The effect of endurance training on the rate of nitrogen elimination in women.

N. LUNDSETT 1, U. WISLØFF 1,2, A. HJELDE 1, A. O. BRUBAKK 1

1Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, 2Department of Cardiology, St. Olavs Hospital, Trondheim, Norway


Lundsett N, Wisløff U, Hjelde A, Brubakk AO. The effect of endurance training on the rate of nitrogen elimination in women. Undersea Hyperb Med 2006;33(4):281-289. Introduction: The rate of nitrogen elimination may be an important factor in evaluating the risk of DCS following dives. The present study determined the reproducibility of a method for evaluating nitrogen elimination (series I), and the effect of chronic training on the nitrogen elimination in healthy young women (series II). Methods: Nitrogen elimination was determined with subjects wearing an AGA full-face mask breathing pure oxygen. To evaluate the reproducibility of the method for nitrogen elimination, three tests were performed in six subjects in series I. Nitrogen elimination in series II was measured before and after the training period. The training protocol (series II) consisted of interval training, three times per week for eight weeks. Four repeated intervals alternated between four minutes at 90-95% of maximum heart rate and three minutes at 50-60%. Results: There was no significant difference between the three repeated tests. Interval training for eight weeks increased maximum oxygen uptake by 22.1%. Endurance training did not influence the total nitrogen elimination at rest. Conclusion: The method for evaluating nitrogen elimination at rest was found to be reproducible. Improved aerobic capacity does not increase the rate of nitrogen elimination at rest.

INTRODUCTION

Decompression sickness (DCS) is characterized by bubble formation as a result of supersaturation, mainly by inert gases (1). The most commonly used diving gas is air, where nitrogen (N₂) is the inert gas. There is evidence that N₂ bubbles grow from small gas-filled bubbles, micronuclei (~1 μm in diameter) that are attached to hydrophobic surfaces, like the blood vessel endothelium, fascia and tendons (2). The risk of DCS increases with the amount of N₂-accumulated in body fluids. It is assumed that most of these bubbles are removed by the lungs and that only few bubbles enter the arterial circulation (3, 4). There is, however, concern that diving may lead to permanent damage to the central nervous system, even in absence of clinical symptoms of DCS (5, 6).

Several studies indicate a reduced incidence of DCS in well-trained humans (7), pigs (8), and mice (9). These studies suggest that chronic exercise may influence bubble formation.

The exact mechanism for the long-term training-protective effect is not known, and several possible factors have been suggested. First, it is well established that chronic training leads to increased capillary density in skeletal muscles (10, 11), which improves blood perfusion both at rest and during exercise (12, 13, 14), and may lead to an increase in the rate of N₂ elimination. Second, an increase in pulmonary diffusion capacity could be a response to aerobic endurance training, since both maximum cardiac output and pulmonary blood flow increase with endurance training (15). However, there is no agreement of whether this adaptation takes place (15). Third, chronic training may change the properties of the blood vessel endothelium and affect bubble formation.
(Wisløff and Brubakk 2001). Thus, aerobic endurance training may alter several variables that are central in nitrogen elimination.

From this, several lines of research can be followed to determine the effect of chronic training on the risk of developing DCS. The present study had two aims: first, to determine the reproducibility of the established method for evaluating N\textsubscript{2} elimination, and second, to investigate whether increased aerobic capacity improves N\textsubscript{2} elimination at rest.

**MATERIALS AND METHODS**

The study consisted of two experimental series. In *series I*, we determined the reproducibility of a method for measuring N\textsubscript{2} elimination. In *series II* we determined the effects of increased maximum oxygen uptake on the rate of N\textsubscript{2} elimination. All tests were performed in a laboratory with a temperature of 21.5 ± 0.4 °C and a relative humidity of ~50%.

**Subjects**

A total of 21 female subjects (22.9±1.3) participated in this study, divided into *series I* (n=6) and *series II* (n=15). The participants were students recruited from the School for Medical Laboratory Technicians at the Sør-Trøndelag University College, Norway. Each subject reviewed the consent forms approved by the Human Research Review Committee before inclusion. Subjects were included if they were non-smokers and had a maximal oxygen uptake below 45 mL·kg\textsuperscript{-1}·min\textsuperscript{-1} (17). Subjects were not allowed to consume food and alcohol six hours and 48 h, respectively, prior to the N\textsubscript{2} elimination tests.

