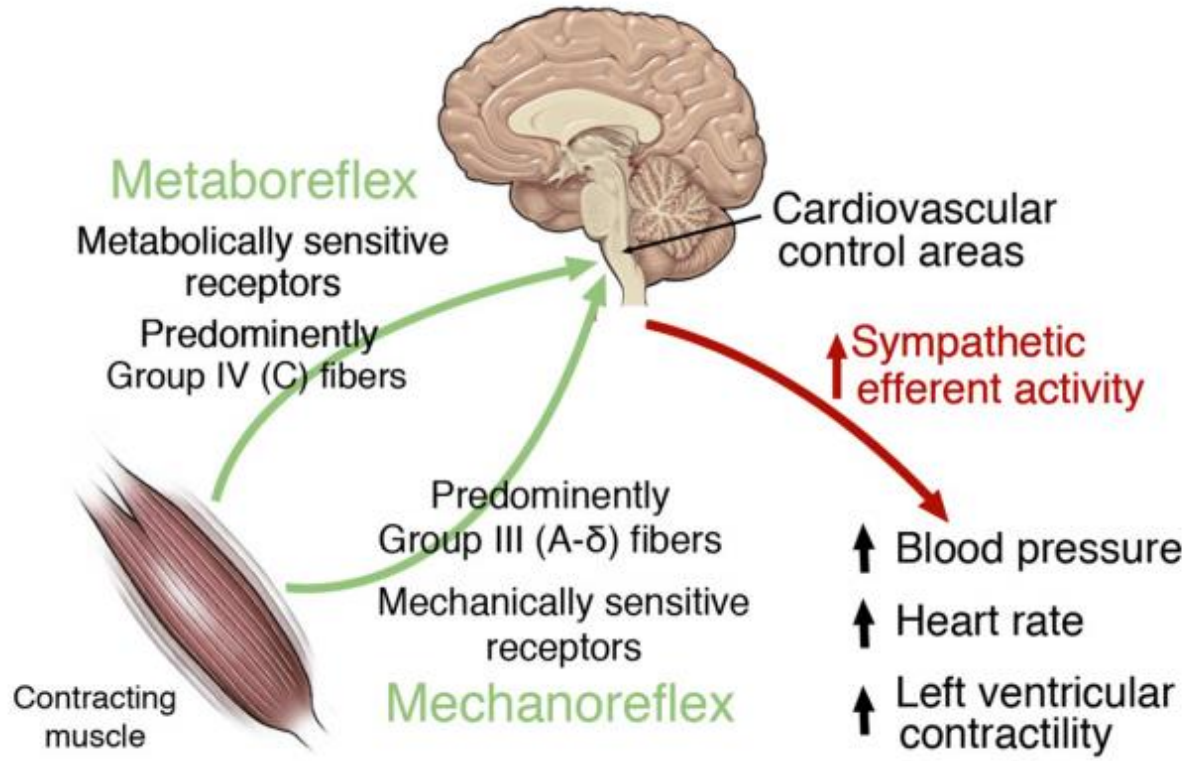
Central Command

Central command is a feed-forward mechanism that activates cardiovascular centers in the brain, and plays an important role for regulating both sympathetic and parasympathetic activity both at the onset, and during exercise [24, 32]. Since central command is a feed-forward component, it can independently act upon the cardiovascular

system before peripheral feedback can be reached to the higher brain centers. However, central command can also receive information from peripheral afferent nerves, and modify this feedback by sending signals through efferent nerves to further adjust cardiovascular changes [24].

Exercise Pressor Reflex

The exercise pressor reflex (EPR) is a peripheral control mechanism that provides sensory feedback to the brain [23, 33, 35, 37]. At the onset of exercise, receptors located in the skeletal muscles activate thinly myelinated afferent nerve fibers (group III fibers) that are predominately activated by mechanical distortion (pressure or stretch) in the contracting muscle, also known as the muscle mechanoreflex. Additionally, unmyelinated afferent nerve fibers, (group IV fibers) predominantly activated by the build-up of metabolic byproducts in the contracting muscle, are chemically stimulated and is known as the muscle metaboreflex [22, 32, 33]. Due to the nature of the muscle contraction, the mechanoreflex is activated immediately at the onset of exercise, and is reliant on the increase in HR, whereas the metaboreflex requires a longer activation time due to the time needed for the build-up of metabolites, and relates to peripheral vasoconstriction [32]. However, the stimulation of *both* the mechanoreflex and metaboreflex arising from the working skeletal muscle, evoke the EPR mediated cardiovascular responses (Figure 2) during exercise [26,34].



Mitchell, 2017.

Figure 2. A diagram sourced from [22], showing how the EPR is activated with both metabolic (muscle metaboreflex) and mechanical (muscle mechanoreflex) stimulation. These signals ascend to the cardiovascular control centers in the brain, which then raise sympathetic nerve activity, resulting in increases in blood pressure, heart rate, and contractility.

Arterial Baroreflex

The aortic arch and carotid sinus contain several pressure sensors called the baroreceptors. These sensory afferent nerve endings provide negative feedback in response to the stretching of arterial walls due to the changes in the blood pressure [28, 30, 35]. Baroreceptor sensors transduce the mechanical stretching of arterial walls into reflex signals through afferent nerves that ascend to the CNS. These signals then descend via both sympathetic and parasympathetic efferent pathways to the heart and

vasculature, which respond to acute moment-to-moment changes in blood pressure [30, 32]. Importantly, the baroreflexes act as modulators to constantly maintain beat-to-beat blood pressure and blood flow changes at rest, and resets during exercise [36].

Blood pressure responses to static handgrip exercise and post-exercise ischemia

Static handgrip exercise involves contracting of the skeletal muscle, without causing changes in muscle length, and is one of the best suited models for studying the influence of both central command and the EPR on blood pressure responses during exercise in humans [22]. In response to sustained static (isometric) exercise, there are large increases in MAP and HR, with no change in stroke volume [37]. Activation of the EPR enhances sympathetic activity and it was discovered by Alam & Smirk [26] that the pressor response to exercise can increase independently of central command; which has been further supported in animal models [7]. In humans, cardiovascular and autonomic responses (independent of central command and the muscle mechanoreflex) can be measured by isolating the muscle metaboreflex. Specifically, a handgrip dynamometer is squeezed at a percent of their maximal voluntary contraction and held constant for a two-minute duration. Then, a sphygmomanometer cuff is inflated suprasystolic on the upper arm seconds before the end of exercise. Since the cuff inflates rapidly, this traps the metabolites produced during muscle contraction (PEI; post-exercise ischemia), allowing for prolonged stimulation of the metaboreceptors and isolation of the metaboreflex. The associated cardiovascular responses are then measured post-cuff inflation [27, 38], and remains elevated for as long as the forearm is kept ischemic [38]. In healthy humans, the

metaboreflex increases in MAP remains above resting values, [26, 37], whereas HR returns down to resting values during PEI [39-41].

Phosphate and Blood Pressure

A growing number of animal studies have linked high Pi intake to hypertension, which is a well-known risk factor and precursor for CVD [6, 7]. Healthy rats fed a four-week high Pi diet has recently been shown to have both elevated systolic and diastolic blood pressure at rest. They also found that the addition of phosphate binders in the diet abolished resting elevations, suggesting a direct effect of dietary Pi on blood pressure [6]. In addition, only one study has tested the influence of dietary Pi on the CNS control of blood pressure regulation during exercise [7], and reported novel findings that chronic exposure to a high Pi diet in healthy animals induces sympathetic over-activation during EPR stimulation. They found that consuming a high Pi diet over a twelve-week period enhanced both cardiovascular and sympathetic activity in response to EPR activation during electrically-stimulated muscle contractions. Additionally, high dietary Pi also potentiated BP, HR, and renal SNA in response to passive muscle stretch (mechanoreflex) and capsaicin administration (metaboreflex) suggesting enhanced EPR function. Importantly, these findings contribute novel evidence that can further explain possible mechanisms by which high Pi consumption directly contributes to the pathogenesis of Pi-induced hypertension, both at rest [6], and during exercise [7].

