General discussion
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The aim of this thesis was to understand what makes an athlete a champion. Hereto, we aimed to unravel the critical physiological determinants of physical performance. A major objective was to understand how athletes obtain both a high sprint and high endurance performance and why it may be so difficult to achieve this, even though many sports require a combination of sprint and endurance (e.g. cycling, rowing, hockey, and speed-skating). We collected detailed physiological profiles of cyclists and rowers at different levels of organization in the body, to understand the interplay between physiological systems that contribute to physical performance. Moreover, we implemented these insights into a training strategy to enhance physical performance.

In this general discussion, we will discuss the following topics:

- **Sprint versus endurance**: To understand why it may be difficult to combine a high sprint and endurance, we first zoom in to prime determinants of sprint and endurance at the muscle fiber level. Then, we zoom out to the whole-body level, discussing whether our athletes were able to combine a high sprint and endurance performance. Subsequently, we show that two simple measures of whole-body sprint and endurance performance likely provide a useful tool for coaches and athletes to characterize athletes within the sprint-endurance continuum.

- **Comprehensive physiological profile**: To understand how an athlete is able to achieve his/her (combined) sprint and endurance performance, we collected comprehensive physiological profiles of cyclists and rowers. Using these profiles, we extracted key determinants of physical performance. These determinants could be used as targets for training and talent identification and are discussed in more detail.

- **Training strategy**: To understand how training can alter prime muscle fiber determinants of sprint and endurance, we investigate how skeletal muscle adapt to a promising new training strategy of living at altitude and training low (LHTL) combined with repeated-sprint training in normoxia or hypoxia. We discuss how this training strategy may help to improve these prime muscle fiber determinants of sprint and endurance in field-hockey players. Moreover, we provide some perspectives on the skeletal muscle adaptations in response to training.

- **Technological developments**: To allow advanced monitoring of training adaptations, we have further developed a 3D ultrasound imaging technique, for assessment of muscle volume and muscle architecture, and discuss the use of near-infrared spectroscopy, for detection of a (mis)match between oxygen supply and oxygen demand within the muscle during exercise. These techniques may improve the monitoring of training adaptations in future studies.

- **Future directions for research, training and monitoring**
- **Practical applications**
Sprint versus endurance
Inverse relationship of skeletal muscle properties for sprint and endurance
During physical performance, an athlete produces power by contraction of his skeletal muscles. Physiological properties of skeletal muscle may illustrate why combining high sprint and endurance performance is difficult, i.e. fiber size and oxidative capacity (fiber $\hat{\text{V}}\text{O}_{2\text{max}}$) are inversely related across animal species (Figure 8.1)$^{1,2}$. As mentioned in Chapter 1, a large muscle fiber size contributes to high maximal muscle force and power generating capacity and consequently to a high sprint performance. High endurance performance requires muscle fibers with a high oxidative capacity, which is important to ensure prolonged delivery of energy rich phosphates using oxygen within the mitochondria. In Chapter 6, we have shown that also in the cyclists there was an inverse relationship between muscle fiber size and oxidative capacity similar to that of untrained animals$^{1,2}$, although these athletes succeeded to obtain higher combinations of muscle fiber size and oxidative capacity (i.e. their curve was shifted upward with respect to the curve of the untrained animals, Figure 8.1)$^{1,2}$. So, as illustrated by the inverse relationship, also for athletes, it may be difficult to combine a high sprint and endurance performance.

![Figure 8.1. Muscle fiber size and muscle fiber oxidative capacity ($f\hat{\text{V}}\text{O}_{2\text{max}}$) are inversely related in cyclists ($r=-0.50$, $p<0.05$, as also shown in Figure 6.4). Their inverse relationship is displayed with respect to the inverse relationship across animal species reported in literature$^{1,2}$.](image)

Inverse relationship of whole-body sprint and endurance
Whole-body normalized sprint and endurance performance were inversely related in the cyclists in Chapter 6, as already suggested by the inverse relationship between fiber size and oxidative capacity. Similarly, the rowers in Chapter 4 demonstrated an inverse relationship between normalized sprint performance and aerobic power or $\hat{\text{V}}\text{O}_{2\text{max}}$. Whole-body sprint and endurance scale with the body size of an organism$^3$ and therefore were normalized to lean body mass$^{2/3}$ according to$^4$, to investigate intrinsic properties of the neuromuscular system. In sum, the data suggest that both rowers and cyclists did have difficulty combining high sprint and endurance.

Whole-body sprint and endurance could be compared between both sports if the same
measures are used for rowers and cyclists (i.e. Wingate peak power output and $\dot{V}O_{2\text{max}}$, respectively). Strong inverse relationships were observed between these measures of endurance and sprint in male Olympic rowers as well as male cyclists ($r = -0.94$ and $r=-0.74$, respectively; Figure 8.2), revealing similar slopes. However, Olympic rowers displayed higher values for endurance, but similar values for sprint compared to the cyclists.

Could this be related to methodological differences? The athlete’s aerobic power or $\dot{V}O_{2\text{max}}$ was obtained during an incremental rowing or cycling test, respectively. One may expect to find relatively higher $\dot{V}O_{2\text{max}}$ values in the rowers on the rowing ergometer, because they use both arms and legs, yet experienced rowers have been shown to elicit similar $\dot{V}O_{2\text{max}}$ values on a rowing or bicycle ergometer\textsuperscript{5,6}. Therefore, this does not seem to explain differences in $\dot{V}O_{2\text{max}}$ values between our rowers and cyclists. Our measure of sprint performance was the athlete’s peak power produced during a 30-s Wingate cycling test\textsuperscript{7}. Of note is that also the rowers were tested on a bicycle ergometer, because such an ergometer is designed to accurately measure accelerations with high temporal resolution, more so than a rowing ergometer. The rowing ergometer would substantially underestimate peak power output as it measures velocity of the rotating flywheel, but does not capture changes in kinetic energy. Moreover, the recovery phase during the rowing stroke is relatively long (~half of the stroke)\textsuperscript{8} and may preclude rowers from reaching their ‘true’ peak power production. The similar values for sprint suggest that also the rowers were able to elicit their peak power production on the bicycle ergometer. Could the higher values for endurance in the male rowers compared to cyclists also be due to differences in training status?

Figure 8.2. Wingate peak power output and maximal oxygen uptake were inversely related in male Olympic rowers (▼) and male cyclists (●). Peak power output was obtained from a 30-s Wingate cycling test in both rowers and cyclists. $\dot{V}O_{2\text{max}}$ was obtained from an incremental rowing and cycling exercise test, respectively. Measures were normalized to LBM$^{2/3}$ to scale for differences in body size. Three of the cyclists (no. 1-3) fall within the confidence interval of the rowers’ inverse relationship (see text).
A practical tool to assess and monitor training status?

Comparison of the relationship between aerobic and peak power in rowing and cycling shows that this relationship is shifted upward in rowers compared to cyclists. This likely relates to training status of the athletes: all male rowers were Olympic athletes, whereas the male cyclists were (inter)national to Olympic athletes. Heterogeneity in training status of the cyclists is also illustrated by the larger confidence intervals of the regression line as well as the somewhat lower correlation coefficient. Note that the higher heterogeneity in our cyclists was not unexpected.

