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NEW INSIGHTS INTO RESPIRATORY MUSCLE TESTING AND TRAINING ADAPTATIONS

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presented by

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1. SUMMARY

The recognition that fatiguing respiratory muscles can impair whole-body exercise performance has received growing attention in exercise physiology in the past decades. This interaction between respiratory muscles and whole-body exercise is currently believed to be explained by the so-called respiratory muscle metaboreflex, resulting in a reduction of blood flow towards locomotor muscles induced by fatiguing respiratory muscles. Respiratory muscles, similar to other skeletal muscles, can be trained to increase their resistance to fatigue and to be less limiting to whole-body exercise performance. However, the combined action of respiratory muscles is a result of a complex interaction of the different inspiratory and expiratory muscles, making the development of respiratory muscle training (RMT) and testing regimens a challenging matter. Therefore, it is not surprising that no established respiratory muscle performance test exists which challenges the respiratory system in its functional integrity and that only little is known about the detailed physiological mechanisms involved in the different training adaptations of different RMT regimens.

In light of the current knowledge, the study described in Chapter 4 aimed to develop a functional incremental respiratory muscle test (IncRMT) involving inspiratory and expiratory muscles that tests respiratory muscle performance and possibly predicts maximal oxygen consumption ($\dot{V}O_{2peak}$) achieved during cycling. With the combination of forced vital capacity and total work of breathing during the IncRMT, whole-body $\dot{V}O_{2peak}$ could be predicted with R^2=0.79, showing not only a connection between respiratory muscles and whole-body fitness but also making the IncRMT a potential tool to assess the risk for peri-operative complications in immobile patients instead of the usual incremental cycling test. As with whole-body exercise training, different RMT regimens exist, the most established RMT regimens being inspiratory strength training (RMST) and respiratory muscle endurance training (RMET). Recently, a new time-saving RMT regimen combining strength and endurance aspects was developed, termed respiratory muscle sprint-interval training (RMSIT) but currently the training effects of RMSIT are unknown. Thus, the effects of one month of RMSIT and RMET on training improvements and IncRMT performance were investigated in Chapter 5. Work of breathing during training sessions

and IncRMT performance increased to the same extent in both training groups despite a lower training volume with RMSIT.

Not only are changes in respiratory muscle performance with RMT of great interest but also how RMT translates into in whole-body exercise performance. For this purpose, the effects of one month RMSIT and RMET on whole-body exercise performance and potential mechanisms underlying the interaction between respiratory muscles, leg muscles and whole-body exercise performance were analysed in the study described in Chapter 6. RMSIT resulted in a lower level of leg muscle fatigue in males after a work-matched constant load cycling test (CLT), a potential indication of a reduced respiratory muscle metaboreflex. RMET, on the other hand, improved 12-km time trial performance, also in males only. These results indicate a sex-specific training response with females profiting less from RMT than males, possibly due to naturally less fatiguing and thus exercise-limiting respiratory muscles in females. The reason why RMSIT affected activation of the metaboreflex more than RMET, and RMET, but not RMSIT, improved exercise performance at a level where the metaboreflex is supposedly not activated anymore, remains to be further investigated.

Ideally, during the above studies, $\dot{V}O_2$ could have been assessed during the IncRMT to shed more light on the mechanism of respiratory muscle improvement. However, the measurement of $\dot{V}O_2$ during hyperpnoea with partial rebreathing and added resistance, as performed during IncRMT, RMSIT and RMET, is not possible with the current commercially available techniques due to the rebreathing system resulting in fast changing inspiratory air conditions, i.e. gas composition and pressure. Hence, an important physiological parameter for RMT evaluation was unavailable. Chapter 7 therefore describes the development of a test setup to calculate $\dot{V}O_2$ during normocapnic hyperpnoea which was validated with the Douglas bag method, the gold standard for $\dot{V}O_2$ measurements. This setup enabled the calculation of $\dot{V}O_2$ during IncRMT, RMSIT and RMET, broadening the physiological understanding of respiratory muscle physiology.

Bringing results from common subjects of Chapter 5 and 6 together and pooling RMSIT and RMET for analysis of RMT effects, a significant correlation between changes in respiratory muscle oxygen consumption, assessed by the newly developed tool described in Chapter 7, and changes in leg muscle fatigue in the work-matched CLT was found. This relationship further indicates the presence of the

respiratory muscle metaboreflex and presents a mechanistic explanation for changes in whole-body exercise performance.

All in all, this work provides new insights into respiratory muscle testing, improvements in respiratory muscle performance after two different training regimens, RMSIT and RMET, and it sheds more light on physiological mechanisms linking respiratory muscle performance and whole-body exercise performance.

