Summary
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How do athletes achieve their exceptional performance? A question easily asked, but much more difficult to answer. To understand what makes an athlete a champion, we aimed to unravel the physiological determinants of physical performance and to implement these insights into training strategies to improve physical performance. Whereas physical performance is commonly distinguished in endurance or sprint performance, most sports require a combination of sprint and endurance (such as cycling, rowing, hockey, and speed-skating). So far, the critical physiological determinants for achieving both a high sprint and high endurance performance remain unknown. Moreover, maximization of (combined) sprint and endurance performance is complex, because their physiological determinants exist at different biological levels, which interact, and as (determinants of) sprint and endurance performance have shown to be mutually exclusive.

In this thesis, we focused on athletes, who have optimized their physical performance. To understand what makes an athlete a champion, the following aims were addressed: 1) testing and improving technological tools to characterize skeletal muscle determinants non-invasively 2) obtaining insight in the key physiological determinants of the athlete’s physical performance using a comprehensive physiological profile, and 3) assessment of skeletal muscle adaptations to a training strategy with high potential for improving both sprint and endurance.

Technology
Non-invasive techniques may be useful for advanced monitoring of training adaptations and for quantification of skeletal muscle determinants of physical performance.

NIRS provides non-invasive measures of the balance between oxygen delivery and oxygen consumption within the muscle. In Chapter 2, we tested whether it is feasible to detect the exercise intensity at which a mismatch occurs between O₂ supply and demand, by obtaining the Δ[O₂HbMb-HHbMb]-breakpoint using NIRS in a group of 40 subjects (10 trained female cyclists, 10 trained male cyclists, 11 endurance-trained males and 9 recreationally-trained males). In addition, we determined the reproducibility of NIRS signals and exercise thresholds and assessed the confounding effect of adipose tissue thickness on NIRS signals. The results of this study show that the Δ[O₂HbMb-HHbMb]-breakpoint can be reproducibly obtained and is potentially a suitable exercise threshold for revealing when anaerobic energy production starts to increase in the muscle. However, continuous-wave NIRS measurements were shown to be strongly affected by adipose tissue thickness. In contrast, the first ventilatory threshold, a rather indirect measure of these changes in energy status of the muscle, discriminates better across sexes and training status, showed a higher reproducibility, and was not affected by adipose tissue thickness. Therefore, the first ventilatory threshold is currently favoured, however, if effects of adipose tissue thickness on NIRS measurements can be diminished or corrected for, then NIRS may provide a valuable tool to assess QO₂/VO₂ matching in vivo, with a high practical use in the field of sports or rehabilitation.

Muscle volume and muscle architecture can non-invasively be obtained by 3D ultrasound imaging approaches. However, these approaches have proven cumbersome, time consuming and technically limited (only small segments of large muscles could be reconstructed). In Chapter 3, we showed that with our modifications of the 3D ultrasound technique, substantial improvements...
could be obtained in processing speed (~99%) and size of the reconstruction volume, so that morphology and dimensions of large muscles (such as the VL) could also be measured. We have shown that 3D ultrasound imaging allows reproducible and valid measurements of muscle morphology (r>0.98), but is not as expensive, time-consuming and spatially constrained as Magnetic Resonance Imaging (MRI). Thereby, the 3D ultrasound technique is a cost-effective alternative to the MRI technique, enabling assessment of muscle volume and muscle architecture. This technique may be useful in the clinic as well as in sports, to obtain insight in the physical status of an athlete or patient or to determine effects of training or treatment strategies. Moreover, it may be useful to monitor how muscle morphology and physical performance are related during training.

Physiological profile
To better understand how athletes achieve their exceptional performance, we obtained key physiological determinants of whole-body physical performance and assessed how much of the variance in performance they explained.

