The Effect of Nitrate on the Oxygen Availability during Exercise in Effort Angina Pectoris

Hiroshi YAMABE, M.D., Katsuya KOBAYASHI, M.D., and Hisashi FUKUZAKI, M.D.

SUMMARY

Respiratory responses during exercise were observed to determine whether improvement of oxygen availability in working skeletal muscle is attributable to increased aerobic capacity after administration of nitrate in patients with effort angina. After isosorbide dinitrate (ISDN) administration, the aerobic capacity increased 3.3 ml/min/Kg (20%) as compared with the control test (p<0.001), but the anaerobic threshold (AT), a good indicator of oxygen availability, was unchanged, and the respiratory quotient at the peak of exercise was elevated. These findings suggest that oxygen availability in skeletal muscle was not altered after ISDN, and increased exercise load accompanied increased anaerobic glycolysis. It was concluded that the nitrate-induced increase in aerobic capacity was not dependent upon the change in oxygen availability in skeletal muscle but rather upon the elevated anginal threshold.

Additional Indexing Words:
Anaerobic threshold Ventilation Metabolism

It is well known that nitrate can increase exercise tolerance and aerobic capacity in patients with effort angina. Nitrate also improves elevated left ventricular filling pressure, decreased stroke volume and inadequate responses of cardiac output during exercise. However, the effects of nitrate on oxygen availability remain unknown, but they may be a factor in the increased aerobic capacity observed after nitrate administration. This study was undertaken to elucidate the oxygen availability after nitrate administration by analysis of respiratory responses during exercise in patients with stable effort angina.
SUBJECTS AND METHODS

Subjects
Ten male patients with stable angina, including 4 patients with post-infarction angina, were subjects in this study. Their ages were 59±10 years ranging from 40 to 72 years. Body weights were 63±10 Kg from 48 to 78 Kg. No patients had either congestive heart failure or developed exertional dyspnea due to compromised respiratory function.

Methods

Study protocol: The exercise test was a symptom-limited maximal treadmill test using a 1 min incremental protocol (Fig. 1). The endpoint of exercise was progressive chest pain, marked dyspnea or fatigue. A 12 lead electrocardiogram was recorded before and immediately after exercise. In addition, the ST segment was continuously monitored at 0.08 sec after the J-point in leads aV\(_2\), V\(_3\) and V\(_5\) with a computer assisted system for exercise (CASE, Marquette Co., Ltd.). The onset of ST depression was determined from the trendgram of ST level (Fig. 2). Heart rate (HR) was also measured continuously. Patients underwent exercise tests 3 times on separate days, including two tests under no medication and one test 30 min after oral administration of 10 mg isosorbide dinitrate (ISDN). One baseline unmedicated test was performed during first session. The second baseline test and the ISDN test were then undertaken at random order. The second baseline test was defined as the control test in order to avoid the learning effect.

Measured variables: The respiratory indices were measured by a RM-200 system (Minato Medical Science Co., Ltd.) with the breath-by-breath method. A face mask with 150 ml of dead space was applied to a patient. It was directly open to room air. The expiratory and inspiratory flows were measured by a hot-wire flowmeter attached to the outlet of a face mask. Gas samples

![Fig. 1. Schematic illustration of the 1 min incremental protocol in this study. The protocol for multistage exercise utilized in this study is a 1 min incremental protocol which is appropriate for determination of anaerobic threshold (AT). This protocol is modified from Bruce's protocol as indicated in this schema. It starts from the level of 1.1 mile/hour walking speed with 0% gradient for 3 min and increases its workload by 1/3 of each corresponding stage of Bruce's protocol per minute.](image-url)
were sucked into a mass spectrometer EL 1100 (Perkin Elmer Co., Ltd.) at a rate of 60 ml/min, and oxygen and carbon dioxide contents were analyzed continuously. Subsequently, these values were put into a RM-200 on-line system for computer analysis to calculate the oxygen intake and carbon dioxide production at each breath. The measured variables were minute oxygen intake (\(VO_2\) ml/min/Kg), minute carbon dioxide production (\(VCO_2\) ml/min/Kg), respiratory quotient (RQ), minute ventilation (\(VE\) l/min), oxygen removal (\(VO_2/VE\)) and \(VCO_2/VE\). These values were recorded on a printer every 30 sec during and after exercise. The value for 5 min in the sitting position before exercise is a resting level. The anaerobic threshold (AT; expressed as \(VO_2\)) was visually determined as the initial breakpoint at which an increment of VE exceeded that of \(VO_2\) during exercise (Fig. 3). Blood pressure (BP) was measured using a sphygmomanometer.
Fig. 3. The method of measuring anaerobic threshold (AT). The VE was plotted on the vertical line corresponding to the VO₂. AT is determined as the initial breakpoint at which an increase of VE exceeds that of VO₂ by visual inspection.