From the male cyclists, we also obtained muscle biopsies and it is almost impossible to obtain these from Olympic athletes. Despite the differences in training status between cyclists and rowers, both displayed similar negative slopes, illustrating that the interference between aerobic and peak power appears to be similar across these sports disciplines and training status. Another interesting finding is that three of the cyclists (no. 1-3 in Figure 8.2) fall within the confidence limits of the Olympic rowers. This may reflect that also these cyclists had a well-developed training status. In fact, one of these cyclists (no. 3) was a team pursuit cyclist competing at the 2016 Olympics. Another cyclist (no. 1) possessed an exceptionally high $\dot{V}O_{2\text{max}}$ of ~80 mL·kg$^{-1}$·min$^{-1}$, that is suggested to be the minimal $\dot{V}O_{2\text{max}}$ for a cyclist to win the Tour de France. The last cyclist (no. 2) revealed exceptionally high capillarization, being able to demonstrate very effective matching of oxygen supply and demand in order to utilize his full oxidative capacity at $\dot{V}O_{2\text{max}}$. A topic that is addressed in Chapter 5.

In sum, outcomes of these two simple exercise tests – a Wingate test and maximal incremental test – could serve as a practical tool for coaches and athletes to reveal an athlete’s training status (i.e. compare his sprint and endurance performance to the ‘benchmark’ data in Figure 8.2), assess how well athletes combine sprint and endurance and distinguish whether athletes can be characterized as sprint-type or endurance-type athletes within the sprint-endurance continuum (Figure 8.2). Implementing this tool in common sports practice (e.g. in cycling and rowing) will likely help coaches and top-level athletes to improve performance by facilitating future talent identification, tuning athletes to their preferred sports discipline and by optimizing and monitoring adaptations to individual training strategies.

Comprehensive physiological profile: implications for training and talent identification

To understand how athletes achieve their (combined) sprint and endurance performance, we collected comprehensive physiological profiles of cyclists and rowers. With these profiles we could identify key physiological determinants of physical performance and identify the athlete’s physiological strengths and weaknesses. As mentioned previously, maximal power production of an organism scales with body size. Sprint, endurance and combined sprint and endurance performance were normalized to lean body mass$^{2/3}$ in accordance with$^{1}$. By this normalization, we removed effects of differences in body size on performance of athletes and were able to investigate the intrinsic functional properties of the neuromuscular system. Therefore, we extracted key determinants of normalized (combined) sprint and endurance performance, which could be used as targets for training strategies and talent identification (in rowing and cycling) and are discussed in more detail below.
Targets for sprinters

Muscle fiber type

Sprinters need to quickly accelerate and maintain high speeds after their start to achieve a high sprint performance\textsuperscript{10}, producing high peak power outputs. Proportion fast type fibers within muscles is critical to peak power production, as it determines maximal muscle fiber contraction velocity\textsuperscript{11–13}. Sprinters are known for their large percentage of fast type muscle fibers; world-class sprinters display as much as ~70-80\% fast type fibers within their quadriceps (vastus lateralis) muscle\textsuperscript{14,15}. Moreover, optimal pedaling velocity during sprint cycling has been shown to relate to the proportion of fast type fibers\textsuperscript{16} as well as to the proportion of cross-sectional area occupied fast type fibers in m. vastus lateralis\textsuperscript{17}. In Chapter 6, we have shown that in our cyclists the percentage fast type fibers in the m. vastus lateralis is important for sprint performance, explaining 26\% of the variance in normalized peak power output. Moreover, multiple regression analyses showed that fiber type is one of the critical determinants of sprint performance. In our group of cyclists we observed as much as ~60\% fast type fibers, whereas the explained variance might have been higher if we could also have obtained muscle biopsy samples from world-class sprinters (i.e. assuming a larger range in peak power values and percentages fast type fibers). A major question from sports practice is: Are we able to change our muscle fiber type composition through training? “The short (disappointing) answer is; Not really” (for review see\textsuperscript{18}). Though humans show large variation in muscle fiber type composition, training seems to induce only small changes therein. Generally, resistance training leads to conversion of IIX into IIA fibers, whereas absence or decrease in resistance training converts IIA into IIX fibers, leaving the percentage type I fibers unaffected\textsuperscript{14,18}. Percentage IIX fibers may even be boosted to higher proportions following detraining (and potentially tapering) after a period of resistance training\textsuperscript{18,19}. Conversions of type II into type I fibers with training, and vice versa, are less well-documented\textsuperscript{14,18}, but sprint training may increase percentage IIA fibers in elite sprinters from 35\% to 52\% at expense of their percentage type I and IIX fibers\textsuperscript{20}. Alternatively, athletes can induce hypertrophy of fast type fibers after resistance training to increase their proportion of muscles’ cross-sectional area occupied by fast fibers and potentially improve their peak power and sprint performance.

Muscle morphology

Not only fiber-type distribution, but also muscle volume is important for sprint cycling performance\textsuperscript{21}. A large muscle mass is beneficial for fast accelerations\textsuperscript{10} and a large leg muscle mass has been associated with high peak power production in healthy subjects ($r^2$=0.70)\textsuperscript{22}. In Chapter 4, we demonstrated that in Olympic rowers, an even larger portion of the variance in peak power production was explained by muscle volume of the m. vastus lateralis ($r^2$=0.82). After normalization for body size differences, VL muscle volume still explained variance in sprint performance in Olympic rowers ($r^2$=0.45, Chapter 4) and cyclists ($r^2$=0.38, Chapter 6). This indicates that a large VL volume may be beneficial even after scaling for body size differences. Though we did not examine how athletes maintained speed during sprinting, a large muscle mass may be a disadvantage for reaching high speeds, as it increases inertia\textsuperscript{10}. Training with high-resistance activity is known to substantially increase muscle volume (for review see\textsuperscript{23}). Increases in muscle volume can result from muscle fiber hypertrophy, muscle fiber lengthening and hyperplasia (addition of muscle fibers). Consensus is that number of muscle fibers is determined early in life and that hyperplasia is limited – if any – in mature mammalian
Muscle fiber hypertrophy, however, is considered to be the primary response to long-term resistance training. Muscle fiber lengthening with training is discussed below (in the section Targets for both sprint and endurance). Certainly, increases in muscle volume will improve peak power production by the muscle. However, athletes should consider that increasing muscle volume also impacts the architecture of the muscle.

Muscle architecture describes the arrangement of muscle volume into fascicle length and PCSA (i.e. area of sarcomeres in-parallel). A long fascicle length (i.e. high numbers of sarcomeres in-series) has theoretically been associated with high maximal muscle fiber contraction velocity and has been shown to relate to better sprint performance. PCSA increases with muscle fiber hypertrophy, and is associated with proportional increases in muscle force assuming that specific force (F/PCSA) remains constant. In addition to muscle volume, we obtained muscle architecture from the same 3D voxel array using our advanced 3D ultrasound imaging technique (see Chapter 3). In Chapter 4, we have shown that normalized sprint performance was positively associated with $L_f$ ($r^2=0.45$), but not with PCSA. In Chapter 6, similar observations were made in the subgroup of road and team pursuit cyclists ($L_f: r^2=0.35$). However, in the group with all cyclists, PCSA was positively associated with sprint performance while $L_f$ was not. Likely, this is because of higher sprint performance and different arrangement of the track sprinters, revealing a much larger PCSA but similar $L_f$ compared to the road and team pursuit cyclists. Larger PCSA was associated with a larger FCSA of both type I and II fibers ($r=0.56$ and $r=0.74$, respectively), supporting the consensus that muscle fiber hypertrophy is essential to whole-muscle hypertrophy and the notion of differentiated hypertrophy, with fast type fibers showing ~twofold greater hypertrophy and contributing to a larger proportion of (the variance in) PCSA. Interestingly, $L_f$ was positively associated with knee extensor specific force in both male rowers and male cyclists ($r=0.66, p<0.05$ and $r=0.65, p<0.001$, respectively), illustrating more effective force transmission from the longer muscle fibers to the muscle’s line of pull. Conversely, PCSA and specific force were not related in male rowers and negatively related in male cyclists ($r=-0.46, p=0.135$ and $r=-0.63, p<0.001$, respectively). Possibly, this is because muscle hypertrophy is associated with increases in pennation angle, reducing effective force exertion on the tendon and subsequently hampering the specific force. In support of these results, pennation angle tended to be negatively related to specific force in both rowers and cyclists ($r=-0.46, p=0.054$ and $r=-0.36, p=0.059$ respectively). In addition to changes in pennation angle with training, changes in fascicle length and muscle hypertrophy both affect muscle optimum length, which could impact the operating range of the muscle length-force relation over which muscles are active and therefore may affect the execution of the sport-specific task.

