Increasing critical power and anaerobic work capacity with high-intensity training or bicarbonate supplementation

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INCREASING CRITICAL POWER AND ANAEROBIC WORK CAPACITY WITH HIGH-INTENSITY TRAINING OR BICARBONATE SUPPLEMENTATION

A thesis submitted to attain the degree of

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presented by

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Summary

The critical power (CP) concept is an extensive approach for characterization of endurance performance ability by covering its aerobic and anaerobic parts. CP represents theoretically the highest power output that can be maintained for an indefinite time. The second parameter of the concept is defined as a finite work capacity that is used when exercising above CP ($W'$). In most endurance and team sports disciplines, an increase in CP with a concomitant improvement in $W'$ is aspired. A higher CP is thereby sought to increase fatigue resistance, while a higher $W'$ strengthens coping with power peaks (*e.g.* sprints). Training and nutritional strategies might be used to achieve these desired improvements. On the one hand, sodium bicarbonate (NaHCO$_3$) is known as an efficient ergogenic aid during high-intensity exercise and is therefor promising in enhancing competition performance. On the other hand, classical endurance training leads to improvements in CP, while conventional resistance training leads to increases in $W'$. However, it is known that, especially in trained athletes, the efficiency and effectiveness of classical training modalities is limited. Therefore, we aimed at further improving CP and/or $W'$ with novel effective and efficient training stimuli as well as by the application of an up to the present uninvestigated NaHCO$_3$ supplementation protocol.

In the first study, we addressed for the first time the multiday acute NaHCO$_3$ supplementation. This ingestion protocol is frequently used during multiday competitions and tournaments. In eight well-trained endurance athletes, time-to-exhaustion ($T_{lim}$) at CP was improved with NaHCO$_3$ supplementation compared to placebo on five consecutive testing days. In concomitance to the performance enhancement, pretest [HCO$_3^-$] and blood pH were increased on every testing day to the same extent, respectively. The high salt loading due to NaHCO$_3$ supplementation led to an increase in plasma volume, which might have presumably attenuated a further increase in [HCO$_3^-$]. This mechanism might have limited the performance-enhancing effect of NaHCO$_3$. Consequently, it is advised for athletes during multiday competitions or tournaments at CP intensity to use NaHCO$_3$ as ergogenic aid.
In a second study, we aimed at increasing the effectiveness and efficiency of classical training modalities by overcoming the interference effect. Based on previous research in our laboratory showing that resistance training with superimposed side-alternating whole-body vibration (WBV) and vascular occlusion, termed vibroX, leads simultaneously to endurance and resistance type adaptations, we provided the final proof of concept that vibroX induced these adaptations simultaneously also in endurance-trained males with their associated training-induced energy stress. VibroX increased CP, cross-sectional area (CSA) of myosin heavy-chain (MyHC)-1 and -2 fibers from M. vastus lateralis, and overall capillary-to-fiber ratio, while conventional resistance training did not increase any of these variables. \( W' \) was increased with conventional resistance training and showed a non-significant increase with vibroX. Hence, vibroX increased CP in endurance-trained males and prevented the interference effect that occurred with conventional resistance training.

In the third study, we addressed major disadvantages of conventional aerobic high-intensity interval training (HIT), namely decreases in \( W' \) and muscle fiber atrophy of MyHC-2 fibers. By replacing the low-intensity rest intervals with side-alternating WBV decreases in \( W' \), CSA of MyHC-2A fibers from M. vastus lateralis, maximal voluntary torque, rate of force development, and maximal countermovement jump power were prevented without additional time commitment. In addition, CP, overall capillary-to-fiber ratio, peak cardiac output, and oxidative enzyme activities were similarly increased after conventional aerobic HIT and HIT combined with WBV during rest intervals. Therefore, HIT with WBV during rest intervals is advised for athletes in team and endurance sports, wherein anaerobic performance and/or capacity play a deciding role.

In conclusion, increases in CP were achieved with vibroX as well as with HIT (with or without WBV during rest intervals). \( W' \) was increased following resistance training and the decrease in \( W' \) after conventional aerobic HIT was prevented by the addition of WBV during rest intervals. The novel training modalities induced thereby higher and/or additional adaptations compared to classical modalities. Consequently, training efficiency and effectiveness was improved by applying the novel training modalities. Athletes engaged in team or endurance sports, in which anaerobic performance and capacity play a deciding role, aiming at increasing CP are advised to incorporate vibroX or HIT with WBV during rest intervals into their training routine. Furthermore, \( T_{\text{lim}} \) at CP was improved with daily acute NaHCO\(_3\) supplementation on five consecutive days. During multiday competitions or tournaments with intensities at the threshold
between the heavy and severe intensity domains, it is recommended to ingest daily a single acute dose of NaHCO₃ before competition.
Zusammenfassung


In der ersten Studie befassten wir uns erstmals mit der mehrtägigen akuten Einnahme von NaHCO₃. Dabei handelt es sich um ein Protokoll, welches regelmäßig bei mehrtägigen Wettkämpfen und Turnieren eingesetzt wird. Die Fahrzeit bis zur Erschöpfung ($T_{lim}$) bei CP war bei acht sehr gut trainierten Ausdauersportlern an allen fünf Messtagen mit NaHCO₃-Supplementierung länger im Vergleich zu Placebo. Parallel zur längeren Fahrzeit waren die Vortest-$[\text{HCO}_3^-]$ und der Blut-pH-Wert an allen Tagen höher bei der Intervention verglichen mit dem Placebo. Die hohe Salzzufuhr als Folge der NaHCO₃-Supplementierung führte zu einer
Steigerung des Plasmavolumens, welche womöglich eine weitere Erhöhung der $[\text{HCO}_3^-]$ verhinderte und so ebenfalls die leistungssteigernde Wirkung der Supplementierung limitierte. Zusammenfassend wird Athleten, die in mehrtägigen Wettkämpfen oder Turnieren teilnehmen und deren Wettkampfintensität um CP liegt, angeraten täglich NaHCO$_3$ direkt vor dem Wettkampf einzunehmen.


gleichzeitig auf eine Erhöhung der anaeroben Leistungsfähigkeit und Kapazität abzielt, HIT mit seitenalternierender GKV während den Pausenintervallen angeraten.

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Chapter 1

Introduction

Success in sports relies on a multiplicity of distinct intrinsic and extrinsic factors (Tucker and Collins 2012). Based on the athlete’s intrinsic factors, *i.e.* genetic predisposition, training is the key extrinsic factor that determines success in competition. In endurance sports and most team sports, the physical performance ability represents the key factor for success. Basically, we distinguish between four different components of physical performance ability. On the one hand, exercise intensity determines the type of adenosine triphosphate (ATP) producing reactions, namely aerobic metabolism during low-intensity and anaerobic metabolism during high-intensity exercises. On the other hand, it is differentiated between the ability to achieve a peak value, *i.e.* performance, and the ability to sustain a submaximal value for prolonged time, *i.e.* capacity. Therefore, the four components of physical performance ability are: aerobic performance, aerobic capacity, anaerobic performance, and anaerobic capacity. The characteristics of the sports discipline define the exact contribution of these distinct components to competition performance. Accordingly, training routines are planned in the manner to improve these components based on the sports disciplines characteristics. It is obvious that effectiveness and efficiency of training determine training progress and are therefore crucial factors for competition performance. However, the effectiveness and efficiency of classical training modalities are limited. In this context, especially well-trained athletes might reach a performance plateau by using classical exercise stimuli. With the objective of developing performance ability to the maximum novel training modalities are necessary to achieve an advantage in competition.

For the accurate determination of actual values at distinct time points and time responses of these deciding factors during the training routine and for optimally planning of training
schedules, physical performance tests are indispensable. There are a multitude of different concepts and associated physical performance tests to characterize performance ability. The most used concepts for the determination of endurance performance ability aim on the maximal metabolic steady state or approximations therefrom (e.g. anaerobic threshold, lactate threshold, and gas exchange threshold; Faude et al. 2009, Meyer et al. 2005). The main focus of these concepts concentrates on aerobic performance, while aerobic capacity as well as anaerobic performance and capacity are covered insufficiently. To cover all factors of human endurance performance ability, additional tests or different concepts are necessary.

1.1 The critical power concept

The cornerstone for an adequate concept was set almost ninety years ago by Hill (1925), who recognized that there is a hyperbolic relationship between average competition speed of athletic world records in running and swimming and the time needed to cover the distance. Hill (1925) concluded that a variety of different fatigues cause exercise cessation within this distinct velocity-time relationship. Forty years later, a similar relation was found for maximum work and limited time of work in single muscle groups (Monod and Scherrer 1965). Consequently, it was assumed that every muscle could sustain a given power above a certain threshold only for a limited amount of time. Monod and Scherrer (1965) termed the threshold critical power (CP) and defined it as the maximum work rate that a muscle can sustain for a very long time without fatigue. Subsequently, the CP concept was adapted to cycling exercise (Moritani et al. 1981) indicating that not only local peripheral fatigue but also systemic fatigue is characterized by a hyperbolic power-time relationship.

![Power-time relationship](image)

**Figure 1.1:** Power-time relationship. CP, critical power; $W''$, finite work capacity above CP.
The power-time relationship (Fig. 1.1) is characterized on the one hand, by the asymptote to the hyperbola, i.e. CP, and on the other hand, by the curvature constant \( W' \). Theoretically, CP stands for the power that can be sustained for an infinite long time and \( W' \) for a finite amount of work that is drawn on during exercise at intensities above CP (irrespective of the exact exercise intensity). This implicates that the CP concept is only applicable for exercise intensities at or above CP. Exercise at intensities below CP can inevitably be maintained for an indefinite time.

In a physiological context, CP stands for the threshold between the heavy and severe intensity domains (Jones and Poole 2005). The heavy intensity domain is characterized by power outputs leading to delayed steady states for blood lactate concentration and oxygen uptake (\( \dot{V}O_2 \)). Exercise in the severe intensity domain leads to progressively increasing blood lactate concentrations and \( \dot{V}O_2 \) values until \( \dot{V}O_{2\text{peak}} \) is attained resulting in exercise cessation. Therefore, CP represents theoretically the highest power output at which a metabolic steady state can be achieved. In practice, exercise at CP can be maintained for 20 to 40 min (Brickley et al. 2002), mainly because of reductions in intrinsic energy stores or because of attaining maximal rates of thermoregulatory cooling mechanisms.

Based on the theoretical definition, CP should be congruent with the anaerobic threshold, which is defined as the highest power output where blood lactate concentrations attain a steady state (Urhausen et al. 1993). However, there is a controversy if CP is equal to the anaerobic threshold or if it overestimates it. On the one hand, Poole et al. (1988) reported \( \dot{V}O_2 \), blood lactate and blood pH steady states during cycling at CP, while cycling above CP (CP +5% peak power of an incremental test) resulted in ascending values up to maximum. Furthermore, phosphocreatine concentration ([PCr]), inorganic phosphate concentration, and muscle pH estimated with \(^{31}\)P magnetic resonance spectroscopy attained steady states after 3 min of one-legged knee extension exercise with a workload 10% below CP, while with exercise 10% above CP [PCr] and muscle pH decreased until exhaustion and inorganic phosphate concentration increased progressively (Jones et al. 2008). It is important to mention that workloads during one-legged knee extension differed on average by only 4 W (Jones et al. 2008) indicating that CP represents a strong demarcation between the intensity domains. In addition, a previous study in our laboratory revealed identical values for CP and the ventilatory threshold, i.e. the inflection point in the expiratory minute volume (\( \dot{V}E/\dot{V}O_2 \)-curve determined in an incremental step test (unpublished data). On the other hand, six of eight participants in a study by Jenkins and Quigley (1990) were not able to maintain CP for 30 min during cycling. This contradicts the definition of the maximal lactate steady state that blood lactate concentration does not increase more than 1 mmol \( \cdot \) l\(^{-1} \) from the 10\(^{th} \) to 30\(^{th} \) min of exercise (Urhausen et al. 1993). In addition,
further studies directly compared CP with the maximal lactate steady state during cycling and showed that CP overestimated the maximal lactate steady state by ~10-15% (Dekerle et al. 2003, Pringle and Jones 2002).

1.2 Mathematical basis of the critical power concept

Mathematically, the hyperbola of the power-time relationship is defined as (Whipp et al. 1982):

\[ W' = (P - CP) \cdot t \]  

(1.1)

Accordingly, a given power output above CP \( P \) can only be maintained for a limited time \( t \) yielding into the finite work capacity \( W' \). Alternatively, to ease the analysis and plotting of the relationship, often the linear \( 1 \cdot \text{time}^{-1} \) model (Hill 1993) is used:

\[ P = \frac{W'}{t} + CP \]  

(1.2)

In this linear model, CP represents the intercept of the regression line with the \( y \)-axis and \( W' \) describes the slope of the regression line.

An advantage of the CP concept over most determinations of anaerobic, ventilatory or lactate thresholds is its mathematical basis. The equations can be solved with two values for power and limited time, respectively, whereby CP and \( W' \) are determined. A mathematical model leaves no scope for interpretation. In contrast, in most other models, the determination of the threshold through an expert bears the risk of large intersubjective variation. Furthermore, the determinations of thresholds using incremental tests are reliant on inflection points in the response curve of the investigated parameter. In some exceptional cases where an inflection point is not present, determination of the threshold is not possible. In comparison to the determination of the maximal lactate steady state, the short duration of the tests and the fix amount of determining tests are advantageous.

To handle the fact that the above-described 2-component model might overestimate the true maximal metabolic steady state, a 3-component model was described that should settle this overestimation (Morton 1996). In this model, the above-mentioned equation is supplemented with the factor \( k \) that represents the time asymptote at \( t = k \):

\[ W' = (P - CP) \cdot (t - k) \]  

(1.3)

To avoid a factor that represents negative time \( (k) \) and is unusual as a physiological character, Morton (1996) transformed the equation to:
\[ t = \frac{W'}{(P - CP)} + \frac{W'}{(CP - P_{\max})} \]  

(1.4)

As part of this transformation, Morton (1996) introduced the maximal instantaneous power \( (P_{\max}) \). According to this model, CP was significantly lower and \( W' \) was markedly higher than determined with the 2-component model (Morton 1996). However, there is one major reason that precluded the acceptance of the 3-component model in the scientific community. According to the 3-component model, an all-out effort is the only mathematically optimal competition strategy (Morton 2009). As this strategy might be reasonable for extreme short competitions, it may be doubted whether this strategy will be successful in competitions of intermediate and long durations. Therefore, most scientists favor the 2-component model, because competition strategy is not restricted therein (Fukuba and Whipp 1999). In addition, the benefit of the third parameter is questioned, as the 3-component model leads to estimates of CP and \( W' \) that are inferior correlated to physiological variables, e.g. work rate at ventilatory threshold, than those calculated from the 2-component model (Chatagnon et al. 2005).

1.3 Determination of critical power

1.3.1 Constant-load tests for the determination of critical power

Classically, the two parameters of the critical power concept are determined by at least 3 constant-load tests at different power outputs (Moritani et al. 1981). To increase the accuracy of determination often a fourth constant-load test is included. Using the afore-mentioned 2-component linear and/or hyperbolic equations, CP and \( W' \) are calculated. To assure that the duration of the determining constant-load tests do not fall below or exceed certain thresholds most procedures include an initial incremental test for the ascertainment of power outputs of the following constant-load tests. It is thereby advised that the duration of the determining constant-load tests is between 1 and 10 min (Poole 1986). Tests shorter than 1 min bear two risks of adulterating determination of CP and \( W' \). First, the steep slope of the power-time relationship for short exercise times leads to a mathematical incertitude. Second, insufficient force of the participants might reduce cycling time at exaggerated power outputs (Poole 1986). In contrast, performance in prolonged constant-load tests might be influenced by participant’s motivation to maintain the power output despite increasing fatigue, whereby reliability of the test outcome is reduced (Hopkins et al. 2001).
1.3.2 All-out tests for the determination of critical power

The classical determination of CP and $W'$ implies a large expenditure of time. In the course of simplifying the determination of CP and $W'$, the main focus was on reducing the number of determining tests. The use of all-out tests was thereby promising. The theoretical approach of the all-out tests is to deplete $W'$ at the beginning of exercise. During the first seconds up to a minute of an all-out effort, PCr utilization and anaerobic glycolysis allows a rapid ATP re-synthesis. Consequently, power output peaks during the first seconds and falls thereafter hyperbolically, mainly because of a reduction in the ATP re-synthesis rate. At the end of the all-out test, *i.e.* after depletion of $W'$, the participant is only able to maintain CP, whereby ATP production mainly relies on oxidation of substrates. In a first step, all-out test duration of 90 s was investigated (Dekerle *et al.* 2006). However, using this protocol end test power, *i.e.* average power during the last 30 s of the all-out test, overestimated CP significantly. In a subsequent step, all-out test duration was extended to 3 min (Burnley *et al.* 2006). It was shown that end test power of the 3-min all-out test corresponds to CP (Vanhatalo *et al.* 2007). Moreover, cumulated work above CP during the all-out test, *i.e.* the area under the power-time-curve and above CP, coincides with $W'$ (Vanhatalo *et al.* 2007). However, despite the practicability of this CP determination, the testing modality suffered from two disadvantages. First, $V\dot{O}_2$peak was not achieved during the all-out test (Vanhatalo *et al.* 2007). Hence, an important variable of endurance performance ability remained hidden. Second, determination of the resistance on the pedals during the all-out test was based on the outcome of a previous incremental cycling test (Burnley *et al.* 2006). Therefore, the main goal to determine CP and $W'$ with a single test was not achieved.

1.3.3 Wingate-tests for the determination of critical power

In an effort to minimize expenditure of time for the determination of CP, we tested a 3-min Wingate-test with a resistance of 5% body mass as predicting trial (unpublished results). End test power of the Wingate-test significantly correlated with CP without differences in the mean of these variables. End test power and CP were also similar to the ventilatory threshold determined during an incremental step test. In addition, stroke volumes measured at the end of the Wingate-test and at peak power of an incremental step test were equal. However, cumulated work above CP was significantly lower than $W'$ and there was no correlation between these variables. In addition, cardiac output at the end of the 3-min Wingate-tests was significantly lower compared to peak cardiac output at peak power of the step test. The lower cardiac output at the end of the Wingate-test was based on a significantly lower heart rate compared to peak
heart rate in the step test (unpublished results). Our results are supported by the report of Clark et al. (2013), who showed that end test power between a 3-min all-out test and a 3-min Wingate-test were similar. In contrast to our result, cumulated work above CP was not different between the 3-min all-out test and the 3-min Wingate-test (Clark et al. 2013). However, cumulated work above CP was less reliable and there was a poor correlation between the values determined from the two different tests. Consequently, a 3-min Wingate-test is not suitable for the comprehensive determination of human endurance performance.

1.3.4 Application of the critical power concept in performance diagnostics and training

As described above, the CP concept does not only provide information about aerobic performance but also about anaerobic capacity. This represents a large advantage compared to incremental tests that are often used for endurance performance diagnostics. In addition to CP and $W'$, the procedure for CP determination applied in our laboratory provides peak values for $\dot{V}O_2$, cardiac output, stroke volume, and heart rate. Therefore, the test battery of the CP concept allows a comprehensive characterization of human endurance performance ability. The exact and comprehensive knowledge of an individual’s performance ability and adaptability to a training stimulus is crucial in planning training routines, namely because of two reasons. First, every individual reacts and adapts different on a specific training stimulus (Timmons 2011). Second, the ability to train is not causally linked to the ability to adapt (Toigo and Boutellier 2006). Both parameters of the CP concept can be used for the description of single training interventions. On the one hand, exercise around CP or at a certain percentage therefrom can be used to attain defined physiological responses with the goal of correspondent training adaptations. On the other hand, interval trainings can be planed with the goal to deplete $W'$ during high-intensity intervals at a given intensity for a given time. Therefore, training sessions can be planed adapted to every individual athlete, whereby the largest training outcomes can be expected.

1.4 Increasing performance with conventional training modalities

1.4.1 Modification of CP and $W'$ with conventional training modalities

An increase in CP is achieved after 8 weeks of continuous moderate-intensity cycling endurance training consisting of three 30-40 min training sessions per week (Jenkins and Quigley 1992). This training modality has no significant effect on $W'$ (Jenkins and Quigley 1992). However, $W'$ decreased after continuous endurance training on average by $\sim 25\%$ (Jenkins and Quigley 1992).
Up to date, increases in $W'$ have only been shown with two types of exercise. First, $W'$ improves by $\sim 50\%$ after 8 weeks of sprint cycling training with long rest intervals (Jenkins and Quigley 1993). Second, resistance training for the legs leads to an increase in $W'$ during cycling by $\sim 35\%$ after 6 weeks (Bishop and Jenkins 1996) and by $\sim 50\%$ after 8 weeks of training (Sawyer et al. 2014). In addition, it is known that resistance training does not alter CP (Bishop and Jenkins 1996, Sawyer et al. 2014).

1.4.2 Specificity of training

On the continuum of training modalities, there is on the one extreme endurance training and on the opposite extreme resistance training. These two extremes are not only characterized by different exercise tasks but lead also to completely distinct training adaptations. It is scientifically well established that endurance training lead to mitochondrial biogenesis, an increase in $\dot{V}O_{2\text{max}}$, and improved capillarization of the skeletal muscle (Hawley 2002). In contrast, resistance training leads to myofiber hypertrophy, increases in “strength”, and improved neuronal function (Narici et al. 1996). These distinct training adaptations are emphasized by the selective activation of different molecular signaling pathways following the two training modalities and the concomitant upregulation of a variety of distinct genes (only those with relevance for the present thesis are mentioned below).

Messenger ribonucleic acid (mRNA) expression indicates that energy stress during conventional endurance training leads acutely to the activation of 5'-adenosine monophosphate activated protein kinase (AMPK; Wang et al. 2009), the main regulator of cellular energy homeostasis. AMPK activates peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α, Fig. 1.2). This activation is supported by an increase in PGC-1α mRNA expression after endurance exercise (Leick et al. 2010). PGC-1α acts as a master regulator for several downstream targets involved in mitochondrial biogenesis. In addition, PGC-1α also regulates vascular endothelial growth factor (VEGF, Fig. 1.2). An increase in VEGF mRNA expression following endurance exercise (Ringholm et al. 2011) is associated with exercise-induced angiogenesis.

An acute bout of resistance training results in the activation of the protein kinase B (AKT)-mammalian target of rapamycin (mTOR)-pathway (Fry et al. 2011) that is involved in muscle fiber hypertrophy (Fig. 1.2). AKT activates mTOR through inhibition of the negative regulator tuberous sclerosis complex (TSC). In addition, AKT inhibits the forkhead box proteins (FOXO) and hence, downregulates atrophy signaling by reduced expression of atrogin-1 and
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muscle RING-finger protein-1 (MuRF-1). mTOR acts as a key integrator of multiple upstream signals additional to AKT and activates p70 ribosomal S6 kinase (p70S6K). p70S6K controls several downstream targets involved in translation initiation. Activated mTOR and p70S6K inhibit eukaryotic elongation factor 2 kinase (eEF2K), which represents an inhibitor in translation elongation by phosphorylating and inactivating eEF2. Moreover, resistance exercise reduces mRNA expression of myostatin as well as TSC 1 and 2 (Drummond et al. 2009) that are responsible factors for muscle atrophy and inhibition of protein synthesis, respectively (Fig. 1.2).

