Alveolar-Membrane Diffusing Capacity Limits Performance in Boston Marathon Qualifiers

Kaleen M. Lavin¹, Allison M. Straub¹, Kathleen A. Uhranowsky¹, James M. Smoliga³, Gerald S. Zavorsky¹,²

¹Human Physiology Laboratory, Marywood University, Scranton, Pennsylvania, United States of America, ²The Commonwealth Medical College, Scranton, Pennsylvania, United States of America, ³Department of Physical Therapy, High Point University, High Point, North Carolina, United States of America

Abstract

**Purpose:** (1) to examine the relation between pulmonary diffusing capacity and marathon finishing time, and (2) to evaluate the accuracy of pulmonary diffusing capacity for nitric oxide (DLNO) in predicting marathon finishing time relative to that of pulmonary diffusing capacity for carbon monoxide (DLCO).

**Methods:** 28 runners [18 males, age = 37 (SD 9) years, body mass = 70 (13) kg, height = 173 (9) cm, percent body fat = 17 (7) %] completed a test battery consisting of measurement of DLNO and DLCO at rest, and a graded exercise test to determine running economy and aerobic capacity prior to the 2011 Steamtown Marathon (Scranton, PA). One to three weeks later, all runners completed the marathon (range: 2:22:38 to 4:48:55). Linear regressions determined the relation between finishing time and a variety of anthropometric characteristics, resting lung function variables, and exercise parameters.

**Results:** In runners meeting Boston Marathon qualification standards, 74% of the variance in marathon finishing time was accounted for by differences in DLNO relative to body surface area (BSA) (SEE = 11.8 min, *p* < 0.01); however, the relation between DLNO or DLCO to finishing time was non-significant in the non-qualifiers (*p* = 0.14 to 0.46). Whereas both DLCO and DLNO were predictive of finishing time for all finishers, DLNO showed a stronger relation (*r*² = 0.30, SEE = 33.4 min, *p* < 0.01) compared to DLCO when considering BSA.

**Conclusion:** DLNO is a performance-limiting factor in only Boston qualifiers. This suggests that alveolar-capillary membrane conductance is a limitation to performance in faster marathoners. Additionally, DLNO/BSA predicts marathon finishing time and aerobic capacity more accurately than DLCO.

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* E-mail: zavorsky@marywood.edu

Introduction

In 2010, nearly one half million runners in the United States completed a marathon, representing about 0.2% of the U.S. population over 18 years of age. Many marathoners aspire to qualify for the Boston Marathon, participation in which is restricted to a relatively small percentage of runners by age and gender-graded qualification standards. In 2011, approximately 40,000 runners qualified for the 2012 or 2013 Boston Marathon, representing about 10% of all runners who finished a marathon in the United States. These statistics are readily available to the public online at MarathonGuide.com.

Due to its aura and relative popularity, marathon running has been examined in several scientific studies, with particular attention given to the metabolic [1] and physiological [2,3,4,5,6] correlates of running a fast marathon. Although these correlates are multifactorial and widely debated [7], it is well established that aerobic capacity (VO₂max) is an important determinant of marathon performance. Approximately 40 to 77% of the variance in marathon performance is attributable to VO₂max [5,8,9,10,11].

In addition to aerobic capacity, marathon-specific endurance is related to performance, such that the ability to sustain a higher percentage of VO₂max is correlated with a faster marathon [12]. On average, top runners (=136 minutes for men, =158 minutes for women) run at 85 to 90% VO₂max [5], while those in the 156 to 240 minute range run at approximately 75 to 85% of VO₂max [10,13].

Aerobic capacity is dependent on the integrated function of major organ systems, including the heart, lung, and skeletal muscle [14]. Unlike the heart and skeletal muscle, the lung does not readily adapt to endurance training [15,16], possibly limiting VO₂max [15]. Even in those who are highly aerobically fit, heavy exercise may cause arterial oxygen pressure to drop ≤80 mm Hg, while the alveolar-to-arterial oxygen pressure difference may increase to ≥25 mmHg [17]. In this way, arterial oxyhemoglobin saturation is reduced, leading to a decrease in VO₂max and, subsequently, endurance performance [18,19,20].

