Maximal oxygen uptake is proportional to muscle fiber oxidative capacity from chronic heart failure patients to professional cyclists

Adapted from:
Abstract

$\dot{V}O_{2\max}$ during whole-body exercise is presumably constrained by oxygen delivery to mitochondria rather than by mitochondria’s ability to consume oxygen. Humans and animals have been reported to exploit only 60-80% of their mitochondrial oxidative capacity at $\dot{V}O_{2\max}$. However, ex vivo quantification of mitochondrial overcapacity is complicated by isolation or permeabilization procedures. An alternative method for estimating mitochondrial oxidative capacity is via enzyme histochemical quantification of succinate dehydrogenase (SDH) activity. We determined to what extent $\dot{V}O_{2\max}$ attained during cycling exercise differs from mitochondrial oxidative capacity predicted from SDH activity of m. vastus lateralis in chronic heart failure patients, healthy controls and cyclists. $\dot{V}O_{2\max}$ was assessed in 20 healthy subjects and 28 cyclists and SDH activity was determined from biopsy cryosections of m. vastus lateralis using quantitative histochemistry. Similar data from our laboratory of 14 chronic heart failure patients and 6 controls were included. Mitochondrial oxidative capacity was predicted from SDH activity using estimated skeletal muscle mass and the relationship between ex vivo fiber $\dot{V}O_{2\max}$ and SDH activity of isolated single muscle fibers and myocardial trabecula under hyperoxic conditions. Mitochondrial oxidative capacity predicted from SDH activity was related ($r^2=0.89$, $p<0.001$) to $\dot{V}O_{2\max}$ measured during cycling in subjects with $\dot{V}O_{2\max}$ ranging from 9.8 to 79.0 mL·kg$^{-1}$·min$^{-1}$. $\dot{V}O_{2\max}$ measured during cycling was on average 90±14% of mitochondrial oxidative capacity. We conclude that human $\dot{V}O_{2\max}$ is related to mitochondrial oxidative capacity predicted from skeletal muscle SDH activity. Mitochondrial oxidative capacity is likely marginally limited by oxygen supply to mitochondria.

New and noteworthy

$\dot{V}O_{2\max}$ during whole-body exercise is presumably constrained by oxygen delivery to mitochondria rather than by mitochondria’s ability to consume oxygen. However, mitochondrial oxidative overcapacity remains unclear due to complicated isolation and permeabilization procedures. In the present study, human $\dot{V}O_{2\max}$ attained during cycling exercise is related and ~90% of mitochondrial oxidative capacity predicted from skeletal muscle succinate dehydrogenase activity. This mitochondrial oxidative overcapacity is substantially lower than previously reported from isolation and permeabilization procedures.
$\dot{V}O_{2\text{max}}$ is proportional to muscle fiber oxidative capacity, from heart patients to prof. cyclists

Reference: van der Zwaard et al., *J Appl Physiol*, 2016a
van der Zwaard et al., *J Appl Physiol*, 2016b

What limits whole-body $\dot{V}O_{2\text{max}}$?

### Muscle fiber $\dot{V}O_{2\text{max}}$
- Biopsy from vastus lateralis muscle
- Fiber $\dot{V}O_{2\text{max}}$ predicted from SDH activity (respiratory chain complex II)

### Whole-body $\dot{V}O_{2\text{max}}$
- Maximal incremental cycling test
  - $9.8 < 18 \text{ ml/kg/min not able to live independently}$
  - $79 \geq 80 \text{ ml/kg/min to win the Tour de France}$

### Oxygen Supply

**Marginal O$_2$ supply limitation**

**Whole-body $\dot{V}O_{2\text{max}}$: $90\%$ of muscle fiber $\dot{V}O_{2\text{max}}$**

Individual differences may relate to differences in O$_2$ supply

$\dot{V}O_{2\text{max}}$ is proportional to muscle fiber oxidative capacity in humans

Oxidative capacity is likely marginally limited by O$_2$ supply to the mitochondria
Introduction
Maximal oxygen uptake (\( \dot{V}O_{2\text{max}} \)) is used to quantify cardiorespiratory fitness\(^1\). \( \dot{V}O_{2\text{max}} \) is critical for endurance performance\(^1,2\), but also predicts loss of independence in elderly\(^3\) and mortality in chronic patients and healthy subjects\(^4\). Moreover, \( \dot{V}O_{2\text{max}} \) has been widely used to assess effects of training interventions. Although \( \dot{V}O_{2\text{max}} \) is generally considered a strong predictor of physical performance, factors limiting \( \dot{V}O_{2\text{max}} \) are still subject to controversy.

Ever since A.V. Hill et al.\(^5\) have postulated the concept of \( \dot{V}O_{2\text{max}} \) in the 1920s, exercise physiologists have debated factors that limit \( \dot{V}O_{2\text{max}} \). \( \dot{V}O_{2\text{max}} \) may be limited by any factor related to the \( O_2 \) pathway from atmosphere to the mitochondria. Hill et al.\(^5\) already speculated that \( \dot{V}O_{2\text{max}} \) is limited by the rate of \( O_2 \) supply by the cardiorespiratory system. Currently, the consensus is that \( \dot{V}O_{2\text{max}} \) is constrained by oxygen delivery to the mitochondria and not by mitochondria’s ability to consume oxygen\(^1,6-9\). This presumption is supported by the observation that \( \dot{V}O_{2\text{max}} \) and performance increase when \( O_2 \) supply is marginally enhanced by acute hyperoxia\(^10,11\) or blood reinfusion\(^12\). In addition, \( O_2 \) supply limitations are less pronounced in exercises with smaller muscle groups\(^13\).

Even though it is presumed that mitochondria’s ability to consume oxygen is not limiting \( \dot{V}O_{2\text{max}} \), mitochondrial volume density in locomotory muscles of animals is closely related to body-mass-specific \( \dot{V}O_{2\text{max}} \) (\( r^2 = 0.97 \))\(^14,15\). Not only mitochondrial density, but also capillary density, heart’s pumping capacity and lung diffusion capacity are scaled in proportion to \( \dot{V}O_{2\text{max}} \)\(^14,15\). These findings support the concept of “symmorphosis”, postulating that physiological determinants of the \( O_2 \) cascade that contribute to \( \dot{V}O_{2\text{max}} \) are proportional\(^16\). Also in humans, \( \dot{V}O_{2\text{max}} \) is closely related to mitochondrial volume density (\( r = 0.82 \))\(^17\) and oxidative enzyme activity (\( r = 0.72-0.79 \))\(^18,19\) in locomotory muscles. Humans, however, have been suggested not to conform as closely to symmorphosis as other animal species because of their ‘excess’ mitochondrial oxidative capacity\(^20,21\). This ‘excess’ oxidative capacity is, however, less substantial considering that human bipedal locomotion involves less active muscle mass compared to animal quadrupedal locomotion\(^21\). Therefore, comparative data suggest that mitochondrial oxidative capacity scales with \( \dot{V}O_{2\text{max}} \) of an organism even though the extent of excess oxidative capacity remains unclear.

