Contribution of oxygen extraction fraction to maximal oxygen uptake in healthy young men

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Abstract
We analysed the importance of systemic and peripheral arteriovenous O2 difference (a- vO2 difference and a-vfO2 difference, respectively) and O2 extraction fraction for maximal oxygen uptake (VO2max). Fick law of diffusion and the Piiper and Scheid model were applied to investigate whether diffusion versus perfusion limitations vary with VO2max. Articles (n = 17) publishing individual data (n = 154) on VO2max, maximal cardiac output (Qmax; indicator-dilution or the Fick method), a-vO2 difference (catheters or the Fick equation) and systemic O2 extraction fraction were identified. For the peripheral responses, group-mean data (articles: n = 27; subjects: n = 234) on leg blood flow (LBF; thermodilution), a-vfO2 difference and O2 extraction fraction (arterial and femoral venous catheters) were obtained. Qmax and two-LBF increased linearly by 4.9-6.0 L · min −1 per 1 L · min −1 increase in VO2max (R2 = .73 and R2 = .67, respectively; both P < .001). The a- vO2 difference increased from 118-168 mL · L −1 from a VO2max of 2-4.5 L · min −1 followed by a reduction (second-order polynomial: R2 = .27). After accounting for a hypoxemia-induced decrease in arterial O2 content with increasing VO2max (R2 = .17; P < .001), systemic O2 extraction fraction increased up to ~90% (VO2max: 4.5 L · min−1) with no further change (exponential decay model: R2 = .42). Likewise, leg O2 extraction fraction increased with VO2max to approach a maximal value of ~90-95% (R2 = .83). Muscle O2 diffusing capacity and the equilibration index Y increased linearly with VO2max (R2 = .77 and R2 = .31, respectively; both P < .01), reflecting decreasing O2 diffusional limitations and accentuating O2 delivery limitations. In conclusion, although O2 delivery is the main limiting factor to VO2max, enhanced O2 extraction fraction (≥90%) contributes to the remarkably high VO2max in endurance-trained individuals.
1 | INTRODUCTION

Under resting conditions in humans, the O₂ uptake (VO₂) is 3-5 mL·kg⁻¹·min⁻¹, and only a small fraction is consumed within the skeletal muscles.¹ However, during incremental exercise, the pulmonary VO₂ increases gradually and can reach a maximum (VO₂max) of ~90 mL·kg⁻¹·min⁻¹ depending on gender, age, body weight, genetics, training status and health.²,³

According to the Fick equation, VO₂max is determined by the product of the maximal cardiac output (Q max) and the arterial to mixed venous O₂ difference (a-VO₂ difference). Q max multiplied by the arterial O₂ content (CaO₂) sets the upper limit of systemic O₂ delivery, which is the principal limitation to VO₂max during exercise recruiting a large muscle mass, at sea level.⁴-⁶ Despite extensive research since the 1950s on the factors limiting VO₂max, it is still debated whether peripheral O₂ extraction capacity contributes to limiting VO₂max.⁷,⁸

Several original studies⁴,⁵,⁹-¹² and review articles⁶,¹³-¹⁵ have addressed this topic in recent decades, yet no study has aimed to statistically analyse all the existing data on the association between VO₂max and its limiting factors. This kind of analysis is warranted, as the original studies often used homogenous groups with a small number of subjects (<10) since they applied costly and invasive techniques involving catheterization to determine Q max (indicator-dilution techniques or the direct Fick method), regional blood flows (thermodilution or indicator-dilution techniques) and O₂ extraction fraction (calculated by the Fick equation or directly measured through arterial and venous catheters). Consequently, the statistical power is often too low to detect small but meaningful differences between subjects, groups with different training status and before and after training, thus precluding a definite conclusion.

It is documented that the a-VO₂ difference at VO₂max is only slightly different between untrained and endurance-trained individuals,¹⁶,¹⁷ suggesting that peripheral adaptations to endurance training have only a minor impact on VO₂max. However, the CaO₂ difference is determined not only by the peripheries' ability to extract O₂, reflected in the mixed venous O₂ content (a-VO₂) but also by the CaO₂, which sets the upper limit for the a-VO₂ difference during maximal exercise. The CaO₂ is set by the haemoglobin concentration ([Hb]) and the O₂ saturation of Hb (SO₂), which may change with training and is acutely modified during exercise. For instance, endurance training causes plasma volume expansion¹⁸ that can lead to haemodilution and a lower O₂ carrying capacity of the blood.¹⁶ A high Q max shortens the time for alveolar/capillary gas equilibration at the lung causing exercise-induced arterial hypoxemia that further reduces the CaO₂.¹⁹,²⁰ Therefore, it may be that the a-VO₂ difference does not increase substantially after endurance training because of a concurrent training-induced lowering of CaO₂, whereas the systemic O₂ extraction fraction (ΔO₂ extraction: a-VO₂ difference/CaO₂) may improve.

Another aspect of this discussion is whether the measurement techniques are sensitive enough to detect meaningful changes in the a-VO₂ difference. Most studies have not measured a-VO₂ difference directly but calculated it using the Fick equation (VO₂max/Q max).¹⁶,¹⁷,²¹-²⁴ The reason why so few studies have measured a-VO₂ difference directly during maximal exercise is because of the need for right heart catheterization. Therefore, studies measuring the arterial to femoral venous O₂ difference (a-vfO₂ difference) and leg O₂ extraction fraction directly using peripheral catheters may be more sensitive in evaluating whether the O₂ extraction capacity changes with endurance training.

It is important to note that the factors limiting VO₂max may change over the course of training. For instance, the maximal mitochondrial respiratory capacity (OXPHOS) measured in permeabilized muscle fibres ex vivo and VO₂max is associated in untrained, but not in trained individuals.²⁵ These and other data²⁶ suggest that peripheral factors contribute to limit VO₂max in the untrained state, but their influence may diminish with increased VO₂max and training status.

In the present study, we critically reviewed and statistically analysed the previously published data on the association between VO₂max and O₂ extraction fraction, in men, by focusing on catheterization studies. Two approaches were used: Part 1) articles containing individual data on pulmonary VO₂max, Q max (indicator-dilution techniques or the Fick method), a-VO₂ difference (mostly calculated) and O₂ extraction fraction measured during whole-body maximal exercise (running, cycling) were included; Part 2) to investigate the relationship between limb VO₂ and peripheral O₂ extraction fraction, mean data from studies reporting leg blood flow (LBF), a-vfO₂ difference and leg O₂ extraction fraction (catheters) measured during whole-body maximal exercise (running, cycling, cross-country skiing) were included. To investigate whether the limiting factors vary with VO₂max, we employed the Fick law of diffusion to calculate the muscle O₂ diffusing capacity (D MO2) and subsequently used the Piiper and Scheid model to calculate the relative roles of perfusion versus diffusion limitations to VO₂max.²⁷ Finally, we discuss the potential mechanisms behind the elevated O₂ extraction fraction observed after endurance training.

