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LIST OF ORIGINAL PUBLICATIONS

- I Purge, P., Valiulin, D., Kivil, A., Müller, A., Tschakert, G., Jürimäe, J., Hofmann, P. (2021). The Effect of Lower Body Anaerobic Pre-Loading on Upper Body Ergometer Time Trial Performance. *Sports*, 9(6): 79. https://doi.org/10.3390/sports9060079.
- Valiulin, D., Purge, P., Mäestu, J., Jürimäe, J., Hofmann, P. (2022). Effect of Short-Duration High-Intensity Upper-Body Pre-Load Component on Performance among High-Level Cyclists. *Sports*, 10(3): 32. https://doi.org/10.3390/sports10030032.
- III Valiulin, D., Purge, P., Hofmann, P., Mäestu, J., Jürimäe, J. (2021). Can We Improve the Functional Threshold Power Test by Adding High-Intensity Priming Arm-Crank? *Journal of Functional Morphology and Kinesiology*, 6(4): 88. https://doi.org/10.3390/jfmk6040088.

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ABBREVIATIONS

10% Δ 10% of the difference between the work rate at the VT₂ and V·O_{2max}

above VT₂

BMI body mass index

CI_{95%} confidence interval at a 95% level

FTP20 20-minute Functional Threshold Power Test

HIE high-intensity exercise

HR heart rate La lactate

[La] lactate concentration

mLSS maximal lactate steady state

MPT_{high} maximal performance test with common warm-up added by high-

intensity priming load

MPT_{low} maximal performance test with common warm-up

P_{max} maximal power PPO peak power output

RER respiratory-exchange-ratio RPE rating of perceived exertion $V^{*}CO_{2}$ carbon dioxide output

 $V^{\cdot}O_{2max}$ maximal oxygen consumption VT_1 first ventilatory threshold VT_2 second ventilatory threshold

1. INTRODUCTION

An adequate warm-up is a key aspect of a successful and sustainable performance with better perception and lower risk of traumas (McGowan et al., 2015). Finding a balance between warm-up-induced activation and physiological benefits remains crucial for every pre-competitive preparation. In this context, maintaining the muscles in an optimal pre-start condition plays an important role before each competition (McGowan et al., 2015).

A traditional warm-up process, consists of light endurance activity and stretching, is the basic requirement for a successful performance (Burkett et al., 2005; Hajoglou et al., 2005; Richendollar et al., 2006). Active warm-up is responsible for physiological changes, the combined effect of which is capable of improving competitive performance (Bishop et al., 2001). The main effects are increased muscle temperature, nerve conduction rate, speeded metabolism kinetics, oxygen uptake kinetics ($V^{\circ}O_2$), muscle post-activation potentiation, and psychological preparedness (Bishop et al., 2001; McGowan et al., 2015). Since athletic skills and physiological properties have increased rapidly in the recent years, the focus has moved to better physiological preparation (Burnley et al., 2011; Wilkerson et al., 2004), that could speed up $V^{\circ}O_2$ kinetics and increase peak power output (PPO) values without spending crucial energy and time.

High-intensity warm-ups are found to have a greater effect on V'O₂ kinetics, increase time to exhaustion and enable a greater aerobic contribution (Burnley et al., 2011, 2005; Jones et al., 2003). In addition, high-intensity warm-ups significantly elevate blood lactate concentration [La] which per se could accelerate V'O₂ at the start of exercise by forcing the organism to stimulate mitochondrial respiration (Burnley et al., 2001). This effect is suggested from an inhibition of lactate production due to an inverse gradient for lactate due to already elevated blood [La] from non-dominant muscles (Parolin et al., 1999). An interaction between lactate (La) producing and La consuming muscle cells and their interaction with the body system can be explained by the lactate shuttle theory (Brooks, 2018, 2009). The latter broadens the knowledge regarding La as glyconeogenetic precursor, energy substrate and key regulator, and explains the distribution of glucose and lactate to well-oxygenated fast-twitch glycolytic and slow-twitch oxidative muscle fibres (Brooks, 2018, 2009).

It is suggested that short-term performance, which is dependent on the ability to break down high-energy phosphate stores, will be negatively affected by an intense warm-up that decreases the availability of high-energy phosphates (Bishop et al., 2001). On the other hand, exercise longer than 2–3 min is primarily limited by aerobic energy contribution which may be enhanced by intense warm-up procedures (Fujii et al., 2019). It has previously been substantiated that blood [La] is related to carbon dioxide output ($V^{\circ}CO_2$) (Iaia et al., 2010) and thus to anaerobic effort (Bishop et al., 2001; Gray and Nimmo, 2001; Robergs et al., 1991). A major problem with high-intensity, long-duration exercise is tolerating the side effects of the highly anaerobic first 40–60 s of exercise, which causes

rapid increase in heart rate and ventilation. Therefore, it was suggested that an early accelerated increase in $V^{\circ}O_2$ (Martin et al., 1975) and a lower net La production within the working muscle improves performance (Tschakert and Hofmann, 2013).

Commonly used warm-up techniques are based on isolated preparation of performance-related muscles added to a general warm-up. In contrast, more general warm-up approaches can impact muscles systemically without raising local muscle acidosis or using glycogen depot (Purge et al., 2017). This thesis intends to research the extent to which high-intensity warm-up could influence a subsequent endurance performance.

2. LITERATURE REVIEW

Maximal athletic performance requires an overall systemic preparation, which is reinforced by a preceding warm-up session. The scientific community has agreed on the positive effects of warm-up and absence of warm-up is considered to have a detrimental effect. Although warm-ups can be different, the aim is to prepare musculoskeletal, neural, metabolic, and psychological mechanisms to stimulate performance of muscle contraction (McGowan et al., 2015).

Metabolic conditioning is an emerging trend intended to improve the basic warm-up effects. Competition puts the greatest strain on all the body's functioning mechanisms, while requiring the biggest effort from both local and systemic levels. ATP turnover efficiency rises with tissue oxygenation rates, and higher oxygen (O₂) concentration allows the possibility to use slow but high-energy density substrates such as fat or intracellular lactate to provide ATP (Gladden, 2004). Exploiting metabolic mechanisms such as priming can provide higher O₂ levels, speed up V:O₂ kinetics, and increase power output and submaximal activation of aerobic mechanisms (Bohnert et al., 1998; Chorley and Lamb, 2019).

High-intensity performance requires a high $V \cdot O_{2\text{max}}$ for endurance athletes, but the true challenge is to sustain a high fraction of $V O_{2max}$ for a prolonged time (Ghosh, 2004). V'O_{2max} alone was not shown to be a good predictor of endurance performance when athletes of similar endurance ability are compared (Morgan et al., 1989). In fact, economy at first (VT₁) and second (VT₂) ventilatory thresholds plays a crucial role, since athletes with nearly identical VO_{2max} values can perform at different levels (Ghosh, 2004). Aerobic training often improves submaximal thresholds such as VT₁ and the VT₂ as well as other physiological variables without a concomitant increase in $V^{\cdot}O_{2max}$ (Allen et al., 1985). The VT_2 representative of the maximal lactate steady state (mLSS), has a superior relationship to endurance performance compared to $V O_{2max}$ and is suggested to be a better indicator of aerobic endurance (Beneke, 1995; Billat et al., 2003). VT₂ and mLSS describe a maximal metabolic steady state in which lactate production and utilization are in a fine systemic balance (Billat et al., 2003; Tschakert and Hofmann, 2013) and improvements of VT₂ were shown to enhance endurance performance (Bacon et al., 2013). Studies have shown that athletes are capable of maintaining VT₂ intensity for an impressive amount of time. Some research limited the capacity to 60 min (Gavin et al., 2012; Morgan et al., 2018) but others have raised discussions about the accuracy of this statement, questioning its physiological basis (Jones et al., 2019). Considering athletes' individuality and the different research methods, work duration at mLSS intensity is suggested to be about ~20–60 min (Tschakert et al., 2022) but may be dependent on exercise mode and muscle mass involved (Gavin et al., 2012; Morgan et al., 2018). However, as the power increases above VT₂ intensity, the duration dramatically decreases. Identifying the critical point of maximal metabolic steady state and predicting performance capability is a main concern for many athletes, coaches, and sports scientists. Therefore, VT₂ and V O_{2max} are both used to select a tailored

exercise workload (Bailey et al., 2009; Burnley et al., 2011; Tschakert and Hofmann, 2013).

High-intensity warm-ups are found to have a greater effect on physiological condition (Burnley et al., 2011, 2005; Jones et al., 2003); however, the selected intensity should not induce fatigue or it should be eliminated during the recovery phase before the sports performance. Stronger athletes have shown a faster recovery and greater effect from high-intensity warm-up (Seitz et al., 2014), thus efficient recovery time after warm-up may still vary depending on the individual athlete, affecting subsequent performance and physiological parameters (Hodgson et al., 2005; Seitz and Haff, 2016). Competitive experience and age of the athlete influence the rating of perceived exertion (Barroso et al., 2014), that can vary from day to day despite the same training duration and same intensity due to the specificity of individual characteristics (physical and psycho-social) that might affect the internal load of each individual athlete (Haddad et al., 2017). Therefore, optimizing performance depends on the warm-up intensity, individual factors and recovery time between the warm-up activity and maximal performance (Hodgson et al., 2005; Seitz and Haff, 2016). This is the reason why well-trained athletes tend to use an individualized warm-up, based on athletic experience and current well-being (Palmer et al., 2009).

High-intensity warm-up component, i.e. priming, can induce a significantly elevated [La] in a short period of time – thus affecting metabolic function and creating a negative [La] gradient such as La uptake rather than La efflux on a systemic level. For performances that require (sub)maximal aerobic metabolism, metabolic conditioning can be achieved both by moderate and heavy-intensity exercises equally (Burnley et al., 2005). Overall metabolic status achieved by priming inhibits La production during subsequent time trial (Birnbaumer et al., 2018; Purge et al., 2017). Lower V^*CO_2 values from spirometry measurements relate to lower La production per each watt (Iaia et al., 2010), and consequently lower anaerobic effort (Bishop et al., 2001; Gray and Nimmo, 2001; Robergs et al., 1991). Decreased net La production during performance inhibits anaerobic metabolism and expected to enhance aerobic metabolism (Müller et al., 2013; Parolin et al., 1999).

According to previous studies, systemic changes can be generated by any muscle group, regardless of muscle mass or localization (Bogdanis et al., 1994; Bohnert et al., 1998; Spendier et al., 2020). Additionally, systemic changes provoked by non-sport specific muscles give an opportunity to improve subsequent maximal performance efficiency while keeping sport-specific muscles unaffected, and without raising local muscle acidosis or using glycogen depot (Müller et al., 2013; Purge et al., 2017). Previous studies have reported that preloaded high-intensity exercise conducted by the upper body affected glycogenolysis and significantly reduced $V^{\circ}CO_2$ during subsequent maximal performance test (Purge et al., 2017). Previously, a variety of studies have investigated such priming effects by non-dominant muscles, e.g. leg exercise priming for subsequent arm and upper-body exercise (Birnbaumer et al., 2018; Bogdanis et al., 1994; Müller et al., 2013; Purge et al., 2017).

Studies have shown that pre-loading by the arms can be compared to prior leg sprint exercise (Bogdanis et al., 1994; Bohnert et al., 1998) and induce similar V'O₂ levels up to 45 min after high-intensity exercise (Burnley et al., 2006). Purge et al. (2017) found that pre-loaded high-intensity exercise conducted by the upper body affected glycogenolysis and significantly reduced V CO₂ during 7–8 min of all-out performance in rowers. Despite clear evidence of physiological changes, there have also been contradictory findings stating that non-target muscle priming does not have a beneficial effect on subsequent performance (Purge et al., 2017). Possible reasons were inhibited anaerobic energy contribution, overly intensive warm-up, and insufficient recovery after warm-up, as well as modified pacing strategies (Purge et al., 2017). However, physiologically beneficial performance enhancement could only be detected in small-muscle-group exercise (Birnbaumer et al., 2018). Birnbaumer et al. (2018) showed that anaerobic metabolic pre-conditioning significantly improved pull-up exercise compared to a standard warm-up program performed by legs. Bohnert et al. (1998) investigated upperbody pre-loading, which is qualitatively comparable to lower-body pre-loading, although lower in magnitude. According to this approach, Müller et al. (2013) found that arm crank exercise before leg exercise significantly inhibited anaerobic energy contribution during the leg workout. Less anaerobic energy in the same amount of total work means more aerobic energy was produced and consumed. Priming induced by non-sport specific limbs may be a new preparation method, prescribed as metabolic conditioning for endurance sports where aerobic metabolism could be more useful (Birnbaumer et al., 2018; Purge et al., 2017). Although excessively intense exercise before a high-intensity bout may reduce performance level through decreased muscle glycogenolysis and La production (Bangsbo et al., 1993; Gaitanos et al., 1993), and lower levels of muscle glycogen and pH, the effects of anaerobic pre-load with non-dominant muscles remain unclear.