Recently, alveolar-capillary membrane diffusing capacity (measured at rest) has been shown to be very closely related to VO₂max in fit and...
obese individuals [21,22] and to longevity in heart disease patients [23]. Specifically, when measured at rest, pulmonary diffusing capacity for nitric oxide (DLNO, mL NO.min⁻¹.mmHg⁻¹) – a surrogate for alveolar-membrane diffusing capacity for carbon monoxide (DmCO) [24,25] – has been shown to be related to aerobic capacity in fit men and women, such that for every 1 unit increase in DLNO, \( \dot{V}O_2_{2max} \) increases by 0.3 mL O₂.kg⁻¹.min⁻¹ [21]. The ratio of DLNO to DmCO is debated. It has been said that DmCO = DLNO ± 2.42, or, more recently, DmCO = DLNO ÷ 2.06 to 2.26. As long as the ratio is kept consistent within a study, any percent change in DmCO or pulmonary capillary blood volume is still valid. Furthermore, alveolar membrane conductance is the main pulmonary diffusing capacity component representative of fitness, with the exception of pulmonary capillary blood volume (VC) and the blood transfer conductance (Ω) for CO (ΩCO). There are mixed data as to whether DLCO or DLNO is a more valid predictor of aerobic capacity, but overall diffusion capacity does appear to be significantly correlated with aerobic capacity in fit subjects [21,22]. Nitric oxide (NO) has been shown to bind more strongly than CO to hemoglobin [26], leading to a higher value for membrane conductance and a diffusion measurement more reflective of total membrane diffusion. An additional benefit of measuring DLNO simultaneously with DLCO is a reduction in time and effort of the procedure.

As there is a relation between DLNO and \( \dot{V}O_2_{2max} \), and between \( \dot{V}O_2_{2max} \) and marathon running performance, it follows that DLNO may be related to marathon performance, such that pulmonary diffusing capacity introduces a limitation that influences marathon performance. However, it is likely that this correlation may be observed in only well-trained marathoners, who are more likely to experience pulmonary limitations to oxygen availability. Pulmonary limitations have been observed in elite athletes, and as many as 50% of highly-trained individuals experience low oxygen concentrations in the blood (hypoxemia), potentially due to diffusion limitation [27]. Whereas hypoxemia is most frequently observed at exercise intensities near maximal exertion, it is possible that endurance events such as a marathon place constraints on the working lung muscles regardless of the submaximal speed at which most runners compete. Indeed, Amann et al. [28] found that pulmonary limitations were capable of significantly decreasing performance in a 5 kilometer cycling time trial. This effect is likely intensified during a marathon, which, although run at a slower pace, is more than eight times as long. Another study shows that seasoned runners experience significant decreases in diffusing capacity following completion of a marathon [29], suggesting an important role for the lungs in an event that requires submaximal speed but maximal overall effort.

Based on these observations, the purpose of this study was to compare the relation between DLNO and DLCO (indexed to body surface area, BSA) and marathon running performance. It was hypothesized that pulmonary diffusion limitation would exist in faster runners, defined herein as those meeting qualification standards for the Boston Marathon (Boston Qualifiers, BQ). Qualification for this prestigious event is dependent on one’s performance relative to age and gender standards, thus eliminating the concerns associated with grouping subjects based on marathon time alone (e.g. creating a younger or predominantly male group). Furthermore, the Steamtown Marathon is a certified qualification course and frequently qualifies over 20% of its participants for the Boston event. This local event therefore provides the opportunity to study differences between sub-elite and more recreational marathoners using a less ambiguous distinction than running pace.

In analyzing these data, the relation between marathon finishing time and DLNO or DLCO in both groups was examined using a linear regression model. No significant relation between these factors was expected in non-qualifiers (non-BQ), whereas qualifiers (BQ) were expected to show a significant correlation between finishing time and DLNO/DLCO. Additionally, it was hypothesized that DLNO would show a stronger relation with marathon finishing time than would DLCO, in agreement with previous findings [21,30].

**Methods**

Twenty-eight endurance trained subjects (18 males, 10 females), reported for preliminary testing 2 to 3 weeks before the 2011 Steamtown Marathon in Scranton, PA. Institutional Review Board-approved informed written consent and a Physical Activity Readiness Questionnaire were obtained from all subjects before participation. Anthropometrics (body mass, height, BSA) and age were obtained, and percent body fat (BF %) was measured using Dual Energy X-ray Absorptiometry (DEXA) (Lunar Prodigy™, GE Medical Systems, Madison, WI).

