Effect of Moderate-Intensity Exercise, Whole-Body Periodic Acceleration, and Passive Cycling on Nitric Oxide Release Into Circulation*

Marvin A. Sackner, MD; Emerance Gummels, MS; and Jose A. Adams, MD

Study objective: To determine if a 3-min bout of moderately intensive supine bicycle exercise, whole-body periodic acceleration (pGz), and passive motorized cycling cause nitric oxide (NO) release into the circulation, as detected by dicrotic notch descent on the diastolic limb of a finger pulse wave.

Participants: Fourteen healthy adults underwent two levels of supine bicycle ergometry that caused heart rate to rise to 56% (light moderate exercise) and 67% (heavy moderate exercise) of maximum predicted heart rate, and a single bout of pGz. Several months later, 9 of the 14 subjects underwent passive motorized cycling.

Methods: The ECG and finger pulse wave were recorded. The dicrotic notch position was computed from the amplitude of the digital pulse wave (a) divided by the height of the dicrotic notch above the end-diastolic level (b) and designated the a/b ratio. Increase of the a/b ratio due to dicrotic notch descent reflects the vasodilator action of NO on resistance vessels. The last 30 s of baseline, exercise or pGz, and recovery periods were analyzed.

Results: Compared to baseline, light moderate exercise produced a nonsignificant rise of the a/b ratio. Both heavy moderate exercise and pGz produced statistically significant rises of peak and mean a/b ratios over baseline. Heavy moderate exercise produced a greater mean a/b ratio than pGz, but the peak a/b ratio did not differ between the two. Episodic rises and falls of a/b ratios were more common during pGz than exercise. Passive motorized cycling did not alter the a/b ratio.

Conclusions: Dicrotic notch descent occurs during a brief bout of moderate cycling exercise, consistent with NO release into circulation. pGz produces comparable descent, but passive motorized cycling does not. In terms of the beneficial effects of NO, this suggests that pGz might serve as a substitute in subjects who are physically incapable of exercising.

Key words: dicrotic notch; exercise nitric; oxide; periodic acceleration

Abbreviations: a = amplitude of the digital pulse wave; ANOVA = analysis of variance; b = height of the dicrotic notch or wave above the end-diastolic level; BMD = bone mineral density; eNOS = endothelial nitric oxide synthase; FMD = flow-mediated vasodilatation; NO = nitric oxide; pGz = whole-body periodic acceleration

Exercise increases shear stress to the endothelium causing up-regulation of endothelial nitric oxide synthase (eNOS), thereby releasing nitric oxide (NO) into the circulation. This effect has been mainly detected in humans by finding increased plasma or serum nitrite/nitrate, which are metabolites of NO, or by demonstrating increased flow-mediated vasodilatation (FMD). Several weeks of chronic exercise training produce both effects,1–5 with the qualification that high-intensity exercise (75% maximal oxygen consumption) does not increase FMD, whereas moderate intensity exercise (50% maximal oxygen consumption) does increase FMD.6 The situation with single bouts of exercise is less clear. Following an acute bout of high-intensity, dynamic exercise, serum or plasma nitrite/nitrate may show a sharp rise of nitrite/nitrate in the order of 14 to 40 μmol/L.7,8

*From the Division of Pulmonary Disease and Critical Medicine (Dr. Sackner) and Department of Neonatology (Dr. Adams), Mount Sinai Medical Center, Miami Beach; and Non-Invasive Monitoring Systems (Ms. Gummels), North Bay Village, FL. Dr. Sackner owns approximately 33% of Non-Invasive Monitoring System shares. Ms. Gummels owns approximately 0.2% of Non-Invasive Monitoring System shares. Dr. Adams is a member of the Scientific Advisory Board and owns approximately 0.2% of Non-Invasive Monitoring System shares.

Manuscript received January 31, 2005; revision accepted April 7, 2005.