Figure 1.2: Simplified schematic illustration of the signaling pathways activated after endurance exercise (blue) and resistance exercise (orange) as well as those leading to protein breakdown (purple; modified from Hoppeler et al. 2011). For further information, see text. AMP, adenosine monophosphate; AMPK, 5'-adenosine monophosphate activated protein kinase; AKT, protein kinase B; ATP, adenosine triphosphate; eEF2, eukaryotic elongation factor 2; eEF2K, eukaryotic elongation factor 2 kinase; FOXO, forkhead box proteins; mTOR, mammalian target of rapamycin; MuRF-1, muscle RING finger protein-1; p70S6K, p70 ribosomal S6 kinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor.
1.4.3 Concurrent training and interference effect

Based on the different training adaptations and distinct selective activation of molecular signaling pathways, there are at least two problems that arise from these distinct training modalities. First, it is not possible to train for all adaptations simultaneously. It is known that an interference effect occurs if resistance and endurance training are combined (Hickson 1980). Concurrent resistance and endurance training attenuates muscle fiber hypertrophy in comparison to resistance training (Kraemer et al. 1995). In addition, the increases in one-repetition maximum (1RM; Gergley 2009) and maximum jumping power (Chtara et al. 2008) are reduced with concurrent training compared to the gains following exclusive resistance training. In contrast, aerobic adaptations, e.g. $\dot{V}O_{2max}$, are not affected by concurrent training (Dudley and Djamil 1985, Hickson 1980). On a molecular level, it is assumed that the interference effect is based on an acute upregulation of AMPK as result of the high-energy turnover following endurance training (Atherton et al. 2005, Yeo et al. 2010; Fig. 1.2). AMPK activates TSC2 (Atherton et al. 2005) and eEF2K (Thomson et al. 2008). TSC2 inhibits phosphorylation of mTOR and prevents activation of translation initiation factors. eEF2K phosphorylates eEF2 and thereby inhibits its function in translation elongation (Fig. 1.2). Based on this intracellular signaling as explanation for the occurrence of the interference effect, it is assumed that the effect is locally limited to the exercised muscle groups.

Second, based on the interference effect, endurance and resistance training should be separated in the weekly training routine for enhanced training adaptations. However, also with splitting of the trainings modalities from the same to alternate days, it is not possible to completely overcome the interference effect (Dudley and Djamil 1985, Bell et al. 2000). Nonetheless, already the splitting of training modalities to alternate days results in a larger expenditure of time. As the limited amount of time to prepare for the next competition or the next season is a critical factor for every athlete, the most effective and efficient training modality is the method of choice. Saving of time enables the athlete to invest more time in regeneration procedures or in additional/alternative trainings. Based on the interference effect, especially endurance athletes might suffer from an inability to increase muscle mass as result of their high energy turnover. As an increase in muscle mass might be a critical factor in several tasks, e.g. final sprint or time trials, the interference effect might determine victory or defeat in this population group.

Most of the studies reporting an interference effect were conducted with young and trained participants. In contrast, the interference effect seems to be less prominent in the untrained
(McCarthy et al. 1995) and the elderly (Wood et al. 2001). In the untrained, the attenuation of the interference effect relies on increased anabolic molecular signaling after an identical bout of resistance exercise compared to trained individuals (Nader et al. 2014). In the elderly, AMPK is phosphorylated after a single bout of resistance exercise without affecting phosphorylation of mTOR and without reducing mixed muscle fractional synthesis rate (Drummond et al. 2008). Hence, resistance training seems to bear a higher energy stress in the elderly compared to younger individuals but this additional stress has no influence on protein synthesis.

1.5 Increasing performance with novel training modalities

1.5.1 Increasing performance with novel exercise stimuli

Classical training stimuli are not applicable to increase efficiency or effectiveness of training. Furthermore, up to date, no combination of classical training stimuli was able to inhibit the interference effect. Therefore, to increase efficiency and effectiveness of training and to allow a comprehensive training effect, there is the need for novel training stimuli that provide these benefits. In addition, well-trained athletes might have reached a performance plateau using the classical training modalities. For these athletes, new exercise stimuli acting on the individual response matrix might activate or reinforce signaling pathways leading to higher training adaptations.

1.5.1.1 Whole-body vibration

Whole-body vibration (WBV) is one novel training stimulus that has the potential for a comprehensive training effect. WBV training leads to an increase in maximal muscular power with a concomitant increase in rate of force development (RFD; Cheng et al. 2012). The increase in maximal muscular power is furthermore associated with improved jumping height (Rønnestad 2004). In addition, WBV training might lead to muscular hypertrophy (Bogaerts et al. 2007). Irrespective of these neuronal and muscular adaptations, it was shown that vibration superimposed to cycling exercise increases serum content of VEGF (Suhr et al. 2007). As VEGF is the master regulator of capillarization, it is speculated that WBV training increases capillary-to-fiber ratio. It is reasonable that a higher capillarization leads to a higher exchange area between muscle cell and blood. This increased exchange area might enhance endurance performance through improved transport of oxygen and nutrients to the muscle as well as transport of metabolites and heat away from the muscle (Saltin et al. 1986).
The underlying reasons for these training adaptations following WBV are widely unidentified. This lack of knowledge is mainly due to the unknown exact mechanistic function of WBV. It is assumed that vibration leads to a reflex contraction in the exposed muscle and to relaxation of the antagonists, termed the tonic vibration reflex (Hagbarth and Eklund 1966). The presence of stretch reflexes was supported by an elongation of the muscle tendon complex length by ~1% of its resting length during side-alternating WBV at 6 Hz (Cochrane et al. 2009). It is proposed that neurogenic potentiation based on this reflex activity might improve performance. On this note, several acute effects have been measured and are proposed to explain the adaptations to side-alternating WBV. On the one hand, electromyography activity of the lower limb muscles is increased with side-alternating WBV (Perchthaler et al. 2013, Pollock et al. 2010). With an adequate knee angle and vibration amplitude electromyography activity up to 75% of maximal voluntary contraction was recorded (Perchthaler et al. 2013). On the other hand, motor unit recruitment might be altered with side-alternating WBV. In single motor units, it was shown that recruitment thresholds of lowest threshold motor units were increased, while recruitment thresholds of higher threshold motor units were decreased after WBV (Pollock et al. 2012).

1.5.1.2 Vascular occlusion
The second auspicious novel training stimuli is vascular occlusion. Low-load resistance training with vascular occlusion of the trained extremity results in similar training adaptations compared to high-load resistance training without vascular occlusion. These adaptations incorporate muscle fiber hypertrophy (Takarada et al. 2000) and improvements in “strength” (Moore et al. 2004). Additional support for muscle fiber hypertrophy is lent by an increased mixed muscle fractional synthetic rate 3 h after blood flow restricted exercise (Fry et al. 2010, Fujita et al. 2007). These effects were proved in elderly (Takarada et al. 2000) and young people (Kubo et al. 2006a) as well as in athletes (Abe et al. 2005). Furthermore, activation of signaling pathways involved in translation initiation and translation elongation as well as reduced activation of pathways involved in protein breakdown supports muscle fiber hypertrophy. Low-load resistance training with vascular occlusion leads to phosphorylation of mTOR (Fry et al. 2010) and p70S6K (Fujita et al. 2007) pointing to an increment in translation initiation. On the contrary, mRNA expression of FOXO3a and atrogin-1 (Manini et al. 2011), which both are involved in muscle fiber atrophy mechanisms, are decreased after low-intensity resistance exercise with vascular occlusion.

Low-intensity exercise with vascular occlusion also enhances at least three variables that are characteristic for endurance exercise or endurance training. First, low-intensity resistance
exercise with blood flow restriction increases mRNA expression of VEGF in young participants (Larkin et al. 2012), which might promote capillary formation. Second, low-intensity walking exercise with vascular occlusion increases $\dot{V}O_{2\text{peak}}$ by 11.6% in basketball players after 24 training sessions (Park et al. 2010). Third, low-load resistance training with blood flow restriction improves oxygen availability during exercise and increases task-specific endurance capacity after 16 training sessions (Kacin and Strazar 2011). Therefore, exercise with vascular occlusion of the legs represents an auspicious modality to achieve a comprehensive training effect. In all mentioned studies in this and the previous paragraph, vascular occlusion was only applied during exercise. Immediately after exercise cessation, the pressure of the cuffs was released resulting in re-perfusion of the trained extremity.

1.5.1.3 Sustaining vascular occlusion

A further possible application of vascular occlusion is sustaining the cuff pressure after exercise cessation. It is proposed that this procedure results in further metabolic trapping. The intermediate catabolic products (e.g. metabolites and ions) remain in the trained extremity and are supposed to activate additional signaling pathways or amplify activated pathways. Up to date, this application was not used as an individual exercise stimulus and therefore it might only be conjectured which effects can be attributed to this stimulus. Until now, it was applied as part of the vibroX (vibration + occlusion + resistance exercise; Item et al. 2011, 2013) training. A duty cycle of vibroX training consisted of high-intensity resistance exercise with superimposed WBV and vascular occlusion until muscular failure and sustaining of vascular occlusion for additional 3 min after exercise cessation. Based on the corresponding result (Item et al. 2011), it is assumed that sustained vascular occlusion might be accountable for an improvement in capillarization, an increase in oxidative enzyme activity, and inducing myosin heavy chain (MyHC)-1 fiber hypertrophy. Furthermore, mRNA expression of PGC-1α and VEGF are increased 3 h after vibroX (Item et al. 2013), whereby sustaining vascular occlusion might be accountable for these activations.

1.5.1.4 High-intensity interval training

Interval trainings are part of the training routine in athletes for decades. Only in the recent years, the focus of the scientific community was traced back to the physiological mechanisms of this training modality. High-intensity interval training (HIT) results in higher adaptations of the cardiovascular system, i.e. $\dot{V}O_{2\text{max}}$ and stroke volume, than long slow distance training or training at the lactate threshold (Helgerud et al. 2007). Out of the multiplicity of HIT protocols, short high-intensity intervals (15-30s) with similar short rest intervals (Stepto et al. 1999) or
high-intensity intervals of 4 min duration with 3 min rest intervals (Helgerud et al. 2007) result in the highest training adaptations. In addition, these training protocols lead to a large training effect with a considerable short expenditure of time. The effectiveness of HIT was proved along all population groups from men after heart failure (Wisløff et al. 2007) up to well-trained athletes (Driller et al. 2009). In accordance with these mentioned results, conventional HIT increases CP (Gaesser and Wilson 1988, Poole et al. 1990, Vanhatalo et al. 2008). In the studies of Gaesser and Wilson (1988) as well as Vanhatalo et al. (2008) concomitant to the increase in CP $W'$ showed a slight decline after training. More importantly, there was a significant negative correlation between $\Delta CP$ and $\Delta W'$. While increasing CP with endurance training, the activation of the slow gene program in concomitance with a more oxidative metabolism (Hood et al. 2011) seems to have a negative impact on $W'$. This reduction in $W'$ despite the increase in CP might have a crucial impact on competition performance in athletes engaged in team sports and endurance sports in which anaerobic performance and capacity (e.g. sprinting) plays a deciding role in succeeding. Therefore, the focus of new training modalities should concentrate on the maintenance or increase of $W'$ simultaneously to an improvement in CP.

The adaptations to conventional HIT do not only adversely affect $W'$ but also the share of muscle fiber types. HIT leads to a decrease in MyHC-2X fiber proportion (Simoneau et al. 1986) and to a strong tendency for a reduction in cross-sectional area (CSA) of MyHC-2A fibers (Kohn et al. 2011). The reduced share of MyHC-2 fibers leads inevitably to a reduced maximal muscular shortening velocity (Larsson and Moss 1993) and hence, it reduces also maximal muscular power. Muscle fiber atrophy in response to HIT relies on both, a decreased myofibrillar muscle protein synthesis and an increased muscle protein breakdown (Rennie and Tipton 2000). The decrease in myofibrillar muscle protein synthesis is associated with activation of AMPK after aerobic HIT (Yeo et al. 2010). Consequently, a novel HIT modality aiming at increasing CP should prevent muscle fiber atrophy and/or increase neuronal function to maintain or even enhance anaerobic performance and capacity.

1.5.2 Combination and superimposition of exercise stimuli

As described previously, it is not possible to attain a comprehensive training effect using the classical exercise modalities. As efficiency and effectiveness are the key players in introducing a novel training modality, new paths need to be taken. Based on the afore-mentioned literature, WBV and vascular occlusion represent training stimuli that might enable a comprehensive training effect by simultaneously increasing aerobic performance, aerobic capacity, anaerobic performance, and anaerobic capacity. A training modality, based on superimposing these
training stimuli to a classical training modality, leading to a comprehensive training effect is vibroX (Item et al. 2011). VibroX training induces MyHC-1 and -2 fiber hypertrophy concomitant with increases in capillarization and endurance capacity in sedentary females (Item et al. 2011). Furthermore, this training modality increases mRNA abundance of PGC-1α and VEGF in recreationally active males (Item et al. 2013) even though it is classically characterized as a resistance training modality. Therefore, there is the promise that the superimposition of classical training modalities, i.e. resistance or endurance training, with novel training stimuli might enhance training effects and result in the best of cases in a most comprehensive training effect. In addition, it remains to be investigated whether the superimposition of exercise stimuli is necessary or whether the sole combination of certain exercise stimuli within a training session is sufficient in achieving the intended training adaptations.

1.6 Increasing performance with nutritional stimuli

In addition to the physical performance ability, several substances have been assigned a beneficial effect on performance. According to current scientific knowledge and the joint position statement of the American Dietetic Association, the Dietitians of Canada, and the American College of Sports Medicine (2009), five ergogenic aids have an evidence-based positive effect on performance. These supplements are bicarbonate (HCO$_3^-$), caffeine, creatine, sports drinks/gels/bars, and protein/amino acids. All of these five supplements have received plenty of scientific attention. Accordingly, the effects of HCO$_3^-$ on performance are widely investigated. It is scientifically well described that a dose of HCO$_3^-$ increases blood pH and [HCO$_3^-$]. The increased [HCO$_3^-$] gradient between cytosol and blood leads to a higher transport rate of protons (H$^+$) out of the muscle cell. This mechanism prolongs intact muscle function by maintenance of intramyocellular pH. Up to date, it was shown that HCO$_3^-$ is effective from ~1 min (Van Montfort et al. 2004) up to 60 min (McNaughton et al. 1999b). During short exercises of less than 1 min, the effectiveness of HCO$_3^-$ supplementation is controversially discussed (Shelton and Kumar 2010). H$^+$-efflux time from intramyocellular compartment to blood might thereby surpass the upper limit for a significant reduction in intramyocellular pH during exercise. Based on the theoretical mechanism, HCO$_3^-$ should only be an ergogenic aid in exercise that comes along with disturbances in intramyocellular acid-base homeostasis, i.e. exercises at intensities higher than CP or the anaerobic threshold. The improved performance in a 60 min time trial (McNaughton et al. 1999b) might therefor be explained by the distinct power profile of such exercise. However, to our knowledge, no study investigated the effect of
NaHCO\textsubscript{3} supplementation during exercise at CP or the anaerobic threshold, yet. Therefore, the physiological responses following supplementation at these intensities remain hidden. There is also another large knowledge gap on HCO\textsubscript{3}\textsuperscript{−} supplementation. Within the large samples of studies investigating the effects of HCO\textsubscript{3}\textsuperscript{−}, only two supplementation procedures were discussed, \textit{i.e.} acute loading vs. chronic loading. None of the studies so far has investigated the effects of multiple doses on continuative days. This procedure is frequently used in multiday competitions or tournaments. In addition, it is well known that salt loading, which occurs during the chronic and multiday acute ingestion protocols, increases plasma volume (Heer \textit{et al.} 2009). However, none of the studies investigating the chronic supplementation mode has focused on this parameter and the associated effects on [HCO\textsubscript{3}\textsuperscript{−}], yet.

1.7 Objectives

Based on promising results of nutritional and novel exercise stimuli on human performance and the components of the CP concept, we aimed at expanding the current knowledge of exercise enhancement with the investigation of a further supplementation protocol and with the investigation of novel high-intensity training modalities. First, we aimed at investigating the effects of a multiday acute NaHCO\textsubscript{3} ingestion protocol on time-to-exhaustion (\textit{T}_{\text{lim}}) at CP. Therein, a special focus was on day-to-day alterations in physiological variables during the consecutive intake days (Chapter 2).

Based on the promising results from our laboratory that vibroX training simultaneously induces myofiber hypertrophy and improves endurance performance and capacity, we aimed at the final proof of concept that these adaptations holds also true in endurance-trained athletes. By the addition of vibroX training to a large volume of endurance training, possible effects due to an untrained state of the participants can be eliminated and the mechanisms of vibroX can be consolidated (Chapter 3).

Following the knowledge that CSA of MyHC-2 fibers as well as anaerobic capacity and performance are decreased after conventional aerobic HIT, we aimed at modifying a HIT protocol to prevent any negative effects on performance. Therefore, we aimed at investigating the effects of HIT with side-alternating WBV in lieu of the cycling rest intervals on the parameters of the CP model (Chapter 4).
Chapter 2

*Multiday acute sodium bicarbonate intake improves endurance capacity and reduces acidosis in men*

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2.1 Introduction

Competitive sports performance is strongly dependent on optimal muscle function. During cycling exercise across the heavy and severe intensity domains (Jones et al. 2010), energy is provided more and more by anaerobic glycolysis. This leads to an increased rate of accumulation of metabolites, which have been linked with muscle fatigue (e.g. P\text{\textsubscript{i}}, ADP, H\textsuperscript{+}, and extracellular K\textsuperscript{+}). Cycling exercise at the threshold between the heavy and severe domain, i.e. at ‘Critical Power’ (CP), can, in contrast to the theoretical concept (Monod and Scherrer 1965), only be sustained for as long as 20 to 40 min (Brickley et al. 2002) before task failure. Furthermore, it was shown that CP overestimates the highest possible metabolic steady state (Jenkins and Quigley 1990, Pringle and Jones 2002) and, consequently, that exercise at or above CP is associated with a decline in muscle and blood pH (Jones et al. 1977, Jones et al. 2008). An activity-induced decrease in intracellular pH has been suggested to limit exercise because it inhibits glycogenolysis and glycolysis (Hollidge-Horvat et al. 2000), increases muscular K\textsuperscript{+}-release (Fabiato and Fabiato 1978) and inhibits sarcoplasmatic Ca\textsuperscript{2+}-release (Donaldson et al. 1978, Lannergren and Westerblad 1991). Furthermore, it induces a metabolic acidosis that might impair muscle function (Fitts 1994) and compromise performance. To blunt the fall in intracellular pH and prolong time-to-exhaustion ($T_{\text{lim}}$), nutritional modulation might be a promising avenue. With respect to endurance exercise, to date especially sodium bicarbonate (NaHCO\textsubscript{3}) has gained much attention. However, the mechanisms by which NaHCO\textsubscript{3} ingestion may enhance performance are not fully understood. It is believed that NaHCO\textsubscript{3} ingestion leads to an increase in blood bicarbonate concentration ([HCO\textsubscript{3}\textsuperscript{-}]), which in turn increases extracellular buffer capacity. More precisely, it is proposed that the higher [HCO\textsubscript{3}\textsuperscript{-}] gradient between blood and the intramyocellular compartment enhances H\textsuperscript{+}-efflux out of the muscle cell, thereby delaying the fall in intracellular pH (Forbes et al. 2005), which in turn may delay an impairment in optimal muscle function and performance (Bishop et al. 2004, Mainwood and Worsley-Brown 1975). Therefore, NaHCO\textsubscript{3} supplementation would be expected to improve $T_{\text{lim}}$ at CP if muscle pH is a limiting factor for exercise tolerance.

Basically, three types of NaHCO\textsubscript{3} supplementation protocols can be applied: acute (single dose), chronic (multiple dose) and multiday acute supplementation (one dose per day before competition for consecutive days of competition). During the acute delivery mode participants take one single dose (mostly 0.3 g·kg\textsuperscript{-1} body mass NaHCO\textsubscript{3}) 60 to 90 min before the start of competition. During the chronic delivery mode participants take a daily amount of NaHCO\textsubscript{3} (mostly 0.5 g·kg\textsuperscript{-1} body mass), divided in 2 to 3 portions, for several days before competition takes place. On the day of competition, no NaHCO\textsubscript{3} is consumed (McNaughton and Thompson
The multiday acute delivery mode comprises the ingestion of acute doses on consecutive days of competition. In contrast to the chronic loading protocol, acid-base balance is perturbed on every day during the multiday acute delivery mode. This fact leads to major differences regarding the acid-base status and accordingly the underlying mechanisms as well as the effectiveness of the different delivery modes. While the acute and chronic supplementation protocols are scientifically well described, data on the effects of multiday acute supplementation are lacking. There are several studies, which investigated NaHCO$_3$ ingestion during tournament-like sports, but only for single events. For example, it was shown that NaHCO$_3$ supplementation increases tennis performance (Wu et al. 2010) but does not affect prolonged intermittent cycling exercise performance (Price and Cripps 2012). However, up to date, no study investigated the effect of a consecutive multiday supplementation on consecutive multiday performance. Since consecutive, acute-load daily use of NaHCO$_3$ might represent an interesting option to increase performance during multiday competitions or tournaments that involve exercise in the heavy and severe intensity domains, further research is warranted. In particular, scientific knowledge is limited with respect to the recovery of the body’s acid-base balance after high-intensity exercise with NaHCO$_3$ supplementation and consequently, the initial positions on the following days remain elusive.

Thus, the purpose of this randomized, placebo-controlled, double-blind interventional crossover study was to investigate if multiday acute NaHCO$_3$ supplementation in well-trained endurance athletes leads to changes in $T_{lim}$ at CP during constant-load cycle ergometer trials on a day-to-day basis with daily acute NaHCO$_3$ vs. placebo supplementation for 5 days. Furthermore, we aimed to investigate if differences in $T_{lim}$ can be explained by alterations in [HCO$_3^-$] and if the high amount of ingested Na$^+$ influences plasma volume (PV) and thus [HCO$_3^-$]. Given that exercise at or above CP leads to muscle and blood acidification (Jones et al. 1977, Jones et al. 2008), and that [HCO$_3^-$] increases extracellular buffer capacity (Forbes et al. 2005), we hypothesized that consecutive, acute-load daily supplementation of NaHCO$_3$ increases $T_{lim}$ relative to placebo. We assumed that an increase in [HCO$_3^-$] after the first intake is responsible for the rise in $T_{lim}$. Since during multiday NaHCO$_3$ intake, a high amount of Na$^+$ is ingested and absorbed, detrimental effects on endurance performance are possible. In fact, a higher [Na$^+$] leads to water retention and thereby results in PV expansion (Heer et al. 2009). An increase in PV decreases blood ion concentrations, and as such results in a diminished [HCO$_3^-$], which in turn could counteract the benefits associated with NaHCO$_3$ intake. It is therefore questionable, whether [HCO$_3^-$] can be increased beyond the concentration reached after the first day of supplementation on all subsequent days of supplementation. Consequently, we hypothesized
that PV expands following a high Na\(^+\) intake, limiting any further increase in [HCO\(_3^-\)], and consequently \(T_{\text{lim}}\), beyond that observed after the first day of supplementation.