2. ZUSAMMENFASSUNG

Durch die Erkenntnis, dass ermüdende Atmungsmuskulatur die sportliche Leistungsfähigkeit beeinträchtigt, wurde in den letzten Jahrzehnten der Physiologie der Atmungsmuskulatur immer mehr Aufmerksamkeit geschenkt. Der Zusammenhang von Atmungsmuskulatur und sportlicher Leistungsfähigkeit wird anhand des respiratorischen Metaboreflex erklärt, welcher bei intensiver körperlicher Belastung eine Reduktion des Blutflusses in die Extremitäten aufgrund einer Ermüdung der Atmungsmuskulatur voraussagt. Die Atmungsmuskulatur kann, wie die übrige Skelettmuskulatur, durch spezifisches Training ermüdungsresistenter werden, was wiederum die sportliche Leistungsfähigkeit steigert. Der Atmungsvorgang besteht aus einer komplexen Interaktion von mehreren Ein- und Ausatmungsmuskeln, was die Entwicklung spezifischer Atmungstrainings-Regimes und insbesondere das Testen dieser Muskulatur erschwert. Aufgrund dieser Komplexität ist es nicht verwunderlich, dass kein etablierter Leistungstest der Atmungsmuskulatur existiert und nur wenig über physiologischen Trainingsanpassungen der Atmungsmuskulatur bekannt ist.

Aufgrund dessen fokussiert die Studie im Kapitel 4 auf die Entwicklung eines funktionellen respiratorischen Stufentests (IncRMT), analog dem etablierten Fahrradstufentest, mit dem Ziel die Leistungsfähigkeit der Atmungsmuskulatur zu testen und, wenn möglich, die maximale Sauerstoffaufnahme ($\dot{V}O_{2peak}$) des ganzen Körpers beim Fahrradfahren vorauszusagen. Anhand der forcierten Vitalkapazität, in Kombination mit der geleisteten Atmungsarbeit während des IncRMT, konnte in der Tat eine gute Voraussage des $\dot{V}O_{2peak}$ erreicht werden (Korrelation R^2=0.79). Dies zeigt nicht nur die bereits erwähnte Abhängigkeit der Leistungsfähigkeit vom Zustand der Atmungsmuskulatur, sondern macht den IncRMT ein potentielles Mittel, um zum Beispiel peri-operative Komplikationen in immobilen Patienten vorherzusagen, anstelle des üblichen $\dot{V}O_{2peak}$ -Tests auf dem Fahrrad.

Analog zu Ganzkörpertrainings existieren auch verschiedene Atmungsmuskeltrainingsregimes, wobei Atmungskrafttraining (RMST) und Atmungsausdauertraining (RMET) am meisten etabliert sind. Vor kurzem wurde ein neues, zeitsparendes Atmungsmuskel-Sprint-Intervalltraining (RMSIT) entwickelt, welches Kraft und Ausdauer kombiniert. Die Trainingseffekte von RMSIT sind jedoch noch unbekannt.

Daher wurden in der Studie des Kapitels 5 anhand des IncRMT mögliche Effekte von einem Monat RMSIT und RMET auf die Atmungsleistungsfähigkeit untersucht. Die Atmungsleistungsfähigkeit während den Trainings-Einheiten und während des IncRMT verbesserte sich mit beiden Trainingsregimes im gleichen Masse, wobei das Trainingsvolumen mit RMSIT signifikant reduziert war.

Nicht nur Veränderungen der Atmungsleistungsfähigkeit nach dem Training sind von Bedeutung, sondern auch wie sich diese Verbesserung auf die Ganzkörperleistungsfähigkeit auswirkt. Daher wurden in der im Kapitel 6 beschriebenen Studie die Effekte eines Monates RMSIT und RMET auf die Ganzkörperleistungsfähigkeit und auf mögliche mechanistische Zusammenhänge zwischen Atmungs- und Ganzkörperleistungsfähigkeit analysiert. RMSIT führte zu einer reduzierten Beinermüdung, insbesondere bei Männern, nach gleicher Arbeitsleistung in einem intensiven constant-load Fahrradtest (CLT). Dies ist ein Hinweis auf einen reduzierten respiratorischen Metaboreflex. RMET hingegen, nicht aber RMSIT, verbesserte bei Männern die Leistung in einem 12-km Zeitfahrtest bei einer Intensität, bei welcher der Metaboreflex nicht aktiv sein soll. Frauen verbesserten sich mit keinem der Trainings bei keinem der Leistungstests. Diese Ergebnisse weisen auf eine geschlechtsspezifische Atmungstrainingsantwort hin, wobei Frauen weniger vom Atmungstraining profitieren als Männer. Möglicherweise ist die Atmungsmuskulatur bereits vor dem Atmungstraining weniger leistungslimitierend bei Frauen, aufgrund einer ermüdungsresistenteren Atmungsmuskulatur. Die Ursache, weshalb Männer der RMSIT Gruppe weniger Beinermüdung zeigten, jedoch im Zeitfahren die Leistung nicht verbesserten und sich die Männer der RMET Gruppe nur im Zeitfahren verbesserten, bleibt unklar.

