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TRANSLATIONAL PHYSIOLOGY

Periodic acceleration: effects on vasoactive, fibrinolytic, and coagulation factors

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Adams, Jose A., Jorge Bassuk, Dongmei Wu, Maria Grana, Paul Kurlansky, and Marvin A. Sackner. Periodic acceleration: effects on vasoactive, fibrinolytic, and coagulation factors. *J Appl Physiol* 98: 1083–1090, 2005. First published October 22, 2004; doi:10.1152/japplphysiol.00662.2004.—Cellular and isolated vessel experiments have shown that pulsatile and laminar shear stress to the endothelium produces significant release of mediators into the circulation. Periodic acceleration (pG_z) applied to the whole body in the direction of the spinal axis adds pulses to the circulation, thereby increasing pulsatile and shear stress to the endothelium that should also cause release of mediators into the circulation. The purpose of this study was to determine whether addition of pulses to the circulation through pG_z would be sufficient to increase shear stress in whole animals and to acutely release mediators and how such a physical maneuver might affect coagulation factors. Randomized control experiments were performed on anesthetized, supine piglets. The treatment group (pG_z) ($n = 12$) received pG_z with a motion platform that moved them repetitively head to foot at ± 0.4 *g* at 180 cpm for 60 min. The control group ($n = 6$) was secured to the platform but remained on conventional ventilation throughout the 4-h protocol. Compared with control animals and baseline, pulsatile stress produced significant increases of serum nitrite, prostacyclin, PGE_2, and tissue plasminogen activator antigen and activity, as well as D-dimer. There were no significant changes in epinephrine, norepinephrine, cortisol, and coagulation factors between groups or from baseline values. Pulsatile and laminar shear stress to the endothelium induced by pG_z safely produces increases of vasoactive and fibrinolytic activity. pG_z has potential to achieve mediator-related benefits from the actions of nitric oxide and prostaglandins.

nitric oxide; prostaglandins; endothelin; pulsatile shear stress; endothelium

PERIODIC ACCELERATION is a novel method of cardiopulmonary support and ventilation. Periodic acceleration is achieved by a motion platform that supports the body and moves the supine body in a headward-footward repetitive motion at frequencies of 1–6 Hz and a displacement of the platform of 1–4 cm. The latter generates acceleration forces of 0.2–1 G_z. This back-and-forth motion is similar to that which is observed when a baby carriage is pushed back and forth, generating similar G_z forces. Periodic acceleration of the body also produces changes in intrapleural pressure. Our laboratory has shown that periodic acceleration is able to ventilate paralyzed sedated animals with normal and diseased lungs (1, 3). Furthermore, regional blood flow is increased in all organs during periodic acceleration, with a modest increase in cardiac output. Periodic acceleration also causes significant increase in serum nitrite and elicits additional pulsations in the vascular stream including beat frequency. The increase in regional blood flow and pulsatility increases shear stress and circumferential stretch on the vascular endothelium (2). In isolated perfused vessels, our laboratory and others have shown that flow induced shear stress, and combined flow and pulsatile shear stress significantly increase nitric oxide production (4, 26).

Increased shear stress to the endothelium causes release of several mediators such as nitric oxide, prostacyclin, and tissue plasminogen activator that affect vasomotor tone, fibrinolysis, and coagulation (6, 15, 46). In isolated, perfused blood vessels or endothelial layers, increased flow over the endothelium increases shear stress to the endothelium, thereby increasing production of nitric oxide (4, 45). In isolated blood vessels, pulsatile circumferential stress is frequency encoded (26).

Almost pure increase of pulsatile shear stress in animals and humans can be accomplished by rapid atrial pacing, intra-aortic balloon counterpulsation, external counterpulsation (10, 50, 52), and periodic acceleration (2–4).

Although the acute effects of pulsatile shear stress on vasoactive and fibrinolytic factors have been reported in isolated vessels and endothelial cell preparations, data for whole animal models are unavailable. The purpose of this study was to determine the acute effects on these parameters in a whole animal preparation by increasing both shear stress and pulsatile circumferential stress through periodic acceleration.

MATERIALS AND METHODS

Animal preparation. These studies were approved by the Institutional Animal Care and Use Committee and comply with the Animal Welfare Act. Eighteen juvenile piglets, weighing between 10 and 14 kg, were anesthetized with intramuscular ketamine (10 mg/kg) and an intravenous propofol (10 mg/kg) bolus, followed by titration until a surgical plane was reached. Propofol was administered as a continuous infusion of 0.2 $mg \cdot kg^{-1} \cdot h^{-1}$ and maintained throughout the experiment. Skeletal muscular paralysis was induced with pancuronium bromide at 0.1 mg/kg and supplemented throughout the experiment along with sedation as necessary. Endotracheal intubation was carried out with a 5.0 cuff endotracheal tube. Measurement of airway pressure was obtained at its proximal end with a variable reluctance pressure transducer (model MP45, full scale range ± 50 cmH_2O,

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Validyne Engineering, Northridge, CA). The airway pressure transducer was oriented so that the diaphragm of the transducer was 90°, relative to the *z*-axis of the animal. The femoral artery was cannulated for measurement of mean arterial blood pressure (MAP) via a pressure transducer (Transpac, Abbott Critical Care Systems, North Chicago, IL) and for arterial blood sampling. All fluid-filled transducers were stabilized at the level of the heart and away from the motion platform. A right atrial catheter was placed via the left external jugular vein for administration of fluids and drugs. Arterial blood gases were measured with a blood-gas analyzer (Rapid Lab TM348, Bayer Diagnostics, Tarrytown, NY).