## 2.2 Methods

### 2.2.1 Participants

Eleven well-trained male cyclists and triathletes volunteered to participate in this study. The participants were recruited from different cycling or triathlon clubs. Two of them were excluded from the analysis because they contravened our instructions. One participant did not refrain from high-intensity exercise and the other markedly increased the training volume during or before the second testing sessions (see below). Another participant had to abort the measurements because of illness. The physical characteristics of the remaining eight participants were (mean ± SD) age 31.4 ± 8.8 years, height 184.6 ± 6.5 cm, body mass 74.1 ± 7.4 kg, peak power output \((P_{\text{peak}})\) during ramp test 402.0 ± 29.1 W, peak oxygen uptake \((\dot{V}O_2^{\text{peak}})\) 61.0 ± 4.3 ml·kg\(^{-1}\)·min\(^{-1}\). These athletes were all involved in their early preparation phase of training (pre-season). During this phase, the training consisted of constant-load rides at low-intensity. The participants were instructed to maintain their individual, low-intensity training programs. Additionally, they were advised to refrain from any high-intensity exercise during the testing sessions and to continue their nutritional habits. The determination of CP after the wash-out phase served to ascertain that no training effect occurred during the first phase of the study. None of the participants included was currently using buffer substances or any other ergogenic agents that may have compromised the administration of NaHCO\(_3\). Participants were fully informed about the purposes, benefits and risks associated with this study and completed a routine health questionnaire before giving written informed consent. This study was approved by the Swiss Federal Institute of Technology Zurich (ETH) ethics committee and was conducted in accordance with the Declaration of Helsinki.

### 2.2.2 Experimental overview

Using a randomized, placebo-controlled, double-blind interventional crossover design, all participants completed two exercise periods, each consisting of ten testing sessions (Fig. 1). These periods were separated by at least one week and on average 2.3 ± 2.1 weeks of washout, during which the participants maintained their low-intensity training programs. During both periods, the first five tests were conducted to determine CP and consisted of one incremental test and four constant-load tests to volitional exhaustion. The determination of CP was followed
by a five-day intervention period, which was conducted either with NaHCO₃ or sodium chloride (NaCl) supplementation. On each day during the intervention period, a constant-load trial at CP was performed. All tests were carried out under temperature-controlled laboratory conditions (19-24 °C) and at the same time of day. The participants had a 23 h 34 min ± 53 min and 23 h 22 min ± 45 min rest period between the single tests during the placebo and NaHCO₃ trials, respectively. All test devices were calibrated before, and whenever indicated after each test under the terms of the manufacturer’s recommendations. An independent researcher randomly assigned the two conditions to the participants and administered the non-distinguishable placebo or NaHCO₃ tablets without revealing the ingredient. The investigator performing the tests was also blinded to the treatment. No feedback on test performance was given to the participants until all trials had been finished.

2.2.3 Supplementation

NaHCO₃ was administered orally as tablets (Bullrich Salz Magentabletten, delta pronatura Dr. Krauss & Dr. Beckmann, Egelsbach, Germany). The NaHCO₃ and placebo tablets (NaCl, delta pronatura Dr. Krauss & Dr. Beckmann, Egelsbach, Germany) were matched by shape and taste. During the two conditions either 0.3 g·kg⁻¹ body mass of NaHCO₃ or 0.045 g·kg⁻¹ body mass of NaCl (McNaughton 1992, Stephens et al. 2002) had to be ingested 90 min before (Siegler et al. 2010) each of the five consecutive constant-load trials. Each supplement was consumed during a 15-min period with 0.75 dm³ still water to minimize gastrointestinal discomfort or any other adverse effects (Hollidge-Horvat et al. 2000, Vanhatalo et al. 2010b). One NaHCO₃ tablet contained 850 mg of NaHCO₃, whereas one placebo tablet contained 130 mg of NaCl, which assured the intake of equal number of pills during the varying conditions (i.e. 0.35 tablets·kg⁻¹ body mass). If a participant’s body mass was such that they required to consume a non-round number of tablets, the participants were instructed to consume the number of pills rounded to the nearest whole pill to required to obtain the dose. To minimize falsification of the pill count,
participants were given an unknown (to them) number of pills in excess of needs and were asked to return any remaining pills at the end of the study.

2.2.4 Determination of ‘Critical Power’

Five cycle ergometer tests were performed to determine CP (Hill 1993). On the first visit, the seat and handle bar of the cycle ergometer (Ergoselect 200 K, Ergoline, Bitz, Germany) were adjusted. These settings were adopted for all consecutive trials. Participants started with a ramp cycle ergometer test to determine \( P_{\text{peak}} \) and \( \dot{V}O_{2\text{peak}} \). After a 3-min rest, the ramp test started at 100 W and involved power increases of 9 W every 18 s (30 W-min\(^{-1}\)) until volitional exhaustion. For all tests, participants were asked to maintain a cadence of 80 revolutions per min throughout the test. Volitional exhaustion, i.e. task failure, for all cycling tests was defined as the point in time when participants stopped pedaling or the cadence fell below 75 revolutions per minute for > 5 s. On each of the following testing days, one constant-load trial at different power output was completed to determine CP. After a 3-min rest, participants started with a 5-min warm-up at 75 W (Brickley et al. 2007). The power was then increased immediately to 85%, 90%, 95% or 105% of \( P_{\text{peak}} \) in a randomized order (modified from Brickley et al. (2007) including the 85% stage). These endurance capacity tests were conducted until task failure. Using the \( T_{\text{lim}} \) from these tests, CP was then calculated from the linear power−time\(^{-1}\) equation (Hill 1993).

2.2.5 Constant-load cycling trials at ‘Critical Power’

During each of the two intervention periods, five constant-load trials at CP were completed on five consecutive days. These trials started with a 3-min rest and were followed by a 5-min warm-up at 75 W. Subsequently, power was immediately increased to the previously calculated CP and participants were encouraged to maintain the given cadence for as long as possible.

2.2.6 Gas exchange and heart rate analysis

Participants were equipped with a facemask, which covered their mouth and nose (Hans Rudolph, Shawnee, KS, USA). The facemask was connected with an anti-bacterial filter (PALL PRO1087, Pall, East Hills, NY, USA) to an Innocor™ device (Innocor™, Innovision, Odense, Denmark). Pulmonary gas exchange and ventilation were continuously measured breath by breath throughout all ergometer trials. Throughout all cycling tests, heart rate was recorded (Polar S610i, Polar Electro, Kempele, Finland). \( \dot{V}O_{2\text{peak}}, \dot{V}O_{2} \) during the constant-load trials at CP (\( \dot{V}O_{2\text{CLT}} \)), carbon dioxide output during the constant-load trials at CP (\( \dot{V}CO_{2\text{CLT}} \)), respiratory
exchange ratio during the constant-load trials at CP (RER_{CLT}) and heart rate during the constant-load trials at CP (HR_{CLT}) were determined as the highest mean over a 10-s period. The \( \dot{V}O_2 \) slow component was calculated as the difference between the changes in \( \dot{V}O_2 \) between min 2 and task failure and between min 2 and 6.

2.2.7 Blood analysis

For the analysis of [HCO\(_3^\-)\], [Na\(^+\)], pH and actual base excess (ABE) 125 μl blood from the same earlobe were always obtained 75 min after the NaHCO\(_3\) ingestions and 15 min before the constant-load trials at CP on 1 and day 5. Blood was collected in a heparinized glass capillary tube and analyzed using a clinical blood gas analyzer (ABL 505, Radiometer, Copenhagen, Denmark). Venous blood samples (4 ml) were collected from the cubital vein before the constant-load trials at CP on days 1 and 5 (medica, Medizinische Laboratorien Dr. F. Kaeppeli, Zurich, Switzerland). These blood samples were analyzed for hemoglobin concentration and hematocrit, which were used to calculate changes in PV according to Dill and Costill (1974).

2.2.8 Body composition measurement

A densitometer (Lunar iDXA™, GE Healthcare, Madison, WI, USA) was used for the determination of total lean body mass and lean soft tissue mass of the legs. Dual-energy X-ray absorptiometry (DXA) measurements were performed just before the constant-load trials every second day throughout the intervention periods to assess leg lean mass as an indicator of glycogen content. According to the DXA two-component soft tissue model, lean soft tissue mainly consists of water, proteins, glycogen and soft tissue minerals (Pietrobelli et al. 1996). Water and glycogen content are further interconnected since each gram of glycogen binds 3-4 g of water (Olsson and Saltin 1970). To ensure a similar provision of carbohydrates in the immediate post-exercise period, participants were given 0.75 dm\(^3\) of a regeneration drink (57 g carbohydrates·portion\(^{-1}\), Carbo Basic Plus, Winforce, Menzingen, Switzerland) instantly after completion of each constant-load trial.

2.2.9 Statistical analysis

To assess differences in \( T_{lim} \), blood values, gas exchange, heart rate, and body composition a two-way repeated-measures ANOVA having two levels of condition (NaHCO\(_3\) and placebo) and five levels of time (5 days of testing) was used. The assumption of sphericity was tested using Mauchly’s test. If the assumption of sphericity was violated, the degrees of freedom were corrected using the Greenhouse-Geisser estimates of sphericity. When \( F \) ratios were significant,
post hoc comparisons of main effects were performed using a Student’s paired t-test with Bonferroni correction. PV data were not normally distributed and thus log-transformed before using the described analysis. All data are presented as means ± SD. The effect size is denoted as $\eta^2_p$ (partial eta-squared). The level of significance was set at $P < 0.05$. The statistical analyses were conducted using the software SPSS Statistics 20.0 (SPSS, Chicago, IL, USA).

2.3 Results

As judged by the leftover pill count, average compliance with NaHCO$_3$ and placebo supplementation was 100%. $T_{\text{lim}}$ increased by 23.5% following NaHCO$_3$ ingestion ($F_{(1,7)} = 35.45, P = 0.001, \eta^2_p = 0.84$; Fig. 2.2a). However, there was neither an effect of time ($F_{(4,28)} = 1.1, P = 0.375, \eta^2_p = 0.14$) nor an intervention x time interaction ($F_{(4,28)} = 0.74, P = 0.464, \eta^2_p = 0.01$; Fig. 2.2b). No differences in CP, as measured before the first and second supplementation period, could be found (306.8 ± 21.4 W vs. 309.0 ± 30.4 W; $F_{(1,7)} = 0.15, P = 0.708, \eta^2_p = 0.02$). Also, no difference could be found between CP as determined before the NaHCO$_3$ and placebo intervention (304.3 ± 25.6 W vs. 311.5 ± 26.5 W; $F_{(1,7)} = 1.99, P = 0.202, \eta^2_p = 0.22$).

![Figure 2.2](image)

**Figure 2.2:** a) Mean ± SD time-to-exhaustion ($T_{\text{lim}}$) with NaHCO$_3$ and placebo, respectively, $**P < 0.01$; b) $T_{\text{lim}}$ with NaHCO$_3$ (solid line) and placebo (dashed line) on the 5 days of testing are presented as group mean ± SD ($n = 8$).

The NaHCO$_3$ intervention resulted in a significantly higher [HCO$_3^-$] relative to placebo ($F_{(1,7)} = 118.71, P < 0.001, \eta^2_p = 0.94$; Tab. 2.1). However, there was neither a main effect for time ($F_{(1,7)} = 0.05, P = 0.835, \eta^2_p = 0.01$) nor an intervention x time interaction ($F_{(1,7)} = 0.04, P = 0.855, \eta^2_p = 0.01$). [Na$^+$] increased after NaHCO$_3$ ($F_{(1,7)} = 12.44, P = 0.012, \eta^2_p = 0.68$) but remained
constant with placebo supplementation. [Na⁺] did not significantly change over time (F(1,7) = 0.49, P = 0.509, \( \eta_p^2 = 0.08 \)) with either condition. The mean ABE were significantly higher during the NaHCO₃ compared to the placebo trials (F(1,7) = 100.42, P < 0.001, \( \eta_p^2 = 0.94 \)), but not between days of testing (F(1,7) = 0.01, P = 0.920, \( \eta_p^2 = 0.00 \)). Blood pH was increased with NaHCO₃ supplementation (F(1,7) = 42.04, P < 0.001, \( \eta_p^2 = 0.86 \)), showing no change between the testing days (F(1,7) = 0.01, P = 0.920, \( \eta_p^2 = 0.00 \)). The re was a main effect for a PV increase during interventions (F(1,7) = 18.12, P = 0.004, \( \eta_p^2 = 0.72 \)), as well as a significant intervention x time interaction (F(1,7) = 22.05, P = 0.002, \( \eta_p^2 = 0.76 \)).

**Table 2.1.** [HCO₃⁻], [Na⁺], ABE, pH and PV 75 min after supplement ingestion on the first and the fifth day of testing with either NaHCO₃ or placebo supplementation.

<table>
<thead>
<tr>
<th></th>
<th>NaHCO₃</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>[HCO₃⁻] (mmol·l⁻¹)</td>
<td>32.4 ± 1.8***</td>
<td>32.6 ± 2.7***</td>
</tr>
<tr>
<td>[Na⁺] (mmol·l⁻¹)</td>
<td>142.1 ± 3.9*</td>
<td>142.4 ± 3.0*</td>
</tr>
<tr>
<td>ABE (mmol·l⁻¹)</td>
<td>8.4 ± 1.7***</td>
<td>8.3 ± 2.3***</td>
</tr>
<tr>
<td>pH</td>
<td>7.49 ± 0.02***</td>
<td>7.48 ± 0.02***</td>
</tr>
<tr>
<td>PV (%)</td>
<td>55.5 ± 2.3</td>
<td>62.6 ± 3.8††</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 8). [HCO₃⁻], blood bicarbonate concentration; [Na⁺], blood sodium concentration; ABE, actual base excess; PV, plasma volume. \( *P < 0.05 \), ***\( P < 0.001 \) relative to placebo at the same time point; ††\( P < 0.01 \) relative to day 1.

The NaHCO₃ ingestion resulted in a significant intervention x time interaction for total lean body mass (F(1,7) = 7.77, P =0.027, \( \eta_p^2 = 0.53 \); Tab. 2.2). In addition, total lean body mass raised over the five consecutive testing days in both conditions (F(2,14) = 10.97, P = 0.001, \( \eta_p^2 = 0.61 \); Tab. 2.2). Lean soft tissue mass of the legs did not change neither during the interventions (F(1,7) = 3.16, P = 0.119, \( \eta_p^2 = 0.31 \)) nor across the days of testing (F(2,14) = 1.38, P = 0.283, \( \eta_p^2 = 0.17 \); Tab. 2.2).

**Table 2.2.** Total lean body mass (TLBM) and lean soft tissue mass (LSTM) of the legs on the different days of testing with either NaHCO₃ or placebo ingestion.

<table>
<thead>
<tr>
<th></th>
<th>NaHCO₃</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLBM (kg)</td>
<td>60.7 ± 4.8</td>
<td>60.5 ± 5.3</td>
</tr>
<tr>
<td>LSTM (kg)</td>
<td>21.1 ± 2.5</td>
<td>21.0 ± 2.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 8). \( *P < 0.05 \) relative to placebo; ††\( P < 0.01 \) relative to day 1.
\( \dot{V}O_{2,\text{CLT}} \) and \( \dot{V}CO_{2,\text{CLT}} \) did not differ between the interventions (\( F(1,7) = 1.453, P = 0.267, \eta_p^2 = 0.172 \) and \( F(1,7) = 1.32, P = 0.323, \eta_p^2 = 0.139 \); Tab. 2.3) or between the days of testing (\( F(2,14) = 0.631, P = 0.667, \eta_p^2 = 0.387 \) and \( F(2,14) = 0.145, P = 0.964, \eta_p^2 = 0.139 \)). None of the daily \( \dot{V}O_{2,\text{CLT}} \) (data not shown) differed from \( \dot{V}O_{2\text{peak}} \) (\( F(2,14) = 0.081, P = 0.923, \eta_p^2 = 0.011 \)). There was no difference in the \( \dot{V}O_2 \) slow component between the NaHCO\(_3\) and placebo intervention (0.08 ± 0.31 vs. 0.03 ± 0.28 l·min\(^{-1}\) for the NaHCO\(_3\) and placebo intervention, respectively; \( P = 0.504 \)). RER\(_{\text{CLT}}\) also was not different between interventions (\( F(1,7) = 2.947, P = 0.130, \eta_p^2 = 0.296 \)) and days of testing (\( F(2,14) = 0.821, P = 0.523, \eta_p^2 = 0.105 \)). HR\(_{\text{CLT}}\) decreased during the 5 testing days (\( F(4,28) = 5.97, P = 0.001, \eta_p^2 = 0.46 \); Tab. 2.3) but there was no main effect for condition (\( F(1,7) = 0.04, P = 0.852, \eta_p^2 = 0.01 \)).

**Table 2.3.** Peak values during the CLT at CP for \( \dot{V}O_2\), \( \dot{V}CO_2\), RER and HR on the first and fifth day of testing with either NaHCO\(_3\) or placebo supplementation.

<table>
<thead>
<tr>
<th></th>
<th>NaHCO(_3)</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 5</td>
</tr>
<tr>
<td>( \dot{V}O_{2,\text{CLT}} )</td>
<td>4.64 ± 0.39</td>
<td>4.66 ± 0.30</td>
</tr>
<tr>
<td>( \dot{V}CO_{2,\text{CLT}} )</td>
<td>4.63 ± 0.47</td>
<td>4.67 ± 0.19</td>
</tr>
<tr>
<td>RER(_{\text{CLT}})</td>
<td>1.07 ± 0.04</td>
<td>1.08 ± 0.05</td>
</tr>
<tr>
<td>HR(_{\text{CLT}})</td>
<td>177.4 ± 8.5</td>
<td>172.8 ± 9.0**</td>
</tr>
</tbody>
</table>

Values are mean ± SD (\( n = 8 \)). CLT, constant-load trials; CP, 'Critical Power'; \( \dot{V}O_2\), oxygen uptake; \( \dot{V}CO_2\), carbon dioxide output; RER, respiratory exchange ratio; HR, heart rate. **\( P < 0.01 \) relative to day 1.

**2.4 Discussion**

Several new findings have been observed in this randomized, placebo-controlled, double-blind interventional crossover investigation. First, multiday NaHCO\(_3\) supplementation for 5 days increased \( T_{\text{lim}} \) at CP on each day relative to placebo in highly trained athletes. Second, there was no difference in the increased \( T_{\text{lim}} \) over the 5 days of supplementation with NaHCO\(_3\) or NaCl. Third, the increase in \( T_{\text{lim}} \) was paralleled by increases in [HCO\(_3^-\)], pH and ABE. Fourth, [HCO\(_3^-\)] and [Na\(^+\)] in the blood stabilized over time in the NaHCO\(_3\) condition. Fifth, calculated PV increased during the NaHCO\(_3\) more than in the placebo intervention.
We found that NaHCO3 supplementation led to an increase in $T_{lim}$ at CP and that the improvement in $T_{lim}$ was paralleled by an increase in blood $[\text{HCO}_3^-]$, pH and ABE, indicating that the alteration in $T_{lim}$ appears to be linked to an elevated extracellular buffer capacity. In fact, it has been shown that an increased $[\text{HCO}_3^-]$ gradient between the intra- and extramyocellular compartment leads to an amplified $\text{H}^+$-efflux from the muscle cell and delays the fall in intramyocellular pH (Bishop et al. 2004, Hollidge-Horvat et al. 2000). We observed a trend for higher $[\text{La}^-]$ during the constant-load tests following NaHCO3 supplementation ($P = 0.070$, data not shown), supporting the notion that the increased $\text{H}^+$-concentration resulted from a lactate-proton symport. A fall in intramyocellular $[\text{H}^+]$ is associated with muscle fatigue due to 1) an inhibition of glycogenolysis and glycolysis (Hollidge-Horvat et al. 2000), 2) increased muscular $\text{K}^+$ release, 3) lesser contractility of the heart muscle (Fabiato and Fabiato 1978), 4) inhibition of the sarcoplasmatic calcium release (Donaldson et al. 1978) and 5) inhibition of the actin-myosin interactions (Lannergren and Westerblad 1991). Thus, delaying the fall in intramyocellular pH might postpone the fatigue process and prolong intact muscle function. Indeed, our results showed that the ingestion of NaHCO3 induced metabolic alkalosis, which in turn enhanced $T_{lim}$ at CP and thus improved high-intensity exercise in the range of 10 to 20 min duration.

As hypothesized, $T_{lim}$ at CP could be increased with NaHCO3 supplementation. This is in contrast to the theoretical model, which states that an intramyocellular metabolic steady state exists at exercise intensities up to CP. However, our results support the notion that CP overestimates the metabolic steady state (Jenkins and Quigley 1990, Pringle and Jones 2002). Furthermore, our result that NaHCO3 increased $T_{lim}$ at CP extends previous findings showing that NaHCO3 supplementation increases exercise above CP relative to placebo (Bishop et al. 2004, McNaughton et al. 1999a). In the latter studies, short high-intensity tests, during which intramyocellular pH falls rapidly from the beginning of exercise, were completed. During these types of tests, the finite work capacity above CP ($W'$) is drawn on after the start of exercise and becomes reduced. In light of our findings, these results might be interpreted to mean that NaHCO3 simply increases $W'$. However, Vanhatalo et al. (2010b) showed that NaHCO3 does not increase $W'$ during a 3-min all-out test, and concluded that changes in intramyocellular pH might not influence $W'$ in this particular test setting, and that for short all-out exercise, [PCr] dynamics is more important in determining $W'$. In our constant-load trials at CP, $W'$ was supplied to a large extent by anaerobic glycolysis. Therefore, we assume that NaHCO3 supplementation increases $W'$ in conditions where acidification occurs during exercise. Our result that the estimated $\dot{V}O_2$ slow component was not different between the two interventions lends further credence to this notion, although the influence of NaHCO3 on the $\dot{V}O_2$ slow
component remains ambiguous (reduction: Berger et al. (2006); no change: Santalla et al. 2003)). In our study, the identical \( \dot{V}O_2 \) slow component for both, the NaHCO\(_3\) and placebo condition, indicated that \( \dot{V}O_2 \)peak was attained at the same point in time. Based on the fact that the depletion of \( W' \) coincides with the attainment of \( \dot{V}O_2 \)peak (Burnley and Jones 2007), our results indicate that NaHCO\(_3\) ingestion did not increase the rate of \( W' \) utilization but rather \( W' \) itself. Further support for our assumption comes from another study, where average power in a 60 min cycling time trial was found to be higher with NaHCO\(_3\) as compared to placebo (McNaughton et al. 1999b). During a 60 min time trial, power output will fluctuate around CP with power peaks occurring e.g. at the start and during (final) sprints. In these occasions, i.e. when exercising above CP, \( W' \) will be reduced. Consequently, a higher \( W' \) can increase performance during tests of longer duration, especially if pacing strategies are implemented.

