

Symmorphosis and skeletal muscle $\dot{V}_{O_2\max}$: *in vivo* and *in vitro* measures reveal differing constraints in the exercise-trained and untrained human

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Key points

- The concept of symmorphosis predicts that the capacity of each step of the oxygen cascade is attuned to the task demanded of it during aerobic exercise at maximal rates of oxygen consumption ($\dot{V}_{O_2\max}$) such that no single process is limiting or in excess at $\dot{V}_{O_2\max}$.
- The present study challenges the applicability of this concept to humans by revealing clear, albeit very different, limitations and excesses in oxygen supply and consumption among untrained and endurance-trained humans.
- Among untrained individuals, $\dot{V}_{O_2\max}$ is limited by the capacity of the mitochondria to consume oxygen, despite an excess of oxygen supply, whereas, among trained individuals, $\dot{V}_{O_2\max}$ is limited by the supply of oxygen to the mitochondria, despite an excess of mitochondrial respiratory capacity.

Abstract The concept of symmorphosis postulates a matching of structural capacity to functional demand within a defined physiological system, regardless of endurance exercise training status. Whether this concept applies to oxygen (O_2) supply and demand during maximal skeletal muscle O_2 consumption ($\dot{V}_{O_2\max}$) in humans is unclear. Therefore, *in vitro* skeletal muscle mitochondrial $\dot{V}_{O_2\max}$ ($\dot{V}_{O_2\max}^{\text{Mito}}$, mitochondrial respiration of fibres biopsied from vastus lateralis) was compared with *in vivo* skeletal muscle $\dot{V}_{O_2\max}$ during single leg knee extensor exercise ($\dot{V}_{O_2\max}^{\text{KE}}$, direct Fick by femoral arterial and venous blood samples and Doppler ultrasound blood flow measurements) and whole-body $\dot{V}_{O_2\max}$ during cycling ($\dot{V}_{O_2\max}^{\text{Body}}$, indirect calorimetry) in 10 endurance exercise-trained and 10 untrained young males. In untrained subjects, during KE exercise, maximal O_2 supply ($\dot{Q}O_{2\max}^{\text{KE}}$) exceeded ($462 \pm 37 \text{ ml kg}^{-1} \text{ min}^{-1}$, $P < 0.05$) and $\dot{V}_{O_2\max}^{\text{KE}}$ matched ($340 \pm 22 \text{ ml kg}^{-1} \text{ min}^{-1}$, $P > 0.05$) $\dot{V}_{O_2\max}^{\text{Mito}}$ ($364 \pm 16 \text{ ml kg}^{-1} \text{ min}^{-1}$). Conversely, in trained subjects, both $\dot{Q}O_{2\max}^{\text{KE}}$ ($557 \pm 35 \text{ ml kg}^{-1} \text{ min}^{-1}$) and $\dot{V}_{O_2\max}^{\text{KE}}$ ($458 \pm 24 \text{ ml kg}^{-1} \text{ min}^{-1}$) fell far short of $\dot{V}_{O_2\max}^{\text{Mito}}$ ($743 \pm 35 \text{ ml kg}^{-1} \text{ min}^{-1}$, $P < 0.05$). Although $\dot{V}_{O_2\max}^{\text{Mito}}$ was related to $\dot{V}_{O_2\max}^{\text{KE}}$ ($r = 0.69$, $P < 0.05$) and $\dot{V}_{O_2\max}^{\text{Body}}$ ($r = 0.91$, $P < 0.05$) in untrained subjects, these variables were entirely unrelated in trained subjects. Therefore, in untrained subjects, $\dot{V}_{O_2\max}$ is limited by mitochondrial O_2 demand, with evidence of adequate O_2 supply, whereas, in trained subjects, an exercise training-induced mitochondrial reserve results in skeletal muscle $\dot{V}_{O_2\max}$ being markedly limited by O_2 supply. Taken together, these *in vivo* and *in vitro* measures reveal clearly differing limitations and excesses at $\dot{V}_{O_2\max}$ in

untrained and trained humans and challenge the concept of symmorphosis as it applies to O₂ supply and demand in humans.

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Abbreviations BIOPS, biopsy preservation fluid; Body, whole-body; KE, knee extensor; Mito, mitochondrial; $\dot{Q}O_{2\max}$, maximum specific oxygen uptake rate; $\dot{V}O_{2\max}$, maximal oxygen uptake; WR_{\max} , maximum work rate.

Introduction

Despite decades of research, it remains unclear which step, if any, along the O₂ cascade limits maximal oxygen uptake ($\dot{V}O_{2\max}$) (Levine, 2008; Wagner, 2008). Based on the Fick principle, $\dot{V}O_2$ is the product of O₂ supply ($\dot{Q}O_2$) and mitochondrial O₂ extraction (Richardson, 2003), and thus the steps of the O₂ cascade that potentially determine $\dot{V}O_{2\max}$ can be generalized into two components: O₂ supply to the skeletal muscle mitochondria by the cardiopulmonary system and O₂ demand by the skeletal muscle mitochondria. Proponents of the theory of symmorphosis have suggested that the capacity of each step of the O₂ cascade, with the exception of the lungs, is built in strict proportion to the task demanded of it at $\dot{V}O_{2\max}$, regardless of species (i.e. adaptive variation) or variation in body size (i.e. allometric variation), or training status (i.e. induced variation) (Weibel *et al.* 1991; Hoppeler & Weibel, 1998). Thus, neither the capacity for O₂ supply, nor the capacity for O₂ demand would be in excess or limiting at $\dot{V}O_{2\max}$. Although this concept applies well when considering variation in $\dot{V}O_{2\max}$ across many species (Hoppeler & Weibel, 1998), how well symmorphosis pertains to humans is unclear because studies in humans often indicate that O₂ supply or demand are either limiting or in excess at $\dot{V}O_{2\max}$ (Hoppeler & Weibel, 1998; Levine, 2008; Wagner, 2008).

Although many agree that a rate-limiting step probably exists along the O₂ cascade in humans, there is considerable disagreement regarding where the bottleneck lies because of the variety of prior findings. For example, several studies have provided convincing evidence that O₂ supply limits $\dot{V}O_{2\max}$ by documenting that the capacity of mitochondria to consume O₂ exceeds the ability of the cardiopulmonary system to supply O₂ (Margaria *et al.* 1972; Knight *et al.* 1993; Boushel *et al.* 2011). However, other studies have also made an equally convincing case for the concept that mitochondrial O₂ demand can limit $\dot{V}O_{2\max}$ by clearly illustrating that increasing O₂ supply with hyperoxia does not always augment $\dot{V}O_{2\max}$ (Roca *et al.* 1992; Cardús *et al.* 1998).