**KEYWORDS**

arteriovenous oxygen difference, cardiac output, exercise, leg blood flow, limiting factors, maximal oxygen uptake, oxygen diffusion, stroke volume
ANALYSIS OF EXISTING DATA

The strategy to use individual and mean data to investigate the systemic and peripheral responses, respectively, was chosen since a large amount of individual data has been published on systemic responses, whereas we were unable to identify other than mean values in studies investigating peripheral haemodynamics and O₂ extraction fraction. The data were identified through searches conducted in the PubMed database using several combinations of the following search terms: circulation, circulatory, hemodynamic(s), cardiac output, leg blood flow, arteriovenous oxygen difference, oxygen extraction and exercise. Cross-reference checks were also conducted, in addition to separate searches on authors with articles already included in the database. Only exercise modes engaging a large muscle mass that could elicit ̇VO₂max were included (cycling, running and cross-country skiing using the diagonal technique). Data from cross-sectional studies or before and after training interventions that were collected in normoxia on young (<40 years old) and healthy individuals were included. Data collected in hypoxia, after acclimatization to altitude, in altitude natives, in hyperthermia, with atrial pacing, after bed rest and after blood volume manipulations were excluded. The control condition was used when the above forms of manipulations of the cardiovascular system were conducted. Only catheterization studies that used invasive methods to measure ̇Q̇max (indicator-dilution techniques or the Fick method) and LBF (bolus or continuous infusion thermodilution and indicator-dilution techniques) were included. Only individual data from men are used (Part 1). In Part 2, studies that had a sample with a majority of men were used (≥50%). When several papers reported data from the same data collection, only one of the articles was included. If an article used some of the same subjects as previously reported, but with supplementation with new subjects, the data were included. The included articles are presented in Tables 1 and 2 for Parts 1 and 2, respectively.

### 2.1 Calculations

When the data were published in graphs and not in tables or text, ImageJ (v1.50b; National Institutes of Health, USA) was used for data extraction. If not all variables were reported

| Article                          | n  | Exercise | Age  | Method used to measure: | Reported or can be calculated | O₂ extraction | MAP |
|----------------------------------|----|----------|------|-------------------------|-------------------------------|---------------|-----|
| Blomqvist et al116               | 4  | Cycling  | 23-33| ̇Q̇max, ̇VO₂max          | Calculated using Fick equation | –             | Yes |
| Ekblom and Hermansen16           | 14 | Run      | 22-34| ̇Q̇max, ̇VO₂max          | Yes                           | Yes           | Yes |
| Ekblom et al17                   | 8  | Cycling  | 19-27| ̇Q̇max                  | Yes                           | Yes           | Yes |
| Ekblom112                        | 7  | Cycling  | 22-26| ̇Q̇max                  | Yes                           | Yes           | –   |
| Epstein et al117                 | 2  | Run      | 21   | ̇Q̇max                  | Yes                           | Yes           | –   |
| Epstein et al118                 | 4  | Run      | 18-30| ̇Q̇max                  | Yes                           | Yes           | –   |
| Gleser22                         | 6  | Cycling  | 20-23| ̇Q̇max                  | Yes                           | Yes           | –   |
| Hermansen et al21                | 13 | Cycling/Run| 19-34| ̇Q̇max                  | Yes                           | Yes           | –   |
| Mitchell et al23                 | 6  | Run      | –    | ̇Q̇max                  | –                             | –             | –   |
| Robinson et al119                | 5  | Run      | 19-31| ̇Q̇max                  | –                             | –             | Yes |
| Saltin110                        | 4  | Cycling  | 23-26| ̇Q̇max                  | –                             | –             | Yes |
| Saltin and Stenberg109           | 4  | Cycling  | 23-25| ̇Q̇max                  | –                             | –             | Yes |
| Saltin et al85                   | 5  | Cycling  | 19-21| ̇Q̇max                  | –                             | –             | Yes |
| Saltin et al111                  | 4  | Cycling  | 20-21| ̇Q̇max                  | –                             | Yes           | Yes |
| Stenberg et al114                | 6  | Cycling  | 20-36| ̇Q̇max                  | –                             | Yes           | Yes |
| Stenberg et al108                | 5  | Cycling  | 20-39| ̇Q̇max                  | –                             | Yes           | Yes |
| Åstrand et al24                  | 12 | Cycling  | 21-30| ̇Q̇max                  | –                             | Yes           | Yes |

Abbreviations: DB, Douglas bag technique; ID, indicator-dilution method using indocyanine green or Evans blue dye (only used in Mitchell et al23); MAP, mean arterial pressure; n, number of subjects meeting the inclusion criteria. Note that some subjects were investigated on more than one occasion (before/after training, running/cycling).
in the articles, the reported data were used to derive the missing values via the following formulas or combination of formulas if possible:

\[
Pulmonary \dot{V}O_{2\text{max}} = \dot{Q}_{\text{max}} \times a-vO_2 \text{ difference} \quad (1)
\]

\[
Leg \dot{V}O_{2\text{max}} = LBF \times a-vO_2 \text{ difference} \quad (2)
\]

\[
\text{Stroke volume} = \frac{\dot{Q}_{\text{max}}}{\text{heart rate}} \quad (3)
\]

\[
\text{Blood } O_2 \text{ content (e.g., } CaO_2) = 1.39 \times [Hb] \times SO_2 + 0.003 \times PO_2 \quad (4)
\]

\[
\text{Leg } O_2 \text{ delivery} = LBF \times CaO_2 \quad (5)
\]

\[
\bar{O}_2 \text{ extraction} = a-vO_2 \text{ difference}/CaO_2 \quad (6)
\]

\[
O_2 \text{ extraction} = a-vfO_2 \text{ difference}/CaO_2 \quad (7)
\]

Systemic vascular conductance = \frac{\dot{Q}_{\text{max}}}{(MAP - CVP)} \quad (8)

If no arterial partial pressure of O₂ (PO₂) was reported, 100 mmHg was assumed for the calculation of CaO₂ (ie, 3 mL O₂ freely dissolved in blood plasma per 1 L of blood). Central venous pressure (CVP) at \( \dot{V}O_{2\text{max}} \) was taken as 5 mmHg when calculating systemic vascular conductance.

**TABLE 2** Articles reporting mean values of leg oxygen uptake (leg \( \dot{V}O_{2\text{max}} \)), leg blood flow (LBF) and arterial to femoral venous \( O_2 \) difference (a-vfO₂ difference) during maximal exercise (cycling and cross-country skiing using the diagonal technique)

