Increased consumption and vasodilatory effect of nitrite during exercise

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Hon YY, Lin EE, Tian X, Yang Y, Sun H, Swenson ER, Taveira-Dasilva AM, Gladwin MT, Machado RF. Increased consumption and vasodilatory effect of nitrite during exercise. Am J Physiol Lung Cell Mol Physiol 310: L354–L364, 2016. First published December 18, 2015; doi:10.1152/ajplung.00081.2015.—This study investigated the effects of aerobic-to-anaerobic exercise on nitrite stores in the human circulation and evaluated the effects of systemic nitrite infusion on aerobic and anaerobic exercise capacity and hemodynamics. Six healthy volunteers were randomized to receive sodium nitrite or saline for 70 min in two separate occasions in an exercise study. Subjects cycled on an upright electronically braked cycle ergometer 30 min into the infusion according to a ramp protocol designed to attain exhaustion in 10 min. They were allowed to recover for 30 min thereafter. The changes of whole blood nitrite concentrations over the 70-min study period were analyzed by pharmacokinetic modeling. Longitudinal measurements of hemodynamic and clinical variables were analyzed by fitting nonparametric regression spline models. During exercise, nitrite consumption/elimination rate was increased by ~137%. Cardiac output (CO), mean arterial pressure (MAP), and pulmonary artery pressure (PAP) were increased, but smaller elevation of MAP and larger increases of CO and PAP were found during nitrite infusion compared with placebo control. The higher CO and lower MAP during nitrite infusion were likely attributed to vasodilation and a trend toward decrease in systemic vascular resistance. In contrast, there were no significant changes in mean pulmonary artery pressures and pulmonary vascular resistance. These findings, together with the increased consumption of nitrite and production of iron-nitrosyl-hemoglobin during exercise, support the notion of nitrite conversion to NO resulting in systemic vasodilation. However, at the dosing used in this protocol achieving micromolar plasma concentrations of nitrite, exercise capacity was not enhanced, as opposed to other reports using lower dosing.

nitrite store; incremental exercise test; pharmacokinetics; hemodynamics; vasodilation

UNTIL RECENTLY, NITRITE WAS considered to be only an end product of nitric oxide (NO) metabolism. However, recent evidence indicates that nitrite (NO₂⁻) is abundant in blood and tissues and may be the biggest source for intravascular and tissue NO. Over the last decade, it has been proposed that nitrite plays an important physiological role in signaling, blood flow regulation, and hypoxic nitric oxide-mediated responses (9). During physiological and pathological hypoxia, nitrite is converted to NO via reactions with heme globins (e.g., hemoglobin, myoglobin, cytoglobin, and neuroglobin) and molybdopterin-containing enzymes (e.g., xanthine oxidoreductase, aldehyde oxidase, sulfite oxidase, and mitochondria amidoxime reducing component) (9, 25–28, 34, 36, 38, 41). These reactions allow a graded conversion of nitrite to NO in response to oxygenation and pH state and allow for hypoxic/hypercapnic/acidotic vasodilation and modulation of hypoxic mitochondrial respiration.

During exercise, there is a lag in the rate at which oxygen uptake (V̇O₂) rises to meet energy demand. It is uncertain whether this limitation is due to inadequate oxygen (O₂) delivery to working muscle, limitations at the rate at which mitochondria can generate ATP to meet demand, or a combination of both. Both of these limitations may be modulated by NO. During exercise, NO will both vasodilate smooth muscle and inhibit mitochondrial respiration. The latter effect could either decrease oxygen extraction by limiting the ability of mitochondria to utilize oxygen, or paradoxically increase oxygen utilization by inhibiting mitochondrial proximal to blood vessels, an effect that facilitates oxygen diffusion to distal tissue and mitochondria (NO-dependent facilitated oxygen diffusion) (35).