In 1972, Klausen and colleagues investigated positive effects that could be induced by earlier activation of aerobic mechanisms and provide a benefit to cycling performance. Physiological changes such as speeded V O₂ kinetics and lower net [La] increase are confirmed by numerous studies (Bishop et al., 2001; Gerbino et al., 1996; Müller et al., 2013; Wilkerson et al., 2004). From an athletic perspective physiological changes may act as favorable conditions, although not guaranteeing improved competitiveness (Carter et al., 2005; Ferguson et al., 2007; Sousa et al., 2014). Although half a century has passed since the primary investigation, only marginal performance related effects have been discovered (Bishop et al., 2001; Gerbino et al., 1996; Müller et al., 2013; Wilkerson et al., 2004), there is still some need regarding the optimal dosage of such a conditioning protocol before integrating such priming activities into competitive sports. The most detailed suggestions according implementation of priming have been concluded by Caritá and colleagues (2015). Heavy priming exercise with a duration of 6–10 minutes can improve tolerance to severe exercise of maximum and supramaximal intensity (> 100% V'O_{2max}; duration between 2–5 minutes), but no priming is suggested before completing severe exercise of submaximal intensity (< 100% V O_{2max}; duration between 9–12 minutes). Severe priming exercise of lower intensities (duration between 15–18 minutes) with sufficient recovery time of 9–20 minutes can be beneficial for subsequent submaximal performance ($<100\%\ V\cdot O_{2max}$) and improve exercise tolerance (Caritá et al., 2015). Additionally, priming could serve as a re-warm-up as Yanaoka and colleagues (2020) have shown a positive effect from 1-minute cycling exercise at 90% of $V\cdot O_{2max}$ at completing a subsequent >10 minute time trial.

Resource distribution throughout the distance, i.e. pacing, plays an important role in ensuring the best possible performance outcome (Abbiss and Laursen, 2008). Bailey et al. (2011) reported that utilizing a fast-start pacing strategy improved both the overall V O₂ uptake and cycling exercise performance. It is a topic for discussion whether priming can alter a traditional pacing effect, and if it should cover all the distance with moderate intensity changes or just a part with high-intensity spurt. Two studies have attempted to investigate the effects of combining priming with pacing strategies in detail (Brock et al., 2018; Caritá et al., 2014). In both studies primed fast-start conditions performed the fastest, although insignificantly in the Caritá et al. (2014) study, since all primed pacing strategies showed the same beneficial effect. However, it was reported that the presence of priming exercise is principally compared to fast-start pacing (Caritá et al., 2014). Consequently, the fast-start can improve and not by any means decrease performance. These findings give an idea of the positive effects of pacing, although the magnitude and distribution over the distance need to be discussed.

2.1 Previous research for methodological set up

Overview articles were selected from the PubMed Central and EBSCO Discovery databases using a close search with further manual filtering (date: 26.03.2022). The search was conducted to put specific topic-descriptive words into abstract or title such as – prior exercise; high intensity; priming; preload; performance; effect. General words to describe physiological and measurable component were added for "all-fields" selection – warm up; lactate; aerobic/anaerobic; maximal; split/trial/sprint. PubMed Central (1) and EBSCO Discovery (2) advanced searches were carried out using following formulas:

((((prior exercise[Abstract] OR high intensity[Abstract] OR
"priming"[Abstract] OR preload*[Abstract] OR pre-load*[Abstract]) AND
(performance[Abstract] OR effect*[Abstract]))) OR ((prior exercise[Title]
OR high intensity[Title] OR "priming"[Title] OR pre-load*[Title])) AND (performance[Title] OR effect*[Title]))) AND (("warm
up" OR "warm-up") AND lactate AND ("aerobic" OR "anaerobic") AND
("maximal" OR "all out" OR "all-out" OR "peak") AND ("split" OR "trial"
OR "sprint"))

(1)

TI (prior exercise OR high intensity OR "priming" OR preload* OR preload*) AND TI (performance OR effect*) AND TX ("warm up" OR "warm-up") AND TX lactate AND TX ("aerobic" OR "anaerobic") AND TX ("maximal" OR "all out" OR "all-out" OR "peak") AND TX ("split" OR "trial" OR "sprint") Full Text

Research articles describing physiological changes such as performance alterations were manually filtered out from articles with a measurable competitive component such as time, distance, or number of repetitions.

2.1.1 Inclusion and exclusion criteria

The main criterion was the presence of two warm-up protocols, with common warm-up and priming component added, which were completed with a counterbalanced crossover design using a test-retest method. The results of subsequent performance meant giving an adequate measure to both warm-up efficiencies among healthy male participants.

Cyclic sports and exercises until exhaustion were included. Team games are usually multi-variable, and an improved effect can be caused by different factors. The search was focused on endurance sports lasting ≥ 3 minutes in order to be able to relate maximal performance to aerobic capacity (Viru, 1990), excluding sprint distances with a relatively low aerobic contribution. Pre-loading before shorter efforts < 2 minutes is usually situated to post-activation-potentiation effect, which should be considered as a neurological improvement (McGowan et al., 2015).

Studies which had no relation to human physiology were excluded. As metabolic preconditioning is seeking acute effects on endurance sports, training programs and motor learning specific studies were omitted. Studies including additional performance enhancement such as supplement use or physiological variables as blood flow restriction, ischemic preconditioning, and hypoxia modelling, were also excluded, although these methods could also offer an interesting and comparable effect.

2.1.2 Search results

As a result of the search PubMed Central showed 506 articles and EBSCO Discovery databases 341 full-text articles. After title and abstract revision studies which respond to all inclusion criteria were selected and analyzed, their references also filtered and matching studies added to the list. Detailed and systematic processing of methods and performance left in only a minor part of the total studies (13), which can be seen in Table 1.

Thirteen articles fulfilled all criteria. The main cyclic endurance sports were cycling (9), rowing (2), running (1) and modified pull-up exercise (1). Performance distance of targeted sports was not specified in 7 cases, instead they asked subjects to continue until exhaustion with specified intensity. In 7 articles a statistically significant performance improvement (p < 0.05) was achieved.

2.1.3 Summary from previous research

Former studies on metabolic pre-conditioning investigated the effects of different intensity warm-up protocols on physiological aspects. Being productive in finding effective methods to increase $V \cdot O_2$ kinetics or PPO (Gerbino et al., 1996; Müller et al., 2013; Tomaras and MacIntosh, 2011), researchers still remain cautious as competitive performance contains more factors than just physiological parameters. Even though objective measures prove athletes' preparedness, individual responses and perception need to be taken into consideration. As speeded up $V \cdot O_2$ kinetics, increased body and muscle temperature, increased PPO are prerequisites for successful performance, they truly serve the purpose of enhancing athletes' perception before subsequent effort. Feeling energized, fast, and powerful is the subjective part of the warm-up that should not be forgotten.

Studies which did not show performance improvements (Table 1) after high-intensity priming exercise (HIE) stated that the possible causes were reduced depletable energy resource or prolonged fatigue-metabolite levels (Ferguson et al., 2007). Moreover, fatigue and insufficient time to recover could have detrimental effects (Purge et al., 2017). These conditions could easily be prevented, and athletes' perception enhanced. In (2006), Burnley and colleagues found that priming effect of prior heavy exercise on the V O₂ uptake response persists up to 30–45 min. Knowing this extensive period of time, further research could give athletes some extra recovery time after an HIE priming session or use athletes' self-selected duration based on their readiness without losing any priming effect. Then the results of both Ferguson et al.'s (2007) and Purge et al.'s (2017) studies found to have no improving effect on performance, could have different outcomes.

Although many studies have been conducted on metabolic pre-conditioning research, no conclusive formula has been developed. The results are affected by multiple variables, i.e. pre-conditioning intensity and duration, recovery time and targeted sports intensity and duration. Still no common agreement has been reached (Ferguson et al., 2007). Some study results can show high variability between individuals in the context of the same protocol, and no relation between performance and $V \cdot O_2$ kinetics (Carter et al., 2005; Ferguson et al., 2007; Sousa et al., 2014). In the light of further research more individual intrinsic factors should be investigated. For example, self-selected recovery duration prior to performance trial along with [La] and RPE Borg scale measurements could show a correlation between enhanced performance and efficient [La] level.

In conclusion, cyclic sports with the possibility to use ergometer or repetitive movement should be considered in further investigations of metabolic preconditioning. We suggested using a counterbalanced crossover design with a test-retest method, in which one test would contain high-intensity pre-load with non-sport-specific muscle groups. Subsequent self-selected recovery duration with [La] and RPE Borg scale measurements and using sport-specific performance distance or all-out trial until exhaustion, based on previous research.

Table 1. Selected studies for metabolic pre-conditioning topic analysis.

Research	Pre- Pre- conditioning conditioning activity duration	Pre- conditioning duration	Pre- conditioning intensity	[La] before the start	Recovery before MPT	Targeted sports	Targeted sports duration	Statistical significance
Jones et al., 2003; $n = 7$	cycling	6 minutes	$50\% \Delta$ (half- way between the gas exchange threshold and V-O _{2peak})	$2.4 \pm 0.3 \text{ mmol \cdot L}^{-1}$	7 min passive + 3 min active	cycling	Until exhaustion with abrupt intensity increase to 100% VO _{2peak}	Significant increase in time to exhaustion $(p < 0.05)$
Carter et al., 2005; $n = 11$	cycling	3×73 sec	90% V · O _{2max}	$1.7 \pm 0.2 \text{ mmol} \cdot \text{L}^{-1}$	6 min active	cycling	Until exhaustion at critical power	Significant increase in time to exhaustion $(p < 0.05)$
Ferguson et al., 2007 ; $n = 6$	cycling	6 minutes	275 ± 34 W	$8.6 \pm 1.4 \text{ mmol·L}^{-1}$	2 min active	cycling	Until exhaustion with ramp increment to maximal power	Not significant decrease in time to exhaustion $(p > 0.05)$
Bailey et al., 2009; $n = 8$	cycling	6 minutes	70% V О _{2тах}	$9.3 \pm 1.8 \text{ mmol·L}^{-1}$ and 8.0 ± 1.6 mmol·L ⁻¹	9 and 20 (\geq 9 min).	cycling	Until exhaustion with abrupt intensity increase to 80% V O _{2peak}	Significantly improved exercise tolerance at $80\%\Delta$ in the 70-9-80 and 70-20-80 conditions $(p < 0.05)$
Palmer et al., 2009; $n = 8$	cycling	5 min + 5 min	100 W and 50% Δ (halfway between the gas exchange threshold and V Ozpeak) respectively	4.8 mmol·L ⁻¹	1 min active + 12 min passive	cycling	4000 m at performance intensity	Not significantly faster performance $(p > 0.05)$