Table I. Results of Two Exercise Tests under No Medication

<table>
<thead>
<tr>
<th></th>
<th>HR (bpm)</th>
<th>BP (mmHg)</th>
<th>VO₂ (ml/min/Kg)</th>
<th>VCO₂ (ml/min)</th>
<th>Vₚ (l/min)</th>
<th>VO₂ at ST depression (ml/min/Kg)</th>
<th>VO₂ at angina (ml/min/Kg)</th>
<th>AT* (ml/min/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st test</td>
<td>112±23</td>
<td>159±21</td>
<td>16.9±5.5</td>
<td>15.8±6.6</td>
<td>35.4±12.8</td>
<td>11.0±4.0</td>
<td>15.6±6.1</td>
<td>13.2±1.4</td>
</tr>
<tr>
<td>2nd test</td>
<td>111±21</td>
<td>156±14</td>
<td>16.5±5.5</td>
<td>15.3±6.3</td>
<td>33.7±13.8</td>
<td>10.9±4.1</td>
<td>14.9±6.0</td>
<td>13.5±1.5</td>
</tr>
</tbody>
</table>

HR = heart rate; BP = blood pressure; VO₂ = oxygen intake; VCO₂ = carbon dioxide production; Vₚ = minute ventilation; AT = anaerobic threshold. * n=6.

Table II. Comparison of the Circulatory and

A. at rest

<table>
<thead>
<tr>
<th></th>
<th>Exercise time (min)</th>
<th>HR (bpm)</th>
<th>BP (mmHg)</th>
<th>VO₂ (ml/min/Kg)</th>
<th>VCO₂ (ml/min/Kg)</th>
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</thead>
<tbody>
<tr>
<td>control test</td>
<td>—</td>
<td>73±13</td>
<td>121±10</td>
<td>3.8±0.6</td>
<td>3.2±0.4</td>
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<tr>
<td>ISDN test</td>
<td>—</td>
<td>82±18</td>
<td>103±15</td>
<td>3.8±0.7</td>
<td>3.2±0.6</td>
</tr>
<tr>
<td>p-value</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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</tr>
</tbody>
</table>

B. at the peak of exercise

<table>
<thead>
<tr>
<th></th>
<th>Exercise time (min)</th>
<th>HR (bpm)</th>
<th>BP (mmHg)</th>
<th>VO₂ (ml/min/Kg)</th>
<th>VCO₂ (ml/min/Kg)</th>
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</thead>
<tbody>
<tr>
<td>control test</td>
<td>6.9±2.6</td>
<td>111±21</td>
<td>156±14</td>
<td>16.5±5.5</td>
<td>15.3±6.3</td>
</tr>
<tr>
<td>ISDN test</td>
<td>8.7±2.0</td>
<td>123±21</td>
<td>157±17</td>
<td>19.8±4.8</td>
<td>19.1±5.7</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001</td>
<td>0.01</td>
<td>ns</td>
<td>0.001</td>
<td>0.05</td>
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</table>

HR = heart rate; BP = blood pressure; VO₂ = oxygen intake; VCO₂ = carbon dioxide production.
Statistical analysis: The statistical analysis to compare the ISDN test with the control test was performed by Student's t-test for paired data. A p-value of <0.05 was considered statistically significant. The values in this study were expressed as the average for 1 min and indicated by the mean±SD.

Results

Coronary arteriography revealed one vessel disease in 4 patients, two vessel diseases in 3 patients and three vessel diseases in 3 patients, according to the AHA criteria of 75% or greater luminal stenosis. Left ventricular ejection fraction was 59±13% (35–78%). Pulmonary function test exhibited that % vital capacity was 91±14% (71–120%) and % forced expiratory volume 1.0 was 81±7% (69–94%). Three patients showed slightly disturbed pulmonary function.

Table I shows the results of two exercise tests under no medication. All patients terminated the two exercise tests due to chest pain. The average values of the hemodynamic and respiratory indices, the onset of ST depression and anginal threshold were the same level for the first and the second test. The AT could not be detected in 6 of 20 exercise tests under no medication (4 of 10 first tests and 2 of 10 second tests) because chest pain progressed at a lower level exercise before reaching AT. AT was same level in matched 6 patients between the first and the second test.