The animals were maintained at 38°C with a thermostatically controlled warming pad. The piglet was placed on a motion platform (prototype AT 101, Non-Invasive Monitoring Systems, North Bay Village, FL) in the supine posture with the front and hind legs tied securely to the platform to couple it closely to platform. The endotracheal tube was connected to a pressure-limited ventilator (Bear Cub BP-200, Inter Med): frequency 14–20 breaths/min, peak inspiratory pressure 18–24 cmH_2O, and positive end-expiratory pressure 5 cmH_2O. Initial settings on the mechanical ventilator were adjusted to achieve arterial P_{CO_2} at ~35–45 mmHg. All animals were paralyzed with pancuronium bromide (0.1 mg/kg). All animals received 100% O_2 during the entire protocol. No heparin or anticoagulants were used during the entire experimental protocol.

Periodic acceleration was applied with the motion platform as previously reported (1–4). Briefly, a plywood platform was fixed to a linear displacement direct-current motor (model 400, 12v; APS Dynamics, Carlsbad, CA). The motor was powered by a dual-mode power amplifier (model 144, APS Dynamics) connected to a sine wave controller (model 140-072; Non-Invasive Monitoring Systems). The controller permitted control of frequency, linear displacement of the platform, and duty cycle of the motor. The unit has a maximum weight capacity of 30 kg and is capable of operating at a frequency between 0.5 and 10 Hz with ±0.1 to ±1.5 *g*. The animals were secured to the platform, and acceleration was continuously measured with an accelerometer. During periodic acceleration, the endotracheal tube of the paralyzed animals was connected to a bias flow of 100% O_2, and continuous positive airway pressure of 5 cmH_2O was applied to maintain oxygenation and functional residual capacity. During periodic acceleration, there was no other method of ventilatory support other than the ventilation imparted by the motion platform.

Arterial blood gases were measured every 30 min or as needed, and an electrocardiogram was continuously monitored in a three-wire lead configuration. The analog signals from the transducers, accelerometer, and ECG were continuously recorded on a data-acquisition processor (Powerlab, Grand Junction, CO).

Experimental design. The initial postinstrumentation stabilization period of 30 min consisted of maintaining the animal on a pressure-limited ventilator set with peak inspiratory pressures of 15–18 cmH_2O, positive end-expiratory pressure of 5 cmH_2O, frequency of 15–20 breaths/min, and inspired O_2 fraction of 1.0. Thereafter, settings of the ventilator were adjusted to maintain normal arterial blood gases. Baseline measurements of hemodynamic parameters and blood gases were obtained on all animals. A total of 18 animals were randomized to *1*) periodic acceleration (pG_z) or *2*) control group. Periodic acceleration was applied at a frequency of 3 Hz with ±0.4 *g*. Periodic acceleration was also used as the means of ventilatory support in these paralyzed piglets (1). Periodic acceleration was applied for 1 h, and blood gases were obtained every 30 min. After completion of periodic acceleration, the endotracheal tube was connected to the pressure-limited ventilator for 3 h. The control group was secured to the periodic acceleration platform, but the latter was not turned on; this group remained on conventional mechanical ventilation for the entire 4-h protocol period. Blood was collected for analysis, and the animals were euthanized with an overdose of pentobarbital at the end of the 4 h.

Arterial samples were collected for analysis of vasoactive, fibrinolytic, and coagulation factors at baseline, after 60 min of periodic acceleration, and 180 min after cessation of periodic acceleration. Blood was placed into tubes containing 3.8% sodium citrate for immediate analysis of prothrombin time, activated partial thromboplastin time, thrombin time, plasminogen activator inhibitor, Factor VII, and Factor VIII. Blood for tissue plasminogen activator antigen (t-PA antigen) was collected in Stabilyte (Biopool, Ventura, CA). Blood was collected in a glass tube, allowed to clot, and then centrifuged to separate the serum and freeze it. The serum was analyzed for PGE_2, 6-keto-PGF_1-α (prostacyclin stable metabolite), endothelin-1, cortisol, epinephrine, and norepinephrine.

Prothrombin time, activated partial thromboplastin times, and fibrinogen were determined by an automated method Electra 1600 C (Beckman Coulter, Fullerton, CA). Thrombin time was determined by the electromechanical method of Start 4 (Diagnostica Stago, Parsippany, NJ). Plasminogen activator inhibitor was performed by a two-stage indirect enzymatic assay, using the Spectrolyse kit (Biopool) (17). D-dimer was measured by using a monoclonal antibody to D-dimer and the spectrophotometric assay of IL-Test D-dimer (Beckman Coulter, Fullerton, CA) (21, 32). t-PA antigen and tissue plasminogen activator activity (t-PA activity) were assayed with a plasma enzyme immunoassay (Biopool TintElize, Biopool) (7, 21, 30, 41). The Hemostasis Reference Laboratory (Hamilton, Canada) carried out the aforementioned tests.