Previous studies have demonstrated that NO production increased during exercise (5, 37), and regional inhibition of NO production from endothelial NO synthase (NOS) reduced exercise-dependent blood flow by ~10%. NOS inhibitors such as N G-nitro-L-arginine methyl ester (L-NAME) have been shown to decrease exercise tolerance (20), and the NO precursor L-arginine has been shown to increase exercise tolerance (4). The importance of NO in the regulation of coronary and systemic vasodilator tone has been demonstrated experimentally by inhibiting its synthesis. Traverse et al. (39) observed in dogs that coronary NO production increased with exercise, suggesting that NO may be a limiting factor in exercise tolerance for people with coronary disease. Cannon and colleagues (8) have shown that eNOS inhibition reduced regional and coronary blood flow during exercise by ~25%. It has also been noted by Schaefer et al. (32) that in cardiac transplant patients with reduced exercise tolerance, there was limited NO production during exercise. In a more recent study, nitrite has been administered to sheep by inhalation and has conclusively demonstrated that under hypoxic conditions the vasoactive effect of nitrite was potentiated in the pulmonary circulation, further substantiating a deoxyheme protein involvement in nitrite reduction to NO (17).
Considering the potential role of nitrite bioconversion to NO during hypoxia, it is likely that nitrite plays an important role in modulating exercise physiology. With systemic exercise, as studied in our protocol, we suspected that lower systemic arterial and venous pH and reduction in partial pressure of oxygen in working muscle tissues would create an even greater demand for NO and utilization of nitrite as an intravascular store. We therefore hypothesized that, during aerobic and in particular high-intensity exercise, nitrite in blood would be converted to NO and modulate muscle blood flow, mitochondrial respiration, and oxygen diffusion to ultimately enhance maximal oxygen consumption. The objectives of the study were to investigate the effects of aerobic-to-anaerobic exercise on nitrite stores in the human circulation and to evaluate the effects of systemic nitrite infusion on aerobic maximal exercise capacity and pulmonary and systemic hemodynamics. Utilization of nitrite reduction to NO in regions of tissue stress or low oxygen tension/pH would present potential unique therapeutic opportunities for a number of diseases characterized by endothelial dysfunction (loss of endothelial NO bioavailability) such as coronary artery disease, congestive heart failure, and hemoglobinopathies.

MATERIALS AND METHODS

Study design. The study was approved by the National Heart, Lung, and Blood Institute Institutional Review Board. This was a randomized, single-blinded, placebo-controlled, crossover trial. Six healthy volunteers were recruited and participated in the study after giving written, informed consent. They included four men and two women, all between the ages of 22 and 31. Three subjects were White (one Hispanic) and three were Black. The mean (range) height, weight, and body mass index of the subjects were 1.71 (1.60–1.85) m, 76.8 kg, and 24.0 (21.7–26.9) kg/m², respectively. Prior to participating in the exercise study, volunteers were evaluated by a clinical examination and baseline clinical laboratory testing to ensure that they had normal pulmonary function tests, electrocardiograms, metabolic panel (including serum electrolytes, glucose, and renal and hepatic functions) and blood cell counts. In addition, the subjects underwent a prestudy exercise study to establish workload increments for the main study.

During the study, an internal jugular central venous line was inserted for the purpose of floating a Swan-Ganz catheter to measure pulmonary pressures and to collect central hemodynamic measurements and mixed venous (pulmonary artery) blood. A brachial arterial line was inserted to monitor arterial blood pressure and to collect arterial blood. An antecubital venous catheter was inserted in the contralateral arm to the arterial line for the purpose of drug infusion. We used topical anesthesia with 2% lidocaine in the catheterization of the pulmonary and radial arteries. For each volunteer, sodium nitrite was infused (loading infusion 15 μmol/min for 20 min, followed by maintenance infusion 5.5 μmol/min for another 50 min) through this catheter on one day, and an equivalent volume of saline was administered on the other day so that subjects would serve as their own controls. Because of the short half-life of nitrite and its effects, the subjects were randomized to receive the two treatments and performed the exercise in two consecutive days. The dosing regimens of sodium nitrite were designed by pharmacokinetic simulation to achieve a mean whole blood nitrite concentration of 5 μmol/l in humans, based on the human pharmacokinetic parameter values obtained from allometric scaling of nitrite pharmacokinetic data from animals in the literature (30, 31, 33). The target concentration of 5 μmol/l was chosen because our preliminary data suggested that nitrite pharmacological effects would be observed within the concentration range of 1–10 μmol/l. Sodium nitrite was prepared by the Pharmaceutical Development Section of the Clinical Center Pharmacy Department and administered under Investigational New Drug No. 70,411.