We also found that five bolus intakes on five consecutive days did not result in an increase of \( T_{lim} \) beyond the value observed after the first intake. Thus, multiday administration of NaHCO\(_3\) did not lead to a cumulative effect on endurance capacity. Accordingly, [HCO\(_3\)-], blood pH, and ABE after multiday NaHCO\(_3\) administration also remained unchanged relative to the initial rise after the first bolus. The most obvious explanation would be that during each CP-trial a certain amount of NaHCO\(_3\) was used, leading to lower values for [HCO\(_3\)-], pH and ABE post vs. pre test. During the following 24 h of recovery, the body would then be expected to re-establish the resting values. On the following day, the participants then would start the CP trial at similar (complete recovery) or lower [HCO\(_3\)-], blood pH, and ABE (incomplete recovery) relative to the first day, whereby an additional increase in performance would not be expected. Although we did not measure [HCO\(_3\)-], pH and ABE before supplementation on the following days, these two described cases can be most likely excluded. The reason for this is that [Na\(^+\)] also did not increase during the consecutive 5 days of NaHCO\(_3\) supplementation despite the fact that Na\(^+\), unlike HCO\(_3\)-, was not used as a buffer during the CP trials, and that the high amount of ingested Na\(^+\) could not be used completely through sweating. The predicted sweating rate during exercise of 1 dm\(^3\)·h\(^{-1}\) water, with a sweat [Na\(^+\)] of 50 mEq·dm\(^3\) (Montain et al. 2006) would have led to a Na\(^+\) loss of \( \sim 0.36 \) g. This calculated sweat-induced loss of Na\(^+\) corresponds to \( \sim 20\% \) of the daily Na\(^+\) intake during the placebo intervention. Regarding the substantially higher Na\(^+\) intake during the NaHCO\(_3\) intervention, the sweat-induced loss of Na\(^+\) was negligible during this intervention.

As shown in this study, the NaHCO\(_3\) intervention led to an increase in [Na\(^+\)] and plasma osmolality after the first bolus administration. This increase was counteracted by an expansion
in PV. The increase in PV was to such an extent that pre-exercise blood \([\text{HCO}_3^-]\), \(\text{pH}\), and ABE remained constant during the 5 days of testing. This proposed mechanism of PV expansion has already been described by Máttar et al. (1974), who showed that plasma \([\text{Na}^+]\) and plasma osmolality were increased after \(\text{NaHCO}_3\) injections in acute cardiac resuscitation. Other mechanisms to counteract increases in \([\text{Na}^+]\) and plasma osmolality comprise a shift of fluid from the intra- to the extramyocellular compartment (He et al. 2005), a stimulation of arginine vasopressin secretion (Robertson et al. 1976), which leads to an intensified water retention from the kidneys (Roos et al. 1985), and a stimulation of the thirst center whereby more fluid is consumed (Robertson et al. 1976). In accordance with our results, McNaughton et al. (1999a) found an increase in plasma \([\text{Na}^+]\) after the first of five doses of \(\text{NaHCO}_3\) but no further increase of plasma \([\text{Na}^+]\) on the following days. The elevation of PV in the present study is mirrored by the measured increase in DXA whole-body lean mass. In the DXA two-component soft tissue model, lean mass comprises water, proteins, glycogen, and non-bone minerals (Pietrobelli et al. 1996). As increases in protein, glycogen, and non-bone minerals can virtually be excluded (see below), the increase in whole-body lean mass must have resulted from an increase in whole body water, which led to an expansion in PV. Our findings are in accordance with the report of Lands et al. (1996) who found a significantly higher value for DXA-derived whole-body lean mass after saline infusion given to healthy male participants. Finally, our finding that \(\text{HR}_{\text{CLT}}\) was reduced lends further credence to our result that PV increased as a consequence of \(\text{NaHCO}_3\) supplementation, because PV expansion simultaneously increases stroke volume and reduces sympathetic nervous activity, leaving \(\dot{V}\text{O}_2,\text{CLT}\) unaffected (Kanstrup and Ekblom 1982).

In our study, DXA-derived leg lean mass did neither change between interventions nor over time (Tab. 2). As with each gram of glycogen stored in muscle tissue 3-4 \(\text{g}\) of water is bound (Olson and Saltin 1970), and body water is present within the lean soft tissue compartment (Pietrobelli et al. 1996), a decrease in leg lean mass in such a short time (2 days) would indicate a loss of glycogen. In turn, glycogen loss would implicate incomplete regeneration, which would manifest itself in a reduced anaerobic work capacity and, accordingly, decreased performance (Miura et al. 2000). Since our participants displayed neither a reduction in leg lean mass nor performance, the provided regeneration drink and the participants’ daily nutritional intake were sufficient to restore glycogen from day to day, allowing them to perform maximally on each day.

Our results have at least two practical implications. First, since the \([\text{HCO}_3^-]\) gradient between intramyocellular compartment and blood did not decrease over time, \(\text{NaHCO}_3\) can be taken
daily in multiday competitions or tournaments lasting \( \leq 5 \) d without the risk of reducing performance. Second, the apparent PV expansion in response to the high ion intake (see above) blunted any further increase in \([\text{HCO}_3^-]\). If the same mechanism would be true for the chronic supplementation protocol, the effectiveness of this protocol should be questioned, as it seems that \([\text{HCO}_3^-]\) cannot be increased limitless, \textit{i.e.} that it probably reaches a ceiling. The observed ceiling effect was probably based on a metabolic compensation mechanism preventing a disproportionate increase in \([\text{HCO}_3^-]\). A respiratory compensation mechanism is unlikely to have occurred in our study because there were no differences between the NaHCO\(_3\) and placebo intervention for \(\dot{\text{V}}\text{CO}_2\) \((P = 0.903, \text{data not shown})\) and RER \((P = 0.556, \text{data not shown})\) during the resting measurements before the constant-load tests. Of further note is that the standard chronic protocol comprises a daily dose of \(0.5 \, \text{g NaHCO}_3 \, \text{kg}^{-1} \text{body mass}\) (Douroudos \textit{et al.} 2006), which might accentuate the increase in PV and possible side effects. Thus, one adequate dose of NaHCO\(_3\) administered before the competition should be effective in mediating all of the performance-enhancing effects without the need of a “loading phase”. In this context, our results expand the findings of McNaughton and Thompson (2001) as well as Siegler \textit{et al.} (2010), who compared different acute and chronic protocols and found that there are no differences between these ingestion protocols with respect to exercise performance.

It may be argued that the present findings could be limited by 1) differences in performance ability throughout the study period and 2) decreasing motivation. Regarding the first point we have shown that CP was neither different between the first and second intervention period nor before the NaHCO\(_3\) and placebo condition. An increase in CP from the first to the second intervention would have indicated a training effect, whereas a decrease in CP would have indicated incomplete recovery. Hence, we can assume that the participants had the same performance ability throughout the study, allowing a comparison of \(T_{\text{lim}}\) between the two conditions. Regarding the second point, decreasing motivation in a single participant would be evident from a decrease in \(T_{\text{lim}}\) within or between interventions. Considering the single variations in \(T_{\text{lim}}\) irrespective of condition, during which no distinct increases or decreases in \(T_{\text{lim}}\) over time \textit{(i.e. from the second to the fifth test day)} were identified, a decreasing motivation can be excluded for all participants. In addition, \(\dot{\text{V}}\text{O}_{2,\text{CLT}}, \dot{\text{V}}\text{CO}_2,\text{CLT}\) and RER\(_{\text{CLT}}\) were not different between conditions and days of testing. This indicates that the participants’ effort was constant during the whole study period.
2.5 Conclusions

In conclusion, multiple acute, consecutive day NaHCO₃ supplementation led to an increase in $T_{\text{lim}}$ at CP after the first bolus intake. However, while $T_{\text{lim}}$ remained elevated in the NaHCO₃ condition, it was not further altered with prolonged NaHCO₃ supplementation. The increase in $T_{\text{lim}}$ was accompanied by a higher [$HCO_3^-$] gradient between the blood and the intramyocellular compartment, which stabilized over time in the NaHCO₃ intervention. In contrast to the theoretical CP-model, where metabolites should reach a steady state during exercise at CP, and consequently, buffer substances should be ineffective in enhancing $T_{\text{lim}}$, we showed that in practice $T_{\text{lim}}$ can be increased with NaHCO₃ supplementation. Furthermore, the high amount of ingested Na⁺ caused a sustained elevation in PV, which inhibited a further increase in [$HCO_3^-$], and consequently limited the performance-enhancing effect. Therefore, this study indicates that NaHCO₃ can be taken daily in multiday competitions or tournaments to maintain performance ability throughout the whole duration of the competition.
Chapter 3

*High-load resistance exercise with superimposed vibration and vascular occlusion increases critical power, capillaries and lean mass in endurance-trained men*

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3.1 Introduction

Training regimens of endurance athletes typically consist of both long, continuous, low-intensity exercise and shorter high-intensity interval trainings (HIT), which are designed to improve central (e.g. cardiac output) and peripheral (e.g. arterial-venous oxygen difference) components (Daussin et al. 2007). Taken together, these adaptations increase several parameters of aerobic function, such as critical power (CP), maximal O$_2$ consumption ($\dot{V}O_{2\text{max}}$), gas exchange threshold (GET, “anaerobic threshold”), and exercise efficiency.

It is widely accepted that adding resistance exercise to a regular regimen of endurance exercise in trained athletes brings further positive effects on endurance performance, as first described by Hickson et al. (1988). For the sake of clarity, concurrent endurance and resistance training in this context means that endurance athletes perform their regular weekly endurance training regimen, and add 2-3 sessions of resistance training per week to their individual training routine (note that recovery time between resistance exercise sessions $\geq$48 h). The exact temporal sequence of endurance and resistance training sessions usually is unknown. It was first suggested that the positive effects of resistance exercise on endurance performance are based on alterations in motor unit recruitment (Hickson et al. 1988). More recently, however, Kubo et al. (2012) proposed that improvements in neural function and increases in tendon stiffness might also represent possible explanations. Despite the findings indicating that endurance performance is improved when regular endurance training is supplemented with resistance exercise, it appears that CP is not influenced by the addition of resistance exercise to the regimen (Bishop and Jenkins 1996). In accordance with the first mentioned aspect, experts agree that it is beneficial for most endurance-trained individuals to supplement their endurance training routine with resistance exercise, as it promotes substantial benefits for the individual, e.g. cycling economy (Rønnestad et al. 2011).

Despite these benefits, there is at least one issue that should be considered. According to the interference effect (Hickson 1980), endurance exercise has a negative impact on adaptations to resistance exercise, especially on gains in lean mass. This mechanism seems to appear only in young and trained individuals. On a molecular level, it is suggested that 5’-adenosine monophosphate-activated protein kinase (AMPK) phosphorylates and activates tuberous sclerosis protein 2 (TSC2), which inhibits the mammalian target of rapamycin complex 1 (mTORC1). The downregulation of the key component in integrating resistance training-activated signaling pathways (mTOR) inhibits the myofibrillar protein synthesis (Atherton et al. 2005, Inoki et al. 2003). In most cases, the interference effect is even positive for endurance
athletes, because they can improve their endurance performance ability without gaining weight, which has to be carried for the competition distance. However, there are cases in which endurance athletes may wish to gain lean mass (e.g. road cyclists want to improve their time trial performance or cross country skiers want to improve their sprint performance). The addition of resistance training to their high volume of endurance training leads mostly not to the desired training effect. In these cases, as a result of the high endurance stimulus AMPK is upregulated and hence, the myofibrillar protein synthesis is reduced or inhibited (Inoki et al. 2003). These athletes would profit from a novel training modality that allows both a gain in lean body mass and an increase in endurance performance. Furthermore, it is well accepted that—especially in the case of trained endurance athletes—resistance training generally neither increases $\dot{V}O_{2\text{max}}$, nor mitochondrial content nor capillarization (Aagaard et al. 2011, Hickson et al. 1988). To further advance endurance athlete performance there is a need for new, effective, and time-efficient training stimuli that are capable of providing endurance performance benefits from resistance exercise, and which will serve to further increase (otherwise possibly capped) structural and functional endurance-type adaptations, which, in turn, affect CP.

We have recently described a novel training modality termed “vibroX”, which might be able to accommodate these needs mentioned above (Item et al. 2011, 2013). It simultaneously combines heavy resistance exercise, side-alternating whole-body vibration, and sustained vascular occlusion. In untrained females (Item et al. 2011), vibroX elicits a concomitant increase in muscle fiber cross-sectional area (CSA), overall capillary-to-fiber ratio, thigh lean mass, and endurance capacity. Additionally, we found that compared to resistance exercise per se, vibroX acutely activates angiogenic and metabolic gene programs, which are normally activated after endurance but not resistance exercise in recreationally trained men (Item et al. 2013). Altogether, these results point to a unique role of vibroX in simultaneously mediating endurance- and resistance-type adaptations in untrained or resistance-trained individuals, and thus vibroX has the potential of being considered to be a “total conditioning” stimulus. Moreover, this unique training regimen has been found to be extremely time efficient. In our first study, concurrent adaptations were achieved with a muscle contraction time of approximately 9 min per week for a total of 8 weeks (time commitment per training session: 12-15 min; Item et al. 2011). Although we have already shown the benefits of vibroX in young healthy untrained women and young healthy recreationally trained men, a final proof of concept demonstrating that vibroX also works in trained endurance athletes is warranted.
Thus, in this study, we aimed to test whether 8 weeks of vibroX would simultaneously improve CP, capillarization, and myofiber size in young, trained male endurance athletes matched for CP at baseline. We chose CP as the functional endpoint of endurance performance, since this approach is believed to be functionally more valuable than the sole measurement of discrete physiological values such as GET and $\dot{V}O_{2\text{max}}$ (Jones et al. 2010, Whipp and Ward 2009) or apparently distinct entities such as “short-term and long-term endurance capacity”. Another reason for choosing CP as the primary outcome variable was that supplementation of endurance exercise with classical resistance exercise is not believed to impact this variable (Bishop and Jenkins 1996). Based on our previous results (Item et al. 2011, 2013) and comprehensive work on the effects of resistance exercise in athletes, ranging from well-trained to highly-trained athletes levels of skill and performance (Aagaard and Andersen 2010, Hickson et al. 1988, Rønnestad et al. 2012), we hypothesized that only vibroX would increase CP, overall capillary-to-fiber ratio, myofiber CSAs and thigh lean mass simultaneously.

### 3.2 Methods

#### 3.2.1 Participants

Twenty-six endurance-trained males volunteered to participate in this study. The participants were recruited from different cycling, triathlon and academic sport clubs. Two participants aborted the study because of illness or personal reasons. After the pretests, participants were assigned pairwise, according to their CP·kg$^{-1}$ body mass, to a vibroX or resistance training group. None of the participants had been involved in structured resistance training prior to the study, and the participants had no or little experience in squat exercise. Study participants were all involved in their early preparation phase of training (pre-season). They were instructed to maintain their individual training routine relating to training frequency, as well as training intensity, and were advised not to include new or additional high-intensity exercise during the study period. Three participants were excluded from the final analysis because they contravened our instruction not to stop their individual training during the study period. Thus, the resulting number of participants was $n = 11$ and $n = 10$ for the vibroX and resistance training group, respectively. There were no statistical significant differences in physical and performance characteristics between the vibroX and the resistance training group before training (Tab. 3.1). Participants were fully informed about the purposes, benefits and risks associated with this study and completed a routine health questionnaire before giving written informed consent to
their participation in this study. This study was approved by the ethics committee of the canton Zurich and was conducted in accordance with the declaration of Helsinki.

Table 3.1. Physical characteristics and pre training values for the vibroX and resistance training (RT) groups.

<table>
<thead>
<tr>
<th></th>
<th>vibroX (n = 11)</th>
<th>RT (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [y]</td>
<td>26.7 ± 3.5</td>
<td>28.4 ± 4.8</td>
</tr>
<tr>
<td>Height [cm]</td>
<td>181.5 ± 6.6</td>
<td>178.8 ± 6.3</td>
</tr>
<tr>
<td>Body mass [kg]</td>
<td>77.9 ± 8.1</td>
<td>75.5 ± 7.0</td>
</tr>
<tr>
<td>CP [W]</td>
<td>286.4 ± 37.8</td>
<td>274.3 ± 43.9</td>
</tr>
<tr>
<td>CP·kg⁻¹[W·kg⁻¹]</td>
<td>3.70 ± 0.53</td>
<td>3.65 ± 0.62</td>
</tr>
<tr>
<td>1RM [kg]</td>
<td>128.1 ± 20.2</td>
<td>130.3 ± 39.2</td>
</tr>
<tr>
<td>MVT [Nm]</td>
<td>169.4 ± 24.8</td>
<td>166.5 ± 29.5</td>
</tr>
<tr>
<td>RFD 30 ms [Nm·s⁻¹]</td>
<td>1447.8 ± 526.5</td>
<td>1272.8 ± 405.6</td>
</tr>
<tr>
<td>RFD 200 ms [Nm·s⁻¹]</td>
<td>1088.9 ± 280.0</td>
<td>978.7 ± 280.1</td>
</tr>
<tr>
<td>SJ Pmax,rel [W·kg⁻¹]</td>
<td>49.4 ± 4.0</td>
<td>51.6 ± 6.0</td>
</tr>
<tr>
<td>CMJ Pmax,rel [W·kg⁻¹]</td>
<td>55.2 ± 6.4</td>
<td>60.2 ± 6.0</td>
</tr>
<tr>
<td>Overall CTF ratio</td>
<td>1.82 ± 0.20</td>
<td>1.86 ± 0.19</td>
</tr>
<tr>
<td>MyHC-1 fiber CSA [10⁻⁹ m²]</td>
<td>4.51 ± 0.66</td>
<td>4.82 ± 1.16</td>
</tr>
<tr>
<td>MyHC-2 fiber CSA [10⁻⁹ m²]</td>
<td>5.78 ± 0.67</td>
<td>6.33 ± 1.03</td>
</tr>
<tr>
<td>Thigh lean mass [kg]</td>
<td>13.6 ± 1.9</td>
<td>13.4 ± 1.2</td>
</tr>
</tbody>
</table>

Values are mean ± SD: CP, critical power; 1RM, one-repetition maximum; MVT, maximal voluntary torque; RFD, rate of force development; SJ, squat jump; CMJ, countermovement jump; Pmax,rel, relative maximal power; CTF, capillary-to-fiber; MyHC, myosin heavy chain; CSA, cross-sectional area.

3.2.2 Experimental procedures

The study consisted of pre- and post-tests for all participants and 8 weeks of either vibroX or resistance training (Fig. 3.1). On the first testing day, a percutaneous muscle biopsy was obtained from the M. vastus lateralis using a 6-mm Bergström needle (Dixons Surgical Instruments, Essex, UK) as previously described (Item et al. 2013). On the second testing day, participants completed an incremental ramp cycle ergometer test to determine peak power (Ppeak) and peak oxygen uptake (VO2peak). On that occasion, seat and handle bar of the cycle ergometer (Ergoselect 200 K, Ergoline, Bitz, Germany) were adjusted. These settings were adopted for all of the consecutive trials. After a 3 min rest, the ramp test started at 100 W and
involved power increases of 9 W every 18 s (30 W·min⁻¹) until volitional exhaustion. The participants were free to choose the cadence but the chosen revolutions per min (rpm) had to be maintained throughout all the following cycling tests. Volitional exhaustion, i.e. task failure, for all cycling tests was defined as the point in time when participants stopped pedaling or the cadence fell more than 5 rpm for > 5 s. On the following testing days, constant-load cycle ergometer tests at 85, 90, 95 and 105% $P_{\text{peak}}$ [modified from Brickley et al. (2007) by including a 85% stage] were completed in a randomized order to determine CP (Hill 1993, Moritani et al. 1981). After a 3 min rest, participants started with a 5-min warm-up at 75 W (Brickley et al. 2007). The power was then increased immediately to the given power output. These endurance capacity tests were conducted until volitional exhaustion as defined above, whereupon time to exhaustion ($t_{\text{lim}}$) was recorded. CP and the finite work capacity above CP ($W'$) were calculated by applying the linear power-time⁻¹ equation ($P = W' \cdot t^{-1} + \text{CP}$, Hill 1993). The coefficient of determination $R^2$ for CP was 0.98 ± 0.01 and 0.99 ± 0.02 for the pre- and posttests, respectively. The typical error expressed as a coefficient of variation for CP determined in our laboratory was 3.8%. The familiarization sessions were composed of three attempts for knee extension (dynamometry) and three attempts for both countermovement jumps (CMJ) and squat jumps (SJ). Furthermore, the participants performed two sets of six repetitions of squatting without additional weight. Particular attention was paid to exercise anatomy, form, and movement speed.

3.2.3 Equipment and measurements

Cycling tests. During all cycling tests, participants were equipped with a facemask, which covered their mouth and nose (Hans Rudolph, Shawnee, KS, USA). The facemask was connected with an anti-bacterial filter (PALL PRO1087, Pall, East Hills, NY, USA) to an
Innocor™ device (Innocor™, Innovision, Odense, Denmark). Pulmonary gas exchange and ventilation were continuously measured breath by breath throughout all ergometer trials. $\text{VO}_{2\text{peak}}$ was determined as the highest mean over a 10-s period.

**Knee extension, one repetition maximum (1RM) and jumping test.** Knee extensor maximal voluntary torque (MVT) and rate of force development (RFD) were tested using a commercially available dynamometer (Con-Trex® MJ, Physiomed Elektromedizin, Schnaittach/Laipersdorf, Germany). After the body of the participants was stabilized with straps and handles, they performed 3 maximal knee extensions ($\omega = 3.14 \text{ rad} \cdot \text{s}^{-1}$) separated by 1 min to assess MVT. Subsequently, the lever arm of the dynamometer was fixed at a knee angle of 110° (full extension = 180°) and the participants were advised to extend their leg as fast and as powerful as possible. RFD was calculated for two time intervals: 0-30 and 0-200 ms. Onset of muscle contraction was defined as baseline + 7.5 N·m (Aagaard et al. 2002). Squat 1RM measurement was performed according to Niewiadomski et al. (2008) over the individual’s maximal range of motion. Briefly, the warm-up protocol entailed 8 repetitions at 50% of estimated 1RM and 3 repetitions at 70% of estimated 1RM with a 3 min rest interval between sets. Subsequently, starting with the estimated 1RM, maximal attempts were made to determine 1RM with a 1 min rest interval between attempts (Matuszak et al. 2003). The load was successively increased to the point where participants were not able to successfully execute a squat. For the determination of jumping power during CMJs and SJs, three vertical jumps (separated by 30 s of rest) were performed per jumping maneuver on a Leonardo Mechanograph® force plate (Novotec Medical, Pforzheim, Germany). For the detection, storage and analysis of data, we used the manufacturer’s software (Leonardo Mechanography GRFD version 4.4, Novotec, Pforzheim, Germany). CMJs were performed with freely moving arms, whereas the SJs were performed with the hands resting on the waist.