At \( \dot{V}O_{2\text{max}} \), animals have been suggested to exploit only 60-80% of their mitochondrial oxidative capacity\(^22\). This estimation results from comparisons of in situ measured \( \dot{V}O_{2\text{max}} \) with electrically stimulated muscle and ex vivo mitochondrial oxidative capacity that is quantified from isolated mitochondria by polarographic measures of muscle mitochondrial respiration\(^22\). In humans, mitochondrial oxidative capacity has been quantified from isolated mitochondria or permeabilized muscle fibers obtained from biopsy samples\(^20,23\). Such experiments show that at \( \dot{V}O_{2\text{max}} \) during cycling exercise humans use only 64-73% of the mitochondrial oxidative capacity in active lower limb muscles\(^20,23\). The excess mitochondrial oxidative capacity is higher than what is expected based on the mitochondrial oxygen tension during maximal exercise (3.1 Torr based on myoglobin saturation)\(^24\) and the Michaelis constant for oxygen of the mitochondria (0.5 Torr)\(^25\). This discrepancy may be explained by mitochondrial inhibition, for instance by nitric oxide\(^26\), or by methodological issues related to isolation or permeabilization procedures\(^27\). Isolation of mitochondria alters mitochondrial morphology and function, which is presumably less of a problem in permeabilized fibers, but the incubation buffers differ from in vivo conditions\(^27\). An alternative method to determine mitochondrial oxidative capacity is quantitative histochemistry.
Maximal oxygen uptake is proportional to muscle fiber oxidative capacity.

of succinate dehydrogenase (SDH) activity, a citric acid cycle enzyme and complex II of the electron transport chain. SDH activity obtained from homogenized muscle tissue strongly relates to mitochondrial content (similar to other mitochondrial biomarkers e.g. citrate synthase, complex I and IV activity) as well as mitochondrial oxidative capacity in permeabilized fibers (similar to complex IV activity). Here we determined SDH activity by quantitative enzyme histochemistry as this method allows for muscle fiber specific comparison with other histochemical assays (e.g. myosin heavy chain typing, capillary density and muscle fiber size). This method has previously been calibrated showing that SDH activity is proportionally related to ex vivo $\dot{V}O_{2\text{max}}$ in intact single muscle fibers and myocardial trabeculae in hyperoxia. The predictive relationship has only been investigated for SDH activity, but illustrates that SDH activity provides a quantitative measure of mitochondrial oxidative capacity even though it may not be rate limiting for the maximal flux through the Krebs cycle. Therefore, within a muscle biopsy, mitochondrial oxidative capacity of individual muscle fibers can be estimated from SDH activity by quantitative enzyme histochemistry, avoiding isolation or permeabilization procedures.

The aim of this study was to quantify to what extent mitochondrial oxidative capacity predicted from SDH activity in biopsies of m. vastus lateralis differs from $\dot{V}O_{2\text{max}}$ attained during cycling exercise in chronic heart failure patients, healthy subjects and elite cyclists. We hypothesized that in humans SDH activity of m. vastus lateralis is related to cycling $\dot{V}O_{2\text{max}}$ and that humans exploit 60-80% of their mitochondrial oxidative capacity.

**Methods**

**Subjects**

48 subjects (20 healthy untrained subjects and 28 cyclists ranging from recreationally trained to professional) volunteered to participate in this study (Table 5.1). Cyclists competed at (inter)national level, except for 4 amateur cyclists. Previously published data from our laboratory of 14 chronic heart failure (CHF) patients and 6 healthy subjects were re-examined (see details below). Therefore, 68 subjects were included in our analysis. Prior to participation, experimental procedures and risks of the study were explained and all subjects provided written informed consent. The study was conducted according to the principles of the Declaration of Helsinki and was approved by the medical ethics committee of the VU medical center, Amsterdam, the Netherlands (NL43423.029.13 and NL49060.029.14).

**Whole-body $\dot{V}O_{2\text{max}}$ during cycling**

Subjects performed a maximal incremental exercise test on a cycle ergometer to voluntary exhaustion, despite verbal encouragement (respiratory exchange ratio of 1.20 ± 0.07). $\dot{V}O_{2\text{peak}}$ was calculated as the highest 30-s value achieved during the maximum-effort incremental test and is considered a valid index of $\dot{V}O_{2\text{max}}$ in subjects exercising to their limit of exercise tolerance. Respiratory data were analyzed breath-by-breath using open circuit spirometry and expressed at STPD (Cosmed Quark CPET, Cosmed S. R. L., Rome, Italy). Prior to every test, the gas analyzer and volume transducer were calibrated according to manufacturer's instructions. Subjects were instructed to avoid strenuous exercise and alcohol consumption within 24 hours before the test and caffeinated beverages and meals within 3 hours before the test.
Skeletal muscle biopsy

Biopsy samples were obtained from the m. vastus lateralis using a modified Bergström needle technique. The vastus lateralis muscle (part of the m. quadriceps femoris) acts as knee extensor and for cycling exercise is predominantly involved in the pushing phase and power-producing phase of the pedal cycle. Biopsy sites were locally anesthetized with a 2% lidocaine solution and an incision of <1 cm was made through the skin and fascia latae. The biopsy needle was inserted ~15 cm above the patella to a depth of approximately 4 cm. Biopsy samples were carefully removed and aligned according to their muscle fiber arrangement using a magnifying glass. Subsequently, samples were frozen in liquid nitrogen. After freezing, biopsy samples were placed in a cryostat and cut in 10 μm thick sections at -20°C. Sections were collected on polylysine-coated slides.

SDH histochemistry

SDH activity was determined using quantitative histochemistry, which has been described in detail elsewhere. In short, biopsy sections were incubated at 37°C in a medium consisting of 0.4 mM tetrabromo blue tetrazolium (Sigma, St. Louis, MO), 75 mM sodium succinate, 5 mM sodium azide, and 37.5 mM sodium phosphate buffer, pH 7.6. Biopsy sections were incubated for 20 minutes. Images were made with ×10 or ×20 objectives and absorbance was measured by microdensitometry with an interference filter at 660 nm using NIH ImageJ. Weighed average SDH activity was determined from spatially averaged SDH activity of over 40 randomly selected individual cells, including the subsarcolemma mitochondria. The randomly selected cells mirrored the distribution of high oxidative (i.e. type I) and low oxidative (i.e. type II) fibers within the biopsy section (i.e. SDH activity of individual fibers can be used to discriminate between fiber types similar to myofibrillar ATPase activity). Reproducibility of quantitative SDH histochemistry was assessed by comparison of absorbance values of two subsequent measurements of the same muscle tissue.