Idealerweise hätte in den oben erwähnten Studien $\dot{V}O_2$ während des IncRMT gemessen werden können, um mehr Klarheit über die Mechanismen der Veränderungen aufgrund des Atmungstrainings zu erhalten. Allerdings ist die Messung von $\dot{V}O_2$ während Hyperpnoe, welche beim IncRMT, bei RMSIT und RMET mit partieller Rückatmung und z.T. mit zusätzlichem Widerstand erfolgt, mit den verfügbaren Ergospirometriegeräten nicht möglich gewesen, wodurch ein aussagekräftiger physiologischer Parameter nicht erfasst werden kann. Kapitel 7 fokussiert daher auf die Entwicklung eines Messaufbaus, um $\dot{V}O_2$ während normokapnischer Hyperpnoe zu bestimmen und mit dem

Goldstandard, der Douglas Bag – Methode, zu validieren. Dies erweitert die Möglichkeiten, die Physiologie der Atmungsmuskulatur weiter zu untersuchen.

Wurden die Daten von Personen, welche in beiden Studien (Kapitel 5 und 6) teilnahmen, zusammengebracht und die Daten von RMSIT und RMET gepoolt analysiert, zeigte sich eine signifikante Korrelation zwischen Veränderungen des Sauerstoffverbrauchs der Atmungsmuskulatur, gemessen mit dem Messaufbau getestet im Kapitel 7, und der Beinermüdung nach dem CLT. Dieser Zusammenhang ist ein weiterer Hinweis darauf, dass eine Veränderung des respiratorischen Metaboreflexes eine mechanistische Erklärung für die Verbesserung intensiver Leistungen nach Atmungstraining sind.

Diese Arbeit schafft neues Wissen über die Möglichkeit die Atmungsmuskulatur zu testen, über Verbesserung der Leistungsfähigkeit der Atmungsmuskulatur nach zwei unterschiedlichen Trainingsregimes, RMSIT und RMET, und untersucht mögliche Mechanismen, welche den Zusammenhang zwischen der Leistungsfähigkeit der Atmungsmuskulatur und der sportlichen Leistungsfähigkeit erklärt.

3. GENERAL INTRODUCTION

Respiratory muscle mechanics and metaboreflex

The main functions of the respiratory system are to deliver oxygen to, and clear carbon dioxide from, the body through diffusion between lungs and cardiovascular system. To achieve this purpose, a complex interaction between respiratory muscles and rib cage produces negative pressures in the thoracic cavity (inspiration) to suck oxygen rich ambient air into the lungs and positive pressures (expiration) to push carbon dioxide rich air out. At rest, expiration is a passive process which results from the elastic recoil of the lungs and the chest wall. Inspiration on the other hand is achieved by active contractions of the diaphragm (M. diaphragmaticus), pulling the lungs towards the stomach, as well as the external intercostals (Mm. intercostales externi) lifting the rib cage, both acting together to increase lung volume and therefore create negative pressures. If ventilatory demand is increased, expiration becomes an active process involving progressive activation of the abdominal muscles (M. rectus abdominis), pushing the diaphragm back up, and the internal intercostals (Mm. intercostales interni) lowering the rib cage. Furthermore, to make inspiration faster during high ventilatory demands, the sternocleidomastoids (Mm. sternocleidomastoidei) and scalene muscles (Mm. scaleni) are additionally recruited.

Muscle fibre type distribution in the adult human diaphragm was reported to be about 55% slow twitch fibres, 21% fast twitch oxidative, and 24% fast twitch glycolytic (Polla *et al.*, 2004) . To supply the large amount of oxidative fibres more efficiently, the diffusion distance between capillary vessels and muscle fibres is smaller in the diaphragm compared to other skeletal muscles (Polla *et al.*, 2004). This distinct physiological structure contributes to an increased fatigue resistance of the diaphragm to ensure an adequate gas exchange even after exhaustive exercise. It was long thought that the respiratory system was overbuilt for high ventilatory demands because in healthy individuals, task failure of the diaphragm would result in inevitable death at exhaustion (Dempsey *et al.*, 2003). However, it was shown that during high intensive whole-body exercise respiratory muscles, the diaphragm and accessory muscles, develop fatigue which may in fact limit exercise performance (Johnson *et al.*, 1993; Mador *et al.*, 1993; Taylor *et al.*, 2006). Currently the most accepted model linking respiratory muscle performance to