**Body composition.** A densitometer (Lunar iDXA™, GE Healthcare, Madison, WI, USA) was used for the determination of body composition and thigh lean mass. The delineation in region of interest for the thigh was done manually using the integrated software (encore, GE Healthcare, Madison, WI, USA; version 11.40.004) as follows: ROI upper boundary = horizontal line just below the ischium, ROI lower boundary = horizontal line between femur and tibia, ROI lateral boundaries = outer leg cuts.

### 3.2.4 Muscle biopsy analysis

Tissue sections were cut at 8-μm thickness in a cryostat maintained at −25 °C, and mounted on Fisherbrand Superfrost/Plus microscope slides (Fisher Scientific, Pittsburgh, PA, USA). The
serial cryocut cross-sections were stained using the myofibrillar adenosintriphosphatase (mATPase) method as previously described (Item et al. 2011). At least 350 muscle fibers were classified according to their myosin heavy chain (MyHC) isoform into MyHC-1, MyHC-2A and MyHC-2X. For the analysis of oxidative and glycolytic enzyme activities, we incubated the sections in media containing succinate dehydrogenase (SDH) and glycerol-3-phosphate dehydrogenase (GPDH), respectively. At least 350 muscle fibers were counted for each of these two staining procedures. As marker for muscle capillaries, the monoclonal mouse anti-human CD31 endothelial antibody (DAKO, Carpinteria, Canada, 1:600 dilution) was used. Overall capillary-to-fiber ratio was calculated by dividing the number of CD31-positive cells by the number of muscle fibers. At least 50 muscle fibers were counted for the analysis of the capillary-to-fiber ratio (McCall et al. 1998, Porter et al. 2002). We used the NIH Image J Software (version 1.44o, National Institutes of Health, Bethesda, MD, USA) for all fiber analyses. Fiber CSAs were determined by fully encircling the borders of the mATPase stained cells with Adobe Photoshop Pro CS6 (Adobe Systems Incorporated, San Jose, California, USA) of at least 50 fibers per MyHC isoform (McCall et al. 1998). Fiber circularity was calculated using the formula $(4\pi \cdot \text{CSA})/\text{perimeter}^2$ and only fibers with a circularity higher than 0.7 were considered for analysis (perfect circle = 1.0). Because of the low number of counted MyHC-2X fibers, CSA data were pooled to the 2 major fiber types (MyHC-1 and MyHC-2).

3.2.5 Training regimen

The participants reported twice per week to the laboratory for the supervised training sessions (Fig. 1). The vibroX training consisted of loaded (Multipower®, Technogym, Gambettola, Italy) parallel back squat exercise with superimposed whole-body vibration and sustained vascular occlusion, as described previously (Item et al. 2011, 2013). Briefly, squats were performed on a Galileo® vibration plate (Novotec, Pforzheim, Germany) oscillating at 30 Hz while tourniquet cuffs (0.09 m width, 0.76 m length; VBM, Sulz a.N., Germany) inflated to 200 mmHg (26.7 kPa) were affixed to the inguinal fold region of the thigh. The suprasystolic pressure employed here was the highest pressure that was tolerated by the participants in this setting. One duty cycle consisted of squats until volitional exhaustion, 3 min resting with the pressure of the cuffs maintained, and 1 min resting with cuffs deflated to 100 mmHg (13.3 kPa). Resistance training was conducted analogous to vibroX but without whole-body vibration and vascular occlusion. The participants in the resistance training group were given a break of 3 min between exercise sets [according to the American College of Sports Medicine (2009)]. Load was set to 70% 1RM at the beginning of the training period and was reduced by approximately 10 % for each of the following sets to induce volitional muscle failure within 60-100 s of exercise. The load
magnitude was adjusted progressively during the training period to maintain time under tension between 60 and 100 s (Item et al. 2013, Toigo and Boutellier 2006). During the training period, the participants performed two duty cycles or sets of the respective training during the first 4 weeks and three cycles or sets for the remaining of the training period. The technical exercise execution of the squats consisted of a 4 s eccentric action, a smooth 1-2 s transition phase and a 4 s concentric action. After each training, the participants were given a protein shake containing 20 g of whey protein (Nutriathletic Muscle Growth Formula, Scientifecs, Schwyz, Switzerland).

3.2.6 Statistical analysis

Data are presented as mean values ± standard deviations (SD). Normality of data was visually ascertained by Q-Q-plots. For the detection of significant differences between groups over time, a univariate general linear model was applied. For this analysis, the differences post–pre (Δ) of each variable was compared between the groups. Significant differences within groups from pre to post intervention were displayed by parameter estimates. This analysis tested the null hypothesis that Δ parameter was 0. If Δ parameter had a P-value lower than the level of significance, the null hypothesis was rejected meaning that Δ parameter was significantly different from 0. A linear regression analysis was used to determine which training-induced improvement best predicted the increase in CP. Pearson correlations were performed to assess the relationships between the variables. The muscle biopsies of two participants could not be analyzed for the mATPase, SDH and GPDH stainings as well as the CSAs due to technical failures. The level of significance was set at P < 0.05. All statistical analyses were performed using the software SPSS Statistics 20.0 (SPSS, Chicago, IL, USA).

3.3 Results

Starting from a non-significant higher absolute baseline value (Tab. 1), CP increased by 7.9 ± 7.5 W in the vibroX group, while it remained unchanged in the resistance training group (Fig. 3.2). Overall capillary-to-fiber ratio increased by 8.2 ± 6.8% in the vibroX group, while the resistance training group showed no change (−1.0 ± 7.2%, P = 0.528), resulting in a significant group x time interaction (Fig. 3.3a). The increase in overall capillary-to-fiber ratio was the only predictor for the change in CP (adjusted $R^2 = 0.605$, $P = 0.008$) in the vibroX group. Thigh lean mass increased by 3.1 ± 1.7% in the vibroX group, whereas a slight, non-significant increase was observed in the resistance training group (+1.2 ± 2.0%, $P = 0.074$; Fig. 3.3b), yielding a significant group x time interaction. MyHC-1 and MyHC-2 CSAs were increased by 24.7 ± 19.9
and 22.2 ± 16.5%, respectively, in the vibroX group, but did not significantly change in the resistance training group (+3.6 ± 28.2%, \( P = 0.643 \) and +14.9 ± 29.1%, \( P = 0.119 \) for MyHC-1 and MyHC-2 CSA, respectively; Fig. 3.3c, d), with no differences between the groups. There was a correlation between the increase in overall capillary-to-fiber ratio and the gain in thigh lean mass in the vibroX group \( (R^2 = 0.301, \ P = 0.010; \) results not shown). Furthermore, the change in MyHC-1 CSA correlated with the change in thigh lean mass in the vibroX group \( (R^2 = 0.631, \ P = 0.006; \) results not shown) but not the resistance training group. MyHC-2A fiber proportion increased in the vibroX group, and also tended to increase in the resistance training group (Tab. 3.2). MyHC-1 fiber and MyHC-2X fiber proportions did not significantly change from pre- to post-training in both groups. There were no effects of the two training interventions on GPDH and SDH activity (Tab. 3.2).

![Figure 3.2: Critical Power (CP) pre (white bars) and post (black bars) training in the vibroX and resistance training group. Bars and error bars represent mean values and SD, respectively, for 21 participants (vibroX: \( n = 11 \), resistance \( n = 10 \)). \(*\text{**} P < 0.01\), significant differences within group pre vs. post; \(* P < 0.05\), significant differences between groups pre vs. post.]

\( P_{\text{peak}} \) increased by 5.9 ± 6.1% in the vibroX group, while there was a non-significant increase of 2.7 ± 5.9% in the resistance training group (pre vs. post: vibroX: 383.9 ± 45.8 vs. 405.2 ± 41.9 W; resistance training: 379.9 ± 44.0 vs. 389.8 ± 47.4 W). \( \dot{V}O_{2\text{peak}} \) (pre vs. post: vibroX: 4.58 ± 0.54 vs. 4.63 ± 0.59 l·min\(^{-1}\), \( P = 0.136 \); resistance training: 4.16 ± 0.46 vs. 4.22 ± 0.45 l·min\(^{-1}\), \( P = 0.162 \)) and relative \( \dot{V}O_{2\text{peak}} \) (pre vs. post: vibroX: 59.0 ± 6.2 vs. 58.9 ± 6.2 ml·min\(^{-1}\)·kg\(^{-1}\), \( P = \)
0.874; resistance training: $55.2 \pm 4.2$ vs. $55.9 \pm 4.5$ ml·min$^{-1}$·kg$^{-1}$, $P = 0.156$) were not altered in response to the two training interventions. No testing group x time interaction was detected for $V_O^{2\text{peak}}$ ($P = 0.983$).

![Figure 3.3](image)

**Figure 3.3:** a) Overall capillary-to-fiber ratio, b) thigh lean mass, c) myosin heavy chain type 1 (MyHC-1) fiber cross-sectional area (CSA), and d) MyHC-2 fiber CSA pre (white bars) and post (black bars) training in the vibroX and resistance training group. Bars and error bars represent mean values and SD, respectively, for 21 (Fig. 3.3a, b; vibroX: $n = 11$, resistance $n = 10$) or 19 participants (Fig. 3.3c, d; vibroX: $n = 9$, resistance $n = 10$). *$P < 0.05$, **$P < 0.01$, significant differences within group pre vs. post; "$P < 0.05$, ""$P < 0.01$, significant differences between groups pre vs. post.

1RM increased in both training groups (vibroX: $+22.2 \pm 10.6\%$, resistance training: $+31.8 \pm 13.4\%$; Fig. 3.4a), with a tendency towards a higher increase in the resistance training group ($P = 0.057$). MVT increased in both groups (vibroX: $+3.1 \pm 8.5\%$, resistance training: $+4.9 \pm 4.6\%$) but the increase was significant in the resistance training group only (Fig. 3.4b). SJ relative maximum power remained unchanged in the vibroX group while it significantly
increased in the resistance training group (+2.0 ± 4.8% vs. +4.7 ± 5.9%, respectively; Fig. 3.4c). CMJ relative maximum power significantly increased in the vibroX training group by 5.0 ± 8.3%, while the increase did not reach statistical significance in the resistance training group (+3.1 ± 3.7%; Fig. 3.4d). RFD during the first 30 ms remained unaffected by both training interventions (P = 0.867 and P = 0.091 for the vibroX and resistance training group, respectively; Fig. 3.4e). RFD during the first 200 ms remained constant for vibroX (+3.0 ± 6.8%, P = 0.563) but increased by 14.0 ± 19.5% in response to resistance training (Fig. 3.4f). No significant group differences were found for all these variables. W′ was not significantly elevated from pre to post vibroX (+5.1 ± 9.6%; pre vs. post: 17.8 ± 4.2 vs. 18.5 ± 3.7 kJ) while it increased by 10.5 ± 14.9% (pre vs. post: 16.7 ± 3.8 vs. 18.2 ± 3.5 kJ) in the resistance training group. No testing group x time interaction was detected for W′ (P = 0.406). During the first 4 weeks, average time under tension per training session was 135 ± 18 s and 161 ± 6 s in the vibroX and resistance training group, respectively (P < 0.001). In the remaining 4 weeks, average time under tension per training session was 188 ± 17 s and 218 ± 10 s for the vibroX and resistance training group (P < 0.001).

### Table 3.2. Distribution of the myosin heavy chain (MyHC) types, glycerol-3-phosphate dehydrogenase (GPDH) activity, and succinate dehydrogenase (SDH) activity pre and post training period in the vibroX and resistance training group.

<table>
<thead>
<tr>
<th></th>
<th>vibroX (n = 9)</th>
<th>resistance training (n =10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>MyHC-1</td>
<td>58.7% ± 6.7%</td>
<td>56.6% ± 7.0%</td>
</tr>
<tr>
<td>MyHC-2A</td>
<td>36.6% ± 8.6%</td>
<td>40.7% ± 5.6%*</td>
</tr>
<tr>
<td>MyHC-2X</td>
<td>4.7% ± 4.3%</td>
<td>2.7% ± 3.3%</td>
</tr>
<tr>
<td>GPDH activity</td>
<td>47.9% ± 9.4%</td>
<td>50.3% ± 9.4%</td>
</tr>
<tr>
<td>SDH high activity</td>
<td>60.9% ± 6.0%</td>
<td>60.9% ± 5.3%</td>
</tr>
<tr>
<td>SDH weak activity</td>
<td>38.5% ± 5.9%</td>
<td>38.2% ± 5.3%</td>
</tr>
<tr>
<td>SDH no activity</td>
<td>0.7% ± 1.1%</td>
<td>1.0% ± 2.2%</td>
</tr>
</tbody>
</table>

Values are mean ± SD. *P < 0.05; †P = 0.051 pre vs. post.
Figure 3.4: a) One repetition maximum (1RM), b) maximum voluntary torque (MVT), c) squat jump (SJ) relative maximum power ($P_{\text{max,rel}}$), d) countermovement jump (CMJ) relative maximum power ($P_{\text{max,rel}}$), e) rate of force development (RFD) during the first 30ms of contraction, and f) RFD during the first 200ms of contraction pre (white bars) and post (black bars) training in the vibroX and resistance training group. Bars and error bars represent mean values and SD, respectively, for 21 participants (vibroX: $n = 11$, resistance $n = 10$). *$P < 0.05$, ***$P < 0.001$, significant differences within group pre vs. post.
3.4 Discussion

This study provides evidence that in trained endurance athletes undergoing regular pre-season training, the addition of vibroX increased CP, capillary-to-fiber ratio and thigh lean mass. Conversely, in the resistance training group, all these parameters remained unaffected by the addition of conventional resistance training. The gain in CP was positively correlated with the gain in capillarization, and the gain in thigh lean mass was paralleled by increases in MyHC-1 and MyHC-2 fiber CSAs and strength. The present data extend our previous findings (Item et al. 2011, 2013) to trained endurance athletes and thus establish the final proof of concept that in young healthy individuals it is possible to modify a resistance exercise stimulus through superimposition of vibration and sustained vascular occlusion to mediate adaptations otherwise known to occur with endurance exercise only.

In this study, vibroX but not conventional resistance training, induced muscle hypertrophy, as shown by the specific increases in MyHC-1 and MyHC-2 fiber CSAs and thigh lean mass. Our finding that supplementation of endurance exercise with vibroX promoted muscle hypertrophy is intriguing, since in young men, adaptations to resistance training are usually negatively affected by concurrent endurance exercise, i.e. simultaneous endurance exercise impairs “strength” and muscle size gains compared with resistance exercise alone (Hickson 1980, Nader 2006) – a phenomenon known as concurrent training or interference effect. Similarly, when endurance exercise is supplemented with resistance exercise in young male trained endurance athletes, typically no increase in muscle fiber CSA and leg lean mass is observed (Aagaard et al. 2011, Hickson et al. 1988). Here, we clearly showed that vibroX is capable of overcoming this interference effect. It further appears that muscle hypertrophy in the vibroX group was primarily driven by the increase in MyHC-1 fiber size, since we found a positive correlation between MyHC-1 fiber hypertrophy and the increase in thigh lean mass. This finding is not surprising, given the fact that MyHC-1 fiber proportion in the M. vastus lateralis of our participants was clearly dominant over MyHC-2 fiber proportion. Conversely, we did not expect that MyHC-1 fibers in trained endurance athletes would undergo further hypertrophy as a result of vibroX training, because these fibers are innervated by small motoneurons with low recruitment thresholds and thus, they are believed to be activated during endurance-type exercise. Remarkably, the advantage of the vibroX training group in relation to muscle fiber hypertrophy was achieved with a significant lower time under tension compared to the resistance training group. However, average time under tension per training session was, for both training groups, in the predetermined range of 60 to 100 s per set. Therefore, we assume that this difference in
training volume had no effect on the outcome. The reduction in time under tension irrespective of the applied load was possibly due to the ischemic pain.

The blunted hypertrophy in response to conventional resistance training with concurrent endurance exercise might potentially be explained by conditions causing significant cellular energy stress (such as may be the case during high-volume and/or high-intensity endurance exercise), which will promote an increased activity of 5′-AMP activated protein kinase (AMPK). AMPK is a heterotrimeric serine/threonine kinase, composed of a catalytic subunit (α) and two regulatory subunits (β and γ) (Steinberg and Kemp 2009). It serves as an energy sensor and is activated by an elevation in the AMP/ATP ratio, i.e. enhanced binding of AMP to the γ subunit leads to an increase in the phosphorylation of the Thr172 residue on AMPK, and subsequently induces an increase in AMPK activity (Steinberg and Kemp 2009). It has been shown that activation of AMPK inhibits energy consuming anabolic processes such as protein synthesis, and stimulates catabolic energy producing processes such as protein degradation (Steinberg and Kemp 2009). Taken together, there is evidence suggesting that AMPK may negatively affect skeletal muscle mass accretion in the setting of concurrent training by blunting the increase in protein synthesis and increasing protein degradation (Goodman et al. 2011).

Contrary to conventional resistance training, vibroX produced a robust hypertrophic response, which might theoretically either indicate that the addition of vibration and occlusion inhibited AMPK interference or that alternate pathways mediating muscle hypertrophy were activated. An inhibition of AMPK interference might represent a potential mechanism. However, we are not aware of any study investigating this potential mechanism. In contrast, we previously showed, using the same training protocols, that vibroX strongly induces peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) without concomitantly increasing the canonical PGC-1α downstream targets mediating mitochondrial biogenesis in recreationally trained men (Item et al. 2013). Interestingly, Ruas et al. (2012) have recently identified a form of PGC-1α (PGC-1α4) that results from alternative promoter usage and splicing of the primary transcript. Although it is highly expressed in exercised muscle, it does not regulate most known PGC-1α targets such as the mitochondrial OXPHOS genes. This is in principle consistent with our previous observation (Item et al. 2013). Ruas et al. (2012) showed in vitro and in vivo that PGC-1α4 specifically induces insulin-like growth factor 1 (IGF1) and represses myostatin in the context of resistance exercise, resulting in muscle hypertrophy. In the light of the findings that vibroX specifically and acutely increases PGC-1α mRNA abundance and induces muscle
hypertrophy despite concurrent endurance exercise, more research is needed to elucidate the possible roles of PGC-1α splice variants in mediating muscle size adaptation during vibroX.

As shown by linear regression analysis, we found a positive correlation between the increases in CP and capillarization after vibroX training. Notably, these increases occurred without concomitant elevations in $\dot{V}O_2^{\text{peak}}$ and/or skeletal muscle oxidative enzyme activities. These findings are in line with the notion that in endurance-trained muscles, the augmented capillarization serves other purposes than the oxygenation of tissue, namely a better uptake of substrates and/or removal of fatigue-related metabolites and heat (Saltin et al. 1986). Consequently, the enhanced CP observed following vibroX training may, in part, have been the result of better conditions for the uptake and utilization of blood-borne substrates and/or removal of fatigue-related metabolites. Interestingly, it also appears that the increased size of the capillary-to-fiber interface after vibroX was functionally (in terms of CP) more relevant than a decreased diffusion distance consequent to increased capillary density. The reason for this is that vibroX also induced myofiber hypertrophy.

Recently, we showed in recreationally resistance trained young men that an acute bout of vibroX does not increase hypoxia-inducible factor-1α (HIF-1α) mRNA abundance nor the expression of HIF-1α target genes (e.g. HIFα prolyl hydroxylase domain 3 [PHD3], lactate dehydrogenase A [LDHA], or phosphofructokinase [PFK]), in spite of a marked increase in the expression of vascular endothelial growth factor (VEGF), PGC-1α, and oxidative stress markers (Item et al. 2013). Given the known sensitivity of PGC-1α signaling to reactive oxygen species (Kang et al. 2009, St-Pierre et al. 2006), we speculate that VEGF expression was induced in a HIF-1 independent manner, possibly through oxidative stress dependent activation of PGC-1α. Our present finding that vibroX significantly increased capillarization in trained endurance athletes provides converging evidence that vibroX promotes capillarization through non-canonical signaling pathways. The reason being, that in trained endurance athletes most adaptations in terms of capillarization presumably have already occurred, and no further significant effects are to be expected by their classical training routine. Unlike vibroX, resistance training alone was unable to increase the capillary-to-fiber ratio in endurance-trained individuals participating in this study. This finding is in accordance with a recently published study by Aagaard et al. (2011), showing that capillary-to-fiber ratio did not increase following 16 weeks of endurance training supplemented with resistance exercise in elite cyclists.
The increase in $W'$ was only statistically significant in the resistance training group and twofold greater than in the vibroX training group. This was surprising, because an increase in thigh lean mass, which is believed to influence $W'$ (Miura et al. 2002), was observed solely in the vibroX group. Furthermore, the share of MyHC-2A fibers, which is also believed to influence $W'$, increased in the vibroX training group and tended to increase in the resistance training group at the expense of MyHC-2X and MyHC-1 fibers, even though both decreases were not statistically significant. However, the increase in $W'$ in the resistance training group was paralleled by increases in MVT and RFD. Therefore, the augmentation in $W'$ was probably due to improved neuronal function. The discrepancy in adaptation between the two training groups might be explained by a ceiling effect. There is a balance between CP and $W'$, both parameters representing distinct parts of energy supply (oxidative and glycolytic, respectively). In an individual athlete, it is not possible to increase both parameters limitless (e.g. a marathon runner won’t achieve the same $W'$ as a 100 m sprinter and vice versa for CP). The idea of a ceiling effect might be supported by our findings that $W'$, MVT, and RFD at the beginning of the study tended to be lower in the resistance training group as compared to the vibroX group but were not different any more after the exercise period. The notion that a ceiling effect exists is further supported by the results of Bishop and Jenkins (1996). In their study, untrained males exhibit similar gains in 1RM, comparable to our resistance training group (+28.6% vs. +30.0%, respectively), but the magnitude of the increase in $W'$ was markedly higher (+34.9% vs. +6.5%, respectively). The above-mentioned slight increase in MyHC-2A fiber proportion might have benefited the overall outcome of the cycling tests, but presumably only to a small extent. This finding is in line with the results of Aagaard et al. (2011), who showed that even in elite competitive cyclists, proportional distribution of MyHC-2A increases after a resistance training intervention. Similar to the results of previous studies investigating the effects of additional resistance training in endurance-trained athletes (Aagaard et al. 2011, Hickson et al. 1988, Losnegard et al. 2011), resistance training alone increased 1RM, MVT, RFD, and SJ relative maximum power in this study, demonstrating that our resistance training protocol was a valid and effective control condition.

