THE POTENTIAL ROLE OF ARTERIOLAR VASODILATOR RESPONSIVENESS IN ORTHOSTATIC INTOLERANCE

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BACKGROUND

Humans experience profound physiological adaptations during space flight, including bone demineralization, neuroendocrine and immune system responses, neurovestibular responses, muscle atrophy, and cardiovascular adaptations (52, 55). The weightlessness environment causes deprivation of normal locomotive function (hypokinesia), deprivation of normal weight-bearing function (hypodynamia), and muscle atrophy. Among the effects of space flight on landing is inability to maintain sufficient blood pressure to the head when standing (orthostatic hypotension). General symptoms of orthostatic intolerance include lightheadedness, fatigue, and fainting (52). Without hydration in space, the hypotension is initially due to total body hypovolemia (low body fluid). Significant evidence suggests that cardiovascular adaptations may be occurring that are rendered inappropriate on return to earth (5, 19, 54, 58). One such adaptation to weightlessness may be up-regulated vasodilatory response of the peripheral resistance vasculature. This study investigates the potential role of enhanced vasodilator responsiveness of arterioles from skeletal muscle in causing orthostatic hypotension.

ETIOLOGY OF ORTHOSTATIC HYPOTENSION

On earth, orthostatic hypotension results from inadequate compensatory responses to the gravitational shifts in blood that occur when a person moves from a horizontal to a vertical position, especially following a prolonged bed rest. The decrease in blood pressure that results

from blood pooling in the leg veins upon standing up is normally detected by the baroreceptors, which rapidly initiate compensatory responses to restore blood pressure to its proper level. When a person who has been bedridden begins to stand upright, gravity causes blood to pool in the lower extremities. The subsequent orthostatic hypotension and decrease in brain blood flow are responsible for the dizziness or actual fainting that occurs.

Experiments in Humans

The primary cause of orthostatic hypotension in the first days of space flight is thought to be the weightlessness-induced hypovolemia. Immediately upon leaving a gravity environment, interstitial fluid and blood caudally shift in response to mechanisms that are designed to return fluids to the heart through a gravity-induced pressure gradient. Such mechanisms include venous valves and the lymphatic system. The headward fluid shift activates the baroreceptors in the hypervolemic chest cavity. The brain subsequently releases vasopressin, and the heart releases atrial naturetic peptide which triggers release of fluid from the kidneys. The amount of total body fluid stabilizes within several days (11). The total fluid volume is maintained at a constant level (which is a hypovolemic level relative to that on earth). On return to earth, body fluid redistributes to the lower body under the influence of terrestrial gravity and the same fluid distribution as the pre-flight fluid distribution. Since blood volume is low, mean arterial pressure is not adequately maintained.

The tendency toward low blood pressure and syncopal symptoms during standing, head-up tilt, or lower body negative pressure is well documented in humans who have been exposed to periods of actual or simulated weightlessness (6, 8, 10, 12, 27, 29, 32, 45). Orthostatic hypotension induced by weightlessness is associated with reduced plasma volume (6, 9, 10), decreased left ventricular-end-diastolic volume with consequent lowering of stroke volume and cardiac output (11, 45), and attenuated cardiac baroreflex response (10, 12). Heart rate increases to offset the decrease in stroke volume (52). Postflight tests of orthostatic tolerance showed

increased heart rate and decreased pulse pressure as compared with preflight measurements (7, 50, 51). The net result is that cardiac output is only slightly lower or in many following exposure to a weightlessness environment:

Cardiac Output (not significantly changed) = ⇧Heart Rate x ⇩ Stroke Volume

The documented decrease in plasma volume contributes to orthostatic intolerance. Upon return to terrestrial gravity, fluid distribution in orthostasis returns to preflight levels with less total blood volume, and subsequently maintenance of adequate cephalic pressure relies more heavily on the cardiovascular system than pre-flight. This hypovolemia-induced strain on the cardiovascular system contributes to orthostatic hypotension.

Evidence is mounting that orthostatic hypotension is not solely attributed to hypovolemia (5, 54, 58). A study by Vernikos et al (58) identified oral rehydration with bullion or salt tablets as an effective strategy in reversing orthostatic hypotension. However, the study revealed that the rehydration with salt tablets became less effective as exposure to weightlessness increased, suggesting that other factors besides hypovolemia become increasingly important in orthostatic hypotension during a space mission. This conclusion is in accord with others (5) whose findings indicate that hypovolemia in humans does not fully explain the decrement in orthostatic tolerance and work capacity that accompanies exposure to microgravity.

Since cardiac output is unchanged or only slightly lowered with rehydration, hypovolemia does not adequately explain the increase in hypotension with increasing exposure to microgravity. Therefore, total peripheral resistance must be compromised in weightlessness:

⇩ Mean Arterial Pressure = Cardiac Output (not changed) x ⇩ Total Peripheral Resistance

Arteriolar resistance by far offers the greatest percentage of the total peripheral resistance. This means that arterioles immensely influence the maintenance of mean arterial pressure. By constricting, arterioles reduce the blood flow to a particular tissue, which increases the main driving pressure head to other organs, and in particular the brain. In effect, sympathetically induced arteriolar responses help to maintain the appropriate driving pressure to other organs. Since mean arterial pressure depends on the cardiac output and the degree of arteriolar vasoconstriction, arteriolar dilation in one tissue results in arteriolar constriction in other tissues to maintain an adequate arterial blood pressure to provide a driving force to push blood not only to the vasodilated tissue but also to the brain, which requires a constant blood supply.

Evidence is mounting that a compromised ability to elevate vascular resistance is an important component of orthostatic hypotension. In the study by Vernikos et al (58), the subjects who did not faint maintained their ability to vasoconstrict. This result supports the claim that arteriolar resistance is a factor in orthostatic hypotension. Schmid et al (47) found that forearm vascular resistance was reduced after 12 days of bed rest, a model used to simulate microgravity in humans. The reduction in resistance remained during intraarterial infusions of norepinephrine. Mulaugh et al (39), used echocardiography to show that inadequate vasoconstrictor responses significantly contributed to the post-flight orthostatic intolerance. Buckey et al (7) investigated a broad spectrum of cardiovascular regulatory mechanisms during pre- and post-flight stand tests in crewmembers having flown 9-14 days. Upon return to earth, the subjects fell into two groups: those that could and those that could not finish the stand test. The distinguishing characteristic of the non-finishing group was a compromised ability to increase vascular tone and subsequently increase total peripheral resistance. In addition, heart rate was higher in the non-finishers and venous pooling and stroke volume were similar between the two groups. The authors suggested that the diminished vasoconstrictor response could occur by a number of factors, including

baroreceptor responsiveness, central integration of reflex responses, afferent input, efferent output and end organ (vascular) responsiveness.

Shoemaker et al (50) investigated the effect of head-down-tilt bed rest for 14 days on supine sympathetic discharge and cardiovascular hemodynamics at rest. While heart rate at rest was greater post-bed rest, mean arterial pressure was unchanged. Aortic stroke distance during post-bed rest was lower that pre-bed rest levels. Muscle sympathetic nerve activity burst frequency was reduced in the post- compared with the pre-bed rest condition. Bed rest did not alter forearm blood flow, forearm vascular resistance, or total peripheral resistance. Thus, reductions in muscle sympathetic nerve activity with head-down-tilt bed rest were not associated with a decrease in forearm vascular resistance. These results imply that the inability to elevate vascular resistnace does not involve changes in sympathetic nerve activity.