Targets for endurance athletes

Gross efficiency

To achieve a high endurance performance, an athlete needs to produce a high power output that can be sustained for a prolonged period of time. Endurance athletes display high rates of oxygen consumption to ensure prolonged energy delivery, likely provided by a high number of mitochondria in the muscle, which are supplied with sufficient amounts of oxygen and that work efficiently. Gross efficiency determines how much speed or power is achieved with this rate of energy consumption, and therefore, gross efficiency is an important determinant of endurance performance. In our cyclists in Chapter 6, gross efficiency explained 36% of the variance in
endurance performance. Gross efficiency, obtained at an exercise intensity of \(77\pm8\% \text{VO}_2\text{max}\), was related to the percentage slow type fibers, similar to previous observations in competitive cyclists\(^{30}\). Endurance athletes typically have high proportions of slow type fibers, which are suggested to display greater mechanical efficiency at common cycling cadences (i.e. 60-120 rpm)\(^{29,31}\). We also found that gross efficiency was positively associated with capillarization, possibly because a higher oxygen supply capacity facilitates matching of oxygen supply and demand, providing the mitochondria with sufficient amounts of oxygen also at higher exercise intensities. When controlling for exercise intensity, partial correlation analyses show that significant correlations disappear between gross efficiency and capillarization or fiber type. This suggests that these muscle fiber properties indirectly influence gross efficiency by allowing steady state \(\text{O}_2\) consumptions at higher work rates. In support of these results, gross efficiency has shown to be strongly dependent on work rate \(\left(r^2=0.91\right)\), as the effect of baseline energy expenditure diminishes with increasing work rate\(^{32}\). Gross efficiency can be assessed reliably\(^{33}\), is similar when obtained from 3-min or 6-min stage incremental tests\(^{34}\), does not display a circadian rhythm\(^{33}\), and is lower at (simulated) altitude compared to sea level\(^{35}\). Moreover, recent findings suggest that gross efficiency is not constant, as it decreases during exercise performance\(^{36}\). An interesting research question for future studies is therefore to investigate whether this drop in gross efficiency during cycling performance is related to muscle fiber properties, such as muscle fiber type or capillarization.

**Matching of oxygen supply and demand**

During endurance performance, athletes display high rates of oxygen consumption to provide the required energy to sustain the high-intensity exercise. This performance \(\dot{\text{VO}}_2\) is a critical determinant of endurance performance and is considered to depend on both \(\dot{\text{VO}}_2\text{max}\) and \(\dot{\text{VO}}_2\) at the lactate or ventilatory threshold\(^{29}\), i.e. when oxidative metabolism becomes insufficient to deliver all energy. In Chapter 6, we showed that 92% of the variance in endurance performance was explained by performance \(\dot{\text{VO}}_2\) and oxygen supply capacity within the circulation (mean corpuscular hemoglobin concentration and \([\text{O}_2\text{HbMb}]\)). As expected, most of the variance in performance \(\dot{\text{VO}}_2\) was explained by \(\dot{\text{VO}}_2\text{max}\) and \(\dot{\text{VO}}_2\) at LT2 \((R^2 = 0.93)\). However, our study also shows that VL muscle fiber \(\dot{\text{VO}}_2\text{max}\), the interaction of \([\text{Mb}]\) with C/F and PCSA explained a large proportion of the variance in performance \(\dot{\text{VO}}_2\) \((R^2 = 0.67\), see Equation 6.2\). These results suggest matching between \(\text{O}_2\) supply and demand at the muscle fiber level \((\dot{\text{Q}}_2/\dot{\text{VO}}_2\text{ matching})\) and demonstrate the detrimental effect of muscle hypertrophy (i.e. increase in PCSA) on average oxygen consumption during the endurance performance. The beneficial effect of a more effective \(\dot{\text{Q}}_2/\dot{\text{VO}}_2\) matching was also illustrated by a higher \(\text{O}_2\text{HbMb}\) concentration during the endurance performance obtained by NIRS. In sum, high endurance performance requires effective matching of \(\text{O}_2\) demand and \(\text{O}_2\) supply, preferably with a small PCSA.

In Chapter 2, we investigated whether non-invasive NIRS measurements could also be used
to determine the exercise intensity at which a mismatch between $O_2$ supply and demand starts to occur, since NIRS provides non-invasive measures of changes in tissue oxygenation, which is a net effect of oxygen delivery and consumption in the muscle\(^4\) and therefore may more directly reflect when oxygen supply becomes insufficient. Although the $[O_2HbMb-HHbMb]$ breakpoint was reproducible ($ICC = 0.80–0.88$) and differed across sexes, it did not differ across training status and NIRS signals were substantially affected by adipose tissue thickness. Therefore, the $[O_2HbMb-HHbMb]$ breakpoint may currently not be suitable for detection of this critical exercise threshold, and the use of ventilatory thresholds is recommended. If an athlete improves this exercise threshold towards higher intensities, suggesting better $\dot{Q}O_2/\dot{V}O_2$ matching, the athlete will likely display a better endurance performance.

In the previous paragraphs, we discussed $\dot{Q}O_2/\dot{V}O_2$ matching at submaximal intensities. However, we have also addressed $\dot{Q}O_2/\dot{V}O_2$ matching at maximal exercise intensity in Chapter 5, comparing the whole-body $\dot{V}O_{2max}$ obtained during maximal incremental cycling exercise with the theoretical $\dot{V}O_{2max}$ predicted from mitochondrial oxidative capacity in muscle biopsy samples. If oxygen supply was not sufficient to achieve the predicted $\dot{V}O_{2max}$, there would be a mitochondrial oxidative overcapacity, illustrating potential limitations in oxygen supply to the muscle mitochondria at maximal exercise. In other words, we investigated the mitochondrial oxidative overcapacity that exists at $\dot{V}O_{2max}$ and assessed this for cyclists, untrained control subjects and chronic heart failure patients. In Chapter 5, it is shown that the mitochondrial oxidative capacity explains a substantial portion of the variance in $\dot{V}O_{2max}$ ($R^2=0.89$) and that these two variables are proportionally related. All groups, including cyclists, controls and CHF patients, displayed a similar oxidative overcapacity, i.e. they used $-90\%$ percentage of their oxidative capacity at $\dot{V}O_{2max}$, indicating that humans do not fully exploit their oxidative enzyme capacity. With regard to oxygen supply, it was previously shown that higher $\dot{V}O_{2max}$ was related to a higher muscle deoxygenation (i.e. more oxygen extraction from the blood at maximal exercise, $r=0.44$\(^4\)). Moreover, additional analyses in our cyclists revealed that a higher muscle deoxygenation at maximal incremental exercise was also related to a lower mitochondrial oxidative overcapacity (i.e. less oxygen supply limitation) ($r=0.43$, $p<0.05$ for [HHbMb] and $r=-0.41$, $p<0.05$ for $[O_2HbMb]$). For future research, it may be very interesting to see how breathing hyperoxic air instead of normoxic air during the incremental exercise test could improve oxygen supply of the athlete and whether this results in an improved oxygenation, higher $\dot{V}O_{2max}$ and lower mitochondrial oxidative overcapacity. This may also be a good strategy to differentiate athletes with a more central or peripheral limitation of the $\dot{V}O_{2max}$ (i.e. higher $\dot{V}O_{2max}$ in hyperoxia or similar $\dot{V}O_{2max}$ in hyperoxia compared to normoxia, respectively).