**Test procedure series I**

**N\textsubscript{2} elimination**

Upon entering the laboratory, the subjects were resting in an armchair for 15 minutes, and then in a supine position breathing compressed ambient air (AGA, Oslo, Norway) for five minutes wearing an tight-fitting AGA full-face mask (Interspiro, SE) for familiarization to the test equipment. The subjects started to breathe 99.95% oxygen (O\textsubscript{2}) and the expired gas from the mask was collected in a bag with volume of 2L. A one-way valve was connected to the bag to avoid inflow of ambient air. A vacuum pump (Pfeiffer, Balzers, Asslar, Germany), with a capacity of about 400mL/min, sampled the gas mixture directly from the bag, every 5 seconds for a total collection time of 23 minutes, and transported the gas to the analysers for measurements of the different gases. N\textsubscript{2}, O\textsubscript{2} and CO\textsubscript{2} obtained from the bag were analysed by a N\textsubscript{2}- (Morgan Nitrogen Analyzer, P.K. Morgan Ltd, UK), O\textsubscript{2}- (PM 1111E, Servomex Ltd, UK) and a CO\textsubscript{2} (17515A, Vacumetrics Inc, USA) analyser with an accuracy of 1%, 0.1% and 0.03%, respectively. To determine the repeatability of the nitrogen elimination recordings, the procedure was repeated 3 times, each time separated by 48h.

**Minute ventilation**

Minute ventilation (V\textsubscript{E}) was measured during the N\textsubscript{2} elimination tests using a portable system for metabolic measurement (Metamax, Cortex, Germany). The data was recorded every 20 seconds during a 23 minute period.

**Heart rate**

The subjects resting heart rates (HR) were determined using short-range radio telemetry, stored and read off using a computer with Polar software program (Polar Sport Tester, Polar Electro, Finland). An electrode belt fastened to the chest registered HR as a mean of 5 second intervals and transmitted signals to a receiver fastened to the subjects arm.
Gas analyses equipment

The gas analysers were calibrated using 99.9999% O₂ and 100% N₂, additionally the N₂-analysers was calibrated with a gas mixture of N₂ (77.79%), O₂ (20.01%) and CO₂ (2.02%) and one gas mixture of N₂ (1.42%) and O₂ (98.58%). The analysers were checked for possible drift, by using each of the above calibrating gases, immediately after each test. All data were recorded on a computer using the Lab View software program (National Instruments, USA).

Test procedure series II

The individuals in series II went through a 2-day test procedure before and after, pre-test and post-test respectively, 8 weeks of endurance training.

Test-day one

Upon entering the laboratory, body height, body-mass, hemoglobin concentration ([Hb]) and hematocrit (Hct) were measured for normative data comparisons. For [Hb] and Hct determination, a blood-sample was collected by venopuncture and analysed by standard procedure (Cobas Micros, Bergman Diagnostics, Norway).

Body fat

Body fat was measured using a standard skin-fold caliper (Holtain Ltd, UK). The measurement was performed over the skin covering the Musculus triceps brachii and the Musculus subscapularis, and was repeated 3 times. The average was used in subsequent calculation. Percent body fat was estimated using the formula:

\[
\text{% Body fat} = 0.55(A) + 0.31(B) + 6.13
\]

where A = triceps fat fold (mm) and B = subscapular fat fold (mm). The predicted fat mass using this particular method is within 3-5% of body fat measured by hydrostatic weighing (18).

Measurements of maximal oxygen uptake

Maximal oxygen uptake (\(V_{O_2}^{\text{max}}\)) and maximum heart rate (\(H_{R_{max}}\)) were determined 5 days prior to the 8 week training period. Oxygen uptake (\(V_{O_2}\)) was measured using Metamax (Cortex, Germany) consisting of a mask, transmitter and receiving unit. The subjects ran on a motorized treadmill (Challenger LE5000, Jaeger EOS Sprint, Germany) with 3° incline and a speed of ~7 km/h for 20 minutes as a warm-up period before the measurement of \(V_{O_2}^{\text{max}}\) and \(H_{R_{max}}\). Thereafter, the treadmill speed was increased by 1 km/h every minute to a level that brought the subject close to exhaustion within approximately 5 minutes. Inclination was constant at 3°. Immediately after \(V_{O_2}^{\text{max}}\) determination, each subject ran for 2 minutes at an intensity of 50-60% of \(V_{O_2}^{\text{max}}\) followed by a supramaximal intensity, resulting in exhaustion within about 3 minutes (19. 2001). Heart rate was determined using short range radio telemetry (Polar Sport Tester, Polar Electro, Finland). The highest HR recorded during the last minute of the supramaximal run was accepted as the \(H_{R_{max}}\).