Part III. Vascular Function and Phosphate

Endothelium

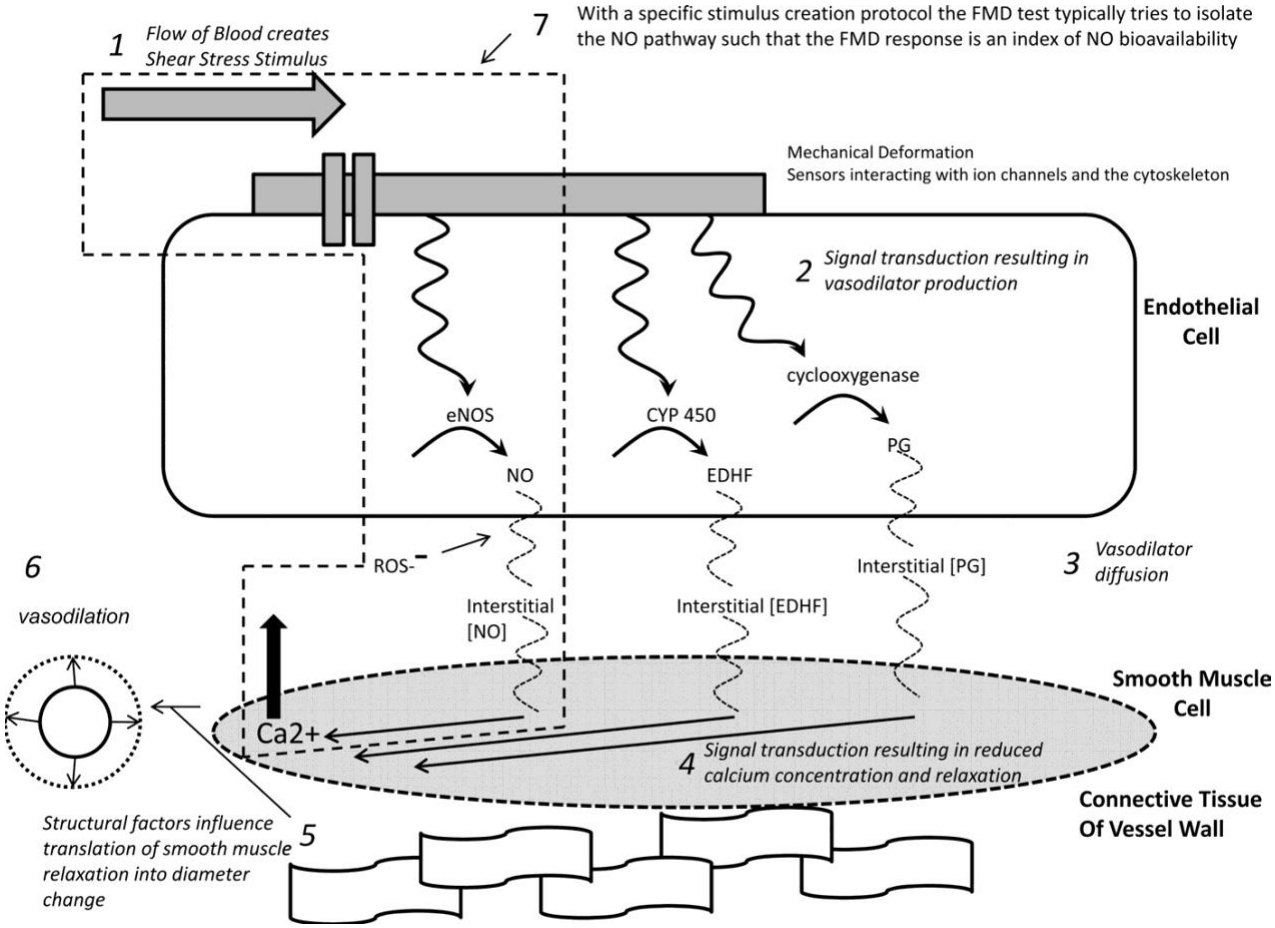
The endothelium is a single-layer of endothelial cells that lines the entire vascular system, acting as a dynamic barrier, and modulator of metabolic and regulatory pathways vital for maintaining cardiovascular homeostasis [42, 43]. By producing a vast array of vasoactive substances, the endothelium regulates the tone of underlying vascular smooth muscle, maintains a non-adhesive luminal surface, and controls inflammatory and immune responses in the vascular wall [42, 44].

Shear Stress and the Vasodilatory Response

Endothelial cells are sensitive to shear stress, which is the frictional force created by blood flow through an artery [43]. In response to these increases in shear stress, the endothelium produces several vasodilating substances. One specific and well-studied chemical compound is Nitric Oxide (NO), a powerful, vasodilating substance [45], which signals the relaxation of local vascular smooth muscle [43]. NO is synthesized from L-arginine by nitric oxide synthase, and is released when increases in blood flow (thus shear stress) via reactive hyperemia causes conduit artery vasodilation [46]. This increase in blood flow and wall shear stress and the vasodilatory response is known as flow-mediated dilation (FMD).

Flow-Mediated Dilation (FMD)

FMD is a noninvasive, widely used technique for assessing endothelial dependent function by measuring the degree of conduit artery vasodilation in response to hyperemic-mediated increases in shear stress, following a period of cuff occlusion [47]. Briefly, increases in arterial blood flow produces an increase in shear on the endothelium and subsequently stimulates the release of NO (Figure 3). To measure FMD, ultrasound assessment is the most common technique to measure endothelial function and the brachial artery is the most ideal location due to the size of the diameter and ease of access [48]. Following a period of resting baseline measures, a sphygmomanometer cuff, placed around the forearm and distal to the ultrasound probe, is rapidly inflated to suprasystolic pressure for 5 minutes [44, 47]. This causes tissue ischemia and dilation of downstream resistance vessels via auto-regulatory mechanisms. When the cuff is released, a sudden increase in blood flow (reactive hyperemia) through the brachial artery fills the dilated resistance vessels, which exerts shear stress on the endothelium. The resulting dilation, which peaks at 60-90 seconds after cuff release, is highly dependent on NO activity. FMD is expressed as the maximum percentage change in vessel diameter after cuff release relative to baseline vessel diameter, with a low percentage indicating poor endothelial function [48].



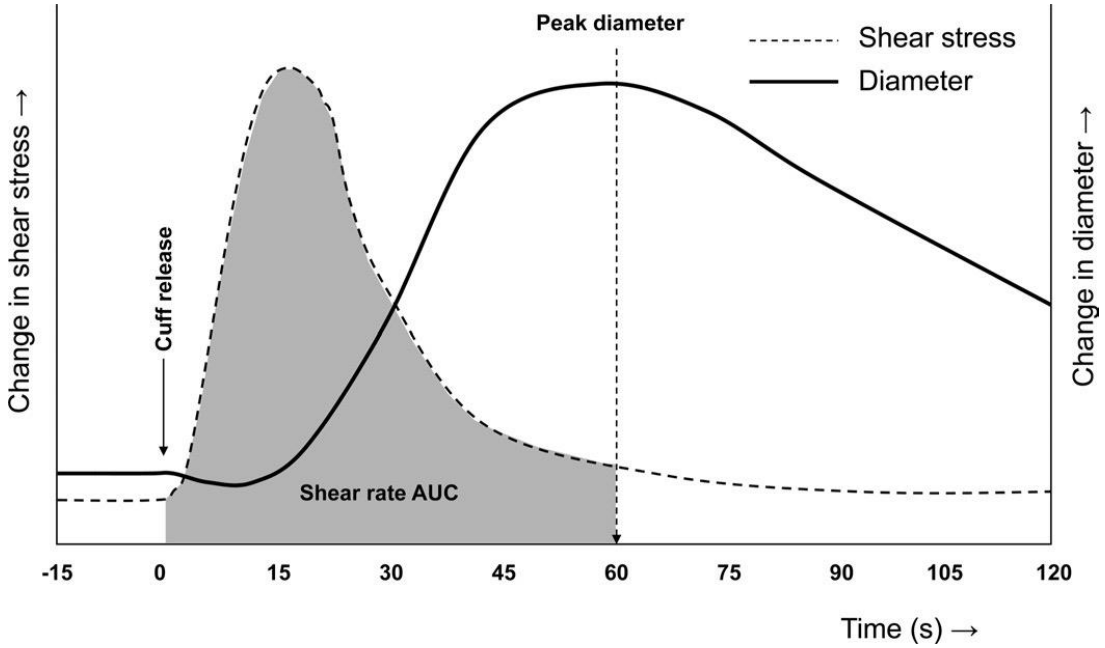
Thijssen 2010.

Figure 3. A diagram showing step-by-step how the increase in blood flow and shear stress on the endothelium transduces signals which results in the production and release of vasodilators. These signals result in reduced calcium concentration and relaxation of the local smooth muscle cells below, which results in smooth muscle relaxation and overall dilation of the vessel in response to NO bioavailability.

Macrovascular and Microvascular Function

FMD can be used to assess both macrovascular (conduit artery) and microvascular (resistance vessels) function (Figure 4). It is well known that the mechanisms of the FMD response are highly sensitive to the nature of the shear stress stimulus [46]. Microvascular function, or the shear stimulus is calculated as 8 x velocity/diameter, which includes only the area-under-the-curve from the moment of

occlusion cuff release, to the point in which hemodynamic values return to baseline values. Once the cuff is released after a 5- minute ischemic stimulus, there is an immediate vast increase in shear rate, which can last up to 45 seconds. As mentioned previously, this transient increase in shear rate stimulates NO release, which then allows for conduit artery dilation 30-60 seconds following cuff release. Macrovascular function is expressed as %FMD = (peak diameter–baseline diameter)/baseline diameter * 100 [47].