For training purposes, it may be very difficult to pinpoint specific training interventions to improve this $\dot{Q}O_2/\dot{V}O_2$ matching, as the matching of oxygen demand and supply is complex and subjected to many influences. At the whole-body level, polarized training (i.e., $-75–80\%$ at low intensity, $-5–10\%$ at moderate intensity, and $-15–20\%$ at high intensity) may be a promising training strategy to improve $\dot{V}O_{2max}$ and $\dot{V}O_2$ at LT2, even in elite athletes\(^4\). The low-intensity exercise is suggested to enhance stroke volume, plasma volume and metabolic efficiency, whereas the high intensity exercise is thought to improve both oxygen supply capacity and oxygen demand within the muscle\(^4\). It would be very interesting to study how these whole-body adaptations with polarized training relate to cardio-respiratory and skeletal muscle adaptations. Another promising strategy to improve oxidative capacity and oxygen supply capacity within the muscle
is the combination of living high and training low with repeated-sprint training in normoxia or hypoxia. In the section Training strategies to enhance physical performance we will discuss skeletal muscle adaptations with this training strategy in more detail.

**Targets for both sprint and endurance**

**Matching of oxygen supply and demand**

One of the main objectives of this thesis was to understand how athletes obtain both a high sprint and high endurance performance and why it may be so difficult to achieve this, even though many sports require a combination of sprint and endurance. As displayed by Figure 8.1, part of this difficulty may be understood by looking at skeletal muscle properties. Muscle fiber size and oxidative capacity (fiber \( \dot{V}_O_2^{max} \)) have shown to be inversely related across animal species\(^1,2\), and in Chapter 6, we have shown that also in our cyclists there is such an inverse relationship. Despite this inverse relationship, our athletes succeeded to obtain higher combinations of fiber size and oxidative capacity than untrained humans (as part of the inverse relationship across animal species\(^1,2\)) and chronic heart failure patients\(^43\). Hill-type model predictions show that increases in oxygen demand (i.e. the product of FCSA and fiber \( \dot{V}_O_2^{max} \cdot iSDH \) activity) may occur, but only when accompanied by improved oxygen diffusion from capillary blood to the core of the muscle fiber\(^1\). The oxygen diffusion depends on the solubility of oxygen in muscle, diffusion coefficient for oxygen in sarcoplasm, interstitial oxygen tension (PO\(_2\)) and the concentration of intracellular oxygen carrier myoglobin\(^44,45\). If oxygen demand exceeds oxygen supply, the muscle fiber will become hypoxic. Enhanced oxygen supply may accommodate sustained increases in oxygen demand (iSDH activity), by elevating interstitial PO\(_2\) above the PO\(_2\) at which the center of the muscle fiber becomes hypoxic at \( \dot{V}_O_2^{max} \) (PO\(_{2\text{crit}}\)). As illustrated by Figure 8.1, our cyclists were able to obtain a higher PO\(_{2\text{crit}}\) than that of untrained animals including humans\(^1\). It was expected that high oxygen demands and high PO\(_{2\text{crit}}\) were matched by enhanced oxygen supply, due to 1) enhanced oxygen supply towards the muscle fiber 2) enhanced oxygen supply within the muscle fiber, or 3) relocating mitochondria to the sarcolemma\(^1\).

The question is now whether our data support this expectation. In our cyclists, a high subsarcolemmal mitochondrial enzyme activity was indeed confirmed by visual inspection of Figure 6.1. Moreover, higher oxygen demand (iSDH activity) was associated with oxygen supply capacity towards the muscle fiber (particularly capillarization), but not with myoglobin

![Figure 8.3](image-url)

**Figure 8.3.** PO\(_{2\text{crit}}\), the PO\(_2\) at which the center of the muscle fiber becomes hypoxic at \( \dot{V}_O_2^{max} \), was calculated with myoglobin and was proportionally related to capillary-to-fiber ratio (A) and capillaries around the fiber (CAF), but not to myoglobin concentration (C) in our cyclists.
concentration (see Chapter 6). Similarly, Figure 8.3 shows that $PO_{2\text{crit}}$ increases proportionally with capillarization, but was not related to Mb concentration. For capillarization, many factors contribute to the diffusion of oxygen, such as number of capillaries, capillary length, capillary diameter, number of open or closed capillaries. Here, we used capillaries around the fiber and capillary-to-fiber ratio as markers of capillarization (see Figure 8.3). Capillarization was exceptionally well-developed in our cyclists, displaying 7.2 capillaries per fiber, similar to that of elite cyclists 46. This value was higher than that of our hockey players (5.4 capillaries per fiber, see Chapter 7) and untrained humans (4 capillaries per fiber) 13,47. Interestingly, the Mb concentration was very low in our cyclists (0.38 mM). This value was lower than that of healthy control subjects (0.48 mM, unpublished data from our lab), elite hockey players (0.52 mM, see Chapter 7), chronic heart failure patients (0.56 mM, cf. Ref 48) and healthy elderly (0.59 mM, cf. Ref 48). Moreover, we observed small variation in [Mb] between cyclists (coefficient of variation ~10%). What happens if we use both [Mb] and capillarization to explain variance in oxygen demands using a multiple regression analysis? These analyses show that variance in iSDH activity could only be explained by capillary-to-fiber ratio in the cyclists from Chapter 6 ($r^2=0.34$, $p<0.01$). However, in the elite hockey players from Chapter 7, variance in iSDH activity was explained by both their capillary-to-fiber ratio and Mb concentration ($R^2=0.51$, $p<0.01$). Previous data on a heterogeneous group of chronic heart failure patients and healthy elderly has also shown that variance in iSDH activity was largely explained by capillary-to-fiber ratio ($r^2=0.58$, $p<0.001$; [Mb] was not measured in this study) 43. Therefore, high oxygen demands, illustrated by high combinations of fiber size and fiber $\dot{V}O_{\text{2max}}$, need to be accommodated by enhanced oxygen supply capacity, for example by enhanced capillarization and/or myoglobin concentrations.