Pulmonary function tests, consisting of spiroometry maneuvers to identify obstructive or restrictive patterns, were conducted according to established guidelines [31]. The maneuver to determine DLNO and DLCO was also performed according to established guidelines [31], with a 5 to 6 second breath-hold [21]. Because this one-step maneuver allows simultaneous measurement of DLNO and DLCO, pulmonary capillary blood volume (VC) was then calculated as follows: DmCO was computed as DLNO ÷ 2.42. The \( 1/ΩCO \) was determined from Roughton and Forster [33] as \( 0.73+0.058P_{PAO2} \cdot [14.6/Hb]\), where alveolar oxygen pressure \( P_{PAO2} \) was 100 mmHg, and the hemoglobin concentration [Hb] was set as 14.6 g. dL⁻¹ for males, and 13.4 g. dL⁻¹ for females [32]. As such, \( 1/ΩCO \) was 1.310 for males and 1.427 for females. VC was then obtained by solving for it using the following equation [33]:

\[
\frac{1}{DLCO} = \frac{1}{DmCO} + \frac{1}{ΩCO-Vc}
\]

Reference equations were then used to compare each marathon runner’s lung function to normative data from the standard population [34,35].

After the lung function tests were completed, running economy testing was performed on treadmill at a 2% grade to simulate outdoor running conditions at three different sub-maximal running speeds, each lasting 5 minutes. The treadmill was calibrated before the first subject was tested. Throughout testing, heart rate (HR) was measured using a Polar heart rate monitor (Model S610, Polar Electro USA, Lake Success, NY). Metabolic data were collected using breath-by-breath analysis (Sensormedics Vmax 229D, Viaysys, CA).

Assuming that steady state exercise had been achieved within the first three minutes, means for metabolic data for the last two minutes of each stage were computed. Running economy for each stage was computed as the \( \dot{V}O_2 \) required to travel one kilometer and expressed as mL O₂.min⁻¹.km⁻¹. Mean running economy for the three speeds was then computed to serve as a measure of overall running economy.

The final stage of the running economy test protocol was followed immediately by a graded exercise test, in which treadmill speed was increased by 0.5 mph every minute until volitional exhaustion. Several cardiorespiratory parameters, including maximum respiratory exchange ratio \( (RER_{max}) \), maximum heart rate
(HR_max), maximum expired ventilation (VE_max), and VO_{2max} were measured, and the treadmill speed at which VO_{2max} was obtained was recorded \(v_{VO_{2max}}\). If this speed was sustainable for less than a full minute, the highest speed sustained for 60 seconds was also recorded. Marathon-specific endurance \(\%v_{VO_{2max}}\) was calculated by dividing the runner’s mean speed for the Steamtown marathon by \(v_{VO_{2max}}\) with higher values indicating performance at a greater relative physiologic intensity. Since \(VO_2\) could not be directly measured during the race, average \(VO_2\) for the marathon was calculated from the slope of the regression line between speed and \(VO_2\) for each subject, using the three speeds of the running economy test. Average speed for the marathon was then entered into each subject’s own equation to solve for oxygen consumption.

Univariate ANCOVAs or independent \(t\)-tests (with a confidence interval of 95%) were used to determine whether differences in finishing time, \(VO_{2max}\) (both absolute, in L min^{-1}, and relative, in mL kg^{-1} min^{-1}), and lung function parameters existed between BQ and non-BQ groups. Age and gender served as covariates for the ANCOVA. Step-wise multiple linear regressions were conducted to determine variables most closely related to finishing time for the entire sample, as well as for each group separately. DLNO and DLCO normalized to body surface area (BSA) were included in regression analyses to account for the effects of body mass and height on lung size. Other predictor variables entered into regression analysis include gender, age, body mass, body fat percentage, \(VO_{2max}\) (L O_2. min^{-1}), running economy (mL O_2. kg^{-1}. km^{-1}), DLNO, DLCO, and marathon specific endurance. To address the significance of diffusing capacity, the relation between DLNO or DLCO and finishing time was further explored in a bivariate regression analysis for both BQ and non-BQ groups. The data were analyzed by SPSS Version 19.0, (SPSS Inc., Chicago, IL). Statistical significance was declared when \(p<0.05\).

### Results

A total of 392 Steamtown Marathon finishers (22% of marathon participants) met qualification standards for the 2012 or 2013 Boston Marathon. Of the 28 subjects in this study, 10 runners (6 males, 4 females) (36%) qualified for Boston. Anthropometric measurements (Table 1) indicated that significant differences exist between BQ’s and non-BQ’s with respect to age and body fat percentage \(\%_{BF}\) \((p<0.05)\).