Although the source of disagreement, in terms of what limits $\dot{V}O_{2\max}$, remains unclear, it has been suggested that physical activity/exercise training may alter the

source of limitation (Wagner, 2008). The possibility that the limitation to $\dot{V}O_{2\max}$ may shift with endurance training directly conflicts with the concept of symmorphosis (Weibel *et al.* 1991; Hoppeler & Weibel, 1998). Nevertheless, it has been well-documented that, when humans undergo endurance training, the resulting gain in mitochondrial capacity far exceeds the accompanying increase in $\dot{V}O_{2\max}$ (Gollnick *et al.* 1973; Holloszy & Coyle, 1984). Thus, in contrast to the concept of symmorphosis, it is possible that exercise training may result in a mitochondrial reserve capacity that shifts the bottleneck at $\dot{V}O_{2\max}$ away from the mitochondria toward processes related to O₂ supply.

In the present study, we utilized a unique combination of *in vitro* and *in vivo* measures of oxygen supply and demand, in both untrained and endurance exercise-trained humans, aiming to determine whether O₂ supply and demand are consistently well-matched to $\dot{V}O_{2\max}$. Based on previous literature (Roca *et al.* 1992; Cardús *et al.* 1998), we hypothesized that, although O₂ supply and demand would be relatively well matched in untrained subjects, they would ultimately be limited by mitochondrial O₂ demand. Conversely, considering the disproportionate rise in mitochondrial capacity associated with endurance exercise training (Gollnick *et al.* 1973; Holloszy & Coyle, 1984), we further hypothesized that, in trained individuals, mitochondrial O₂ demand would far exceed O₂ supply at $\dot{V}O_{2\max}$, causing the fit individuals to be limited by O₂ supply. If correct, these hypotheses will challenge the applicability of the concept of symmorphosis to the O₂ cascade by revealing clear and very different, limitations and excesses at $\dot{V}O_{2\max}$ in untrained and endurance-trained humans.

Methods

Subjects

Following approval by the Institutional Review Board at the University of Utah and the Salt Lake City Veterans' Hospital, and in accordance with the *Declaration of Helsinki*, 10 young, healthy untrained males and 10 young, healthy, endurance-trained male subjects were recruited for the present study and provided their informed, written

consent. The untrained subjects were selected based upon a self-reported lack of moderate-to-vigorous physical activity over the past 2 years, whereas trained subjects reported regularly participating in moderate-to-vigorous physical activity and endurance training (e.g. cycling, running, cross-country skiing) over the past 2 years.

Muscle O_2 supply and muscle $\dot{V}_{O_2\max}$ *in vivo*

Familiarization sessions and whole-body $\dot{V}_{O_2\max}$ ($\dot{V}_{O_2\max}^{\text{body}}$) protocol. Prior to the main experiment, subjects underwent multiple familiarization visits to become accustomed to performing knee extensor (KE) exercise on a custom built knee extension ergometer and cycling exercise on a commercially available cycle ergometer (Lode, the Netherlands). Once familiar with the exercise modalities, the maximum work rate (WR_{\max}) that a subject could sustain for 1 min at the end of maximal graded cycle and KE exercise test was assessed. Specifically, for both cycling and KE exercise, subjects were given a 2–3 min warm-up, which was followed by an increment in work rate (KE: 5 W, cycling: 25 W) every 1 min until task failure (i.e. inability to maintain 60 rpm during KE exercise or falling from a self-selected cadence of > 60 rpm to < 50 rpm during cycling). To ensure a test duration of 8–12 min, the starting workload for both exercise modalities was tailored to each subject's ability. Additionally, $\dot{V}_{O_2\max}^{\text{body}}$ was assessed during the cycling WR_{\max} test utilizing a gas-calibrated indirect calorimetry system (Parvomedics Inc., Sandy, UT, USA), and was defined as the highest rate of O_2 consumption averaged over 30 s prior to cessation of exercise, whereas the respiratory exchange ratio exceeded 1.1 (Garten *et al.* 2014). Note that, during preliminary visits, KE and cycling tests were separated by at least 48 h, such that subjects were well rested.

KE $\dot{V}_{O_2\max}$ protocol. After resting from exercise for at least 24 h, and experiment-related activity for at least 48 h, subjects performed KE exercise at 25%, 50%, 75%, 90% and 100% of WR_{\max} for 2–3 min each (Richardson *et al.* 1995), during which muscle VO_2 of the leg was assessed by the direct Fick method. Specifically, muscle VO_2 was calculated as the product of femoral blood flow and the arterial-venous O_2 difference ($\dot{V}_{O_2} = \text{femoral blood flow} \times \text{arterial-venous } O_2 \text{ difference}$). Femoral blood flow was assessed via Doppler ultrasound of the common femoral artery, proximal to the exercising quadriceps femoris, whereas arterial and venous O_2 content were assessed by a blood gas analyser (GEM 4000; Instrumentation Laboratories, Bedford, MA, USA) on blood drawn through catheters placed directly in the common femoral artery and vein, as described below.

Subsequently, $\dot{Q}O_2$ to the muscle was calculated as the product of femoral blood flow and arterial O_2 content.

Doppler ultrasound assessments. Femoral artery blood flow was measured with ultrasound Doppler and averaged during the last minute of each workload. Specifically, measurements of common femoral artery blood velocity and artery diameter, 2–3 cm proximal to the superficial/deep bifurcation, were taken using a Logiq 7 ultrasound Doppler system in duplex mode (General Electric Medical Systems, Milwaukee, WI, USA) equipped with a linear array transducer function at an imaging frequency of 14 MHz. Artery diameter, measured as internal diameter, was assessed during the peak of the electrocardiogram R-wave (average of four or five measurements) for each workload. Femoral artery blood velocity was assessed with the same probe with a Doppler frequency of 5 MHz operated in the high-pulsed repetition frequency mode (2–25 MHz), as described previously (Barrett-O'Keefe *et al.* 2013). Blood velocity was assessed with an insonation angle of no more than 60° (Rizzo *et al.* 1990), whereas the sample size was maximized and centred according to vessel size and position in real time. All ultrasound data were calculated using Logiq 7 software. Ultimately, femoral blood flow ($\text{ml} \cdot \text{min}^{-1}$) was calculated using the equation:

$$\text{Femoral blood flow} = [(\text{mean blood velocity}) \times \pi (\text{vessel radius})^2 60 \text{ s}]$$

where mean blood velocity is expressed in cm s^{-1} and radius is expressed in cm.