In another head-down-tilt bed rest study, Shoemaker et al (51) reported that total excess reactive hyperemic forearm blood flow was diminished by bed rest and that the ability of the cold pressor test to lower forearm blood flow was less in the post- than in the pre-bed rest test, despite similar increases in mean arterial pressure. The authors suggested that regulation of vascular dilation and the interaction between dilatory and constrictor influences were altered with bed rest. Thus, these data suggest that modifications in vascular tone could be the result of changes in the contractile properties of vessels, the modifications could also be due to alterations in vasodilator mechanisms.

In the present study on vasodilatory properties of 1A rat arterioles, it is hypothesized that vascular β2-adrenoceptors are up-regulated so that the associated enhanced dilation would exacerbate orthostatic hypotension. Evidence to support this claim includes studies that have reported that the β2-adrenoceptor blocker propranolol prevents pre-syncopal symptoms induced by tilt tests after exposure to simulated weightlessness (37, 46).

Experiments in Rats

Using hindlimb unloading by tail suspension as a model of the effects of weightlessness, rats have been used to study both mechanistic changes and vascular remodeling of the cardiovascular system. Overton and Tipton (42) found that hindlimb unloading decreased cold pressor response and reduced mesenteric vascular resistance to infusions of phenylephrine. In studying effects of simulated weightlessness on visceral blood flow, McDonald, Delp, and Fitts (35) found that during treadmill walking, hindlimb unloaded rats revealed a diminished response in the normal reduction in blood flow. In addition, blood flow was elevated to inactive muscle primarily composed of fast twitch glycolytic muscle fibers (the white portion of the gastrocnemius muscle). These authors (35, 36, 42) suggested that the attenuation of arterial vasoconstrictor properties was a consequence of either a reduction in the number of vascular smooth muscle α–receptors or a modification of the α-receptor-second messenger signal transduction mechanism. To address this hypothesis, Delp et al (16) investigated vascular responsiveness of thoracic and abdominal aortae from hindlimb unloaded and control rats to several vasoconstrictor agonists, including the α–adrenoceptor agonists norepinephrine and phenylephrine. They reported that simulated weightlessness reduced maximal contractile tension evoked by all constrictor agonists. The authors suggested that altered vascular responsiveness may not be because of a modification of α–receptor number or signal transduction, but to compromised ability of the smooth muscle contractile apparatus to generate force.

In a subsequent study by Delp (16), it was found that hindlimb unloading produced an inability to elevate vascular resistance through myogenic autoregulation and vasoconstrictor agonist stimulation. The diminished vasoconstrictor response appeared to be caused by smooth muscle atrophy and the corresponding loss of contractile proteins rather than alterations in the receptor-second messenger signal transduction mechanism, which is in agreement with previous results (18). Arterioles isolated from postural muscles of the hindlimb (i.e., the soleus muscle)

showed no significant reduction in myogenic and vasoconstrictor reactivity. The authors suggest that several mechanisms are involved in initiating adaptations in the skeletal muscle vasculature.

Smooth muscle cell atrophy may contribute to the diminished contractile tension observed in large conduit arteries and arterioles from rats exposed to simulated weightlessness. Atrophy of both cardiac and skeletal muscle results from exposure to weightlessness in rats (34). In addition, changes in hydrostatic pressure have been shown to induce trophic responses in smooth muscle cells. For example, smooth muscle cell hypertrophy occurs in maturing giraffe due to increases in blood pressure (28). Smooth muscle atrophy may occur as result of plasma volume loss and reductions in blood pressure.

In studying the remodeling of rat skeletal muscle arterioles, Delp et al (Delp, Colleran, Wilkerson, Muller-Delp, unpublished observations) have found that hindlimb unloading reduced resistance artery medial thickness with no change in diameter in the gastrocnemius muslce and reduced the media outer perimeter and vessel diameter with no change in medial thickness in the soleus muscle. The authors suggest that reductions in transmural pressure cause the remodeling of gastrocnemius muscle arterioles and reductions in wall shear stress induce the remodeling of soleus muscle arterioles. It remains to be determined whether hindlimb unoading alters intrinsic vasodilator properties of resistance vasculature.

Weightlessness-induced adaptations in peripheral arteriolar reactivity, such as the diminished myogenic and vasoconstriction mechanisms observed by Delp et al. (16), could contribute to orthostatic intolerance when normal pressure gradients are introduced. Decrements in contractile function or enhancements in dilation would lead to decreases in peripheral resistance. As outlined in the previous section, a relative dilation would pass a greater pressure head to the venous side of the circulation, possibly contributing to venous distention, pooling, and orthostatic hypotension. Based on previously observed alterations in muscle blood flow following hindlimb unloading (35), it was hypothesized that dilator properties would be enhanced in arterioles from muscle composed of slow twitch oxidative muscle fibers such as soleus muscle.

Rat Hindlimb Unloading

Numerous studies have used hindlimb unloading with tail suspension to simulate the cardiovascular adaptations induced by zero gravity. The hindlimb unloading of rats simulates weightlessness in two important ways: first, the tail suspension effects a gravity-induced caudal shift of fluids (43), and second, unloading of the hindlimb produces postural muscle atrophy.

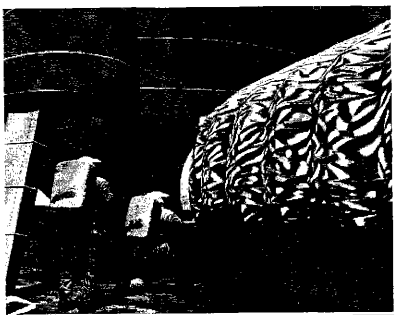
Muscular adaptations and cardiovascular deconditioning exhibited in hindlimb unloaded animals closely mimics those induced by real or simulated weightlessness in humans. Muscular adaptations include muscle atrophy (19), increased capillary density (40), greater glycogen and ATP usage (56), and conversion of slow myosin heavy chains to fast isoforms (53). These adaptations are dependent on the composition of the muscle fiber. Cardiovascular adaptations include a temporary increase in central venous pressure that normalizes within 24 hours (34, 49), natruiresis and diuresis (15), reduced blood volume (23, 43), tachycardia (35), orthostatic hypotension (Delp, Colleran, Wilkerson, Muller-Delp, unpublished observations) and a decreased exercise capacity (20, 43). Thus, these studies show that hindlimb unloading by tail suspension can be used to simulate the effects of weightlessness on the cardiovascular system as a whole, and thus, provides a useful model to investigate arteriolar smooth muscle and endothelial cell function in muscles composed of fast-twitch glycolytic and slow-twitch oxidative muscle fibers.

RATIONALE

In the latest research announcement, NASA requested research to how weightlessness affects vascular responsiveness to vasodilatory stimuli. This study was designed to determine how simulated weightlessness affects vasodilatory responsiveness of skeletal muscle arterioles in vitro to several dilatory agonists acting through the nitric oxide synthase, β2-adrenergic receptor and other vasodilar mechanisms.

Future NASA Missions

Future exploration mission planning and surface structure design are driving the requirements of countermeasures (techniques to counteract the physiological adaptations induced by weightlessness), and subsequently NASA is funding scientific inquiry in determining the mechanisms of orthostatic hypotension. Current NASA protocol requires an emergency egress maneuver from the space shuttle which may not be achievable by most astronauts. The crew return vehicle, which returns the crew from the International Space Station to earth in the event of an emergency, may impose physical requirements that are challenging to debilitated astronauts. Long duration interplanetary missions may expose the crew to six months or more of weightlessness before arrival at their destination (30). Inflatable structure and pneumatic technology might pose addition human physical requirements for the crew on arrival at their destination (21, 48). Mission scenarios and spacecraft design may not afford recovery time for the crew. Thus, an understanding of the mechanisms underlying orthostatic hypotension and is critical in order to develop effective countermeasures so this debilitating condition will not interfere with the successful completion of mission objectives.