Research	Pre- conditioning activity	Pre- conditioning duration	Pre- conditioning intensity	[La] before the start	Recovery before MPT	Targeted sports	Targeted sports duration	Statistical significance
Burnley et al., 2011; $n = 10$	cycling	6 minutes	50% Δ (half-way between the gas exchange threshold and critical power)	$1.8 \pm 0.5 \text{ mmol} \cdot \text{L}^{-1}$	7 min passive + 3 min active	cycling	Until exhaustion at 70% Δ (between the gas exchange threshold and peak work rate) with abrupt intensity increase	Significant increase in time to exhaustion $(p < 0.05)$
Sousa et al., 2014; $n = 6$	rowing	6 minutes	50% Δ (half-way between the gas exchange threshold and critical power)	$5.9 \pm 1.2 \text{ mmol} \cdot \text{L}^{-1}$	7 minutes	rowing	Until exhaustion with abrupt intensity increase to 100% V Ozpeak	Significant decrease in time to exhaustion $(p < 0.05)$
Christensen and Bangsbo, 2015 ; $n = 12$	cycling	5 splits of different duration and recovery	all splits of different intensity	$5.1 \pm 1.7 \text{ mmol} \cdot \text{L}^{-1}$	20 minutes	cycling	4-minute maximal performance	Not significantly decreased mean power $(p > 0.05)$
Purge et al., 2017 ; $n = 9$	arm crank pre-load	25 seconds	30g·kg ⁻¹ body weight / nearly max	$8.4\pm2.3~mmol\cdot L^{-1}$	9 minutes	rowing	2000 m at performance intensity	Significant decrease of performance $(p < 0.05)$
Birnbaumer et al., 2018; $n = 9$	running	25 second shuttle run	maximal peak power	$9.28\pm1.98~mmol\cdot L^{-1}$	8 minutes	pull-up exercise	All-out performance	Significant increase of performance $(p < 0.05)$
Brock et al., 2018; $n = 9$	cycling	3 minutes	70% Δ (70% between the gas exchange threshold and V Ozpeak)	$2.7 \pm 0.5 \text{ mmol} \cdot \text{L}^{-1}$	20 minutes	cycling	4000 m at per- formance intensity with 12 sec all-out start	Significant increase of performance $(p < 0.05)$

Research	Pre- conditioning activity	Pre- Pre- nditioning conditioning activity duration	Pre- conditioning intensity	Pre- Pre- Pre- Research conditioning conditioning conditioning conditioning [La] before the start activity duration intensity	Recovery before MPT	Targeted sports	Targeted Targeted sports sports	Statistical significance
González- Mohíno et al., 2018; $n = 11$	running	6 × 6 second hill strides (60 sec re- covery between)	105% V'О2max	$ 6 \times 6 \operatorname{second} \\ $	10 min passive + 5 min active + 3 min passive	running	Until exhaustion with constant speed at 105% V O _{2max}	Significant increase in time to exhaustion $(p < 0.05)$
Chorley and Lamb, 2019 ; $n = 10$	cycling	3 × 10 seconds (30 secorecy between)	70% of peak power	$4.9 \pm 1.4 \text{ mmol} \cdot \text{L}^{-1}$	5 minutes	cycling	cycling 4000 m at per- formance intensity	Not significantly improved time $(p > 0.05)$

References listed in the sequence of publication; level of significance p < 0.05

3. AIMS

The purpose of this study was to determine whether high-intensity priming exercise (HIE) executed by non-sport specific muscles improves outcomes of subsequent sport-specific performance test in male highly trained endurance athletes.

Specific objectives of the study are listed as below:

- 1. to assess the metabolic response to high-intensity priming exercise and estimate the magnitude of the anaerobic energy contribution to the total energy supply during subsequent maximal performance test.
- 2. to measure differences in time or mean power in maximal performance test in primed and non-primed conditions.
- 3. to determine an optimal recovery time before maximal performance test after HIE.
- 4. to control whether standardized pacing during maximal performance test can compensate the deficit of anaerobic energy contribution induced by prior HIE.

4. METHODS

4.1 Experimental overview

Participants attended the laboratory on three occasions in all studies within a 3-week period, with each visit being separated by at least 3 recovery days. On the first visit participants' body composition was measured and they were introduced to the testing equipment. To exclude any health risks associated with the maximum stress, all subjects had to perform a specialist-supervised incremental cycle ergometer test as the first exertion. Two gas exchange thresholds (VT₁, VT₂) were determined by an experienced researcher considering a disproportionate increase in $V \cdot CO_2$ relative to $V \cdot CO_2$, ventilation breakpoints (V-slope), visual inspection of individual plots and respiratory-exchange-ratio (RER) value. The highest mean measured O_2 during 30 s period was considered $V \cdot O_{2max}$. On two subsequent occasions maximal performance tests (MPTs) were performed. One with traditional warm-up and a second one with additional preload done by non-sport specific musculature.

All participants volunteered to participate in this study and were required to give their written informed consent at the first laboratory visit. Participants were instructed to arrive at the laboratory in a rested and fully hydrated state at least 2 h postprandial. They were asked to avoid ingesting alcohol and caffeine 24 h before each laboratory visit. In addition, maximal efforts no less than 3 days before the laboratory visit were allowed.

Inclusion criteria were that all participants were experienced national level male athletes (skiers in *Paper I* and cyclists in *Paper II*, *III*), being familiar with indoor competition procedures on particular equipment and have been training for at least 5 years. Exclusion criteria were illness or injury. Current studies were approved by the local University ethics committee (257/T-16 and 290/T-17) in accordance with the Declaration of Helsinki (Hellmann et al., 2014).

In order to objectively compare performance in two maximal performance tests under primed (MPT_{high}) and unprimed (MPT_{low}) condition. Participants performed both protocols in a randomized order. Participants were instructed to complete both maximal tests as quick as possible. Characteristics of participants are described in Table 2.

Table 2. Participants' characteristics.

Characteristic		Mean ± SD	
	Paper I	Paper II	Paper III
Participants (n)	13	15	11
Age (years)	18.3 ± 2.9	23.3 ± 3.6	18.8 ± 0.9
Height (m)	1.81 ± 0.05	1.81 ± 0.07	1.82 ± 0.05
Body mass (kg)	70.8 ± 7.3	76.2 ± 10.0	73.0 ± 6.6
BMI $(kg \cdot m^{-2})$	21.7 ± 1.8	23.2 ± 2.0	22.0 ± 1.9
Total lean mass (kg)	55.7 ± 5.9	58.6 ± 6.8	57.5 ± 4.9
Incremental protocol	(40W + 20 W/min)	(60W + 20 W/min)	(100W + 30 W/3min)
$V \cdot O_{2max} (mL \cdot kg^{-1} \cdot min^{-1})$	57.3 ± 5.3	65.4 ± 6.7	67.9 ± 5.1
$V^{\cdot}\mathrm{O}_{2\mathrm{max}}\ (\mathrm{L}\cdot\mathrm{min}^{-1})$	4.1 ± 0.6	4.9 ± 0.5	5.0 ± 0.6
$P_{max}(W)$	270.1 ± 42.1	404.2 ± 42.8	369.0 ± 47.9
Ventilatory threshold $VT_1(W)$	127.0 ± 25.5	218.7 ± 33.9	181.4 ± 43.3
Ventilatory threshold VT ₂ (W)	186.2 ± 28.2	310.8 ± 39.3	295.6 ± 39.9

BMI – body mass index, maximal oxygen uptake (V O_{2max}), maximal power (P_{max}), first ventilatory threshold (VT_1), second ventilatory threshold (VT_2).

4.1.1 Experimental Tests in Paper I

The test started with an initial work rate of 40 W with increments of 20 W after every min until volitional exhaustion (Purge et al., 2017). Experimental all-out tests in an indoor environment were maximally converged to competitive situations using a wind-resistance-braked skiing ergometer. This mode of exercise is conducted by repeatedly pulling down the handles from above the shoulders that are connected to a pulley system. It is characterized by the work of mainly the upper body (Fukuda et al., 2013).

A 1000 m of double poling was completed in the shortest time possible. Athletes were allowed to choose ergometer resistance level between 4 and 6 but were not allowed to check for stroke rate and pace during the trial. Power and stroke frequency were recorded continuously to analyze pace strategy and speed for different parts of distance. Two protocols were performed to measure the effect of a high-intensity anaerobic warm-up phase on all-out cross-country ski endurance performance. Both protocols were aiming to measure parameters of MPT which in one case was following a low-intensity warm-up (MPT_{low}) with a 14 min duration of recovery between warm-up and workout. In the second protocol (MPT_{high}), an additional 25 s high-intensity anaerobic cycling pre-load protocol was performed 5 min after the end of warm up, leaving approximately 9 min for recovery before the 1000 m all-out skiing ergometer MPT (Fig. 1). The order of tests was randomized for each participant to minimize learning effects and exclude a possible advantage of one or other protocol sequence. The warm-up in MPT_{low} consisted of a 20 min workload at 50% V O_{2max} from the incremental

exercise test. In the experimental setting a 25 s high-intensity anaerobic lower body cycling pre-load HIE was added to cause a significant increase in blood [La].

Blood [La] was determined for both conditions at rest (baseline), after the warm-up (immediately after, + 3', + 5'), after the anaerobic priming load (immediately after, + 1', + 2', + 3', + 4', + 5', +7', + 9'), and during recovery (immediately after MPT, + 1', + 3', + 6', + 9', + 12', + 15') (total of 19 samples in MPT_{high} and 12 in MPT_{low} protocol). Heart rate (HR) and gas-exchange were measured continuously as showed in Measurements chapter.

4.1.2 Experimental Tests in Paper II

Two maximal 4000 m cycling tests with upper-body pre-load (MPThigh) or common warm-up conditions (MPT_{low}) were compared (Fig. 1). MPT_{low} and MPT_{high} protocols started with a 20-min warm-up at 40% of V O_{2max}. A 25 s high-intensity all-out arm crank effort on an upper-body hand-crank ergometer was added in the MPT_{high} condition. The 25 s was suggested to induce a lactate increase to a level 8–10 mmol·L⁻¹ to induce a sufficient subsequent inhibition without negative side-effects of the induced acidosis (Purge et al., 2017). In both testing conditions, subjects were allowed to recover from the warm-up until they reported readiness for the subsequent maximal performance. Maximal performance bouts started with a 12 s all-out start. Ten seconds before beginning each MPT, subjects were instructed to take a standing position and adjust the crank angle to their preferred position, as it was documented during the first laboratory visit. Subjects were provided with a 3 s count-down before the 12 s all-out start. Following the initial 12 s all-out upright cycling, subjects were instructed to take a seated position for the remaining test duration. Strong verbal encouragement was provided during both maximal tests. Remaining distance was the only landmark that cyclists were provided, and subjects were unaware of the elapsed time or implemented power (W), the power display and time were covered to exclude any numerical comparison.

Blood [La] was determined for both conditions at rest (baseline), after the warm-up (immediately after, +3', +5'), after the anaerobic priming load (immediately after, +3', +4', +5', +7', +9', immediately before MPT), and during recovery (immediately after MPT, +3', +4', +5', +7', +9', +11', +13', +15') (total of 20 samples in MPT_{high} and 14 in MPT_{low} protocol). Heart rate (HR) and gas-exchange were measured continuously as showed in Measurements chapter.

4.1.3 Experimental Tests in Paper III

Functional threshold power (FTP) is a frequently used additionally important performance quality indicator for cyclists which is highly correlated to mLSS (Denham et al., 2020) and relative power exertion during a mass-start bike race (Sørensen et al., 2019). It is the maximum mean power, which can be sustained for an approximately 1 h period and is a good predictor of overall performance capacity (Denham et al., 2020). FTP can be evaluated by a time efficient 20 min FTP test (FTP20), which predominantly relies on aerobic metabolism (Denham et al., 2020). So, more cycling specific 20 min FTP test with longer distance and fixed pace was performed as MPT in order to explore the effects of priming while excluding any additive pacing effect. The protocol was created considering correlation findings from *Paper II* regarding recovery time and consistent with prior research (Burnley et al., 2005; Jones et al., 2003). Since the main interest of this thesis was priming effect on the performance, an all-out spurt in the end of MPT was added as the measurable variable.

MPT_{low} and MPT_{high} (Fig. 1) protocols started with a similar 20 min warm-up at 40% of $V \cdot O_{2max}$. A 25 s high-intensity all-out arm crank effort on an upper-body hand crank ergometer was added for the MPT_{high} protocol. In both testing conditions, participants were required to recover from the warm-up for a minimum of 10 min plus self-determined duration, until they reported readiness for the subsequent maximal performance test.

The 20 min maximal performance bouts started with 17 min of constant work at a controlled pace of 10% Δ [VT₂ plus 10% of the difference between the work rate at the VT₂ and V·O_{2max}] to cover the possible standard error from determining VT₂ and ensure a gradual increase in blood [La] (Jones et al., 2003). During the final 3 min of the FTP20 performance, athletes were required to perform their self-paced maximal final spurt. They were provided with a 3 s countdown before the start and instructed to increase the pace to achieve the maximal exertion by the end of the 20 min. Strong verbal encouragement was provided during both maximal tests. Remaining time was the only information that cyclists were provided, and participants were made unaware of implemented power (W); the power display was covered to exclude any numerical comparison.

