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General introduction
Limits of human physical performance
What makes an athlete a champion? Winning the Tour de France. It is the dream of most cyclists, though few can say they actually accomplished this extraordinary performance. This year, in 2017, Christopher Froome managed to win his fourth Tour de France, putting him a hair's breadth away from all-time record holders Eddy Merckx, Jacques Anquetil, Bernard Hinault and Miguel Indurain. To win the Tour de France, one has to push the limits of human physical performance: three weeks of cycling for more than 3500 kilometres, climbing mountains of up to 20% gradients, but also reaching very high speeds during time trials and during sprints towards the finish line at the end of a race. Physical performance is influenced by many factors, such as coordination, tactics, aerodynamics, environmental conditions, mental strength to endure pain and adequate recovery. However, the athlete's physiology is key to his or her potential for reaching optimal performance. For endurance performance, this potential is explained by efficiency, maximal oxygen uptake (\(\dot{V}O_{2\text{max}}\)), to use oxygen for prolonged energy delivery, and the lactate threshold, reflecting the sustainable power beyond which the athlete cannot exercise comfortably for a prolonged time\(^1\). Although elite athletes may score exceptionally high on one of these three determinants, it is uncommon to score high on all three determinants, even among elite athletes. Physiological testing of Chris Froome, however, revealed that he possesses a unique combination of a high gross efficiency, an exceptionally high \(\dot{V}O_{2\text{max}}\) and high sustained power at his lactate threshold\(^2\). This exceptional physiology likely attributed to Froome’s four victories on the Champs-Elysées in Paris (see Figure 1.1). To understand what makes an athlete a champion, the major question is: Does the athlete’s physiology provide insights in how athletes deliver their extraordinary performances?

![Chris Froome rides towards his fourth Tour de France win in front of the Arc de Triomphe.](image)

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Aim
The aim of this dissertation thesis was to understand what makes an athlete a champion? Hereto, we aimed to unravel the physiological determinants of physical performance and to implement these insights into training strategies to enhance physical performance.
Physical performance is commonly distinguished in endurance or sprint performance; however, 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.

Maximization of (combined) sprint and endurance performance is complex, because their physiological determinants exist at different biological levels, which interact. Moreover, (determinants of) sprint and endurance performance have shown to be mutually exclusive. These challenges require a new approach - such as incorporating a comprehensive physiological profile of the athlete - to understand the interplay between physiological systems that contribute to physical performance.

In this thesis, we focus on elite athletes, who have optimized their physical performance. To understand what makes an athlete a champion, we have 1) obtained the athlete’s physical performance and his/her comprehensive physiological profile, 2) implemented technological developments to non-invasively characterize skeletal muscle determinants and 3) assessed skeletal muscle adaptations to a novel training strategy.

- **Physiological profile.**
  We obtained the athlete’s physiological profile to investigate and integrate physiological determinants from multiple biological levels in order to obtain a more detailed understanding of physical performance; which provides insights in the key determinants, the most optimal combination of determinants, and the relative contribution of determinants to (combined) sprint and endurance performance.

- **Technological developments.** Elite athletes want to avoid using invasive procedures to quantify their physiological determinants and monitor training adaptations, and therefore, we have adopted non-invasive technologies to study skeletal muscle determinants, which are incorporated in the physiological profile. 
  **Near-infrared spectroscopy (NIRS)** was used to determine the (mis)match of oxygen supply and oxygen demand during exercise. 
  **3D ultrasound imaging** was developed to quantify muscle mass and muscle architecture.

- **Training strategies.**
  Oxygen supply capacity may help to improve the combination of sprint and endurance performance. Training in hypoxic conditions has been shown to improve oxygen supply capacity and to increase the oxidative capacity of the muscle fibers. Therefore, we investigated training adaptations to a promising training strategy including living at altitude and training low combined with repeated-sprint training in normoxia and hypoxia. Here, we assessed changes in skeletal muscle fiber size, oxidative capacity and oxygen supply capacity.

We strongly encourage sport scientists and coaches to assess performance and a comprehensive physiological profile in top-level athletes. These two allow identification of critical determinants of (combined) sprint and endurance performance, investigating how physiological determinants...
from various biological levels (interact to) explain performance. Such physiological determinants can then be used as training targets for the development of training strategies to improve physical performance. Moreover, these insights may lead to benchmark data, allowing identification of young talent and monitoring of talent development. For individual athletes this approach will reveal his/her strength and weaknesses, and it allows coaches to adjust training strategies for the individual athlete and monitor training adaptations more specifically. Our belief is that this approach will enable individualized training, which will open new doors for coaches and top-level athletes to accomplish new important marginal gains in physical performance. But first, let us get into this physiological profile in more detail.