**Preliminary results of myoglobin regulation in cyclists and healthy controls.**

From these previous findings in our cyclists, the question arises why these cyclists had a relatively low Mb concentration in their quadriceps muscles. For this purpose, we performed an additional study, which will be briefly summarized and of which the main results will be highlighted. Using biopsy samples of the m. vastus lateralis, we performed additional analysis of the regulation of Mb in the 28 cyclists and compared these with values of a group of 20 healthy control subjects (unpublished data from our lab). Hereeto, we investigated the [Mb protein], Mb mRNA expression levels, total mRNA expression levels per mg muscle tissue and the number of myonuclei (that produce the mRNA strands). First, [Mb] was obtained from histochemistry of biopsy cross-sections, in accordance with the methods in Chapter 6 and 7. Second, to obtain Mb mRNA expression levels, biopsy samples were homogeneized and cDNA was amplified using real-time qPCR. Relative myoglobin mRNA concentrations were quantified with the following SYBR green primers: 5’–AATGGCAATTTGGCTGAAC-3’ (forward primer) and 5’–GGTGACCCTTAAAGAGCCCTGAT-3’ (reverse primer) relative to the housekeeping gene 18S. Total mRNA concentration was divided by total muscle tissue weight. Myonuclei were detected with immunofluorescence staining with 4’,6-diamidino-2-fenylindool (DAPI) and myonuclear density (MD) was obtained, which is the number of myonuclei per unit volume of cytoplasm.

The results in Figure 8.4 show that not only Mb concentration was lower in the cyclists compared to controls, but also the mRNA expression of Mb (relative to housekeeping gene
Figure 8.4. Myoglobin regulation in cyclists and controls. A) Functional Mb concentration, B) Mb mRNA expression (relative to housekeeping gene 18S) C) Myonuclear domain and D) total mRNA per mg muscle tissue.

18S) was lower. In contrast, total mRNA expression per muscle tissue was similar and myonuclear domain was higher in the cyclists. Moreover, Mb protein concentration and Mb mRNA expression were positively related ($r=0.37$, $p<0.05$).

Despite the high capacity for transcription in the cyclists (indicated by their high MD), these results show a lower Mb mRNA expression level in the cyclists and suggest a lower Mb mRNA expression per myonucleus. Therefore, it seems that transcription of Mb mRNA expression is likely a limiting factor in the regulation of Mb concentration in the cyclists. It remains to be established why the Mb mRNA expressions levels were lower in the group of cyclists. Possibly, the low Mb mRNA expression levels were because the cyclists’ training did not incorporate sufficient high-intensity exercise or exercise in hypoxic conditions. Previous studies have shown that exercise in normoxia and to a larger extent exercise in hypoxia induce local tissue hypoxia that activates translocation of NFATs to the nucleus stimulating Mb mRNA transcription$^{49,50}$. An alternative explanation for the lower Mb mRNA expression levels in these cyclists may also be mRNA degradation by microRNAs in response to resistance and/or endurance exercise$^{51,52}$. Future studies need to investigate how these factors affect the regulation of Mb and how myoglobin concentrations may accommodate increases in oxygen demands and help to improve physical performance. Insights in the mechanisms underlying the low Mb is required to develop training of nutritional interventions to increase the Mb or prevent Mb mRNA or protein degradation. Based on the contribution of Mb to the diffusion of oxygen$^{53}$, it is likely that the cyclist will benefit from this.
The previous paragraphs illustrate that matching of oxygen demand and oxygen supply capacity at the muscle fiber level is important to achieve combinations of a large fiber size and high oxidative capacity in order to ‘escape’ the inverse relationship reported for not specifically trained animal species. Matching of oxygen demand and supply was also illustrated by performance $\dot{V}O_2$ (see section Targets for endurance athletes), which was one of the critical determinants of combined sprint and endurance performance (Chapter 6). Performance $\dot{V}O_2$ could largely be explained by fiber $\dot{V}O_{2\text{max}}$ and the interaction of [Mb] with C/F and PCSA, highlighting the importance of the interplay between these muscle fiber properties and demonstrating the detrimental effect of muscle hypertrophy (i.e. increase in PCSA) for combining a high sprint and endurance performance. Training strategies to combine high sprint and endurance performance may therefore need to focus on the interplay between oxidative capacity, FCSA, PCSA and oxygen supply capacity, such as capillarization and myoglobin concentration (which will be discussed in the section Training strategies to enhance physical performance).

Number of muscle fibers

As hypothesized in the introduction, athletes with a large number of muscle fibers may be able to obtain both a high fiber $\dot{V}O_{2\text{max}}$ and a large PCSA, consisting of muscle fibers of a relatively small cross-sectional area. This way, athletes may still obtain a large muscle volume, necessary for high sprint performance, but also obtain a high fiber $\dot{V}O_{2\text{max}}$ that is beneficial to a high endurance performance. Estimated VL muscle fiber number was, however, not related to combined sprint and endurance performance in Chapter 6. Further analysis of the data from Chapter 6 shows that estimated muscle fiber number was negatively related to iSDH activity ($r=-0.55$, $p<0.01$) and FCSA ($r=-0.57$, $p<0.01$), but was not related to PCSA or muscle volume ($p>0.05$). This suggests that subjects with a large number of muscle fibers did not necessarily have a larger muscle volume or a larger PCSA, but did have less need for combining a high FCSA and fiber $\dot{V}O_{2\text{max}}$ as they achieved a given PCSA with smaller FCSAs. As such, these subjects likely also have less need for high oxygen supply capacity (see previous paragraphs). Indeed, additional analyses show that estimated fiber number was or tended to be inversely related to oxygen supply capacity in the circulation (mean corpuscular volume: $r=-0.38$, $p<0.05$, mean corpuscular hemoglobin: $r=-0.35$, $p=0.069$) and tended to be associated with lower deoxygenation during the endurance performance ([HHbMb]: $r=-0.36$, $p=0.063$). Future studies are warranted to investigate the interactions between estimated muscle fiber number, muscle volume, PCSA, oxygen demands and oxygen supply capacity in more detail, including their adaptations during longitudinal training studies. Note that number of muscle fibers seems to be determined early in life and that the addition of muscle fibers – if any – is limited in mature mammalian muscle.

Muscle fiber length

Whole-muscle architecture is important for combined sprint and endurance performance (see Chapter 6). In the introduction, we suggested that when a muscle is characterized by relatively small but long muscle fibers, the adverse effect of a large FCSA on muscle fiber $\dot{V}O_{2\text{max}}$ may be avoided. In comparison to short and large muscle fibers, these long and small muscle fibers have more surface area for capillary blood supply and a shorter diffusion distance for oxygen to the mitochondria. Such muscle architecture may therefore help to attain high $\dot{V}O_2$ (keeping FCSA and PCSA rather small), but at the same time the long fascicles will induce a high contraction...
velocity\textsuperscript{24} and attribute to sprint performance. Our results in \textit{Chapter 6} show that $L_f$ explained 13-25\% of the variance in $PO_{\text{peak}} + PO_{\text{TT}}$, whereas PCSA was not related to $PO_{\text{peak}} + PO_{\text{TT}}$. $L_f$ and not PCSA, was positively associated with knee extensor specific force, illustrating more effective force transmission from the muscle fibers to the muscle’s line of pull, likely because $L_f$ was associated with smaller pennation angles (see section \textit{Targets for sprinters}). Moreover, PCSA did indeed negatively affect performance $\dot{V}O_2$ (see Equation 6.2), which was a main determinant of combined sprint and endurance performance of our cyclists. These results suggest that a long fascicle rather than a large PCSA is beneficial for achieving high $PO_{\text{peak}} + PO_{\text{TT}}$, thereby mitigating the effect of the inverse relationship between FCSA and muscle fiber $\dot{V}O_{2\text{max}}$.