Blood analysis. Three milliliters of blood were drawn at rest and during the last minute of each exercise stage through catheters placed in the common femoral artery and vein. Next, ~1 ml of blood was analysed for arterial and venous total haemoglobin (tHb) content, haemoglobin saturation (HbO_2) and P_{O_2} using a GEM 4000 blood gas analyser and co-oximeter (Instrumentation Laboratories, Bedford, MA, USA). Arterial and venous blood O_2 content (ml dl^{-1}) were calculated using the equation:

$$\text{Blood } O_2 \text{ content} = [(1.39 \text{ ml } O_2 \cdot \text{g Hb}^{-1}) \cdot (\text{tHb}) \cdot (\text{HbO}_2 \text{ saturation})] + [(0.003 \text{ ml } O_2 \cdot \text{dl}^{-1}) \cdot (P_{O_2})]$$

where total Hb (tHb) is expressed in g dL^{-1} , HbO_2 saturation is expressed as a percentage and P_{O_2} is expressed in mmHg. The remainder of blood drawn was taken and preserved for further analysis.

Determination of leg muscle mass. Muscle volume of the lower limb was assessed by multiple measurements of thigh and leg circumference and skin fold thickness as described

by Layec *et al.* (2014), which strongly correlates with volume measured by magnetic resonance imaging across a broad spectrum of subjects ($r^2 = 0.89\text{--}0.98$). Muscle mass of the thigh and leg were then determined by assuming a muscle density of $1.049 \text{ kg}\cdot\text{L}^{-1}$ (Kemp *et al.* 2015). Finally, the mass of the quadriceps femoris was calculated as described by Jones & Pearson (1969), at the same time as applying the correction factor recommended by (Rådegran *et al.* 1999).

Determination of mitochondrial $\dot{V}_{O_2\text{max}}$ *in vitro*

Muscle biopsy. Subjects reported to the laboratory for a muscle biopsy (~1 week before or after the exercise/catheter portion of the study) in a fasted state and having refrained from vigorous exercise for 24 h. Muscle samples of the vastus lateralis were obtained by a percutaneous needle biopsy, 15 cm proximal to the knee at a depth of ~3.5 cm, under sterile conditions (Richardson *et al.* 2004). Immediately after the muscle sample (~150 mg) was biopsied, ~20% of the sample was immersed in ice-cold biopsy preservation fluid (BIOPS) for respiratory analysis (Pesta & Gnaiger, 2012), whereas the other portion was snap frozen and stored at -80°C for histological and biochemical analyses.

Mitochondrial respiration. Muscle samples were prepared and permeabilized as described by Pesta & Gnaiger (2012). Briefly, BIOPS-immersed fibres were carefully separated with fine-tip forceps and subsequently bathed in a BIOPS-based saponin solution comprising $50 \mu\text{g}$ of saponin $(\text{ml BIOPS})^{-1}$, for 30 min. Following saponin treatment, muscle fibres were rinsed twice in ice-cold mitochondrial respiration fluid (MIR05) for 10 min each rinse. The wet weight of each muscle sample (~3–4 mg) was then assessed on a calibrated scale after excess fluid had been removed from the sample by dabbing the muscle several times with a paper towel.

Muscle fibres were then placed in the temperature-controlled respiration chamber (Oxytherm, Hansatech Instruments, Norfolk, UK) in 2 ml of MIR05 solution and warmed to 37°C . After allowing the muscle 10 min to equilibrate, mitochondrial $\dot{V}_{O_2\text{max}}(\text{Mito } \dot{V}_{O_2\text{max}})$ was assessed by sequentially adding malate (2 mM) + glutamate (10 mM), ADP (5 mM) and succinate (10 mM) to the respiration chamber. Finally, cytochrome C (10 μM) was added to the chamber to verify membrane integrity as recommended by Pesta & Gnaiger (2012). Each respiration state or step lasted as long as required to produce a steady-state respiration rate, which was ~3 min in most cases. Note that, prior to each experiment, the respirometer was calibrated, in accordance with the manufacturer's instructions, with two known levels of O_2 concentration: MIR05

Table 1. Subject characteristics

	Untrained	Trained	<i>P</i>
Subjects (<i>n</i>)	10	10	
Age (years)	25 ± 4	24 ± 2	0.77
Height (cm)	178 ± 2	174 ± 2	0.23
Body mass (kg)	78 ± 4	70 ± 2*	0.04
Body mass index (kg m^{-2})	25 ± 1	23 ± 1	0.20
Body $\dot{V}_{O_2\text{max}}$ (l min^{-1})	2.9 ± 0.2	4.1 ± 0.2*	<0.001
Body $\dot{V}_{O_2\text{max}}$ ($\text{ml kg}^{-1} \text{min}^{-1}$)	38 ± 2	59 ± 1*	<0.001
Cycling WR_{max} (W)	243 ± 38	359 ± 63*	<0.001
KE exercise WR_{max} (W)	45 ± 7	70 ± 5*	<0.001
Total leg muscle mass (kg)	6.35 ± 0.3	6.46 ± 0.3	0.45
Quadriceps muscle mass (kg)	1.94 ± 0.1	2.0 ± 0.1	0.64

*Significantly different compared to untrained subjects.

equilibrated with room air at a known barometric pressure and MIR05 with the O_2 in the chamber removed by the introduction of sodium dithionite. Subsequently, background respiration inherent to the experimental set-up was measured and taken into account when assessing overall mitochondrial respiration. Because the respirometer offered the greatest temperature stability at 37°C but exercise generally results in an increase in muscle temperature from 37°C to 38°C or more (Saltin *et al.* 1968; Kenny *et al.* 2003), respiration rates were obtained with the muscle sample at 37°C and then mathematically adjusted, based on a Q_{10} of 2 (multiplication factor for O_2 consumption at a 10°C difference), to yield predicted values at 38°C (Rasmussen *et al.* 2001; Boushel *et al.* 2011). The respiration data for each of the two separate muscle fibre samples were then averaged.