The graded exercise test to volitional exhaustion lasted 10.1(1.2) minutes including the final 5-min running economy bout. The \(VO_{2max}\) in L O_2. min^{-1} from that test was not different between groups; however, relative \(VO_{2max}\) (mL O_2. kg^{-1}. min^{-1}) was significantly different between the two groups \((p<0.01)\) such that BQ’s had a mean (standard deviation) \(VO_{2max}\) about 11.2(5.8) mL O_2. kg^{-1}. min^{-1} greater than that of non-BQ’s. There was a non-significant trend \((p = 0.06)\) for body mass to differ between groups. During the graded exercise test, BQ’s attained a maximal treadmill speed 20% faster than non-qualifiers \((p<0.05)\). Additionally, BQ’s completed the marathon at a higher percentage of \(VO_{2max}\) \((75.2\%\) \(v_{VO_{2max}}\)) than did non-BQ’s \((67.4\%, p<0.001)\). For both groups combined, there was a significant bivariate relation between \(VO_{2max}\) (mL O_2. kg^{-1}. min^{-1}) and marathon finishing time \(adjusted r^2 = 0.47, SEE = 37.5 min, p<0.05\).

Percent of predicted values for a lung function tests were not significantly different between groups \(Table 2\). Three subjects in each group had a DLNO greater than the upper limit of normal (ULN). Two BQ’s had a DLCO above the ULN, while 5 non-BQ’s had a DLCO that surpassed the ULN. Chi-square analysis reveals that there is not a significant difference in the proportion of subjects with an abnormally high diffusion capacity (DLCO or DLNO) between groups \(data not included\).

Mean finishing time for all subjects was 220.0 minutes, \(range = 142.6 to 288.9\). Weight loss from the marathon was comparable between the two groups \(1.0 (1.4) kg for BQ’s; 0.9 (0.8) kg for non-BQ’s, \(p = 0.746\)\). Ten of the twenty-eight participants \(6 males, 4 females\) qualified for the Boston Marathon \(average time = 2:10.4(23.1) min\); the average time for the remaining 18 \(12 males, 6 females\) was 242.2(28.3) minutes. Finishing time was significantly faster in BQ’s when controlling for age and gender \((p<0.01)\). Step-wise linear regression determined that finishing time for all subjects was dependent on maximum treadmill speed and specific endurance \(adjusted r^2 = 0.97, SEE = 6.9 min, p<0.05\); however, 80% of the variance in finishing time is accounted for by differences in maximum treadmill speed sustained for 60 seconds alone \(adjusted r^2 = 0.80, SEE = 17.7 min, p<0.05\).

In BQ’s, the strongest relation identified was between finishing time and DLNO normalized to BSA. For non-BQ’s, finishing time was best predicted by maximum treadmill velocity sustained for 60 seconds and specific endurance, where 74% of the variance in finishing time is accounted for by differences in maximum treadmill velocity. The relation between DLNO normalized to BSA and finishing time was not significant for non-BQ’s \((p = 0.127)\). A significant difference was found between the correlation coefficients of the two linear regressions (two-tailed \(z = -2.15, p = 0.03)\). When regression lines of DLNO normalized to BSA versus finishing time plotted on the same axes, the regressions intersect at a point corresponding to a finishing time of 178.1 minutes \(Figure 1\), suggesting that the relation between DLNO normalized to BSA and marathon time begins to change around 237 m. min^{-1} pace \(6 minutes, 47 seconds per mile)\. DLCO normalized to BSA was also correlated to finishing time in BQ subjects, to a lesser extent. For non-BQ, no significant relation between the variables is evident \((p = 0.46)\. These regressions intersect at the point corresponding to 184.3 minutes \(Figure 2)\.

### Discussion

The novelty of this study lies in that it shows that the relation between pulmonary factors \(measured at rest\ and marathon performance may differ between athletes of different skill level. In particular, this study was able to isolate an approximate time point at which the relation between pulmonary diffusing capacity for nitric oxide and marathon finishing time changes for trained endurance athletes, pinpointing a pace at which lung function becomes limiting to performance. Whereas runners qualifying for the Boston Marathon, because of their overall faster pace, are limited by DLNO, non-qualifiers probably experience a more mechanical limitation, such as leg turnover \(related to maximum treadmill velocity)\.