| Article | \( n \) | Age (\( \bar{x} \)) | Method used to measure: | Pulmonary \( \dot{V}O_2 \) | LBF | a-vfO₂ difference | O₂ extraction | Reported or can be calculated |
|---------|-------|----------------|------------------------|-----------------|-----|-----------------|---------------|-----------------------------|
| Bender et al\(^{102} \) | 7♂ | 22 | Custom | TD-B | Measured via arterial and femoral venous blood sampling | Yes |
| Calbet et al\(^{27} \) | 4♂4♀ | 24 | Med. Graph. CPX | TD-C | Yes |
| Calbet et al\(^{30} \) | 3♂ | 24 | Amis 2001 | TD-C | Yes |
| Calbet et al\(^{52} \) | 10♂ | 24 | Quark b2 | TD-C | Yes |
| Calbet et al\(^{5} \) | 9♂ | 33 | Quark b2 | TD-C | Yes |
| Calbet et al\(^{51} \) | 9♂ | 31 | Quark b2 | TD-C | Yes |
| Calbet et al\(^{56} \) | 11♂ | 22 | Vmax 29 | TD-C | Yes |
| Cardinale et al\(^{10} \) | 4♂4♀ | 24 | Oxycon Pro | TD-C | Yes |
| Cardus et al\(^{104} \) | 13♂5♀ | 23 | Custom | TD-C | Yes |
| Gonzalez-Alonso et al\(^{11} \) | 8♂ | 24 | OCM-2 | TD-C | Yes |
| Harms et al\(^{105} \) | 7♂ | 29 | Custom | TD-C | Yes |
| Klausen et al\(^{30} \) | 6♂ | 23 | Douglas bag tech. | TD-C | – |
| Knight et al\(^{103} \) | 7♂ | 29 | Custom | TD-C | Yes |
| Knight et al\(^{27} \) | 12♂ | 29 | Custom | TD-C | Yes |
| Lundby et al\(^{53} \) | 8♂ | 26 | Quark b2 | TD-C | Yes |
| Lundby et al\(^{54} \) | 8♂ | 27 | Quark b2 | TD-C | Yes |
| Lundby et al\(^{107,113} \) | 6♂ | 26 | Custom | TD-C | Yes |
| Mortensen et al\(^{9} \) | 13♂ | 28 | Quark b2 | TD-C | Yes |
| Mortensen et al\(^{1} \) | 10♂ | 27 | Quark b2 | TD-C | Yes |
| Munch et al\(^{15} \) | 10♂ | 27 | Quark CPET | TD-C | Yes |
| Poole et al\(^{155} \) | 6♂ | 26 | Custom | TD-C | Yes |
| Roca et al\(^{28} \) | 6♂ | 24 | Custom | – | Yes |
| Roca et al\(^{12} \) | 8♂4♀ | 22 | Custom | TD-C | Yes |
| Proctor et al\(^{120} \) | 11♂ | 21 | TrueMax 2400 | TD-C | Yes |
| Rud et al\(^{35} \) | 4♂4♀ | 23 | Douglas bag tech. | TD-C | Yes |
| Trangmar et al\(^{16} \) | 9♂ | 26 | Not reported | TD-C | Yes |
| van Hall et al\(^{106} \) | 5♂1♀ | 26 | Med. Graph. CPX | TD-C | Yes |

Abbreviations: ID-B, bolus indicator-dilution method (I-labelled human albumin); \( n \), number of subjects; TD-B, bolus-infusion thermodilution method; TD-C, continuous-infusion TD.
$\dot{V}O_2$ and mean capillary PO$_2$ were calculated as previously described,\textsuperscript{28,29} using the measured arterial and femoral venous PO$_2$. $\dot{V}O_2$ is recognized as a compound variable in the mitochondria] is recognized as a compound variable integrating several steps in the O$_2$ cascade, including the dissociation of O$_2$ from Hb, and diffusion through the erythrocyte membrane, plasma, capillary wall, interstitial space, sarclemma, cytoplasm (myoglobin facilitated or by diffusion) and into the mitochondria for utilization by the cytochromes. The equilibration index $Y$, which quantitatively describes perfusion versus diffusion limitations to $V_0_2$, was calculated according to Piiper and Scheid.\textsuperscript{27}

2.2 | Statistical analyses

Data are presented as mean±standard deviation, if not otherwise stated. Regression was analysed using simple linear regression, second-order polynomials and exponential decay models ($y = a \cdot e^{-k \cdot x} + \text{plateau}$), all using least squares as the fitting method. Regression lines/curves are presented with 95% confidence bands representing the likely location of the true curve. The alpha-level was set to ≤0.05 and values between >0.05 and ≤0.10 were considered to indicate trends. GraphPad Prism (v. 8.0.1; GraphPad Software, CA, USA) and Microsoft Office Excel 2013 (Microsoft Corporation, WA, USA) were used for statistical analysis.

2.3 | Part 1: Systemic responses during maximal exercise (individual data)

$\dot{Q}_{\text{max}}$ increased by 4.9 L·min$^{-1}$ for each L·min$^{-1}$ increase in $\dot{V}O_2$ (Figure 1A; $P < .001$), explained by a linear increase in stroke volume (Figure 1B; $P < .001$).

The calculated a-$V_0_2$ difference ($\dot{V}O_{2\text{max}}/\dot{Q}_{\text{max}}$) showed an inverse J-shaped curve, reaching the highest level between 4.5-5.0 L·min$^{-1}$ before declining at higher $\dot{V}O_{2\text{max}}$ (Figure 1C). After accounting for the decrease in CaO$_2$ with increasing $\dot{V}O_{2\text{max}}$ (Figure 1E; $P < .001$), the calculated $\dot{O}_2$ extraction fraction increased up to a $\dot{V}O_{2\text{max}}$ of ~4.5-5.0 L·min$^{-1}$ and then approached a maximal value at ~90% (Figure 1D) when restricting the exponential decay model to plausible physiological limits ($\dot{V}O_{2\text{max}} > 6.7$ L·min$^{-1}$). The linear decrease in CaO$_2$ was explained by arterial hypoxemia (decreased arterial SO$_2$; Figure 1F; $P < .001$) and a non-significant negative relationship between [Hb] and $\dot{V}O_{2\text{max}}$ (Figure 1G; $P = .232$). The calculated $\text{CtO}_2$ gradually decayed and approached a minimum at ~10-15 mL·L$^{-1}$ in the subjects with the highest $\dot{V}O_{2\text{max}}$ (Figure 1H).

Systemic vascular conductance was strongly positively correlated with $\dot{V}O_{2\text{max}}$ (Figure 2B; $P < .001$). There were no significant associations between mean arterial pressure (MAP) and $\dot{V}O_{2\text{max}}$ (Figure 2A; $P = .289$) or with $\dot{Q}_{\text{max}}$ ($y = -0.2x + 125; R^2 = .004; n = 119; P = .475$).

When controlling the regression between the individual data of $\dot{V}O_{2\text{max}}$ and the calculated $\dot{O}_2$ extraction fraction with mean values from studies measuring $\dot{Q}_2$ extraction fraction directly using the Fick method (right heart catheterization), or indirectly using the Fick equation ($\dot{Q}_{\text{max}}$-indicator-dilution or transpulmonary thermodilution), most values fell close to the regression curve (Figure 3).

2.4 | Part 2: Peripheral responses during maximal exercise (mean data)

LBF and two-LBF rose by 4.6 and 5.7 L·min$^{-1}$ for each L·min$^{-1}$ increase in leg and pulmonary $\dot{V}O_{2\text{max}}$ respectively (Figure 4A-D; both $P < .001$). Leg and pulmonary $\dot{V}O_{2\text{max}}$ displayed a linear relationship ($y = 1.27x - 2.01; R^2 = .85; n = 28; P < .001$). The directly measured leg a-$V_0_2$ difference and leg O$_2$ extraction fraction were best explained by exponential decay models and increased gradually with the increase in leg and pulmonary $\dot{V}O_{2\text{max}}$ to approach a maximum at ~180-190 mL·L$^{-1}$ and ~90-95% respectively (Figure 4B,C,E,F). These relationships were equally strong when $\dot{V}O_{2\text{max}}$ was standardized to body weight (Supporting material Figure 1). Note that leg a-$V_0_2$ difference was not lower for the subjects with the highest $\dot{V}O_{2\text{max}}$ as observed for the systemic a-$V_0_2$ difference (Figure 1C), possibly since only one subject group exceeded a $\dot{V}O_{2\text{max}}$ of 4.7 L·min$^{-1}$, where this occurred for the systemic responses (see Figure 1C). In connection, no association was evident between pulmonary $\dot{V}O_{2\text{max}}$ and CaO$_2$ for these data ($y = 1.07 + 195; R^2 < .01; n = 30; P = .701$).