Thijssen, 2011.

Figure 4. A diagram showing a typical FMD response following cuff release. Microvascular function, which assessed by reactive hyperemia, and is the immediate increase in shear stress, which stimulates dilation in the conduit artery. Macrovascular function is the measure of peak diameter in response the shear stress stimulus.

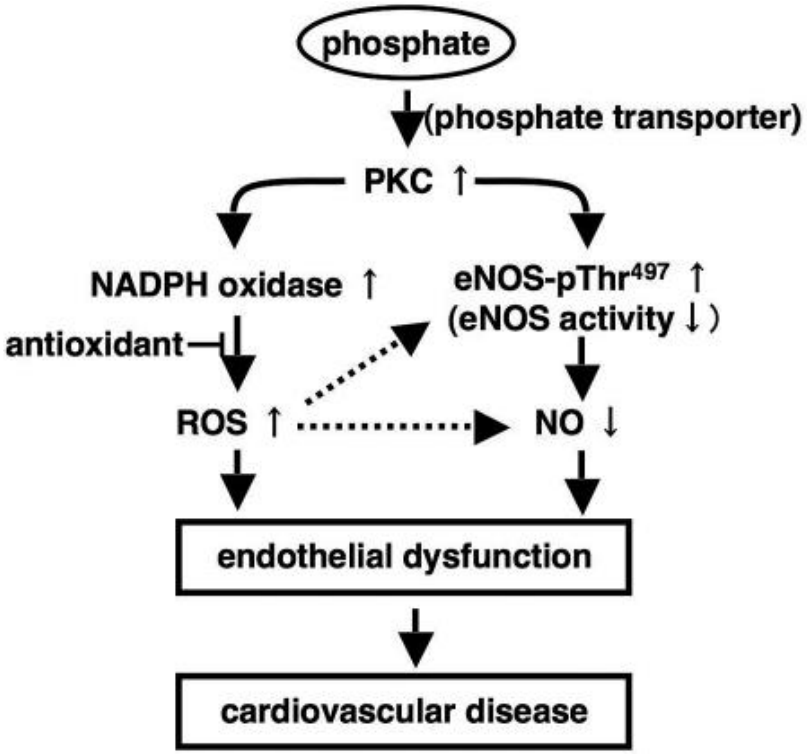
Arterial Stiffness

Arterial stiffness is defined as the thickening and stiffening of arterial walls, is highly associated with hypertension and the development of CVD [49]. The severity of arterial stiffness depends on structural elements within the arterial walls, such as elastin and collagen, which are imperative for allowing arterial distention during blood flow impedance [50]. A number of techniques can be used to assess arterial stiffness noninvasively, from the peripheral circulation. The most widely used “gold-standard” techniques are pulse wave velocity (PWV), and pulse wave analysis (PWA), which has been shown to be associated with microvascular endothelial function [49]. PWV measures the aortic (central) velocity in which the pressure waveform travels between two vessels, (carotid and femoral arteries) using an applanation tonometry [50]. PWA measures brachial artery pressure waveforms, by simply using a blood pressure cuff. Waveforms recorded from these peripheral sites are used to calculate central aortic waveforms and augmentation index using a validated generalized transfer function [50]. Augmentation index is a measure of peripheral arterial stiffness, representing the percentage of pulse pressure due to backward traveling waves within the central arteries [54].

Endothelial Function and Phosphate

Endothelial dysfunction is characterized by a reduction in the bioavailability of vasodilators, especially NO, leading to impaired endothelium-dependent vasodilation [49, 51]. Importantly, endothelial dysfunction is also a marker of atherosclerotic risk, which is a major preceding risk factor that contributes to CVD [49, 51].

Importantly, Shuto *et al*. [9] investigated whether Pi could directly be linked to an increased risk of CVD, and performed novel experiments to explain possible mechanisms associated with post-prandial increases in serum phosphate. In vitro, experiments using bovine aortic endothelial cells demonstrated that high Pi loading inhibited NO production through increased reactive oxygen species (ROS) production and endothelial nitric oxide synthase (eNOS) phosphorylation via protein kinase C (PKC), resulting in impaired endothelium-dependent vasodilation (Figure 5). In humans, it was demonstrated that an acute Pi intake of 1,200 mg of Pi resulted in significantly impaired endothelial function, measured by brachial FMD in healthy men; and also showing a negative relationship between FMD and serum phosphate levels [9]. The same research group also studied the time- and-dose dependent effects of Pi intake on endothelial function, which confirmed their previous findings that phosphate loading rapidly inhibits FMD in healthy subjects following 800 mg and 1,200 mg of Pi, just two hours post-Pi-consumption [8]. In contrast, a recent group repeated this study, but found no effect of high Pi ingestion on FMD [10], and did not find significant increases in serum phosphate. This reason for this discrepancy is unclear, but further are studies are needed identify the possible effect of Pi loading on endothelial function.



Shuto, 2009.

Figure 5. A diagram showing the possible pathway in which phosphate loading directly impairs endothelial function. Increase in reactive oxygen species (ROS), from increased protein kinase C (PKC) and NAD(P)H oxidase, which leads to a decrease in NO production through the phosphorylation of endothelial nitric oxide synthase (eNOS).

Part V. Summary

Phosphate is an essential micronutrient required for critical biological reactions of the human body such as cellular metabolism, energy storage, and formation of bones. However, like sodium, Pi has become nearly indispensable in food manufacturing, and remains a large component in the American diet. Due to the dietary increase of Pi, chronic consumption of high Pi is associated with an increased risk of cardiovascular diseases, even in the general population. Although the exact mechanism(s) remain unknown, it has been reported that Pi-induced hypertension and endothelial dysfunction may be key contributors. The influence of high phosphate consumption on the autonomic control of circulation in response to exercise is a novel topic of research that should be further examined in humans. Secondly, there are equivocal reports that acute high phosphate consumption impairs endothelial function in young, healthy men. The following chapter further examines the effect of acute high phosphate consumption on blood pressure responses during isometric handgrip exercise, and muscle metaboreflex activation pre- and post- consumption of 2,000 mg of phosphate; an amount well over the recommended allowance, but representative of a typical intake found in the American diet. Vascular function was also measured using different methods. Flow-mediated dilation was used to measure conduit artery (macrovascular) function, and reactive hyperemia to measure resistance vessel (microvascular) function. Pulse-Wave Velocity and Pulse- Wave analysis (PWA) via augmentation index measured arterial stiffness. Blood samples were also obtained at three different time points (Baseline, 60-minutes post, 120-minutes post) to quantify serum phosphate concentrations.

Chapter 2

THE EFFECT OF ACUTE PHOSPHATE INTAKE ON MUSCLE METABOREFLEX ACTIVATION AND VASCULAR FUNCTION

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ABSTRACT

Emerging evidence suggests that long term high consumption of inorganic phosphate (Pi) is associated with an increased risk of cardiovascular disease. Importantly, with the growing abundance of processed foods, dietary intake of Pi in the United States has doubled the daily-recommended allowance. Recently, Mizuno *et al*. demonstrated that chronic high dietary Pi consumption results in significantly exaggerated increases in mean arterial pressure (MAP) in response to exercise and muscle metaboreflex activation in rodents. Whether acute high Pi influences MAP responses in humans remains unknown. Notably, acute high Pi consumption has been shown to impair vascular function in healthy men, but a recent study does not support this finding. Therefore, the goal of this research was to investigate whether acute high Pi consumption affects exercise and muscle metaboreflex activation, and whether vascular function is also affected. To investigate, subjects performed static handgrip (HG) at 35% maximal voluntary contraction, followed by post-exercise ischemia (PEI) to isolate the muscle metaboreflex. Vascular function was assessed via brachial artery flow-mediated dilation (FMD), and arterial stiffness was assessed using pulse wave velocity (PWV). Pi group (N= 13; 23 ± 1 yrs; mean ± SEM) ingested a monosodium phosphate drink containing 2,000 mg of phosphorus and 1,520 mg of sodium, and a control group (N=5; 23 ± 2 yrs) ingested a drink containing 1,520 mg of sodium, to match the sodium amount in the Pi drink. After 60 minutes, measures were repeated and blood was drawn at 60 and 120 min post. Serum phosphate was significantly elevated at 60 min (baseline, 3.2 ± 0.2 mg/dL; 60 min, 4.5 ± 0.2 mg/dL; $p<0.01$) and 120 min (4.9 ± 0.3 mg/dL; $p<0.01$) post- Pi, but did not change with control

(p=0.51). Metaboreflex-induced increases in MAP were not different following Pi (P=0.192) or Control (P=0.472). In contrast, macrovascular function only was significantly attenuated after Pi drink, (pre, 5.1± 0.5%; post, 3.2 ± 0.5%; p<0.01), with no changes with Control drink. For microvascular function, there were no differences pre vs. post with the Pi *(P* =0.721) or Control drink (*P*=0.302) and there were no differences in arterial stiffness following Pi drink (P=0.163) or Control drink (P=0.394). In summary, acute high Pi consumption did not affect the pressor response during exercise or muscle metaboreflex activation, or arterial stiffness, whereas macrovascular function was impaired. These data suggest a preserved pressor response, but peripheral macrovasculature is vulnerable to acute high Pi in young healthy men.