**Physiological determinants of performance: an integrative approach**

Physical performance is commonly understood in terms of endurance or sprint performance. Endurance performance is reflected by the power that an athlete can sustain for a prolonged duration, whereas sprint performance depends on the maximal force and/or power generating capacity of the athlete. The athlete produces this power by contracting his skeletal muscles, however, performance determinants exist on many biological levels, such as the molecular, cellular, whole-muscle, organ and whole-body level, which may interact.

For endurance performance, major whole-body determinants are the athlete’s efficiency, $\dot{V}O_{2\text{max}}$ and lactate threshold (as mentioned in previously)\(^1\). Whole-body gross efficiency is robustly measured\(^3\,^4\) and relates to the proportion of slow type muscle fibers within the muscles\(^5\). Slow muscle fibers possess slow myosin heavy chain proteins that are more efficient at lower contraction velocities\(^6\), and therefore are presumed to be more efficient at common pedaling frequencies during cycling\(^1\). Whole-body $\dot{V}O_{2\text{max}}$ and the lactate threshold result from the demand and supply of oxygen within the skeletal muscles. Oxygen demand depends on the muscle fiber’s capacity to utilize oxygen (i.e. fiber $\dot{V}O_{2\text{max}}$) reflected by the maximal aerobic enzyme activity\(^7\,^8\). Oxygen supply to the muscle fiber depends on lung diffusion, ventilation capacity, pumping capacity of the heart (i.e. heart rate and stroke volume), concentration of hemoglobin carrying oxygen in the blood, muscle capillary density and concentration of myoglobin carrying oxygen within the muscle fiber\(^9\,^10\,^11\). Therefore, many physiological determinants from various biological levels interact to contribute to endurance performance.

Sprint performance requires fast accelerations\(^12\). To induce fast accelerations, the athlete needs a high whole-body power generating capacity, such as induced by a high lean body mass and large muscles\(^13\), as well as a well-developed whole-body neuromuscular coordination (e.g. a high voluntary muscle activation)\(^14\). In order to maintain a high sprint performance, fatigue should be minimized, as fatigue compromises the power generating capacity of the muscle\(^15\). At the whole-muscle level, size is important for power generating capacity\(^13\). This muscle size is composed of muscle fiber length and physiological cross-sectional area (PCSA). A long muscle fiber (i.e. high numbers of sarcomeres in-series) has theoretically been associated with a high maximal muscle fiber contraction velocity\(^16\). Contraction velocity is also associated with the proportion of fast type muscle fibers and pennation angle of muscle fibers. However, power is not only a function of velocity, but also a function of force. A large PCSA (i.e. summation of all fiber cross-sectional areas in parallel) together with a high specific force (force per PCSA) contribute to the maximal force generating capacity. Maximal force generating capacity therefore relates to
the fiber cross-sectional area (FCSA) and number of muscle fibers arranged in parallel, but also to factors that may influence specific force, namely the proportion of fast type muscle fibers, muscle moment arm and pennation angle of the muscle fibers\textsuperscript{17}. Other factors such as anaerobic metabolism and lactate clearance also impact sprint performance\textsuperscript{18}. Therefore, many physiological determinants from various biological levels interact to contribute to sprint performance.

So, it has been illustrated that there are many physiological determinants of sprint and endurance performance that exist at different biological levels, which interact. Though physical performance is commonly distinguished in endurance or sprint performance; physical performance often depends on a combination of sprint and endurance (such as in cycling, rowing, hockey, and speed-skating). But how is this illustrated in a sport such as cycling?

**Figure 1.2. Cyclists of different disciplines have different physiological characteristics.** Olympic track sprinter Robert Förstemann (left) has to deliver a high peak power output and possesses exceptionally large muscles, whereas Olympic road cyclist Chris Froome (right) aims to sustain high peak power output for prolonged time and possesses smaller muscles, but is capable of using high amounts of oxygen to ensure prolonged delivery of energy. © Martijn Krullaars (left) © ANP (right).