Statistical analysis

Differences in O_2 supply, O_2 consumption and O_2 extraction during KE exercise were assessed with two-way, repeated measures ANOVA, which, when significant, was followed by Tukey's *post hoc* test (SPSS, version 17; SPSS Inc., Chicago, IL, USA). Differences in variables between groups, such as subject characteristics and maximum values, were tested with an independent samples *t* test. Correlations between variables were assessed with Pearson product-moment correlations. Alpha was set at 0.05 *a priori*. Data are reported as the mean ± SE.

Results

Subject characteristics

Ten healthy, untrained males and ten healthy, endurance-trained males completed the present study. As shown in Table 1, subjects in both groups were well

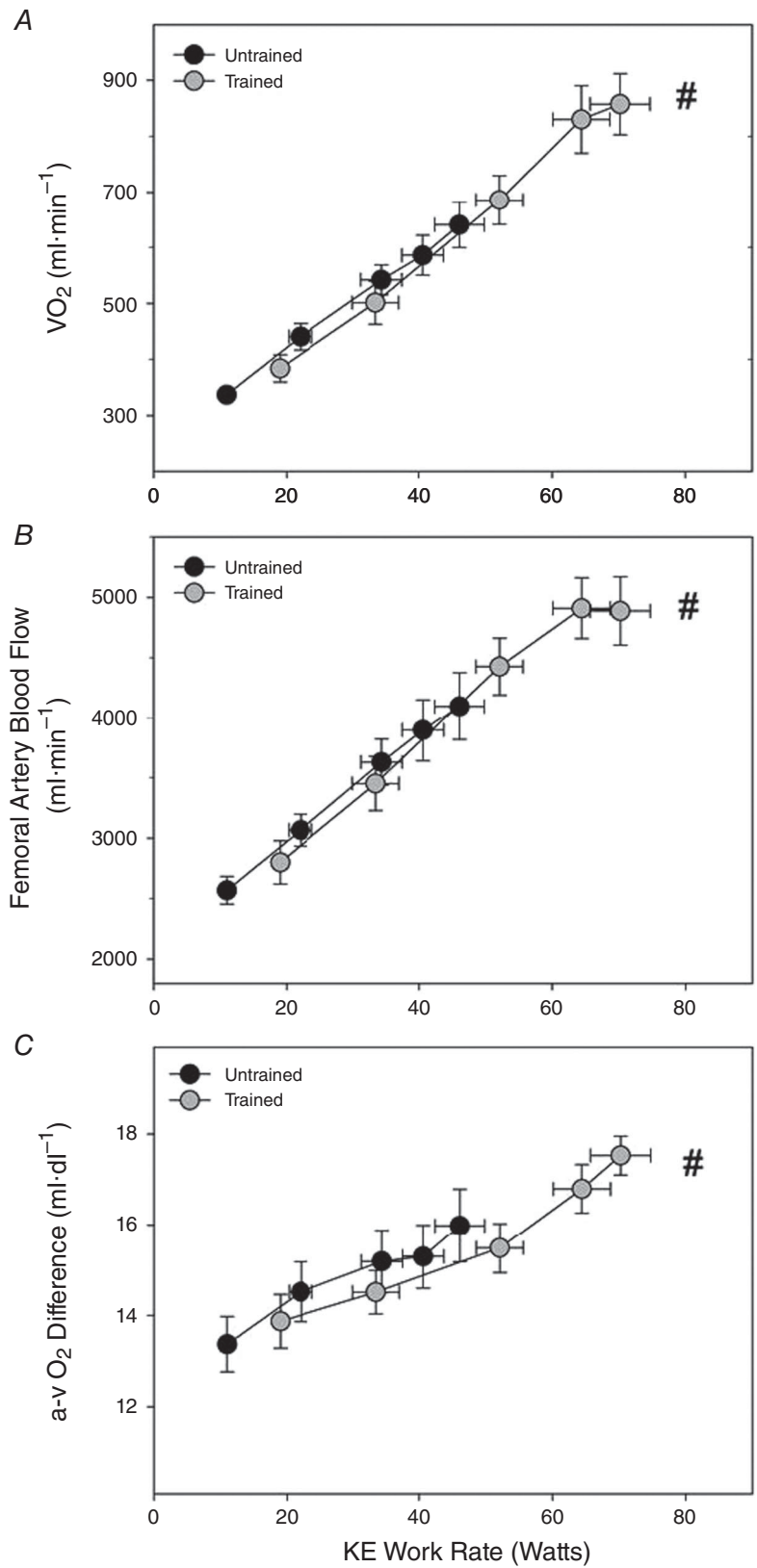


Figure 1. Cardiovascular and metabolic responses during KE exercise in untrained and endurance-trained males

A, muscle O_2 consumption ($\dot{V}O_2$) assessed as the product of (B) femoral artery blood flow and (C) arterial-venous O_2 difference across the exercising limb. Exercise was performed at 25%, 50%, 75%, 90% and 100% of WR_{max} but plotted as absolute WR to allow the appropriate comparisons.

#Significantly different from untrained subjects at maximal KE exercise.

matched, with the exception that, by experimental design, there was a clear difference in exercise capacity. Specifically, $\dot{V}_{O_2\max}$ was in the range 31–44 ml kg⁻¹ min⁻¹ in untrained subjects and 54–66 ml kg⁻¹ min⁻¹ in trained subjects. This difference in $\dot{V}_{O_2\max}$ was also apparent in absolute terms (i.e. $\dot{V}_{O_2\max}$ in L·min⁻¹) and was reflected in both cycle and KE exercise WR_{\max} . Thus, although untrained subjects weighed more than trained subjects, absolute $\dot{V}_{O_2\max}$ was also lower in the untrained group, which, when combined with the fact that body mass index was not different between groups, indicates that the difference in $\dot{V}_{O_2\max}$ was not merely a result of differences in body mass but, instead, was a consequence of real differences in aerobic capacity.