Mars Habitat Setup

Current Countermeasures

Countermeasures presently employed on the space shuttle and the Russian space station Mir include oral rehydration and aerobic exercise training with ergometers and treadmills.

Lower Body Negative Pressure Device

Aerobic training has been shown to effectively maintain aerobic capacity (52). Oral rehydration loses effectiveness as mission duration lengthens (58). Studies of in-flight training (9) and ground-based models (27) report that aerobic exercise produces no significant change in orthostatic tolerance. Lower Body Negative Pressure devices have been tested in space but are inadequate in providing a whole body pressure differential that is needed to prevent the effects of weightlessness on the cardiovascular system (32). The mechanisms of orthostatic intolerance must be elucidated to allow for better suited exercise-based and pharmacological countermeasures for long duration space flight.

OBJECTIVE

This study determines whether arteriolar vasodilator are up-regulated by simulated microgravity in skeletal muscles and may contribute to the orthostatic hypotension that occurs following space flight. Vasodilation induced through endothelium-dependent and endothelium-independent mechanisms are examined. As a secondary objective, possible mechanisms leading to diminished aerobic capacity will be examined.

THE CARDIOVASCULAR SYSTEM

The purpose of the cardiovascular system is to supply the body tissues with blood in amounts commensurate with their requirements for oxygen and nutrients. The highly elastic arteries transport blood from the heart to the tissues and serve as a pressure reservoir to continue driving blood forward when the heart is relaxing and filling. The mean arterial blood pressure is closely regulated to delivery an adequate blood supply to the tissues. The caliber of the highly muscular arterioles control the amount of blood that flows through a given tissue. Arteriolar caliber is subject to neural and hormonal control so that the distribution of the cardiac output can be rapidly adjusted to best serve the body's needs at any moment.

Blood Pressure Regulation

Regulation of mean arterial blood pressure is accomplished by controlling cardiac output and total peripheral resistance. Elaborate and complex mechanisms integrate in agonistic and antagonistic manners to regulate mean arterial pressure.

In concept:

Mean Arterial Pressure (MAP) = Cardiac Output (CO) x Total Peripheral Resistance (TPR)

Cardiac output, the volume of blood pumped by each ventricle per minute, is a measure of the flow rate of blood through the system. Blood flow depends on the frequency of contractions (heart rate) and the volume of blood ejected per contraction (stroke volume):

Cardiac Output (CO) = heart rate (HR) x stroke volume (SV)

So that

$$MAP = HR \times SV \times TPR$$

Heart rate is controlled primarily by autonomic influences on the sinoatrial node, the pacemaker of the heart. Minute to minute changes in heart rate are achieved mainly by changing

the slope of the pacemaker potential via nerves or hormones. Long term mechanisms are related to changes in extracellular electrolyte concentrations.

Stroke volume, the amount of blood pumped out by each ventricle during a beat, has both intrinsic and extrinsic controls that serve to increase the strength of contraction of the heart. Intrinsically, the cardiac muscle fibers respond to increased length before contraction so that increased venous return results in an increased stroke volume. Extrinsically, sympathetic stimulation via cardiac sympathetic nerves or epinephrine serve to enhance the heart's contractility (strength of contraction per end-diastolic volume).

Control of Peripheral Resistance

In humans, cardiac output is not significantly altered after space flight (Buckey, '96). Consequently, insufficient mean arterial pressure is most likely due to an inability to properly regulate total peripheral resistance. Vascular resistance is altered by changing the degree of vascular tone of vascular smooth muscle. The amount of resistance represents a balance between locally driven needs for matching tissue blood flow to tissue metabolism and a centrally directed need to maintain central arterial pressure. Regional resistance is adjusted in such a way that a given tissue receives the blood flow that is required for the maintenance of its metabolic activity. Total peripheral resistance is adjusted in such a way that mean arterial blood pressure is regulated at a desired level. Central nervous regulation of resistance is governed by receptors that respond to changes in arterial blood pressure. As a result, tissues that are metabolically active have low resistance to blood flow because the vasodilator actions of metabolites counteract centrally directed sympathetic vasoconstrictor influences; blood vessels in tissues that are not active are constricted by sympathetic nerves. In orthostatis (abruptly changing to an upright position), maintaining sufficient pressure and correspondingly maintaining adequate blood flow to the brain is the predominant function for elevating peripheral vascular resistance. Vascular tone is increased in the periphery to redirect blood to the central cavity and head region.

Vascular Smooth Muscle

Vascular smooth muscle is the tissue responsible for determining total peripheral resistance and the distribution of blood throughout the body. Smooth muscle fibers constitute a large percentage of the composition of the arteriolar wall. Relaxation of the vascular smooth muscle increases the diameter of the blood vessels, which subsequently decreases the resistance in the vessel. Vascular smooth muscle contracts via interaction of actin and myosin. Increased myoplasmic Ca2+ elicits contraction through voltage-gated calcium channels (electromechanical coupling), through receptor-mediated calcium channels (pharmacomechanical coupling) in the sarcolemma, and by release from the sarcoplasmic reticulum. Pharmacomechanical coupling, which occurs in response to humoral stimuli, is the predominant mechanism for eliciting contraction and relaxation. Substances that cause pharmacomechanical coupling include acetylcholine, adenosine, nitric oxide, catecholamines, CO2, histamine, angiotensin and prostaglandins.

Arterioles

The muscular arterioles, which have the highest resistance to flow in the vasculature, regulate regional blood flow to the capillary beds. These arteries have thick muscular walls that allow the vessel to vastly change its internal radius and resistance in response to changes in the level of contraction in their smooth muscle layer. Contractile impulses from sympathetic nerves, transmural pressure, and numerous local vasoconstrictor and vasodilator substances control the degree of arteriolar contraction.

Endothelium Mediated Regulation

Vasodilation occures through several mechanisms, a few of which involve the endothelium. The endothelium elicits vasoactive responses of the vascular smooh muscle.

Vasodilation Mechanisms

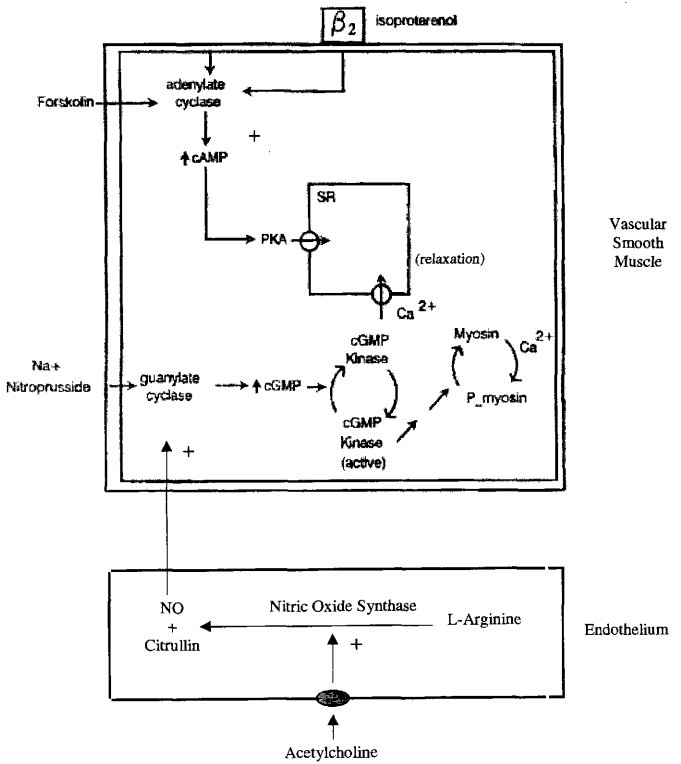


Figure 1

Blood, being relatively viscous, produces a friction force with the vessel wall in the form of shear stress (3):