Volunteers cycled on an upright electronically braked cycle ergometer 30 min after the start of the infusion (i.e., 10 min into the maintenance infusion), with a ramp protocol designed to attain exhaustion in 10 min. The exercise test started with a 2- to 3-min warmup (low load) to acquaint the volunteer with the cycle ergometer (Vmax 229 Cardiopulmonary Exercise System; Sensormedics, Yorba Linda, CA) and prepare him/her for the intensity in the first state of the test. The initial power output was 25 W, followed by increases of 25–40 W based on fitness every minute until maximal tolerance is reached, when the exercise test was terminated. A cooldown period occurred for 3 min when the volunteer continued to pedal at the work rate equivalent to the first stage of exercise testing or lower. The volunteer continued to be monitored for 30 min of recovery. Figure 1 depicts the study design of the protocol.

Arterial and mixed venous blood, systemic arterial blood pressure, and pulmonary artery and wedge pressures were sampled at baseline, after the loading dose, at 10 min into the maintenance infusion, at 2, 4, 6, 8, 10 min into exercise, and at 5, 10, 30 min into recovery (Fig. 1). VO2, carbon dioxide production (VCO2), and expired minute ventilation (Ve) were measured continuously by ergospirometry. Arterial and venous blood samples were collected in diethyletheraminpentectactic acid tubes with N-ethylmaleimide and ferricyanide to stabilize nitrite in lysed red blood cells (10). An aliquot of whole blood was immediately removed and frozen on dry ice and the remaining blood centrifuged at 5,000 rpm for 2 min. Plasma and red blood cell pellet aliquots were removed and frozen immediately on dry ice. All samples were stored at −70°C until analysis.

Measurement of nitrite and HbNO concentrations. Whole blood and plasma nitrite concentrations were determined by chemiluminescence technique (42) by using the Sievers NO-analyzer (NOA 280, GE Analytical Instruments, Boulder, CO). Ion-nitrosyl-hemoglobin (HbNO) concentrations in erythrocytes were analyzed by electron paramagnetic resonance.

Pharmacokinetic modeling. A two-compartment pharmacokinetic model with microparameterization (Fig. 2A) was used to analyze the consumption/elimination and distribution kinetics of whole blood nitrite with the software program SAAM II (Saam Institute, University of Washington, Seattle, WA). With a fixed volume of distribution of 1.12 liters, the pharmacokinetic parameters k10 (elimination rate constant), k12 (transfer rate constant from central to peripheral compartment), and k21 (transfer rate constant from peripheral to central compartment) at rest, during exercise, and after exercise were estimated for the mean whole blood nitrite concentrations obtained from all subjects. For comparison, nitrite concentrations in the absence of exercise were simulated by using the estimated rate constants obtained at rest.

Statistical analyses. The differences in maximal work, maximal oxygen uptake (VO2,max), anaerobic threshold (AT), maximal heart rate (HRmax), and oxygen pulse between saline and nitrite infusion...
were analyzed by paired t-tests. The correlation between two variables was assessed by Spearman’s rank correlation.

The changes of HbNO concentrations in arterial and venous blood during nitrite infusion in the exercise study were analyzed by linear mixed effects modeling, with a different model fitted for the arterial and venous blood separately.

For the repeated measurements of various continuous variables, the mean curves and difference during nitrite and control infusion were analyzed by nonparametric regression spline models. Cubic B-splines with fixed knots at the start of exercise and recovery were used in the regression models to allow fitted mean trajectories to have flexible shape with possible change points due to exercise status. The statistical significance of the difference at each time point between nitrite and control was determined by the 95% confidence band generated from resampling-subject bootstrap. The statistical significance was set at a two-sided $P < 0.05$. Analyses were performed with the R statistical software, version 3.2.2 (R Foundation for Statistical Computing).