Cardiovascular and metabolic responses to KE exercise

As shown in Fig. 1A, muscle mass-specific \dot{V}_{O_2} during KE exercise increased with each increase in WR ($P < 0.05$) until WR_{\max} , except for the change in \dot{V}_{O_2} from 90% to 100% of KE exercise WR_{\max} , which did not achieve significance in either untrained or trained subjects. As expected, trained subjects achieved significantly greater rates of muscle O₂ consumption compared to untrained subjects during KE exercise, both in absolute (untrained: 641 ± 41 ml·min⁻¹, trained: 858 ± 55 ml·min⁻¹) or relative terms (Fig. 1A). Femoral artery blood flow (Fig. 1B) followed a very similar pattern to \dot{V}_{O_2} , with clear increments at each WR, except from 90% to 100%

of KE exercise WR_{\max} in trained subjects, with blood flow at WR_{\max} ultimately being significantly greater in trained subjects compared to untrained subjects. The arterial-venous O₂ difference (Fig. 1C) did not exhibit an increase with each KE exercise WR. In untrained subjects, extraction failed to increase beyond that achieved at 50% of WR_{\max} . Interestingly, although also failing to increase O₂ extraction with each stepwise increase in workload, trained subjects exhibited a greater propensity for extracting O₂ by increasing the arterial-venous O₂ difference at WR_{\max} beyond that achieved at 75% WR_{\max} . Ultimately, the arterial-venous O₂ difference was significantly greater at KE exercise WR_{\max} in trained subjects compared to untrained subjects (17.5 and 15.9 ml·dl⁻¹ respectively, $P < 0.05$).

O₂ supply and mitochondrial O₂ demand during KE and cycling exercise

As is commonly observed with endurance training, muscle samples from endurance-trained subjects exhibited a significantly greater $\dot{V}_{O_2\max}$ than untrained subjects (743 ± 35 and 364 ± 16 ml kg⁻¹ min⁻¹, respectively, $P < 0.05$) (Fig. 2A). As shown in Fig. 2, in untrained subjects, $\dot{V}_{O_2\max}$ (340 ± 21 ml kg⁻¹ min⁻¹) was not significantly different from $\dot{V}_{O_2\max}$ ($P > 0.05$), whereas maximum O₂ supply during KE exercise ($\dot{Q}O_{2\max}$, 462 ± 37 ml·kg⁻¹·min⁻¹) exceeded $\dot{V}_{O_2\max}$ ($P < 0.05$). By contrast, in trained subjects, $\dot{Q}O_{2\max}$ (557 ± 35 ml kg⁻¹ min⁻¹) and subsequently $\dot{V}_{O_2\max}$

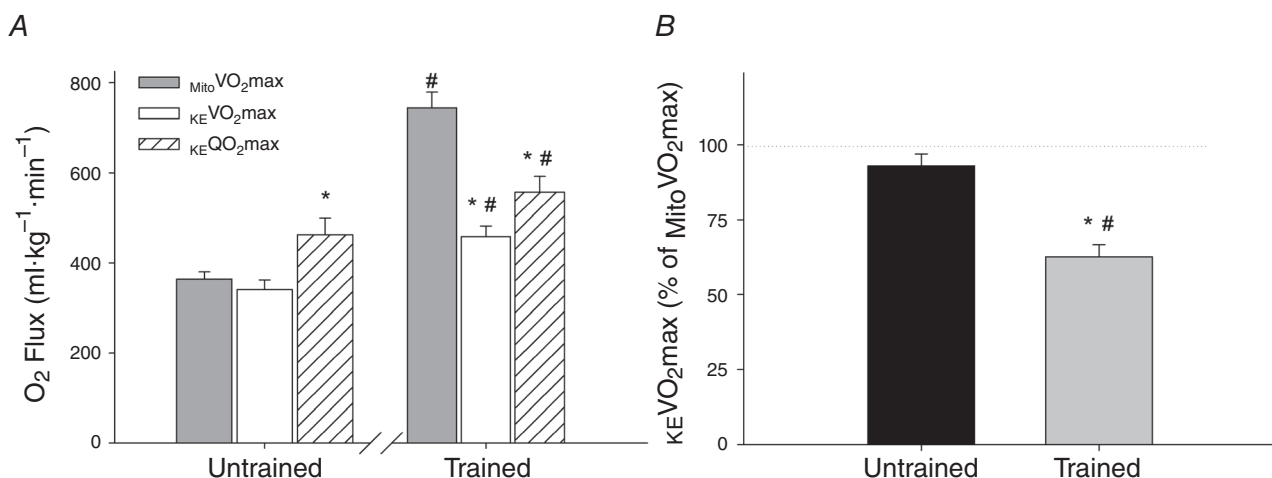


Figure 2. Utilization of mitochondrial respiratory capacity during maximal KE exercise

A, maximal oxygen consumption ($\dot{V}_{O_2\max}$) and supply ($\dot{Q}O_{2\max}$) during maximal KE exercise in trained and untrained subjects compared to the maximal mitochondrial oxygen consumption ($\dot{V}_{O_2\max}$) of permeabilized fibres from the vastus lateralis in the same subjects. B, percentage utilization of mitochondrial capacity (% $\dot{V}_{O_2\max}$) during maximal KE exercise in untrained and endurance-trained subjects. $\dot{V}_{O_2\max}$ is normalized by the mass of the sample of muscle, whereas $\dot{V}_{O_2\max}$ and $\dot{Q}O_{2\max}$ are normalized by the muscle mass of the quadriceps femoris. The dotted line in (B) represents the theoretical point where the full respiratory capacity of the mitochondria is utilized during KE exercise (i.e. 100% $\dot{V}_{O_2\max}$). #Significantly different from untrained subjects. *Significantly different from $\dot{V}_{O_2\max}$.

($458 \pm 24 \text{ ml kg}^{-1} \text{ min}^{-1}$) were both significantly lower than $\text{Mito } \dot{V}_{O_2\max}$ ($P < 0.05$). When considered as a percentage of $\text{Mito } \dot{V}_{O_2\max}$, $\text{KE } \dot{V}_{O_2\max}$ of trained subjects reached only $63 \pm 4 \%$ of $\text{Mito } \dot{V}_{O_2\max}$, whereas the $\text{KE } \dot{V}_{O_2\max}$ of untrained subjects reached $94 \pm 4 \%$ of $\text{Mito } \dot{V}_{O_2\max}$ (Fig. 2B). Of note, an increase in $\text{Mito } \dot{V}_{O_2\max}$ was associated with a proportionate increase in $\text{KE } \dot{V}_{O_2\max}$ among untrained subjects ($r = 0.69$, $P < 0.05$) but not in trained subjects ($r = 0.01$, $P = 0.74$) (Fig. 3). Interestingly, as shown in Fig. 4, untrained subjects exhibited a strong positive relationship between $\text{Mito } \dot{V}_{O_2\max}$ and $\text{Body } \dot{V}_{O_2\max}$, whereas, again in contrast to untrained subjects, trained subjects exhibited no relationship between $\text{Mito } \dot{V}_{O_2\max}$ and $\text{Body } \dot{V}_{O_2\max}$ ($r = 0.12$, $P = 0.74$).