Temperature affects SDH activity in rats, mice and fish, but the effect of temperature on SDH activity has not been assessed in humans. In the present study, we assessed the relationship

Table 5.1. Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>CHF patients</th>
<th>Controls</th>
<th>Cyclists</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>26 †</td>
<td>28</td>
</tr>
<tr>
<td>Age (year)</td>
<td>61 ± 10</td>
<td>38 ± 11 *</td>
<td>25 ± 7 *</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>78.0 ± 10.2</td>
<td>90.5 ± 11.6 *</td>
<td>77.4 ± 8.1*</td>
</tr>
<tr>
<td>BMI</td>
<td>25.6 ± 2.7</td>
<td>26.9 ± 2.2</td>
<td>22.4 ± 1.9 *</td>
</tr>
<tr>
<td>Muscle mass (kg)</td>
<td>27.2 ± 4.7</td>
<td>33.9 ± 4.2 *</td>
<td>32.6 ± 2.3*</td>
</tr>
<tr>
<td>Muscle mass as percentage body mass (%)</td>
<td>34.7 ± 2.9</td>
<td>37.5 ± 2.8 *</td>
<td>42.3 ± 2.1 *</td>
</tr>
<tr>
<td>( VO_{2\text{max}} ) (mL·kg⁻¹·min⁻¹)</td>
<td>19.2 ± 4.5</td>
<td>40.1 ± 7.2 *</td>
<td>62.7 ± 7.9 *</td>
</tr>
<tr>
<td>SDH activity at 37°C (ΔA₆₆₀·μm⁻¹·s⁻¹)</td>
<td>8.6 ± 1.8 \times 10^6</td>
<td>13.7 ± 2.0 \times 10^6 *</td>
<td>17.9 ± 2.1 \times 10^6 *</td>
</tr>
<tr>
<td>SDH activity at T₉₉ (ΔA₆₆₀·μm⁻¹·s⁻¹)</td>
<td>8.4 ± 1.7 \times 10^6</td>
<td>16.8 ± 2.4 \times 10^6 *</td>
<td>21.9 ± 2.6 \times 10^6 *</td>
</tr>
<tr>
<td>Fiber ( VO_{2\text{max}} ) (nmol·mm⁻³·s⁻¹)</td>
<td>0.051 ± 0.01</td>
<td>0.101 ± 0.015 *</td>
<td>0.132 ± 0.015 *</td>
</tr>
</tbody>
</table>

Notes: Values are means±SD; n, no. of subjects. CHF, chronic heart failure; BMI, body mass index; \( VO_{2\text{max}} \), maximal oxygen uptake per kg body mass; SHD, succinate dehydrogenase; \( ΔA_660 \), change of absorbance at 660 nm; T₉₉, temperature quadriceps at maximal exercise. *p <0.01, significantly different from CHF patients (*) and control subjects (#). †Data for 6 controls and 14 CHF patients were obtained from Bekedam et al.
Maximal oxygen uptake is proportional to muscle fiber oxidative capacity. Between incubation temperature and SDH activity in human muscle tissue sections that were incubated at 32°C, 37°C and 42°C (Figure 5.3). Subsequently, the temperature quotient $Q_{10}$ - the increase in SDH activity with a 10°C increase in temperature - was determined.

**Temperature-adjusted SDH activity**

For comparison of SDH activity to whole-body $\dot{V}O_{2\max}$, SDH incubation temperature should equal quadriceps muscle temperature at maximal exercise. Previously, similar data on SDH activity and $\dot{V}O_{2\max}$ during cycling have been obtained in 14 chronic heart failure (CHF) patients and 6 healthy controls, but SDH activity was not adjusted to muscle temperature at maximal exercise. In the present study, SDH activity was adjusted to temperature using the $Q_{10}$ for human tissue and estimated quadriceps muscle temperature at maximal effort, being 36.5°C in CHF patients and ~41°C in healthy subjects and cyclists.

**Figure 5.1. Succinate dehydrogenase (SDH) staining using quantitative histochemistry.** A) healthy control subject. B) cyclist. Cross sections of the human vastus lateralis muscle incubated for SDH activity at 37°C. Section thickness 10 μm, incubation time 20 min. Scale bar 100 μm.

**Figure 5.2. Reproducibility of SDH activity measurements.** SDH activity was measured on two separate occasions without incubation (■) and after 20 min incubation in high oxidative fibers (▲) and low oxidative fibers (△) of the same muscle tissue. The solid line represents the line of identity. SDH activity of 120 muscle fibers in total was determined from absorbance measurements at 660 nm ($A_{660}$). Values are mean ± SD.

**Figure 5.3. Relationship between SDH activity and incubation temperature.** Average SDH activity of high and low oxidative fibers was measured after 20 min incubation (▲) at 32, 37 and 42°C. The SDH activity was significantly different between 32, 37 and 42 °C (*p < 0.001). SDH activity of 240 muscle fibers in total was determined from absorbance measurements at 660 nm. Values are mean ± SD.
Skeletal muscle fiber $\dot{V}O_{2\text{max}}$

Previously, it has been shown that paired determination of SDH activity by quantitative histochemistry and ex vivo maximum rate of oxygen consumption of intact single fibers under hyperoxic conditions are proportionally related in Xenopus (both determined at 20°C)\textsuperscript{30} and rat myocardial trabeculae (both determined at 38°C)\textsuperscript{29}. Therefore, skeletal muscle fiber $\dot{V}O_{2\text{max}}$ (in nmol mm\textsuperscript{-3} s\textsuperscript{-1}) was calculated as $6,000 \times$ the temperature-adjusted SDH staining rate (in change of absorbance at 660 nm per μm section thickness per s incubation time) as described previously\textsuperscript{29,30}.