Table II shows the comparison of control and ISDN test. Under resting condition (Table II–A), HR was high and BP was significantly low after ISDN administration. However, all of the respiratory indices did not change after ISDN. At the peak of exercise (Table II–B), the exercise time was prolonged from 6.9±2.6 min to 8.7±2.0 min (p<0.001). The HR

<table>
<thead>
<tr>
<th>RQ</th>
<th>$V_E$ (l/min)</th>
<th>$VO_2/V_E$ (peak $F_E$)</th>
<th>$VO_2/V_E$ (maximunm)</th>
<th>$VCO_2/V_E$</th>
<th>$VO_2$ at ST depression (ml/min/Kg)</th>
<th>$VO_2$ at angina (ml/min/Kg)</th>
<th>AT* (ml/min/ Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.85±0.07</td>
<td>10.2±1.3</td>
<td>23.5±3.8</td>
<td>—</td>
<td>19.9±2.4</td>
<td>—</td>
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</tr>
<tr>
<td>0.85±0.04</td>
<td>10.0±1.3</td>
<td>23.7±2.4</td>
<td>—</td>
<td>19.8±2.6</td>
<td>—</td>
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<tr>
<td>0.90±0.07</td>
<td>33.7±13.8</td>
<td>31.5±4.8</td>
<td>34.2±3.9</td>
<td>28.5±3.6</td>
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<tr>
<td>0.95±0.07</td>
<td>41.9±14.1</td>
<td>30.2±4.9</td>
<td>34.0±2.9</td>
<td>28.7±3.1</td>
<td>14.6±4.0</td>
<td>18.5±5.5</td>
<td>13.4±1.4</td>
</tr>
<tr>
<td>0.05</td>
<td>0.001</td>
<td>0.05</td>
<td>ns</td>
<td>ns</td>
<td>0.001</td>
<td>0.01</td>
<td>ns</td>
</tr>
</tbody>
</table>

Respiratory Indices between Control and the ISDN Test

$V_E$=minute ventilation; RQ=respiratory quotient; AT=anaerobic threshold. * n=8.
increased from 111±21 bpm to 123±21 bpm (p<0.001) after ISDN, while the BP was unchanged (156±14 mmHg vs 157±16 mmHg). After ISDN, the peak VO₂, which indicates the aerobic capacity, increased 20% from 16.5±5.5 ml/min/Kg to 19.8±4.8 ml/min/Kg (p<0.001), and the VCO₂

![Graph](image)

**Fig. 4.** Comparison of the maximum VO₂/VE, and VO₂/VE and RQ at peak of exercise between control test and ISDN test. The maximum VO₂/VE was unchanged after ISDN. However VO₂/VE at peak of exercise was significantly decreased and RQ was significantly increased after ISDN. These findings suggest that increased anaerobic glycolysis is relevant to the increased energy metabolism after ISDN.

![Graph](image)

**Fig. 5.** Correlation between increase in aerobic capacity and changes in anginal threshold and AT after ISDN. The increase in anginal threshold correlated significantly with the increase in aerobic capacity after ISDN (r= 0.86, p<0.001), while the change in AT did not contribute to the increased aerobic capacity after ISDN.
increased 25% from 15.3±6.3 ml/min/Kg to 19.1±5.7 ml/min/Kg (p<0.001). Thus, RQ increased from 0.90±0.07 to 0.95±0.07 (p<0.05) after ISDN. The VE increased 24% from 33.7±13.8 l/min to 41.9±14.1 l/min (p<0.001). On the other hand, the maximum VO₂/VE was not altered after ISDN (34.2±3.9 vs 34.0±2.9), while VO₂/VE at the peak of exercise decreased significantly from 31.5±4.8 to 30.2±3.9 (p<0.05) after ISDN (Fig. 4). The VCO₂/VE was not altered after ISDN (28.5±3.6 vs 28.7±3.1). The onset of ST depression increased 34% from 10.9±4.1 ml/min/Kg to 14.6±4.0 ml/min/Kg (p<0.001) after ISDN, and the anginal threshold increased 23% from 14.9±6.0 ml/min/Kg to 18.5±5.5 ml/min/Kg (p<0.01). Anginal pain appeared in all patients, but it was not aggravated during ISDN test in 3 patients. Since AT did not appear in 2 patients in the control test, although it appeared in all cases in ISDN test, AT was compared in 8 patients. After ISDN, AT did not change significantly (13.1±1.4 ml/min/Kg vs 13.4±1.4 ml/min/Kg). Thus, as indicated in Fig. 5, the increased aerobic capacity was well correlated with the increased anginal threshold after ISDN (r=0.86, p<0.001), however the change in AT did not contribute to the increase in aerobic capacity.