Blood [La] was determined for both conditions at rest (baseline), after the warm-up (immediately after, + 3', + 5'), after the anaerobic priming load (immediately after, + 3', + 4', + 5', + 7'), during the test (immediately before, + 5', + 10', + 15'), and during recovery (immediately after MPT, + 3', + 4', + 5', + 7', + 9', + 11', + 13', + 15') (total of 21 samples in MPT_{high} and 16 in MPT_{low} protocol). Heart rate (HR) and gas-exchange were measured continuously as showed in Measurements chapter.

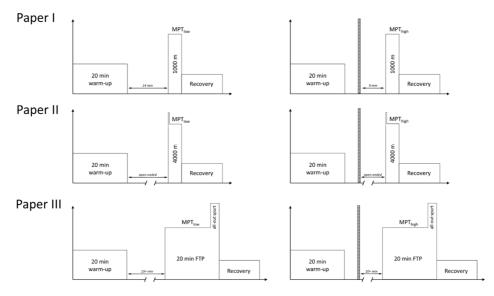


Figure 1. Schematic difference between maximal performance test with common warm-up (MPT $_{low}$ – left side column) and a protocol with added by a 25 s high-intensity all-out arm crank exercise (MPT $_{high}$ – right side column).

4.2 Measurements

On the first visit participants' anthropometric parameters with precision of 0.1 cm of height (Martin metal anthropometer) and 0.05 kg of body mass (A&D Instruments, Abingdon, UK), and body composition using dual-energy X-ray absorptiometry (DEXA) (Hologic Discovery DXA; Massachusetts, USA) were measured.

Heart rate (Polar Electro Oy; Espoo, Finland) and spirometric breath-by-breath functions (Cortex Metamax 3B, Cortex Biophysik; Leipzig, Germany) were measured and analyzed. The analyzer was calibrated before the test with barometric pressure, ambient air, humidity readings and gases of known concentrations. Incremental and experimental tests were performed: in *Paper I* on a C2 ski ergometer (SkiErg, Concept2, Inc., Morrisville, VT, USA, 2009®); in *Paper II* Cyclus2 ergometer (RBM Elektronik-Automation GmbH, Germany) and Wattbike Pro (Wattbike Limited; Nottingham, UK), respectively; in *Paper III* both tests were performed on Cyclus2 ergometer. Pre-loading exercise either by lower-body (*Paper I*: break weight 70 g·kg⁻¹ body weight) or upper-body (*Paper II* and *III*: break weight 35 g·kg⁻¹ body weight) was performed on a Monark Ergomedic 849E (Vansbro, Sweden).

Blood [La] (EKF-Diagnostic; Barleben, Germany) was determined for both conditions at rest, after the warm-up, after the anaerobic priming load, during the test (in *Paper III*), and during recovery. Arterialized blood samples (20 μ L) were taken from a pre-warmed fingertip (from a hyperemised earlobe in *Paper I*). The finger (earlobe in *Paper I*) was always cleansed with alcohol, and the first drop

of blood was removed to prevent contamination of the sample. At the same time as the [La] measurements, participants were asked to evaluate their overall- and muscle fatigue using the Borg rating of perceived exertion (0 to 10-point in *Paper I* and 6 to 20-point in *Paper II*, *III*) scale (Borg, 1978).

All data were analyzed by means of computer support using standard software (MetaMax-Analysis 3.21, Cortex, Leipzig, Germany).

4.3 Statistical analysis

All data variables were checked for normal distribution using the Shapiro-Wilk test, visual inspection of descriptive statistics, and z-score. Parametric methods such as paired samples t-test for comparison of means and 2-way repeated measures ANOVA test for comparison of time course with p < 0.05 as the level of significance were applied for parametric data. Wilcoxon signed-rank test and Kruskal-Wallis test were applied in the case of nonparametric data.

Data are expressed as mean \pm standard deviation (SD), presented with effect size (η^2) and confidence intervals (CI). Effect size based on Cohen's classification 0.01 < d < 0.2 was considered as 'very small', 0.2 < d < 0.5 'small', 0.5 < d < 0.8 'medium', 0.8 < d < 1.2 'large', 1.2 < d < 2.0 'very large' and d > 2.0 'huge' (Cohen, 1988). Partial Eta squared for two variables in repeated measures $\eta^2 < 0.04$ was considered 'small', $\eta^2 = 0.25$ 'moderate', and $\eta^2 > 0.64$ 'strong' (Ferguson, 2009). Correlations were determined using Pearson's correlation coefficient (r). The magnitudes of the correlation coefficients were stratified into groups comprising negligible (r < 0.30), low (0.30 < r < 0.50), moderate (0.50 < r < 0.70), high (r > 0.7) (Hopkins et al., 2009; Mukaka, 2012).

A prior power analysis was performed using G*Power© software (version 3.1.9.2, 2017) for comparison between two independent means. Data management and analysis were executed using Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA).

5. RESULTS

5.1 Priming effect on 1000m Ski-ergometer test (Paper I)

Ski ergometer maximal performance during MPT_{low} was not faster compared to the MPT_{high} condition (225.1 \pm 17.6 s vs. 226.1 \pm 15.7 s; p > 0.05). MPT_{low} was slower in the first 400m (p > 0.05) but tended to be faster in the last 600m. However, correlation analysis showed that slower start during first 200m was more beneficial for MPT_{high} and resulted in a shorter total time (r = -0.410, p < 0.05) and vice versa (Fig. 2), indicating some pacing effects.

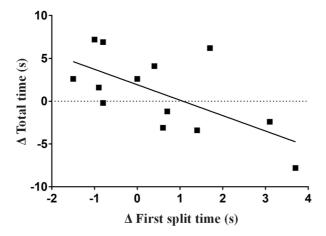


Figure 2. The relationship between first 200m split time (difference between MPT_{low} and MPT_{high}) and final difference in overall time in a maximal 1000-m all-out Ski-ergometer time trial performance test with low intensity (MPT_{low}) and with 25 s all-out cycling preload exercise (MPT_{high}) (r = -0.410; p < 0.05).

The 25 s lower-body high-intensity priming exercise significantly increased [La] before the start 8.2 ± 2.2 mmol·L⁻¹ and 1.4 ± 0.3 mmol·L⁻¹, in MPT_{high} and MPT_{low} respectively. Although, maximal [La] after MPT was 14.7 ± 4.6 mmol·L⁻¹ in MPT_{high} and 11.8 ± 2.4 mmol·L⁻¹ in MPT_{low}, the net [La] increase was reduced by approximately 50% in MPT_{high} (p < 0.05). Net [La] changes during recovery were similar for both protocols; however, remaining 13.5% higher in MPT_{high} trial even 15 min after the maximal test. Thus, considering [La] data in relation to power (W⁻¹) during MPT, less total anaerobic energy was produced in MPT_{high} (p < 0.001) although split by split analysis considering net V CO₂ increase in relation to W⁻¹ no significant differences occurred (p > 0.05; $d_{200} = 0.04$; $d_{400} = 0.5$; $d_{600} = 0.5$; $d_{800} = 0.4$; $d_{1000} = 0.2$). V O₂ uptake value was significantly higher in the first 400m of MPT_{high} (p < 0.05), while V CO₂ value was significantly lower in the first 200m (p < 0.05) and in the fourth and fifth 200m split.

Additionally, rating of perceived exertion before tests (RPE_{before}) was significantly (p < 0.05) lower in MPT_{low} compared to MPT_{high} and also after all-out exercise (Table 3).

Table 3. The results of maximal 1,000-m all-out Ski ergometer time trial performance test (MPT) per 200m splits with low intensity warm up (MPT $_{\rm low})$ and additional high 25 s all-out cycling pre-load (MPT $_{\rm high}).$

Participants $(n = 13)$	Time200m (s)	Time400m (s)	Тіте _{600т} (s)	Times00m(s)	Time1000m (s)	Time (s)	$\mathbf{RPE}_{\mathrm{before}}$	RPEafter
$ m MPT_{low}$	44.8 ± 4.2	44.9 ± 4.4	45.6 ± 4.0	45.7 ± 3.1	44.1 ± 2.6	225.1 ± 17.6	0.6 ± 0.8	8.9 ± 0.9
$ m MPT_{high}$	44.3 ± 3.7	44.4 ± 3.5	45.8 ± 3.4	46.6 ± 3.2	45.2 ± 3.5	226.1 ± 15.7	1.4 ± 1.2	9.5 ± 0.7
Dif	0.5 ± 1.6	0.6 ± 1.6	-0.2 ± 1.4	-0.8 ± 1.5	-1.1 ± 1.9	-0.9 ± 4.6	-0.8 ± 1.2	-0.7 ± 1.1
d	0.28	0.21	0.05*	0.07	0.07	0.47	0.04*	0.05*
Effect size	0.12	0.11	0.05	0.29	0.42	90.0	1.0	0.67

*Significantly different from MPT $_{\text{low}}$ (p < 0.05); Effect size 0.01 < d < 0.2 was considered as 'very small', 0.2 < d < 0.5 'small', 0.5 < d < 0.8 'medium', 0.8 < d < 1.2 'large', 1.2 < d < 2.0 'very large' and d > 2.0 'huge' (Cohen, 1988).

The RPE immediately after MPT, was significantly higher in MPT_{high} (9.5 \pm 0.7) compared to MPT_{low} (8.9 \pm 0.9), which means a significantly greater internal load was experienced by MPT_{high} (p < 0.05). Even though the internal load was greater, participants were able to perform with a minimal total time difference compared to MPT_{low} (1 \pm 4.6 s; p > 0.05).

5.2 Priming effect on 4000m cycling sprint (Paper II)

A 4000 m MPT was completed in 328.9 \pm 17.4 s by MPT_{high} condition which was significantly slower compared to 323.6 \pm 16.2 s in MPT_{low} (p < 0.05; d = 0.7) with a mean performance alteration of 5.3 \pm 7.7 s (1.6%, p <0.05; CI_{95%}= [0.1, 9.5]). Moreover, MPT_{high} condition performed slower in all 500m splits, although insignificantly (except 1500m–2000m split; p = 0.026; d = 0.6). No significant difference was measured between MPT all-out start power in both conditions, $10.5 \pm 2.1 \text{ W} \cdot \text{kg}^{-1}$ and $9.7 \pm 2.5 \text{ W} \cdot \text{kg}^{-1}$ in MPT_{low} and MPT_{high}, respectively (p > 0.05, d = 0.5, CI_{95%}= [-1.6, 0.2]) (Table 4).

Self-selected recovery lasted 522.0 ± 122.7 s in the MPT_{low} condition, compared to 792.6 ± 237.2 s in MPT_{high}. Data analysis showed that longer self-selected recovery time before the maximal performance tests was significantly related to a better maximal performance time in MPT_{high} (r = -0.550; p < 0.05), contrary shorter recovery in MPT_{low} condition related to faster time trial (r = 0.619; p < 0.05) (Fig. 3).

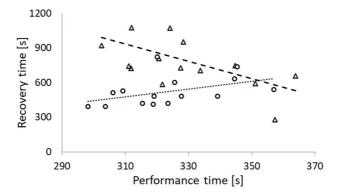


Figure 3. Correlation between recovery time (s) and performance time (s). Dashed line together with triangle-shaped data points indicate MPT_{high} trial (r = -0.550; p < 0.05) and dotted line together with circle-shaped data points MPT_{low} trial (r = 0.619; p < 0.05).