$$\tau = 4\eta Q/\pi r^3$$

Since flow is directly proportional to shear stress, an increase in flow produces more shear stress on the vessel wall. It is hypothesized that the shear stress-induced mechanical deformation of the endothelium wall activates channels in the endothelium which initiate a cascade of events leading to vasodilation (1,2,3). Of great importance in dilation by this mechanism is the formation and release of the endothelium-derived relaxing factor (EDRF), which has been identified as nitric oxide. Nitric oxide is released from the endothelium in response to the shear stress consequent to the increase in velocity of flow. In the endothelium, it is thought that nitric oxide synthase converts L-Arginine to nitric oxide and citrullin (see Figure 1). The nitric oxide then diffuses to the smooth muscle and directs a cascade of events that lead to calcium sequestering in the sarcoplasmic reticulum. Stimulation of the endothelial cells by acetylcholine and other agents also causes the production and release of NO. Acetylcholine works through a same signal-transduction mechanism with numerous common features to flow-induced dilation, and so acetylcholine is used in this study to examine the nitric oxide mechanism.

Evidence exists to support the claim that certain vasodilatory mechanisms involve the endothelium (3). In arterioles denuded of endothelium, neither the NO-related vasodilatory agents nor increased flow induce vasodilation.

Vasodilator agents such as adenosine, CO2, and potassium are released from parenchymal tissue and act locally on the resistance vessels (2, 3). Prostacyclin relaxes vascular smooth muscle via an increase in the cyclic adenosine monophosphate (cAMP) concentration.

The endothelium is also capable of synthesizing endothelin, a very potent vasoconstrictor peptide which may be linked to hypertension, congestive heart failure, and atherosclerosis.

Endothelium Independent Dilation

The sympathetic nervous system influences the cardiovascular system mainly by altering the pattern and rate of efferent discharge and by changing the rate of liberation of catecholamines from the adrenal glands (1,3,4). Catecholemines (norepinephrine and epinephrine) are released in the blood and bind to specific β2 receptors on the surface of the vascular smooth muscle and cause dilation by a mechanism that is independent of the endothelium (see Figure 1). In the present study, isoproterenol was used to bind to β2-adrenergic receptors. This action directs an increase in cyclic AMP by adenylate cyclase, which leads to calcium sequestration and relaxation of the vascular smooth muscle. The resulting alteration in total and regional peripheral resistance and capacitance influences cardiac output as well as distribution.

Nitroprusside increases cGMP and subsequently induces dilation, but it acts directly on the vascular smooth muscle and therefore acts independently of the endothelium (see Figure 1). Forskolin activates adenylate cyclase directly in the smooth muscle without β2-adrenergic receptor binding.

Autoregulation

Autoregulation of the cardiovascular system represents the processes that arterioles employ to adjust vascular resistance to maintain a relatively constant blood flow in response to changes in perfusion (mean arterial) pressure (2,3). Arterioles maintain constant flow by proportionally altering vascular resistance in response to a change in arterial pressure; that is, flow is maintained because arterial resistance increases in response to an increase in perfusion pressure and decreases in response to a decrease in pressure. This response implies that the

arterioles actively contract or relax in response to an increase or decrease in arterial pressure, respectively. This type of autoregulation occurs through a myogenic mechanism.

Myogenic Response

The myogenic mechanism refers to a contraction of vascular smooth muscle that is elicited by an application of force to the muscle (1,3). In vivo the contraction is elicited by an increase in the intravascular or transmural pressure. The term can also be applied to the relaxation of vascular muscle that follows a reduction in the applied force or transmural pressure. According to the myogenic hypothesis, changes in arterial pressure alter the amount of tension in the vessel wall, which in turn stimulates release or suppression of stretch-activated arterial smooth muscle Ca2+ channels. For blood flow to remain constant at an elevated arterial pressure, the average arteriolar radius must decrease to a value less than that which existed prior to increase in arterial pressure. A stretch receptor would not be sensitive to this required radius. It has been suggested that wall tension (pressure x raduis) might be the sense variable responsible for myogenic autoregulation of blood flow.

$$\sigma \text{ (wall stress)} = Pr \text{ (pressure x radius)} / w \text{ (wall thickness)}$$

Transmural pressure, the difference in pressure between the extravascular space and the intravasuclar space, varies proportionally with pressure. A local increase in arterial pressure applies a greater pressure gradient between the intravascular space and the extravascular space. This altered pressure gradient is not returned to normal until the proper radius is achieved.

The myogenic response appears to explain autoregulation, although a tension sensor mechanism has not been fully elucidated.

Metabolic Regulation

The metabolic regulation mechanism proposes that the metabolic activity of local tissue regulates blood flow (2,3). A stimulus that results in an O2 supply that is inadequate for the

requirements of the tissue results in the formation of vasodilator metabolites. Lactic acid, CO2, and hydrogen ions fall short of producing the dilation observed under the physiological conditions of increased metabolic activity. Changes in O2 tension can evoke changes in the contractile state of vascular smooth muscles, but direct measurements of PO2 in the arterioles indicated that over a wide range of PO2, there is no correlation between O2 tension and arteriolar diameter. A change in local metabolites could cause inadequate vascular tone when astronauts return to earth.

MATERIALS AND METHODS

The methods in this study were approved by the Texas A&M University Institutional Animal Care and Use Committee. The investigation conforms with the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* [DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].

Animals

13 male Sprague-Dawley rats weighing approximately 200 grams were obtained (Charles River) and housed in a temperature-controlled (23+/- 2 C) room with a 12-h light-dark cycle. Water and rat chow were provided ad libitum. The rats were randomly assigned to either a hindlimb unloaded (HU, n=5) or control (C, n=8) group. The rats were weighed before hindlimb suspension. The hindlimbs of the *HU* rats were partially elevated with a harness attached to the tail. Two narrow strips of adhesive material (moleskin) were cut and placed on the proximal end of the ventral side of the tail, adjacent and parallel to both sides of the caudal artery to avoid compression of the tail artery. The tail was wrapped (Co-Flex bandage, Andover) and a previously molded plastic caste (X-lite splint, AOA/Kirshner Medical) was placed around the proximal two-thirds of the tail. The caste was laced with acrylic thread. A hook was attached to

the casted tail harness and then connected by a chain to a swivel apparatus at the top center of the cage. The tail was marked at the proximal end of the caste to monitor caste slippage. The height of the hindlimb elevation was adjusted to prevent the hindlimbs from touching supportive surfaces, resulting in a suspension angle of approximately 35-40°. The forelimbs maintained contact with the floor surface, which afforded the animals 360° horizontal circular translation. The HU rats were hindlimb unloaded for 2 weeks, a duration previously demonstrated to induce muscle atrophy (53), resting and exercise tachycardia (35), alterations in muscle blood flow (35, 57), and altered aortic vasomotor responses (18, 19). After the 2 week unloading period, the animals were weighed and anesthetized with sodium pentobarbital (35 mg/kg, I.P.) without allowing the hindlimbs to become weight bearing, and the gastrocnemius-plantaris-soleus muscle group was carefully dissected free from the hindlimbs and placed in a chilled (4 °C) filtered physiological saline buffer solution (PSS) (pH 7.4).