**Performance requires both sprint and endurance**

Cycling almost completely covers the sprint-endurance continuum, with cycling events ranging from 10-s sprint events up to 6-h road stages. Sprint events are featured by extremely high power production\textsuperscript{19} and elite track sprinters are characterized by their high peak power outputs (~1500-2000 W)\textsuperscript{20–22}. In contrast, road stages mostly consist of moderate intensity cycling (~200 W)\textsuperscript{23} for long duration, or consist of prolonged power production during time trials (>430 W for time trial specialists in short time trials)\textsuperscript{24}, which both predominantly rely on energy delivery with oxygen. Still, road cyclists are also capable of producing short, high-power sprints during breakaways (>1000 W)\textsuperscript{25} or end spurts (~1250 W)\textsuperscript{22} in order to win the road stage. Therefore, road cyclists also benefit from development of their sprint performance. Moreover, short-lasting track events, such as the team sprint and 1000-m time trial, already require substantial energy delivery with oxygen (up to 40% and 50% of energetic demands, respectively). Therefore, track sprinters also benefit from development of their endurance performance\textsuperscript{19}. In all disciplines, athletes are challenged to optimally combine their sprint and endurance performance, even though their relative contribution may differ between disciplines. So, cycling is a useful model to study how athletes combine sprint and endurance performance. Of note is that sprint performance of road cyclists is not as high as that of track sprinters and vice versa for endurance performance. Therefore,
it seems difficult to obtain both a high sprint and endurance performance simultaneously, but why?

**Combining high sprint and endurance performance is a challenge**

Training for both sprint and endurance is not easy. Endurance athletes perform a lot of endurance training, whereas sprinters perform many hours of resistance training. However, combining both resistance and endurance training may hamper the build-up of muscle mass and consequently that of maximal force generating capacity (and sprint performance). When athletes perform resistance and endurance training concurrently instead of resistance training only, muscle hypertrophy and strength gains are limited\(^{26,27}\). This is known as the “interference effect”\(^{28}\). The interference is more pronounced if an individual performs many and/or long endurance training sessions\(^{29,30}\). Therefore, the interference effect of concurrent training is particularly relevant for elite athletes, who train frequently (often twice a day) and reach large training volumes.

Prime determinants of physical performance in humans are skeletal muscle endurance and maximal power generating capacity\(^{31,32}\). As previously mentioned, a large muscle fiber size contributes to its maximal force and power generating capacity and consequently to sprint performance. High skeletal muscle endurance requires muscle fibers with a high oxidative capacity, which are important for prolonged delivery of ATP using oxygen within the mitochondria. Studying these muscle fiber properties in more detail, reveals that fiber size (FCSA) and oxidative capacity (fiber \(\dot{V}O_2_{max}\)) are inversely related across multiple animal species (see Figure 1.3)\(^{31,32}\). With an increase in muscle fiber size, the mitochondrial oxidative capacity of the muscle fiber will decrease, and vice versa. This inverse relationship across species is in line with the interference effect found in humans and may explain why combining high sprint and endurance performance is a challenge already at the muscle fiber level.

![Figure 1.3. Fiber size and mitochondrial oxidative capacity of a muscle fiber are inversely related across animal species. Data adopted from Van Wessel et al.\(^{30}\) and Van der Laarse et al.\(^{31,32}\).](image)

Is it still possible to combine a large fiber size with high oxidative capacity at the muscle fiber level? The inverse relationship between fiber \(\dot{V}O_2_{max}\) and fiber size indicates that oxygen demand
(oxygen consumption by the mitochondria) and oxygen supply (transport of oxygen towards the mitochondria) are matched at the muscle fiber level. In order to attain a high maximal oxygen consumption in the muscle fibers, an athlete depends on the amount of oxygen that is available to his mitochondria, which is determined by 1) the amount of oxygen that is diffused in the lungs and transported in the blood by hemoglobin, 2) the diffusion of oxygen from the small vessels and capillaries to the muscle fiber, and 3) the transport of oxygen in the muscle fiber to the mitochondria by intracellular myoglobin. For muscle fibers with a larger cross-sectional area, oxygen needs to travel a larger distance (larger diffusion distance) from the outside of the cell to the core of the muscle fiber, which requires a better oxygen supply to ensure availability of oxygen to mitochondria in the centre of the muscle fiber. In other words, larger muscle fibers require a higher interstitial oxygen tension on the outside of the cell (PO2crit) to provide all mitochondria with enough oxygen and to prevent local hypoxia within the muscle fiber when maximally activated. In contrast, insufficient oxygen supply may lead to prolonged exposure to local hypoxia, which presumably reduces protein synthesis rate. This may eventually lead to a reduction of contractile proteins, so that muscle fiber cross-sectional area (and diffusion distance) reduces. This way, a new equilibrium is set. A possible strategy to obtain a combination of both a high fiber \( \dot{V}O_{2\max} \) and large fiber size is therefore to optimize the oxygen transport to the mitochondria, which may help to avoid interference between sprint and endurance. This requires adjustments of all elements within the oxygen transport cascade from ambient air to the mitochondria: the stronger the elements of the oxygen transport cascade, the higher the combination of muscle fiber size and oxidative capacity may become.