Variables evaluated included oxygen uptake ($V\dot{O}_2$), mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), central venous pressure (CVP), pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PCWP), systemic vascular resistance (SVR), pulmonary vascular resistance (PVR), SVR/PVR ratio, mixed venous oxygen saturation ($S\text{vO}_2$), arterial and venous oxygen saturation, arteriovenous (AV) gradient of oxygen saturation, glucose, lactate, pH, methemoglobin level, and nitrite AV gradient in plasma and in whole blood. Because of the small number of observations, CO values obtained by thermodilution were used for the first 30 min of the study when the subjects were at rest, and PVR and SVR were derived from these CO values. For the rest of the study from 30 min onward, CO values were calculated via the Fick equation based on direct measurement of oxygen consumption, and PVR and SVR were calculated by using the CO values obtained from the Fick equation.

RESULTS

Consumption/elimination and distribution kinetics of whole blood nitrite during and after exercise. The mean observed and model predicted whole blood nitrite concentrations with and without exercise are illustrated in Fig. 2B. Mean nitrite concentration reached a level of $\sim 8$ µmol/l at the end of the loading infusion at 20 min, and was reduced to around 5.5 µmol/l after 10 min of maintenance infusion. Nitrite concentration then rapidly declined during exercise, dropped to a level 32% below that without exercise at 40 min, and slowly in-
increased after exercise. Table 1 summarizes the pharmacokinetic parameter estimates at rest, during exercise, and after exercise. The elimination of nitrite at rest and during recovery was first order with an identical rate constant of 0.118 min⁻¹. During exercise, nitrite consumption/elimination appeared to be zero order with a rate of 12.3 μmol/min. The efflux (from blood to tissue) ratios (i.e., \(k_{12}/k_{21}\)) during the three different time periods were 1.31, 0.00, and 1.15, respectively.

Formation of HbNO from nitrite during incremental exercise. HbNO concentrations were determined in arterial and venous blood to assess the formation of NO at four time points: 30 min into nitrite infusion prior to the exercise, pre-AT, post-AT, and during recovery at 70 min of the study. AT was determined by using the ventilator response to exercise and was the inflection point at which \(V_{E}/V_{O2}\) began to continuously increase.

Thirty minutes after infusion of nitrite at rest, HbNO concentrations rose to mean ± standard deviation (SD) values of 3.80 ± 0.726 and 4.70 ± 0.791 μmol/l in arterial and venous blood, respectively (Fig. 3). In arterial blood, HbNO continued to increase pre- and post-AT and reached a mean concentration of 5.1 μmol/l during recovery at 70 min. The overall change of arterial HbNO concentration was statistically significant (\(P = 0.0001\)). On the other hand, lower HbNO elevations were observed during exercise and recovery in venous blood, partly because of the higher mean value prior to the exercise. HbNO concentration increased during exercise, reached a maximum level of 5.28 μmol/l post-AT, and stabilized thereafter during recovery. The changes of HbNO in venous blood over the four time points was not statistically significant (\(P = 0.087\)).

Nitrite effect on incremental exercise test. The overall mean ± SD maximal work rate for all subjects during the study was 215 ± 64.2 W, \(V_{O2\ max}\) was 2.72 ± 0.750 l/min, \(HR_{\ max}\) was 183 ± 17.6 beats/min, and oxygen pulse was 15.1 ± 4.65 ml/beat. The mean AT was 1.43 ± 0.344 l/min and was 54.3 ± 17.7% of the predicted \(V_{O2\ max}\). There were no significant differences in these parameters between nitrite infusion and control (Table 2).

There were no significant differences in \(\dot{V}_{O2}\) values during exercise between the two treatment arms (Fig. 4A). \(\dot{V}_{O2\ max}\) was reduced in four of six subjects in during nitrite treatment compared with saline, with a mean ± SD reduction of 0.114 ± 0.050 l/min. \(\dot{V}_{O2\ max}\) was increased 0.064 l/min in one subject during nitrite infusion and was the same for another subject during the two study periods.