PGE_2 was determined by a specific enzyme immune assay (EIA), PGE_2 EIA kit (Cayman Chemical, Ann Arbor, MI) (22, 34). The stable metabolite of prostacyclin, 6-keto-PGF_1-α, was assayed with a specific EIA 6-keto-PGF_1-α kit (Cayman Chemical) (20, 43). Endothelin-1 was assayed by an EIA endothelin-1 kit (Cayman Chemical) (12, 33). Epinephrine and norepinephrine were determined by high-pressure liquid chromatography. Cortisol levels were determined by radioimmunoassay. The Endocrine Reference Laboratory (Pennsylvania State University, Hershey, PA) performed the above assays.

Serum nitrites were determined as an indirect estimate of nitric oxide using the methods of Berkels et al. (8) and Zhang and Broderick (55). Nitrite was determined by an amperometric nitric oxide electrode (World Precision Instruments, Sarasota, FL). A chemical titration calibration was performed with use of an acidic iodide solution (0.1 mol/l H_2SO_4, 0.14 mol/l K_2SO_4, 0.1 mol/l KI) against varied volumes of KNO_2. NO was formed stoichiometrically and measured directly. A standard curve was constructed from the preceding with a plot of picoamperes vs. nitric oxide in nanomoles. The quantity of nitric oxide was converted to nitrite and expressed as nanomoles per liter.

In eight piglets in the pG_z group and six in the control group, Factor VII and Factor VIII activities were measured during the same time periods that the blood for coagulation and vasoactive factors were collected.

Statistical analysis. All data that followed a Gaussian distribution frequency were analyzed by one-way ANOVA with Newman-Keuls correction for multiple comparisons or the unpaired *t*-test. Data that were not normally distributed were analyzed with nonparametric analysis by the Kruskal-Wallis ANOVA and median test and comparison between groups. Data are expressed as means and SD, and statistical significance was set at $P < 0.05$.

RESULTS

Hemodynamics and blood gases. Figure 1 depicts representative femoral arterial waveforms at baseline and those during periodic acceleration. The added pulses with periodic acceleration are superimposed on the natural pulse. Arterial blood gases and acid-base status remained normal throughout the entire protocol for both groups. There were no significant differences in blood gases from baseline during or after peri-

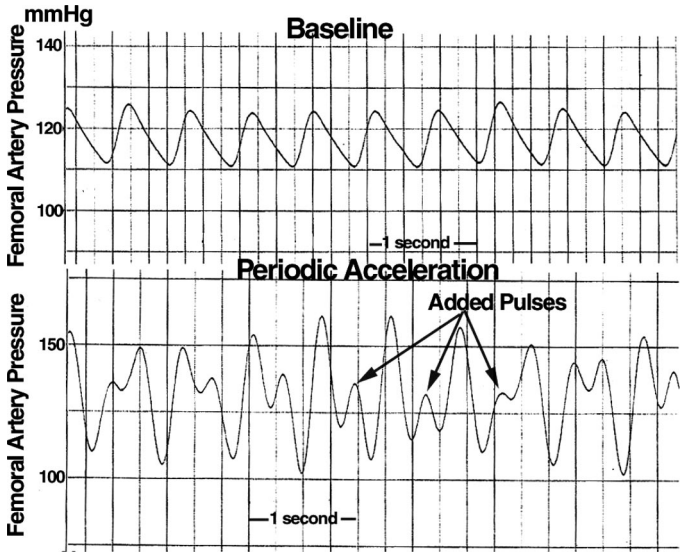

Fig. 1. Representative tracings of femoral artery blood pressure tracings. *Top*: baseline period. *Bottom*: periodic acceleration (pG_z). Note the presence of added pulses to the tracings due to pG_z.

odic acceleration or in the control group. Figure 2 depicts MAP and heart rate during the study. Mean MAP decreased from 107 mmHg at baseline to 88 mmHg during periodic acceleration ($P < 0.05$) and returned to baseline values after periodic acceleration was discontinued. There was a significant difference between control and pG_z groups at 30 min. Heart rate did not change during periodic acceleration or control period.