The primary purpose of this study was to compare the correlation of DLNO and DLCO to marathon running performance. The results demonstrate that there was a significant slope \(indicating a strong correlation\) between DLNO and DLCO \(normalized to BSA\ versus marathon finishing time only in runners that qualified for the Boston Marathon, with these variables showing a stronger predictive relation to finishing time than either \(VO_{2max}\ or running economy\. DLNO was shown to be the strongest predictor of finishing time, such that every 1 mL min^{-1}, mmHg^{-1} m^{-2} increase in DLNO at rest projects that finishing time will decrease by about 1.4 minutes \(with a range of 0.8 to 2.1 min)\. These results strongly suggest that alveolar-capillary membrane conductance may be performance-limiting in
runners that complete a marathon in 3 hours or faster, as shown by the intersection of regression lines for BQ and non-BQ groups (Figure 1 and 2). These figures also demonstrate that DLNO relative to BSA is a more accurate predictor of finishing time than DLCO, as the former correlation shows a larger adjusted $r^2$ and a lower standard error of the estimate.

The physiological mechanism closely relating DLNO to marathon performance in BQ’s is speculative, given that these subjects ran at approximately 75% of $\dot{V}O_2\text{max}$, a value consistent elsewhere in those with similar running abilities [10,13]. Though arterial oxygen pressure and the alveolar-to-arterial oxygen pressure difference was not measured throughout the race in this study, others have shown hypoxemia is not induced in fit athletes running half of a full marathon at $\sim$75% $\dot{V}O_2\text{max}$ [36]. Nonetheless, we suggest that, in well-trained runners, there is a $\dot{V}O_2$ threshold at which pulmonary diffusion limits oxygen consumption. In other words, these individuals run the marathon at a speed at which $\dot{V}O_2$ is high enough that gas diffusion at the alveolar-capillary membrane becomes a physiological bottleneck, and those with greater alveolar-capillary membrane conductance are able to maintain greater arterial oxygen saturation. Similarly, non-BQ’s likely complete the marathon at a $\dot{V}O_2$ at which pulmonary diffusion is not limiting; this may explain the lack of relation between DLNO and performance in this group. Thus, individuals who have superior alveolar-capillary membrane conductance (high DLNO measurements), and yet do not reach a “heart” or “muscle limitation,” (i.e., non-BQ’s) would not have

### Table 1. Anthropometric Data for Boston Qualifiers and non-Qualifiers.

| MEASUREMENT                          | BQ (n = 10) | Non-BQ (n = 18) | Total (n = 28) |
|--------------------------------------|-------------|-----------------|---------------|
| **ANTHROPOMETRICS**                  |             |                 |               |
| Age (yr)*                            | 33 (9)      | 40 (7)          | 37 (9)        |
| Weight (kg)                          | 64.1 (11.1) | 73.2 (13.3)     | 69.9 (13.2)   |
| Height (cm)                          | 170.7 (9.0) | 174.0 (9.2)     | 172.8 (9.1)   |
| Body Fat (%)*                        | 13.1 (7.12) | 19.1 (6.7)      | 17.0 (7.3)    |
| **CARDIOPULMONARY VARIABLES AT MAXIMAL EXERCISE** |       |                 |               |
| $\dot{V}O_2\text{max}$ (L/min)      | 3.75 (0.64) | 3.56 (0.64)     | 3.63 (0.64)   |
| $\dot{V}O_2\text{max}$ (mL/kg/min)* | 59.4 (8.3)  | 48.7 (3.0)      | 52.5 (8.1)    |
| RERmax                               | 1.17 (0.07) | 1.16 (0.06)     | 1.16 (0.06)   |
| VEmax (L/min)                        | 119.91 (21.10) | 112.03 (18.46) | 114.85 (19.43) |
| HRmax (bpm)                          | 187 (12)    | 178 (12)        | 181 (12)      |

Data are reported as mean (SD) values and range.

* denotes significant difference ($p<0.05$) between BQ and non-BQ subjects. Controlling for age and gender using an ANCOVA did not affect the outcome of statistical analyses.

Running Economy calculated at average speed for group; BQ = 222.2 (30.0) m/min, non-BQ = 177.2 (20.4) m/min.