INTRODUCTION

Inorganic phosphate (Pi) is a common additive used in most processed foods for its diverse properties as a preservative and flavor enhancer. However, as a result of the growing abundance of Pi additives over the last 20 years, the average dietary intake of Pi in the United States has more than doubled the daily-recommended allowance of 700 mg/day [11]. This raises a public health concern, as chronic consumption of Pi beyond nutrient requirements is associated with an increased risk of cardiovascular disease (CVD), such as atherosclerosis [20, 21], coronary artery disease [52], hypertension [7], and stroke [53].

Investigation of the potential underlying mechanism(s) by which high Pi intake affects the cardiovascular system in animal models and clinical studies have been limited. Four weeks of a high Pi diet has been shown to increase resting systolic and diastolic blood pressure in rodents [6], and after twelve weeks of a high Pi diet, Mizuno *et al.* [7] demonstrated exaggerated pressor responses to electrically induced activation of the exercise pressor reflex (EPR). The EPR is a reflex initiated by the stimulation of mechanical and metabolic skeletal muscle afferents, resulting in increases in mean arterial pressure (MAP), which increases muscle perfusion [22, 54]. This elevation in MAP is essential during exercise, however, an exaggerated EPR has been shown to be related to adverse cardiovascular events [55, 56]. Additionally, in the same study they reported an enhanced MAP response when isolating the muscle metaboreflex, a functional sensory component of the EPR, which is activated when the accumulation of metabolites during exercise stimulates chemically sensitive muscle afferents [22]. This animal study

was the first to report direct evidence that chronic high Pi consumption induces an overactive EPR response, however, there have been no further studies which have extended these findings from rodents to humans, and it remains unknown whether an *acute* high dose of Pi is enough to increase MAP during exercise or during muscle metaboreflex activation in healthy adults.

Additionally, endothelial dysfunction may also be a possible key contributor to the pathogenesis of CVD risk with high Pi consumption. Shuto *et al*. [9] demonstrated that exposing endothelial cells to Pi loading causes a direct impairment in NO production. However, very few studies have investigated the effect of high Pi consumption on endothelial function in healthy adults, with equivocal results reported. Nishi *et al.* [8] demonstrated that acute high Pi intake in young healthy men caused an impairment in conduit artery (macrovascular) function, measured by flow-mediated dilation (FMD). In contrast, a recent study found no effect of acute high Pi consumption on FMD [10]. Moreover, no previous studies have reported whether acute high Pi consumption impairs microvascular reactivity, measured by reactive hyperemia (RH). Therefore, assessments of both macro- and microvascular function may provide valuable insights into the mechanism(s) of acute high Pi consumption on vascular function in healthy individuals.

Arterial stiffness has also been demonstrated to be related to brachial artery FMD vasodilation [57]. Additionally, a recent study has reported a correlation between higher serum phosphate levels and arterial stiffness among individuals with normal kidney function [58]. Arterial stiffening is associated with an increased risk of CVD, as arterial calcification and thickening of vessel walls leads to atherosclerosis [59]. However, a direct

link between acute high Pi intake, arterial stiffness, and endothelial function remains unknown.

Therefore, the purpose of this study was to test the hypothesis that acute high Pi consumption augments the pressor response during exercise and isolated muscle metaboreflex activation in young healthy humans. Furthermore, we hypothesized that both macrovascular and microvascular function, would be attenuated, and arterial stiffness will increase following Pi loading.

METHODS

Fourteen healthy men (age 22 ± 1 yrs; height 179 ± 2 cm; weight 83 ± 3 kg) (mean ± SEM) participated in the study. On experimental days, subjects were instructed to consume a light meal at least 2 hours before arriving to the lab; abstaining from caffeine for 12 hours, and alcohol or exercise for 24 hours. All study procedures and protocols conformed to the Declaration of Helsinki and were approved by the University of Texas at Arlington Institutional Review Board (IRB # 2017-0447). After receiving a detailed verbal and written explanation of the intended experimental protocol, each subject provided written informed consent prior to participation.

Experimental Measures:

Subjects were instrumented with a standard lead II electrocardiogram (model Q710, Quinton Instrument, Bothell, WA) for continuous measures of heart rate (HR), and a strain-gauge pneumograph (Pneumotrace, UFI, Morro Bay, CA) was placed over the abdomen to monitor respiratory movements. Beat-to-beat arterial blood pressure was measured using photoplethysmography from a finger on the right hand (Finometer; Finapres Medical Systems; Amsterdam, Netherlands), and blood pressure values from the Finometer were confirmed using a brachial automated sphygmomanometer on the subject's left arm (Welch Allyn, Skaneateles Falls, NY). Brachial artery diameter was imaged via duplex Doppler Ultrasound Machine (GE Logiq P5, Milwaukee, WI), as previously described [60, 61]. Continuous blood velocity was simultaneously obtained using the same probe in pulsed-wave mode, corrected at an insonation angle of 60°, with

the cursor set midvessel, encompassing the entire lumen but not extending beyond the vessel walls. Subjects were positioned supine in a quiet, temperature-controlled room (21-22° C) during all experimental visits.

EXPERIMENTAL PROCEDURES

Isometric Handgrip and Post Exercise Ischemia (PEI)

Each subject performed maximal voluntary contractions (MVC) with the left hand to calculate relative work rates of 35% MVC for the experimental protocol. A custom-built handgrip device (Stoelting Co., Wood Dale, IL) was squeezed at maximal effort 3-5 times, and the highest value was designated as the MVC. The subjects then rested quietly for 10 min while cardiovascular hemodynamic measures were recorded (Powerlab, AD Instruments, Colorado Springs, CO). Immediately following, a 2-min run-in period was recorded, and a bout of isometric handgrip was performed for 2 min. Subjects maintained force via visual feedback from a computer screen. An occlusion cuff was rapidly inflated on the upper arm above suprasystolic pressure (220 mmHg) 5s before cessation of the handgrip exercise, and remained inflated for 2 min and 15s to isolate muscle metaboreflex activation. Using the Borg scale, subjects were asked to report a Rate of Perceived Exertion (RPE) between 6 and 20 at the end of each exercise trial.

Macrovascular and Microvascular Function

For each subject, brachial artery endothelial function and reactive hyperemia were assessed using the flow-mediated dilation (FMD) technique. After a 10-min supine rest, baseline blood velocity and diameter were measured for 3-5 min. Then, a blood pressure cuff placed ~2 cm distal to the antecubital fossa was inflated to suprasystolic pressure (220 mmHg) for 5 min. Continuous blood velocity and vessel diameter were measured 30 seconds before, and up to 3 min following the release of the cuff. The skin was marked

for probe placement to ensure measurements were made from the same location pre- and post-Pi or control drink.

Arterial Stiffness

To assess arterial stiffness, Pulse-wave velocity (PWV) and Pulse Wave Analysis (PWA) were measured using SphygmoCor (Atcor Medical, Sydney Australia). For PWV, subjects were instrumented with a thigh cuff placed closest to the inguinal crease, and securely taped to assure it remained in place for the duration of the study. The carotid pulse was then palpated and marked at the strongest location, and distance measurements were made at three locations (carotid to sternal notch, sternal notch to femoral cuff, femoral artery to femoral cuff). A hand-held tonometry probe was used to obtain an arterial blood pressure waveform by placing slight pressure on the carotid artery at the marked location. At the start of the measurement, the leg cuff was inflated, and the tonometer was placed on the carotid for approximately 20 seconds, or until the waveforms became consistent. A laptop computer system was used to display a real time recording of the carotid and femoral pulse waveforms. Pulse transit time was calculated between the distances, and averaged over two repeated measurements. For PWA, a brachial cuff around the arm measured peripheral pressure waveforms and generated a corresponding central waveform from which augmentation index (AIx) was derived using a validated transfer function [62].