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Correspondence to: Marvin A. Sackner, MD, 555 NE 34th St, Miami, FL 33137; e-mail: Artchive@msn.com

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This large elevation is most likely due to activation of inducible NO synthase from the oxidative stress accompanying strenuous exercise rather than sole activation of eNOS.9 No data on nitrite/nitrate measurements using sensitive assay techniques have been reported during an acute bout of moderate-intensity exercise. However, activation of eNOS by intra-arterial injection of acetylcholine increased serum or plasma nitrite by approximately 300 to 575 nmol/L and correlates well with changes in forearm blood flow.10,11 Therefore, for determining acute changes of NO during exercise, serum or plasma nitrite measured with an assay sensitive in the nanomoles-per-liter range is necessary. On completion of a bout of acute dynamic exercise, FMD is increased consistent with up-regulated eNOS.12–14

A potential method for demonstrating NO release into the circulation during exercise is measurement of the descent of the dicrotic notch or wave of the finger pulse wave. This occurs because NO dilates resistance vessels, thereby delaying pulse wave reflection.15–17 One purpose of this article is to determine whether the descent of the dicrotic notch of the finger pulse is a function of intensity for an acute bout of moderate-intensity, supine dynamic exercise. Another purpose is to compare the dicrotic notch position during active exercise to two means for producing passive exercise, whole-body periodic acceleration (pGz) and passive motorized cycling. pGz associated with increased pulsatile shear stress to the endothelium produces descent of dicrotic notch due to NO release, but the magnitude of this change relative to dynamic exercise has not been quantified.17

**Materials and Methods**

**Subjects**

Eight healthy men and six healthy women (mean ± SD age, 53 ± 12 years) participated in the comparison of bicycle ergometry and pGz on position of dicrotic notch of the finger pulse. Six to 8 months after the preceding study, four of the men and five of the women in this study (mean age, 54 ± 11 years) participated in the effects of passive assisted cycle ergometry on the dicrotic notch position of the finger pulse.

**Methods**

The Institutional Review Board of Mount Sinai Medical Center of Greater Miami approved the study protocol, and written informed consent was obtained from all participants. None of the subjects received financial remuneration for their participation. A supine bicycle ergometer (846T Imaging/Ergometer Table; Quinton Instrument Company; Seattle WA) was used for the exercise studies. pGz was accomplished with a motion platform device that has a gyros-like appearance driven by a two-flywheel motor assembly. It has a handheld controller that regulates cycles per minute and acceleration. The supine subject laid on a mattress onto the platform for repetitive sinusoidal head-to-foot movements delivered at approximately 140 cycles per minute and approximately ± 2.2 m/s^2 (Motion Platform; AT 101; Acceleration Therapeutics Division, NonInvasive Monitoring Systems; North Bay Village, FL). The device is 222 cm in length, 77.5 cm in width, and weighs 211 kg. A footboard, 112 cm in height, for strapping the subject’s feet enclosed in shoes is utilized to couple the body to the motion platform during periodic acceleration. Supine passive bicycle exercise was accomplished with a motorized exercise device that moved the legs passively at 60 cycles per minute (MOTOmed letto; Rech; Betzenweiler, Germany).

Descent of the dicrotic notch of the digital pulse down the diastolic limb reflects the vasodilator action of NO on the resistance vessels owing to delay of pulse wave reflection. The change of dicrotic notch or wave position is computed by measuring the a/b ratio, which is the amplitude of the digital pulse wave (a) divided by the height of the dicrotic notch or wave above the end-diastolic level (b); alternately, the b/a ratio may be reported.15,17 In the current study, the dicrotic notch rather than the dicrotic wave is utilized to compute the a/b ratio since the peak of the reflective wave particularly at baseline is usually difficult to detect in elderly subjects.