Like the systemic responses, the measured femoral venous O$_2$ content (CvO$_2$) decreased gradually with increasing pulmonary $\dot{V}O_{2\text{max}}$ until reaching a minimum of ~10 mL·L$^{-1}$ (Figure 5A). Likewise, the femoral venous SO$_2$ and PO$_2$ decreased gradually to approach ~5% and ~10 mmHg at the highest $\dot{V}O_{2\text{max}}$ respectively (Figure 5B,C).

$\text{D}O_2$ was positively correlated with leg $\dot{V}O_{2\text{max}}$ ($y = 27x - 6; R^2 = .92; n = 21; P < .001$), pulmonary $\dot{V}O_{2\text{max}}$ (Figure 6A; $P < .001$) and leg O$_2$ extraction fraction ($y = 1.7x - 110; R^2 = .80; n = 21; P < .001$). Interestingly, the equilibration index $Y$, which quantitatively describes diffusion versus perfusion limitations to muscle $V_0_2$ (where $Y < 0.1$ indicates pure diffusion limitation, 0.1 < $Y < 3$ indicates mixed diffusion limitation and $Y > 3$ indicates pure perfusion limitation),\textsuperscript{27} was well above 1.0 for all subject groups (Figure 6B) and increased progressively with leg $\dot{V}O_{2\text{max}}$ ($y = 0.28x + 1.40; R^2 = .37; n = 21; P = .003$), pulmonary $\dot{V}O_{2\text{max}}$ (Figure 6B; $P = .008$) and leg O$_2$ extraction fraction ($y = 0.023x - 0.129; R^2 = .53; n = 21; P < .001$). The equilibration index $Y$ was also correlated with pulmonary $\dot{V}O_{2\text{max}}$
Maximal oxygen uptake (L · min⁻¹)

Cardiac output (L · min⁻¹)

Cycling
Running

\( y = 4.9x + 5.7 \)
\( R^2 = .73, n = 154 \)

Arterial O₂ content (mL · L⁻¹)

\( y = -7.0x + 224 \)
\( R^2 = .17, n = 115 \)

Stroke volume (mL · beat⁻¹)

\( y = 26x + 30 \)
\( R^2 = .72, n = 148 \)

Arterial O₂ saturation (%)

\( y = -1.06x + 99.4 \)
\( R^2 = .29, n = 45 \)

a-vO₂ difference (mL · L⁻¹)

\( y = -6.1x^2 + 59.5x + 23 \)
\( R^2 = .27, n = 154 \)

Mixed venous O₂ content (mL · L⁻¹)

\( y = 210e^{-0.41x} - 3 \)
\( R^2 = .41, n = 115 \)

Systemic O₂ extraction (%)
standardized to body weight ($R^2 = .38; P = .003$; Supporting material Figure 2). Therefore, the leg muscles were more perfusion than diffusion limited, even for subjects with the lowest $\dot{V}O_2_{\text{max}}$, and were progressively more perfusion/O$_2$ delivery limited with a gradually higher $\dot{V}O_2_{\text{max}}$. This can also be illustrated by applying the Piiper and Scheid model to calculate the fractional extent to which $\dot{V}O_2_{\text{max}}$ is expected to change if DMO$_2$ or LBF are modified$^{27}$; Figure 6C shows that an individual’s $\dot{V}O_2_{\text{max}}$ is less sensitive to any change in DMO$_2$ if the $\dot{V}O_2_{\text{max}}$ is already high, which is caused by the little remaining O$_2$ available for extraction in the femoral venous (ie, end-capillary) blood. For instance, according to this theoretical model and using the relationship in Figure 6C; if a subject with a $\dot{V}O_2_{\text{max}}$ of 5 L·min$^{-1}$ changed his DMO$_2$ by 20%, he would only change his $\dot{V}O_2_{\text{max}}$ by ~6% (20% × 0.3). Conversely, the same subject would increase $\dot{V}O_2_{\text{max}}$ by ~14% after a 20% increase in LBF (20% × 0.7).

### SUMMARY OF FINDINGS

To our knowledge, the present investigation is the first to critically review the existing research on the association between $\dot{V}O_2_{\text{max}}$ and systemic and peripheral O$_2$ extraction fractions in healthy young men. Our findings are as follows:

1. Pulmonary and leg $\dot{V}O_2_{\text{max}}$ were best explained by $\dot{Q}_{\text{max}}$ and LBF, respectively, agreeing with most previous studies where these variables have been directly manipulated.
2. The systemic O$_2$ extraction fraction increased with $\dot{V}O_2_{\text{max}}$ until approximately 4.5-5.0 L·min$^{-1}$. Above this value, the O$_2$ extraction fraction was typically around ~90%.
3. The measured leg O$_2$ extraction fraction increased with leg and pulmonary $\dot{V}O_2_{\text{max}}$ to approach a maximal value at ~90-95%, strengthening the findings from the calculated systemic O$_2$ extraction fraction. This strongly suggests that O$_2$ extraction increases after endurance training and contributes to a high $\dot{V}O_2_{\text{max}}$.
4. The calculated $CvO_2$ and the measured $Cv_fO_2$ indicate a minimum value at ~15 and ~10 mL·L$^{-1}$, respectively, associated with a femoral venous SO$_2$ and PO$_2$ of ~5% and ~10 mmHg respectively. At this point, further peripheral O$_2$ extraction may no longer be possible as a result of diffusional limitations and/or because the remaining O$_2$ represents blood perfusing the least active muscle regions of the leg, connective tissue, bone marrow, adipose tissue and skin, which are characterized by a lower O$_2$ extraction.
5. The progressive increase in the equilibration index $Y$ with pulmonary and leg $\dot{V}O_2_{\text{max}}$ indicates that the muscles become gradually more perfusion/O$_2$ delivery limited with increasing $\dot{V}O_2_{\text{max}}$.