While we demonstrated that vibroX is a potent new training stimulus, we can only speculate about some of the mechanisms, which may mediate its effects. Specifically, the role and contribution of the single vibroX components are still unknown. On the one hand, the compressive cuff during vascular occlusion restricts arterial inflow, resulting in hypoxia and greater metabolic acidosis, while concomitantly blocking venous outflow resulting in continual stimulation of the afferents (Manini and Clark 2009). Blood flow restricted exercise thus may
result in greater stimulation of chemosensitive sensory nerves arising from the active musculature (namely class III and IV afferents), which in turn result in an acute increase in serum growth hormone and catecholamines. In addition, β-adrenergic receptor density is approximately threefold greater in type 1 than in type 2 fibers (Martin et al. 1989, 1992). Together with the possibility that β-adrenergic stimulation might induce a PGC-1α signaling (possibly through PGC-1α4) this might constitute a hypothetical mechanism, which could help to explain why type 1 fiber hypertrophy occurred during vibroX training. On the other hand, we speculate that the vibration-induced reflex activity was associated with the recruitment of distinct, task-related subpopulations of motor units (“functional motor unit pools”) (Burke 2002). There are indeed examples in which two or more functional motor unit pools share a single muscle compartment, and that the motoneurons of these functional pools are intermixed within the same spinal motor nucleus (Burke 2002). In theory, vibration-induced reflexes during vibroX could therefore lead to task-specific recruitment of additional motor units that otherwise are not recruited during squat exercise. It is clear, however, that adaptations to vibroX are significantly more (in terms of quantity and quality) than the sum of the adaptations usually observed for its single components, and these gains may be achieved with a minimal training time commitment. Moreover, given that vibroX produces universal (contrary to specific) effects, further research is warranted to elucidate the mechanisms underlying adaptations/effects and to establish optimal dose-response relationships. In addendum, we would like to point out that, until now, vibroX was only applied in healthy, untrained to trained participants and that the safety in other groups of participants, especially patients, has not been evaluated, yet.

3.5 Conclusions

In conclusion, this study has provided the final proof of concept that modification of resistance exercise by superimposing side-alternating whole body vibration and sustained vascular occlusion induced further improvements in CP, capillarization and hypertrophy in trained endurance athletes, all of which had not been observed with resistance training alone. The increment in CP was positively correlated with the increase in overall capillary-to-fiber ratio. The gain in thigh lean mass was mainly due to MyHC-1 fiber hypertrophy. Increases in $W'$ and dynamometry parameters were only detected after resistance training, while both groups showed improvements in 1RM and maximum jumping power. On the basis of these findings, we recommend that trained endurance athletes who aim at further improving their endurance performance should consider vibroX as an additional new training modality.
Chapter 4

High-intensity interval training with vibration during rest intervals prevents fiber atrophy and decreases in anaerobic performance without compromising the gains in aerobic performance

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4.1 Introduction

High-intensity interval training (HIT) consists of a variety of high-intensity and rest intervals. HIT is known to be a very efficient way to improve cardiovascular variables (Gibala \textit{et al.} 2012) and aerobic function, \textit{e.g.} critical power (CP; Gaesser and Wilson 1988, Poole \textit{et al.} 1990, Vanhatalo \textit{et al.} 2008), leading to improved endurance performance. Because of the large training effect, HIT is used in a variety of sports, encompassing a wide range from classical endurance sports to team sports. Accordingly, many HIT protocols were established which usually differ in regards to intensity (\textit{e.g.} in terms of \% peak power), duration and specific sequence of the exercise intervals. With respect to cycling, it was shown that 4 min high-intensity intervals interspersed with 1.5 min low-intensity intervals leads to the highest increase in 40 km cycling time trial speed as well as peak power (Stepto \textit{et al.} 1999). Furthermore, the highest peak heart rate and peak oxygen uptake ($\dot{V}O_{2peak}$) measurements were attained with 4 min high-intensity intervals relative to 1, 2 and 6 min intervals with a 1:1 work to rest ratio (Seiler and Sjursen 2004). Subsequently, Helgerud \textit{et al.} (2007) found that a 4x4 min HIT, which includes 3 min of low-intensity intervals increases $\dot{V}O_{2peak}$ and stroke volume more than long slow distance running or running at the lactate threshold. Helgerud \textit{et al.} (2007) also stated that from a practical view a 4x4 min HIT was easier to administer than HIT with shorter high-intensity intervals and shorter rest intervals. Finally, irrespective of the interval lengths, HIT leads to a more oxidative metabolism (Hood \textit{et al.} 2011, Larsen \textit{et al.} 2013) and to a reduction in proportion of myosin heavy chain (MyHC)-2X fibers (Simoneau \textit{et al.} 1986). These adaptations might further increase fatigue resistance and thus benefitting endurance performance.

Despite the proven benefits, there appear to be some pitfalls in using HIT. For example, the shift to a more oxidative metabolism and the alteration in MyHC fiber type distribution might reduce anaerobic performance and capacity. Previous studies have shown that countermovement and squat jump power were reduced after HIT (Breil \textit{et al.} 2010), and that HIT led to a reduction in MyHC-2 fiber cross-sectional area (CSA; Kohn \textit{et al.} 2011). Notably, a reduced share of MyHC-2 fibers is associated with a lower maximal muscular shortening velocity (Larsson and Moss 1993). For a given physiological muscle cross-sectional area with given fiber type area distribution, the reduction in maximal shortening velocity will lead to a reduction in maximum muscle power. Further evidence for the possible decrease in anaerobic capacity is that the finite work capacity above CP ($W'$) tended to decrease after only 4 weeks of HIT in favor of the increase in CP, as shown by the significant negative correlation between $\Delta CP$ and $\Delta W'$ (Vanhatalo \textit{et al.} 2008). Notably, these studies have shown that both the decrease in $W'$ and
maximum muscle power prove to be disadvantageous in all team sports as well as in some endurance sports (e.g., hill climbing, sprinting), despite the observed gain in endurance performance by applying HIT.

To avoid the decreases in $W'$ and maximum muscle power that come with HIT, the option of adjusting the rest interval between the high-intensity bouts has been proposed. Spencer et al. (2006) suggested that high-energy phosphates might be restored by implementing a reduction in exercise intensity during the rest intervals and/or by an elongation of these low-intensity intervals. Thereby, the contribution of the anaerobic metabolism to the total energy output would be increased (Spencer et al. 2006). However, both adjustments would lead to a longer time to re-attain $\dot{V}O_{2\text{peak}}$ during the next interval and thereby to a reduction in the time spent at or near $\dot{V}O_{2\text{peak}}$. Because the time spent at or near $\dot{V}O_{2\text{peak}}$ is the main driver for cardiovascular adaptation (Midgley and McNaughton 2006), an elongation or reduction of the intensity of the rest intervals as an alternative would prove to be less effective. Passive rest intervals would only aggravate this problem, since the fractional amount of time spent at or near $\dot{V}O_{2\text{peak}}$ would be of insufficient length to be of significant benefit (Midgley and McNaughton 2006). Furthermore, performance in subsequent high-intensity intervals is not impaired by higher rest interval intensities as long as it does not surpass an upper threshold (Spencer et al. 2008, Thevenet et al. 2008). Therefore, it is clear that the sole modification of cycling exercise intensity during the rest intervals will not attenuate the decrease in anaerobic performance without compromising the gain in aerobic performance. In addition, the studies that investigated the effects of the rest intervals on training adaptations, aimed primarily at increasing aerobic adaptations or making training more bearable with attaining an identical training effect. To our knowledge, there have been no studies presented that investigated whether the modification of rest intervals might prevent the afore-mentioned decreases in anaerobic performance and capacity. Further, it must be pointed out that in most applied HIT-protocols, the warm-up, rest intervals, and cool-down phases account for more than 50% of total exercise time. As the rest intervals are as time-consuming as the high-intensity intervals, it would prove advantageous to increase effectiveness of these phases.

Whole-body vibration (WBV) is a training stimulus that might attenuate the afore-mentioned anaerobic loss. In fact, WBV training has been shown to increase several markers of anaerobic performance: leg press power (Bosco et al. 1999), jumping height (Cochrane and Stannard 2005), and one-repetition maximum (Mileva et al. 2006). Furthermore, improvements in knee extension power were paralleled by increased muscle mass (Bogaerts et al. 2007, Roelants et al.
Therefore, WBV might have the potential to reduce HIT-specific decreases in anaerobic performance/capacity. A further aspect that might be relevant to HIT is that WBV reduces pain sensation (Rittweger 2010). In fact, HIT is associated with high ratings of subjectively perceived exertion (Seiler and Hetlelid 2005). This factor presents special challenges involving attitudes surrounding HIT usage. If WBV performed during the rest intervals significantly reduces leg pain sensation during the high-intensity cycling intervals, the implementation of these high-intensity intervals could be facilitated by reducing negative perceptions that may lead to motivational deficits.

For a given vibration type (side-alternating vertical sinusoidal vibration vs. vertical synchronous vibration) and amplitude, acute effects of WBV are dependent on vibration frequency. With respect to side-alternating vertical sinusoidal vibration (as provided by the Galileo® platform), low frequencies (<20 Hz) allow complete tension-relaxation cycles (with an average duration of complete tension-relaxation cycle ≈ 50 ms) to occur, while with frequencies above 20 Hz, relaxation of the muscle is not complete at the onset of the next stimulus. Accordingly, it is known that higher vibration frequencies lead to higher relative $\dot{V}O_2$ (Rittweger et al. 2002) and that with frequencies from 5 to 30 Hz, electromyography activities also increase in some (but not all) muscles of the lower body (Pollock et al. 2010). In particular, electromyography activity is similar in the thigh muscles, when vibration frequencies >15 Hz are applied (Pollock et al. 2010). Furthermore, based on our experience, Galileo® WBV at 18 Hz is less demanding and is associated with a lower rating of perceived exertion than WBV at 30 Hz. Provided that the similar acute and training effects are achieved, the lower vibration frequency might thereby facilitate execution of HIT. Currently, however, little is known about the differences in training adaptations as well as acute physiological variables (e.g. pain sensation) with different vibration frequencies.

In our current study, we investigated whether the replacement of the active rest intervals during a 4x4 min HIT with Galileo® WBV could prevent the decrease in anaerobic capacity and performance, specifically $W^\prime$, peak knee extensor torque, maximum jumping power and maximum rate of force development (RFD), without compromising the gains in CP and $\dot{V}O_2$peak. In addition, we asked whether the Galileo® WBV intervals would decrease the rating of perceived exertion during the high-intensity cycling intervals. Based on the reported training adaptations following WBV and acute effects of different rest interval intensities, we hypothesized that the combination of HIT and Galileo® WBV leads to similar adaptations in CP and cardiovascular variables as compared to the original 4x4 min HIT-protocol (Helgerud et al. 2004).
2007) but at the same time impedes reductions in $W'$, peak knee extensor torque, maximum jumping power and RFD. Additionally, based on the similar electromyography activity of the thigh muscles with vibration frequencies >15 Hz, we hypothesized that 18 and 30 Hz lead to similar training adaptations despite the difference in the tension-relaxation cycle. Training adaptations for the combination between HIT and Galileo® WBV were controlled by including a group performing Galileo® WBV only, using the same duty cycle as for the HIT groups.

4.2 Methods

4.2.1 Participants

Thirty-six recreationally active males volunteered to participate in this study. They were assigned to one of four training groups matched for CP·kg$^{-1}$ lean mass: 1) Conventional 4x4 min HIT (HIT), 2) HIT with WBV at 18 Hz being applied in lieu of conventional rest intervals (HITVIB18), 3) HIT with WBV at 30 Hz instead of conventional rest intervals (HITVIB30), and 4) only WBV at 30 Hz (VIB30) with the same duty cycle as used for the conventional rest intervals. Prior to this study, the participants were instructed to maintain their individual training routine in terms of training frequency and training intensity, and were advised not to include new or additional high-intensity exercise during the study period. Three participants withdrew from the study due to personal reasons. The resulting number of participants was $n = 8$, $n = 8$, $n = 9$ and $n = 8$ for the HIT, HITVIB18, HITVIB30 and VIB30 group, respectively. The physical characteristics of the participants were as follows (mean ± SD): age 26.0 ± 5.2, 27.6 ± 3.3, 27.5 ± 4.5, and 27.8 ± 3.6; height 180.0 ± 4.2, 181.2 ± 5.9, 180.6 ± 8.1, and 179.0 ± 9.7 cm; body mass 79.2 ± 11.2, 79.3 ± 7.0, 81.8 ± 10.2, and 79.9 ± 11.5 kg; $\dot{V}O_{2peak}$ 49.2 ± 8.2, 48.4 ± 5.1, 48.9 ± 6.1, and 48.1 ± 4.9 ml·min$^{-1}$·kg$^{-1}$ body mass for the HIT, HITVIB18, HITVIB30 and VIB30 group, respectively. There were no statistically significant differences in physical and performance characteristics among the groups prior to the training period. Participants were fully informed about the purposes, benefits and risks associated with the study, and completed a routine health questionnaire before giving written informed consent to participate in the study. The study was approved by the ethics committee of the canton Zurich and was conducted in accordance with the declaration of Helsinki.

4.2.2 Experimental procedures

The study consisted of pre- and post-tests for all participants and 8 weeks of either HIT (HIT, HITVIB18, and HITVIB30) or WBV training alone (VIB30). On the first testing day, a
percutaneous muscle biopsy was obtained from the *M. vastus lateralis* using a 6-mm Bergström needle (Dixons Surgical Instruments, Essex, UK) as previously described (Item *et al.* 2013). On the second testing day, participants completed an incremental ramp cycle ergometer test to determine peak power (*P*\(_{\text{peak}}\)), \(\dot{V}O_2\text{peak}\), peak cardiac output and peak stroke volume. On that occasion, seat and handle bar of the cycle ergometer (Ergoselect 200 K, Ergoline, Bitz, Germany) were adjusted. These settings were adopted for usage in all of the consecutive trials. After a 3 min resting measurement, the ramp test started at 100 W and involved power increases of 9 W every 18 s (30 W·min\(^{-1}\)) until volitional exhaustion. The participants were free to choose the cadence, but the initially chosen revolutions per minute (rpm) had to be maintained throughout all the following cycling tests. Volitional exhaustion, *i.e.* task failure, for all cycling tests was defined as the point in time when participants stopped pedaling or the cadence fell more than 5 rpm for \(>5\) s. On the following testing days, a total of four constant-load cycle ergometer tests were completed in a randomized order to determine CP (Hill 1993, Moritani *et al.* 1981), as previously described (Mueller *et al.* 2014). The constant-load tests were separated by at least 24 h of rest. We then calculated CP and the finite work capacity above CP (*W*\(^\prime\)) by applying the linear power-time\(^{-1}\) equation (Hill 1993). The coefficient of determination (R\(^2\)) for CP was 0.99 ± 0.01 and 0.99 ± 0.01 for the pre- and posttests, respectively. The typical error, expressed as a coefficient of variation for CP determined in our laboratory, was 3.8%. Prior to the third constant-load test, participants underwent jumping mechanography measurements, while knee extension dynamometry measurements were conducted prior to the last constant-load test of the pre-test session. During the post testing, all exercise tests were performed in a reversed order.

### 4.2.3 Equipment and measurements

**Cycling tests.** During all cycling tests and the first and twentieth training sessions, participants were equipped with a facemask, which covered their mouth and nose (Hans Rudolph, Shawnee, KS, USA). The facemask was connected with an anti-bacterial filter (PALL PRO1087, Pall, East Hills, NY, USA) to an Innocor™ device (Innocor™, Innovision, Odense, Denmark). Pulmonary gas exchange and ventilation were continuously measured breath by breath during the ergometer trials. \(\dot{V}O_2\text{peak}\) was determined as the highest mean over a 10-s period. Heart rate was continuously recorded during all cycling tests and all training sessions (S610i, Polar Electro, Kempele, Finland). Peak stroke volume was calculated by dividing peak cardiac output by the corresponding heart rate.

**Acute effects during the first and twentieth training sessions.** At the end of each high-intensity interval, participants were asked to rate their individual perception of breathlessness, respiratory
exertion, leg exertion and pain. For this purpose, a visual analog scale, consisting of a horizontal line, was used. The word “none” was placed at the left end of the scale, and “maximal” was placed on the right end of the scale. The visual analog scale was retrospectively scored from 0 to 10. During the last 30s of each interval, a 20µl sample of arterialized venous blood was taken from the earlobe for the measurement of blood lactate concentration. Blood samples were analyzed with a BIOSEN C_line Sport® (EKF-diagnostic, Barleben, Germany).

*Knee extension.* Knee extensor maximal voluntary torque (MVT) and rate of force development (RFD) were tested using a dynamometer (Con-Trex® MJ, Physiomed Elektromedizin, Schnaittach/Laipersdorf, Germany). The body of each participant was stabilized with straps and handles. Afterwards, the participants performed 3 maximal knee extensions ($\omega = 3.14 \text{ rad} \cdot \text{s}^{-1}$) that were separated by 1 min, for the purpose of assessing MVT. Subsequently, the lever arm of the dynamometer was fixed at a knee angle of 110° (full extension = 180°) and the participants were advised to extend their leg as fast and as powerful as possible. RFD was calculated for the time interval between 0 and 200 ms. Onset of torque production was defined as baseline +7.5 N·m (Aagaard *et al.* 2002).

*Jumping mechanography.* Three vertical counter movement jumps (CMJ) with freely moving arms (separated by 30 s of rest) were performed on a Leonardo Mechanograph® force plate (Novotec Medical, Pforzheim, Germany) for the determination of maximal jumping power (CMJ $P_{\text{max}}$) and peak velocity. For the detection, storage, and analysis of data, we used the manufacturer’s software (Leonardo Mechanography GRFD version 4.4, Novotec, Pforzheim, Germany).

*Body composition.* A densitometer (Lunar iDXA™, GE Healthcare, Madison, WI, USA) was used for the determination of body composition and leg lean mass. The delineation in regions of interest was performed automatically by the integrated software (encore version 14.10.022, GE Healthcare, Madison, WI, USA).

### 4.2.4 Muscle biopsy analysis

Tissue sections were cut at 10-µm thickness in a cryostat (CM3050 S, Leica, Wetzlar, Germany) maintained at $-25 \, ^\circ\text{C}$, and mounted on Fisherbrand Superfrost/Plus microscope slides (Fisher Scientific, Pittsburgh, PA, USA). The serial cryocut cross-sections were stained using the myofibrillar adenosintriphosphatase (mATPase) method at pH 4.6 as previously described (Item *et al.* 2011). On average 707 ± 254 muscle fibers per participant were classified according to their myosin heavy chain (MyHC) isoform into MyHC-1, MyHC-2A and MyHC-2X. For the analysis of oxidative and glycolytic enzyme activities, we incubated the sections in media containing succinate dehydrogenase (SDH), cytochrome c oxidase (COX) and glycerol-3-
phosphate dehydrogenase (GPDH), respectively. On average 560 ± 198, 630 ± 237, and 623 ± 235 muscle fibers were counted per participant for the SDH, COX, and GPDH stainings, respectively. As marker for muscle capillaries, the monoclonal mouse anti-human CD31 endothelial antibody (DAKO, Carpinteria, Canada, 1:600) was used. Overall capillary-to-fiber ratio was calculated by dividing the number of CD31-positive cells by the number of muscle fibers. On average 253 ± 110 capillaries per participant were counted for the analysis of the capillary-to-fiber ratio. We used the NIH Image J Software (version 1.46r, National Institutes of Health, Bethesda, MD, USA) for the fiber and capillary counts. Fiber CSA was determined by fully encircling the borders of the mATPase stained cells with Adobe Photoshop Pro CS6 (Adobe Systems, San Jose, California, USA) of at least 50 fibers per MyHC isoform (McCall et al. 1998). Fiber circularity was calculated using the formula $(4\pi \cdot \text{CSA})/(\text{perimeter})^2$, and only fibers with a circularity higher than 0.7 were considered for analysis (perfect circle = 1.0). Because of the low number of counted MyHC-2X fibers in some individuals, CSA data are solely presented for MyHC-1 and MyHC-2A. Similarly, data was pooled to “GPDH high activity” and “GPDH no activity”, since fibers with intermediate GPDH were rare and confined to only very few participants.

4.2.5 Training regimen

The participants reported alternately two and three times per week to the laboratory for the supervised training session. Prior to the three HIT regimens, the participants completed a warm-up of 3 min at 40% $P_{\text{peak}}$. HIT consisted of high-intensity intervals at 75% $P_{\text{peak}}$, alternating with active rest intervals of 4 min duration at 40% $P_{\text{peak}}$ (adapted from Helgerud et al. 2007) on a cycle ergometer (Bike XT, Technogym, Gambettola, Italy). The additional minute of rest interval was implemented to adjust for the transitions from cycle ergometer to vibration plate and vice versa in the HITVIB groups. In the HITVIB18 and HITVIB30 groups, the low-intensity cycling intervals were replaced by standing still with feet shoulder-width apart in a half-squat position on a side-alternating Galileo® vibration plate (Novotec, Pforzheim, Germany) oscillating at 18 (HITVIB18) or 30 Hz (HITVIB30) for 3 min. The vibration amplitudes were 2.56 ± 0.42, 2.56 ± 0.46, and 3.06 ± 0.42 mm for the HITVIB18, HITVIB30 and VIB30 groups, respectively. Amplitudes were not statistically different between the training groups. WBV training was executed identically as the rest intervals in the groups combining HIT and WBV. All WBV participants wore non-slippery socks to avoid any dampening of shoe soles during vibration training. To assure identical fixation of the feet during cycling, the pedals of the ergometer allowed strapping of the feet with shoes (HIT) as well as with non-slippery socks (HITVIB18 and HITVIB30). As soon as the participants of the HIT, HITVIB18, and
HITVIB30 groups were able to sustain all 4 high-intensity intervals successfully at the predetermined level, the power of the warm-up, high-intensity intervals and rest intervals was increased by 3% $P_{\text{peak}}$ for the upcoming training session.

4.2.6 Statistical Analysis

Data are presented as mean values ± standard deviations (SD). Normality of data was visually ascertained by Q-Q-plots. Pre-test values between the training groups as well as acute training values were analyzed with a one-way analysis of variance. For the detection of significant differences between groups over time, a univariate general linear model was applied. For this analysis, the differences post–pre ($\Delta$) of each variable were compared between groups. Significant differences within groups from pre to post intervention were displayed by parameter estimates. This analysis tested the null hypothesis that $\Delta$ parameter was 0. If $\Delta$ parameter had a $P$-value lower than the level of significance, the null hypothesis was rejected meaning that $\Delta$ parameter was significantly different from 0. Pearson correlations were performed to assess the associations between variables. The level of significance was set at $P < 0.05$. All statistical analyses were performed using the software SPSS Statistics 20.0 (SPSS, Chicago, IL, USA).