Mitochondrial oxidative capacity predicted from SDH activity

Oxidative capacity of mitochondria was predicted from temperature-adjusted SDH activity, total skeletal muscle mass (see details below) and body mass (Equation 5.1). This prediction involves the following assumptions: 1) that all oxygen at $\dot{V}O_{2\text{max}}$ is consumed by skeletal muscle mitochondria, 2) that all mitochondria are active at their $\dot{V}O_{2\text{max}}$ during maximal cycling exercise and 3) that SDH activity of the vastus lateralis locomotor muscle represents SDH activity of whole body musculature. As these assumptions can be debated (see details below), another prediction of mitochondrial oxidative capacity is made that also accounts for 1) differences in SDH activity between arm and leg muscles, 2) active skeletal muscle mass during maximal cycling exercise and 3) oxygen consumption of other organs (see details below).

\begin{equation}
\text{Mitochondrial oxidative capacity} = (6000 \cdot \text{SDH} \cdot V_m \cdot \delta^{-1} \cdot 60) \cdot M_m \cdot M_b^{-1}
\end{equation}

where mitochondrial oxidative capacity is calculated at STPD (in mL·kg\textsuperscript{-1}·min\textsuperscript{-1}), SDH is weighed succinate dehydrogenase activity of m. vastus lateralis adjusted for temperature (in ΔA\textsubscript{660}·μm\textsuperscript{-1}·s\textsuperscript{-1}), $V_m$ is molar volume of oxygen at STPD (22.4 L·mol\textsuperscript{-1}), $\delta$ is muscle density (1.04 kg·L\textsuperscript{-1}), $M_m$ is skeletal muscle mass (kg) and $M_b$ is body mass (kg).

Skeletal muscle mass

Total skeletal muscle mass (including respiratory muscles) was estimated from an anthropometric regression model that has been shown to predict total skeletal mass obtained from whole-body magnetic resonance imaging in 244 nonobese subjects\textsuperscript{40}:

\begin{equation}
\text{Skeletal muscle mass (kg)} = 0.224 \cdot M_b + 7.80 \cdot Ht - 0.098 \cdot \text{age} + 6.6 \cdot \text{sex} + \text{ethnicity} - 3.3
\end{equation}

where $M_b$ is body mass (kg), $Ht$ is height (m), sex = 1 for males or 0 for female, ethnicity = -1.2 for Asian (one CHF patient) and 0 for Caucasian.

SDH activity of skeletal muscle

In animals, mean mitochondrial volume density of whole-body skeletal musculature is not significantly different from mitochondrial volume density of the hind limb musculature\textsuperscript{21}. In
human, however, mitochondrial volume density of arm and leg musculature may differ due to bipedal locomotion. In untrained subjects SDH activity of the deltoid muscle was 80% of the SDH activity in m. vastus lateralis, whereas in leg-trained subjects this was 65%. Therefore we assume that SDH activity of arm musculature is 80% of m. vastus lateralis SDH activity in healthy controls and CHF patients and 65% in cyclists. Here, arm muscle mass accounts for 20% of total skeletal muscle mass (i.e. ~6kg).

**Active skeletal muscle during cycling exercise**
During maximal leg cycling exercise, mitochondria in the arm musculature are likely not active at their \( \dot{V}O_{2\text{max}} \). In healthy subjects, oxygen uptake of the arms during leg pedaling at maximal effort (26.1 mL·kg\(^{-1}\)·min\(^{-1}\)) yielded approximately 35% of \( \dot{V}O_{2\text{max}} \) of the arms attained during arm cranking (77.8 mL·kg\(^{-1}\)·min\(^{-1}\))\(^{43} \). Therefore, we assume that during maximal leg cycling exercise mitochondria in the arm musculature are active at 35% of their \( \dot{V}O_{2\text{max}} \).

**Oxygen consumption of other organs**
At maximal effort, oxygen consumption by the brain was assumed to be similar to resting conditions, approximating 20% of measured oxygen consumption in rest (~1 mL·kg\(^{-1}\)·min\(^{-1}\); cf. Ref\(^{44} \)). Maximal myocardial oxygen consumption has been estimated to be 0.45 mM·s\(^{-1}\)\(^{45,46} \), that is 670 mL·kg\(^{-1}\)·min\(^{-1}\) at core temperature during maximal exercise and ~3 mL·kg\(^{-1}\)·min\(^{-1}\) using an average heart mass of 380 g in males and 330 g in females\(^{47} \). Note that oxygen consumption of other organs such as the liver, kidneys, and digestive system is neglected but is assumed to be less than in resting conditions (< 2 mL·kg\(^{-1}\)·min\(^{-1}\); cf. Ref \(^{44} \)).

\[
\text{Mitochondrial oxidative capacity} = (6000 \cdot SDH \cdot V_m \cdot 8^{-1} \cdot 60) \cdot M_m \cdot M_b^{-1} \]
\[
+ (0.2 \cdot \dot{V}O_{2\text{rest}} + \dot{V}O_{2\text{max,heart}} \cdot M_h \cdot M_b^{-1}) \quad (5.3)
\]

where \( \dot{V}O_{2\text{rest}} \) is oxygen consumption at rest, \( \dot{V}O_{2\text{max,heart}} \) is 670 mL·kg\(^{-1}\)·min\(^{-1}\), \( M_h \) is heart mass (330 g in females, 380 g in males). SDH = 1/5 \times 0.35 \times (SDH_{arm}/SDH_{leg}) \times SDH_{VL} + 4/5 \times SDH_{VL}^3, that is SDH activity of arm musculature is not fully active during maximal cycling exercise (35%) and is lower than leg SDH activity (65% in cyclists, 80% in controls and CHF patients) and accounts for ~20% of whole-body skeletal muscle mass, with SDH_{VL} is SDH activity determined from biopsy samples of the m. vastus lateralis.

**Statistics**
All data are presented as individual values or as mean±SD, unless otherwise indicated. Differences between groups were assessed by one-way ANOVAs (between factor group: CHF patients, controls, cyclists). Differences in SDH activity with incubation temperature were assessed by repeated measures ANOVA (within factor temperature: 32, 37 and 42°C). Post hoc comparisons with Bonferroni correction were performed to detect differences between groups and temperatures. The relationships between SDH-activity and whole-body \( \dot{V}O_{2\text{max}} \), SDH-activity and \( \dot{V}O_{2\text{max}} \) normalized for skeletal muscle mass and between mitochondrial oxidative capacity and whole-body \( \dot{V}O_{2\text{max}} \) were assessed by linear regressions. To check whether regression lines differed from the line of identity or regression line from literature, differences in
slope and intercept were tested by confidence intervals of the regression coefficients. Differences were considered to be significant if \( p < 0.05 \).

**Results**

Table 5.1 summarizes average age, body mass, body mass index (BMI), calculated skeletal muscle mass, \( \dot{V}O_{2\text{max}} \), SDH activity at 37°C, SDH activity adjusted for temperature, and calculated muscle fiber \( \dot{V}O_{2\text{max}} \) of CHF patients, healthy controls and cyclists. Even though skeletal muscle mass as percentage body mass was lower in controls compared to cyclists, the absolute skeletal muscle mass was similar in controls and cyclists, which was likely due to a higher body mass and BMI in the control group.