### 3.1 | Oxygen delivery

To match O$_2$ delivery to O$_2$ consumption, $\dot{Q}_{\text{max}}$ and two-LBF increased by ~5-6 L·min$^{-1}$ per 1 L·min$^{-1}$ increase in pulmonary $\dot{V}O_2_{\text{max}}$. These relationships were strong and complied...
with previous research and the “classic” view that O₂ delivery is the primary determinant of whole-body VO₂max.4,7,11 As maximal heart rate showed no apparent relationship with VO₂max, the high stroke volumes (>180 mL·beat⁻¹) explained the large Q̇o₂max in the athletes included in the present analysis (>35 L·min⁻¹), in agreement with previous knowledge.13,16,30

Despite increased Q̇o₂max, MAP was unchanged with increasing VO₂max as a result of increased vascular conductance. Although untrained individuals typically display a rise in MAP from rest to maximal exercise,31 well-trained athletes can display an unchanged MAP or even a small reduction owing to profound peripheral vasodilation.32 Consequently, vasodilation of a well-developed peripheral vascular network likely contributed to the extremely high stroke volumes by minimizing afterload in the subjects with the highest VO₂max. To substantiate, endurance training of each leg separately, to evoke extensive peripheral adaptations without stimulating the central circulation substantially, has been shown to decrease MAP and the total peripheral resistance during two-legged maximal exercise that likely contributed to the remarkably high stroke volumes (>180 mL·beat⁻¹) explained the large Q̇o₂max in the athletes included in the present analysis (>35 L·min⁻¹), in agreement with previous knowledge.13,16,30

Figure 1E). Therefore, the lower CaO₂ may explain why moderately and well-trained individuals can have a similar a-VO₂ difference, despite differing markedly in DₘO₂, mitochondrial mass and capillary density.40,41 Actually, parts of this mechanism are demonstrated experimentally since acute plasma volume expansion increases Q̇o₂max but lowers the CaO₂ and, hence, reduces the a-VO₂ difference during maximal exercise.42,43

Opposite to the a-VO₂ difference, the systemic Ó₂ extraction fraction—ie, the fraction of O₂ that is taken up with the amount available for utilization (a-VO₂ difference/CaO₂)—increased with VO₂max until reaching ~90%. This pattern was confirmed in the leg when measured using catheters, with the O₂ extraction fraction increasing progressively with leg and pulmonary VO₂max until reaching ~90%. Therefore, the calculated systemic Ó₂ extraction fraction (Fick equation) is supported by direct measurements via arterial and femoral venous blood sampling and strongly

3.2 | Oxygen extraction

The calculated systemic a-VO₂ difference showed a large variability for a given VO₂max and was, if anything, lower in those subjects displaying the highest VO₂max (>5 L·min⁻¹) compared to those being moderately to well trained (VO₂max: 4-5 L·min⁻¹). This agrees with previous studies showing only a small difference between non-endurance-trained and active individuals16,17 and no apparent difference between well-trained individuals and elite athletes.16 This has led previous investigators to argue that improved O₂ extraction does not contribute or only minimally contributes to the remarkably high VO₂max observed in elite athletes.14,39 However, these papers may not have considered that endurance training causes plasma volume expansion,18 which often leads to haemodilution and a lower O₂ carrying capacity of the arterial blood.16 Combined with the below-average haemoconcentration from rest to maximal exercise that occurs in well-trained individuals16 and the exercise-induced arterial hypoxemia that often accompanies a high Q̇o₂max,19,20 individuals with the highest VO₂max displayed a substantially lower CaO₂ (~10%) than those with a low VO₂max (<180 mL·L⁻¹ vs >200 mL·L⁻¹; Figure 1E). Therefore, the lower CaO₂ may explain why moderately and well-trained individuals can have a similar a-VO₂ difference, despite differing markedly in DₘO₂, mitochondrial mass and capillary density.40,41 Actually, parts of this mechanism are demonstrated experimentally since acute plasma volume expansion increases Q̇o₂max but lowers the CaO₂ and, hence, reduces the a-VO₂ difference during maximal exercise.42,43

Figure 3 Mean values (±95% confidence limits, where available) of systemic oxygen extraction fraction versus maximal oxygen uptake from studies using the direct (pulmonary artery catheter) or the modified (right atrium catheter) Fick method.4,9,28,32,51,55,61,121-127 the indicator dilution method5,11,16,17,21,22,24,31,42,52-54,57,85,112,114,128-130 and the transpulmonary thermodilution method.56 Broken line is the regression equation obtained from Figure 1D
indicates that the $\overline{O}_2$ extraction fraction is improved with increasing $\dot{V}O_{2\text{max}}$ to a certain level.

In most endurance training studies investigating the interplay between central and peripheral adaptations in improving $\dot{V}O_{2\text{max}}$, $Q_{\text{max}}$ was measured by non-invasive methods (such as inert-gas rebreathing techniques, impedance cardiography and bioreactance) and the Fick equation was used to derive the a-$\overline{V}O_2$ difference (for references, see the meta-analysis by Montero et al\cite{44}). The majority of these studies failed to detect a statistically significant change in the a-$\overline{V}O_2$ difference. However, this finding does not necessarily mean that $\dot{V}O_{2\text{max}}$ was exclusively increased by elevated $Q_{\text{max}}$ for three
reasons. First, when the a-\(\overline{\text{V}}\)O\(_2\) difference is calculated by the Fick equation, a large variation is introduced as a result of measurement error in \(\dot{Q}_{\text{max}}\), especially when non-invasive methods are used. Second, because of the above, maybe in combination with a considerable individual variation in peripheral adaptations such as capillarization, it is likely that these studies are underpowered for detecting small changes in the a-\(\overline{\text{V}}\)O\(_2\) difference. Third, these studies may have failed to detect actual improvements in systemic \(\overline{\text{O}}_2\) extraction fraction when the a-\(\overline{\text{V}}\)O\(_2\) difference was mostly unchanged, as endurance training may have evoked an accompanying reduction in \(\text{CaO}_2\). Therefore, future studies should strive to measure peripheral or systemic \(\overline{\text{O}}_2\) extraction fraction directly, or at least combine the calculations of a-\(\overline{\text{V}}\)O\(_2\) difference with measurement of \(\text{CaO}_2\) (arterial catheter). Actually, in the endurance training studies where peripheral \(\overline{\text{O}}_2\) extraction fraction
was measured directly during maximal exercise (arterial and venous catheters), the vast majority found an increased O₂ extraction fraction after training. 12,30,45-47

A particular case, concerning the relationship between one-leg \( \dot{V}O_{2\text{max}} \) and O₂ extraction fraction (Figure 4C) and between pulmonary \( \dot{V}O_{2\text{max}} \) and two-LBF (Figure 4D) deserves some attention (the white squares). These data were collected during combined upper- and lower-body exercise (cross-country skiing using the diagonal technique) and 6.6 L·min⁻¹ of \( \dot{Q}_{\text{max}} \) was distributed to the two arms. 32 Hence, when combining the locomotor blood flow (arms+legs), the data fall perfectly on the regression line between blood flow and pulmonary \( \dot{V}O_{2\text{max}} \). When redistributing LBF towards other exercising musculature, the erythrocyte capillary mean transit time (MTT) is increased. Therefore, the conditions for Hb-O₂ off-loading are improved, resulting in a slightly higher O₂ extraction fraction for a given leg. 12,30,45-47 These conclusions are similar to those of Gifford et al, 25 who found a clear relationship between OXPHOS measured in permeabilized muscle fibres ex vivo and \( \dot{V}O_{2\text{max}} \) in untrained but not in trained individuals.