Microvessel Preparation

Under a dissecting microscope, the feed artery leading to the soleus (SOL) or superficial white portion of the gastrocnemius muscle (Gw) was identified and cut with microscissors. First-order (1A) arterioles were identified at the point where the feed artery entered the muscle or where a branch from the feed artery entered the muscle. 1A arterioles were dissected from the SOL or GW and transferred to a Lucite vessel chamber containing chilled PSS solution. One end of the microvessel was cannulated with a glass micropipette (40-55 (m in tip diameter), filled with filtered PSS-albumin solution (1 g bovine serum albumin/100 ml), and tied securely to the pipette with 11-0 ophthalmic suture. The other end of each vessel was cannulated with a second micropipette and secured with suture. After cannulation, the isolated vessel in the tissue chamber was transferred to the stage of an inverted microscope (Olympus IX70) coupled with a video camera (Panasonic BP310), video micrometer (Microcirculation Research Institute, Texas A&M

University), video recorder (Panasonic AG-1300), and a data acquisition system (Macintosh/MacLab). Vessels were then allowed to equilibrate for 1 hour at 37°C and 60 cm H2O intraluminal pressure before vasodilator properties were characterized; the bathing solution was replaced every 15 minutes during the equilibration period. Internal diameters were measured continuously throughout the experiment by videomicroscopic techniques (24, 31, 16).

Experimental Design

To maintain constant intraluminal pressure, the micropipettes cannulating the arterioles were connected to reservoir systems. Intraluminal pressure was measured through side arms of the two reservoir lines by low-volume displacement strain gauge transducers (Electromedics). The isolated vessels were pressurized at 60 cm H2O by setting both reservoirs at the same hydrostatic level. To determine vascular responses to vasodilator agonists, concentration response relationships were determined by the cumulative addition of acetylcholine, adenosine, isoproterenal and sodium nitroprusside. Maximal diameter with an intraluminal pressure of 60 cmH2O was determined after a 45 minute incubation in calcium-free PSS buffer and addition of 10-4 M sodium nitroprusside.

Solutions and Drugs

The PSS buffer contained (in mM) 145 NaCl, 4.7 KCl, 1.2 HaH2PO4, 1.17 MgSO4, 2.0 CaCl2, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 MOPS with a pH of 7.4. Calcium-free PSS buffer was similar to the PSS buffer except it contained 2 mM EDTA and CaCl2 was replaced with 2.0 mM NaCl. Concentrated stock solutions of ACH, ADO, ISOP, and NaNP were prepared in PSS buffer.

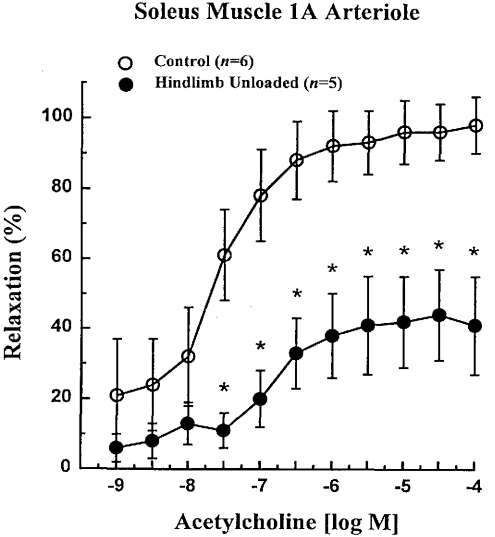


Figure 2

Data are expressed as mean ± SE. * significantly different $p < 0.05$

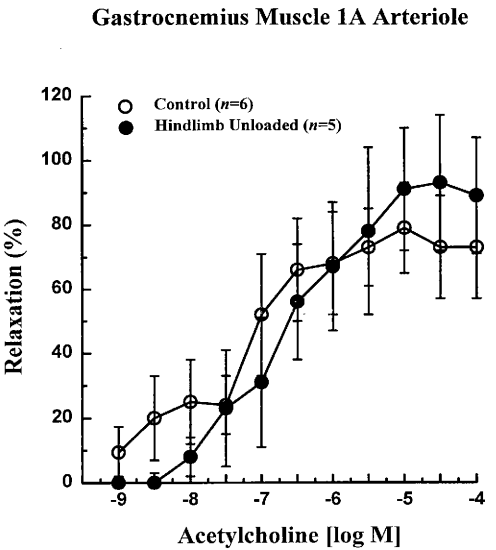


Figure 3

Data are expressed as mean ± SE.

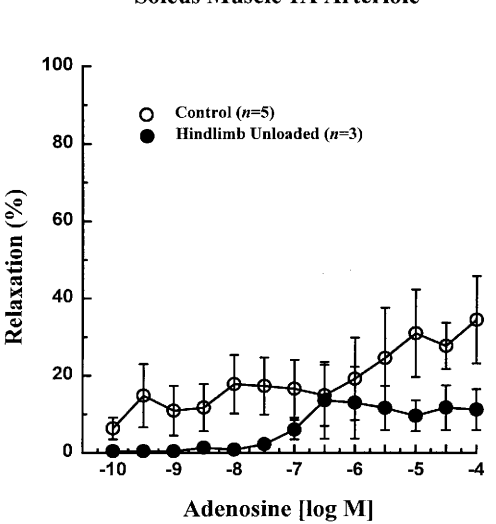


Figure 4

Data are expressed as mean ± SE.

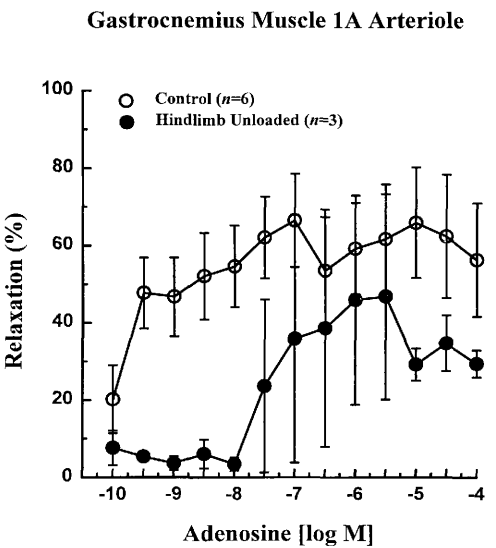


Figure 5

Data are expressed as mean ± SE.

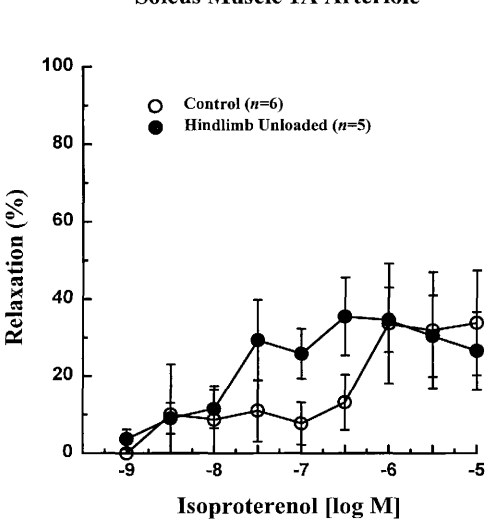


Figure 6

Data are expressed as mean ± SE.