**Histochemical method for SDH activity**

Figure 5.1 shows muscle fiber cross-sections of m. vastus lateralis that were incubated for SDH using quantitative enzyme histochemistry. SDH activity was significantly different between CHF patients, controls and cyclists.

Reproducibility was determined from two measurements of mean SDH absorbance without incubation and in high oxidative (i.e. type I) muscle fibers and low oxidative (i.e. type II) muscle fibers\(^ {36} \) after 20 minutes of incubation (Figure 5.2). It is concluded that average SDH absorbance values were similar for both measurements.

The relationship between SDH absorbance and incubation temperature in human muscle tissue is shown in Figure 5.3. Mean absorbance values of high and low oxidative fibers were significantly different between 32°C, 37°C and 42°C (\( p<0.001 \)). At 42°C, SDH absorbance is 1.76 times higher compared to that at 32°C. Hence, SDH enzyme activity of all subjects was adjusted for muscle temperature using a \( Q_{10} \) of 1.76.

**Relationship between SDH activity and whole-body \( \dot{V}O_{2\text{max}} \)**

Figure 5.4 shows that SDH activity adjusted for muscle temperature is closely related to whole-body \( \dot{V}O_{2\text{max}} \) normalized to body mass (\( r^2 = 0.81, \ p<0.001 \)) and whole-body \( \dot{V}O_{2\text{max}} \) normalized to skeletal muscle mass (\( r^2 = 0.83, \ p<0.001 \)). Without adjustment for muscle temperature, correlation coefficients are slightly lower for \( \dot{V}O_{2\text{max}} \) normalized to body mass (\( r^2 = 0.79, \ p<0.001 \)) and \( \dot{V}O_{2\text{max}} \) normalized to skeletal muscle mass (\( r^2 = 0.80, \ p<0.001 \)). These results indicate that SDH activity is proportional to the \( \dot{V}O_{2\text{max}} \) in humans, irrespective of training status (\( \dot{V}O_{2\text{max}} \) ranging from 9.8 to 79.0 mL·kg\(^{-1}\)·min\(^{-1}\)). Normalization of \( \dot{V}O_{2\text{max}} \) to skeletal muscle mass allows direct comparison with ex vivo measurements from intact animal myocytes in hyperoxic Tyrode solution (represented by the solid line; see methods). In humans, the relationship between temperature-adjusted SDH activity and \( \dot{V}O_{2\text{max}} \) differed significantly from the predicted relationship from ex vivo measurements of animal fibers in hyperoxic conditions (slope < 7.6 × 10\(^6\), CI = [5.3 × 10\(^6\) - 6.7 × 10\(^6\)], \( p<0.05 \); intercept = 0, \( p>0.05 \))\(^ {29,30} \). Therefore, at \( \dot{V}O_{2\text{max}} \), the oxidative enzyme activity is not fully exploited. Note that \( \dot{V}O_{2\text{max}} \) normalized to skeletal muscle mass may even provide an overestimation of the true value, because it also includes oxygen uptake by tissues other than skeletal muscles (Figure 5.4B).
Maximal oxygen uptake is proportional to muscle fiber oxidative capacity.

Figure 5.4. Relationship between SDH activity and whole-body maximal oxygen uptake ($\dot{V}O_{2\text{max}}$) obtained during incremental cycling exercise. A) $\dot{V}O_{2\text{max}}$ normalized to body mass; B) $\dot{V}O_{2\text{max}}$ normalized to skeletal muscle mass. Data is displayed for CHF patients (▲), controls (○) and cyclists (■) and the group averages (large open symbols). In B, the solid line represents the relationship between $\dot{V}O_{2\text{max}}$ and SDH activity from ex vivo measurements of intact animal fibers in hyperoxia (see text). The glycogen-depleted biopsy reported by Bekedam et al. was excluded.

$\Delta A_{660} \cdot \mu m^{-1} \cdot s^{-1} \times 10^{-5}$

$\dot{V}O_{2\text{max}} \text{ (ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})$

$\dot{V}O_{2\text{max}} \text{ (ml} \cdot \text{kg_{muscle}}^{-1} \cdot \text{min}^{-1})$
Relationship between mitochondrial oxidative capacity and whole-body $\dot{V}O_{2\text{max}}$

The relationship between body-mass specific $\dot{V}O_{2\text{max}}$ during leg cycling and mitochondrial oxidative capacity from temperature-adjusted SDH activity and total skeletal muscle mass is shown in Figure 5.5A. In CHF patients, controls, and cyclists, mitochondrial oxidative capacity is proportionally related to $\dot{V}O_{2\text{max}}$ during cycling ($r^2 = 0.88-0.89$, $p<0.001$). The relationship between $\dot{V}O_{2\text{max}}$ and mitochondrial capacity based on Equation 5.1 significantly differed from the line of identity (slope < 1, CI = [0.72 - 0.86], $p<0.05$; intercept = 0, $p>0.05$) and indicates that subjects do not fully use (-85%) the oxidative enzyme capacity at $\dot{V}O_{2\text{max}}$ during cycling exercise. Note that in Figure 5.5A it is assumed that all oxygen at $\dot{V}O_{2\text{max}}$ is consumed by skeletal muscle mitochondria, that all mitochondria are active at their $\dot{V}O_{2\text{max}}$ during maximal cycling exercise and that SDH activity of m. vastus lateralis represents SDH activity of whole body musculature. Figure 5.5B displays the relationship between $\dot{V}O_{2\text{max}}$ and mitochondrial oxidative capacity based on Equation 5.3. This prediction suggests that $\dot{V}O_{2\text{max}}$ during cycling is 90±14% of the mitochondrial oxidative capacity. Even though the relationship in Figure 5.5B tended to differ from the line of identity, the slope was not significantly different from 1 (slope = 1, CI = [0.84 - 1.00], $p>0.05$; intercept = 0, $p>0.05$). Note that there is still considerable individual variation between mitochondrial oxidative capacity (Equation 5.3) and measured $\dot{V}O_{2\text{max}}$ during cycling (CV = 15.2%), which is also illustrated by lower correlation coefficients in separate subgroups (CHF patients $r=0.47$, $p=0.107$; healthy controls $r=0.61$, $p<0.001$, cyclists $r=0.67$, $p<0.001$). Mitochondrial oxidative overcapacity did not differ between CHF patients, controls, and cyclists ($p=0.108$).