4.3 Results

$W'$ remained unaltered for HITVIB18 and HITVIB30, while it decreased in the HIT group with a tendency for a group x time interaction relative to the non-significant increase in the VIB30 group (Fig. 4.1a). CMJ $P_{\text{max}}$ (Fig. 4.1b), MVT (Fig. 4.1c), and RFD (Fig. 4.1d) were decreased in the HIT group and were not altered in the other training groups. In the HIT group, there was a significant decrease in peak velocity during CMJ (pre vs. post: 3.01 ± 0.32 vs. 2.87 ± 0.18 m·s$^{-1}$; $P = 0.007$), while there was no alteration in peak velocity during CMJ in the other training groups (pre vs. post: 2.92 ± 0.27 vs. 2.94 ± 0.23 m·s$^{-1}$, 2.91 ± 0.06 vs. 2.92 ± 0.07 m·s$^{-1}$, and 3.00 ± 0.11 vs. 2.92 ± 0.13 m·s$^{-1}$ for HITVIB18, HITVIB30 and VIB30, respectively). CSA of the MyHC-2A fibers decreased significantly in the HIT group and remained unaltered in the three other training groups (Tab. 4.1). There was a significant correlation between $\Delta W'$ and $\Delta$CSA of the MyHC-2A fibers in the HIT group ($y = 0.23x - 828.7$, $R^2 = 0.596$, $P = 0.025$).
Figure 4.1: a) Finite work capacity above critical power ($W'$), b) maximal power during countermovement jumping (CMJ $P_{\text{max}}$), c) maximal voluntary torque (MVT), and d) rate of force development (RFD) during the first 200 ms of contraction pre (white bars) and post (black bars) training in the high-intensity training (HIT), HIT with 18 Hz whole-body vibration (WBV; HITVIB18), HIT with 30 Hz WBV (HITVIB30), and only 30 Hz WBV training (VIB30) group. Bars and error bars represent mean values and SD, respectively for 33 participants (HIT: $n = 8$, HITVIB18: $n = 8$, HITVIB30: $n = 9$, VIB30: $n = 8$). *$P < 0.05$, **$P < 0.01$, significant differences within group pre vs. post; *$P = 0.067$, difference between groups pre vs. post.

CP (Fig. 4.2a), $P_{\text{peak}}$ (Fig. 4.2b), and overall capillary-to-fiber ratio (Fig. 4.2c) were significantly increased for HIT, HITVIB18, and HITVIB30 without significant differences among these training groups. For CP (Fig. 4.2a) and $P_{\text{peak}}$ (Fig. 4.2b), the significant increases in the three groups involving HIT were significantly different from the VIB30 group. The significant increase in overall capillary-to-fiber ratio in the HITVIB groups was significantly different from the non-alteration in the VIB30 group (Fig. 4.2c). For HIT, HITVIB18, and HITVIB30, $\dot{V}O_2_{\text{peak}}$ (Fig. 4.3a), peak cardiac output (Fig. 4.3b), and peak stroke volume (Fig. 4.3c) increased pre to post training without any differences between the three HIT groups. Peak heart rate decreased for HIT, HITVIB18, and HITVIB30, without any significant group differences (Fig. 4.3d).
Figure 4.2: a) Critical power (CP), b) peak power during incremental cycling ramp test ($P_{\text{peak}}$), c) overall capillary-to-fiber ratio pre (white bars) and post (black bars) training in the HIT, HITVIB18, HITVIB30 and VIB30 group. For more explanations, see legend of Fig. 1. **$P < 0.01$, ***$P < 0.001$, significant differences within group pre vs. post; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, significant differences between groups pre vs. post.
Figure 4.3: a) Peak oxygen uptake ($V_{\text{O}_{2}\text{peak}}$), b) peak cardiac output, c) peak stroke volume, d) peak heart rate pre (white bars) and post (black bars) training in the HIT, HITVIB18, HITVIB30 and VIB30 group. For more explanations, see legend of Fig. 1. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, significant differences within group pre vs. post.

The proportion of MyHC-1 fibers was not altered pre to post training in any of the training groups (Tab. 4.1). The proportion of MyHC-2A fibers increased in the HIT, HITVIB18, and HITVIB30 groups, while the proportion of MyHC-2X fibers decreased significantly in the HIT and HITVIB18 group and showed a strong tendency for a decrease in the HITVIB30 group. There were no differences pre to post in CSA of MyHC-1 fibers in any of the training groups. COX and SDH weak activity were increased in the HIT, HITVIB18, and HITVIB30 group and proportion of fibers with no COX and no SDH activity were decreased for HIT, HITVIB18, and HITVIB30 group. GPDH activity was not altered in any of the training groups pre to post training (Tab. 4.1). There were no group differences for any variables of Tab. 4.1. Total body mass and total fat mass was significantly reduced in the HITVIB30 group only (Tab. 4.2). There were no alterations in total lean mass and leg lean mass in any group (Tab. 4.2).
Table 4.1. Distribution of the myosin heavy chains (MyHC) types, cross-sectional areas (CSA), cytochrome c oxidase (COX) activity, glycerol-3-phosphate dehydrogenase (GPDH) activity, and succinate dehydrogenase (SDH) activity pre and post training period in the conventional 4x4 min high-intensity training (HIT), HIT with whole body vibration at 18 Hz (HITVIB18) or 30 Hz (HITVIB30) instead of conventional rest intervals and the Galileo 30 Hz whole body vibration (VIB30) training group.

<table>
<thead>
<tr>
<th></th>
<th>HIT (n = 8)</th>
<th>HITVIB18 (n = 8)</th>
<th>HITVIB30 (n = 9)</th>
<th>VIB30 (n = 8)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Pre</td>
<td>Pre</td>
<td>Pre</td>
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<tr>
<td></td>
<td>Post</td>
<td>Post</td>
<td>Post</td>
<td>Post</td>
</tr>
<tr>
<td>MyHC-1 (%)</td>
<td>56.2 ± 16.6</td>
<td>55.5 ± 12.7</td>
<td>48.4 ± 13.7</td>
<td>49.0 ± 13.9</td>
</tr>
<tr>
<td>MyHC-2A (%)</td>
<td>31.4 ± 9.0</td>
<td>37.8 ± 8.8*</td>
<td>39.6 ± 9.9</td>
<td>45.1 ± 11.2*</td>
</tr>
<tr>
<td>MyHC-2X (%)</td>
<td>12.4 ± 11.1</td>
<td>6.7 ± 6.7*</td>
<td>12.0 ± 16.3</td>
<td>5.9 ± 7.8**</td>
</tr>
<tr>
<td>CSA MyHC-1 (μm²)</td>
<td>4568 ± 908</td>
<td>4439 ± 981</td>
<td>4341 ± 923</td>
<td>4407 ± 1024</td>
</tr>
<tr>
<td>CSA MyHC-2A (μm²)</td>
<td>5764 ± 1122</td>
<td>5317 ± 798*</td>
<td>5366 ± 1666</td>
<td>5436 ± 1896</td>
</tr>
<tr>
<td>COX high activity (%)</td>
<td>57.3 ± 15.0</td>
<td>57.6 ± 10.8</td>
<td>49.5 ± 13.4</td>
<td>50.7 ± 10.5</td>
</tr>
<tr>
<td>COX weak activity (%)</td>
<td>33.5 ± 9.3</td>
<td>36.8 ± 7.7*</td>
<td>41.1 ± 5.8</td>
<td>442 ± 3.8*</td>
</tr>
<tr>
<td>COX no activity (%)</td>
<td>9.2 ± 10.6</td>
<td>5.6 ± 7.1**</td>
<td>9.4 ± 11.7</td>
<td>5.1 ± 8.2**</td>
</tr>
<tr>
<td>SDH high activity (%)</td>
<td>54.9 ± 14.7</td>
<td>56.6 ± 10.8</td>
<td>48.1 ± 11.3</td>
<td>50.8 ± 8.7</td>
</tr>
<tr>
<td>SDH weak activity (%)</td>
<td>33.6 ± 7.7</td>
<td>39.7 ± 8.1***</td>
<td>42.0 ± 6.3</td>
<td>45.0 ± 6.4*</td>
</tr>
<tr>
<td>SDH no activity (%)</td>
<td>11.5 ± 11.1</td>
<td>3.8 ± 3.5**</td>
<td>9.9 ± 15.0</td>
<td>4.2 ± 6.2*</td>
</tr>
<tr>
<td>GPDH high activity (%)</td>
<td>48.5 ± 16.9</td>
<td>49.7 ± 10.0</td>
<td>56.6 ± 12.2</td>
<td>53.4 ± 11.5</td>
</tr>
<tr>
<td>GPDH no activity (%)</td>
<td>51.5 ± 16.9</td>
<td>50.3 ± 10.0</td>
<td>43.4 ± 12.2</td>
<td>46.6 ± 11.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, significant differences within group pre vs. post training; †P = 0.053 pre vs. post training.

Table 4.2. Body composition pre and post 8 weeks of training in the high-intensity training (HIT), HITVIB18, and HITVIB30 groups and the 30 Hz whole body vibration (VIB30) group.

<table>
<thead>
<tr>
<th></th>
<th>HIT (n = 8)</th>
<th>HITVIB18 (n = 8)</th>
<th>HITVIB30 (n = 9)</th>
<th>VIB30 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Pre</td>
<td>Pre</td>
<td>Pre</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>Post</td>
<td>Post</td>
<td>Post</td>
</tr>
<tr>
<td>Total mass (kg)</td>
<td>79.2 ± 11.2</td>
<td>79.3 ± 11.4</td>
<td>78.4 ± 7.2</td>
<td>81.8 ± 10.2</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>61.5 ± 8.3</td>
<td>61.5 ± 8.4</td>
<td>61.5 ± 7.3</td>
<td>64.2 ± 8.0</td>
</tr>
<tr>
<td>Lean mass legs (kg)</td>
<td>21.8 ± 3.5</td>
<td>21.7 ± 3.6</td>
<td>21.2 ± 2.5</td>
<td>22.6 ± 2.6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>14.5 ± 5.7</td>
<td>13.2 ± 5.4</td>
<td>14.4 ± 3.2</td>
<td>13.3 ± 2.7</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>18.8 ± 6.2</td>
<td>17.4 ± 5.7</td>
<td>19.2 ± 4.9</td>
<td>17.9 ± 3.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD. *P < 0.05, significant differences within training group pre vs. post training.
There were no group differences in average power, average heart rate, and average $\dot{V}O_2$ during the high-intensity intervals during the 1st and 20th training sessions (Tab. 4.3). Average $\dot{V}O_2$ during the rest intervals was higher in the HIT compared to the HITVIB18 and HITVIB30 groups in both training sessions. During the 20th training session, average heart rate during the rest intervals was significantly higher in the HIT than in the HITVIB30 group. There were no group differences in individual perception of breathlessness, respiratory exertion, leg exertion, and pain between the HIT, HITVIB18, and HITVIB30 groups (Tab. 4.3).

<table>
<thead>
<tr>
<th>Table 4.3. Average values for power, heart rate (HR), oxygen uptake ($\dot{V}O_2$), blood lactate concentration ([La$^-$]), and ratings of perceived exertion (PE) during the high-intensity intervals (HI) and rest intervals (RI) in the 1st and 20th training session for the high-intensity training (HIT), HITVIB18, and HITVIB30 groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIT</td>
</tr>
<tr>
<td>(n = 8)</td>
</tr>
<tr>
<td>Power HI (W)</td>
</tr>
<tr>
<td>HR HI (min$^{-1}$)</td>
</tr>
<tr>
<td>HR RI (min$^{-1}$)</td>
</tr>
<tr>
<td>$\dot{V}O_2$ HI (l·min$^{-1}$)</td>
</tr>
<tr>
<td>$\dot{V}O_2$ RI (l·min$^{-1}$)</td>
</tr>
<tr>
<td>[La$^-$] (mmol·l$^{-1}$)</td>
</tr>
<tr>
<td>PE breathlessness</td>
</tr>
<tr>
<td>PE resp. exertion</td>
</tr>
<tr>
<td>PE leg exertion</td>
</tr>
<tr>
<td>PE Pain</td>
</tr>
</tbody>
</table>

Values are mean ± SD. *$P < 0.05$, ***$P < 0.001$, significant differences relative to HIT training group.

4.4 Discussion

Several new findings could be obtained with this study. First, the decreases of anaerobic capacity and performance ($W'$, CMJ $P_{max}$, MVT, RFD, and CSAs of MyHC-2A fibers) after conventional HIT could be prevented, when the active rest intervals during HIT were replaced with Galileo® WBV. Second, the replacement of active rest intervals during HIT with Galileo® WBV did not interfere with the increases in cycling power (CP and $P_{peak}$), cardiovascular variables ($\dot{V}O_2$peak, peak cardiac output, and capillary-to-fiber ratio) and enzyme activities of the classical HIT protocol. Third, the Galileo® WBV intervals had no influence on ratings of perceived exertion during the high-intensity intervals compared to conventional HIT.

The decreases in $W'$, MVT, RFD and CMJ $P_{max}$ in the conventional HIT group is most likely explained by the reduction in CSA of the MyHC-2A fibers. The effect of MyHC-2A fiber
atrophy on the decrease in \( W' \) was supported by the significant correlation between the decrease in fiber CSA and the decrease in \( W' \). It is well established that a lower share of MyHC-2 fibers leads to reduced force and power production and *vice versa* (Cornie *et al.* 2011). The reduced share of MyHC-2 fibers is augmented by the altered MyHC fiber type distribution. The decreased proportion of MyHC-2X fibers, which is an adaptation to exercise *per se*, leads to a reduced maximal shortening velocity (Larsson and Moss 1993). Thereby, maximal power production was reduced further. Our finding that the CSA of MyHC-2 fibers decreased after HIT is supported by the results of Kohn *et al.* (2011), who demonstrated that CSA of MyHC-2 fibers have a strong tendency to decrease after 6 weeks of HIT in well-trained runners.

The decreases in \( W' \), MVT, RFD, and CMJ \( P_{\text{max}} \) were prevented by replacing the rest intervals during conventional HIT with Galileo® WBV, indicating that Galileo® WBV plays a very specific role in mediating this effect. This notion is supported by our finding that the VIB30 group had a non-significant increase in \( W' \) of 1.1 kJ that led to a tendency for a group x time interaction with the HIT group. Furthermore, significant decreases in anaerobic capacity and performance were only present when conventional HIT (*i.e.* including low-intensity cycling active rest intervals) was conducted, while in all three groups, whose procedures included the use of WBV, no decreases were observed. Therefore, Galileo® WBV seems to prevent decreases in anaerobic capacity and performance that were responses to HIT. While we were able to show that additional WBV prevented decreases in these variables, a lack of knowledge on the exact mode of action of WBV allows us only to speculate upon the underlying mechanisms associated with these effects.

First, prevention of MyHC-2A fiber atrophy due to WBV might result from the recruitment of high-threshold motor units, which are not activated by cycling alone. This mechanism is supported by the results of Pollock *et al.* (2012), who showed that the recruitment thresholds of single, higher threshold motor units are reduced after WBV. Thereby, it is assumed that vibration-induced stretch-reflex activity leads to the recruitment of distinct task-specific motor units (Burke 2002). These motor units include most likely MyHC-2A fibers, whose activation through WBV could inhibit atrophy of these fibers. Recently, support for this mechanism was obtained as it was shown that vibration at 30 Hz down-regulates myostatin and atrogin-1 expression *in vitro* and *in vivo* in mice (Ceccarelli *et al.* 2012).

Second, neuronal adaptation might have compensated for the reduction in proportion of MyHC-2X fibers in the combination groups. As muscle fiber characteristics were altered in the same
way in all groups involving HIT towards a more fatigue resistant but slower phenotype, neuronal input had to increase in the HITVIB groups to allow maintenance of similar MVT, RFD, and CMJ $P_{\text{peak}}$ values. An increase in neuromuscular innervation as response to WBV training is very unlikely as electromyography activity relative to power production is decreased after WBV training (Bosco et al. 2000). In turn, neuromuscular efficiency, i.e. better synchronization of motor units (Milner-Brown et al. 1975), as well as improved force production of synergists and inhibition of antagonists (Bosco et al. 2000) might be improved after WBV. These improvements rely on an enhancement of monosynaptic stretch reflexes that are initiated by afferent signals from the muscle spindles to the motoneuron pool and on a depression of inhibitory impacts of Golgi tendon organs, due to their accommodation to vibratory-induced excitation (Issurin 2005). The mechanism of improved neuromuscular efficiency after WBV training is supported by Oosthuyse et al. (2012), who showed that Wingate-test peak power is increased after WBV is added to the usual training routine in cyclists.

The efficiency of the replacement of the active rest intervals during HIT with WBV was proven by the similar training adaptations in cycling power (CP and $P_{\text{peak}}$), cardiovascular variables ($\dot{V}O_{2\text{peak}}$, peak cardiac output, peak stroke volume, overall capillary-to-fiber ratio), as well as by MyHC fiber distribution and enzyme activities between the HITVIB groups and the conventional HIT group. Thus, the decreases in anaerobic performance and capacity were prevented without losses in aerobic performance and capacity. Therefore, both conventional HIT and HIT combined with Galileo® WBV improved central (e.g. peak cardiac output) and peripheral (e.g. oxidative enzyme activities, capillary-to-fiber ratio) factors, determining aerobic function. Consequently, we were able to demonstrate that it is feasible to replace cycling during the active rest intervals with WBV without compromising aerobic training adaptations.

These training adaptations accompany the similar acute effects during the high-intensity intervals. There were no differences between HIT groups (HIT, HITVIB18 and HITVIB30) in average power, average heart rate and average $\dot{V}O_2$. Furthermore, the similar blood lactate concentrations in the three HIT groups point to an equal contribution of anaerobic ATP generation to total energy production in these groups. Our findings thus lend further credence to the notion that the high-intensity intervals are responsible for the aerobic adaptations, as long as time at or near $\dot{V}O_{2\text{peak}}$ is not drastically reduced by extended rest intervals or inappropriate intensities of the rest intervals. Moreover, the non-alterations in GPDH activity with training showed that neither HIT nor WBV had an influence on glycolytic enzyme metabolism. The
ratings of perceived exertion also did not differ between the training groups, which indicated that maintaining a half-squat position on a vibration plate instead of cycling at lower intensities had neither a beneficial nor a disadvantageous effect on respiratory and leg exertion, nor on pain during the subsequent high-intensity intervals.

In contrast to the unchanged acute effects during the high-intensity intervals, average $\dot{V}O_2$, and to some extent average heart rate, were lower in the HITVIB groups during the rest intervals. However, this had no adverse effect on the adaptation of these variables. The active rest intervals during HIT are believed to serve two main functions (Buchheit and Laursen 2013), which were presumably not different between the HITVIB groups and the conventional HIT. First, they should allow a supra-resting blood flow to the leg muscles to allow muscle metabolic recovery. Thereby, we assumed that blood flow to the leg muscles is comparable between WBV in a half-squat position and cycling at 40% $P_{\text{peak}}$. This assumption was based on the results of two independent studies. The first study showed that WBV in a half-squat position at a vibration frequency of 20-30 Hz led to a 4- to 5-fold increase in mean blood cell speed in the femoral artery compared to rest (Lythgo et al. 2009). The second study proved that cycling at 40% $P_{\text{peak}}$ increased femoral blood flow by 4- to 5-fold relative to rest (Calbet et al. 2007). Second, $\dot{V}O_2$ should be maintained also at an increased level to accelerate the time needed to re-attain values at or near $\dot{V}O_2\text{peak}$ and to increase the contribution of the aerobic metabolism to total energy output. Based on the assumption that time at or near $\dot{V}O_2\text{peak}$ is the main driving force for cardiovascular adaptations (Midlgey and McNaughton 2006) and the similar training adaptations in the HITVIB groups and the HIT group, the significant differences in $\dot{V}O_2$-values during the rest intervals had no actual impact on total time at or near $\dot{V}O_2\text{peak}$. Consequently, $\dot{V}O_2$-kinetics to re-attain values at or near $\dot{V}O_2\text{peak}$ during the high-intensity intervals should not have been significantly different between the three groups involving HIT. On the contrary, the contribution of the aerobic metabolism to the total energy output was probably higher in the HIT group. If comparing $\dot{V}O_2$-values for exclusive cycling at rest interval intensity (≈2.0 l·min$^{-1}$) with exclusive WBV (≈1.0 l·min$^{-1}$), there is a large difference for these types of exercise. However, this considerable higher fractional contribution of the aerobic metabolism to total energy yield turnover in the HIT group had no effect on cardiovascular and oxidative enzyme adaptations.

Regarding all tested variables, the two vibration frequencies of 18 and 30 Hz did not induce different training adaptations. These results are in line with similar electromyography and acceleration values with vibration frequencies above 15 Hz in the thigh muscles (Pollock et al.
The equal activation during WBV of the *M. quadriceps*, which is the muscle mainly responsible for force and power production during the exercise tests (knee extension, two-legged jumping, and cycling), led to similar muscular training adaptations. The prevention of decreases in anaerobic capacity and performance due to WBV and the included neuronal adaptation might have been supported by the fatigued state of our participants during WBV. The participants were transferring to the vibration plate after each of the high-intensity intervals. Consequently, they were conducting WBV in a fatigued state, and the muscle activation that might thereby have been increased, may have led to neuronal adaptations. This mechanism is in line with a recent study investigating the effect of fatigue on vertical WBV showing that maximal electromyography activity was significantly higher after fatiguing exercise (Carlucci *et al*. 2013). Summarized, when HIT is combined with side-alternating WBV, vibration frequencies between 18 and 30 Hz will be equally effective in preventing decreases in anaerobic performance and capacity. Under these circumstances, the subjectively perceived exertion might turn the balance to 18 Hz, which is usually better tolerated by participants.

The decrease in CSA of MyHC-2A fibers in the HIT group did not lead to a statistically significant reduction in leg lean mass. The most likely reason for this resides in the dominance of MyHC-1 fibers, for which no significant changes in CSA were measured in this training group. Even though there was on average more than 1 kg decrease in fat mass in all three groups involving HIT, only the decrease in the HITVIB30 group reached statistical significance. Therefore, the higher total energy yield turnover, due to higher power outputs during rest intervals in the HIT group, had no influence on body composition. This result can mainly be explained by the high inter-individual variations in fat mass alteration. The fat mass change ranged from −5.6 to +2.2 kg. It could be expected that the increase in energy expenditure due to training will lead to a decrease in fat mass. Therefore, the increase in fat mass in certain participants might be attributed to a disproportional higher energy intake.

Our results have a major practical impact. The prevention of decreases in anaerobic performance and capacity might be critical in most team sports as well as some endurance sports. In most disciplines, the decision between victory and defeat is based on fatigue resistance in conjunction with anaerobic performance and capacity (*e.g.* sprinting). The combination of aerobic HIT with side-alternating WBV allows an increase in aerobic function without negatively affecting anaerobic capacity and performance. This positive effect is achieved without time loss because the rest intervals between the high-intensity bouts are used more efficiently. Hence, time efficiency and overall effectiveness of training is markedly
increased. For the improvement in fatigue resistance, concomitant with the prevention of reductions in anaerobic performance and capacity, athletes in team sports should include 4x4 min HIT with side-alternating WBV during rest intervals in their training routine.