In addition to this metabolic advantage, long muscle fibers may also be advantageous from a mechanical perspective. This was suggested by the positive correlation between fascicle length and normalized sprint performance in rowers and in a subgroup of road and team pursuit cyclists (\textit{Chapter 4 and 6}). How peak power output is affected by changes in muscle morphology of the vastus muscles, may be investigated by simulation analyses of sprint cycling using a forward dynamic model of the human musculoskeletal system including lower extremity muscle groups\textsuperscript{54,55}. Here, researchers can study how optimal peak power production differs between a reference model and a model including vastus muscles of similar volume, but with increased fiber length and reduced PCSA. It is of interest to assess whether these induced morphological differences shift the optimal peak power production of the muscle towards higher contraction velocities and how this influences whole-body peak power production and optimum pedaling frequency.

Cross-sectional findings in this thesis highlight the importance and positive impact of a vastus muscle architecture that is characterized by long fascicles rather than a large PCSA. However, longitudinal training studies must expand our knowledge on the (training) effects of muscle architecture on (combined) sprint and endurance performance. Optimal fascicle length depends on the number of sarcomeres in-series\textsuperscript{56,57}. There are several training strategies that seem to induce an increase in $L_f$. In competitive soccer and rugby players, 5-wk plyometric sprint-jump training resulted in a 24.9\% increase in $L_f$\textsuperscript{58}. In addition, physically active students which were subjected to explosive resistance training with light loads showed a ~10\% elongation of fascicles\textsuperscript{59}. Moreover, in young males, $L_f$ increased more after 10-wk eccentric resistance training (+12\%) than after concentric resistance training (+5\%)\textsuperscript{60}. It remains to be established how these training strategies translate to the addition of sarcomeres in-series and whether changes in $L_f$ are predominantly induced by range of muscle excursion, contraction velocity, or the length at which a muscle is mostly active\textsuperscript{56,57}. Although an increase in $L_f$ seems beneficial, it should be noted that muscle function alters with an increase in $L_f$, as an increase in number of sarcomeres in-series changes muscle optimum length\textsuperscript{27}, force-length and force-velocity characteristics and shifts peak power production to higher contraction velocities\textsuperscript{24,56}. Future studies may also need to consider the concomitant increases in muscle thickness (hypertrophy) observed during these training interventions\textsuperscript{58–60} and the smaller effects on pennation angle\textsuperscript{58–60}, which may be favorable to a higher knee-extensor specific force. Question is whether training interventions aimed to increase the agonist’s fascicle length also affect the antagonist’s muscle architecture? Moreover, in the design of the training interventions one needs to consider what muscle architecture would be ‘optimal’ for the sport-specific task. Our cross-sectional data illustrates that long fascicles, and not a large PCSA, are advantageous for combined sprint and endurance performance, however,
the question remains how muscle fiber lengthening may be achieved with longitudinal training interventions and how this would affect combined sprint and endurance performance.

**Training strategies to enhance physical performance**

Prime skeletal muscle fiber determinants, such as oxidative capacity, muscle fiber size and oxygen supply capacity, are essential to physical performance (see section *Comprehensive physiological profile*) and illustrate why combining a high sprint and endurance performance may be difficult (see section *Sprint versus endurance*). Therefore, we investigate adaptations of these prime skeletal muscle fiber determinants in response to a promising new training strategy of living at altitude and training low combined with repeated-sprint training in normoxia or hypoxia.

In collaboration with the French Institute of Sport (France), Aspetar (Qatar), the University of Lausanne (Switzerland) and University of Leuven (Belgium), we had the opportunity to investigate skeletal muscle adaptations in response to a well-controlled training intervention in (inter)national field-hockey players. In team sports, such as field hockey, maximal or near-maximal intensity sprints are repeated throughout the match, often with insufficient recovery, and therefore, repeated-sprint ability is a crucial fitness component of athletes engaged in these disciplines\(^6^1\).

To improve repeated-sprint ability, team-sport athletes must concurrently train to improve peak power production required during maximal efforts as well as oxidative metabolism to speed up recovery between efforts\(^6^1^-^6^3\), which may be challenging given the inverse relationship between fiber size and oxidative capacity ([Chapter 6])\(^1^,^2\). Previously, this research group showed that these elite team-sport athletes improved their repeated-sprint ability and incremental field performance after a 2-wk altitude training using a live high-train low (LHTL) regimen with repeated-sprint training in hypoxia and normoxia\(^6^4\). During the intervention, subjects resided at simulated altitude (≥14 h∙d\(^{-1}\) at 2800-3000 m) and performed regular training plus six repeated-sprint sessions in normobaric hypoxia (\(F_{\text{O}_2}\) ~14.2%, 3000 m; LHTLH) or normoxia (\(F_{\text{O}_2}\) 20.9%, 0 m; LHTL) or resided at sea level with regular training only (LLTL). Superior improvements of repeated-sprint ability were observed for the LHTLH group in comparison with the LHTL and LLTL group\(^6^4\). In [Chapter 7](#), we investigated changes in muscle oxidative capacity, fiber size and oxygen supply capacity using immuno- and enzyme histochemistry, which might have attributed to the improvements in repeated-sprint ability and incremental field performance in these team-sport athletes.

Our findings in [Chapter 7](#) show that, in line with the superior improvements in repeated-sprint ability, elite team-sport athletes in the LHTLH group were able to substantially increase their mitochondrial oxidative capacity in type I and II fibers (+37% and +32%, respectively), while maintaining fiber size, after only fourteen days of chronic hypoxic residence combined with six repeated-sprint sessions in hypoxia. Such adaptations enable quick recovery from high-intensity sprints, facilitating fast oxygen consumption recovery kinetics\(^6^5\) and fast resynthesis rate of phosphocreatine\(^6^6\). The LHTL showed a smaller increase in oxidative capacity (+9%) with concomitant increases in fiber size, whereas LLTL showed a decrease in oxidative capacity and increase in fiber size. The expansion of muscle fiber size in LHTL and LLTL may be due to myofibrillar hypertrophy and/or cellular edema. Given the inverse relationships between fiber size and oxidative capacity across animal species\(^1^,^2\) and in our cyclists ([Chapter 6](#)), we have now also displayed these results of [Chapter 7](#) with respect to the two inverse relationships (Figure...
Results of both fiber types were combined into a weighted average using their fiber type distribution. Combining the data from the animal species, our cyclists and field-hockey players shows some very interesting results.

The combination of fiber size and oxidative capacity of our elite team-sport athletes before the intervention fitted remarkably well on the inverse relationship observed in our cyclists (especially for the LLTL and LHTLH groups; Figure 8.5), which may reflect a similar training status in these athletes. Similar combinations of FCSA and oxidative capacity (i.e. similar iSDH values) could be obtained with different capillarization and myoglobin concentrations: cyclists revealed a relatively higher capillarization and a relatively low [myoglobin] (CAF = 7.2, [Mb] = 0.38), whereas hockey players revealed the opposite before the intervention (CAF = 5.4, [Mb] = 0.52). Note that CAF was ~33% higher in cyclists and [Mb] ~33% higher in the hockey players. As discussed above (see section Targets for both sprint and endurance), the variance in iSDH activity was partly explained by capillarization in cyclists ($r^2=0.34$) and by capillarization and Mb concentration in hockey players ($R^2=0.51$). Remaining questions are what combinations of fiber size and oxidative capacity will be achieved by other type of athletes, for example rowers, speed-skaters or runners? Are these values also dependent on the training status of the athlete, and how do these relate to the athlete’s oxygen supply capacity?