DISCUSSION

Energy metabolism in working skeletal muscle depends upon both aerobic ATP production from the TCA cycle and anaerobic ATP production from glycolysis. The ratio of ATP production in these metabolic pathways is affected by the intensity of the workload and the oxygen availability of skeletal muscle. For a low level workload, sufficient ATP is supplied by the TCA cycle. However, when workload exceeds a certain level, anaerobic glycolysis is activated and this results in the elevation of blood lactate. This workload is defined as AT. The AT can be measured by the respiratory method as the point at which VE deviated from a linear relationship to VO₂ during incremental exercise, because increased blood lactate is buffered by HCO₃⁻ in the blood, and results in additional carbon dioxide production which augments the ventilatory drive. The AT is used to evaluate the status of aerobic conditioning, since it reflects the net function of the aerobic environment and oxygen availability in skeletal muscle and is correlated well with the maximal oxygen intake.

We have previously reported that both aerobic capacity and AT are impaired in patients with effort angina, compared to normal age-matched subjects. In patients with effort angina, aerobic capacity can be improved by nitrate, but it is not clear whether nitrate increases AT as well as aerobic
The AT is known to be influenced by several factors such as oxygen transport, activity of the oxygenative enzymes in mitochondria of skeletal muscle, mass of working skeletal muscle and composition of the muscle fibers. It has been postulated that nitrate may improve the oxygen availability in working skeletal muscle resulting in an increased AT. This hypothesis is supported by the findings that nitrate increases cardiac output during exercise in effort angina, resulting in an increase in oxygen transport to skeletal muscle. Furthermore, peripheral venodilating effects of nitrate cause marked blood pooling in the lower extremities, thus, the contact area between capillary blood and skeletal muscle fibers is augmented, consequently increasing oxygen extraction. However, according to our results, AT did not change after ISDN. However, there was a significant, 20% increase in aerobic capacity. This finding suggests that the improved aerobic capacity after ISDN in effort angina cannot be explained as the results of a change in oxygen availability. The unchanged maximum VO$_2$/VE after ISDN was also derived from the unchanged oxygen availability. On the other hand, increased aerobic capacity correlated well with increased anginal threshold. Thus, it is probable that the effects of nitrate on aerobic capacity are mainly attributed to anti-anginal effect. Since AT was unchanged, the increased energy metabolism after ISDN was supported by augmented anaerobic glycolysis, as indicated by the increased RQ and decreased VO$_2$/VE at the peak of exercise after ISDN.

Several mechanisms may explain why AT was unchanged despite the increased aerobic capacity after ISDN. First, cardiac output is not necessarily increased after nitrate due to an extreme reduction of venous return, although anginal attack is alleviated. Second, since blood flow in working skeletal muscle is precisely regulated by energy metabolites in order to respond to the oxygen demand, it may be unchanged after ISDN, even though cardiac output during exercise increases after ISDN. Finally, even when the blood flow in skeletal muscle is increased with resultant increased oxygen transport or when the blood volume in the lower extremities is increased by venodilation after nitrate, it seems aerobic metabolism is not increased without increased activity of the oxygenative enzymes in the mitochondria. Therefore, although AT was not altered by nitrate in this study, it is possible that AT is increased in cases with increased physical activity in daily life, as a result of improved anginal symptoms due to long-term therapy with nitrates. This may be related to improvement in oxygen transport and in the metabolic status of skeletal muscle. In this situation, a further increase in aerobic capacity may also be expected.

There are several limitations of this study. It was reported that an
increase in lactate content in working skeletal muscle precedes an increase in lactate concentration in blood. Thus, the AT determined by the respiratory method may not express a real AT in working skeletal muscle. However, the AT evaluated by the respiratory method undoubtedly reflects a certain level of lactate production with considerable accuracy. Therefore, it seems to be permissible to use the AT as a parameter for evaluating oxygen availability during exercise before and after nitrate administration. It should be noted direct measurements of oxygen transport, oxygen extraction and metabolites in skeletal muscle during exercise are necessary in future studies. Another problem is the determination of the ischemic threshold using a 1 min incremental protocol which is not a steady state exercise. It may have certain gaps compared to the threshold evaluated by a steady state exercise protocol. However, this gap should be small because each workload increment is small. Thus, it is permissible to compare the ischemic thresholds before and after nitrate.

It was concluded that nitrate could improve aerobic capacity in patients with effort angina by increasing anginal threshold, but it could not increase AT which indicates an oxygen availability in skeletal muscle during exercise. Thus, the increased energy metabolism after nitrate accompanied the increased anaerobic glycolysis.

Acknowledgments

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References