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any performance advantage over other individuals who have lower alveolar-capillary membrane conductance. In fact, studies have demonstrated that marathon running causes a significant drop in pulmonary diffusing capacity [29,37]. About 30% of the drop in DLNO (normalized to BSA) post-exercise is accounted for by marathon finishing time [29]. The diminished DLNO with 2 increase in DLNO at rest, marathon finishing time was 0.8 minutes faster (ranging from 1.8 minutes faster to 0.1 minute slower) [29]. Combining these data with those from the present study suggests that 30 to 74% of the variance in DLNO is related to marathon finishing time in BQ's, while no such relation exists in non-BQ's. Additionally, controlling for age and gender does not affect this correlation in either study separately or collectively [29]. Combining the data from these independent studies further supports that pulmonary diffusing capacity is an important contributor to marathon performance in well-trained runners. It is also important to note that while the BQ group represents a very high level, international class runners tend to have even higher values of VO2max and, possibly, an even greater dependence on alveolar-capillary membrane conductance. Although DLNO normalized to BSA was only related to finishing time in BQ's, DLNO did not significantly differ between BQ and non-BQ whether indexed to BSA (p = 0.078) or not (p = 0.80). DLNO is usually higher in fit subjects [21] and in the present study, DLNO was significantly higher than predicted (p = 0.01) [34] or Aguilaniu and colleagues (107% predicted, p = 0.011) [39]. Several subjects in both BQ and non-BQ groups had values above the upper limit of normative data for DLNO (mL.min⁻¹.mmHg⁻¹.m⁻²) at rest is related to marathon finishing time in BQ's, while no such relation exists in non-BQ's. Additionally, controlling for age and gender does not affect this correlation in either study separately or collectively [29]. Combining the data from these independent studies further supports that pulmonary diffusing capacity is an important contributor to marathon performance in well-trained runners. It is also important to note that while the BQ group represents a very high level, international class runners tend to have even higher values of VO2max and, possibly, an even greater dependence on alveolar-capillary membrane conductance.

### Table 2. Pulmonary function measurements for Boston Qualifiers and non-Qualifiers.

|            | BQ (n = 10) | Non-BQ (n = 18) | Total (n = 28) |
|------------|-------------|-----------------|---------------|
|            | Mean (SD)   | Mean (SD)       | Mean (SD)     |
| FVC (L)    | 5.1 (0.9)   | 5.0 (1.0)       | 5.0 (0.9)     |
| FEV1 (L)   | 4.0 (0.7)   | 3.8 (0.7)       | 3.9 (0.7)     |
| FVC/FVC    | 81.2 (10.6) | 99 (8)          | 96 (9)        |
| PEF (L)    | 9.0 (2.2)   | 10.1 (2.0)      | 9.7 (2.1)     |
| FEF25–75   | 5.2 (1.5)   | 4.8 (1.3)       | 5.0 (1.3)     |
| DLNO       | 34.7 (5.9)  | 34.3 (6.6)      | 34.3 (6.7)    |
| DLCO/BSA   | 20.0 (3.1)  | 13.6–23.6       | 13.6–23.9     |
| DLNO       | 179 (27)    | 176 (34)        | 175 (35)      |
| DLNO/BSA   | 103 (14)    | 93 (13)         | 97 (14)       |
| Vc (mL)    | 90 (15)     | 89 (13)         | 90 (14)       |

Data are reported as mean (SD) values and range.

*Denotes a significant difference in observed parameter relative to predicted (p < 0.05).

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FVC: forced vital capacity; FEV1: forced expiratory volume within 1 sec; FEV1/FVC: fraction of inspired air expired within 1 sec; PEF: peak expiratory flow; FEF25–75: forced expiratory flow during 25–75% of 6-second exhale; DLCO: pulmonary diffusing capacity for carbon monoxide, in mL/min/mmHg; DLCO/BSA: DLCO relative to body surface area, in mL/min/mmHg/m²; DLNO: pulmonary diffusing capacity for nitric oxide, in mL/min/mmHg; DLNO/BSA: DLNO relative to body surface area, in mL/min/mmHg/m²; Vc: pulmonary capillary blood volume.

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improve alveolar-capillary membrane conductance above that of untrained individuals, but improvements in DLNO likely plateau well before that of the heart or skeletal muscle.

Generally speaking, the lungs become limiting at a running pace of 6:47 minutes per mile or 236 m.min⁻¹ (a 3 hour marathon). Therefore, athletes performing at or around this pace should be aware of the potential significance of this limitation and its ramifications for performance. It is unknown whether any specific training practices can be implemented to improve pulmonary diffusion and therefore improve marathon performance.