Vasoactive factors. Serum nitrite rose from a mean of 28 (SD 20) nmol/l at baseline to 160 (SD 36) nmol/l during periodic acceleration and remained elevated 180 min after its discontinuance at 163 (SD 39) nmol/l ($P < 0.01$). These changes were significantly different from the control group of 35 (SD 20), 40 (SD 19), and 32 (SD 20) nmol/l at baseline, 60 min, and 180 min, respectively ($P < 0.05$). 6-Keto-PGF_1-α rose from a mean of 154 (SD 25) pg/ml at baseline to 192 (SD 33) pg/ml during periodic acceleration ($P < 0.05$) and remained greater than baseline at 223 (SD 19) pg/ml 180 min after periodic acceleration was discontinued ($P < 0.01$). For the control group, there were no significant differences from baseline or from the pG_z group. PGE_2 rose from mean 246 (SD 68) pg/ml at baseline to 354 (SD 90) pg/ml during periodic acceleration and remained elevated at 451 (SD 108) pg/ml 180 min after its discontinuance ($P < 0.05$). PGE_2 did not significantly change over time in the control group. Figure 3 is a graphical representation of these levels. Endothelin-1 did not significantly change in the control or pG_z groups over time or between groups. Neither serum epinephrine nor norepinephrine values during periodic acceleration and after its discontinuance differed from baseline. Epinephrine levels were 3.6 (SD 1.1), 4.7 (SD 2.7), and 3.4 (SD 1.9) nmol/l at baseline, during periodic acceleration, and 180 min after its discontinuance, respectively ($P > 0.05$). Corresponding norepinephrine levels were 8.3 (SD 5.1), 6.6 (SD 6.9), and 2.0 (SD 1.5) nmol/l, respectively ($P > 0.05$). Similarly, control group epinephrine levels were 4 (SD 2), 3.8 (SD 2), and 3.7 (SD 1) nmol/l at baseline, 60 min, and 180 min, respectively. Norepinephrine in the control group did not differ from periodic acceleration, with levels of 8.5 (SD 3), 7.8 (SD 3), and 5.0 (SD 3) nmol/l at the same time points.

Fibrinolytic factors. Table 1 and Fig. 4 depict the values for coagulation and fibrinolytic factors for both control and pG_z groups over time. Tissue plasminogen activator antigen (t-PA antigen) was mean 0.7 (SD 0.8) ng/ml at baseline and rose to 10.7 (SD 7) ng/ml during periodic acceleration ($P < 0.001$). This increase declined 180 min after discontinuance of periodic acceleration to 2 (SD 3) ng/ml but still remained greater than baseline ($P < 0.05$). In contrast, control group t-PA antigen did not differ from baseline levels of 0.8 (SD 0.7) ng/ml during the 4 h. These levels were not significantly different from periodic acceleration baseline levels. In the pG_z group, t-PA activity was 0.59 (SD 0.26) IU/ml at baseline and rose to 2.6 (SD 1.0) IU/ml ($P < 0.01$) and fell to 0.32 (SD 0.17) IU/ml 180 min after discontinuance of periodic acceleration, which did not differ from baseline ($P > 0.05$). The control group showed no significant change in t-PA activity over time. Plasminogen activator inhibitor activity in the pG_z group was mean 17.1 (SD 2.1) arbitrary units (AU)/ml at baseline and remained unchanged during periodic acceleration at 17.6 (SD 7.9) AU/ml but rose to 34.2 (SD 6.4) AU/ml ($P < 0.01$) 180 min after discontinuance of periodic acceleration, whereas control group values did not significantly change over time. In the pG_z group, D-dimer was mean 212 (SD 94) ng/ml at baseline, rose to 310 (SD 93) ng/ml during periodic acceleration ($P < 0.01$), and was 178 (SD 95) ng/ml 180 min after its discontinuance, which did not differ from baseline. In the control group, there were no significant differences in D-dimer over time.

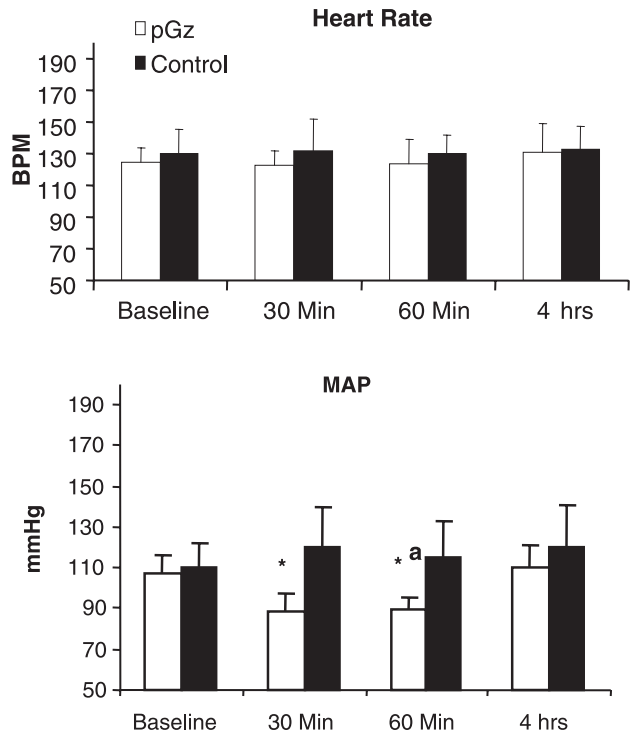

Fig. 2. *Top*: heart rate for pG_z and control animals at baseline, 30 min, 60 min, and 4 h. BPM, beats/min. There were no significant differences in heart rate over time or between groups. *Bottom*: mean arterial blood pressure (MAP). [a]pG_z group significant difference from baseline ($P < 0.05$). There were no significant differences in the control group from baseline or over time. *pG_z significantly different vs. control ($P < 0.05$).