Discussion

The unique combination of *in vitro* and *in vivo* measures of O_2 supply and demand in the present study yielded two related sets of novel findings. First, in contrast to the concept of symmorphosis, the present study reveals that $\text{KE } \dot{V}_{O_2\max}$ is limited by mitochondrial O_2 demand in untrained and O_2 supply in endurance exercise-trained individuals. Second, again contrary to the concept of symmorphosis, the present study documents that the relationship between mitochondrial capacity and $\dot{V}_{O_2\max}$ varies with exercise training status. Taken together, these findings clarify the determinants of $\dot{V}_{O_2\max}$ in

untrained and endurance exercise-trained humans and challenge the invariant matching of structural capacity to functional demand proposed to exist by the concept of symmorphosis.

O_2 supply and mitochondrial O_2 demand limitations during maximal exercise

In theory, if O_2 supply during maximal exercise is sufficient mitochondria should be able to respire at maximal rate (i.e. $\text{Mito } \dot{V}_{O_2\max}$), whereas, if it is O_2 supply that is insufficient, mitochondria would respire at a submaximal rate during maximal exercise (Wagner, 2008). Given the submaximal strain that KE exercise places on central factors (Richardson & Saltin, 1998), we anticipated that, if subjects were to have sufficient O_2 supply to reach $\text{Mito } \dot{V}_{O_2\max}$ during some form of exercise, it would probably be during KE exercise, when pulmonary O_2 diffusion and cardiac output are probably not limiting (Richardson & Saltin, 1998). As shown in Fig. 1, KE exercise yielded high maximal rates of O_2 consumption that were greater in trained compared to untrained subjects. As hypothesized, during maximal KE exercise, untrained subjects appeared to be limited by $\text{Mito } \dot{V}_{O_2\max}$, whereas $\text{KE } \dot{V}_{O_2\max}$ was apparently in excess (Fig. 2B). Conversely, although trained subjects achieved significantly greater rates of O_2 consumption during maximal KE exercise than untrained subjects, the increases in $\text{KE } \dot{V}_{O_2\max}$ and $\text{KE } \dot{Q}_{O_2\max}$ were not proportionate to the large increase in $\text{Mito } \dot{V}_{O_2\max}$, which

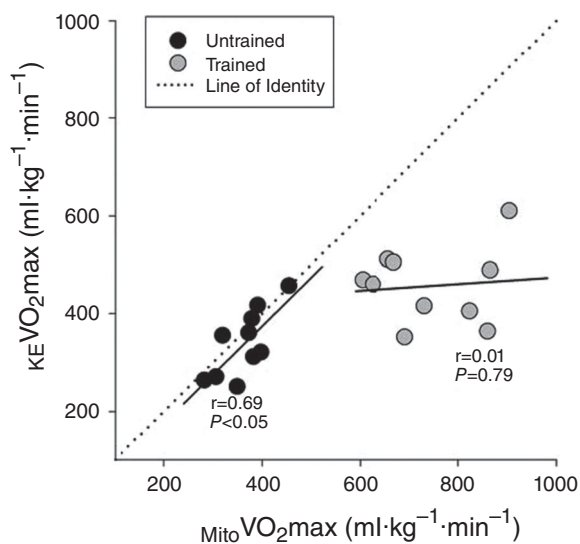


Figure 3. Evidence of a relationship between maximal mitochondrial oxygen consumption ($\text{Mito } \dot{V}_{O_2\max}$) and maximal oxygen consumption during KE exercise ($\text{KE } \dot{V}_{O_2\max}$) in untrained but not trained subjects

$\text{Mito } \dot{V}_{O_2\max}$ is normalized by the mass of the sample of muscle, whereas $\text{KE } \dot{V}_{O_2\max}$ is normalized by the muscle mass of the quadriceps femoris. The dotted line represents the line of identity (i.e. perfect 1:1 relationship between $\text{Mito } \dot{V}_{O_2\max}$ and $\text{KE } \dot{V}_{O_2\max}$).

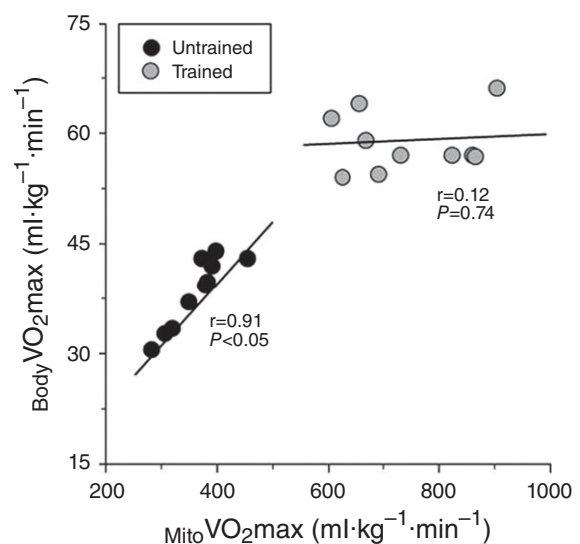


Figure 4. Evidence of a relationship between maximal mitochondrial oxygen consumption ($\text{Mito } \dot{V}_{O_2\max}$) and whole-body oxygen consumption ($\text{Body } \dot{V}_{O_2\max}$) in untrained, but not trained subjects

$\text{Mito } \dot{V}_{O_2\max}$ is normalized by the mass of the sample of muscle, whereas $\text{Body } \dot{V}_{O_2\max}$, which was assessed by indirect calorimetry during cycle exercise, is normalized by the entire body mass.

was almost double that of untrained subjects. Thus, it appears that an insufficient O₂ supply limited trained subjects to only utilizing 63 ± 4% of their far-greater $\dot{V}_{O_{2max}}$ during maximal KE exercise (Fig. 2B).