MPT_{high} condition spirometric values before the all-out start indicate speeded $V^{\circ}O_2$ kinetics (p < 0.05, d = 0.4), accompanied with significantly higher $V^{\circ}CO_2$ values (p < 0.05, d = 0.5), indicating that some buffering of acidosis from the priming was still ongoing. Although during the initial 500 m both parameters net increases were constantly and significantly lower in MPT_{high} compared to MPT_{low} condition (p < 0.006); and also lower, however insignificantly, in mean values in

relation to absolute power (W) (p > 0.05). Thus, no differences in aerobic economy ($5.04 \pm 0.85 \,\mathrm{mL \cdot min^{-1} \cdot W^{-1}}$ in MPT_{low} and $4.96 \pm 0.65 \,\mathrm{mL \cdot min^{-1} \cdot W^{-1}}$ in MPT_{high}) and anaerobic power production could be found during initial 500 m split. Significantly lower anaerobic power exertion, according to net $V \cdot \mathrm{CO}_2$ and mean absolute power, was found during $500-1000 \,\mathrm{m}$ (d = 1.1), $2000-2500 \,\mathrm{m}$ (d = 0.6) and $2500-3000 \,\mathrm{m}$ (d = 0.8) splits and insignificantly lower during other splits (p > 0.05). Despite the differences in net $V \cdot \mathrm{CO}_2$ and net $V \cdot \mathrm{CO}_2$, the total $V \cdot \mathrm{CO}_2$ consumption per W⁻¹ did not show any significant differences between trials (p = 0.765). However, RER data show MPT_{low} as being more anaerobic whereas MPT_{high} RER values remained lower (p < 0.05) during the entire race.

[La] achieved by priming action, was significantly higher in MPT_{high} right before the start ($6.9 \pm 2.1 \text{ mmol} \cdot \text{L}^{-1}$) compared to MPT_{low} ($1.4 \pm 0.5 \text{ mmol} \cdot \text{L}^{-1}$), p < 0.001; d = 2.5; CI_{95%} = [4.3, 6.7]. Although, MPT_{high} [La] remained higher until the end, the total net increase was significantly lower ($11.1 \pm 2.7 \text{ mmol} \cdot \text{L}^{-1}$) compared to $14.9 \pm 2.2 \text{ mmol} \cdot \text{L}^{-1}$ in MPT_{low} (p < 0.001; d = 1.2; CI_{95%} = [-5.5, -2.1]. According to [La] and mean power data, calculations showed that MPT_{low} produced more La_{net} per each W⁻¹, meaning more total anaerobic work was performed (p < 0.05, d = 0.9) (Table 4).

An overall RPE before the start $(8.3 \pm 3.0 \text{ in MPT}_{low} \text{ and } 8.2 \pm 1.9 \text{ in MPT}_{high})$ and immediately after MPT $(18.5 \pm 2.2 \text{ in MPT}_{low} \text{ and } 19.5 \pm 0.9 \text{ in MPT}_{high})$ did not show significant difference (p > 0.05). A muscle type RPE before the start $(8.3 \pm 3.0 \text{ in MPT}_{low} \text{ and } 8.1 \pm 1.8 \text{ in MPT}_{high})$ and immediately after MPT $(18.7 \pm 2.3 \text{ in MPT}_{low} \text{ and } 19.5 \pm 0.9 \text{ in MPT}_{high})$ did not show significant difference (p > 0.05).

Table 4. Effect of prior high-intensity warm-up on MPT (n = 15).

Parameter	MPTlow	MPThigh	<i>p</i> -value	Effect size (95% CI)
Initial 12 s				
Distance (m)	198 ± 14	192 ± 17	0.133	0.4 (-15.3 to 2.2)
Average power (W·kg ⁻¹)	10.5 ± 2.1	9.7 ± 2.5	0.104	0.5 (-1.6 to 0.2)
Peak power (W·kg ⁻¹)	1014 ± 219	974 ± 221	0.391	0.2 (-139.3 to 58.0)
Performance				
Time (s)	323.6 ± 16.2	328.9 ± 17.4	0.019	0.7 (1.0 to 9.5)
Average power (W·kg ⁻¹)	5.6 ± 0.7	5.3 ± 0.7	0.018	0.7 (-0.6 to -0.1)
Labefore (mmol·L ⁻¹)	1.4 ± 0.5	6.9 ± 2.1	< 0.001	2.5 (4.3 to 6.7)
$La_{net} (mmol \cdot L^{-1})$	14.9 ± 2.2	11.1 ± 2.7	< 0.001	1.2 (-5.5 to -2.1)
$La_{max} (mmol \cdot L^{-1})$	16.3 ± 2.2	17.9 ± 2.4	0.017	0.7 (0.3 to 2.9)
$V^{\cdot}\mathrm{O}_{\mathrm{2before}}\;(\mathrm{L}\!\cdot\!\mathrm{min}^{-1})$	0.5 (0.47 –0.66)	0.64 (0.51–0.78)	0.023	0.4 (0.03 to 0.16)
$V^{\cdot}\mathrm{O}_{2\mathrm{max}} \left(\mathrm{L}\!\cdot\!\mathrm{min}^{-1}\right)$	4.9 (4.8–5.2)	4.6 (4.3–5.2)	0.013	0.4 (-0.4 to -0.1)
Recovery time (s)	522 ± 123	792 ± 237	0.003	0.9 (110.2 to 431.0)

 MPT_{low} – maximal performance test without prior loading; MPT_{high} – maximal performance test with prior loading; CI – Confidence Interval for the Difference.

5.3 Priming effect on FTP20 cycling test (Paper III)

Mean constant power for initial 17 min, i.e 10% Δ , of MPT was $4.2 \pm 0.5 \text{ W} \cdot \text{kg}^{-1}$ and for final 3-minute all-out spurt $4.94 \pm 0.27 \text{ W} \cdot \text{kg}^{-1}$ in MPT_{low} and $4.85 \pm 0.39 \text{ W} \cdot \text{kg}^{-1}$ in MPT_{high}, which was not statistically different (p = 0.116; d = 0.5; CI_{95%} = [-0.0, 0.3]) (Fig. 4).

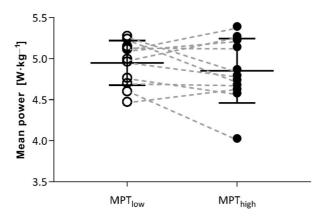


Figure 4. MPT performance mean power output during final spurt of MPT_{low} and MPT_{high}.

Strong correlations occurred between MPT_{low} self-paced finish power and incremental test values of $V \cdot O_{2max}$ (mL·kg⁻¹·min⁻¹) (p = 0.005; r = 0.78), maximal relative power (W·kg⁻¹) (p = 0.011; r = 0.73), and 10% Δ VT₂ (p = 0.016; r = 0.70). Self-paced finish power was not correlated with self-selected recovery time after warm-up, even though recovery time after warm-up was significantly longer in the MPT_{high} condition as the strenuous priming exercise significantly delayed the feeling of readiness from 813 ± 138 s in MPT_{low} to 958 ± 226 s in MPT_{high} (p = 0.011; d = 0.9; CI_{95%} = [42, 249]).

Despite of significantly longer self-selected recovery after warm-up in MPT_{high} the [La] before the start were still significantly higher in MPT_{high} (5.7 ± 0.5 mmol·L⁻¹ versus 1.1 ± 0.1 mmol·L⁻¹; p < 0.001; d = 2.6; CI_{95%} = [3.4, 5.7]). However, recovery was long enough to remove the elevated $V \cdot O_2$ level up to the start. The values at the start did not differ between MPT_{high} with 0.59 ± 0.12 L·min⁻¹ compared to 0.56 ± 0.13 L·min⁻¹ in MPT_{low} (p = 0.640; d = 0.1; CI_{95%} = [-0.11, 0.17]). Between-group effects were also insignificant (p = 0.57; $\eta^2 = 0.73$) throughout the tests. Calculation of metabolic economy was conducted by relating $V \cdot O_2$ to mean power for each split but showed no significant difference between conditions throughout the tests (p > 0.05) (Fig. 5).

Similarly, $V \cdot CO_2$ values were not different at the start between MPT_{high} with 0.53 \pm 0.11 L·min⁻¹ compared to 0.53 \pm 0.13 L·min⁻¹ in MPT_{low} (p = 0.96; d = 0.02) nor net increase throughout the race had any significant difference among two variables between MPT_{high} and MPT_{low} conditions. Although visual inspection of last 3 min of $V \cdot CO_2$ and $V \cdot CO_2$ graphs in Figure 5 can seem to have

a tendency for larger net increase in MPT_{high}, the differences were not significant $(0.195 . The between-group effect was insignificant <math>(p = 0.15; \eta^2 = 0.15)$ throughout the test. RER values demonstrated a steady-state (Fig. 5) from minutes 10 to 17 of the maximal performance up to the final spurt and reached its peak by the end of performance with no significant between-group effect $(p = 0.39; \eta^2 = 0.10)$.

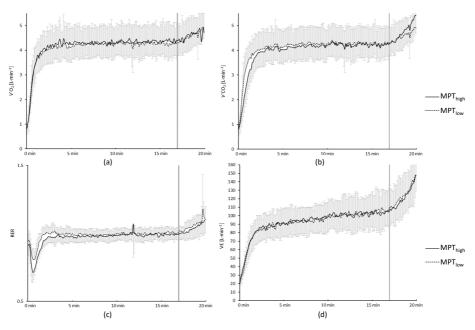


Figure 5. (a) Oxygen uptake $(V \cdot O_2)$; (b) carbon dioxide output $(V \cdot CO_2)$; (c) ventilation $(V \cdot E)$; (d) respiratory exchange ratio (RER), after a usual low intensity warm up (MPT_{low}) and an additional 25 s all-out arm crank priming pre-load (MPT_{high}).

However, net [La] increase in MPT_{high} was 5.4 ± 2.6 mmol·L⁻¹ and 10.8 ± 2.9 mmol·L⁻¹ in MPT_{low} (p < 0.001; d = 2.16; CI_{95%} = [3.7, 7.1]). Considering [La] data in relation to W⁻¹, less total anaerobic energy was produced in MPT_{high} (p < 0.001). Also split-by-split net [La] increase per W⁻¹ showed that anaerobic energy production in MPT_{low} was not significantly different only from 10' to 15', other 5' splits showed significant shift towards anaerobic contribution compared to MPT_{high} (p < 0.019; 0.9 < d < 1.6) (Table 5).

Table 5. Physiological changes throughout MPT's (n = 11).

Stage	Parameter	MPT_{low}	MPT_{high}	<i>p</i> -value	Effect size (95% CI)
Warm-up	Mean power (W·kg ⁻¹)	147.6 ± 19.1	147.6 ± 19.1		
	Recovery time (s)	813 ± 138	958 ± 226	0.011	0.94 (41.7 to 249.2)
Before	$V^{\cdot}O_2(L \cdot min^{-1})$	0.6 ± 0.1	0.6 ± 0.1	0.640	0.14 (-0.1 to 0.2)
start	$V^{\cdot}\mathrm{CO}_{2}\left(\mathrm{L}\cdot\mathrm{min}^{-1}\right)$	0.5 ± 0.1	0.5 ± 0.1	0.955	0.02 (-0.1 to 0.1)
	$[La] (mmol \cdot L^{-1})$	1.1 ± 0.4	5.6 ± 1.7	< 0.001	2.67 (3.4 to 5.7)
MPT 5'	$V^{\cdot}O_2$ (L·min ⁻¹)	3.7 ± 0.6	3.7 ± 0.5	0.434	0.25 (-0.1 to 0.3)
	$V^{\cdot}\mathrm{CO}_{2}\left(\mathrm{L}\cdot\mathrm{min}^{-1}\right)$	3.9 ± 0.8	3.4 ± 0.5	0.098	0.55 (-0.1 to 1.1)
	$[La] (mmol \cdot L^{-1})$	5.2 ± 0.8	6.8 ± 1.1	< 0.001	1.55 (0.9 to 2.4)
	Net [La] increase (mmol· L^{-1})	4.0 ± 0.8	1.2 ± 0.7	< 0.001	1.72 (1.7 to 4.0)
MPT 10'	V'O ₂ (L·min ⁻¹)	4.3 ± 0.6	4.2 ± 0.5	0.580	0.17 (-0.1 to 0.2)
	$V^{\cdot}CO_2$ (L·min ⁻¹)	4.2 ± 0.6	4.2 ± 0.5	0.413	0.26 (-0.1 to 0.3)
	$[La]\ (mmol \cdot L^{-1})$	6.2 ± 1.1	6.6 ± 1.8	0.191	0.42 (-0.3 to 1.2)
	Net [La] increase (mmol·L ⁻¹)	1.2 ± 0.2	-0.2 ± 1.6	0.011	0.93 (0.4 to 2.4)
MPT 15'	V'O ₂ (L·min ⁻¹)	4.3 ± 0.7	4.3 ± 0.5	0.451	0.23 (-0.1 to 0.3)
	$V^{\cdot}CO_2$ ($L^{\cdot}min^{-1}$)	4.3 ± 0.7	4.2 ± 0.5	0.331	0.31 (-0.1 to 0.3)
	$[La] (mmol \cdot L^{-1})$	6.6 ± 1.5	6.9 ± 2.1	0.530	0.20 (-0.7 to 1.3)
	Net [La] increase (mmol·L ⁻¹)	0.4 ± 0.8	0.2 ± 0.9	0.713	0.11 (-0.8 to 1.1)
MPT 20'	$V^{\cdot}O_2$ ($L \cdot min^{-1}$)	4.6 ± 0.6	4.6 ± 0.5	0.804	0.08 (-0.2 to 0.3)
	$V^{\cdot}CO_2$ (L·min ⁻¹)	4.9 ± 0.6	4.8 ± 0.6	0.456	0.23 (-0.2 to 0.4)
	$[La] (mmol \cdot L^{-1})$	11.6 ± 2.7	9.8 ± 3.4	0.073	0.60 (-0.2 to 3.8)
	Net [La] increase (mmol·L ⁻¹)	5.0 ± 2.1	2.9 ± 2.3	0.015	0.88 (0.5 to 3.7)
Perfor- mance	Average power _{steady} (W·kg ⁻¹)	4.2 ± 0.5	4.2 ± 0.5		
	Average power _{all-out} $(W \cdot kg^{-1})$	4.94 ± 0.27	4.85 ± 0.39	0.308	0.32 (-0.1 to 0.3)
	$[La_{net}] (mmol \cdot L^{-1})$	10.8 ± 2.9	5.4 ± 2.6	< 0.001	2.16 (-7.1 to -3.7)
	$[La_{max}] (mmol \cdot L^{-1})$	11.9 ± 2.8	11.1 ± 2.7	0.200	0.41 (-0.5 to 2.3)