3.4 Why is not all the O₂ extracted from the blood?

The entire \( \dot{Q}_{\text{max}} \) cannot be directed to the skeletal muscles during exercise. Other organs like the brain, heart, splanchnic organs and skin need perfusion and O₂ delivery to maintain homeostasis. \( \dot{Q}_{\text{max}} \) must also serve the O₂ demand of the respiratory muscles and the muscles in the trunk and the arms that stabilize the subject’s position on the cycle ergometer, and these tissues are characterized by a substantially lower O₂ extraction than the legs during maximal exercise. 5,50 As a mean of those investigations measuring \( \dot{Q}_{\text{max}} \) and LBF simultaneously (Table 3), the non-leg blood flow was 6.4 L·min⁻¹ and was unaffected by the level of \( \dot{Q}_{\text{max}} \) (\( y = 0.002x + 6.4; \) \( R^2 < .001; n = 12; P > .999 \)). The O₂ extraction was calculated to be 68% on average for all non-leg tissues (head, trunk and arms), explaining why the O₂ extraction fraction of the central circulation was slightly lower than in the legs (79% vs 84%, respectively; Table 3). A mean difference of 5 percentage points might be a small underestimation since the studies using right heart catheterization 4,9,28,29,51,55 combined with arterial and femoral venous catheters indicated a mean difference of 8 percentage points. A difference of 5%-8% points fits well, since the O₂ extraction fraction of the arms, myocardium, brain and trunk range from 40% to 80% during exercise. 5,50-58-60 Therefore, the \( \dot{C}vO_{2} \) can never reach the same level as the \( C_{v}O_{2} \) during exercise involving

### Table 3

Data from studies measuring pulmonary O₂ uptake, cardiac output (indicator-dilution, Fick method or transpulmonary thermodilution), leg blood flow (thermodilution) and leg arteriovenous O₂ difference (a-vO₂ difference; catheters) simultaneously during maximal exercise. From these measurements, O₂ extraction fraction was calculated for the central circulation and the non-leg tissue (combined trunk, arms and head).

|                         | Central circulation (mean ± SD) | Two-leg circulation (mean ± SD) | Non-leg tissue circulation (mean ± SD) |
|-------------------------|---------------------------------|---------------------------------|---------------------------------------|
| Blood flow (L·min⁻¹)    | 25.0 ± 2.4                      | 18.6 ± 3.0                      | 6.4 ± 1.7                             |
| Arterial O₂ content (mL · L⁻¹) | 203 ± 10                      | 203 ± 10                       | 203 ± 10                              |
| O₂ delivery (L·min⁻¹)   | 5.03 ± 0.60                     | 3.77 ± 0.63                    | 1.26 ± 0.32                           |
| O₂ uptake (L·min⁻¹)     | 4.02 ± 0.65                     | 3.19 ± 0.65                    | 0.83 ± 0.24                           |
| a-vO₂ difference (mL · L⁻¹) | 160 ± 17                      | 172 ± 14                       | 137 ± 48                              |
| O₂ extraction fraction (%) | 79 ± 8                        | 84 ± 5                         | 68 ± 26                               |
| Venous O₂ content (mL · L⁻¹) | 42 ± 18                       | 31 ± 10                        | 66 ± 52                               |
| O₂ delivery not utilized (L·min⁻¹) | 1.01 ± 0.36                    | 0.58 ± 0.07                    | 0.43 ± 0.32                           |

**Note:** \( n = 12 \) (articles) 4,5,9,11,31,51-57 or \( n = 117 \) (subjects).
the legs and was calculated to reach a minimum of ~15 mL · L⁻¹ in subjects having a \( \dot{V}O_{2\text{max}} \) of 6 L·min⁻¹ (Figure 1H). To our knowledge, the lowest \( \text{CcO}_{2} \) measured at sea level using right heart (atrium) catheterization is 20.1 mL · L⁻¹ (group mean) in athletes with a \( \dot{V}O_{2\text{max}} \) of 5.1 L·min⁻¹. A slightly lower value was measured in one of these cross-country skiers (15.5 mL · L⁻¹), and a mean value of 18.6 mL · L⁻¹ has been measured in moderately trained individuals after acclimatizing to 6500 metres above sea level⁶¹; indicating that 15 mL · L⁻¹ or lower is approachable.

The highest recorded leg \( O_2 \) extraction fraction was 93% (group mean)⁵⁹ and the regression models indicated a plateau at ~95% within physiological limits for pulmonary \( \dot{V}O_{2\text{max}} \). Hence, a minimum of ~10 mL \( O_2 \) remains in each litre of femoral venous blood associated with a \( P_O_2 \) of ~10 mmHg, even for the best trained individuals. In this situation, a \( P_O_2 \) gradient persists between the blood and myoglobin (myoglobin/intracellular \( P_O_2 \): ~1–2 mmHg), where myoglobin-facilitated diffusion should proceed given the high myoglobin \( O_2 \) affinity (myoglobin \( P_aO_2 \): ~5 mmHg) and the low myoglobin \( SO_2 \) at maximal exercise.⁶² However, according to the Fick law of diffusion, the diffusive flux is directly proportional to the \( P_O_2 \) gradient and will, thus, gradually decrease along the capillary and be very small when approaching low capillary \( P_O_2 \) values such as 10 mmHg. It has also been shown that the primary site of resistance to \( O_2 \) diffusion is between the capillaries and the sarcoplasm and it has been estimated that the “critical capillary \( P_O_2 \)” needed to overcome this resistance may be as high as 10–20 mmHg.⁶²–⁶⁵ The remaining \( O_2 \) may, therefore, represent diffusional limitations across the combined capillary wall, interstitium and sarcolemma barriers together with a MTT that is too short for complete Hb-\( O_2 \) off-loading. This is supported by the need for an infinitesimal \( P_O_2 \) gradient for \( O_2 \) to diffuse from the sarcoplasm to cytochrome c oxidase⁶⁶ and the estimate that a mitochondrial \( P_O_2 \) of ~1 mmHg may be sufficient to support maximal mitochondrial respiration.⁶⁷,⁶⁸ The remaining \( O_2 \) may also represent muscle metabolism-perfusion mismatch⁶⁹,⁷⁰ and an inevitable lower \( O_2 \) extraction from the blood perfusing the skin, connective tissue, fat and bone marrow of the leg causing venous admixture. In this context, the end-capillary \( P_O_2 \), assessed using video microscopy, was found to be lower than the \( P_O_2 \) both in the venule (\( O_2 \) micro-electrode) and vein (blood gas) draining the muscle region of interest.⁷¹ Hence, the lowest femoral venous \( P_O_2 \) values of ~10 mmHg indicates an even lower end-capillary \( P_O_2 \) in the capillaries adjacent to the most metabolically active muscle regions during maximal exercise, possibly approaching ~5 mmHg. Therefore, no matter which kind of limitation prevails, it is highly unlikely that leg \( O_2 \) extraction fraction can improve much further, and that a theoretical threshold of ~95% exists because of the above diffusional and distributional limitations and barriers.

4 | THE MECHANISMS EXPLAINING THE IMPROVEMENTS OF \( O_2 \) EXTRACTION WITH TRAINING

The systemic \( \bar{O}_2 \) extraction fraction may increase through two main mechanisms with training: (a) by directing a higher fraction of \( Q_{\text{max}} \) to the exercising muscles and (b) by increasing the peripheral \( O_2 \) extraction fraction.