Discussion

The present study showed that SDH activity and mitochondrial oxidative capacity predicted from SDH activity are related to $\dot{V}O_{2\text{max}}$ measured during cycling ($r^2 = 0.81$, $p<0.001$ and $r^2 = 0.89$, $p<0.001$, respectively) across chronic heart failure patients, healthy untrained controls and cyclists ($\dot{V}O_{2\text{max}}$ ranging from 9.8 to 79.0 mL·kg$^{-1}$·min$^{-1}$). During cycling exercise, $\dot{V}O_{2\text{max}}$ was on average 90±14% of the mitochondrial oxidative capacity, indicating that humans do not fully exploit their oxidative enzyme capacity.

Symmorphosis in humans: mitochondrial oxidative capacity is proportionally related to $\dot{V}O_{2\text{max}}$

Our results confirmed that $\dot{V}O_{2\text{max}}$ scales proportionally with SDH activity ($r^2=0.81$) and mitochondrial oxidative capacity ($r^2=0.89$) across heart failure patients, healthy controls, and cyclists. These results are in line with previous cross-sectional studies that have shown that in heterogeneous healthy populations the body-mass-specific $\dot{V}O_{2\text{max}}$ is related to SDH activity ($r=0.79$)$^{19}$ and mitochondrial volume density ($r=0.82$)$^{17}$. Note that in more homogeneous populations, correlations between SDH activity and $\dot{V}O_{2\text{max}}$ were found to be substantially lower ($r=0.23$)$^{48}$. Our subgroups also showed lower correlations, which in combination with our coefficient of variation indicated considerable individual differences in mitochondrial oxidative overcapacity (see Unexplained variance of mitochondrial oxidative capacity and $\dot{V}O_{2\text{max}}$). However, mitochondrial oxidative overcapacity did not differ between CHF patients, controls, and cyclists, which supports existence of symmorphosis in humans. CHF impairs $\dot{V}O_{2\text{max}}$ and
Maximal oxygen uptake is proportional to muscle fiber oxidative capacity.

Figure 5.5. Relationship between mitochondrial oxidative capacity predicted from SDH activity and skeletal muscle mass and the measured VO$_{2\text{max}}$ during incremental cycling exercise. A) mitochondrial oxidative capacity based on Equation 5.1; B) mitochondrial oxidative capacity based on Equation 5.3. Data is displayed for CHF patients (▲), controls (○) and cyclists (■) and the group averages are shown (large open symbols). In A, mitochondrial oxidative capacity is predicted based on the assumption that all oxygen is consumed by skeletal muscle mitochondria and that SDH activity of the vastus lateralis locomotory muscle represents SDH activity of whole body musculature. In B, mitochondrial oxidative capacity takes into account 1) the differences in SDH activity between arm and leg muscles, 2) active skeletal muscle mass at maximal cycling exercise and 3) oxygen consumption of other organs (see text). The glycogen-depleted biopsy reported by Bekedam et al. was excluded.
mitochondrial oxidative capacity due to the limited cardiac output\textsuperscript{49}, as a result of reduced $O_2$ flux to the mitochondria from impaired red blood cell (RBC) flux and velocity, reduced percentage of flowing capillaries supporting RBC flux during rest and exercise, impaired $\dot{Q}_O_2/V_0_2\text{max}$ matching, low microvascular $P_0_2$ and high fractional oxygen extraction\textsuperscript{49–52}. Our results indicate that these impairments in both $V_0_2\text{max}$ and mitochondrial oxidative capacity are proportional in CHF patients and therefore support existence of symmorphosis in humans. Also trained cyclists did conform to the proportional relationship between $\dot{V}_0_2\text{max}$ and mitochondrial oxidative capacity. In humans, however, individual increases in $\dot{V}_0_2\text{max}$ induced by endurance training are generally not proportional to changes in oxidative enzyme activity of the skeletal muscle involved in endurance training (cf. Ref\textsuperscript{53,54}). This discrepancy may be explained by the time course of (acute) training adaptations. For instance, $\dot{V}_0_2\text{max}$ tends to increase by 15-30% during the first 2-3 months of endurance training, while 40-50% increases in $V_0_2\text{max}$ may occur over 9-24 months of training\textsuperscript{54}. However, concomitant changes in the citric acid cycle and respiratory chain enzymes are much faster, displaying half-lives in the order of 1-3 weeks\textsuperscript{53,54}. Therefore, increases in $\dot{V}_0_2\text{max}$ and mitochondrial capacity are likely disproportional during acute training adaptations (<9 months), but may become proportional with chronic training adaptations (>9 months), such as in our trained cyclists with an extensive training history (120 months on average). Also note that trainability of $\dot{V}_0_2\text{max}$ may markedly vary between subjects and is strongly related to heritability\textsuperscript{55}. Thus, mitochondrial oxidative capacity scales with $V_0_2\text{max}$ and largely accounts for differences in body-mass-specific $\dot{V}_0_2\text{max}$ in this heterogeneous population.

Animals conform to the concept of symmorphosis assuming that all parts of the $O_2$ pathway that contribute to $\dot{V}_0_2\text{max}$ are designed proportionally\textsuperscript{16}. Across sedentary and athletic animal species, it was shown that mitochondrial volume density is proportional to body-mass-specific $\dot{V}_0_2\text{max}$ explaining 97% of its variance\textsuperscript{14}. Athletic animals presented a 2.5-fold greater body-mass-specific $\dot{V}_0_2\text{max}$, which was matched by a 2.5-fold larger mitochondrial volume density in their muscles\textsuperscript{14}. Therefore, the maximal rate of $O_2$ consumption by skeletal muscle has been considered to be invariant across animals (4-5 mL$O_2$-mL$^{-1}$ mitochondria-$\text{min}^{-1}$)\textsuperscript{14}. Our cross-sectional observation suggests that also in a heterogeneous population of human subjects, $\dot{V}_0_2\text{max}$ was matched by mitochondrial oxidative capacity: both $\dot{V}_0_2\text{max}$ and SDH or predicted oxidative capacity were ~1.5-fold greater in cyclists compared to controls, ~3-fold greater in cyclists compared to CHF patients and ~2-fold greater in controls compared to CHF patients. Our results contradict previous findings\textsuperscript{56}, which challenged the existence of symmorphosis in humans and suggested that the relationship between $\dot{V}_0_2\text{max}$ during cycling and mitochondrial oxidative capacity (from permeabilized fibers) varied with exercise training status. In this previous study\textsuperscript{56}, measurements in untrained subjects did conform to the concept of symmorphosis, while trained subjects did not. However, this conclusion is based on data of only 4 trained subjects and on a smaller observed range of $\dot{V}_0_2\text{max}$ (31-66 mL·kg$^{-1}$·min$^{-1}$). In the present study, mitochondrial oxidative capacity is proportional to $\dot{V}_0_2\text{max}$ explaining 81-89% of its variance when observing a larger range of $\dot{V}_0_2\text{max}$ (9.8-79.0 mL·kg$^{-1}$·min$^{-1}$). Hence, similar to animals, a higher oxygen uptake demand is met by increasing mitochondrial enzyme activity, while oxidative capacity per mitochondria remains invariant across human individuals as well. Note that the observed marginal mitochondrial oxidative overcapacity does not challenge the concept of symmorphosis, as small excess in mitochondrial capacity at maximal exercise may serve as a safety factor\textsuperscript{16}. Thus, our data support the concept of symmorphosis in a heterogeneous population of human subjects.
indicating that mitochondrial oxidative capacity is proportional to $\dot{V}O_{2\text{max}}$.