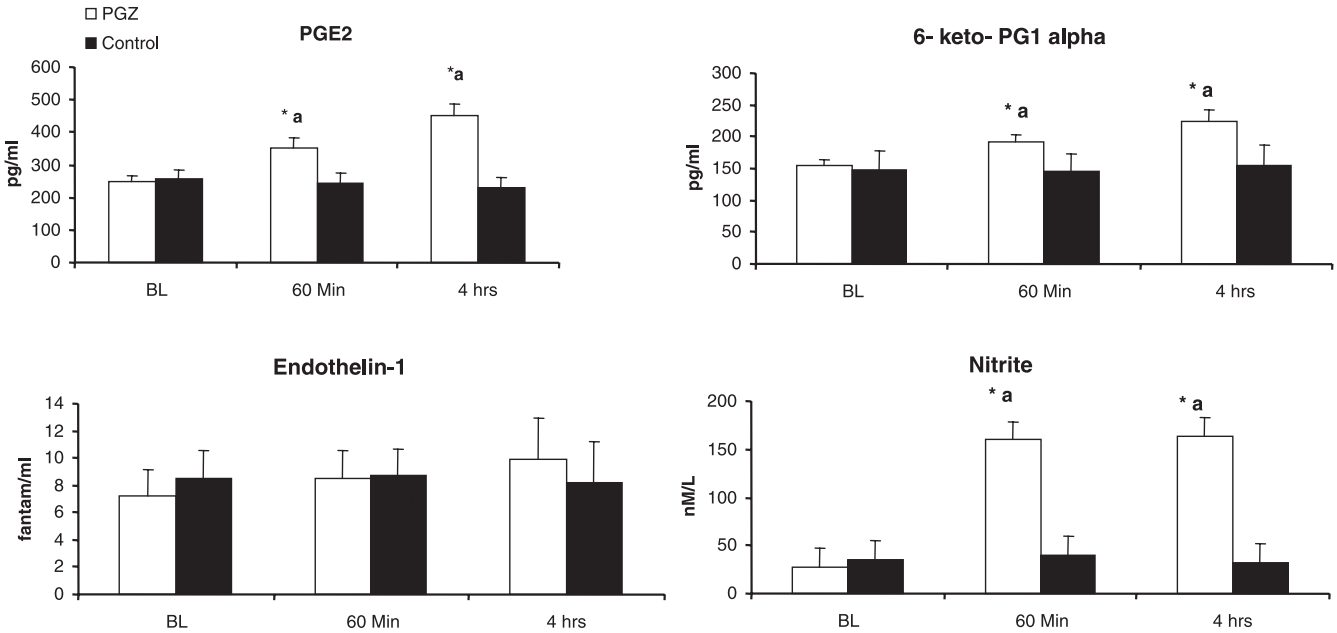

Fig. 3. Vasoactive factors PGE_2, 6-keto-$PGF_{1\alpha}$ (stable metabolite of prostacyclin), endothelin-1, and nitrite during baseline (BL), at 60 min, and at 4 h for pG_z and control groups. *pG_z vs. control ($P < 0.05$); [a]time vs. baseline ($P < 0.05$).

Coagulation factors. There were no significant differences from baseline during periodic acceleration or after its discontinuance 180 min later in prothrombin time, activated plasma thromboplastin time, fibrinogen, or thrombin time as listed in Table 1. Factor VII and Factor VIII were measured and no significant changes were found.

Cortisol. In the pG_z group, there were no significant differences in cortisol levels from baseline to periodic acceleration or after its discontinuance. Serum cortisol values were 17.4 (SD 8.0), 15.0 (SD 4.0), and 9.7 (SD 8.4) μg/dl at baseline, during periodic acceleration, and 180 min after discontinuance of periodic acceleration, respectively ($P > 0.05$). The control group had cortisol levels of 18 (SD 3), 19 (SD 5), and 12 (SD 7) μg/dl at baseline, during periodic acceleration, and 180 min after discontinuance of periodic acceleration, respectively. These values did not differ from the pG_z group and also did not vary over time.

DISCUSSION

Effects of adding pulses to circulation. Addition of sinusoidal pulses to the circulation of an intact animal through periodic acceleration causes acute release of the same mediators that appear in the blood during exercise or flow dilatation of an extremity. The latter takes place owing to increased shear stress to the endothelium that deforms the endothelial cells, causing them to release nitric oxide, prostacyclin, and tissue plasminogen activator among others. In 1961, Hoover et al. (25) showed that periodic acceleration added pulses to the circulation of anesthetized dogs without significantly increasing cardiac output. More recently, Adams et al. (2) found that addition of pulses to the circulation of anesthetized piglets through periodic acceleration significantly altered distribution of regional blood flows, presumably through endothelial release of vasodilator mediators. In both preceding studies, a beat frequency phenomenon was observed as a result of the combined effect of two waves (natural and added pulses) with nearly equal frequencies adding and then subtracting from each other as phase between the two varied.