In theory, if mitochondrial respiratory capacity is important in determining $\dot{V}_{O_{2max}}$ in untrained and not in trained subjects, a strong relationship would be expected between $\dot{V}_{O_{2max}}$ and $\dot{V}_{O_{2max}}$ among untrained subjects but not trained subjects. Indeed, in the present study, mitochondrial capacity was well-matched to $\dot{V}_{O_{2max}}$ in untrained subjects, whereas it was entirely unrelated to $\dot{V}_{O_{2max}}$ among trained subjects (Fig. 3). Interestingly, despite the fact that cycling has a greater probability of eliciting limitations in O₂ supply than KE exercise, (Richardson & Saltin, 1998), this pattern persisted when comparing $\dot{V}_{O_{2max}}$ and $\dot{V}_{O_{2max}}$. Specifically, among untrained subjects, a greater $\dot{V}_{O_{2max}}$ was mirrored by a proportionally greater $\dot{V}_{O_{2max}}$ but, in contrast, among trained subjects, a greater $\dot{V}_{O_{2max}}$ did not translate into a greater $\dot{V}_{O_{2max}}$. Indeed, among trained subjects, $\dot{V}_{O_{2max}}$ was entirely unrelated to $\dot{V}_{O_{2max}}$, suggesting that something else, probably O₂ supply, plays a major role in determining $\dot{V}_{O_{2max}}$ (Fig. 4). Thus, it appears that, during both small and large muscle mass exercise, mitochondria are limiting in untrained subjects, and in excess in trained subjects.

These differing constraints to $\dot{V}_{O_{2max}}$ in trained and untrained humans during maximal exercise may help to explain the varied effects of hyperoxia on $\dot{V}_{O_{2max}}$ (Knight *et al.* 1993; Cardús *et al.* 1998; Pedersen *et al.* 1999; Richardson *et al.* 1999). For example, several studies utilizing untrained subjects have found that augmenting the O₂ supply with hyperoxia has no effect on $\dot{V}_{O_{2max}}$ (Pedersen *et al.* 1999) or cycling $\dot{V}_{O_{2max}}$ (Roca *et al.* 1992; Cardús *et al.* 1998). In light of the current data, these previous findings make sense because untrained subjects already appear to be operating at $\dot{V}_{O_{2max}}$ under normoxic conditions. By contrast, several other studies examining the effect of hyperoxia on $\dot{V}_{O_{2max}}$ in endurance-trained individuals have reported that increasing O₂ supply with hyperoxia can significantly augment $\dot{V}_{O_{2max}}$ by ~17% (Richardson *et al.* 1999) and cycling $\dot{V}_{O_{2max}}$ by ~8% (Margaria *et al.* 1972; Knight *et al.* 1993). This finding is also in agreement with the current data, which indicate that trained subjects, who exhibit a significant mitochondrial reserve capacity under normoxic conditions, have the additional mitochondria in place to consume the extra O₂ made available by hyperoxia. Importantly, the divergence in the determination of $\dot{V}_{O_{2max}}$ in untrained and trained subjects observed in the present study has also been observed in longitudinal studies of subjects before and after endurance training, supporting the idea that training is at the root of the differing constraints to $\dot{V}_{O_{2max}}$ (Roca *et al.* 1992).

Although the present study is unique in terms of how the roles of O₂ supply and demand in untrained and trained subjects were compared, it is certainly not the only one to compare indices of mitochondrial O₂ demand with $\dot{V}_{O_{2max}}$ *in vivo*. For example, Rasmussen *et al.* (2001) and Blomstrand *et al.* (1997) compared multiple measures of muscle oxidative capacity with $\dot{V}_{O_{2max}}$, whereas Boushel *et al.* (2011) made a similar comparison with cycling $\dot{V}_{O_{2max}}$. As was the case for untrained subjects in the present study, both Rasmussen (2001) and Blomstrand (1997) reported that *in vitro* measures of skeletal muscle mitochondrial capacity closely paralleled $\dot{V}_{O_{2max}}$. However, somewhat unexpectedly, Rasmussen *et al.* (2001) also found that $\dot{V}_{O_{2max}}$, assessed in isolated mitochondria, was lower than $\dot{V}_{O_{2max}}$. As acknowledged in this prior work, this observation, which disagrees with the findings of the present study, is physiologically improbable and is potentially the result of altered mitochondrial function, inherent to the isolated mitochondria technique (Picard *et al.* 2011).

More recently, utilizing a similar design to the present study, Boushel *et al.* (2011) reported that, in sedentary subjects, the $\dot{V}_{O_{2max}}$ of the lower limb during cycling exercise did not reach the level of $\dot{V}_{O_{2max}}$, suggesting that they were O₂ supply limited. This is in disagreement with the present study, which suggests that $\dot{V}_{O_{2max}}$ was probably limiting in untrained subjects during both KE and cycling exercise. The discrepancy between the present study and that of Boushel *et al.* (2011) could be a consequence of differences in the aerobic fitness between untrained subjects in these two studies (i.e. $\dot{V}_{O_{2max}}$), which was not reported by Boushel *et al.* (2011), or may be related to an overestimation of the amount of muscle mass of the lower limb engaged during cycling exercise, which Boushel *et al.* (2011) assumed to be 100%. Importantly, it should be restated that the observation that hyperoxia augments $\dot{V}_{O_{2max}}$ in trained (Knight *et al.* 1993) but not untrained individuals (Roca *et al.* 1992; Cardús *et al.* 1998) supports the findings of the present study. Also in support of our findings, Jacobs *et al.* (2013) recently reported that the exercise training-induced increase in $\dot{V}_{O_{2max}}$ of previously sedentary subjects was related to augmented mitochondrial respiratory capacity and not improved O₂ supply. Thus, there is considerable evidence to support the contention that untrained individuals are limited mitochondrial O₂ demand, whereas trained individuals are limited by O₂ supply.

Symmorphosis and determinants of $\dot{V}_{O_{2max}}$ in untrained and trained subjects