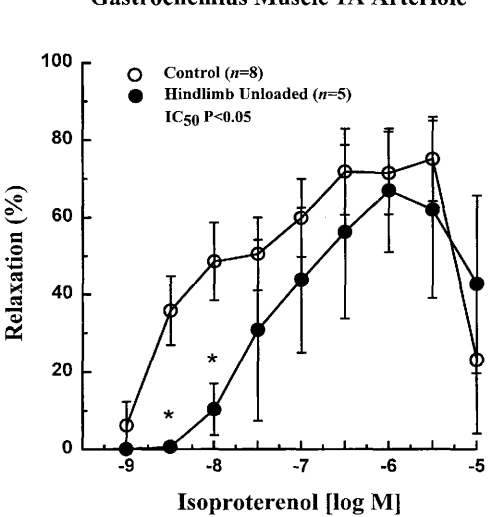


Figure 7

Data are expressed as mean ± SE. * significantly different $P < 0.05$

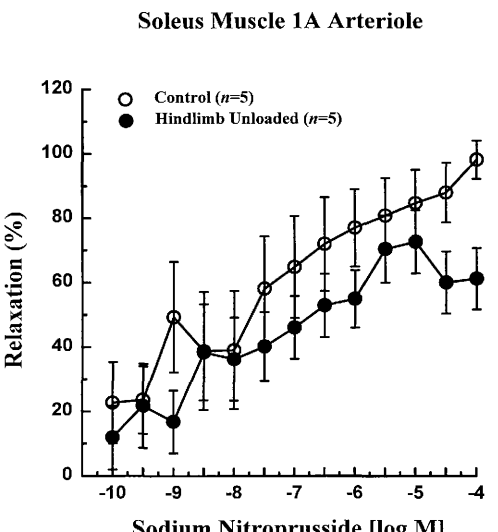


Figure 8

Data are expressed as mean ± SE.

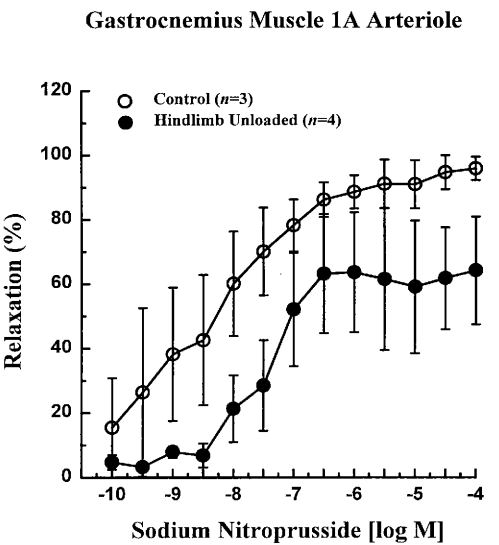


Figure 9

Data are expressed as mean ± SE.

Statistical Analysis

The vasodilator data were evaluated using repeated-measures analysis of variance with one within (agonist concentration) and one between (experimental groups) factor.

RESULTS

Vasodilator Responses

Acetylcholine produced dose-dependent increases in intraluminal diameter in vessels from gastrocnemius and soleus muscles. Arterioles from soleus muscle of hindlimb unloaded rats showed a decrease in percent dilation relative to control rats. Hindlimb unloading did not produce a significant change in sensitivity to acetylcholine. Percent dilation did not differ in arterioles from gastrocnemius muscle in control and hindlimb unloaded rats.

Concentration increases in extracellular isoproterenol produced increases in intraluminal diameter in arterioles from gastrocnemius and soleus muscle fibers. Dilatory responses were slightly decreased in gastrocnemius arterioles from hindlimb unloaded rats. Isoproterenol produced no significant difference in dilation for soleus muscle vessels from control and hindlimb unloaded rats.

DISCUSSION

The purpose of this study is to determine the effect of hindlimb unloading produces a significant change in vasodilatory responsiveness of skeletal muscle arterioles. The data indicate that hindlimb unloading does not enhance the dilation of resistance vessels. Acetylcholine-induced vasodilation was diminished in soleus muscle 1A arterioles and isoproterenol-induced vasodilation was reduced in gastrocnemius muscle 1A arterioles. Decreased dilation of the resistance vessels would serve to maintain upper body pressure. Thus, applying the HU model to

humans in space, weightlessness-induced alterations in vasodilatory properties of resistance vessels would not contribute to the hypotension experienced by humans returning to earth.

The diminished dilatory response to acetylcholine in soleus muscle agrees with the hypothesized decrease in shear stress. Soleus muscle, a postural muscle normally receives blood flow at a rate of approximately 100 *ml/min/*100g (McDonald). During hindlimb unloading, the postural muscle is unloaded and blood flow to the soleus decreases to less than 10 ml/min/100g (McDonald). Since gastrocnemius muscle blood flow is less metabolically active than soleus muscle, the alteration in shear stress seen in soleus aterioles does not occur in the gastrocnemius muscle arterioles. Correspondingly, acetylcholine-induced dilation is also unaltered. Further research has begun to investigate whether the alteration in the dilatory mechanisms in soleus muscle occurs in the endothelium or in the smooth muscle.

Shear stress is directly proportional to blood flow through the vessel and the radius of the vessel. During exercise, the blood flow to skeletal muscle increases, and the vessels feeding these muscles subsequently enlarge. During tail suspension, blood flow to soleus muscle decreases (), which may cause diminished production of nitric oxide synthase in the endothelium. In addition, structural remodeling of the soleus muscle vasculature may occur. Delp et al (Delp, Colleran, Wilkerson, Muller-Delp, unpublished observations) found that hindlimb unloading reduces the maximal diameter of soleus muscle arterioles without altering the medial wall thickness and that the alteration can be attributed to a decrease in wall shear stress.

The correlation between diminished dilation to acetylcholine and diminished shear stress in soleus muscle arterioles provides a possible mechanism for weightlessness-induced diminished aerobic capacity. The reduction in blood flow to normally metabolically active muscles in simulated weightlessness (McDonald) and the corresponding reduction in endothelium-dependent dilation found in this study could alter peripheral resistance to the extent that aerobic capacity is compromised.

In an extension of this study that is currently underway by the author, dose response curves to sodium nitroprusside are being produced using methods identical to the methods in this study. Sodium nitroprusside, an exogenous drug which increases cGMP and produces vasodilation, acts directly on the vascular smooth muscle and is not endothelium-mediated. If nitroprusside does not indicate a marked decrease in percent dilation, the alteration is most likely endothelium-based. If nitroprusside produces a dose response curve similar to that for acetylcholine in soleus, the down-regulation of vasodilation probably occurs in the vascular smooth muscle surrounding the endothelium (see Figure 1). Currently the sodium nitroprusside data is incomplete and conclusions regarding this vasodilator cannot be made at this time.

The data from adenosine, while currently incomplete, is being investigated for dose response trends. However, the preliminary data indicate that vasodilation induced by this metabolite in both soleus and gastronemius muscle arterioles is diminished by hindlimb unloading.

In the dose response curves for isoproterenol in the gastrocnemius muscle, both control and hindlimb suspension percent relaxation significantly decreases at 10-5 M. This effect may be caused by a change in receptor specificity of the isoproternol. Up to a certain concentration, isoproterenol preferentionally binds to β receptors, and in vascular tissues to $\beta 2$ receptors. At high concentrations, isoproterenal begins to bind to α receptors, which induce constriction of the vessel.