Despite the already high combination of fiber size and oxidative capacity before the intervention, athletes in LHTL and LHTLH groups were able to further improve this combination after the intervention: LHTLH increased their oxidative capacity and LHTL increased both fiber size and oxidative capacity, whereas LLTL moved along the curve of the cyclists by increasing their fiber size and reducing their oxidative capacity (Figure 8.5). After pooling data of LHTLH, LHTL and LLTL, additional analyses revealed that the change in iSDH activity from pre to
post-1 was positively associated with changes in capillarization (CAF: \( r=0.55, p<0.05 \); and C/F: \( r=0.55, p<0.05 \)), but not with changes in Mb (\( p>0.05 \)). Likely, this is because \([\text{Mb}]\) decreased in the LHTLH group from pre to post-1, which may relate to hemoglobin synthesis in LHTLH\textsuperscript{64} that poses high demands for iron and may eventually lead to reductions in skeletal muscle iron stores, reducing functional Mb protein expression. Taken together, the biopsy studies of the cyclists and hockey players show that these athletes are able to maintain a skeletal muscle fiber size and oxidative capacity such that their integrated SDH is higher than one would expect based on the inverse relation across species\textsuperscript{1,2}, with substantial variation in capillarization and \([\text{Mb}]\). The question is what combinations of fiber size and oxidative capacity athletes can achieve if they are able to substantially improve both their capillarization and Mb concentration, and how they may do this.

Simultaneous increases in FCSA and oxidative capacity are difficult to obtain, and therefore skeletal muscle adaptations for the optimal combination of both sprint and endurance performance appear to be complex, requiring sophisticated modulation of training intensity, timing and mode. A schematic overview of potential regulatory pathways involved in the complex skeletal muscle adaptations is provided (Figure 8.6), as a simplified summary of previous research\textsuperscript{1,47,49,50,65–80}. During endurance exercise, sustained contractile activity and low energy status (i.e. increased AMP:ATP ratios) induce signaling via calcium and AMPK (i.e. ‘5 adenosine monophosphate activated protein kinase) to activate PGC-1\( \alpha \), which plays an important role in the mitochondrial biogenesis and regulation of the oxidative capacity within the muscle\textsuperscript{47,72,74,77,78}. During resistance exercise, mechanosensing of high-force contractions induces activation of the IGF / Akt / mTOR pathway, stimulating synthesis of contractile elements within the muscle\textsuperscript{47,69,70}. In addition, Akt inhibits protein degradation via down-regulation of ubiquitin ligases\textsuperscript{68}, causing a positive balance between protein synthesis and degradation that will lead to muscle fiber hypertrophy (increased FCSA). Hypoxia (preferably combined with exercise) is an important stimulus for adaptations

Figure 8.6. The interplay between skeletal muscle adaptations for fiber size, oxidative capacity and oxygen supply capacity is complex and relates to many signaling pathways and interactions. Sports practitioners and scientists should consider this complexity and the observed interferences with training when designing training strategies for optimal (combined) sprint and endurance performance (see text). Arrows indicate positive association or excitation, perpendicular lines indicate negative association or inhibition.
in oxygen supply capacity. If the muscle senses a lower cellular oxygen tension ($P_{O_2}$), HIF-1α is stabilized and induces transcription of genes for red blood cell formation, capillary growth and glycolytic energy metabolism. Chronic hypoxic residence has been shown to increase red blood cell mass, even in well-trained athletes. However, only high-intensity training in hypoxia leads to transcription of vascular endothelial growth factor (VEGF) and myoglobin (Mb) via NFAT, which enhance capillary growth and $O_2$ transport within the muscle fibers, respectively. Magnitude of increases in Mb protein are not the same as those in functional Mb, as Mb protein may already increase before the incorporation of iron, necessary for Mb to become functional. Similarly, there may be a discrepancy between transcription and translation during angiogenesis, i.e. with endurance training at supramaximal intensities VEGF mRNA expression may be increased, whereas increases in capillarization and VEGF protein expression may be abolished. Interference between pathways may occur between AMPK and mTOR, reducing muscle fiber hypertrophy. Hypoxia may attenuate the rate of translation and therefore diminish muscle hypertrophy or even result in muscle atrophy (for review see Ref). Prolonged residence in severe chronic hypoxia ($\geq$40 days and at $>$5000 m) has been shown to result in about 15–25% reductions in FCSA. The mechanisms for atrophy with exposure to hypoxia are not only depend on severity and duration of hypoxic exposure (i.e. hypoxic dose), but certainly are more complex, potentially also involving changes in protein turnover rate and regulation of muscle protein synthesis and breakdown (for review see Ref). Recently, it has also been shown that the IGF pathway may inhibit signaling for oxygen supply capacity, i.e. phosphorylation of mTOR inhibits activation of myoglobin mRNA expression. Therefore, sports scientists and sports practitioners should consider the complexity of these skeletal muscle adaptations and find ways to modulate frequency, intensity, duration and mode of endurance and resistance training to minimize interference between both modalities and to reach optimal combinations of fiber size and oxidative capacity to obtain optimal combinations of sprint and endurance performance.

**Technological developments**

To allow advanced monitoring of training adaptations, we have incorporated technological developments of non-invasive tools within this thesis, addressing near-infrared spectroscopy (NIRS) and a 3D ultrasound imaging technique. These techniques may not only provide important physiological determinants of physical performance, but may also improve the monitoring of training adaptations in future studies.

NIRS provides non-invasive measures of the balance between oxygen delivery and consumption in the muscle, and therefore may help to 1) quantify the oxygen supply capacity of the athlete, 2) prescribe training and 3) monitor training intensities. In Chapter 2, we have used near-infrared spectroscopy (NIRS) to determine occurrence of a mismatch between $O_2$ supply and demand, i.e. when oxygen supply becomes insufficient for the muscle to deliver all energy with use of oxygen, resulting in higher reliance on (intra)muscular glycogen stores and accelerated exhaustion. Although the $\Delta [O_2HbMb-HHbMb]$ breakpoint is potentially a suitable exercise threshold revealing when anaerobic energy production starts to increase in the muscle, the first ventilatory threshold being a rather indirect measure of these changes in energy status of the muscle, discriminates better across sexes and training status, showed higher reproducibility,
and was not affected by adipose tissue thickness. If the effects of adipose tissue thickness on NIRS tissue saturation and symmetry of the \([O_2HbMb]\) and \([HHbMb]\) amplitude can be diminished or corrected for, then NIRS may provide a valuable tool to assess \(\dot{Q}_{O_2}/\dot{V}_{O_2}\) matching in vivo, with a high practical use in the field of sports or rehabilitation.

3D ultrasound imaging approaches have previously been used\(^{90-93}\), but have proven cumbersome, time consuming and technically limited (only small segments of large muscles could be reconstructed). In Chapter 3, we show that with our modifications of the 3D ultrasound technique, substantial improvements in processing speed (~99%) and reconstructions of large muscles (such as the VL) could be provided. We showed that the 3D ultrasound is a reproducible and valid technique for measurements of muscle morphology (r>0.98), whereas it is less expensive, less time-consuming and less spatially constrained than 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 from the same 3D voxel array.