This study is limited by its small sample size; a higher power to detect differences in group means would likely be achieved by recruiting more participants. Nevertheless, it is frowned upon to conduct a post-hoc power analysis after data collection has occurred [40,41], thus we did not perform one. Instead, confidence intervals have provided confidence intervals in Figures 1 and 2. Additionally, small group sizes result in large variances; as such, the standard errors of the estimate for both regressions are large and overlapping, obscuring estimation of a clear range of intersection at which DLNO indexed to BSA begins to predict finishing time.

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The genetic basis of DLNO could be further studied to determine how this parameter might change as one adapts to training. Furthermore, longitudinal and interventional studies are recommended to determine if any specific type of training can optimize pulmonary diffusion capacity and therefore improve endurance running performance. More extensive understanding of the relationship outlined in this study will allow us to confirm the validity of diffusing capacity for nitric oxide as a fitness predictor.

In conclusion, this study found that DLNO indexed to BSA is a better predictor of marathon finishing time in runners qualifying for the Boston Marathon than are more commonly used variables, such as VO₂max or running economy, but this relation was not observed for non-BQs. This suggests that alveolar-capillary membrane conductance can be pulmonary limitation in well-trained runners.
Author Contributions
Conceived and designed the experiments: GSZ JMS. Performed the experiments: KML AMS KAU. Analyzed the data: KML GSZ.