Although the a/b ratio can be computed from the raw pulse wave during supine active and passive exercise, in most subjects it cannot be computed during pGz because added pulses caused by acceleration and deceleration of the body and motion artifacts obscure the dicrotic notch position. Therefore, it is necessary to employ an ECG R-wave–triggered ensemble-averaging computer program to reveal the dicrotic notch and eliminate the added pulse waves and motion artifacts. A five-beat ensemble average is applied to all the pulse waves for consistency. To aid detection of the dicrotic notch position, ensemble-averaged pulse waves and their second derivatives are displayed on a computer screen, and the peak of the largest upward deflection in diastole of the second derivative taken as the point for that location. The computer program calculates the a/b ratio based on this algorithm. If the end-diastolic level moves to the next pulse waveform as judged by an end-diastolic level higher than the dicrotic notch, then an arbitrary value of 100 is assigned to the a/b ratio; otherwise, the value would be indeterminate. For example, if b = 0, then the a/b ratio would compute as infinite. The arbitrary value of 100 is a realistic one since an a/b value of 96 was determined from an actual pulse tracing in the current investigation. The computer-aided detection point for dicrotic notch position requires visual confirmation by an operator. If the dicrotic notch marker, the diastolic peak of the second derivative, and the dicrotic notch of the raw pulse do not line up with a vertical cursor on the computer screen, then the a/b ratio of this pulse is computed by hand. This occurred in 4 of the total of 4,904 beats analyzed in this study. Either a pulse oximeter (Minox; Ohmeda; Louisville, CO) or photoelectric-plethysmograph (Model 2122I Bioamplifier; UPI; Morro Bay, CA) were used to obtain the pulse wave, as described previously.17

Three-lead ECG electrodes were placed on the chest, and a reusable photoelectric plethysmograph sensor was placed on the index finger. Lead II ECG and the finger pulse waveform were recorded continuously throughout the various procedures.

**Study Design**

Subjects were placed on the bicycle ergometer table or motion platform in random order. Seven subjects cycled first, and seven subjects received pGz first. The interval between these procedures was ≈ 15 min. The exercise protocol consisted of 5 min of
rest in the supine posture on the bicycle ergometer table with the feet on the bicycle pedals. The men then pedaled at approximately 42 W and the women pedaled at 33 W for approximately 3 min and then rested for 5 min followed by another rest period of 5 min. A second exercise load of approximately 75 W for men and 59 W for women was undertaken followed by a recovery period of 5 min. These exercise loads were selected to provide two levels of moderate exercise intensity.

Subjects were placed on the motion platform, rested 5 min, and underwent a 3-min application of pGz with settings of approximately ± 2.2 m/s², and approximately 140 cycles per minute. This was followed by a 5-min recovery period.

On another day 6 to 8 months later, 9 of the 14 subjects were placed on the motorized, passive bicycle, and their feet were strapped into the pedals. The subjects rested 5 min, and the pedals were activated at 60 revolutions per minute for approximately 3 min followed by a 5-min recovery period.

For 30 s at the end of each period to approximate a steady state, e.g., baseline, cycling or pGz, and recovery, the following values were computed: (1) mean heart rate, (2) mean and median a/b ratio, (3) peak a/b ratio, and (4) the ratio of median to mean a/b ratio.

Statistical Analysis

Statistical analysis of the data was accomplished with statistical software running on a personal computer (Statistica 7.0; StatSoft; Tulsa, OK). Results were expressed as mean ± SD, and comparison of means with 95% confidence levels was carried out using analysis of variance (ANOVA) followed by post hoc analysis with the Newman-Keuls test. Significance between means was taken as p < 0.05.

The ANOVA setup for the 14 subjects who underwent cycling and pGz on the same day was carried out in the same statistical design. For 9 of the 14 subjects who underwent passive motorized cycling 6 to 8 months later, ANOVA was carried out as a separate statistical design.

RESULTS

Although a clean finger pulse wave that allowed detection of the dicrotic notch was obtained in all 14 subjects during bicycle ergometry, all baseline and recovery periods, as well as in 3 of the 14 subjects during pGz, a five-beat ensemble-averaging procedure was applied to all data for consistency (Fig 1, 2). There were no statistical differences among the baseline and recovery mean heart rates for the two levels of moderate exercise and pGz. Based on maximum heart rate predictions for age and sex, light moderate exercise corresponded to 56 ± 8% of maximum predicted heart rate and heavy moderate exercise corresponded to 67 ± 8% of the subjects in this investigation. The mean heart rate during both exercise levels was significantly greater than all the baseline and recovery values as well as pGz (p < 0.001). The mean heart rates among pGz, baseline, and recovery periods did not differ. The heart rate did not significantly differ between heavy moderate exercise (111 ± 16 beats/min) and light moderate exercise (94 ± 14 beats/min) [Fig 3].