**Future directions for research, training and monitoring**

**Sprint and endurance performance**

Though physical performance is commonly distinguished in endurance or sprint performance; future studies should address combined sprint and endurance performance, for example using our approach in Chapter 6. Sprint and endurance performance can be measured sport-specific to facilitate high specificity of the results or can be studied sport-transient to compare performances of different types of athletes. For the latter, cycling would be a good option as it is a generic movement that can be well standardized and for which performance is less sensitive to differences in technique compared to other sports. It should be confirmed by other studies whether the monitoring tool incorporating measures of the athlete’s sprint and endurance performance (described in Figure 8.2) is useful to quantify differences in training status both within and between athletes of different sports and to monitor training adaptations of individual athletes. In addition, long time monitoring with this tool will show its potential for talent identification. Critical physiological determinants for achieving (both a) high sprint and high endurance performance may need to be studied in more detail across various sports disciplines, using a comprehensive approach to understand the interplay between physiological systems that contribute to physical performance. Importantly, the question how training (e.g. concurrent resistance and endurance training) may alter the comprehensive physiological profile of athletes and their (combined) sprint and endurance performance needs further investigation.

**Oxygen supply and demand matching**

Future studies are warranted to investigate the interplay between fiber size, fiber number, oxidative capacity and oxygen supply capacity in different types of athletes and to assess their adaptations in response to training interventions, such as endurance training in hypoxic conditions or concurrent resistance and (polarized) endurance training. Furthermore, the mechanisms and interactions for structural adaptations in capillarization, myoglobin and hemoglobin should be further explored as well as assessment of mitochondrial oxidative overcapacity to improve our knowledge on the oxygen supply limitations during exercise. The nature of this overcapacity may
be understood in light of differences in $\dot{V}O_{2\text{max}}$ in hyperoxic versus normoxic conditions. These insights ought to be supplemented with knowledge on the spatial and temporal dynamics of $\dot{Q}O_2/\dot{V}O_2$ matching during exercise (e.g. with assessment of cardiac output, leg blood flow, mixed venous desaturation, oxygenation, capillary blood flow, vasodilation, open/closed capillaries). If the effects of adipose tissue thickness (including the impact of wavelength and temperature) and asymmetry between the [$O_2HbMb$] and [$HHbMb$] signals can be diminished or corrected for, then NIRS may provide a valuable non-invasive tool to assess $\dot{Q}O_2/\dot{V}O_2$ matching in vivo and even provide a proxy-measure of $P0_{2\text{crit}}$. Additionally, it would be very interesting to see how the oxygen supply limitations and $\dot{Q}O_2/\dot{V}O_2$ matching from chronic heart failure patients to professional athletes contribute to oxygenation- and $\dot{V}O_2$-kinetics, which could be very valuable within the context of daily-life activities.

**Muscle morphology**

As previously mentioned, future research should explore how muscle fiber lengthening by addition of sarcomeres in-series may be achieved with longitudinal training interventions and how this impacts combined sprint and endurance performance. One may need to consider concomitant changes in pennation angle, muscle hypertrophy as well as adaptations in whole-muscle specific force. The question is whether training interventions aimed to increase the agonist’s fascicle length also affect the antagonist’s muscle architecture and what muscle architecture is ‘optimal’ for the sport-specific task. In light of sprint performance, maintenance of high speeds may be a new focus point, which may be investigated in relation to muscle morphology (e.g. in context of inertia). To gain better insights in how a high knee extensor specific force may be obtained, specific measurements of optimal knee joint angle and optimal fascicle length are warranted, whereas 3D ultrasound imaging may enable estimation of muscle moment arms. Moreover, one may need to consider regional differences in muscle thickness, pennation angle and fascicle length along the length of the muscle belly, and assess differences in muscle morphology between active versus passive state, as well as measure muscle morphology of more of the important muscles involved in the sport-specific task. From a practical point of view, a mobile set-up consisting of smaller camera system and compact ultrasound device may be very useful to measure at the site of sports practice or in the clinic. As 3D ultrasound imaging is an excellent tool to monitor changes in muscle architecture and volume, we pursue its implementation in sports practice to assess training adaptations and in the clinic for diagnostics and monitoring the effects of disease and aging and medical interventions.

**Practical applications**

- **Practical tool to quantify athletic performance.** Performance measures obtained from two simple exercise tests – a Wingate and incremental exercise test – can be used to characterize athletes within the sprint-endurance continuum and assess how well athletes combine sprint and endurance performance, illustrating an athlete’s training status (Figure 8.2). Implementing this tool in common sports practice will likely help coaches and top-level athletes to improve performance by facilitating future talent identification, tuning athletes to their preferred sports discipline and by optimizing and monitoring adaptations to individual training strategies. For example, the Royal Dutch Cycling
General discussion

Union can organize national selection days using two bicycle ergometers to perform a Wingate and incremental exercise test to screen for future talents per cycling discipline.

- **Comprehensive physiological profile.** To understand how athletes achieve this athletic performance, assessment of the comprehensive physiological profile of the athlete is likely very helpful. The profile will illustrate the athlete’s strengths and weaknesses with respect to their sport-specific discipline, which can then be used as input for individualized training strategies. Comparison of (adaptations in) these physiological determinants to that of benchmark physiological data from athletes of a certain training status and discipline can help to monitor progress of training interventions and talent development. At the start of the season, coaches could perform exercise testing, blood sampling and 3D ultrasound imaging to obtain key physiological characteristics of their athletes and test after the (pre) season how the determinants and performance of their athletes have changed.

- **Benchmark data.** This thesis contains benchmark data for performance of Olympic rowers and (inter)national to Olympic cyclists, such as displayed in the monitoring tool. Moreover, the thesis contains benchmark data for physiological determinants of Olympic rowers, (inter)national to Olympic cyclists, and (inter)national hockey players. These values can be used as a reference for sports practitioners in the context of talent identification or monitoring of training adaptations.

- **Training targets.** Critical determinants of sprint, endurance and combined sprint and endurance performance have been obtained in this thesis. Sprint performance requires fast type muscle fibers, long muscle fibers and a large muscle volume, whereas endurance performance benefits from high gross efficiency and high performance $\dot{V}O_2$. Combined sprint and endurance performance is enhanced with a large muscle volume, long muscle fibers, high efficiency and high performance $\dot{V}O_2$, which relates to a well-developed $Q_{O_2}/\dot{V}O_2$ matching. These targets for training and talent identification have been discussed in detail above, including considerations for training interventions. Sports scientists and practitioners need to optimize their training strategies to enhance these training targets.

- **Training strategies.** Skeletal muscle fiber determinants are essential to physical performance, and illustrate why combining a high sprint and endurance performance may be difficult. 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 normoxia or hypoxia. As illustrated by Figure 8.5, this may be a very valuable training strategy for concurrent improvements in fiber size and oxidative capacity, facilitating peak power production as well as oxidative metabolism, and therefore, be very suitable for enhancement of (combined) sprint and endurance performance. For sports practice, it would be very valuable to incorporate such a training strategy during altitude camps. The effectiveness of such a training strategy in athletes could be assessed with measurements of hemoglobin in the blood and the difference between $\dot{V}O_{2_{max}}$ in normoxia and hyperoxia (indication for central or peripheral limitations - see above).

- **Technological advances.** To allow advanced monitoring of training adaptations, we have incorporated technological developments of non-invasive tools within this thesis. Near-infrared spectroscopy requires further research to correct for the effects of adipose tissue and asymmetry of the [O$_2$HbMb] and [HHbMb] signals, before this potentially valuable and practical technique could be used for assessment of $Q_{O_2}/\dot{V}O_2$ matching.
during exercise and training. 3D ultrasound imaging has proven to be an excellent tool to monitor muscle architecture and volume and has great potential for implementation in sports practice for assessment of training adaptations and in the clinic for diagnostics and monitoring the effects of disease and aging.
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