Metabolic changes during and after exercise. Figure 4, B–E, illustrates the pH and concentration profiles of glucose, lactate, and methemoglobin during the study period. Arterial pH decreased from 7.4 ± 0.014 to 7.24 ± 0.066 during exercise and increased back to 7.36 ± 0.077 30 min after exercise. Glucose and lactate rapidly increased during exercise and decreased toward the baseline during recovery. There were no differences in these variables between nitrite and saline infusion. Methemoglobin rose from a mean baseline of 0.7 ± 0.17% to a plateau of 1.0 ± 0.18% 20 min after the initiation of nitrite. Thereafter, it remained significantly elevated for the rest of the study period compared with the control infusion (Fig. 4E).

Hemodynamic effects of nitrite infusion during exercise and recovery. Figure 5 depicts the mean curves and differences in MAP, CO, and PAP, respectively, during nitrite and saline infusion at rest and during exercise and recovery. MAP increased during exercise, peaked at around 38 min, dropped below baseline at around 50 min, and returned back to near baseline at 70 min. Significantly lower MAP was observed during nitrite infusion compared with saline control at 20–45 min, with a largest mean difference of 7.7 ± 4.2, SD mmHg during exercise at 30 min (\(P = 0.006\)). CO increased during exercise, peaked at around 38 min, and returned close to baseline value at 50 min. CO tended to be higher during exercise and recovery when nitrite was infused, although the difference between the nitrite treatment and the saline control was not statistically significant. PAP exhibited similar changes as those described above for MAP; it went up from a baseline of 15.7 ± 5.3 mmHg to 28.6 ± 4.4 mmHg at its maximal value at around 36 min. Contrary to lower MAP values during nitrite administration than control, PAP peak was significantly higher during nitrite infusion at 38–40 min with a mean difference of 5.3 ± 4.9 mmHg, possibly reflecting an increase in CO related to nitrite infusion.

Figure 6 illustrates the mean curves of CVP, PCWP, HR, PVR, SVR, and PVR/SVR ratio, respectively, throughout the study period. CVP started to decrease at the beginning of the warmup period and further declined during exercise until 10 min after exercise. Similarly, PCWP tended downward during exercise until 50 min, when both CVP and PCWP increased toward the baseline during recovery. In contrast, HR rose during exercise from 79 ± 6 beats/min to a peak of 176 ± 21 beats/min at the end of the exercise at around 38 min, which at that point decreased back to baseline within 10 min after exercise.
exercise. PVR and SVR both decreased during exercise. Because the reduction was larger for SVR than PVR, the PVR/SVR ratio increased during exercise. There were no differences in these variables between nitrite infusion and saline control in this study.

The changes in oxygenation throughout the study period are depicted in Fig. 7. Arterial oxygen saturation decreased slightly during exercise and returned to baseline at end of the study. However, the decline was slightly higher and lasted longer during nitrite infusion, resulting in a significant difference in arterial oxygen saturation between nitrite and control during recovery at 40–60 min (Fig. 7A). As expected with vigorous exercise, venous oxygen saturation decreased rapidly from 74 ± 5 to 37 ± 8% during exercise and increased within 10 min during recovery for both nitrite and saline administration. Similarly, SvO₂ declined from 73 ± 0.5% to a nadir of 57 ± 11% before returning to baseline values. Although the extent of this decline appeared to be smaller when nitrite was infused, there were no differences in SvO₂ between the two treatment periods. The changes of oxygen saturation AV gradient followed those of venous oxygen saturation but in a different direction (Fig. 7D); oxygen saturation AV