References
1. Rapoport BI (2010) Metabolic factors limiting performance in marathon runners. PLoS Comput Biol 6: e1000960.
2. Coyle EF (2007) Physiological regulation of marathon performance. Sports Med 37: 306–311.
3. Joyner MJ, Ruiz JR, Lucia A (2011) The two-hour marathon: who and when? J Appl Physiol 110: 275–277.
4. La Torre A, Vernillo G, Agnello L, Berardelli C, Rampinini E (2011): Is it time to consider a new performance classification for high-level male marathon runners? J Strength Cond Res 25: 3242–3247.
5. Billat VL, Demarle A, Slawinski J, Paiva M, Koralsztein JP (2001) Physical and training characteristics of top-class marathon runners. Med Sci Sports Exerc 33: 2089–2097.
6. Sjodin B, Wedenham J (1985) Applied physiology of marathon running. Sports Med 2: 83–99.
7. Stellingwerff T, Zehnder AE, Perrey S, Shephard RJ, Schubert MM, et al. (2011) Commentaries on Viewpoint: The two-hour marathon: Who and when? J Appl Physiol 110: 278–293.
8. Hagans RD, Upton SJ, Duncan JJ, Gettman LR (1987) Marathon performance in relation to maximal aerobic power and training indices in female distance runners. Br J Sports Med 21: 3–7.
9. Florence S, Weir JP (1997) Relationship of critical velocity to marathon running performance. Eur J Appl Physiol Occup Physiol 75: 274–278.
10. Maughan RJ, Leiper JB (1983) Aerobic capacity and fractional utilisation of aerobic capacity in elite and non-elite male and female marathon runners. Eur J Appl Physiol Occup Physiol 52: 80–87.
11. Loftin M, Sothern M, Koss C, Tuuri G, Vanvrancken C, et al. (2007) Energy expenditure and influence of physiologic factors during marathon running. J Strength Cond Res 21: 1108–1110.
12. Millet GY, Bardi Jc, Kerhove H, Morin JB, Vincent L, et al. (2011) Physiological and biological factors associated with a 24-h treadmill ultra-marathon performance. Scand J Med Sci Sports 21: 54–61.
13. Maron MB, Hoyvath SM, Wilkinson JE, Gliner JA (1976) Oxygen uptake measurements during competitive marathon running. J Appl Physiol 40: 836–838.
14. ATS/ACCP (2003) ATS/ACCP Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med 167: 211–277.
15. Dempsey JA, Fregosi RF (1985) Adaptability of the pulmonary system to changing metabolic requirements. Am J Cardiol 55: 59D–67D.
16. Wagner PD (2005) Why doesn’t exercise grow the lungs when other factors do? Exerc Sport Sci Rev 33: 3–8.
17. Dempsey JA, Hanson PG, Henderson KS (1984) Exercise-induced arterial hypoxaemia in healthy human subjects at sea level. Journal of Physiology, London 355: 161–175.
18. Hammers CA, Chargaren SR, Nickle GA, Pegelow DF, Nelson VB, et al. (2000) Effect of exercise-induced arterial O2 desaturation on VO2max in women. Med Sci Sports Exerc 32: 1101–1108.
19. Romer LM, Haverkamp HC, Levering AT, Pegelow DF, Dempsey JA (2006) Effect of exercise-induced arterial hypoxemia on quadriiceps muscle fatigue in healthy humans. Am J Physiol Regul Integr Comp Physiol 290: R365–375.
20. Koskelou MD, McKenzie DG (1994) Arterial hypoxia and performance during intense exercise. Eur J Appl Physiol 68: 80–86.
21. Zavorsky GS, Wilson B, Harris JK, Kim DJ, Carl F, et al. (2010) Pulmonary diffusion and aerobic capacity: Is there a relation? Does obesity matter? Acta Physiol (Oxf) 198: 499–507.
22. de Bisschop C, Martinot JB, Leurquin-Sterk G, Faoro V, Guenard H, et al. (2012) Improvement in lung diffusion by endothelin A receptor blockade at high altitude. J Appl Physiol 112: 20–25.
23. Guazzi M, Pontone G, Brambilla R, Agostoni P, Reina G (2002) Alveolar-capillary membrane gas conductance: a novel prognostic indicator in chronic heart failure. Eur Heart J 23: 467–476.
24. Tamhane RM, Johnson RL, Jr., Hsia CC (2001) Pulmonary membrane diffusing capacity and capillary blood volume measured during exercise from nitric oxide uptake. Chest 120: 1850–1856.
25. Ceridono ML, Beck KC, Olson TP, Bilezikian JA, Johnson BD (2010) Calculating alveolar-capillary conductance and pulmonary capillary blood volume: comparing the multiple- and single-inspired oxygen tension methods. J Appl Physiol 109: 643–653.
26. Borland CD, Higgotham TW (1969): A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. Respir Physiol 2: 56–63.
27. Powers SK, Dodd S, Lawler J, Ludby G, Kirtley M, et al. (1988) Incidence of exercise induced hypoxemia in elite endurance athletes at sea level. Eur J Appl Physiol Occup Physiol 50: 295–302.
28. Amann M (2012) Pulmonary system limitations to endurance exercise performance in humans. Exp Physiol 97: 311–318.
29. Manier G, Monard J, Techoueyres P, Varrene N, Guenard H (1994) Pulmonary diffusion limitation after prolonged strenuous exercise. Respir Physiol 93: 143–153.
30. Puri S, Baker BL, Dutka DP, Oakley CM, Hughes JM, et al. (1995) Reduced alveolar-capillary membrane diffusing capacity in chronic heart failure. Its pathophysiological relevance and relationship to exercise performance. Circulation 91: 2769–2774.
31. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, et al. (2005) Standardisation of spirometry. Eur Respir J 26: 319–338.
32. Macintyre N, Crago RO, Vieggi G, Johnson DG, van der Grinten CP, et al. (2005) Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J 26: 720–735.
33. Roughton FJ, Forrer RF (1957) Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 11: 290–302.
34. Zavorsky GS, Cao J, Murias JM (2008) Reference values of pulmonary diffusing capacity for nitric oxide in an adult population. Nitric Oxide 18: 70–79.
35. Hankinson JL, Odenrentz JC, Fedan KB (1999) Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med 159: 179–187.
36. Hansson P, Claremont A, Dempsey J, Reddan W (1982) Determinants and consequences of ventilatory responses to competitive endurance running. J Appl Physiol 52: 613–623.
37. Miles DS, Doerr CE, Schonfeld SA, Sinks DE, Gotshall RW (1983) Changes in pulmonary diffusing capacity and closing volume after running a marathon. Respir Physiol 52: 349–359.
38. Zavorsky GS, Lands LC (2005) Lung diffusion capacity for nitric oxide and carbon monoxide is impaired similarly following short-term graded exercise. Nitric Oxide 12: 31–38.
39. Aguilaniu B, Maitre J, Glenet S, Gegout-Petit A, Guenard H (2008) European guidelines and explanations. American Psychologist 54: 594–604.
40. Wennberg JL, Odenrentz JC, Fedan KB (1999) Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med 159: 179–187.
41. Wilkinson I, Reference TFS (1996) Statistical methods in psychology journals - Guidelines and explanations. American Psychologist 54: 394–404.
42. Dennis SC, Neakes TD (1999) Advantages of a smaller body mass in humans when distance-running in warm, humid conditions. Eur J Appl Physiol Occup Physiol 79: 280–284.