 $MPT_{low}-maximal\ performance\ test\ without\ prior\ loading;\ MPT_{high}-maximal\ performance\ test\ with\ prior\ loading;\ CI-Confidence\ Interval\ of\ the\ Difference.$

Reported RPE for overall readiness which felt convenient to start the exertion was indicated by 7.1 ± 1.4 in MPT_{low} compared to 8.0 ± 1.5 points in MPT_{high} (p = 0.126) according to the Borg rating of perceived exertion (6 to 20-point) scale. Reported muscle type readiness was found significantly (p = 0.031) higher at 8.1 ± 1.7 points in MPT_{high} compared to 7.1 ± 1.4 points in MPT_{low} at the start. The overall RPE immediately after MPT was not significantly different (p > 0.05) in MPT_{low} (19.6 ± 0.9) compared to MPT_{high} (19.2 ± 1.5), similarly the muscle type RPE did not report any significant difference (19.6 ± 0.5) in MPT_{low} and 19.1 ± 1.2 in MPT_{high}) (p > 0.05). Between-group effects during 15 min of recovery after the maximal performance were insignificant for overall fatigue (p = 0.80; $\eta^2 = 0.02$) and muscle fatigue (p = 0.12; $\eta^2 = 0.01$), meaning no subjectively determined benefit related to recovery could be pointed to in either case.

5.4 Priming effect on economy and anaerobic contribution (Paper I, II, III)

Consistent calculations were used among all studies in order to compare the effect of priming on economy and anaerobic energy production. Spirometric values of $V^{\cdot}O_2$ and $V^{\cdot}CO_2$ (mL·kg⁻¹·min⁻¹) were related to relative power (W·kg⁻¹); similarly total net [La] values were related to absolute power (W⁻¹). Difference between calculated economy and anaerobic energy production could be considered as the anaerobic component. The exact values of total MPT and split-by-split calculations are given in Table 6. Considering the effect size above 'large' (d > 0.8) (Cohen, 1988) and significance level of p < 0.05, the data could be summed up as follows.

Total economy was significantly higher in MPT_{low} in *Paper I* (p < 0.05; d = 1.4) and in MPT_{high} in *Paper III* (p < 0.05; d = 1.5). Total anaerobic contribution calculated from V:CO₂ data was significantly higher in MPT_{low} in *Paper I* (p < 0.05; d = 2.0) and *Paper III* (p < 0.05; d = 1.7), no significant difference occurred in *Paper III* (p > 0.05; d = 0.8). Although, an anaerobic contribution calculated from [La] data had a larger effect size and significance, showing inhibited anaerobic energy production in MPT_{high} trial in all three studies (p < 0.001 and d = 2.2 in *Paper I*; p < 0.01 and d = 1.7 in *Paper II*; p < 0.001 and d = 2.2 in *Paper III*).

Split-by-split data is not applicable while 'small' and 'medium' effect size indicate limited practical significance.

Table 6. Economy and anaerobic contribution of each MPT

		Paper I			Paper II			Paper III	
	$\mathrm{MPT}_{\mathrm{low}}$	$ m MPT_{high}$	p-value; effect size (d)	$\mathrm{MPT}_{\mathrm{low}}$	$\mathrm{MPT}_{\mathrm{high}}$	p-value; effect size (d)	$\mathrm{MPT}_{\mathrm{low}}$	$\mathrm{MPT}_{\mathrm{high}}$	p-value; effect size (d)
Total work economy $(mL \cdot min^{-1} \cdot W^{-1})$	14.7 ± 3.3	14.9 ± 3.2	< 0.05; 1.4	13.6 ± 3.6	13.7 ± 3.7	> 0.05; 0.3	14.1 ± 1.1	13.9 ± 1.1	< 0.05; 1.5
Work economy by splits (mL·min ⁻¹ .W ⁻¹)	Min. 9.1 ± 3.3; Max. 17.3 ± 2.4	Min. 9.4 ± 2.5 ; Max. 17.6 ± 2.7	> 0.05; 0.03 < d < 0.2	Min. 5.0 ± 0.8 ; Max. 15.7 ± 1.5	Min. 5.0 ± 0.6 ; Max. 15.6 ± 1.3	> 0.05; $0.06 < d < 0.3$	Min. 11.4 ± 3.3; Max. 14.2 ± 4.1	Min. 11.3 ± 3.2; Max. 14.1 ± 4.1	> 0.05; $0.02 < d < 0.3$
Total anaerobic contribution (mL·min ⁻¹ ·W ⁻¹)	17.1 ± 4.6	14.7 ± 3.4	< 0.05; 2.0	15.4 ± 4.2	14.7 ± 4.2	< 0.05; 1.7	14.4 ± 1.1	13.8 ± 1.6	> 0.05; 0.8
Anaerobic contribution by splits (mL·min ⁻¹ ·W ⁻¹)	Min. 9.5 ± 3.7; Max. 21.0 ± 3.4	Min. 9.4 ± 1.9 ; Max. 20.0 ± 3.3	> 0.05; $0.04 < d < 0.5$	Min. 9.1 ± 3.3 ; Max. 17.3 ± 2.6	Min. 9.4 ± 2.5 ; Max. 17.6 ± 2.9	II, V, VI < 0.05; 0.6 < d < 1.1 I, III, IV, VII, VIII > 0.05; 0.1 < d < 0.6	Min. 12.4 ± 4.3; Max. 15.0 ± 4.3	Min. 10.5 ± 3.0; Max. 14.8 ± 4.3	> 0.05; $0.1 < d < 0.6$
Total anaerobic contribution (mmol·L ⁻¹ ·W ⁻¹)	0.05 ± 0.01	0.03 ± 0.01	< 0.001; 2.2	0.04 ± 0.01	0.03 ± 0.01	< 0.01; 1.7	0.03 ± 0.01	0.02 ± 0.01	< 0.001; 2.2
Net [La] increase by splits (mmol·L ⁻¹)	N/A	N/A		N/A	N/A		$I 4.0 \pm 0.8$ $II 1.2 \pm 0.2$ $III 0.4 \pm 0.8$ $IV 5.0 \pm 2.1$	11.2 ± 0.7 $II - 0.2 \pm 1.6$ $III 0.2 \pm 0.9$ $IV 2.9 \pm 2.3$	I, II, IV < 0.05; $0.9 < d < 1.7$ III > 0.05; 0.1
. 1000	5	:	300	1 \ 1	:		:	000/ 31 0 7 1 1 1 1	Contra

1000m Ski-ergometer test ($Paper\ I$) was divided into 5 splits (200 m each); 4000m cycling sprint ($Paper\ II$) was divided into 8 splits (500 m each); FTP20 cycling test ($Paper\ III$) was divided into 4 splits (5 minutes each); level of significance p < 0.05

6. DISCUSSION

6.1 General findings

The main finding of present thesis was that priming could not improve performance time or mean power throughout the MPT from the statistical point of view in three of our studies. However, every study contained subjects who improved their MPT_{high}, which means that single-subject effect is important. Moreover, priming had a strong effect on [La] before the start, lowering net [La] increase during the performance and respectively the total anaerobic contribution.

The four-minute upper-body maximal exertion performed in *Paper I* is highly related to V'O_{2max} and locomotion's oxygen cost (Losnegard et al., 2011). The distribution of aerobic versus anaerobic energy supply was described to be ~70%/30% (Losnegard et al., 2011), which is similar to what was found for other sports of the same duration (Gastin, 2001). It is a fact that anaerobic energy contribution is important for successful maximal performance, as it can fulfil fast and explosive energy demands (Secher, 1993) but as the race progresses, aerobic energy production becomes more dominant. Energy system contribution in crosscountry skiing was shown to be similar to running, cycling and rowing (Losnegard et al., 2011) at least with the applied duration of maximal exercise in Paper I. On the other hand, HIE preload as applied in our Paper I and Paper III reduced [La] increase, and accordingly anaerobic energy production, while without reducing overall performance. The findings of the current study are consistent with those of Baker and colleagues (2010), who found that aerobic metabolism and energy production are able to support extremely high muscle force application and power outputs – meaning that a preceding high-intensity bout or a preliminary race will not necessarily impair a subsequent time trial. In support of Baker and colleagues (2010), the results from Paper I and Paper III showed a significant reduction of the increase in net [La] in MPT_{high} (Müller et al., 2013) without significant differences for overall peak and mean power which is consistent with Bogdanis et al. (1994).

Significantly slower time was shown by MPT_{high} in *Paper II* (328.9 \pm 17.4 s in versus 323.6 \pm 16.2 s; p < 0.05; d = 0.7), with accompanying changes in participants' spirometric response on performance trials, as their respective $V \cdot O_2$ and $V \cdot CO_2$ curves during performance were significantly different. Moreover, during all 500 m splits $V \cdot O_2$ and $V \cdot CO_2$ both remained lower in the MPT_{high} condition throughout the execution of the protocol, although $V \cdot O_2$ was higher just before the start. The results indicate that in MPT_{high}, less absolute O_2 was consumed, less carbon dioxide exhaled, and a larger part of work was performed aerobically. However, the total amount was insufficient to produce beneficial effects. Therefore, efficient priming mechanisms for such short-duration maximal effort need to be questioned, which is in line with earlier results for all-out rowing exercise (Purge et al., 2017).

In *Paper III*, aerobic energy contribution was facilitated at the expense of anaerobic energy. A more economical power production due to a primed aerobic metabolism could be beneficial in the case of an energy deficit during longer distances among comparable athletes and saving more energetic reserves for the competition state. Perceived exertion data, overall fatigue, and spirometric capacity indicated that the FTP20 is too short to induce energy depletion. Furthermore, Borg RPE values showed statistically significant changes in readiness to perform for muscle fatigue levels, but at the same time reported overall fatigue did not show any statistical difference. Certainly, the practical significance of a 1-point difference is not highly relevant, although it can give an idea of altered perception after priming and that athletes are less dependent on muscle fatigue rather than overall fatigue when determining readiness to perform. This can give some confidence for further studies to feel free to use even higher loads for priming effects as muscle alterations cannot be the primary reason to limit readiness to perform.