**Mitochondrial oxidative overcapacity estimated from SDH activity**

Our subjects used on average 90% of their mitochondrial capacity during maximal cycling exercise, suggesting that mitochondrial oxidative capacity exceeded O$_2$ use by ~11%. Based on the relationship between mitochondrial respiration and cellular PO$_2$, one could calculate at what percentage of their maximal capacity the mitochondria operate during dynamic exercise. Assuming first order Michaelis Menten kinetics for mitochondrial oxygen consumption\textsuperscript{25}, the maximum obtainable $\dot{V}O_2$ relative to the theoretical $\dot{V}O_{2\text{max}}$ (under hyperoxic conditions) is:

$$\frac{\dot{V}O_2}{\dot{V}O_{2\text{max}}} = \frac{1}{1 + \left(\frac{K_m}{P_{O_2}}\right)} \quad (5.4)$$

where $K_m$ is the Michaelis constant for oxygen of the mitochondria (0.5 Torr)\textsuperscript{25} and $P_{O_2}$ is the mitochondrial oxygen tension (3.1 Torr during maximal cycling exercise estimated from myoglobin saturation and the $P_{50}$ of myoglobin for oxygen)\textsuperscript{24}. Following Equation 5.4, $\frac{\dot{V}O_2}{\dot{V}O_{2\text{max}}} = 0.86$. Therefore, during maximal cycling exercise, the mitochondria should theoretically operate at ~86% of their maximal oxidative capacity, which agrees well with reported values reported in the present study. Further mitochondrial inhibition (e.g. by NO) is therefore not necessary to explain the reported oxidative overcapacity. It should be noted that Equation 5.4 assumes homogeneous oxygen tension inside the muscle cells, which is physiologically impossible (for further details see Refs\textsuperscript{57,58}). Note that the use of mitochondrial oxidative capacity (90 ± 14%) is similar to the exploited mitochondrial oxidative capacity (90 ± 15%) when based on the assumption that skeletal muscle mass accounts for ~90% of the total oxygen consumption at maximal exercise\textsuperscript{14} (i.e. instead of estimating oxygen consumption by the heart and brain). Moreover, considering that $\dot{V}O_{2\text{max}}$ is 5-10% higher during running versus leg cycling\textsuperscript{59,60} and with arm musculature being fully active, the exploited mitochondrial oxidative capacity during running is estimated to be comparable to leg cycling (86 ± 13% and 90 ± 14% respectively). Therefore, mitochondrial oxidative overcapacity of the present study appears to be robust and agrees well with mitochondrial overcapacity calculated from cellular oxygen tension.

**Mitochondrial oxidative overcapacity with enhanced oxygen supply**

Our results indicate that circumventing limitations to $\dot{V}O_{2\text{max}}$ can increase $\dot{V}O_{2\text{max}}$ on average by ~11% at most. What happens to maximal oxygen uptake when limitations of O$_2$ supply are reduced? As the O$_2$ pathway from atmosphere to mitochondria is an in-series system, it is evident that every step significantly impacts $\dot{V}O_{2\text{max}}$. Acute interventions that enhance O$_2$ supply have shown to increase $\dot{V}O_{2\text{max}}$. Hyperoxia, for instance, by increasing inspired O$_2$ fraction ($F_{O_2}$) increased whole body $\dot{V}O_{2\text{max}}$ with 2-5%\textsuperscript{11} and leg $\dot{V}O_{2\text{max}}$ with 8%\textsuperscript{10} by enhancing oxygen diffusion at the lungs and from microvessels to mitochondria\textsuperscript{10,11}. Blood reinfusion of 900-1350 mL elevated oxygen carrying capacity of the blood and thereby increased $\dot{V}O_{2\text{max}}$ by 4-9%\textsuperscript{12}. Moreover, $\dot{V}O_{2\text{max}}$ of one leg derived from blood flow and arterio-venous O$_2$ differences measurements was 5-14% higher in one-legged cycling compared to two-legged cycling exercise, because of the higher leg blood flow in one-legged exercise\textsuperscript{61}. The lower leg blood flow
during maximal exercise with two legs compared to one leg is likely due to vasoconstriction
of active muscles that is required to avoid hypotension and increase transit time at the muscle
capillaries. The theoretical model of Wagner, linking lungs, circulation and muscles to \( \dot{V}_O^{2max} \),
predicts that an isolated 20% increase in ventilation, \( F_O^2 \), lung diffusion capacity, muscle \( O_2 \)
diffusion conductance, hemoglobin concentration or blood flow will increase \( \dot{V}_O^{2max} \) by only
1.3-5%. Enhancement of oxygen supply can also be studied together with noninvasive
assessment of post-exercise PCr recovery kinetics with 31P-MRS, as PCr recovery kinetics
have been used to estimate mitochondrial oxidative capacity. Previous studies have shown that
enhanced oxygen supply with hyperoxia and reactive hyperemia induced by cuff occlusion
improved both PCr recovery kinetics and estimated mitochondrial respiration rate, however, the
quantitative interpretation of muscle mitochondrial capacity remains difficult (for review see).
Therefore, evidence indicates that increases in whole-body \( \dot{V}_O^{2max} \) due to enhanced \( O_2 \) supply are
generally within a 10% range and agree well with our findings of excess mitochondrial capacity.