EXPERIMENTAL PROTOCOL

Phosphate Drink

After a 20-minute supine rest, brachial artery FMD was performed to assess macrovascular function (i.e. conduit artery dilation) and microvascular function (i.e. reactive hyperemia). Afterwards, baseline cardiovascular measures were recorded for a 10-minute period, while subjects rested quietly. Following baseline, a 2min run-in was recorded and subjects completed isometric handgrip at 35% MVC, followed by PEI. Subjects then orally ingested a monosodium phosphate drink (Swanson Health, Fargo, ND) consisting of 2,000 mg of phosphorus, and 1,520 mg of sodium, mixed in 8 oz of flavored water (MiO, Kraft Foods). After consuming the drink, subjects rested for 60 min, following which all the pre-phosphate measures were repeated identically. Blood samples were collected at baseline, 60 min, and 120 min post-phosphate intake to measure serum phosphate levels.

Control Drink

In a subset of the same subjects (n=5), we performed control experiments to ensure that the amount of sodium present in the phosphate beverage alone would not affect any of the measurements made. The identical protocol was performed as mentioned above, with the only difference being the drink consumed. The drink for the control protocol consisted of 1,520 mg of sodium only (Morton Salt, Chicago, IL), mixed in 8 oz of flavored water to match the sodium content in the phosphate supplement drink.

DATA ANALYSIS

Resting values for MAP, HR, and stroke volume (SV) were averaged over a 2-minute steady-state period for both pre- and post-Pi and Control. During 35% HG and PEI, the hemodynamic parameters were averaged over the last 60s of HG and PEI, respectively. Cardiac output was calculated as HR × SV/ 1000. For blood flow data, offline edge detection analyses (LabView, National Instruments, Austin, TX) determined the brachial artery diameter (mm) and weighted mean velocity (cm/s) from the captured video output. For assessment of resting hemodynamics, shear rate was calculated as: 8 x mean blood velocity/diameter and blood flow was calculated as: $\pi \times (\text{diameter}/2)^2$ x mean blood velocity x 60. For assessment of macrovascular function, brachial artery FMD was calculated as: %FMD = (peak diameter–baseline diameter)/baseline diameter × 100. Assessment of microvascular function was determined via the sum of trapezoids method. Reactive hyperemic responses were calculated as area under the curve (AUC) from cuff release to the point at which hemodynamics returned to baseline values. For arterial stiffness, PWV and AIx were analyzed using the average of two trials. In addition, because AIx is influenced by heart rate, an index normalized for a heart rate of 75 bpm was used in accordance with Wilkinson *et al.* [62].

STATISTICAL ANALYSIS

Blood Pressure, HR and total vascular conductance (TVC) data were analyzed using two-way repeated measures ANOVA for condition and time effects separately for the phosphate and the control drink protocols (SPSS, version 24). When a significant interaction was present, pairwise comparisons were made using the Bonferroni post-hoc test. FMD, arterial stiffness data were analyzed using paired t-tests. Differences were considered significant at $p < 0.05$ and data are presented as means ± standard error of the mean (SEM).

RESULTS

Effect of Phosphate on Resting Cardiovascular Measures

Following acute high Pi, serum phosphate was significantly elevated from at 60min post (BL, 3.2±0.2 mg/dL to 60 min; 4.5±0.2 mg/dL; $P<0.01$) and remained significantly elevated through the remainder of the study (Figure 1A). Elevations in serum phosphate did not significantly influence BL systolic blood pressure ($P=0.575$), diastolic blood pressure ($P=0.770$), HR ($P=0.715$), or TVC ($P=0.999$) pre vs. post Pi (Table 1). In the subset of individuals who completed the Control visit, we observed no significant elevations in serum phosphate (Figure 1B), and no significant changes in resting cardiovascular measures for systolic blood pressure ($P=0.508$), diastolic blood pressure ($P=0.776$), HR ($P=0.943$), or TVC ($P=0.162$) pre vs. post- Control at BL.

Effect of Phosphate on Isometric Handgrip 35% and PEI.

MAP increased in response to both HG and PEI (time effect $P<0.001$) (Figure 2A), however, this pressor response did not change pre vs. post Pi (treatment effect P=0.192). RPE was similar for the HG performed following Pi drink (Pre, 13.5±0.4; Post, 12.8±0.6; $P=0.231$). In the Control group, there was also an increase in MAP in response to HG and PEI (time effect $P=0.019$), with no difference between pre and post Control (condition effect $P=0.472$). RPE was also not different for HG performed pre vs post Control (Pre, 12.9±0.3; Post, 13.5±0.7; $P=0.426$). For cardiovascular measures during HG and PEI, systolic blood pressure, diastolic blood pressure, HR, and TVC had a significant time

effect in both the Pi and the Control group (Table 1), however there was no treatment effect in either groups.

Effect of Phosphate on Macrovascular and Microvascular Function

Resting baseline diameter, blood flow, and blood velocity pre- and post- Pi consumption are shown in Table 2. There was a significant impairment in macrovascular function assessed by brachial artery FMD at 60 min post-Pi consumption (pre, 5.1 ± 0.5%; post, 3.2 ± 0.5%; $P<0.01$), as presented in Figure 3A, and a significant change in FMD when normalized to shear (pre, 6.0 ± 0.5%; post, 4.1 ± 0.5%; $P<0.01$). However, there was no change in the microvascular function assessed by hyperemic blood velocity AUC (pre, 8232 ± 909 a.u; post, 8518 ± 1072 a.u; $P=0.721$) (Figure 4A) or for peak velocity (pre, 55.8 ± 3.8 m/s; post, 55.3 ± 3.1; $P=0.864$) pre vs. post- Pi consumption. There were no differences between with resting diameter ($P=0.084$) and blood flow ($P=0.176$) or blood velocity ($P=0.080$) pre- and post Pi (Table 1). In Control, macrovascular function assessed by brachial artery FMD did not change 60 min post ($P=0.971$) (Figure 3B) and when normalized to shear, there was still no significant change ($P=0.560$). For microvascular function, there was also no change in hyperemic blood velocity AUC ($P=0.302$) (Figure 4B) or peak velocity ($P=0.444$). There were no changes in resting brachial diameter ($P=0.461$), blood flow ($P=0.189$) or blood velocity ($P=0.222$), pre vs. post Control.

Effect of Phosphate on Arterial Stiffness

Arterial stiffness assessed by PWV, was not affected pre vs. post Pi (*P*=0.163) (Figure 5A). Similarly, AIx assessed by PWA did not show any differences pre vs. post Pi (*P*=0.234) (Figure 5A, *bottom panel*). For Control, there were also no significant differences in PWV (*P*=0.394) (Figure 5B, *top panel*) or AIx (*P*=0.910) (Figure 5B, *bottom panel*) pre vs. post.

DISCUSSION

This study was the first to investigate the influence of acute Pi loading on the pressor response during isometric HG exercise and isolated muscle metaboreflex activation during PEI in humans. The primary findings demonstrate that a single high dose of Pi did not alter MAP in response to exercise and muscle metaboreflex activation. In contrast, acute high Pi resulted in impaired conduit artery vasodilation, assessed by brachial artery FMD, whereas microvascular function, assessed by reactive hyperemia, was not attenuated. Arterial stiffness measured by pulse-wave velocity and pulse-wave analysis was also not different compared to pre-Pi, however serum phosphate significantly increased 60 min post-Pi, and remained elevated through the remainder of the study. Collectively, these data indicate a preserved pressor response during exercise and muscle metaboreflex activation with a single dose of 2,000 mg of monosodium phosphate. However, peripheral macrovasculature is vulnerable in young, healthy subjects free from any known cardiovascular disease risk factors. These data support previous work indicating that Pi loading and serum phosphate elevation may contribute to an increased risk in CVD in healthy individuals, through mechanisms relating to endothelial dysfunction.

The purpose of this study was to extend the findings of a previous study that reported augmented cardiovascular and sympathetic responses to electrical stimulation of the EPR and isolated muscle metaboreflex activation with chronic Pi loading in animals [7]. However, when confirming these findings in young and healthy humans, the influence of acute high Pi loading did not alter resting cardiovascular measures or MAP in response

to HG exercise and PEI. The possible mechanism(s) contributing to our contrasting findings are unclear, however previous studies have reported that Pi influences an exaggerated MAP response by altering baroreflex sensitivity [7], which restrains EPR induced increases in MAP [63]. The baroreflex and EPR monitor each another during exercise [64, 65], therefore it is possible that the baroreflex maintained MAP in response to HG and PEI in our study. Since our subjects were young and healthy, a single high dose of Pi was not enough to influence baroreflex sensitivity. Although this is one possible explanation, the mechanisms underlying Pi-induced enhancements in EPR function remain unknown [7].