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Infusion</th>
<th>Nitrite Infusion</th>
<th>P Value</th>
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</thead>
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<tr>
<td>Maximal work rate, W</td>
<td>213 ± 66.9</td>
<td>216 ± 67.7</td>
<td>0.335</td>
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<tr>
<td>V̇O₂ max, l/min</td>
<td>2.75 ± 0.766</td>
<td>2.68 ± 0.805</td>
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<td>AT, l/min</td>
<td>1.47 ± 0.366</td>
<td>1.40 ± 0.350</td>
<td>0.419</td>
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<tr>
<td>AT, % V̇O₂ max predicted</td>
<td>55.7 ± 18.9</td>
<td>52.8 ± 18.3</td>
<td>0.401</td>
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<tr>
<td>HR max, beats/min</td>
<td>183 ± 15.3</td>
<td>183 ± 21.1</td>
<td>0.950</td>
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<tr>
<td>O₂ pulse, ml/beat</td>
<td>15.3 ± 4.66</td>
<td>15.0 ± 5.09</td>
<td>0.395</td>
</tr>
</tbody>
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V̇O₂ max: maximal oxygen consumption; AT, anaerobic threshold; HR max, maximal heart rate.

The changes in oxygenation throughout the study period are depicted in Fig. 7. Arterial oxygen saturation decreased slightly during exercise and returned to baseline at end of the study. However, the decline was slightly higher and lasted longer during nitrite infusion, resulting in a significant difference in arterial oxygen saturation between nitrite and control during recovery at 40–60 min (Fig. 7A). As expected with vigorous exercise, venous oxygen saturation decreased rapidly from 74 ± 5 to 37 ± 8% during exercise and increased within 10 min during recovery for both nitrite and saline administration. Similarly, SvO₂ declined from 73 ± 0.5% to a nadir of 57 ± 11% before returning to baseline values. Although the extent of this decline appeared to be smaller when nitrite was infused, there were no differences in SvO₂ between the two treatment periods. The changes of oxygen saturation AV gradient followed those of venous oxygen saturation but in a different direction (Fig. 7D); oxygen saturation AV
gradient increased from $29 \pm 5$ to $56 \pm 22\%$ during the exercise period and declined back near baseline 10 min into recovery.

**AV gradient of nitrite concentration and oxygen saturation in the systemic circulation.** Figure 8, A and B, presents the systemic AV gradient of nitrite in plasma and in whole blood, respectively. During nitrite treatment, plasma nitrite levels were higher in arterial than in venous blood, leading to a mean positive AV gradient over the study period. A mean positive AV gradient was also found in plasma nitrite during saline control, although the difference was relatively small in the control arm. Both positive and negative nitrite AV differences were found in the whole blood during nitrite infusion and saline control; the reason for the fluctuations in whole blood nitrite AV gradient is unknown. Overall, no differences in AV nitrite gradients in plasma and in whole blood were observed for both treatment arms.

In the saline control, arterial plasma nitrite concentration correlated inversely with arterial oxygen saturation value ($r_s = -0.38$, $P = 0.004$), whereas plasma nitrite AV difference tended to correlate with oxygen saturation AV gradient ($r_s = 0.229$, $P = 0.106$). No such correlation was observed for venous plasma nitrite and venous oxygen saturation. Of note, our nitrite sampling was not performed across the exercising muscle but reflected whole body levels instead.

**DISCUSSION**

The main findings of this study are 1) exercise to maximal levels in healthy humans is associated with increased conversion of circulating nitrite to NO; 2) the enhanced nitrite consumption leads to lower arterial pressure with an associated trend toward increased CO, but otherwise no changes in

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*Fig. 5. Mean curves and difference in mean arterial pressure (MAP, A), cardiac output (CO, B), and pulmonary artery pressure (PAP) during nitrite infusion and saline control at rest, during exercise, and after exercise. *CO values obtained by thermodilution were used for the first 30 min, and values calculated by the Fick equation based on direct measurement of oxygen consumption were used for the rest of the study period.*
pulmonary vascular hemodynamics; and 3) nitrite administration at the doses used did not enhance incremental exercise maximal work or oxygen consumption.