Test-day two

Nitrogen elimination measurements, minute ventilation and resting heart rate were performed as previously described in the test procedure for series I.

Training protocol

The training program was designed based on the test of \(V_{O_2}^{\text{max}}\) and \(H_{R_{max}}\). The subjects exercised under the supervision of one of the authors three times per week for eight weeks. Each exercise started with a warm-up period of 20 minutes at an exercise intensity corresponding to 60-70% of \(H_{R_{max}}\). Then the
interval period lasted about 25 minutes and consisted of 4 bouts of walking/running up a step hill for 4 minutes with an exercise intensity corresponding to 90-95% of HR_{max} alternated with a 3 minute recovery at 50-60% of HR_{max} between the intervals (Slørdahl et al. 2004). Total duration of the training session was 45 minutes. All subjects used short-range radio telemetry (Polar Sport Tester, Polar Electro, Finland) to control their own HR.

**Statistical methods**

Comparisons of results before and after the training period in series II, were evaluated using a paired t-test. Normality of data in series II was confirmed using the Kolmogorov-Smirnov statistic or Q-Q normal probability plot. However, due to the small number of subjects (n=6) in series I, a non-parametric Friedman test was carried out to evaluate any differences between the 3 repeated measurements within the 6 subjects. Differences were considered significant at p<0.05.

**RESULTS**

Physical and physiological characteristics, except for VO_{2max} of the subjects did not change during the training period (p>0.05) (Tab.1). Maximal respiratory exchange ratio did not differ between the tests and was on average 1.14 ± 0.5.

**Series I**

There was no significant difference between the three repeated measurements in series I. There was no drift in the tests of the gas analysis equipment.

Minute ventilation (L · min^{-1}) in test 1, 2 and 3 is presented in Table 2 (n=6) and demonstrate reproducible data. The table shows mean minute ventilation from 6 individuals at different time periods. Each individual is represented with a mean value of 3 consecutive measurements during one minute up to 5 minutes. From 6-23 minutes the data presented as the average of 3 measurements every second minute.

**Series II**

Eight weeks interval training increased VO_{2max} by 9.1 ± 2.2 mL·kg^{-1}·min^{-1} (Tab.1), corresponding to an average increase of 22.1 ± 5.8% (p<0.0001) during the experimental period.

There was no significant difference in the rate of total body nitrogen elimination at rest before and after the 8 weeks of training. However, a slight increase in N2 elimination was observed during the first 2 minutes (p<0.008) of breathing ~100% O2. For the rest of the observation (2.5-23 min) period there was no significant difference between pre- and post-test values (Fig. 1). Minute ventilation during N2 elimination is presented in Table 3 and shows only minor non-significant variations between pre- and post test.