Our study also tested the effect of acute high Pi on the varying components of vascular function in healthy subjects, including both macrovascular and microvascular responses, and arterial stiffness. The current study is consistent with previous findings that acute high Pi attenuates conduit artery endothelial function [8, 9], but not all [10]. Nishi *et al.* [8] acutely loaded Pi in their healthy subjects in the form of a low phosphate meal (400 mg) consumed with a Pi supplement (800 mg), and found an impairment in FMD and a significant elevation in serum phosphate. Interestingly, Levac *et al*. [10] replicated these findings, and found no impairment in FMD, and no elevation in serum phosphate two hours after consuming 1,200 of Pi in a form of a supplement only [10]. While the reason for this is discrepancy is unclear, it may relate to the differences in their methodological approach. When explaining a possible reason behind their findings, they explained that since Pi was administered in the form of a supplement only, instead of mixed with a meal, this could explain why they saw no change in FMD. However, in the present study we provided 2,000 mg of Pi as supplement, and there was an increase in

both serum phosphate and an impairment in FMD 60min post. This is crucial because although we did not administer a chronic dose of Pi, it may be important to note that the amount, even in a single dose, should be of concern in healthy individuals.

Additionally, we saw different results in the measurements of vascular function from our study, including an impairment in macro-, but not microvascular function. A possible mechanism by which Pi directly impairs endothelial function is through the NO pathway [9]. NO is a key endothelium-derived relaxing factor, which is a key component in the maintenance of vascular tone and reactivity [66]. Pi loading inactivates endothelial nitric oxide synthase (eNOS), an NO producing enzyme [9], and can increase oxidative stress by increasing reactive oxygen species (ROS), which scavenge NO and decreases endothelial vasodilation [9, 67]. Di Marco *et al.* [68] also reported similar findings that high Pi loading causes vascular dysfunction by Pi-induced apoptosis in endothelial cells, which has been previously explained as a possible mechanism by which high Pi loading can lead to atherosclerosis [68]. However, it is not exactly known how Pi directly induces ROS generation, and in the current study there were no cellular measurements of NO or ROS. Previous studies have explained that FMD is largely NO mediated, and reactive hyperemia relies on separate mechanisms involving local myogenic and metabolic pathways within the resistance vasculature [69, 70]. Despite the common use of reactive hyperemia as a measure of vascular health, the underlying mechanisms of vasodilation that contribute to this response are largely unknown [69].

Following acute high Pi consumption, it was surprising that we did not see a change in arterial stiffness, since NO is also a potent regulator of arterial compliance [50]. Arterial stiffness is associated with brachial artery vasodilation [57], and in our study, we

measured aortic PWV, which is a powerful independent predictor of future cardiovascular events and all-cause mortality [71]. When considering the link between these arterial stiffness and endothelial function, it is already known that high Pi concentrations contribute to the pathophysiology of CVD by interfering with endothelial function, causing sustained endothelial stiffening [17]. However, arterial stiffness and endothelial dysfunction represent different aspects of vascular disease; therefore, separate mechanisms must be responsible for the lack of changes in arterial stiffness found in our study.

In the present study, the Pi administered to the subjects was a single dose of monosodium phosphate containing 2,000 mg of Pi, reflecting Pi intake of an individual consuming a fast-food meal three times a day [72]. Inorganic Pi was chosen as the source for Pi loading, as it is the most bioavailable form of Pi with an absorption rate of up to 90-100%, as opposed to organic phosphates found naturally in protein rich foods and vegetables. The elevation in serum phosphate concentrations at 60 min and 120 min post-Pi confirmed that Pi remained present in the body during the entire study. At 60 min post-Pi, our subjects' mean serum phosphate levels were at the upper limit of the clinical normal ranges of 2.4-4.5 mg/dL, and exceeded these values at 120-min post Pi. Increases in serum phosphate concentrations with acute high Pi loading is consistent to previous human studies [8, 9], but not all [10]. This is important to consider because large-scale observational studies [53, 73], have reported higher serum Pi levels are consistently associated with CVD and cardiovascular events among patients with normal kidney function. Serum phosphate levels within the normal reference range have also been

shown to be associated with cardiovascular mortality in the general population in two recent independent studies [19, 74]. In an observation community-based study of over 2,000 Framingham offspring study participants, free of overt CVD and with normal renal function, higher serum phosphate levels were associated with increased cardiovascular risk [19]. A similar association between higher levels of serum phosphate and the risk of death and cardiovascular events has also been demonstrated in survivors of myocardial infarction [74]. This data suggests that the amount of Pi consumed during a single meal may be just as important as the duration of a continuous exposure to high levels of Pi throughout the day and over time.

Chapter 3

Future Direction and Conclusion

FUTURE DIRECTION

The average dietary intake of Pi has doubled the daily-recommended allowance in the last 20 years, and to date, very few studies have investigated the effect of acute high phosphate intake on cardiovascular health. Importantly, high Pi intake increases the risk of developing CVD and all-cause mortality, and our study shows that even a single dose of high phosphate impairs endothelial function in young healthy men. However, further research is needed to investigate whether similar impairments also occur in women, to fully characterize the mechanism of how Pi causes endothelial dysfunction. It has been found previously that estrogen plays an important role in vascular health [67], Moreau *et al.* [67] has previously demonstrated that oxidative stress is a key mechanism mediating large artery stiffening and endothelial dysfunction with aging and with estrogen deficiency in women. Therefore, comparing healthy pre-menopausal and post-menopausal women to age-matched men should be of interest. In addition, not much is known on how phosphate can affect expanded populations with compromised cardiovascular health, such as those already with hypertension and atherosclerosis. A correlational study has previously reported a relationship between higher serum phosphate in the normal range with death and cardiovascular event rates in those with a history of myocardial infarction [74], however there is a lack of clinical studies focusing on these patient populations. Whether or not these populations would be more susceptible to exaggerated pressor responses during exercise and muscle metaboreflex activation with acute Pi loading, despite having normal kidney function, should be of interest. Importantly, despite not finding exaggerated MAP responses to acute Pi loading as in the current study, we did

not have a measurement of muscle sympathetic nerve activity (MSNA). Therefore, we did not know if there was an increase in MSNA during EPR activation following Pi loading in our subjects, similar to the findings in Mizuno's animal model study that found enhanced renal sympathetic activity and MAP during electrical stimulation of the EPR, after 12 weeks of chronic high Pi diet. Additionally, in the present study, current food habits and meal contents of subjects were not documented. A food log would have given us a better idea of the eating patterns of our subjects, as well as a more precise calculation of the amount of daily Pi being consumed, instead of using estimated reference values for age and gender.

CONCLUSION

In summary, the primary findings of this study were that MAP did not change in response to HG exercise or muscle metaboreflex activation after consuming a single high dose of Pi in young, healthy men. In contrast, acute Pi loading resulted in impaired conduit artery vasodilation, assessed by brachial artery FMD, whereas microvasculature, assessed by reactive hyperemia, as well as arterial stiffness was not affected. Specifically, our study confirms previous findings that conduit artery vasodilation is impaired, suggesting that peripheral macrovasculature, but not microvasculature is vulnerable to a single dose of 2,000 mg of phosphate in young healthy, men. Collectively, although evidence shows that brachial artery FMD is attenuated at just 60min post-Pi ingestion, further research is needed to better understand the link between high Pi and cardiovascular dysfunction. Acute and chronic high phosphate loading on serum phosphate, even within the normal range in healthy individuals has clinical implications, as phosphate directly impairs endothelial function, a vascular condition characterized as a decrease in NO bioavailability, which can lead to the development and progression of atherosclerosis, a precursor to myocardial infarction, stroke, and overall cardiovascular disease.

FIGURES

Figure 1.