Nitrite pharmacokinetics at rest and during maximal exercise. Concentration data from one of our previous human studies showed that nitrite pharmacokinetics was best described by a two-compartment model (11), which conceptually consists of a central blood compartment and a peripheral tissue compartment. This model assumes that nitrite elimination occurs primarily in the blood compartment because, mathematically, it is impossible to identify simultaneously an elimination rate constant for both the central and the peripheral compartments. Physiologically, nitrite reduction can occur in blood and in tissues via reactions with hemoglobins and molybdopterin-containing enzymes (9, 25–28, 34, 36, 38, 41). As such, the estimated nitrite elimination rate constant should be viewed as an overall consumption rate for nitrite in the body as a whole. It is noteworthy that, unlike a physiologically pharmacokinetic model, the two-compartment model does not describe any physiological processes that are involved in the anabolism and catabolism of nitrite, NO, or other NO species. Nevertheless, our model is consistent with the data showing that nitrite is converted to NO via reactions with hemoglobin, as evidenced by the continued increases of HbNO concentrations before exercise to pre-AT and post-AT, and acts as a storage form of NO in blood and in tissues (14, 29). Overall, HbNO concentrations were higher in venous blood than in arterial blood, showing more NO production in the venous blood where the oxygen saturation and pH value were lower.
Modeling results revealed that nitrite elimination at rest and during recovery was first order with an identical rate constant of $0.118 \text{ min}^{-1}$. During exercise, this consumption/elimination rate became zero order with a rate of $12.3 \mu \text{mol/min}$, which is roughly equivalent to a first order rate constant of $\sim 0.28 \text{ min}^{-1}$ at an average concentration of $4 \mu \text{mol/l}$. This represents an $\sim 137\%$ rate increase, or an extra $123 \mu \text{mol}$ of nitrite consumed during a 10-min exercise. The efflux ratios from blood to tissue at rest and during exercise and recovery were 1.31, 0.00, and 1.15, respectively, suggesting that nitrite distribution and storage into tissue ceased during exercise and resumed slowly after exercise at a rate $\sim 12\%$ lower than that at rest for $\sim 30$ min after exercise.

In a recent report, Kelly et al. (19) described similar kinetic changes of plasma nitrite during severe-intensity exercise in normoxia after 3 days of dietary nitrate supplementation with 100 ml/day of beetroot juice. Resting nitrite concentration, which was elevated after nitrate supplementation compared with placebo, decreased from $\sim 280$ to $140 \text{ nmol/l}$ (i.e., $50\%$) from the beginning of the severe-intensity exercise until exhaustion. This decrease appears to be slightly larger than the concentration change that we observed during exercise (i.e., $32\%$ decrease), but nitrite concentration was in the micromolar range in our study compared with nanomolar range in this report. In addition, subjects in this study had two bouts of moderate-intensity exercise prior to the severe-intensity exercise; the effect of these previous exercise sessions on plasma nitrite level cannot be ruled out. In another exercise study without the supplementation of intravenous nitrite or dietary nitrate, mean plasma nitrite level decreased by $\sim 12\%$ from $767$ to $674 \text{ nmol/l}$ during exercise and slightly increased 10 min after exercise (12). It appears that the metabolism of nitrite during exercise was enhanced in the presence of nitrite or nitrate supplementation.

Because of its vasodilatory effect, nitrite has been proposed and is under development for use as therapeutics for different diseases and conditions. The use of enteral administration of inorganic nitrite and nitrate as well as topical administration of nitrite has also been proposed (29). Although the actual nitrite dose regimen for a specific disease or condition remains to the
determined, the observed effects of exercise on nitrite elimination and distribution may have potential therapeutic implications on a nitrite dose regimen that can be used. If nitrite is used intravenously for its immediate effect on a disease that is dependent on nitrite concentration in an acute setting, physiological changes similar to those induced by exercise could potentially diminish nitrite pharmacodynamic effects by increasing nitrite elimination and reducing concentration. Increased nitrite dose may be needed in this setting. On the other hand, short-term exercise or exercise-induced physiological changes may have lesser effects on nitrite level if nitrite (or nitrite donors) is used to provide prolonged supplementation for storage as observed in the study by Kelly et al. (19). The effect of prolonged submaximal exercise on nitrite pharmaco-kinetics and its therapeutic use, in either the acute or the chronic use setting, remains to be determined.