In 6 of 14 subjects during pGz, in 1 of 14 subjects during light moderate exercise, and in 3 of 14 subjects during heavy moderate exercise, the dicrotic notch moved to the beginning of the succeeding pulse. In this situation, the a/b ratio was assigned an arbitrary value of 100, since otherwise the value would become infinite. This arbitrary value was based on finding an actual value of 96 in one of the subjects. Default of the a/b ratio to a value of 100 was unrelated to whether cycle exercise was performed before or after pGZ.

During light moderate exercise, heavy moderate exercise, and pGz, the mean a/b ratio rose in all subjects compared to baseline and the recovery periods. The difference between baseline and recovery periods and between baseline and heavy moderate exercise (p < 0.001) and pGz (p < 0.05) was statistically significant, whereas light moderate exercise was not. The mean a/b ratio value of heavy moderate exercise was greater than pGz (p < 0.05) and light moderate exercise (p < 0.002). The mean a/b value for pGz was greater than rest and recovery periods (p < 0.05) but not different from light moderate exercise (Fig 4).

The peak a/b ratio for pGz exceeded light moderate exercise (p < 0.002) and all the baseline and recovery periods (p < 0.0001) but did not differ statistically from heavy moderate exercise (Fig 4). The peak a/b ratio for heavy moderate exercise was greater than all the baseline and recovery periods (p < 0.001) but did not differ statistically from light moderate exercise. The peak a/b ratio for light moderate exercise did not differ statistically from its baseline and recovery periods.

Cycling rises and falls of the a/b ratio occurred most prominently during pGz and to a lesser extent during heavy moderate exercise (Fig 5). This phenomenon lowers the ratio of the median to the mean a/b ratio. Values for pGz were lower than cycling exercise, baseline, and recovery periods (p < 0.0001) [Fig 6]. Values for the two exercise intensities did not differ but were significantly lower than baseline and recovery periods, e.g., for heavy moderate exercise (p < 0.001) and for light moderate exercise (p < 0.05).

There was no significant statistical difference between the mean a/b ratios whether exercise was performed first followed by pGZ or vice versa. For the passive motorized cycling experiments carried out in 9 of the 14 subjects 6 to 8 months after the pGz and dynamic exercise studies, neither the peak not mean a/b ratios differed from baseline or recovery periods.
Discussion

To our knowledge, the present study is the first to call attention to dicrotic notch descent of the finger pulse wave during dynamic exercise. Further, this phenomenon as measured by rise of the a/b ratio appears to be a function of the exercise intensity within the moderate range. As mentioned in our previous article and by others,\textsuperscript{15,17} descent of the dicrotic notch of the pulse wave is a result of the specific dilating action of NO on the resistance blood vessels. This delays arrival of the pulse wave to the
Figure 2. Ten-second section of the 30-s recordings used for analysis of baseline and pGz in a 67-year-old man whose other recordings are depicted in Figures 1. Cyclic descent and ascent of the dicrotic notch during pGz is seen in both the pulse wave and the ensemble-averaged pulse wave. The mean a/b ratio was 1.8 at baseline and rose to 24.2 during the 30 s of pGz. See Figure 1 legend for expansion of abbreviations.

Figure 3. Mean heart rate with 95% confidence limits of the baselines (bl) and recovery (recov) periods and the procedure themselves in the last 30 s of pGz, light moderate exercise (lt mod ex), and heavy moderate exercise (hy mod ex) in 14 healthy subjects. The heart rates rises with exercise but not with pGz.
Figure 4. Mean (solid circles) and peak (solid squares) a/b ratios with 95% confidence limits of the baselines and recovery periods and the procedure themselves in the last 30 s of pGz, light moderate exercise, and heavy moderate exercise in 14 healthy subjects. Although the mean a/b ratio rose with light moderate exercise, it did not differ statistically from baseline or recovery or pGz periods. The mean a/b ratio for pGz was greater than baseline and recovery periods but did not differ statistically from light moderate exercise. The mean a/b ratio for heavy moderate exercise was significantly greater than baseline, recovery, light moderate exercise, and pGz periods. The peak a/b ratio during pGz was significantly greater than baseline, recovery, and light moderate exercise periods but did not differ from heavy moderate exercise. The peak a/b ratio for heavy moderate exercise was greater than baseline and recovery periods but did not differ from light moderate exercise and pGz. See Figure 3 legends for expansion of abbreviations.