The notion that frequency encoding of pulses causes nitric oxide release from endothelium was first reported by Hutcheson and Griffith in 1991 (26). Frequency encoding relates to the endothelial cells' sensitivity to respond to the rate of change of shear stress, within a given range of pulsations. Using a two-vessel preparation, in which one vessel was perfused, and varying frequency from 0.1 to 12 Hz and the effluent perfusate of this vessel bathed a donor vessel, these

Table 1. *Coagulation factors during periodic acceleration and control*

	Baseline		60 min		4 h	
Parameter	pGz	Control	pGz	Control	pGz	Control
PT, s	12.7 (0.1)	13.0 (0.1)	12.9 (0.4)	13.1 (0.3)	12.5 (0.2)	13.5 (0.5)
APTT, s	11.8 (0.6)	12 (0.5)	10.2 (0.4)	11 (0.2)	10.7 (0.5)	11.5 (0.4)
Fibrinogen, mg/dl	227 (11)	235 (25)	202 (10)	230 (35)	190 (17)	228 (25)
Thrombin time, s	19.2 (1.0)	21 (2.0)	18.6 (1.1)	20 (3)	17.2 (0.9)	19 (4)
Factor VII, U/ml	0.51 (0.17)	0.55 (0.3)	0.44 (0.16)	0.56 (0.3)	0.51 (0.17)	0.55 (0.2)
Factor VIII, U/ml	10.8 (3.6)	11.2 (4)	9.1 (2.7)	11 (4)	9.2 (4.6)	10.8 (3)

Values are means with SD in parentheses. pG_z, periodic acceleration; PT, prothrombin time; APTT, activated plasma thromboplastin time.

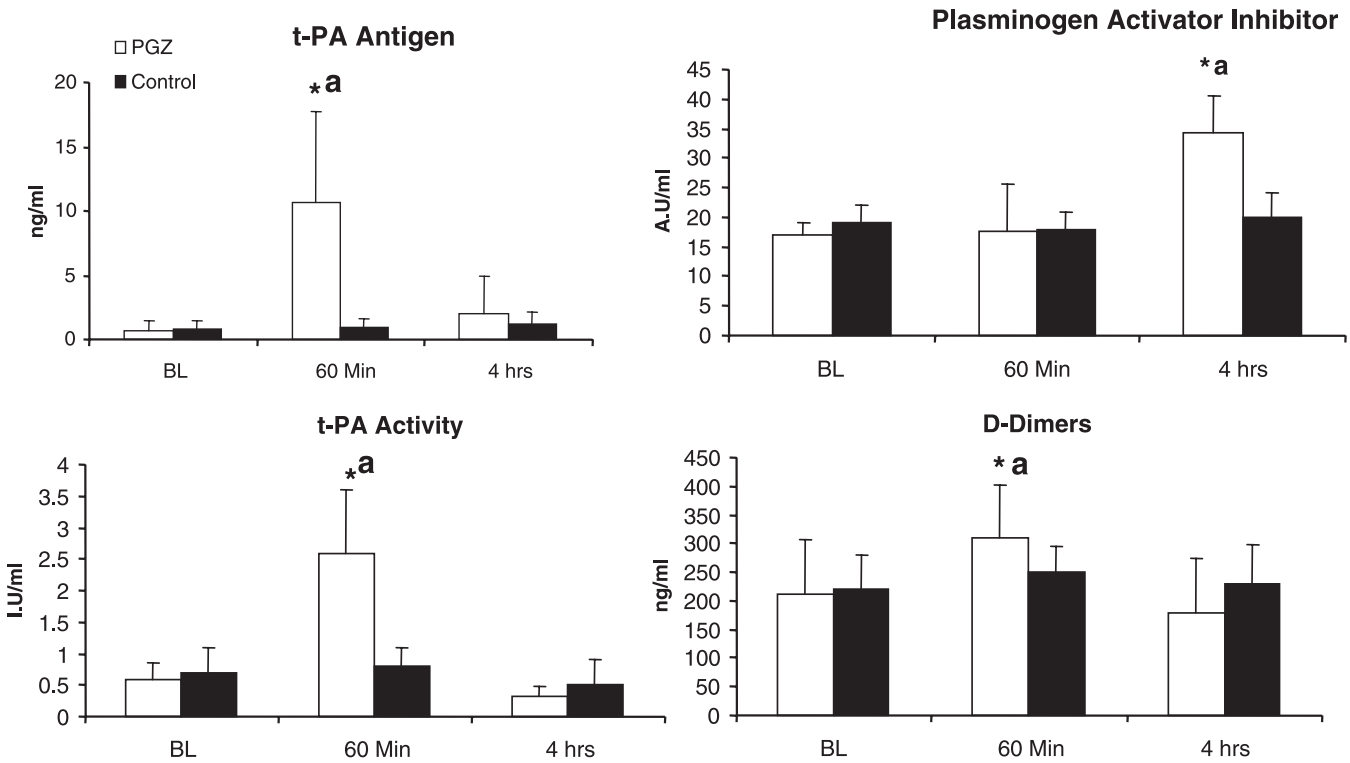

Fig. 4. Fibrinolytic factors tissue plasminogen activator antigen (t-PA antigen), tissue plasminogen activator activity (t-PA activity), plasminogen activator inhibitor, and D-dimers. No significant differences in the control group from baseline or over time. *pG_z vs. control ($P < 0.05$); [a]time vs. baseline ($P < 0.05$).

investigators found relaxation of the donor vessel as a function of increased frequency, with maximal response in the frequency range of 3–3.5 Hz. Incubation of the donor vessel with nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor, or removal of endothelium by rubbing abolished both the frequency and amplitude effects, indicating that these effects were mediated by nitric oxide release. Adams et al. (4) showed that periodic acceleration, when applied to an isolated vessel preparation, produces pulsatile stress, which significantly increases nitric oxide production.