Effects of supplemental nitrite on exercise and cardiovascular physiology. To cope with the increased metabolic and oxygen demands of skeletal muscle during exercise, the cardiovascular system responds by increasing CO and decreasing SVR, leading to moderate elevation in MAP. Our results showed that, despite similar increase in HR, CO tended to be higher and MAP was lower during nitrite infusion, suggesting vasodilation and a decrease in SVR as the main contributing factor for these effects. Although we did not observe differences in SVR between the nitrite infusion and saline control, the small number of subjects and the high variability of the observations might have contributed to the lack of effect. Of note, we were only able to detect relatively large differences with a sample size of six in our study. Our findings, together with the increased nitrite consumption and HbNO formation during exercise, support the notion of nitrite conversion to release NO resulting in vasodilatation. Our results also showed a greater reduction in arterial oxygen saturation during recovery after exercise, likely suggesting an adverse effect of nitrite on ventilation-perfusion matching due to pulmonary vasodilation and inhibition of baseline hypoxic pulmonary vasoconstriction (1).

Recently, studies were performed to determine the effects of dietary nitrate on \( \text{Vo}_2 \) during exercise (2, 3, 6, 21, 23, 24, 40, 43). Healthy volunteers or athletes were supplemented with sodium nitrate or beetroot juice hours to days prior to exercise testing. \( \text{Vo}_2 \) was significantly reduced and plasma nitrite levels were significantly increased during nitrate supplementation compared with placebo. Mechanistically, nitrate supplementation reduced the expression of ATP/ADP translocase, a protein that is involved in proton conductance, and improved mitochondrial oxidative phosphorylation efficiency (phosphate-to-oxygen ratio)
leading to lowered oxygen cost (22). Our study did not demonstrate a significant reduction in $\overline{V_O_2}$, but $\overline{V_O_2}_{max}$ was lower in four of six subjects during nitrite treatment compared with saline control. It is possible that our sample size was too small to allow the detection of a small difference between groups and that a more prolonged administration of nitrite may be needed to induce changes such as ATP/ADP translocase expression. In addition, maximal exercise was not achieved in all subjects, which would further limit the interpretation of the results. Contrary to these previous results, NOS inhibition by l-NAME infusion significantly reduced $\overline{V_O_2}_{max}$ during incremental cycle exercise (18), suggesting additional influence of NO on exercise $\overline{V_O_2}$ by other mechanisms such as the inhibition of cytochrome c oxidase and mitochondrial respiration (7).

Nitrite has been shown to have a number of opposing effects on mitochondrial respiration that might enhance or impair maximal exercise capacity. Inhibition of complex I and IV during hypoxia has been shown to inhibit respiration (34). In contrast, nitrite can increase exercise efficiency ($\Delta \overline{V_O_2}/\Delta W$) via downregulation of uncoupling proteins and reduction of proton leak (34). It is therefore possible that the higher concentrations of nitrite used in this study (approaching 10 $\mu$mol/l) compared with the levels during nitrate exposure (nitrate consumption leads to plasma nitrite levels that are typically less than 1 $\mu$mol/l) may explain the differences in our measured $\overline{V_O_2}_{max}$ values.

In addition to the lack of significant changes in oxygen uptake, there was no difference in work rate between nitrite infusion and saline control in our study. Although the limitations described above also apply to these parameters, this finding is inconsistent with the effect of other NO modulating drugs such as sildenafil, which improved aerobic exercise capacity during experimental hypoxic stress (low inspired oxygen fraction) (13) or agents that impair NO generation (20). Furthermore, exercise performance was improved with peak $\overline{V_O_2}$ and $\Delta \overline{V_O_2}/\Delta W$ significantly increased in patients with pre- and postcapillary pulmonary hypertension receiving NO inhalation and sildenafil, respectively (15, 16). Our results in light of the work of others suggest that the effects of nitrite supplementation on exercise are complex and may depend critically on factors such as total dose, dosing schedules (acute vs. chronic), and level and duration of exercise. Further larger studies with preconditioned exposure to nitrite or greater hypoxic stress in healthy subjects and patient populations may further elucidate the physiological role of nitrite during exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