The data show that vasodilation mediated by β-adrenergic receptors may be down-regulated. It can be hypothesized that symphathetic stress induced by the hindlimb unloading desensitizes the arterioles to symphathic stimulation. The stress induced by hindlimb unloading could increase catecholimine release in the blood. A chronic increase in β receptor activation has been shown to lead to desensitization. To address this hypothesis, forskolin, a pharmacological agent that directly activates adenylate cyclase in the vascular smooth muscle, can be used to

determine whether the β2 receptors are altered or if the signal transduction mechanism is altered. If forskolin produces a dose response curve that is similar in arterioles from control and hindlimb unloaded rats, then the data indicate that β2 receptor sensitivity or density is altered by hindlimb unloading.

CONCLUSION

The primary objective of this study is to determine the role of vasodilatory mechanisms in weightlessness-induced orthostatic hypotension. The results indicate that hindlimb suspension does not significantly enhance dilatory properties of arterioles. Thus, vasodilatory mechanisms of arterioles do not play a significant role in causing orthostatic hypotension. This study is significant in eliminating vasodilatory responsiveness of arterioles as a factor in orthostatic hypotension.

The decrease in vasodilator responsiveness may contribute to the reduced aerobic capactiy (VO2 max) experienced by astronauts. Compromise dilatory response of the peripheral vascular could necessarily diminish total body aerobic capacity.

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REFERENCES

1. Ackermann, Uwe. *Essentials of Human Physiology.* St. Louis, Mosby Year Book: 1992.
2. Bell, David R. *Core Concepts in Physiology.* New York, Lippincott – Raven publishers: 1998.
3. Berne, Robert M., and Matthew N. Levy. *Cardiovascular Physiology.* St. Louis, Mosby: 1997.
4. Bevan, J.A., R.D. Bevan and S.P. Duckles. Adrenergic regulation of vascular smooth muscle. Ed. S. Greiger. *The Handbook of Physiology: The Cardiovascular System,* vol. 2, Bethesda, Maryland: American Physiology Society, pp. 515-534, 1980.
5. Blomqust, C. G. Regulation of the systemic circulation at microgravity and during readaptation to 1G. *Med Sci Sport Exerc* 28: S9-S13, 1996.
6. Blomqvist, C.G. and J.L. Stone. Cardiovascular adjustments to gravitational stress, In: *Handbook of Physiology, Peripheral Circulation and Organ Blood Flow,* ed. J.T. Shepherd and F. M. Abbound. Bethesday, MD: Am Physiol. Soc., 1983, sec. 2, vol. 3, part 2, pp. 1027-1063.
7. Buckey, J.C., L.D. Lane, B.D. Levine, D.E. Watenpaugh, S.J. Wright, W.E. Moore, F.A. Gaffney, and C.G. Blomqust. Orthostatic intolerance after spaceflight. *J Appl Physiol* 81:7-18, 1996.
8. Bungo, M. W., J. V. Charles, and P. C. Johnson. Cardiovascular deconditioning during space flight and the use of saline as a countermeasure to orthostatic intolerance. *Aviat. Space Environ. Med.* 56: 985-990, 1985.
9. Convertino, V.A. Physiological adaptations to weightlessness effects on exercise and work performance. *Exerc. Sport Sci. Rev.* 18: 119-165m 1990.

10. Convertino, V. A. Carotid-cardiac baroreflex: relation with orthostatic hypotension following simulated microgravity and implications for development of countermeasures. *Acta Astronautica* 23: 9-17, 1991.

11. Convertino, V. A. Adaptation of baroreflexes and orthostatic hypotension. In: *Vascular Medicine,* edited by H. Boccalone. Amsterdam: Elsevier Sci. Pub., 1993, pp. 573-577.

12. Convertino, V. A., D. F. Doerr, D. L. Eckberg, J. M. Fritsh and J. Vernikos-Danellis. Head-down bedrest impairs vagal baroreflex responses and provokes orthostatic hypotension. *J. Appl. Physiol.: Respir. Environ. Exerc. Physiol.* 68: 1458-1464, 1990.

13. Convertino, V. A., J. L. Polet, K. A. Engelke, G. W. Hoffler, L. D. Lane, and C. G. Blomquist. Evidence for increased β-adrenoreceptor responsiveness induced by 14 days of simulated microgravity in humans. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42) R93-R99, 1997.

14. Dawson, Thomas. *Engineering Design of the Cardiovascular System of Mammals.* Englewood Cliffs, New Jersey, Prentice Hall: 1991.

15. Deavers, D. R., E. J. Musacchia, and G. A. Meininger. Model for antiorthostatic hypokinesia: head down tilt effects on water and salt excretion. *J. Appl. Physiol.* 49: 576-582, 1980.

16. Delp, M.D., Myogenic and vasoconstrictor responsiveness of skeletal muscle arterioles is diminished by hindlimb unloading. *J. Appl. Physiol.* 86: 1178-1184, 1999.

17. Delp M. D., Armstrong, R. B. Blood flow in normal and denervated muscle during exercise in conscious rats. *Am J Physiol.* 255: H1509-1515, 1988.

18. Delp, M.D., M. Brown, M.H. Laughlin, and E.M. Hasser. Rat aortic vasoreactivity is altered by old age and hindlimb unloading. *J. Appl. Physiol.* 78: 2079-2086, 1995.

19. Delp, M.D., T. Holder-Binkley, M.H. Laughlin, and E.M. Hasser. Vasoconstrictor properties of rat aorta are diminished by hindlimb unweighting. *J. Appl. Physiol.* 75: 2620-2628, 1993.

20. Desplanches, D., M. H. Mayet, B. Sempore J. Frutoses, and R. Flandrois. Effect of Spontaneous recover or retraining after hindlimb suspension on aerobic capacity. *J. Appl. Physiol.* 63: 1739-1743, 1987.

21. Drake, Brett, ed. *Reference Mission Version 3.0. Addendum to the Human Exploration of Mars: The Reference Mission of the NASA Mars Exploration Team.* EX13-98-036: June, 1998.

22. Drexler, H., A. M. Zeiher, E. Bassenge, H. Just, eds. *Endothelial Mechanisms of Vasomotor Control.* Darmstadt: Steinkopff; New York: Springer: 1991.

23. Dunn, C. D., P. C. Johnson, and R. D. Lange. Regulation of hematopoiesis in rats exposed to antiorthostatic hypokinetic/hypodynamia. II. Mechansims of the "anemia." *Avia. Space Environ. Med.* 57: 36-44, 1986.

24. Falcone, J.C., M.J. Davis, and G.A. Meininger. Endothelial independence of myogenic response in isolated skeletal muscle arterioles. *Am. J. Physiol.* 260 (*Heart Circ. Physiol.* 29: H130-H135, 1991.

25. Fox, Robert and Alan McDonald. *Introduction to Fluid Mechanics.* New York: John Wiley and Sons: 1992.

26. Gray S. D., Responsiveness of the terminal vascular bed in fast and slow skeletal muscles to alpha-adrenergic stimulation. *Angiologia* 1971, 8: 285-296.

27. Greenleaf, J. E., C. E. Wade, and G. Leftheriotis. Orthostatic responses following 30-day bed rest deconditioning with isotonic and isokinetic exercise training. *Aviat. Space environ. Med.* 60: 537-542, 1989.