Nitric oxide. Measurement of changes in serum nitric oxide in intact animals during increased shear stress such as exercise is not possible owing to its rapid metabolism in blood. Instead, the stable metabolites of nitric oxide such as nitrite, nitrate, and combined nitrite/nitrate have generally been reported with the Griess reaction assay (54). The significance of changes in these metabolites has been a source of confusion in the literature. Most importantly, the standard Griess reaction is sensitive only to changes of ±1 mmol/l (53), whereas nitric oxide released into the circulation from activation of endothelial nitric oxide synthase (eNOS) amounts to changes in the order of nanomoles per liter (18, 49). Furthermore, normal daily food contains more nitrate than that formed from nitric oxide released by eNOS, and therefore diet-derived nitrate contributes considerably to the concentration in blood (54). Strenuous, prolonged exercise stresses the body and activates inducible nitric oxide synthase present in leukocytes leading to prolonged release of nitric oxide in millimole per liter quantities (36). Thus millimole per liter rise of the stable metabolites of nitric oxide has often been implicitly attributed to eNOS upregulation during acute exercise (13, 27, 37). However, it is more likely that stress of intense exercise leads to activation of inducible nitric oxide synthase with release of large quantities of nitric oxide, leading to formation of high concentrations of nitrogen free radicals (18). During light to heavy exercise of 3-min duration, no change in the stable metabolites of nitric oxide was detected with the standard Griess reaction (48). In addition to problems with measurement of the stable metabolites of nitric oxide in the blood, the metabolite(s) most reflective of nitric oxide released from eNOS has only recently been addressed. Thus serum nitrite, nitrate, and nitrite/nitrate each have been reported to reflect acute release of nitric oxide from eNOS. However, from experiments dealing with decrease or increase of forearm blood flow by injection of N^G-monomethyl-L-arginine, an eNOS antagonist, or acetylcholine, respectively, only serum nitrite shows a close correlation to changes of blood flow. In these experiments, serum nitrite was measured with a high-performance liquid chromatography assay rather than the standard Griess reaction. The changes of serum nitrite were in nanomoles per liter, changes that cannot be detected with the standard Griess reaction. Such concentrations of nitrite do not produce vasodilatation and only serve as a marker of increased blood flow (29). The present study indicates that acute elevation of serum nitric oxide takes place during periodic acceleration. Measurements were obtained by converting serum nitrite into nitric oxide and estimating the levels with a specific nitric oxide electrode (8). There was substantial increase of nitrites from 28 nm/l at baseline to 160 nm/l during periodic acceleration, which remained elevated at 163 nm/l 3 h after discontinuance of periodic acceleration. This might reflect the presence of nitrosothiols, a metabolite of nitric oxide that has vasodilator properties owing to its slow, prolonged release

of nitric oxide into the circulation (40). The mean baseline values of serum nitrite measured with the nitric oxide electrode in piglets were lower than the baseline levels in humans measured with high-performance liquid chromatography, viz., 28 nmol/l vs. 222 nmol/l (31) and 402 nmol/l (29). The reason for this difference might relate to the assay and species differences and requires further study.

Prostaglandins. Both plasma prostacyclin, measured as its metabolite, 6-keto-$PGF_1\alpha$, and PGE_2 significantly rose with periodic acceleration and remained elevated 180 min after its discontinuance. Prostacyclin is formed in the endothelium as well as vascular smooth muscle (5, 15). Laminar shear stress upregulates prostacyclin synthase in isolated conduit vessels, thereby releasing this substance into the circulation (15). Rapid atrial pacing in normal humans to 140 beats/min does not increase prostacyclin blood levels (28). This might relate to the lesser number of pulses of 140 pulses/min within the circulation compared with the total number of pulses in piglets in the present study, a mean of 124 natural pulses/min and 180 added pulses/min to equal up to 304 pulses/min. Although eNOS activation appears to be frequency encoded, such evidence for prostacyclin synthase has not been demonstrated in isolated vessel experiments. The present study suggests that prostacyclin synthase might be frequency encoded in terms of pulsatile stress just like eNOS (26). Periodic acceleration increases PGE_2 in the circulation. The source of PGE_2 in the vascular system is controversial, with evidence that it is and is not present in endothelial cells, although there is agreement of its presence in vascular smooth muscle. Shear stress within the vascular system could produce its release from endothelial cells directly or from vascular smooth muscle by an indirect mechanism. Thus the cytoskeletal attachments of the endothelium to the smooth muscle layer could transmit both shear and circumferential stress to smooth muscle, and/or the intramural circulation within vascular smooth muscle could provide shear stress (5, 47). Blood levels of both prostacyclin and PGE_2 remained elevated 180 min after periodic acceleration was discontinued. This occurred in the presence of elevated serum nitrite levels, presumably owing to circulating nitrosothiol compounds that are known to slowly release nitric oxide into the circulation (40). Stimulation of eNOS in cultured cells with release of nitric oxide increased prostacyclin production through activation of prostaglandin H synthase. Furthermore, prostacyclin production from endothelial cells was blocked by nitro-L-arginine methyl ester in these experiments (14). Thus low levels of nitric oxide in the circulation from nitrosothiols might stimulate prostacyclin and PGE_2 release and explain the prolonged elevations in these metabolites after discontinuance of periodic acceleration.