Figure 5. This depicts the last 30 s recordings of light moderate exercise (left, A), heavy moderate exercise (center, B), and pGz (bottom, C) in a 55-year-old man (upper panels) and a 66-year-old woman (lower panels). There is no episodic rise and fall of the a/b ratio during light moderate exercise in both subjects and during heavy moderate exercise in the 66-year-old woman. In the remaining panels, there is episodic rise and fall of the a/b ratio.
site and delays return of the reflected wave, creating the downward descent on the diastolic limb of the pulse for the dicrotic notch and wave. The a/b ratio, the amplitude of the pulse divided by the height of the dicrotic notch above the end-diastolic level, quantifies this phenomenon. Since this ratio increases after administration of NO donor drugs in a dose-related manner, it is consistent with greater NO production within the moderate-intensity exercise range.

Descent of the dicrotic notch position leading to an increased a/b ratio signifies a physiologically vasoactive effect on the vasculature. This differs from changes of serum nitrite, a metabolic marker measured after interventions designed to increase or decrease NO release. Lauer et al. investigated the accuracy of metabolic markers of NO in humans by correlating changes in forearm blood flow after stimulation of eNOS with acetylcholine (1 to 10 μg/min) that dose-dependently augmented venous nitrite levels up to 71%. This effect parallels an almost fourfold increase in forearm blood flow. Inhibition of eNOS with N(G)-monomethyl-L-arginine, 4 to 12 mmol/min, dose-dependently reduced basal serum nitrite and forearm blood flow by 90%. Infusions of nitrite in volunteers to reproduce the serum changes for this metabolite of NO fail to effect vasodilatation as measured by forearm blood flow measurements. Venous nitrate and total nitrate remain unchanged during these interventions. Forearm blood flow and serum nitrite are highly correlated but not with other metabolites such as serum nitrate and total nitrite/nitrate. Therefore, serum nitrite serves as a marker of acute changes in regional eNOS activity but in concentrations that are vasodilator-inactive.

The increased a/b ratio during moderate exercise is most likely, mainly related to increased laminar shear stress due to increased blood flow. Heart rate rose from a baseline of approximately 64 to 94 beats/min during light-intensity moderate exercise and to 111 beats/min during heavy-intensity moderate exercise. This value is below the pulse rate expected to produce significant pulsatile shear stress to activate eNOS, from 180 to 360 pulses per minute in an isolated blood vessel preparation. In an isolated, perfused blood vessel, there is a threefold increase of NO at the effluent with pulsatile flow compared to nonpulsatile flow. If the vessel with pulsatile flow is subjected to periodic acceleration, the NO at the effluent increases 10-fold relative to nonpulsatile flow.

![Figure 6](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22032/ on 04/11/2017)
In the current investigation, cycle exercise in the supine posture was compared to pGz for the following reasons. With the current technology, pGz can only be carried out in the supine posture; exercise in the upright posture such as running and walking produces different hemodynamic actions than exercise in the supine posture in terms of heart rate and stroke volume. Skeletal muscular contractions are less likely to occur during passive supine cycling as a control than upright cycling. Finally, large added pulsations and motion artifacts present in the finger pulse often associated with entrainment of superimposed pulses during treadmill exercise prevent detection of the dicrotic notch position with an ensemble averaging procedure.21

pGz in healthy subjects and patients with various diseases releases NO into the blood stream as inferred from an increase of the a/b ratio.17 In the current study, it was found that the effect was substantial since increase of the mean and peak a/b ratios during pGz were closer to heavy moderate exercise (67% maximum predicted heart rate) than light moderate exercise (56% maximum predicted heart rate). The peak value of 51 during the last 30 s of the 3-min application of pGz in the current study compares well to the peak value of 53 in 14 healthy subjects with 45-min application of pGz previously reported.17 Although pGz applied to humans produces potent vasodilatation due to NO release equivalent to moderate dynamic exercise, there may be substantial differences relative to exercise in terms of regional organ blood flows and blood coagulation parameters.22,23

In anesthetized swine, there was no change from baseline during pGz or 3 h after its discontinuance in prothrombin time, activated thromboplastin time, fibrinogen, thrombin time, Factor VII, and Factor VIII.24 In exercising humans, these parameters trend to a hypercoagulable state.24–26 Gunga et al26 concluded that in the case of a subject with risk factors such as impaired fibrinolysis, exercise might produce intravascular clotting. Whether this difference between pGz and exercise applies to humans requires further investigation.