Both in trained and untrained subjects, during exercise with a large muscle mass (such as running and cycling), the muscle-specific blood flow (per unit of mass) is restrained as a result of sympathetically mediated vasoconstriction of peripheral vascular beds, caused by a limited \( Q_{\text{max}} \).⁹,³²,⁴⁸ Even in “untrained” leg skeletal muscle, the reserve in vasodilatory capacity is very high and supports 2-3 times larger blood flow per unit of mass, as observed during dynamic one-legged knee extension.⁷² Simply increasing \( Q_{\text{max}} \) (for instance, by training), without any peripheral adaptations, may increase the systemic \( O_2 \) extraction fraction by two mechanisms. First, the recruitment of a larger portion of the already existing capillary network may reduce diffusion distances and thereby increase the \( O_2 \) extraction. This additional recruitment may also serve to maintain MTT despite increased LBF. Second, a larger fraction of \( Q_{\text{max}} \) will flow through the exercising muscles (Figure 7) because the non-exercising tissue blood flow is independent of \( Q_{\text{max}} \) in healthy young subjects (at ~6.4 L·min⁻¹, see section 3,4).⁴,⁵,⁹,¹¹,³¹,⁵¹-⁵⁷ Consequently, even without any peripheral adaptations, the systemic \( O_2 \) extraction fraction may increase when \( Q_{\text{max}} \) and LBF are elevated with training.

FIGURE 7  The fraction of maximal cardiac output (\( Q_{\text{max}} \)) that is directed to the legs during maximal exercise (cycling) as a function of \( \dot{V}O_{2\text{max}} \). The included studies measured \( Q_{\text{max}} \) by using the indicator-dilution method, Fick method or transpulmonary thermodilution, and leg blood flow was measured by thermodilution.⁴,⁵,⁹,¹¹,³¹,⁵¹-⁵⁷ Note that the uppermost data point (0.915; ie, only 2.2 L·min⁻¹ in calculated non-leg blood flow) is supra-physiological, but the correlation was similar after its exclusion (\( R^2 = .42 \))
The peripheral O₂ extraction depends on the interplay between several factors: (a) the kinetics of O₂ off-loading from Hb; (b) the erythrocyte MTT, which is determined by the blood flow, the capillary density, the capillary recruitment and the degree of matching of blood flow distribution to the metabolic demand; (c) the diffusional O₂ conductance over the combined capillary wall, interstitium and sarcolemma barriers; and (d) the muscle oxidative capacity, the mitochondrial P₅₀ and the mitochondrial activation.¹⁰,²⁹,⁷³

A right-shifted O₂-Hb dissociation curve (elevated P₅₀O₂) increases the O₂ extraction fraction in pump-perfused dog muscle.⁷⁴ A close relationship has also been demonstrated between O₂ extraction fraction and in vivo P₅₀O₂ in humans during exercise.²⁹ Very few of the studies included in the present analysis reported the in vivo P₅₀O₂, but it was possible to calculate it from the other blood gas parameters using Kelman’s Equation.⁷² after assuming a femoral venous blood temperature of 39.₀°C at maximal exercise.⁹⁵,⁵⁵,⁷⁶ Based on 15 of the studies presented in Table 2, the P₅₀O₂ was linearly associated with leg O₂ extraction fraction \( R² = .27; n = 15; P = .048 \). Despite this relationship, a high P₅₀O₂ does not seem to be compulsory to achieve high O₂ extraction during whole-body maximal exercise, as demonstrated in experiments using a small dose of carbon monoxide (carboxyhaemoglobin at 6%-7%), which left-shifts the ODC without a negative impact on O₂ extraction fraction.⁵⁶

Increased MTT has the potential to increase O₂ extraction, but whether this occurs after endurance training is determined by the balance between the changes in blood flow and the capillary blood volume. Capillary density typically improves by 10-30% after 4-24 weeks of endurance training, which is similar to the changes in VO₂max for this training duration.⁷⁷-⁷⁹ Moreover, cross-sectional data indicate a similar difference in capillary density to that of VO₂max between untrained and endurance trained men.⁴¹ Therefore, the capillary growth probably maintains the MTT despite elevated \( \dot{Q}_{\text{max}} \) and peripheral blood flow after training. In support, similar improvements in arm blood flow and capillary density have been observed after a period of arm training, causing no change in the calculated MTT.⁴⁷ The arm O₂ extraction fraction was increased in the same study, suggesting that elevated MTT is not the primary mechanism by which O₂ extraction is improved after training. However, this may differ between arms and legs (ie, small vs large muscle mass exercise). Moreover, in the calculation of MTT in the study mentioned above, full capillary recruitment was assumed. Therefore, even though the changes in capillary density and muscle blood flow share magnitudes after endurance training, the MTT may still be increased if the capillary recruitment is altered.

An increased capillary-to-fibre ratio after endurance training increases the number of contact points between the capillary and the muscle fibre. This increases the diffusional surface area that, according to the Fick law of diffusion, increases the diffusive flux in a directly proportional manner. Therefore, the capillary-to-fibre ratio is regarded as a critical determinant of O₂ diffusion from the erythrocytes to the cytoplasm.⁸¹,⁸² As an example, a larger diffusional area and shorter diffusional distance are proposed to contribute to the higher O₂ extraction fraction in the legs than in the arms during exercise.²⁹ Moreover, if the capillary recruitment is changed with training, this may also affect the effective diffusional surface area similarly to de novo capillarization.

During whole-body maximal exercise, the oxidative capacity of skeletal muscle exceeds the O₂ delivery, as illustrated by the twofold higher VO₂ per unit of muscle mass during dynamic one-legged knee extension compared to cycling exercise (approximately 2.5 vs 20 kg active muscle mass, respectively).¹⁰,⁷² Therefore, the leg muscles possess an oxidative reserve capacity at VO₂max during whole-body exercise, which has frequently been used as an argument to indicate that the large improvements in mitochondrial and capillary networks after endurance training are likely only crucial for improvements in endurance performance and do not affect the limiting factors to VO₂max.⁸³ In support of this view, the calculated O₂ extraction fraction is maintained or increases after prolonged bed rest (3-6 weeks), although a substantial reduction in mitochondrial volume density occurs.⁸⁴,⁸⁵ However, the \( \bar{\dot{O}}_2 \) extraction fraction depends on the interactions between several factors. For instance, by acutely decreasing \( \dot{Q}_{\text{max}} \) and LBF using β-adrenergic blockade, a-\( \Delta \)O₂ difference and a-vO₂ difference increase during submaximal and maximal exercise, facilitated by increased erythrocyte MTT.⁸⁶,⁸⁷ This is substantiated by the positive relationship between the ratio of OXPHOS/O₂ delivery and the leg O₂ extraction fraction,⁶⁰ meaning that the balance between muscle oxidative capacity and blood flow (ie, oxidative capacity and MTT) is more critical for O₂ extraction than any of these factors alone. Therefore, as bed rest reduces \( \dot{Q}_{\text{max}} \) dramatically but causes only a minor change in capillary density,⁴⁸,⁸⁵ the MTT is elevated, and the ratio of OXPHOS/ O₂ delivery is probably the same, in favour of increased or maintained O₂ extraction fraction. In contrast, by changing the exercise mode from upright to supine cycling after bed rest, which preserves \( \dot{Q}_{\text{max}} \) at the pre-bed rest level, the calculated a-\( \Delta \)O₂ difference is decreased (154 to 120 mL·L⁻¹).⁸⁸ Similarly, after a dog gastrocnemius muscle was immobilized for 3 weeks, followed by electrical stimulation to VO₂max while being pump perfused to receive a similar O₂ delivery as a control muscle, the O₂ extraction fraction was dramatically reduced.⁸² Therefore, muscle oxidative capacity seems to play a role in determining O₂ extraction, and the bed rest studies need to be evaluated carefully because of the consequences for peripheral MTT.
If $\bar{O}_2$ extraction fraction improves after endurance training, it is probably affected by the balance between central and peripheral adaptations. For instance, after 2 weeks of high-intensity interval training that elevated the cytochrome c oxidase activity by 20% but caused no change in $\dot{Q}_{max}$, $\dot{V}O_{2\text{max}}$ was increased by 8% and was entirely attributed to the improved systemic (calculated a-$\bar{v}O_2$ difference) and leg (increased deoxyhaemoglobin and decreased tissue oxygenation index in Vastus Lateralis, assessed using NIRS) $O_2$ extraction. However, after 3-8 weeks of endurance training, improvements in $\dot{Q}_{\text{max}}$ explain almost the entire increase in $\dot{V}O_{2\text{max}}$, as indicated by meta-regression. If the training lasts longer (>8 weeks), enhancements of $\dot{Q}_{\text{max}}$ decelerate and improvements in a-$\bar{v}O_2$ difference are again evident. Therefore, the peripheral adaptations are probably just sufficient to counteract the “negative influence” of elevated $\dot{Q}_{\text{max}}$ and LBF on MTT in periods with large central adaptations, and improvements in $\bar{O}_2$ extraction fraction is likely only evident when the peripheral adaptations largely surpass those of the central circulation. This can be substantiated by findings from one-legged endurance training that induces robust peripheral adaptations without stimulating the central circulation substantially and commonly improves leg $a$-$v\bar{O}_2$ difference by 5-10 mL $\cdot$ L$^{-1}$. 