Cortisol. Periodic acceleration did not alter cortisol levels. Cortisol levels have been reported to increase in relation to exercise of longer duration than 1 h (39), suggesting that a stress response is not elicited in pigs subjected to periodic acceleration. Furthermore, elevation of cortisol suppresses nitric oxide release and eNOS expression in cultured bovine coronary artery endothelial cells (42).

Fibrinolytic factors. Periodic acceleration caused t-PA antigen to rise to 10.7 ng/ml and t-PA activity to rise to 2.6 IU/ml, which returned to baseline values 180 min after discontinuance. These values are far below those achieved by therapeutic doses of recombinant t-PA administered intravenously. Thus Fong et al. (19) found that recombinant t-PA administered intravenously at 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ to anesthetized dogs produced plasma t-PA activity of ~50 IU/ml and t-PA antigen of ~200 ng/ml. Although the levels of t-PA during periodic acceleration are not in the therapeutic range to lyse a clot, significant fibrinolytic activity was present, as indicated by the 46% increase of D-dimer with periodic acceleration. Increased plasma concentrations of D-dimer reflect the extent of intravascular fibrinolysis of cross-linked fibrin (51). Release of t-PA from the endothelium during periodic acceleration arises from shear stress as well as stimulation by nitric oxide generated from eNOS (35, 46). The results of the present study suggest that pulsatile shear stress release of t-PA from endothelium might be frequency encoded, similar to the circumstances associated with nitric oxide release (26). Plasminogen activator inhibitor activity was not changed by periodic acceleration but rose 100% over baseline 180 min after its termination. Perhaps its delayed rise was a homeostatic mechanism caused by the increase of t-PA activity. Because neither plasminogen activator inhibitors, t-PA antigen, t-PA activity, nor D-dimers changed in the control group over time, the changes observed in these factors are solely related to periodic acceleration.

Coagulation factors. There were no changes in any of the coagulation factors measured at the end of periodic acceleration and 180 min later. These factors included prothrombin time, activated plasma thromboplastin time, fibrinogen, thrombin time, Factor VII, and Factor VIII.

Periodic acceleration and exercise. Release of nitric oxide, prostacyclin, PGE_2, and t-PA from shear stress during periodic acceleration into the circulation also occur from shear stress during aerobic exercise. The former produces pulsatile stress and the latter mostly laminar shear stress to the endothelium. Periodic acceleration was not associated with significant increase of sympathetic nervous system activity as indicated by unchanged serum levels of epinephrine and norepinephrine in contrast to aerobic exercise (11, 39). In addition, exercise is associated with a hypercoagulative state, whereas the present study indicates that periodic acceleration does not produce hypercoagulability (9, 16).

Limitations of study. This work was carried out in anesthetized animals, and the results might differ in human subjects. In terms of vasoactive, fibrinolytic, and coagulation factors, the changes of most of these values during periodic acceleration fell within the range of changes during mild to moderate-intensity exercise (23). Compared with a control group that did not receive periodic acceleration, neither time or anesthesia was a factor that elicited this response. Thus this investigation demonstrates that the intervention that elicited production of these factors was periodic acceleration. The present study assessed a single level of whole body periodic acceleration and frequency and did not investigate dose responsiveness.

In conclusion, in addition to noninvasively supporting ventilation in anesthetized, paralyzed animals, whole body periodic acceleration produces laminar and pulsatile shear stress to the circulation that causes release of endothelial derived factors. Unlike strenuous exercise, in which a hypercoagulable state has been reported (16, 23, 24), periodic acceleration does not induce a hypercoagulable state. Periodic acceleration has clinical and possibly therapeutic implications in a host of diseases in which these endothelial derive mediators will be of

benefit. The vasodilator, antiatherogenic, anti-inflammatory properties of endothelial-released nitric oxide and prostaglandins produced with periodic acceleration offer potential treatment for diseases that have their basis in endothelial dysfunction and inflammation (44).

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DISCLOSURES

J. Adams and J. Bassuk own stock options in Non-Invasive Monitoring Systems (NIMS). M. Sackner is employed by and holds partial ownership in NIMS and holds two patents assigned to NIMS.

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