Rowers need to combine high sprint and endurance capacities. Assessment of muscle properties, such as morphology of knee extensors in rowers, may provide insights in how high sprint and endurance capacities are concurrently established. However, little is known about how muscle morphology relates to these rowing performance measures. In Chapter 4, we used the 3D ultrasound technique to determine how muscle morphology of the m. vastus lateralis relates to 2000-m rowing ergometer performance, sprint capacity and endurance capacity of Olympic rowers. Muscle volume explained a large proportion of the variance in 2000-m rowing ergometer performance (r²=0.85, p<0.001), maximal oxygen uptake (r²=0.65, p<0.0001), and Wingate peak power production (PO_peak: r²=0.82, p<0.001), which suggests athlete rowers may seek to maximize their (vastus lateralis) muscle volume within the limits of their weight class. When normalized for differences in body size, \( \dot{V}O_{2\text{max}} \) and Wingate PO_peak were negatively related in oarsmen (r=-0.94, p<0.001), which indicates that maximizing both capacities appears to be challenging. Normalized endurance capacity was not negatively related to PCSA, which was unexpected: a smaller muscle fiber diameter implies a shorter oxygen diffusion distance to the core of the muscle fiber, which is thought to contribute to a high oxidative metabolism. Normalized sprint capacity was positively associated with fascicle length, but not with PCSA, and therefore athletes may benefit from long fascicles. However, it remains to be established to which extent functional elongation of muscle fascicles can be achieved by training.

Maximal oxygen uptake (\( \dot{V}O_{2\text{max}} \)) quantifies cardiorespiratory fitness, is widely used to assess effects of training interventions, but is also critical for endurance performance, and predicts loss of independence and mortality. \( \dot{V}O_{2\text{max}} \) during whole-body exercise is presumably constrained by oxygen delivery to mitochondria rather than by mitochondria’s ability to consume oxygen. However, factors limiting \( \dot{V}O_{2\text{max}} \) and the extent of these limitations remain subject to controversy. In Chapter 5, we determined to what extent \( \dot{V}O_{2\text{max}} \) 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. The results show that SDH activity and mitochondrial oxidative capacity predicted from SDH activity are proportionally related to \( \dot{V}O_{2\text{max}} \) measured during cycling (r² = 0.81, p<0.001 and r² = 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). Measured whole-body \( \dot{V}O_2 \) was ~90% of the mitochondrial oxidative capacity, which can be explained by limited oxygen supply to muscle mitochondria. This mitochondrial oxidative overcapacity is substantially lower than values that were previously reported from estimates of in vitro muscle fiber segments, after isolation and permeabilization procedures. For future research and sports application, it would be interesting to study whether breathing hyperoxic air instead of normoxic air would result in a higher whole-body \( \dot{V}O_2 \). If this is the case, the oxygen supply towards and/or within the muscle fibers of the athlete is likely limiting to fully employ the oxidative capacity within the muscle fibers. The outcome of such a test provides valuable information regarding the targets for individualized (altitude) training interventions.

Optimizing physical performance is a major goal in current physiology. Basic understanding of combining high sprint and endurance performance is currently lacking, as many studies focus on determinants of either sprint or endurance performance, even though physical performance is rarely a dichotomous function of only sprint or endurance. Moreover, combining high sprint and endurance performance is complex, as adaptations for endurance or peak power are mutually exclusive, particularly in skeletal muscle. In Chapter 6, we identified critical determinants of sprint, endurance, and combined sprint and endurance performance using correlation and multiple regression analyses of physiological determinants at different biological levels. Performance determinants were obtained from whole-body oxygen consumption, blood sampling, knee-extensor maximal force, muscle oxygenation, whole-muscle morphology and muscle fiber histochemistry of m. vastus lateralis in twenty-eight cyclists (24 (inter)national sprint, team pursuit and road cyclists). When normalized for differences in body size, a large extent of the variation in sprint performance was explained by percentage fast type fibers and muscle volume (\( R^2=0.65 \) p<0.001) and normalized endurance performance was almost completely explained by performance \( \dot{V}O_2 \) and oxygen supply in the circulation (i.e. mean corpuscular hemoglobin concentration and muscle oxygenation; \( R^2=0.92 \) p<0.001). Normalized sprint and endurance performance were negatively related (\( r=-0.66, \) p<0.001). Combined sprint and endurance performance was determined by gross efficiency, performance \( \dot{V}O_2 \) and likely by muscle volume and fascicle length (\( p=0.056 \) and \( p=0.059 \), respectively). High performance \( \dot{V}O_2 \) was related to a high oxidative capacity, high capillarization-myoglobin and small physiological cross-sectional area (\( R^2=0.67 \) p<0.001). The results suggest that, in sports such as cycling, fascicle length and capillarization are important training targets to optimize sprint and endurance performance simultaneously. For future application, the comprehensive physiological profile can be used to monitor the progress of training interventions, and reveal the athlete’s physiological strengths and weaknesses with respect to their sport-specific discipline, which is key to talent development and designing individualized training strategies.