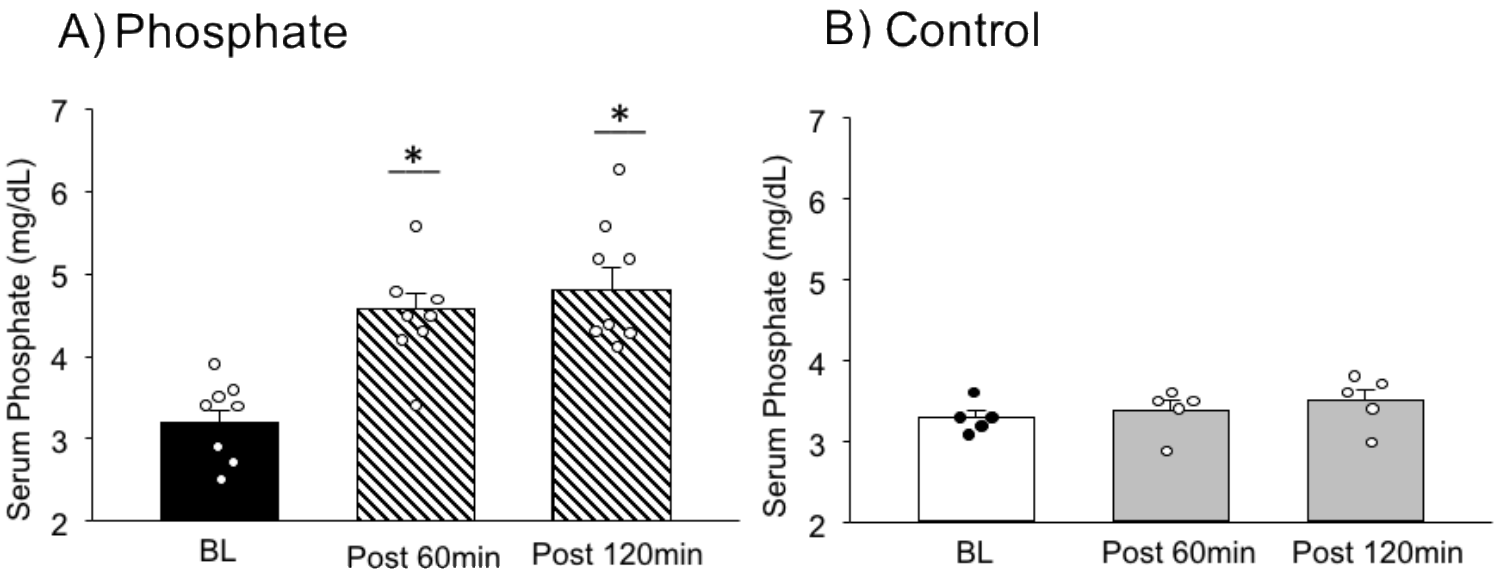

Figure 1. Group means and individual data for serum phosphate at Baseline (BL), 60min and 120min-post for (*A*) Pi consumption and (*B*) Control. *P < 0.05, versus Baseline.

Figure 2.

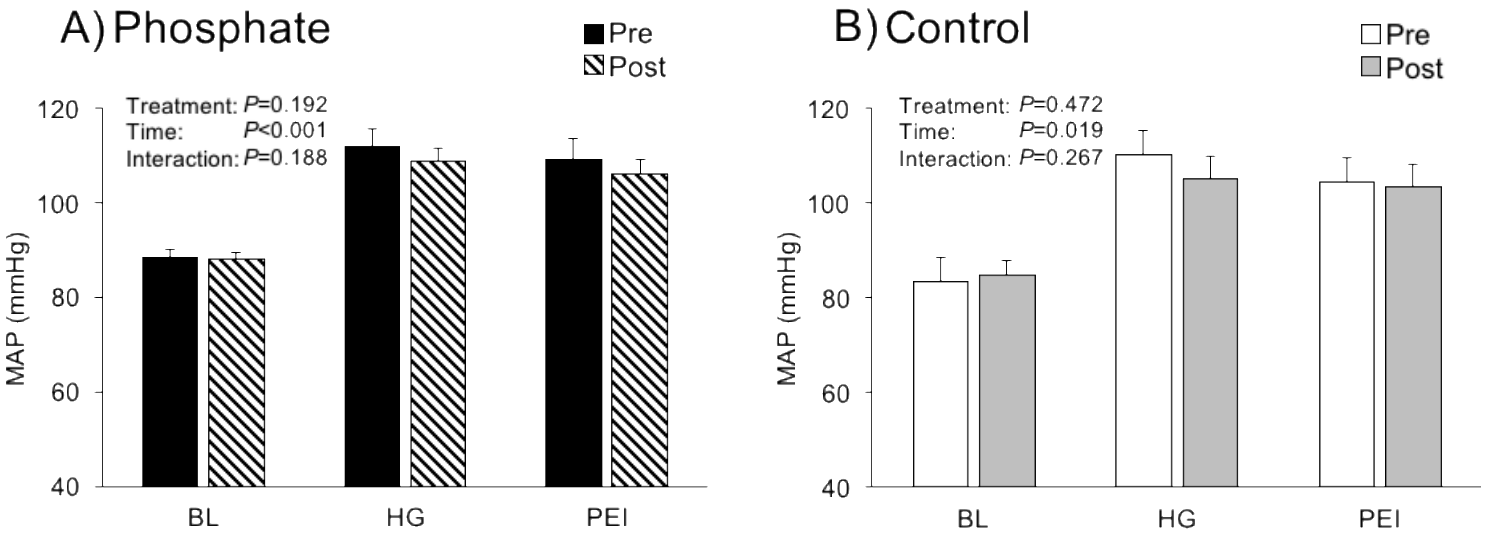

Figure 2. Mean arterial pressure (MAP), during baseline (BL), last 60s of 35% handgrip exercise (HG), and last 60s of post-exercise ischemia (PEI) for pre-and post-Pi consumption (*A*) and Control (*B*).

Figure 3.

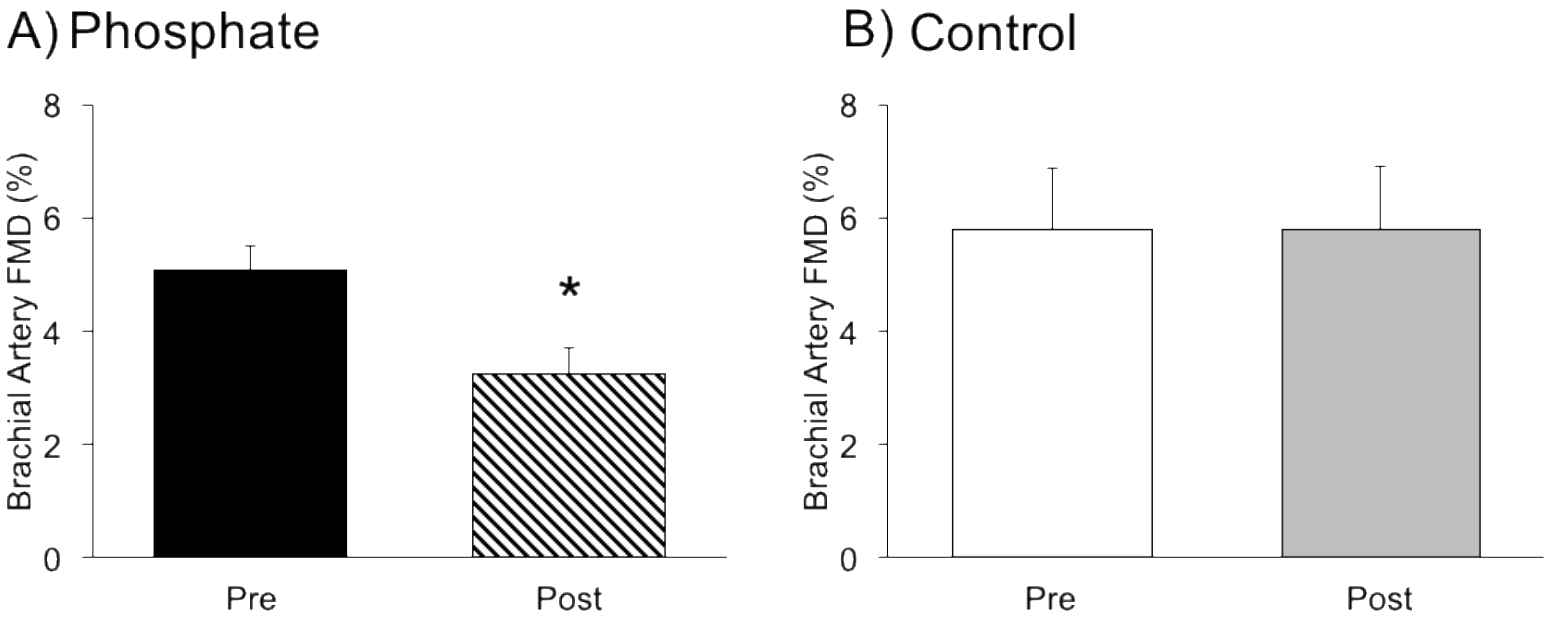

Figure 3. Brachial artery flow-mediated dilation (FMD) pre- and post-Pi consumption (*A*) and Control (*B*); *P < 0.05, versus Pre.

Figure 4.