World-class athletes in sports such as rowing, cycling and speed skating show that it is not impossible to combine both a relatively high peak power production and good endurance performance. Currently, very little is known on how these athletes are able to deliver both a high sprint and endurance performance. There are three potential adaptations or (genetic) predispositions that may help to circumvent or mitigate the effects of the interference between sprint and endurance. 1) A combination of high fiber \( \dot{V}O_{2\max} \) with large (and strong) fibers may be accommodated by a well-developed oxygen supply capacity. This includes oxygen supply by myoglobin within the muscle fiber and oxygen supply towards the muscle fibers by capillaries, blood (hemoglobin and hematocrit), heart and lungs. 2) Athletes may demonstrate a high fiber \( \dot{V}O_{2\max} \) with a relatively small fiber size, but have many muscle fibers. As such, athletes may be able to combine a large muscle volume, necessary for high sprint performance, with a high fiber \( \dot{V}O_{2\max} \). Muscle fiber number seems to be determined early in life and does not change with training. 3) When a muscle is characterized by relatively small but long muscle fibers, the adverse effect of a large FCSA on muscle fiber \( \dot{V}O_{2\max} \) may also be avoided. Such muscle architecture may help to attain high \( \dot{V}O_2 \) (keeping FCSA and PCSA rather small), but simultaneously the long muscle fibers facilitate a high contraction velocity that attributes to sprint performance. Although maximization of both sprint and endurance performance may require (a combination of) high oxygen supply capacity, muscle fiber number and relatively long muscle fibers, it remains to be established to which extent each of these potential factors determine combined sprint and endurance performance.

To date, the optimal physiological profile (that integrates determinants from multiple biological levels) for achieving a high sprint, high endurance or both a high sprint and endurance performance remains unknown. The leading question in this thesis was to investigate how the
comprehensive physiological profile of athletes may help to obtain a more detailed understanding of physical performance, providing insights in the key determinants, the most optimal combination of determinants, and the relative contribution of determinants to (combined) sprint and endurance performance. Optimal physiological profiles found among athletes can serve as benchmarks that may be used for talent identification, but also provide athletes and their coaches with their strengths and weaknesses in comparison to the optimal profile of their discipline. This makes it possible to set training targets that are customized to the individual athlete and will improve monitoring of training adaptations.

**Technological developments**

As part of the physiological profile, we have studied physiological determinants of skeletal muscle using non-invasive technologies. Athletes want to avoid using invasive procedures to quantify their physiological determinants and monitor training adaptations, and therefore, we have addressed two non-invasive techniques in this thesis, namely near-infrared spectroscopy (NIRS) and 3D ultrasound imaging.

NIRS was used to determine the (mis)match of oxygen supply and oxygen demand during exercise. The point at which oxygen supply to the muscle becomes insufficient to deliver all energy with use of oxygen, can be used to quantify the oxygen supply capacity of the athlete and potentially to prescribe and monitor exercise intensities during training. During a maximal incremental exercise test to voluntary exhaustion, at a certain exercise intensity, the oxygen supply becomes insufficient to deliver all energy with use of oxygen. Though this critical exercise intensity is generally determined from ventilation or blood lactate concentrations, NIRS may more directly reflect when oxygen supply becomes insufficient, as it measures the balance between oxygen consumption and oxygen supply within the muscle\(^\text{11,33}\). Moreover, NIRS has the practical advantage to be used in the field, as it is a wireless technology.

3D ultrasound imaging was developed to monitor muscle mass and muscle architecture. This technique allows quantification of muscle fascicle length, physiological cross-sectional area of the muscle and even estimation of the number of muscle fibers (in combination with the average fiber cross-sectional area determined from a muscle biopsy). We assessed validity and reliability of this 3D ultrasound imaging technique for analysis of muscle morphology. 3D ultrasound imaging allows monitoring of muscle mass and architectural adaptations of muscle in response to training and/or injury and is less expensive, less time-consuming and less spatially constrained than Magnetic Resonance Imaging.