**Training strategy**

Prime determinants for endurance or peak power have shown to be mutually exclusive, particularly in skeletal muscle. This is also relevant in team sports; where repeated-sprint ability is a crucial fitness component and where maximal to near-maximal intensity sprints are repeated throughout the match. Team-sport athletes may seek training strategies to simultaneously increase oxidative capacity and muscle fiber size, possibly by enhancing oxygen supply capacity (as we previously suggested). In other words, they need to concurrently train for enhancement
of peak power production required during maximal efforts as well as oxidative metabolism to speed up recovery between efforts. One strategy is to train or live in hypoxia, which lowers the oxygen tension in the muscle and triggers enhancement of the oxygen cascade from air to mitochondria. In Chapter 7, we investigated adaptations in muscle oxidative capacity, muscle fiber size and oxygen supply capacity in team-sport athletes in response to hypoxic residence combined with repeated-sprint training either in hypoxia or normoxia vs. living and training at sea level. Lowland elite field hockey players resided at simulated altitude (≥14 h·d⁻¹ at 2800-3000 m) and performed regular training plus six repeated-sprint sessions in normobaric hypoxia (3000 m; LHTLH; n=6) or normoxia (0 m, LHTL; n=6) or lived at sea level with regular training only (LLTL; n=6). Our findings show that elite team-sport athletes in LHTLH were able to substantially increase the skeletal muscle oxidative capacity in type I and II fibers (+37% and +32%, respectively), while maintaining muscle fiber size. Changes in oxygen supply capacity (i.e. myoglobin and capillarization) were less prominent, showing mostly small-to-moderate effects between groups. A unique observation was that LHTLH and LHTL, but not LLTL, improved their combination of fiber size and oxidative capacity. Therefore, LHTLH or LHTL are adequate training strategies to improve the combination of prime skeletal muscle fiber determinants for peak power production and maximal oxygen consumption. This strategy may also be effective in other sports, and therefore it would be interesting assess the effectiveness of this strategy in other sports as well as in combination with resistance training.

Collectively, these chapters indicate that quantification of physiological determinants is relevant for understanding differences in physical performance of athletes, and that physiological determinants exist at various biological levels, which interact. As physical performance is rarely a dichotomous function of only sprint or endurance, we have provided a measure of combined sprint and endurance performance. Using two simple exercise tests (i.e. Wingate test and maximal incremental test or time trial), one is able to characterize athletes within the sprint-endurance continuum and assess how well athletes combine sprint and endurance performance. The comprehensive physiological profile provides the critical determinants of (combined) sprint and endurance performance, which may be limiting and serve as targets for training. In addition, the profile includes determinants obtained from non-invasive techniques (i.e. 3D ultrasound and NIRS), which can conveniently be implemented in practice with elite athlete populations. Note that the presented data of our Olympic rowers, (inter)national to Olympic cyclists, and (inter) national hockey players may be used as benchmark data. The comprehensive physiological profile can reveal the athlete’s physiological strengths and weaknesses with respect to their sport-specific discipline, and such information is key to talent development, designing individualized training strategies and monitoring of training adaptations. We have reported skeletal muscle adaptations in response to a promising new training strategy of living at altitude and training low combined with repeated-sprint training in hypoxia or normoxia. Future studies that investigate similar or other training strategies (e.g. concurrent endurance and resistance training) may be evaluated using such a comprehensive physiological profile. In conclusion, skeletal muscle fiber determinants are essential to physical performance, and illustrate why combining a high sprint and endurance performance may be difficult. And that is why muscles matter!