4.5 Conclusions

In conclusion, reductions in CSA of MyHC-2A fibers, MVT, RFD and CMJ $P_{\text{max}}$ that were characteristic for the classical 4x4 min HIT protocol were prevented by the addition of side-alternating WBV during the rest intervals. The replacement of low-intensity cycling as active rest intervals during a 4x4 min HIT with WBV led to similar adaptations in cycling power, cardiovascular variables, and enzyme activities. To prevent MyHC-2A fiber atrophy and to prevent decreases in anaerobic performance and capacity, athletes should complement aerobic HIT with side-alternating WBV. This is of particular importance for athletes engaged in team or endurance sports that involve anaerobic tasks (e.g. sprinting).
Chapter 5

General discussion

The present thesis aimed to investigate the influence of HIT or NaHCO₃ on the parameters of the critical power concept. All applied interventions were successful in achieving performance increases. In particular, $T_{\text{lim}}$ at CP was increased with multiday acute NaHCO₃ ingestion without differences in performance among the five testing days. CP was increased with vibroX training in endurance-trained males, while $W'$ was non-significantly elevated following vibroX and significantly increased after resistance training. Furthermore, HIT led to an increase in CP. Aerobic HIT resulted in a decrease in $W'$, while the replacement of the active cycling rest intervals with side-alternating WBV prevented this decrease.

In chapter 1, for the first time the multiday acute NaHCO₃ ingestion protocol was scientifically investigated. $T_{\text{lim}}$ at CP was similarly improved on every testing day with NaHCO₃ supplementation compared to placebo. Likewise, equally increased pretest $[\text{HCO}_3^-]$ were attained on all five days with supplementation relative to placebo. Hence, the application of this supplementation protocol was ensured. We proved thereby that induced alkalosis enhances cycling at CP intensity. Furthermore, we filled the gap of test durations positively influenced by NaHCO₃ supplementation between the previously investigated short high-intensity performances of ~1 min (Vanhatalo et al. 2010a, Van Montfoort et al. 2004) and time trial of 60 min (McNaughton et al. 1999b). The high salt loading due to NaHCO₃ supplementation led to an increase in plasma volume, which might presumably attenuated a further increase in $[\text{HCO}_3^-]$ and precluded a resulting improvement in $T_{\text{lim}}$. Athletes competing in multiday competitions or tournaments with intensities at CP are therefore advised to ingest 0.3 g kg⁻¹ body mass NaHCO₃ 90 min before each competition to improve performance.
In chapter 2, we provided the final proof of concept that vibroX leads to a concomitant increase in endurance and resistance type adaptations. VibroX increased simultaneously CP, CSA of MyHC-1 and -2 fibers, and overall capillary-to-fiber ratio. Remarkably, the increase in CSAs was despite a high volume of endurance training during the individual training routines of the participants. Therefore, vibroX was able to overcome the interference effect, which was present following conventional resistance training. The increase in CSA of MyHC-1 fibers and in particular, the significant correlation between the gain in thigh lean mass and CSA of MyHC-1 fibers receives particular attention in endurance athletes. It is known that muscle fiber type distribution in endurance athletes is dominated by MyHC-1 fibers and that MyHC-1 fibers are less responsive to a hypertrophic stimulus than MyHC-2 fibers (Tesch 1988). Hence, a gain in muscle mass is hard to achieve for endurance athletes. In the light of the fact that a higher muscle mass might be decisive in several competitions, e.g. time trial or final sprint, endurance athletes are reliant on such a training modality. Furthermore, vibroX led to an additional increase in overall capillary-to-fiber ratio although the participants already had high starting values. Hence, vibroX as a new training modality might represent an interesting option for athletes that had reached a performance plateau by using classical training modalities.

In chapter 3, we addressed major disadvantages of conventional aerobic HIT, namely muscle fiber atrophy of MyHC-2 fibers as well as decreases in anaerobic performance and capacity. By using conventional aerobic HIT, additional trainings (e.g. resistance training) would be necessary to compensate for MyHC-2 fiber atrophy and decrements in anaerobic performance and capacity. By replacing the low-intensity cycling rest intervals with side-alternating WBV, these decreases were prevented without additional time commitment. In addition to the prevention of the decrements in $W'$, MVT, RFD, and maximal jumping power by combining of HIT with side-alternating WBV, CP, overall capillary-to-fiber ratio, peak cardiac output, and oxidative enzyme activities were similarly increased compared to aerobic HIT. Therefore, athletes engaged in team sports or endurance sports, in which anaerobic performance and/or capacity plays a crucial role in meeting with success, find herein a new training modality that improves CP without negatively affecting anaerobic variables. Therefore, these athletes are advised to include HIT with side-alternating WBV into their training routine.

Our results implicated that CP is sensitive to increases in overall capillary-to-fiber ratio and to improvements in the cardiovascular system as well as oxidative enzyme activity. The adaptations in the cardiovascular system and increased capillarization improve oxygen transport from the lung to the working muscles. It is reasonable that a higher oxygen supply of the muscle
in conjunction with an increase in oxidative enzyme activity enables a higher contribution of the aerobic system to energy production. Higher muscular oxygen availability as improving factor for CP is supported by the results of Vanhatalo et al. (2010a), who showed that one-legged knee extension CP determined in hyperoxia is higher than determined in normoxia. In their study, the prolonged $T_{lim}$ at an identical work rate of the constant-load trials is attributed to a higher muscle oxygenation and a concomitant slower decrease in [PCr] (Vanhatalo et al. 2010a). The physiological adaptations leading to an increase in CP are in line with several previous studies that reported increases in peak cardiac output (Helgerud et al. 2007), overall capillary-to-fiber ratio (Jensen et al. 2004), and oxidative enzyme activity (Henriksson and Reitman 1977, Hood et al. 2011) after high-intensity trainings. Furthermore, an improvement in CP with HIT is in line with a couple of studies that investigated the effect of conventional cycling HIT on CP showing increases between 10 and 15% after 4 to 7 weeks of training (Gaesser and Wilson 1988, Poole et al. 1990, Vanhatalo et al. 2008). In contrast, conventional resistance training had no influence on CP. This result is supported by the reports of Bishop and Jenkins (1996) as well as Sawyer et al. (2014), who also showed that resistance training for the legs did not increase cycling CP.

Based on the theoretical definition of CP, an intervention that alters blood pH should not have an impact on exercise at CP. Our result of increased $T_{lim}$ with induced metabolic alkalosis indicates that acid-base balance is disturbed during exercise at CP without supplementation. Therefore, our result of prolonged $T_{lim}$ with NaHCO$_3$ supplementation is in line with previous results indicating that CP overestimates the maximal metabolic steady state (Dekerle et al. 2003, Jenkins and Quigley 1990, Pringle and Jones 2002). In addition, Brickley et al. (2007) reported progressively decreasing pH values already at 90% CP. Our finding is contrary to the results of Jones et al. (2008), who showed that muscle pH attains a decreased steady state after 3 min during one-legged knee extension exercise at an intensity slightly below CP and decreases progressively with an intensity above CP. Furthermore, as CP represents theoretically the demarcation between the heavy and severe intensity domains, $\dot{V}_O_2$ should attain submaximal steady states during exercise at CP. In contrast to this theoretical definition, the participants in our study attained $\dot{V}_O_2^{peak}$ during the constant-load trials at CP. Our finding stands in contrast to the results of several studies (Overend et al. 1992, Poole et al. 1988, Soares-Caldeira et al. 2012), which showed that $\dot{V}_O_2$ achieves a steady state during constant-load cycling at CP. Furthermore, $\dot{V}_O_2^{peak}$ was attained only with exercise above CP (Poole et al. 1988).
There is still an ongoing controversy whether CP is congruent with the maximal metabolic steady state or not. Irrespective of this controversy, two factors underline the importance of CP in endurance performance characterization. First, CP correlates with performance during 10 mile (Black et al. 2013) up to 40 km (Smith et al. 1999) time trials, whereby time trials represent the veridical form of endurance performance. In addition, CP predicts time trial performance better than $\dot{V}O_{2\text{peak}}$, gas exchange threshold, and respiratory compensation point (Black et al. 2013) as well as the ventilatory threshold (Smith et al. 1999) that are all well-established parameters for endurance performance characterization. Second, as shown in our studies, CP is sensitive to adaptations in cardiovascular variables and muscle oxidative enzyme activity that are known adaptations to endurance training. In particular, this is strongly supported by the correlation between $\Delta$CP and $\Delta$overall capillary-to-fiber ratio after vibroX training.

The physiological underpinnings of $W'$ are still ambiguous and hence, are broadly discussed. In the first studies on the CP model, $W'$ was mainly characterized as a finite anaerobic energy reserve (Monod and Scherrer 1965, Moritani et al. 1981). Therefore, it was assumed that it is mainly limited by PCr and glycogen availability. This assumption was supported by an increased $W'$ after oral creatine supplementation compared to placebo (Miura et al. 1999) and by a reduced $W'$ after glycogen depleting exercise (Miura et al. 2000). Later, several studies supported the notion that a depletable energy reserve is responsible for exercise cessation above CP (Ferguson et al. 2007, Jones et al. 2008). In this context, a severe intensity priming exercise bout leads to a reduced $W'$ without affecting CP (Ferguson et al. 2007) and exercise above CP reduces [PCr] to a large extent (Jones et al. 2008). However, both research groups stated that in addition to a depletable energy resource an accumulation of metabolites might limit $W'$, too. Therefore, the model of a sole limited anaerobic energy reserve does not characterize $W'$ satisfactorily. This classical view was even more challenged as $W'$ tended to decrease after HIT (Vanhatalo et al. 2008), whereby this training modality should not have impaired PCr (Edge et al. 2013) and glycogen (Burgomaster et al. 2008) stores.

Our results extend this classical view by at least three further factors that determine the magnitude of $W'$. First, based on our results of chapter 2, we assume that NaHCO$_3$ supplementation increases $W'$ during exercise with H$^+$ accumulation. Following this line of reasoning, blood and muscle buffer capacity have an impact on the magnitude of $W'$ as long as anaerobic glycolysis is a main supplier for $W'$. Second, improved neuronal function after resistance training led to an increase in $W'$. It is known that improved neuronal function is a
well-described adaptation to resistance training (Sale 1988). Accordingly, the increases in MVT, RFD, and $W'$ after conventional resistance training without a gain in muscle mass were attributed to improved neuronal function (Chapter 3). Based on our results, we lend further credence to previous studies showing that improved neuronal function after resistance training can be adapted to cycling exercise and improve thereby cycling performance and/or capacity (Aagaard et al. 2011, Sunde et al. 2010). Third, the decrease in CSA of MyHC-2 fibers correlated significantly with the reduction in $W'$ in the HIT group (chapter 4). Hence, we assume that the share of MyHC-2 fibers influences the magnitude of $W'$. Our result of muscle fiber atrophy as explanation for a decrease in $W'$ is partly supported by a significant correlation in CSA of the thigh muscle measured by ultrasonography and $W'$ in untrained males (Miura et al. 2002).

Endurance capacity was increased without a concomitant increase in $\dot{V}O_2_{peak}$ after NaHCO$_3$ supplementation, vibroX training, and resistance training. This stands in contrast to the classical view that endurance performance ability is mainly determined by $\dot{V}O_2_{peak}$ (Saltin and Astrand 1967). However, recent studies showed that a multiplicity of factors, ranging from local muscular to systemic variables, have an influence on endurance performance and capacity (Jacobs et al. 2011). Hence, we provided further evidence that endurance capacity is dependent on at least three more factors than solely $\dot{V}O_2_{peak}$. First, our result on $T_{lim}$ at CP implicates that the blood buffer system plays an important role in maximal exercise up to 15 min. It is assumed that due to the higher alkaline concentration gradient between cytosol and blood an intramyocellular steady state can be maintained for a prolonged time. Therefore, the activity-induced decrease in intramyocellular pH with the associated exercise limiting mechanisms (Fitts 1994), e.g. inhibition of sarcoplasmatic Ca$^{2+}$-release (Donaldson et al. 1978) and increase in muscular K$^+$-release (Fabiato and Fabiato 1978), is protracted. A training- or nutrition-induced improvement in the blood buffer system will therefore lead to an enhanced endurance capacity. We assume that this alteration in the blood buffer system does not affect blood oxygen binding as well as oxygen extraction and hence, that it has no impact on $\dot{V}O_2_{peak}$. According to the Bohr effect, an induced alkalosis shifts the hemoglobin oxygen affinity curve to lower oxygen partial pressures. As the arterial oxygen saturation is almost 100% in healthy resting individuals, the induced alkalosis will not influence this variable. On the contrary, oxygen extraction is slightly hindered by an increased blood pH, presumably favoring oxygen extraction at the beginning of exercise in the control condition. During the cycling test, blood pH values will assimilate among the alkalosis and control condition, whereby the difference in hemoglobin oxygen affinity disappears before achieving $\dot{V}O_2_{peak}$. This assumption is supported by non-alterations in $\dot{V}O_2$.
kinetics between trials with bicarbonate supplementation and placebo (Santalla et al. 2003, Zoladz et al. 1997). In a wider context, muscle buffer capacity should therefore increase endurance capacity possibly to an even greater extent without alterations in $\dot{V}O_{2\text{peak}}$. This mechanism is supported by the data of Weston et al. (1997), showing a significant correlation between muscle buffer capacity and time trial performance as well as a close to significant correlation between training-induced improvement in muscle buffer capacity and the concomitant increase in time trial performance. Furthermore, muscle buffer capacity is increased after 2 weeks of altitude training without a concomitant improvement in $\dot{V}O_{2\text{peak}}$, resulting in enhanced short-term exercise performance (Mizuno et al. 1990).

Second, the increase in overall capillary-to-fiber ratio, which was the only significant predictor of CP improvement following vibroX, had no impact on $\dot{V}O_{2\text{peak}}$. This result supports the notion that capillarization plays other important roles than solely facilitate oxygen exchange between blood and intramuscular compartment. These roles of capillaries are removal of fatigue-related metabolites to maintain an intramyocellular metabolic steady state as well as dissipation of contraction-induced heat away from working muscles to the periphery (Saltin et al. 1986). The former role is strongly associated with the factor presented in the paragraph before, emphasizing the importance of maintaining an intramyocellular metabolic steady state for prolonging performance. Our finding is supported by the results of Coyle and colleagues (1991), who proposed that a higher capillary density accounts for the better time trial performance in cyclists with identical $\dot{V}O_{2\text{peak}}$.

Third, classical resistance training improved endurance capacity without affecting $\dot{V}O_{2\text{peak}}$. In addition to the improved neuronal function after resistance training (see above), enhanced movement economy (Sunde et al. 2010), increased tendon CSA (Rønnestad et al. 2012), and increased stiffness of the tendon-aponeurosis complex (Kubo et al. 2006b) might contribute to improved endurance capacity. High-intensity resistance training is thereby favored over explosive resistance training with lower loads (Rønnestad and Mujika 2013). Summarized, while $\dot{V}O_{2\text{peak}}$ is a main indicator for peak performance, competition performance (i.e. submaximal performance) relies on more than a single factor. Maintenance of an intramyocellular metabolic steady state is thereby a major contributor to prolonging exercise performance.

Our performance and biopsy data support the fact that the novel training stimuli were successful in enabling a comprehensive training effect or prevent decreases in anaerobic performance and
capacity following aerobic HIT. WBV prevented thereby MyHC-2 fiber atrophy and was part of the hypertrophic stimulus during vibroX. Hence, WBV attenuates atrophy signaling and might promote hypertrophic signaling. The attenuation of atrophy signaling is supported by a recent study showing that vibration downregulates myostatin and atrogin-1 gene expression in mice (Ceccarelli et al. 2012). Furthermore, fusion of cultured satellite cells was promoted by applied vibration in vitro (Ceccarelli et al. 2012). The latter point portend also to an induced muscle hypertrophy. A hypertrophic stimulus following WBV training was observed by an increase in myofiber CSA of the M. soleus in young males during 55 days of bed rest with on average less than 10 min of WBV per day as only exercise stimulus (Blottner et al. 2006). In addition, myotube formation is promoted and mRNA expression of the myogenic regulatory factors, myogenin and MyoD, are increased after vibration in myoblasts (Wang et al. 2010) pointing to differentiation of satellite cells.

The hypertrophic response of blood flow restricted exercise is well described. It is known that low-intensity blood flow restricted exercise leads to similar muscular adaptations as high-intensity resistance exercise (Takarada et al. 2000). The gain in muscle mass following blood flow restricted exercise is attributed to an increase in muscle protein synthesis (Fujita et al. 2007) and a decrease in muscle protein breakdown (Manini et al. 2011). The underlying molecular mechanisms induced by low-intensity resistance exercise with blood flow restriction lend further credence for the increase in protein synthesis and prevention of protein breakdown. Low-intensity blood flow restricted exercise increases phosphorylation of p70S6K (Fujita et al. 2007, Wernbom et al. 2013) and mitogen activated protein kinase (MAPK, Wernbom et al. 2013). In contrast, mRNA expression of FOXO3A, atrogin-1, and MuRF-1 are decreased after blood flow restricted exercise (Manini et al. 2011). Furthermore, it was shown that low-load resistance exercise with blood flow restriction results in proliferation of myogenic stem cells and in a concomitant addition of myonuclei in skeletal muscle (Nielsen et al. 2012).

The responsible stimuli for these molecular mechanisms following low-intensity blood flow restricted exercise and the resulting muscle mass gains are largely unknown. Based on previous investigations, three mechanisms were proposed as responsible stimuli. First, it was hypothesized that muscle cell swelling as response to blood flow restricted exercise (Loenneke et al. 2012) is sensed by an intrinsic volume sensor, which might activate mTOR and MAPK pathways. This cell swelling hypothesis is supported by prevention of muscle atrophy with blood flow restriction without exercise during prolonged limb suspension (Clark et al. 2006). Likewise, due to an increased sarcolemmal permeability as response to blood flow restriction
(Wernbom et al. 2012a), macromolecule, metabolite and ion fluxes might be increased for several hours after blood flow restricted exercise and hence, muscle cell swelling might be prolonged (Wernbom et al. 2012b). Second, stimulation of chemosensitive class III and IV afferents from the active musculature during low-intensity blood flow restricted exercise might lead to an increase in serum growth hormone. This growth hormone response is even larger than during higher intensity (70% 1RM) resistance exercise (Reeves et al. 2006). It is supposed that during blood flow restricted exercise growth hormone exerts its function on facilitation of cell fusion independent of insulin-like growth factor-1 (IGF-1), because this type of exercise does not alter serum IGF-1 levels (Fujita et al. 2007). Third, blood flow restriction leads to a precipitate fatigue of MyHC-1 fibers and therefore to an earlier recruitment of MyHC-2 fibers. In this context, electromyography activity during blood flow restricted exercise with a tourniquet cuff pressure of only 100 mmHg and a load of 40% 1RM was almost identical compared to unrestricted exercise with 80% 1RM (Takarada et al. 2000). Therefore, mechanical signaling comparable to the signaling during high-intensity resistance exercise might be the third responsible stimulus.

The endurance type adaptations following low-intensity resistance training with vascular occlusion are assumed to rely mainly on an increased capillarization. This assumption is supported by promoted microvascular filtration capacity (Evans et al. 2010) and improved muscle oxygen delivery (Kacin and Strazar 2011) following low-intensity resistance training with blood flow restriction. Furthermore, this exercise modality is associated with an increase in VEGF mRNA expression (Larkin et al. 2012) and VEGF blood serum concentration (Takano et al. 2005). VEGF expression following vascular occlusion might be attributed to hypoxia, as a concomitant increase in the upstream transcription factor hypoxia-inducible factor (HIF)-1α was reported (Larkin et al. 2012). In contrast to this low-intensity blood flow restricted exercise, HIF-1α mRNA abundance was not increased following vibroX (Item et al. 2013). Hence, we proposed that reactive oxygen species might induce VEGF expression via activation of PGC-1α in a HIF-1α independent manner (Chapter 3). In addition to hypoxia and reactive oxygen species, elevated shear stress during re-perfusion after releasing the tourniquet cuff pressure might be responsible for the increased capillarization after blood flow restricted exercise. It is thereby well known that shear stress is an important mechanical signal in angiogenesis (Egginton et al. 2001).

It must be pointed out that all afore-mentioned results on low-intensity blood flow restricted exercise were achieved with releasing tourniquet cuff pressure right at the end of exercise. Our
application of blood flow restriction with sustaining it for additional 3 min after exercise cessation is a unique characteristic of vibroX. We might speculate that this application led to a further increase in cell swelling. Especially, the release of the occlusion pressure from 200 to 100 mmHg during the 1 min recovery period might have allowed additional blow flow to the legs because the occlusion pressure might have fallen below blood pressure of the *A. femoralis* (Pascarelli and Bertrand 1964). In contrast, venous outflow was improbable as the tourniquet cuff pressure was still considerably higher than blood pressure in the *V. femoralis*.

By replacing the active rest intervals during HIT with WBV, decreases in anaerobic performance and capacity were prevented. However, it was not possible to concurrently increase aerobic and anaerobic variables with the exclusive addition of WBV to HIT. It is proposed that performance increases with exclusive WBV are attained mainly in untrained or less trained participants, while this stimulus is less efficient in well-trained athletes (Manimmanakorn et al. 2013). Therefore, an alternative to simultaneously increase these variables also in well-trained athletes might represent the use of additional weight (Berschin et al. 2003). However, the practicability of resistive WBV during the rest intervals of a HIT is questionable, because it was already challenging for our participants to maintain a static squat position without additional weight. Therefore, a comprehensive training effect could not be attained with the sole combination of two novel training stimuli. This points to the importance of superimposing training stimuli to achieve optimal training adaptations, as proved by vibroX. Furthermore, the vibroX-induced adaptations were quantitatively and qualitatively higher compared to the sum of adaptations following training with the individual stimuli. In a further step, it would remain to investigate that actual the superimposition of stimuli is necessary to attain a comprehensive training effect and that the discrete individual stimuli does not lead to similar effects. However, vibroX is build up of four individual stimuli. Therefore, twenty-four different possibilities exist to combine these stimuli. Unfortunately, in human studies, the breakdown of vibroX in its single components is therefor not feasible.

In conclusion, increases in CP were achieved only when resistance training was superimposed with vascular occlusion and WBV as well as with HIT with or without WBV during rest intervals. Furthermore, $T_{lim}$ at CP was prolonged with daily acute NaHCO$_3$ supplementation on five consecutive days. $W'$ was increased following resistance training and the decrease in $W'$ after aerobic HIT was prevented by the addition of WBV during rest intervals. Consequently, athletes engaged in team or endurance sports, in which anaerobic performance and capacity play a critical role, aiming at increasing CP are advised to incorporate vibroX or HIT with WBV.
during rest intervals into their training routine. During multiday competitions or tournaments with intensities at the threshold between the heavy and severe intensity domains, it is recommended to ingest daily a single acute dose of NaHCO$_3$ before competition.
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