**DISCUSSION**

The major finding of the present study was that a substantial increase in aerobic capacity did not increase the rate of N2 elimination at rest. It is known that chronic endurance training increases skeletal muscle capillarization as well as the plasma volume (10, 11), which has been suggested as mechanisms for increased rate of N2 elimination observed in a previous single-case study (7). Dick et al. (1984) observed that when the diver was untrained he had reduced rate of N2 elimination after dives and the diver experienced itching and post-dive fatigue as a possible sign of decompression sickness (DCS). The reason for the discrepancy between the study of Dick et al. (1984) and the present study is not known, but may be due to inter-subject differences. Nitrogen elimination is
### Table 1. Physical and physiological characteristics of the subjects in *series I* and of the subjects in *series II* at pre- and post-test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Series I</th>
<th>Series II pre-test</th>
<th>Series II post-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>23.0 ± 1.6</td>
<td>22.9 ± 1.0</td>
<td>22.9 ± 1.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.0 ± 1.7</td>
<td>170.0 ± 5.0</td>
<td>170 ± 5.0</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>63.1 ± 5.8</td>
<td>64.3 ± 8.8</td>
<td>64.3 ± 8.4</td>
</tr>
<tr>
<td>Body fat percentage (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting heart rate (beats · min⁻¹)</td>
<td>51.2 ± 4.6</td>
<td>50.2 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>Maximum heart rate (beats · min⁻¹)</td>
<td>194.0 ± 11.5</td>
<td>194.1 ± 11.5</td>
<td></td>
</tr>
<tr>
<td>Resting heart rate during N₂ elimination test (beats · min⁻¹)</td>
<td>62.9 ± 7.2</td>
<td>61.2 ± 10.2</td>
<td></td>
</tr>
<tr>
<td>VO₂max (mL·kg⁻¹·min⁻¹)</td>
<td>42.0 ± 1.2</td>
<td>51.1 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>[Hemoglobin] (g·dL⁻¹)</td>
<td>13.0 ± 1.0</td>
<td>12.7 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39.3 ± 2.6</td>
<td>37.8 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>V̇Emax (L · min⁻¹) at VO₂max</td>
<td>107.2 ± 8.9</td>
<td>103.6 ± 11.2</td>
<td></td>
</tr>
</tbody>
</table>

The data are mean and standard deviations (SD). VO₂max; maximum oxygen uptake, V̇Emax; maximum minute ventilation (n=6 in series I and n=15 in series II). # = significant

### Table 2. Minute ventilation during nitrogen elimination in test 1, 2 and 3 in *series I*.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Test 1 (L · min⁻¹)</th>
<th>Test 2 (L · min⁻¹)</th>
<th>Test 3 (L · min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 minute</td>
<td>6.1 ± 2.1</td>
<td>6.2 ± 1.9</td>
<td>6.2 ± 2.7</td>
</tr>
<tr>
<td>2 minute</td>
<td>6.5 ± 1.2</td>
<td>6.6 ± 1.6</td>
<td>6.0 ± 2.2</td>
</tr>
<tr>
<td>3 minute</td>
<td>6.9 ± 1.9</td>
<td>7.0 ± 2.9</td>
<td>6.4 ± 3.1</td>
</tr>
<tr>
<td>4 minute</td>
<td>7.9 ± 1.7</td>
<td>7.9 ± 1.5</td>
<td>7.3 ± 1.3</td>
</tr>
<tr>
<td>5 minute</td>
<td>7.5 ± 2.0</td>
<td>7.6 ± 1.0</td>
<td>8.1 ± 2.1</td>
</tr>
<tr>
<td>6-23 minutes</td>
<td>7.4 ± 1.9</td>
<td>7.3 ± 1.4</td>
<td>7.7 ± 1.8</td>
</tr>
</tbody>
</table>

The data are mean ± SD, (n=6).

### Table 3. Minute ventilation during nitrogen elimination in pre- and post-test in *series II*.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Pre test (L · min⁻¹)</th>
<th>Post test (L · min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 minute</td>
<td>10.1 ± 3.5</td>
<td>8.9 ± 2.8</td>
</tr>
<tr>
<td>2 minute</td>
<td>10.3 ± 3.2</td>
<td>9.3 ± 2.5</td>
</tr>
<tr>
<td>3 minute</td>
<td>9.7 ± 2.4</td>
<td>8.8 ± 2.1</td>
</tr>
<tr>
<td>4 minute</td>
<td>9.6 ± 2.4</td>
<td>9.0 ± 3.6</td>
</tr>
<tr>
<td>5 minute</td>
<td>10.1 ± 3.2</td>
<td>9.3 ± 3.4</td>
</tr>
<tr>
<td>6-23 minutes</td>
<td>9.5 ± 2.4</td>
<td>9.1 ± 2.5</td>
</tr>
</tbody>
</table>