**Training strategy**

In addition to unraveling physiological determinants of performance, we assessed adaptations of prime skeletal muscle determinants to a training intervention using hypoxic conditions. Hypoxia is likely an essential training stimulus for optimizing both sprint and endurance performance. In hypoxic conditions, such as living at altitude, the oxygen tension is lower and induces additional strain for the oxygen cascade to deliver oxygen to the muscle fibers. Thereby, hypoxic conditions trigger enhancement of the oxygen cascade from air to mitochondria to enhance oxygen supply capacity\(^\text{34,37}\). Living at altitude elicits release of erythropoetin (EPO) that leads to improved oxygen carrying capacity of the blood by red blood cell formation and increased
hemoglobin\textsuperscript{38}. In addition, training in hypoxic conditions has been shown to improve oxygen supply capacity in the muscle by enhancing capillary density and myoglobin concentration, and simultaneously increases the oxidative capacity of the muscle fibers\textsuperscript{34,37}. Repeated-sprint training may even further augment these skeletal muscle adaptations, as it provides an additive training stimulus by lowering the oxygen tension within the muscle\textsuperscript{39,40}. In this thesis, we will determine in elite athletes how prime determinants of physical performance (i.e. skeletal muscle fiber size, and oxidative capacity) and oxygen supply capacity adapt to living at altitude combined with repeated-sprint training in hypoxic or normoxic conditions.

**Aims and outline of the thesis**

The aim of this thesis was to understand what makes an athlete a champion? Hereto, we have 1) tested and improved technological tools to characterize physiological determinants of skeletal muscle non-invasively 2) obtained key physiological determinants of the athlete’s physical performance using a comprehensive physiological profile, and 3) assessed skeletal muscle adaptations to a training strategy with high potential to improve both sprint and endurance.

**Technology**

In the first two chapters, we describe two non-invasive techniques to determine skeletal muscle determinants.

- In Chapter 2, we have tested whether near-infrared spectroscopy could provide non-invasive assessment of oxygen supply capacity during exercise, detecting the exercise intensity at which a mismatch between oxygen supply and demand occurs and oxygen supply becomes insufficient to deliver all energy with use of oxygen.
- In Chapter 3, validity and reliability of the 3D ultrasound imaging technique was tested for analysis of muscle morphology.

**Physiological profile**

We discuss key physiological determinants of whole-body performance in Chapter 4-6.

- In Chapter 4, the 3D ultrasound technique was used to measure muscle morphological parameters of the m. vastus lateralis in Olympic athletes and to assess the relative contribution of muscle volume and architecture to rowing ergometer performance, sprint performance, maximal oxygen uptake and force generating capacity. Moreover, sprint and endurance performance were shown to be negatively related in male rowers.
- In Chapter 5, muscle fiber oxidative capacity of the m. vastus lateralis was obtained in chronic heart failure patients, control subjects and cyclists, and used to calculate whole-body oxidative capacity. Comparison of whole-body oxidative capacity and whole-body maximal oxygen uptake during maximal cycling exercise was performed to indicate oxygen supply limitations at maximal exercise.
- In Chapter 6, we have investigated a comprehensive physiological profile of cyclists from sprint to endurance disciplines and assessed the key determinants, most optimal combination of determinants, and relative contribution of determinants to (combined) sprint and endurance performance. Sprint and endurance performance were shown to be negatively related and a new measure of combined sprint and endurance performance
was introduced in this Chapter.

**Training strategy**

- In Chapter 7, skeletal muscle adaptations of prime determinants of physical performance (i.e. oxidative capacity, fiber size and oxygen supply capacity) were revealed in response to a promising new training strategy of living at altitude and training low combined with repeated-sprint training in normoxia or hypoxia.

**General discussion**

- Chapter 8 integrates and discusses the results of previous chapters, and addresses their implications for sports practice and training.

**Infographics**

In Chapters 2-7, you will find a visual summary at the start of each chapter for a quick overview of the study. *Note:* on the right side of the infographic, it is indicated to what category the study belongs (i.e. technology, physiological profile or training strategy), whether the study includes invasive or non-invasive methods and what biological levels are addressed in the study (i.e. performance, whole-body physiology, blood properties, whole-muscle physiology, or muscle fiber physiology), as explained below:
References