Andersson et al. (2016) found that cross-country skiers tend to use positive pacing strategies with higher accumulated oxygen deficit during the first half of the time trial, while accumulated O_2 deficit was decreased during the final 300 m. Similar results were shown in *Paper I*, athletes reached their $V \cdot O_{2max}$ at the end of the first 400 m split and it was similar in both MPT_{high} and MPT_{low} tests. In the MPT_{high} test the athletes performed better during the first 400 m, although insignificantly. However, MPT_{low} showed significantly faster time during the last 400 m split. The decrease in overall performance in MPT_{high} compared to MPT_{low} was related to a small number of participants starting too fast. The others were able to perform faster although a reduced anaerobic contribution was indicated by a significant decrease in net [La] and in $V \cdot CO_2$. Maximal blood [La] in our study were found at 11.8 ± 2.4 mmol·L⁻¹ after 1000 m in MPT_{low}, and 14.7 ± 4.6 mmol·L⁻¹ in MPT_{high} which is comparable to the results of our previous studies in rowers (Purge et al., 2017). The metabolic pre-conditioning elevated both pre and post all-out exercise [La] but was well within tolerable limits.

6.2 Self-estimated recovery time outcome

While O₂ availability is crucial in maintaining the work level, prior priming has been proven to have a rapidly achievable (Chorley and Lamb, 2019) and relatively long-lasting and up to 45-min effect (Burnley et al., 2006). In earlier studies a 10-min recovery duration after priming was robustly estimated to have a beneficial effect on V O₂ response and a substantial increase in performance (Burnley et al., 2005; Jones et al., 2003). Sprint exercise before a heavy exercise described in prior studies may be compared to the applied HIE in our study as the physiological influence on V O₂ response and lasting lactic acidosis was similar (Burnley et al., 2001). This implies that high-intensity anaerobic pre-load exercise without sufficient recovery may limit athletic performance and maximal effort capacity, as long as maximal anaerobic energy contribution is required (Bishop

et al., 2001; Iaia et al., 2010; Parolin et al., 1999). Our conducted studies aimed to equalize perceived readiness for maximal performance, to eliminate any psychological feeling of distress or fatigue.

Interestingly, the subjects later described the MPT_{high} performance (in *Paper II*) as a "bad-leg day" and was perceived as uncomfortable laboratory temperature or sleepy feeling, which did not occur under the non-primed condition. This "badleg" feeling may be attributed to the obviously decreased anaerobic energy contribution, as shown by other studies (Bogdanis et al., 1994; Müller et al., 2013). As all of them followed the same preparation and regimen before both performance trials, and started from a fully recovered state, we assume the arm-crank loading preceding the time trial to be the main cause. Another cause may be that subjects might have overestimated their readiness or have become additionally sensitive to external factors after having experienced the supramaximal 25 s effort. In a similar study with rowers, Purge et al. (2017) also suggested that athletes who were forced to begin the time trial after 9-min recovery could not achieve their readiness to perform maximally in this study. Despite this limitation and a slower start, rowers were able to perform the second part of the distance at the same speed, thus calling into question the pacing strategy (Purge et al., 2017). Interestingly, our MPT_{high} trial cyclists' split times never achieved the same speed compared to MPT_{low} respective splits. However, the subjects were given the free will to determine their readiness level; we expected to have them fully prepared and recovered from the previous high-intensity short-duration arm-crank exercise.

Rating of perceived exertion was reported during all three studies at the same time with [La] sampling. Shorter recovery time in MPT_{high} trial in *Paper I* caused a significant difference between two conditions in overall RPE before the start $(0.8 \pm 1.2; p < 0.05)$, which referred to certain inequality and possible reason for a modest priming effect. Therefore, to equalize the internal load indicated by RPE, self-selected recovery time was adopted in *Paper II* and *III*. As the result, *Paper II'*'s overall RPE before the start and immediately after MPT were insignificantly different (p > 0.05), although subjective recovery up to 11' was slower in MPT_{high}. Similarly, *Paper III* overall RPE before the start and immediately after MPT did not show any significant difference (p > 0.05). Therefore, self-selected recovery time did more or less equalize the perceived internal load, since RPE in MPT_{high} was still found to be greater immediately after MPT in *Paper II*, although not significant difference occurred (p > 0.05). However, it appears that self-estimated recovery duration cannot guarantee optimal performance.

6.3 Implementation of different pacing strategies

Some details from *Paper I* suggest beneficial effects are not realized due to an unfavorable pacing strategy. Starting the first 200 m split faster than in MPT_{low} resulted in a prolonged total time compared to the MPT_{low} condition, which may be explained by the fact that a lower anaerobic contribution suggested participants to underestimate their condition and start faster. At the same time the 5-min

split analysis from Paper III did not indicate a significant difference between spirometric variable levels, although additional analyses showed significantly elevated V'O₂, RER, and V'E parameters during the first 2 min of MPT_{high}. Moreover, the [La] change during the first 5 min of MPT_{high} was significantly lower, which means that the same performance could be delivered with less anaerobic metabolism. If an increased demand for aerobic energy production could be less strenuous than anaerobic production, then lower overall fatigue during the start phase could be maintained. Apparently, this gives some space for higher intensity at the start and in combination with a suitable choice of intensity a significant improvement may be suggested due to the priming. Therefore, according to Paper I's results, refraining from a faster start is therefore necessary with respect to overall performance time, which could allow athletes to gain the effects of a reduced anaerobic energy contribution during the first minute of high-intensity anaerobic exercise. Contrarily, *Paper III* suggests that a physiologically favorable condition occurs during first 2 minutes of the race. We may argue that forcing an athlete's metabolism towards aerobic metabolism by inhibiting anaerobic glycolysis during the first minute of exercise can preserve the muscles' abilities to perform on a high level for finishing the distance without losing overall performance, although the optimal dosage of pre-load and recovery remains unclear and needs further investigations. Pacing the workload was shown to increase overall performance in the pull-up exercise (Birnbaumer et al., 2018), which indicates that a different pacing strategy needs to be developed by applying such a metabolic pre-conditioning warm-up compared to the usual pacing strategy. However, pacing gives the opportunity to realize the potential from priming. Paper III's findings make it clear that a poor pacing strategy, as suggested by Purge et al., 2017, cannot be the reason for priming a non-beneficial effect as both trials were paced to the same fatigue levels using the same strategy and a fixed pace. During the last 3 min, a cycling competition reaches its culmination where no pacing can be rationalized as an all-out strategy is most common. Pacing should instead be timed at greatest physiological response achieved by priming and should be used as a tool to realize the potential.

According to Caritá et al. (2014), priming is principal compared to fast-start pacing. Therefore, metabolic preparation can have an even more prominent effect than decisions made during maximal performance. In *Paper I* it was decided to exclusively investigate the effects of priming and no pacing was added to MPT. Correlation analysis of completed performance showed contrary/opposite results to previous studies (Bailey et al., 2011; Brock et al., 2018; Caritá et al., 2014), that a slow start was more favorable for the primed condition. Specifically, primed participants' slower first 200m split resulted in a shorter total MPT time. Despite this statistical finding, the slow start for MPT_{high} trial was doubted, since priming-induced spirometric changes were present, particularly in the beginning of the maximal performance, which is in line with Purge and colleagues' (2017) findings. Moreover, it was noted that a high-intensity priming bout could change the perception of subjects and raise a level of caution that did not allow them to start at the same speed. With the intention of avoiding any self-pacing strategies, it was

decided to add all-out start to the beginning of the maximal exertion in *Paper II*, during which the altered spirometric conditions were present. Paper II's pacing regime was adopted from Brock and colleagues (2018), using 12 s all-out start. Brock et al. (2018) has shown the combination of priming and all-out start to be the most beneficial when performed before a time trial and showed better results for such a protocol compared to any other condition (all-out unprimed, self-pacedunprimed and self-paced primed). Their research group used a 3-min submaximal 70Δ gas exchange threshold (GET) leg exercise as a priming activity, and in the current study we tried to achieve the same effect using a low-volume highintensity arm-crank priming exercise. Additionally, initial 12 s should have been completed mainly by an alactic energy production, which is already suggested to be effectively re-stored 10 min after heavy-intensity priming exercise and not so much dependent on oxygenation status (Burnley et al., 2011). This is in line with the results of mean power and distance travelled during the first 12 s, which did not show any significant difference between two conditions. However, overall performance mean power was lower in MPT_{high} (p = 0.018), thus total performance was significantly slower by 5.3 ± 7.7 s. Paper III's pacing strategy was intended to prepare both conditions to the same exertion rate during the first 17 minutes, with prior calculated intensity from incremental test of 10% Δ [VT₂ plus 10% of the difference between the work rate at the VT_2 and $V O_{2max}$, and all-out spurt during last 3 minutes as the competitive component. Both conditions reached an aimed exertion rate as seen from RER values 1.0 ± 0.04 (Fig. 5) in MPT_{high} and 1.0 ± 0.03 in MPT_{low} (p = 0.129; d = 0.5) right before all-out spurt. Thus, priming did not decrease the ability of MPT_{high} to tolerate a 17-minute exertion slightly above the VT₂, although could not perform significantly better during the last 3-minute all-out spurt. However, refraining from all-out start and beginning the distance with equally fixed pace did not spare/reserved anaerobic capability up to the end of MPT in *Paper III*, which was inhibited by priming from the very start. Even though [La] levels after 15 min were not significantly different between both interventions (p = 0.53; d = 0.2) the net [La] increase during the final 5' of MPT was significantly higher in MPT_{low} (p = 0.015). This means that submaximal intensity was too high to potentially restore anaerobic capacity, during the progress of MPT. We suggest that participants were used to a gradual increase of [La] such as in MPT_{low}, whereas in MPT_{high}, [La] values already plateaued at significant levels and anaerobic energy contribution may have been already depleted/inhibited to some extent by the beginning of the final spurt. Although FTP20 is predominantly aerobic (Denham et al., 2020), potentially the duration was still not long enough to bring out beneficial effects of priming.

Importantly, the effect of a high-intensity anaerobic start needs to be elucidated. High-intensity effort increases muscle and blood [La] and decreases pH already by the first minute of a 3–4 min race if starting [La] is low (Martin et al., 1975). It is well known that anaerobic glycolysis is at its highest after about 40–50 s and starts to decrease thereafter due to inhibitory effects of the high [La], low pH situation (Rivera-Brown and Frontera, 2012). It was shown that pre-

elevation of systemic La levels by non-specific muscle exercise inhibited subsequent net La production, an effect which is suggested to be applied in competitive high-intensity exercise to improve performance (Müller et al., 2013). Previous studies have suggested that arterialized blood [La] level <5 mmol·L⁻¹ could have a beneficial effect on subsequent performance as values substantially >5 mmol·L⁻¹ could lead to a reduction in performance (Burnley et al., 2005; Gerbino et al., 1996). Although [La] could be used as an additional fuel for muscle contraction (Brooks, 1998), it is also a limiting factor as decreased pH inhibits anaerobic glycolysis for subsequent performance (Bangsbo et al., 1993). Within the investigated [La] elevation, pre-exercise blood [La] and MPT performance in MPT_{high} were not significantly related although the optimal limits of [La] increase need to be elucidated. Two main effects may be expected from priorly elevated [La], such as a decrease of anaerobic energy production during the first minute of a race and a resulting increased oxidative energy contribution (Birnbaumer et al., 2018; Bogdanis et al., 1994). Developing this idea opens new doors to understanding the metabolic background of competitions longer than 2-3 min. On the one hand the strategy of metabolic pre-conditioning could be helpful in the prologue but on the other the higher [La] levels during recovery may be disadvantageous for the subsequent exercise bouts. However, this intervention may be beneficial as in some cases even an increased performance was found (Birnbaumer et al., 2018) if [La] elevation $(9.3 \pm 2.0 \text{ mmol} \cdot \text{L}^{-1} \text{ before start})$ was induced by muscle groups not involved in the main exercise workout. Thus, specific sport should also be re-evaluated as potentially the priming produced by bigger muscle mass can elevate [La] levels to a greater extent without causing significant fatigue. Priming HIE executed by lower-body, as in *Paper I* and such as applied by Birnbaumer et al. (2018), may be more favorable to successfully increase performance.