Supported by comparative studies that report a constant relationship between the capacity of many steps of the O₂ cascade (e.g. $\dot{V}_{O_{2max}}$) and $\dot{V}_{O_{2max}}$, the concept of

symmorphosis postulates that the capacity of each step of the O_2 cascade is well matched to the demand placed on it at $\dot{V}_{O_2\max}$. Therefore, an invariant ratio, regardless of body size or training status, should exist between the capacity of each step of the O_2 cascade and $\dot{V}_{O_2\max}$ (Weibel *et al.* 1991; Hoppeler & Weibel, 1998). For the most part, the current data from untrained subjects appear to support the concept of symmorphosis, with $\dot{V}_{O_2\max}^{\text{Mito}}$ being well matched and proportional to $\dot{V}_{O_2\max}^{\text{KE}}$ and $\dot{V}_{O_2\max}^{\text{Body}}$ (Figs 3 and 4). Nevertheless, $\dot{Q}_{O_2\max}^{\text{KE}}$, which somewhat exceeded $\dot{V}_{O_2\max}^{\text{Mito}}$, does appear to challenge the matching concept set forth by symmorphosis. However, this somewhat moderate excess may be necessary to maintain a diffusional gradient from blood to muscle cell (Wagner, 2015) and may also serve as a safety factor (Diamond, 1992). By contrast, the data from trained subjects deviate substantially from the concept of symmorphosis. First, as shown in Fig. 2, the capacity for O_2 supply clearly limits $\dot{V}_{O_2\max}^{\text{KE}}$, leaving ~40% of the respiratory capacity of the mitochondria in excess. Thus, the capacity of each step of the O_2 cascade does not appear to be well matched to $\dot{V}_{O_2\max}$ in trained subjects. Second, as shown in Figs 3 and 4, the strong relationships that exist between mitochondrial respiratory capacity and $\dot{V}_{O_2\max}^{\text{KE}}$ and $\dot{V}_{O_2\max}^{\text{Body}}$ in untrained subjects are no longer evident in trained subjects. Thus, in contrast to the concept of symmorphosis (Weibel *et al.* 1991; Hoppeler & Weibel, 1998), training status or 'induced variation' does appear to affect the relationship between $\dot{V}_{O_2\max}$ and the steps of the O_2 cascade.

The reason that the concept of symmorphosis inadequately applies to the O_2 cascade in humans appears to be related to distinct processes (i.e. mitochondrial O_2 demand in untrained subjects and cardiopulmonary O_2 supply in trained subjects) acting as bottlenecks along the cascade. Although the reasons surrounding the O_2 demand limitation of untrained subjects were not specifically examined in the present study, recent evidence suggests that this low $\dot{V}_{O_2\max}^{\text{Mito}}$ may be attributable to differences in the mitochondrial quality, as well as a lower mitochondrial density (Jacobs & Lundby, 2013). Regarding the O_2 supply limitation exhibited by trained subjects, it is recognized that the term ' O_2 supply' covers multiple steps of the O_2 cascade, all of which may contribute to the O_2 supply limitation. Although central limitations, such as pulmonary diffusion and cardiac output, have been reported to limit O_2 supply during maximal exercise (Dempsey *et al.* 2008; Levine, 2008), the capacity of these factors probably was not exhausted during KE exercise, a small muscle mass model (Richardson & Saltin, 1998). Therefore, it appears that peripheral factors such as capillary-muscle diffusion (Richardson *et al.* 1995) or the ability of the vascular system to conduct O_2 -rich blood to the exercising muscle

may contribute to the O_2 supply limitation in trained subjects.

Implications of a mitochondrial reserve capacity

Although mitochondrial respiratory capacity, at maximal KE exercise, in trained subjects appears to be in great excess of metabolic demand, it is doubtful that this reserve capacity serves no purpose. Indeed, Gollnick & Saltin (1982) proposed that increasing mitochondrial enzyme capacity, even without increasing $\dot{V}_{O_2\max}$, would greatly increase endurance performance by increasing fatty acid utilization, thus delaying the onset of blood lactate accumulation and the subsequent sparing of glycogen. Thus, as supported by the recent work of Jacobs *et al.* (2011), the elevated mitochondrial capacity associated with training may be more related to endurance performance (e.g. time trial) than $\dot{V}_{O_2\max}$ or maximal aerobic power.

Additionally, mitochondrial reserve capacity may benefit the cell by allowing the mitochondria to buffer the additional stresses encountered during times of high metabolic demand (Sansbury *et al.* 2011). Studies examining mitochondrial reserve capacity in animal models and cultured cells have reported that cells with a greater mitochondrial reserve are more resistant to oxidative stress (Sansbury *et al.* 2011), defects as a result of mitochondrial toxins (Fern, 2003) and cell death (Nickens *et al.* 2013) than cells with little-to-no reserve capacity. Thus, when confronted with a metabolic challenge, untrained subjects without a mitochondrial reserve capacity may have a greater probability of showing outward manifestations of this stress (e.g. mitochondrial dysfunction, exercise intolerance, etc.) than exercise-trained subjects, who may be able to buffer such an insult with their mitochondrial reserve.

Experimental considerations

In the present study, $\dot{V}_{O_2\max}^{\text{Mito}}$ was assessed in permeabilized fibres with saturating levels of ADP and substrates for complex I and complex II of the electron transport chain. Although this general protocol reportedly yields the highest rates of respiration at the same time as maintaining physiological conditions (Gnaiger, 2009; Pesta & Gnaiger, 2012), and has previously been used to represent $\dot{V}_{O_2\max}^{\text{Mito}}$ (Boushel *et al.* 2011), it is possible that this paradigm and measures do not represent the true maximum capacity of the mitochondria. Nevertheless, in the improbable event that the $\dot{V}_{O_2\max}^{\text{Mito}}$ assessed in the present study was shown not to be maximal, the patterns exhibited in Figs 3 and 4 would still suggest a strong relationship between mitochondrial respiration

and $\dot{V}_{O_{2\max}}$ in untrained but not trained individuals under similar conditions.

Conclusions

The unique combination of *in vivo* and *in vitro* measures utilized in the present study clarifies the determinants of $\dot{V}_{O_{2\max}}$ and identifies two very different sources of limitation to $\dot{V}_{O_{2\max}}$ in untrained and endurance exercise-trained individuals. Among untrained individuals, $\dot{V}_{O_{2\max}}$ appears to be limited and therefore determined by mitochondrial O_2 demand, despite evidence of adequate O_2 supply. By contrast, among trained individuals, O_2 supply appears to limit $\dot{V}_{O_{2\max}}$, despite the presence of a large mitochondrial respiratory reserve capacity. Given this evidence of reserve capacities at $\dot{V}_{O_{2\max}}$ in both untrained and trained individuals, the results of the present study call into question the invariant matching of structural capacity to functional demand that is proposed to exist at $\dot{V}_{O_{2\max}}$ by the premise of symmorphosis.

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Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

JG, RG, MR and RR conceived and designed the experiments. JG, RG, AN, JD, GL MW, JW, TM, CH, CE, JJ, AB, DM, DW, MR and RR collected, assembled, analysed and interpreted data. JG, RG, AN, JD, GL MW, JW, TM, CH, CE, JJ, AB, DM, DW and RR drafted or revised the article. All data were collected in the Utah Vascular Research Laboratory at the University of Utah. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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