The data are mean ± SD, (n=15).
determined by the difference in partial pressure of the nitrogen in the tissue and the breathing gas. Both a decrease in nitrogen content in the breathing gas and a decrease in environmental pressure will increase nitrogen elimination. Another factor that may have influenced our results is the choice of position of the subjects when measuring the N₂ elimination. As cardiac output is increased in the supine position and peripheral resistance is lowered we can not exclude the possibility that this could mask the effects of training and thus explain our negative results. The reason for choosing a supine position during the gas elimination tests was based on previous reports suggesting a higher rate of N₂ elimination in supine versus a sitting position (20), and observations in our lab (21) of better reproducibility when measuring N₂ elimination in a supine versus a sitting position. Furthermore, the blood distribution in the supine position is similar to that observed during immersion. Increased nitrogen elimination during exercise can not be excluded as perfused muscle tissues are expected to eliminate the main part of the nitrogen in the body during the washout period of 23 minutes. Moderate exercise after decompression may increase nitrogen elimination by a factor of three (22) and in the future, studies should measure nitrogen elimination during submaximal or maximal exercise.

Despite a similar rate for “total N₂ elimination” we observed a slight increase in nitrogen elimination during the first 2 minutes of oxygen breathing. To our knowledge no similar observation exists in the literature. Nitrogen elimination observed in the first minutes is related to elimination from lung and fast compartments.

Lung diffusion capacity has not been considered as a limiting factor of gas elimination in healthy subjects at rest (23). However, there are conflicting results in the
literature in regard to how chronic exercise affects the diffusion capacity of the lung, indicating no effect (15) or increased capacity in long distance swimmers (24,25). The reason for contrasting reports of adaptations following chronic training is not known, but might be due to differences in exercise intensity used in the various studies.

Most previous reports show that structural elements involved in lung-blood gas exchange (such as decreased diffusion distance, an increased diffusion area and an increased pulmonary capillary volume) do not change appreciably with endurance training, despite their increased functional importance during exercise (See review in 26). As mentioned, the capillary density in human skeletal muscles increases with endurance training (27,28) but as far as we know, this has not been shown in respiratory muscles. Although the pulmonary area available for diffusion and the characteristics of the alveolar-capillary membrane remain fairly constant, the central blood volume is highly variable in humans (29). An increased blood volume would increase pressure in the capillary bed as well as lead to recruitment of the previously closed pulmonary capillaries and dilatation of existing ones, thereby increasing the functional capillary bed. An increase in blood volume in the lung could thus increase gas elimination in the first minutes of pure O₂ breathing.

**Methodological aspects**

The present study showed that the protocol and the recording equipment used were reproducible for evaluating N₂ elimination in humans. Test results in series I showed no significant differences of gas elimination between the three different tests. The potential effect of habituation to the apparatus and the environment where thus excluded for the tests in series II. All subjects in both series I and II wore clothing identical to what was used in the N₂ elimination tests. The temperature and humidity in the laboratory were kept nearly equal for all the tests. Therefore, we consider that elimination and uptake of N₂ across the skin was approximately identical in all test situations.

**Training protocol**

The present endurance training induced a 22% increase in \( V_{O_{2\max}} \) which confirms the effectiveness of this type of interval training (17). The reason for choosing the high intensity aerobic interval training is the general agreement as to the cardiac output limits \( V_{O_{2\max}} \) in normal subjects (30). As the maximum heart rate does not change following endurance training, the maximum cardiac output is determined by the stroke volume of the heart. The stroke volume continues to increase up to \( V_{O_{2\max}} \) (31). Thus, in order to increase the stroke volume and thereby, \( V_{O_{2\max}} \), it is necessary to exercise at an intensity close to \( V_{O_{2\max}} \) and maximum stroke volume. Theoretically, the present improvement in \( V_{O_{2\max}} \) increased the stroke volume by 23% (32), which is in accordance with observations reported (18) using a similar training regimen.

In conclusion, the present test protocol was found to be reproducible for evaluating N₂ elimination in humans at rest. This study has demonstrated that N₂ elimination rate is not increased by endurance training and increased aerobic capacity. Even if the elimination rate of the inert gas from muscles following training is not significantly affected at rest, which is most relevant for decompression, it may be increased during exercise.

**ACKNOWLEDGMENTS**

This study was supported by the Norwegian Petroleum Directorate, Norsk Hydro, Esso Norge and Statoil under the ‘Dive contingency contract no 4600002328’ with Norwegian Underwater Intervention.
REFERENCES