The mitochondrial volume density can differ by as much as 150% between untrained and well-trained individuals in extreme cases (eg, ~4 vs ~10 vol. %) and can improve by as much as ~40%-55% after 6 weeks of endurance training in previously sedentary individuals. Why does this disproportionate adaptation occur when the muscle already possesses an oxidative reserve capacity? Does it have any physiological meaning for $\dot{V}O_{2\text{max}}$ or is it only important for improvements in, for example, fat oxidation and the lactate threshold, thus improving endurance?

Although an impressive increase in leg $O_2$ extraction fraction from 72% to 82% has been reported after only 9 weeks of intense endurance training in previously sedentary subjects, we propose that remarkable increases in muscle oxidative capacity are needed to achieve the outstanding leg $O_2$ extraction fraction observed in elite athletes (close to 95%). By analogy, the oxidative reserve capacity may act as a “bottomless pit”, keeping the myoglobin $SO_2$ and intracellular $PO_2$ low. This, in turn, maintains the $PO_2$ gradient between the capillary and the muscle cell, promoting $O_2$ diffusion and $O_2$ extraction even at a very low capillary $PO_2$.

Emerging evidence suggests that the mitochondrial volume density is increased while their intrinsic OXPHOS (OXPHOS divided by mitochondrial volume density or citrate synthase activity) is unchanged and sometimes even reduced after training. Since the mitochondrial respiratory rate and the ex vivo mitochondrial p50 increase in parallel, the unchanged or reduced intrinsic OXPHOS after training may permit an increased OXPHOS per unit of muscle mass while preserving (or increasing) the mitochondrial $O_2$ affinity (ie, by keeping the mitochondrial p50 low). Thus, a large pool of mitochondria with high $O_2$ affinity may preserve mitochondrial activation at low $O_2$ availability (low capillary $PO_2$) and promote peripheral $O_2$ extraction, but is yet to be experimentally tested. Moreover, the subsarcolemmal mitochondrial population increases relatively more than the intermyofibrillar population after endurance training. These mitochondrial clusters in close proximity to the capillaries may, speculatively, amplify the $O_2$ concentration gradient, shorten the diffusional distance and, thus, promote $O_2$ diffusion across the sarcolemma and enable further $O_2$ extraction at the end of the capillaries.

As shown in Figure 6C, a subject’s $\dot{V}O_{2\text{max}}$ becomes gradually less sensitive to adaptations improving diffusion when $\dot{V}O_{2\text{max}}$ is already high. Therefore, to raise the $O_2$ extraction fraction even slightly (eg, 2%), it is likely that more substantial improvement in peripheral adaptations is needed. However, a change in leg $O_2$ extraction fraction from, for example, 93% to 95% would only have a small impact on whole-body $\dot{V}O_{2\text{max}}$: for an athlete with a $\dot{V}O_{2\text{max}}$ of 5 L.min$^{-1}$, a two-LBF of 24 L.min$^{-1}$ ($\dot{Q}_{\text{max}}$: ~31 L.min$^{-1}$) and an $CaO_2$ of 190 mL $\cdot$ L$^{-1}$, the $\dot{V}O_{2\text{max}}$ would only increase by ~90 mL $\cdot$ min$^{-1}$ (1.8%). In comparison, an increase of 1 L.min$^{-1}$ in two-LBF would increase $\dot{V}O_{2\text{max}}$ by ~170 mL $\cdot$ min$^{-1}$ (3.4%) if all other factors remained the same.

### 5 | STUDY CONSIDERATIONS

The data were collected from several research groups and published over six decades (1958-2017) using a variety of gas analysers, flow sensors, methods to determine blood $O_2$ content and $PO_2$, and several procedures to analyse the indicator-dilution and blood temperature curves for $\dot{Q}_{\text{max}}$ and LBF measurements respectively. Therefore, for a given $\dot{V}O_{2\text{max}}$, the between-subject variability presented here may be overestimated. Moreover, several different averaging strategies for $\dot{V}O_2$ and the associated variables have likely been applied (rarely stated in the manuscripts). Despite these potential sources of noise, in general, the studies’ mean values converged to similar values. The fact that, despite the combination of several measurements with distinct methods (such as pulmonary gas exchange, thermodilution and blood gas analyses), the integrations of the obtained values fitted into the physiological range and agreed between studies, demonstrates the quality of these studies and the robustness of the analysis presented here.
CONCLUSION AND PERSPECTIVE

In conclusion, measurements of $\dot{Q}_{\text{max}}$ and LBF show that O$_2$ delivery is the primary determinant of whole-body and limb $\dot{V}O_2_{\text{max}}$. However, we also show that a very high O$_2$ extraction fraction contributes to the remarkably high $\dot{V}O_2_{\text{max}}$ in well-trained individuals and elite endurance athletes. To reinforce this conclusion we can, using the regression lines established in the present investigation, compare a typically sedentary subject and an elite endurance athlete with a large difference in $\dot{V}O_2_{\text{max}}$ (3.0 vs 5.5 L·min$^{-1}$): the elite athlete has a 1.83-fold higher $\dot{V}O_2_{\text{max}}$, a 1.60-fold higher $\dot{Q}_{\text{max}}$ and a 1.26-fold higher O$_2$ extraction fraction (Figure 8). However, because of the lower CaO$_2$, the a-vO$_2$ difference is only 1.13-fold higher in the elite athlete. This also stresses that a-vO$_2$ difference and O$_2$ extraction fraction cannot be used interchangeably when evaluating central versus peripheral limitations to $\dot{V}O_2_{\text{max}}$.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

Conception and design of the investigation: ØS, JH, CC, JALC. Literature search and analysis of data: ØS. Interpretation of data: ØS, JH, CC, JALC, BR. Writing the first draft of the manuscript: ØS. Revising and approving the final version: ØS, JH, CC, JALC, BR.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article, as no new data were created in this study.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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