6.4 Priming as a highly individual approach

Correlation analysis in *Paper III* showed that last 3 min relative power in MPT_{low} was correlated with variables from the incremental tests such as $V \cdot O_{2max}$ (mL·kg⁻¹·min⁻¹), maximal relative power (W·kg⁻¹), and 10% Δ VT₂ for the first 17 min. Since MPT_{high} did not correlate with any of these initial values, it shows that initial physiological preparation cannot predict a performance capacity in primed condition and can easily diminish previous training. However, many studies (Bailey et al., 2009; Bohnert et al., 1998; Burnley et al., 2011; Gerbino et al., 1996) have shown the physiological advantages of priming, although they cannot guarantee any improved results during an actual race (Carter et al., 2005; Ferguson et al., 2007; Sousa et al., 2014). In this case, we still suggest pre-load to have beneficial effects, which are highly individual and rather empirical methods are needed to distinguish such effects in every single athlete. For example, in *Paper I* six participants (46.2%) from MPT_{high} completed 1000m Ski-ergometer test in a shorter time; in *Paper II* two participants (13.3%) from MPT_{high} completed

4000m cycle ergometer test in a shorter time; in *Paper III* four participants (36.4%*typo in original article) benefited from the priming and developed higher mean power during last 3 min of the final spurt in our study. A possible explanation for this might be the neuromuscular excitability, which was induced on central level, since priming was performed with non-primary muscle groups. However, previous studies prescribed neuromuscular potentiation mainly to single repetition or sprint exercise, but endurance sport on submaximal level is not common (Seitz and Haff, 2016). However, a clear advantage of this particular low-volume priming method is that it could enable the athletes to have a time-efficient and physiologically beneficial re-warm-up during a competitive event with multiple time trials. A previous study by Yanaoka and colleagues (2020) found that 1-minute cycling at 90% of $V \cdot O_{2max}$ is superior to 15-minute rest period and may increase a subsequent intermittent cycling sprint performance over 10 minutes.

6.5 Limitations and strengths of the dissertation

The main limitation of Paper II and Paper III is the non-specific recovery time before the MPT. Since the effect of prior heavy exercise can be modified by the duration of recovery, it should thus be treated as the independent variable (Burnley et al., 2006). The main reasons for choosing self-selected recovery time is the aim of providing a comparably full readiness among subjects based on their perceived state, since expected maximal performance should be well preconditioned based on athletes' competitive experience and age (Barroso et al., 2014). The previous study shows that effective recovery duration can vary among individuals (Seitz et al., 2014) and we were willing to exclude any insufficient recovery, and provide self-selected conditions that would correspond to those of an ideal competitive preparation, which has also shown to be beneficial (Palmer et al., 2009). Evaluating the pros and cons, it was decided to rely on subjects' self-perception since all of them have been well trained athletes with more than 5 years of competitive experience. The second limitation was found in *Paper I*, when poor performance in some participants could occur because they were not frequently using the skiing ergometer, as many of them prefer to use roller skis as an alternative for the summer season. In the absence of experience of using it for maximal performance, starting and finishing pace strategies were based simply on the first laboratory test. Similarly, some of the participants did not switch power during the final all-out spurt in Paper III though preferring to add more watts. Thirdly, statistical power calculations prior to all studies showed a sufficient number of participants, but the total number of 20 participants would allow us to use additional regression analysis, which could show cause and effect relationship.

The current study's incremental test protocol was different in all three studies (Table 2); however, it did not affect the results. Standard 1-minute incremental step is suggested for further studies. Additionally, our experiment is limited to

the influence of high-intensity anaerobic metabolic preconditioning exercise on just a single subsequent bout of either cross-country specific ergometer 1000m exercise or cycling specific 4000m sprint and FTP20 but did not focus on several repeated exercise bouts such as in a real-life competition (Hébert-Losier et al., 2017).

The main strength of this thesis is the consistent methods of priming with greatly homogenous well-trained athletes that allowed to gather data in a short period. Two protocols were prepared in a way that priming was left as the only variable between two conditions. Calculated intensities for priming, warm-up, and pacing (*Paper III*) guaranteed the expected physiological response (Fig. 5). All protocols contained a competitive component that served as a quantitative measure. In case of individual compatibility with any of the protocols, the methods are cheap and easy to apply without any additional expenses.

7. CONCLUSIONS

Based on the results of the current dissertation, the following conclusions were made:

- 1. Priming lowers net [La] increase and respectively the total anaerobic energy contribution during subsequent maximal performance test. (*Paper I, II, III*)
- 2. Warm-up added by HIE may not improve performance during subsequent maximal performance test; however, positive responses observed at the individual level suggest that some subjects may benefit from this method. (*Paper I, II, III*)
- 3. Self-selected recovery time after HIE should be longer because of additional load to ensure an optimal performance and equal perceived exertion during subsequent maximal performance test. (*Paper II*, *III*)
- 4. Steady pace after warm-up added by HIE improves exercise tolerance and enhances second ventilation threshold due to increased economy during not less than 17 minutes of anaerobic exercise. (*Paper III*)

8. PRACTICAL APPLICATIONS

Metabolic conditioning is an emerging trend improving basic warm-up effects. Based on our study results, anaerobic HIE performed with non-dominant muscles with respect to the main task has the potential to inhibit the net La increase at the start of exercise and the total net La of the performance, while remaining insignificantly different mean power. Consequently, 25 second all-out arm crank exercise or lower-body HIE cycling is sufficient to inhibit anaerobic and facilitate aerobic metabolism. Although it did not improve the performance, it could be used to enhance aerobic efficiency during training process, as pre-loading with opposite muscle groups cannot be recommended prior to time trials.

This research extends our knowledge of systemic metabolism and possibilities to alter anaerobic mechanisms. Exercise therapy is safe intervention and in selected cases is just as effective as medical treatment. High-intensity low-volume priming could serve as an additional treatment effect in the cases of chronic diseases, where exogenous additional lactate could inhibit the particular disease and add an effect to medication, chemo- or radiotherapy.

Our findings could encourage the health systems to create the necessary infrastructure to provide a supervised exercise, while it is important that society supports the active lifestyle. However, there is still a need to define the most optimal type and dose of exercise, but avoiding physical activity poses a greater risk than engaging in physical exercise. The author hope that current thesis could promote the debate and bring something valuable in todays' research.

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SUMMARY IN ESTONIAN

Lühiajalise maksimaalse eelpingutuse mõju maksimaalsele pingutusele vastupidavusalade sportlastel

Klassikaliselt tuntud eelsoojenduse ülesehitus ühendab endas madala intensiivsusega aeroobset harjutust (MPT_{low}), millele järgneb venitus ning spordiala spetsiifiline soojendus (Burkett et al., 2005; Hajoglou et al., 2005; Richendollar et al., 2006). Mõlema eesmärk on lihaste ja keha temperatuuri tõstmine, närvijuhtivuse parandamine, kiirendatud metabolism ning hapnikutarbimise ja füsioloogilise valmisoleku parandamine. Sportlikust soojendusest tingitud aktivatsioon ja tekitatud väsimus peaksid olema omavahel hästi reguleeritud tasakaalus ning viima sportliku saavutusvõime paranemiseni (McGowan et al., 2015).

Viimasel ajal on hakatud uurima kehalises pingutuses mitteosalevate lihasgruppide tugevat, kuid lühiaegset (high-intensity exercise - HIE) eelpingutust (Birnbaumer et al., 2018; Purge et al., 2017), väsitamata spordiala-spetsiifilisi lihasgruppe. Lisades HIE eelpingutust aeroobsele harjutusele (MPT_{high}), võib see aidata häälestada keha süsteemsed mehhanismid eelseisvaks pingutuseks. Mõju avaldub ka väiksemate lihasgruppidega sooritatud HIE eelpingutusest (Bogdanis et al., 1994; Bohnert et al., 1998; Spendier et al., 2020), mis suudab tõsta maksimaalse hapniku omastamist (V'O2) suurte lihasgruppidega teostatud HIE eelpingutusega võrreldavale tasemele (Bogdanis et al., 1994; Bohnert et al., 1998). Eelpingutusest tingitud kõrgenenud [La] pärsib anaeroobse energia produktsiooni, mida sportlikul sooritusel kompenseerivad vastavalt aeroobsed mehhanismid. Seejuures summaarne energia tootmine ei tarvitse olla vähenenud (Müller et al., 2013). Kuigi HIE eelpingutus võib aktiveerida füsioloogilisi näitajaid, ei garanteeri füsioloogiline valmisolek paremat sportlikku sooritust (Carter et al., 2005; Ferguson et al., 2007; Sousa et al., 2014). Samas kiire starditempo valik koos eelpingutusega on näidanud positiivset mõju tulemusele (Brock et al., 2018; Caritá et al., 2014).

Anaeroobse töö energia tootmine põhineb enamjaolt glükoosi lagundamisel ehk glükolüüsil, mille esmasel lagunemisel koguneb lihasesse püruvaat, mis lihase hapnikuvaeguse korral muundatakse laktaadi dehüdrogenaasi toimel laktaadiks. Algselt suureneb [La] lihaste tasandil, mis madalal intensiivsusel edukalt hapniku abil lagundatakse, ning edaspidi vereringes, kui lihase hüpoksiline seisund pole enam võimeline suurt hulka laktaati elimineerima (Gladden, 2004). Vereringe kannab laktaati kui energiaallikat teistele lihastele ning organitele, mis oma hapnikuvarude abil sellest efektiivselt ATP-d sünteesivad (Brooks, 2018, 2009, 1998; Gladden, 2004). Lisaks on laktaat ka glükoneogeneetiline prekursor ning regulaator (Brooks, 2018, 1986).

Uurimustöö eesmärgid

Uurimustöö eesmärk oli kindlaks määrata, kas mitte-spordispetsiifiliste lihaste sooritatud kõrge intensiivsusega eelpingutus (HIE) parandab järgneva spordispetsiifilise maksimaalse jõudluse testi tulemusi kõrgelt treenitud meessoost vastupidavusalade sportlastel.

Uurimustöös püstitati järgmised ülesanded:

- 1. Hinnata HIE eelpingutuse mõju keha metaboolsetele mehhanismidele ja anaeroobse energia tootmise osakaalule kogu energiavarustusest järgneva maksimaalse jõudluse testi ajal.
- 2. Mõõta aja ning võimsuse erinevusi maksimaalse jõudluse testis eelpingutusega ning ilma.
- 3. Määrata optimaalne taastumisaeg enne maksimaalse jõudluse testi pärast HIE eelpingutust.
- 4. Kontrollida, kas standardiseeritud tempo maksimaalse jõudluse testi ajal suudab kompenseerida eelnevast HIE-st põhjustatud anaeroobse energia puudujääki.

Uuritavad ja metoodika

Käesolev doktoritöö koosneb kolmest eraldiseisvast uuringust. Uuringus I osales 13 suusatajat (18.1 \pm 2.9 a; 181 \pm 5 cm; 70.8 \pm 7.6 kg; 57.3 \pm 5.3 mL·kg⁻¹·min⁻¹), uuringus II 15 jalgratturit (23.3 \pm 3.6 a; 181 \pm 7 cm; 76.2 \pm 10.0 kg; 65.4 \pm 6.7 mL·kg⁻¹·min⁻¹) ja uuringus III 11 jalgratturit (18.8 \pm 0.9 a; 182 \pm 5 cm; 73.0 \pm 6.6 kg; 67.9 \pm 5.1 mL·kg⁻¹·min⁻¹).

Uuritavad läbisid võistlusdistantsi kahel korral maksimaalse võimaliku võimsusega, mõlemale eelnes madala intensiivsusega soojendus (20 min), kuid MPT_{high} puhul lisandus ka kõrge intensiivsusega lühiajaline anaeroobne pingutus (25 sek). Uuringute protokollid erinesid taastumisaja, MPT kestvuse ning määratud tempo poolest.

Järeldused

- 1. Eelpingutus vähendab neto [La] kasvu ning vastavalt kogu anaeroobse energiatootmise osakaalu järgneva maksimaalse jõudluse testis. (Uuring I, II, III)
- 2. Soojendusele lisatud HIE ei pruugi järgneva maksimaalse jõudluse testi ajal jõudlust parandada; siiski, üksikisiku tasandil täheldatud positiivsed mõjud viitavad, et mõned katsealused võivad sellest meetodist kasu saada. (Uuring I, II, III)
- 3. Sportlaste endi valitud taastumisaeg pärast HIE-d peaks lisakoormuse tõttu olema pikem, et tagada optimaalne jõudlus ja võrdne tajutud pingutus järgneva maksimaalse jõudluse testi ajal. (Uuring II, III)
- 4. Ühtlane tempo pärast eelpingutusega soojendust parandab soorituse taluvust ning võimekust teisel ventilatsioonilävel tänu suurenenud ökonoomsusele vähemalt 17-minutilise anaeroobse treeningu ajal. (Uuring III)

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