Unexplained variance of mitochondrial oxidative capacity and \( \dot{V}_O^{2max} \)

Even though \( \dot{V}_O^{2max} \) and mitochondrial oxidative capacity are related, there are still considerable
individual differences in mitochondrial oxidative overcapacity. These individual differences may
arise from methodological errors in estimating mitochondrial oxidative capacity. For instance,
the location of biopsy sampling affects fiber type distribution by 2-3% and since SDH activity
is closely related to fiber type, this could also affect weighed SDH activity. Nonetheless, we
showed that SDH activity from quantitative histochemistry is highly reproducible (Figure 5.2).
We could not account for individual variation in muscle temperature, which in healthy controls is
likely to be 1-1.5% and -3.5% in CHF patients. Even though skeletal muscle mass was
estimated from an anthropometric model, the estimated skeletal mass as percentage of body
mass (34.7% in CHF patients, 37.5% in controls and 42.3% in cyclists) was in agreement
with skeletal muscle mass derived from MRI measurements in subgroups of 468 subjects
with similar age (33.8%, 39.1% and 42.3% respectively). Errors in oxygen consumption by
the brain and heart (e.g. due to differences in heart mass and mitochondrial volume density
between CHF patients, controls and cyclists) may have attributed to the unexplained variance
between measured \( \dot{V}_O^{2max} \) and mitochondrial oxidative capacity. However, note that maximal
oxygen consumption of the heart (~3 mL·kg\(^{-1}\)·min\(^{-1}\)) and oxygen consumption of the brain (~1
mL·kg\(^{-1}\)·min\(^{-1}\)) only marginally contributed to the mitochondrial oxidative capacity in control
subjects and cyclists. Moreover, we could not account for individual variation in SDH activity
differences between arm and leg musculature and submaximally active mitochondria in arm
musculature during leg cycling exercise. \( \dot{V}_O^{2max} \) has shown to be obtained reproducibly within
our laboratory (ICC=0.98, p<0.001; unpublished results) and all subjects terminated exercise
due to voluntary exhaustion. Although \( \dot{V}_O^{2peak} \) attained during the maximum-effort incremental
test to voluntary exhaustion is considered a valid index of \( \dot{V}_O^{2max} \) that is not different from
\( \dot{V}_O^{2peak} \) attained during supra-maximal exercise, small differences in maximal effort may have
contributed to individual differences in mitochondrial oxidative overcapacity, as underestimates
of \( \dot{V}_O^{2max} \) may have occurred in some subjects. Taken together, unexplained variance between
mitochondrial oxidative capacity and \( \dot{V}_O^{2max} \) is only 11% (\( r^2 = 0.89 \)) in the present study, and
therefore it is likely that possible effects of methodological errors are (partially) cancelled out in
our group of human subjects.
A portion of unexplained variance between mitochondrial capacity and \( \dot{V}O_2_{\text{max}} \) may also be attributed to physiological differences between individuals. These differences may arise from limitations to the convective \( O_2 \) supply, for instance arterial and venous saturation, systemic hematocrit, hemoglobin content, cardiac output and end-diastolic heart volume\(^1\,^2\,^8\). Also, limitations to diffusive \( O_2 \) supply may explain individual differences in mitochondrial oxidative overcapacity at maximal exercise. Oxygen diffusion at the muscle level is determined by oxygen transport from sarcolemma to mitochondria by myoglobin and by blood-myocyte \( O_2 \) flux set by the oxygen tension gradient between the capillaries and sarcolemma, which depends on (changes in) red blood cell (RBC) velocity and capillary hematocrit (i.e. RBC flux), on capillary density and the proportion of flowing capillaries\(^1\,^8\,^49\,^71\). Moreover, differences in mitochondrial oxidative overcapacity may be investigated in light of individual fiber type distribution as the vasomotor control may be modulated as a function of fiber type and/or oxidative capacity of the muscle fibers\(^51\,^72\), displaying better \( \dot{Q}O_2 / \dot{V}O_2 \) matching and thus higher microvascular \( PO_2 \) in high versus low oxidative muscle fibers or muscle parts\(^51\). Furthermore, individual differences may arise from the effectiveness in energy distribution within the muscle cells, which depends on metabolite facilitated diffusion (i.e. oxygen transport by the oxy-deoxy myoglobin shuttle and ATP diffusion by the creatine kinase shuttle) and on membrane potential conduction via the mitochondrial reticulum\(^73\). Since SDH activity using quantitative enzyme histochemistry is determined in individual muscle fibers, it can be related to other fiber specific variables (e.g. myosin heavy chain type and muscle fiber size, capillary density, diffusion distance or myoglobin concentration). Future studies should investigate whether differences in determinants of convective and diffusive \( O_2 \) supply or energy distribution may also account for part of the unexplained variance in the relationship between mitochondrial oxidative capacity and \( \dot{V}O_2_{\text{max}} \), i.e. explain differences between individual subjects with similar \( \dot{V}O_2_{\text{max}} \) but different SDH activity.

Use of mitochondrial oxidative overcapacity

Even though oxygen use during maximal cycling exercise is on average ~90% of the oxidative capacity, a greater proportion of the mitochondrial oxidative capacity may be used when oxygen supply is not limiting. For instance, during exercise with smaller muscle groups (without cardiac output limitation), blood flow heterogeneity may facilitate matching of oxygen supply to oxygen demand in the active muscle groups, while this does not come at the expense of \( \dot{Q}O_2 / \dot{V}O_2 \) matching in another region\(^51\,^72\). Also at submaximal exercise intensities, a higher mitochondrial oxidative capacity may enable mitochondria to operate at a lower percentage of their maximal oxidative rate for a given oxygen consumption. Consequently, following Michealis Menten kinetics of mitochondria, \( \dot{V}O_2 \) kinetics will be faster and result in glycogen sparing and faster adaptations of steady state during submaximal exercise. In addition, lower substrate concentrations are required and mitochondria are less likely to become hypoxic, thereby reducing damage from oxidative stress induced by hypoxia. Moreover, hypoxia in the muscle fibers will likely occur at higher exercise intensity and therefore could increase intensity at the lactate or ventilatory threshold (i.e. intensity at which aerobic ATP resynthesis can no longer match ATP use in the working muscles), which is an important determinant of endurance performance in subjects with similar \( \dot{V}O_2_{\text{max}} \). Therefore, with a higher mitochondrial oxidative capacity, a higher rate of ATP production may be sustained during endurance performance\(^74\). As mitochondria conform to Michaelis Menten kinetics, it is highly unlikely that mitochondria operate at 100% of their maximal oxidative
capacity, as this requires very high intracellular $\text{PO}_2$ (that would likely require an increase in the $P_{50}$ of the blood and enhanced diffusion capacity in the lungs) and extremely high substrate concentrations (that potentially could have an osmotic effect similar to lactate accumulation and thereby increase blood viscosity).

**Conclusions**

Human maximal oxygen uptake attained during cycling exercise is related to mitochondrial oxidative capacity predicted from skeletal muscle succinate dehydrogenase activity. Measured whole-body $\dot{V}O_2\text{max}$ is ~90% of the mitochondrial oxidative capacity, which can be explained by limited oxygen supply to muscle mitochondria.
References


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Chapter 5A

Maximal oxygen uptake is proportional to muscle fiber oxidative capacity