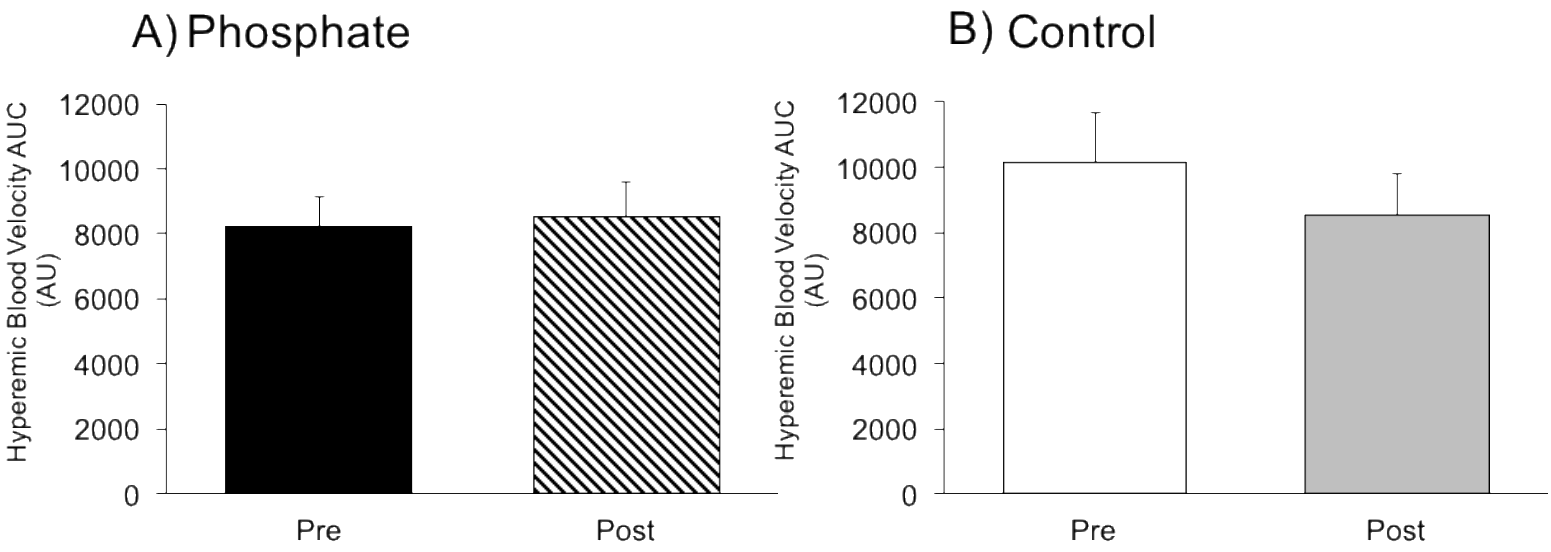

Figure 4. Hyperemic blood velocity pre-and post- Pi consumption (*A*) and Control (*B*).

Figure 5.

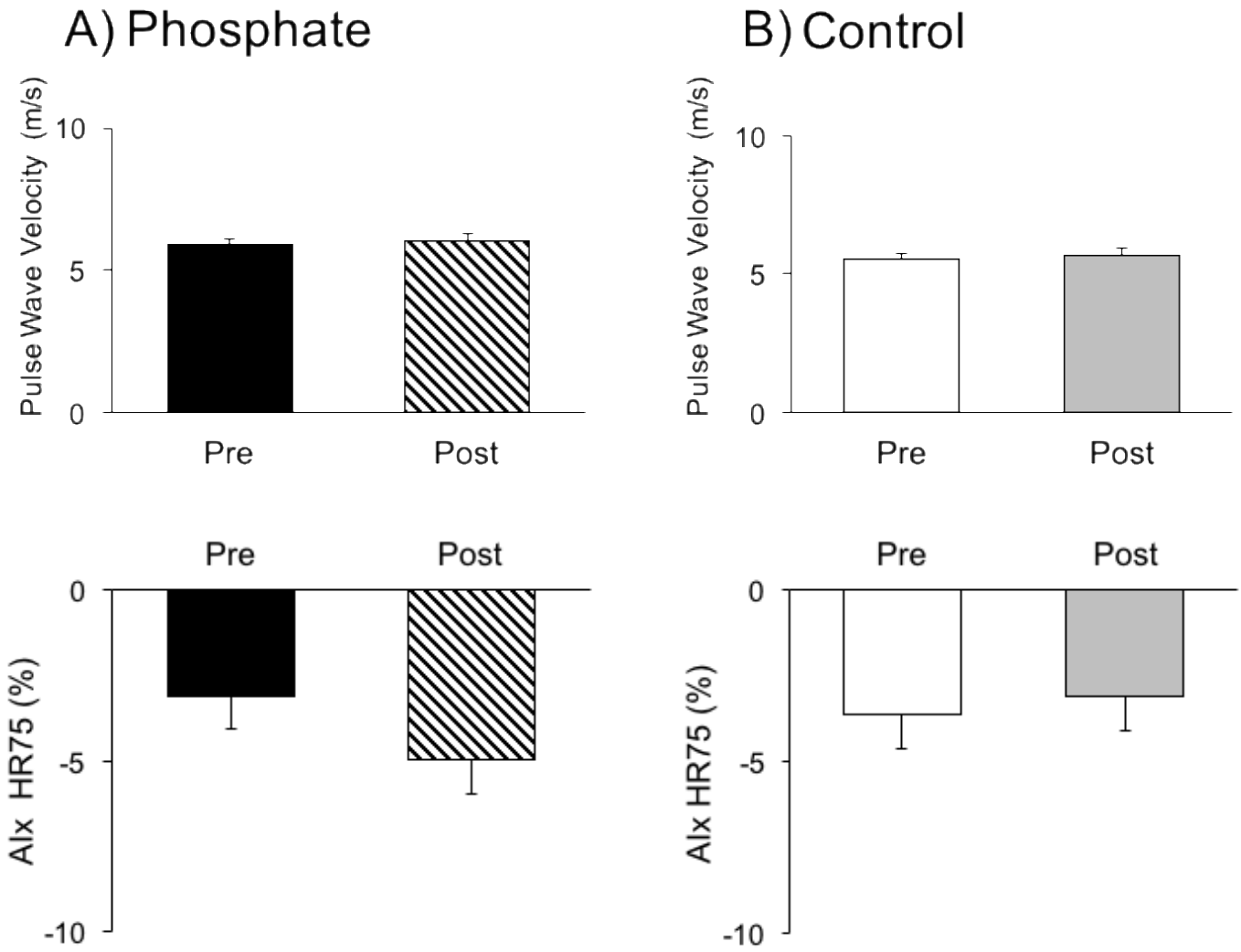

Figure 5. Group means for pulse wave velocity (*top panel*) and augmentation index corrected to a heart rate of 75 beats per min (AIx HR75; *bottom panel*) for pre-and post-Pi consumption (*A*) and Control (*B*).

TABLES

Table 1. *Cardiovascular Measures During HG 35% and PEI*

	PRE			POST			
Characteristics	BL	HG	PEI	BL	HG	PEI	*P* Value
Systolic Blood Pressure (mmHg)							
Phosphate	127±3	148±5	147±5	128±2	150±3	147±4	Time: 0.000 Treatment: 0.739 Interaction: 0.759
Control	122±5	148±7	146±8	124±4	147±7	142±6	Time:0.024 Treatment: 0.625 Interaction: 0.316
Diastolic Blood Pressure (mmHg)							
Phosphate	68±2	88±3	82±3	68±1	85±2	80±2	Time:0.000 Treatment: 0.226 Interaction: 0.079
Control	65±3	92±8	79±4	65±2	88±6	77±3	Time: 0.012 Treatment: 0.287 Interaction: 0.290
Heart Rate (beats/min)							
Phosphate	60±2	81±3	65±3	60±3	83±3	66±3	Time: 0.000 Treatment: 0.168 Interaction: 0.216
Control	55±2	76±3	55±3	55±2	79±4	55±3	Time: 0.032 Treatment: 0.382 Interaction: 0.640
TVC (mL/min/mmHg)							
Phosphate	76±5	83±6	78±6	76±3	87±4	81±4	Time: 0.047 Treatment: 0.455 Interaction: 0.498
Control	76±8	85±9	70±8	80±7	99±10	77±8	Time: 0.001 Treatment: 0.169 Interaction: 0.242

Data are mean±SEM; Pre, before drink consumption; Post, after drink consumption; BL, baseline; HG, handgrip 35% MVC, PEI, post-exercise ischemia; TVC; total vascular conductance.

Table 2. *Resting Brachial Artery Hemodynamics*

Characteristics	Pre	Post	*P* value
Diameter (mm)			
Phosphate	4.29 ± 0.16	4.34 ± 0.16	0.084
Control	3.96 ± 0.29	3.94 ± 0.29	0.461
Blood Velocity (cm/sec)			
Phosphate	13.6 ± 2.3	10.0 ± 1.5	0.080
Control	11.4 ± 2.5	8.6 ± 3.1	0.222
Blood flow (mL/min)			
Phosphate	110 ± 18	89 ± 14	0.176
Control	88 ± 20	59 ± 18	0.189

Data are mean± SE; Pre, before drink consumption; Post, after drink consumption.

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Biographical Information

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