28. Hargens A. R., Steakai J., Johansson C., Tipton C. M. Tissue fluid shift, forelimb loading, and tail tension in tail-suspended rats. *Physiologist (Suppl).* 1984; 27:S37-S38.

29. Hoffler, G. W. Cardiovascular studies of U.S. space crews: an overview and perspective. In: *Cardiovascular Flow Dynamics and Measurements*, edited by N. H. C. Hwang and N. A. Normann. Baltimore: University Park Press, 1977, pp. 335-363.

30. Hoffman, Steven and David Kaplan, ed. *Human Exploration of Mars: The Reference Mission of the NASA Mars Exploration Study Team.* NASA Special Publication 6107: July 1997.

31. Jasperse, J.L., and J.H. Laughlin. Flow-induced dilation of rat soleus feed arteries. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H2423-H2427, 1997.

32. Johnson, R. L., G. W. Hoffler, A. Nicogossian, S. A. Bergman, and M. M. Jackson. Lower body negative pressure: third manned Skylab mission. In: *Biomedical Results from Skylab,* edited by R. S. Johnson and L. F. Dietlein. Washington, DC: National Aeronautics and Space Administration, 1977, P. 284-312. (NASA SP-377)

33. Kroemer, K. H., H. J. Kroemer and K. E. Kroemer-elbert. *Engineering Physiology.* Albany, Van Nostrand Reinhold: 1997.

34. Maurel D., Ixart G., Barbanel G. Mekaouche M., Assenmacher I. Effects of acute tilt from orthostatic to head-down antiorthostatic restraint and of sustained restraint on the introcerebroventricular pressure in rats. *Brain Res.* 1996, 736: 165-173.

35. McDonald, K.S., M.D. Delp and R.H. Fitts. Effects of hindlimb unweighting on tissue blood flow in the rat. *J. Appl. Physiol.* 72: 2210-2218, 1992.

36. McDonald, K. S., M. D. Delp, Fatigability and blood flow in the rat gastrocnemius-lantaris-soleus after hindlimb suspension. *J. Appl. Physiol.* 73: 1135-1140, 1992.

37. Melada, B.A., R.H. Goldman, J.A. Leutscher, and P.G. Zager. Hemodynamics, renal function, plasma renin and aldosterone in man after 5-14 days of bedrest. *Aviat. Space Environ. Med.* 46: 1049-1055, 1975.

38. Moffit, J. A., Foley, C. M. Schadt, J. C. Attenuated baroreflex control of sympathetic nerve activity after cardiovascular deconditioning in rats. *Am. J. of Physiol.* V. 274 No. 5: 1397-1405, 1998.

39. Muluagh, S.L., J.B. Charles, J.M. Riddle, T.L. Rehbein, and M.W. Bundo. Echocardiographic evaluation of the cardiovascular effects of short duration space flight. *J. Clin. Pharmacol.* 31:1024-1026, 1991.

40. Musacchia X. J., Steffen J. M., Fell R. D, Dombrowski M. J. Skeletal muscle response to spaceflight, whole body suspension, and recovery in rats. *J Appl Physiol.* 1990, 69: 2248-2253.

41. Musacchia X. J., Deavers D. R., Meininger G. A., Fluid/electrolyte balance and cardiovascular responses: Head-down tilted rats. *Physiologist (Suppl).* 1990, 33: S47-S47.

42. Overton, J.M. , and C.M. Tipton. Effect of hindlimb suspension on cardiovascular responses to sympathomimietics and lower body negative pressure. *J. Appl. Physiol.* 68:355-362, 1990.

43. Overton, J.M., C.R. Woodman, and C.M. Tipton. Effect of hindlimb suspension on VO2max and regional blood flow responses to exercise. . *Appl. Physiol.* 66:653-659, 1989.

44. Provost, S. B., Tucker B. J. Effect of 14 day head-down tilt on renal function and vascular and extracellular fluid volumes in the conscious rat. *Physiologist (Suppl).* 1992; 35: S-105-S-106.

45. Sandler, H. Cardiovascular effects of inactivity. In: *Inactivity: Physiological Effectrs,* edited by H. Sandler and J. Vernikos. Orlando, FL: Academic, 1986, pp. 1-9.

46. Sandler, H., D.J. Goldwater, R.L. Popp, L. Spacavento, and D.C. Harrison. Beta blockade in the compensation for bedrest cardiovascular deconditioning: Physiological and pharmacological observations. *Am. J. Cardiol.* 55:114D-120K, 1985.

47. Schmid, P.G., M. McCally, T.E. Piemme, and J. A. Shaver. Effects of bed rest on foremarm vascular responses to tyramine and norepinephrine. In: *Hypogravic and Hypodynamic Environments,* edited by R. H. Murray and M. McCally. Washington DC:NASA, 1971, SP-269, pp. 211-223.

48. Schneider, William C. *Mars Combo Lander Design Study* (Team project presentation). July 31, 1998.

49. Shellock F.G., Swan. JH. J., Rubin S. A. Early central venous pressure changes in the rat during two different levels of head-down suspension. *Aviat Space Envron Med.* 1985; 56: 791-795.

50. Shoemaker, J. K., C. S. Hogeman, U. A. Leuenberger, M. D. Herr, K. G. Gray, D. H. Silber, and L. I. Sinoway. Sympathetic discharge and vascular resistance after bed rest. *J. Appl. Physiol.* V. 81 pp. 612-617, 1998.

51. Shoemaker, J. K., C. S. Hogeman, D. H. Silber, M. D. Herr, K. G. Gray, D. H. Silber, and L. I. Sinoway. Head-down-tilt bed rest alters forearm vasodilator and vasoconstrictor responses. *J. Appl. Physiol.* Vol. 82 No. 5 pp. 1756-1762, 1998.

52. Tischler, Marc E. Space travel, biochemistry and physiology. Ed. R. Dulbecco. *Encyclopediea of Human Biology*, vol. 8, San Diego: Academic Press, pp. 9 97-106, 1997.

53. Thomason, D.B., and F.W. Booth. Atrophy of the soleus muscle by hindlimb unweighting. *J. Appl. Physiol.* 68: 1-12, 1990.

54. Watenpaugh, D.E., and A.R. Hargens. The cardiovascular system in microgravity. Ed. M.F. Fregly and C.M. Blatteis. *The Handbook of Physiology: Environmental Physiology,* vol. 1, New York: Oxford University Press, pp. 631-674, 1996.

55. West, J. B. Spacelab: the coming of age of space physiology research. J. Appl. Physiol. 57: 1625-1631, 1984.

56. Witzmann, F. A., K. H. Kim, and R. H. Fitts. Effect of hindlimb immobilization on the fatigability of skeletal muscle. *J. Appl. Physiol.* 73: 90S-93S, 1992.

57. Woodman, C.R., L.A. Sebastian, and C.M. Tipton. Influence of simulated microgravity on cardiac output and blood flow distribution during exercise. *J. Appl. Physiol.* 79: 1762-1768, 1995.

58. Vernikos, J., M.V. Dallman, G. van Loon, and L.C. Keil. Drug effects on orthostatic intolerance induced by bedrest. *J. Clin. Pharmacol.* 31: 974-984, 1991.

59. Vissing S. F., Scherrer U., Victor R. G. Relation between sympathetic outflow and vascular resistance in the calf during perturbations n central venous pressure. Evidence for cardiopulmonary afferent regulation of calf vascular resistance n humans. *Circ Res.* 1989, 65: 1710-1717.