Episodic rises and falls of the a/b ratio were often observed during pGz and the dynamic exercise periods. Presumably this relates to pulsatile bursts of NO from eNOS stimulation that have been observed with in vivo NO electrode measurements after eNOS stimulation.27–29 This lowers the median to mean a/b ratio, a finding more prominent during pGz than exercise. The physiologic basis for the difference between of pGz and dynamic exercise is unclear, but it suggests that pulsatile shear stress might be more efficient means than laminar for enhancing NO activity. Tsoukias et al30 developed a mathematical model to explain the benefits of pulsatile bursts of NO release into the circulation. They commented that the endothelial production of NO is in close proximity to RBCs, which could scavenge a significant amount of NO. They formulated a mathematical model for NO transport in an arteriole to test the hypothesis that transient, burst-like NO production can facilitate more efficient NO delivery to vascular smooth muscle and reduce NO scavenging by blood. The model simulations predicted the following: (1) the endothelium can maintain a physiologically significant amount of NO in smooth muscle despite the presence of NO scavengers such as hemoglobin and myoglobin; (2) under certain conditions, transient NO release presents a more efficient way for activating soluble guanylate cyclase increasing cyclic guanosine monophosphate formation several fold; and (3) frequency- rather than amplitude-dependent control of cyclic guanosine monophosphate formation is possible.

If their mathematical model is correct, then there should be some advantage of increasing pulsatile shear stress in real-life situations, such as running, for more efficient delivery of NO. Each strike of the ground during running adds a pulse to the circulation. In running athletes during warm-up, strides range from 130 to 165/min, from 140 to 175/min during submaximal speed, and from 165 to 205/min during sprinting.31 As demonstrated in the current study, added pulses do not occur during cycling and therefore cycling might be a less efficient means of producing the beneficial effects of NO through exercise. This may be pertinent to bone metabolism because NO is involved in coordinating specific phases of osteoblast differentiation and bone formation. For example, eNOS gene knockout mice show marked reductions of total bone mineral density (BMD).32 In athletes, running is associated with increased total BMD compared to control subjects, whereas cycling is associated with less total BMD.33 Thus, more efficient eNOS regulation of osteoblast metabolism might occur with running because added pulses to the circulation provide increased pulsatile shear stress. In the current study, passive motorized cycling did not cause release of NO as measured by change of a/b ratio of the finger pulse. No data have been reported on the hemodynamic effects of supine passive cycling but for the upright posture, cardiac output hardly changes and therefore neither laminar or pulsatile shear stress would be expected to increase.34,35

The current study pertains to 3-min episodes of moderate exercise or pGz, and the effects on the dicrotic notch position could differ with more intense exercise and greater duration. However, strenuous exercise produces oxidative stress that might
down-regulate eNOS activity and decrease bioavailability of NO from this source.9–36,37 As reported in our previous study,15 the effects of pGz in humans lasted throughout its entire duration of 45 min. The magnitude of descent of the dicrotic notch as a function of intensity and duration of exercise might provide another measure of endothelial function, but this requires further investigation.

Conclusions

Increased bioavailability of NO during exercise can be obtained by measurement of the dicrotic notch descent of the finger pulse wave. This appears to be a function of exercise intensity within the moderate range over a brief period. Passive exercise with pGz applied with a motion platform produces descent of the dicrotic notch lying between 56% and 67% of predicted maximum heart rate. In terms of the beneficial effects of NO released during exercise, this suggests that application of pGz might be complimentary to dynamic exercise or serve as a substitute in those subjects who might be physically unable to moderately exercise.

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