whole-body exercise performance is the so-called respiratory muscle metaboreflex (Figure 3.1), which suggests, supported by recent evidence, that during intense exercise, a competition for the finite available blood flow takes place between the respiratory muscles and the exercising limbs, causing respiratory muscles to fatigue which then, via afferent feedback from fatiguing inspiratory and expiratory muscles (Harms *et al.*, 2000; Sheel *et al.*, 2001; Romer *et al.*, 2006; Wüthrich *et al.*, 2013), leads to an increased sympathetic outflow to locomotor muscles, resulting in vasoconstriction and ultimately to a reduced oxygen supply and metabolite removal, exacerbating contractile fatigue in the exercising limbs.

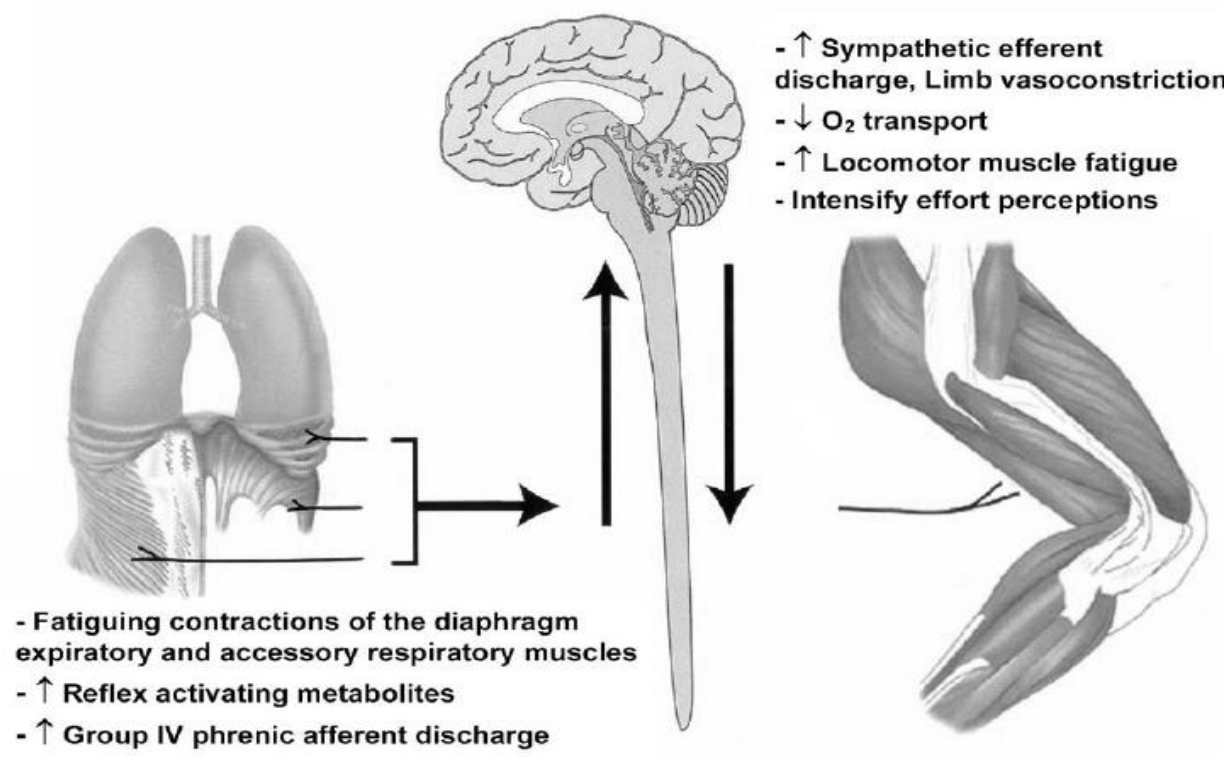

Figure 3.1: Schematic illustration of the proposed respiratory muscle metaboreflex and its effects (Dempsey *et al.*, 2006).

Specific training of respiratory muscles (RMT) was shown to improve physical exercise performance (Gosselink *et al.*, 2011; Illi *et al.*, 2012; Eichenberger *et al.*, 2013). One of the current hypotheses brought forward to explain this improvement is an improved fatigue resistance of respiratory muscles (Verges *et al.*, 2007) which then reduces or delays the vasoconstrictory effects induced by the respiratory metaboreflex. However, RMT-induced changes in the development of the respiratory

muscle metaboreflex have never been directly measured. Furthermore, respiratory muscle fatigue is believed to occur only with a high ventilatory and metabolic demand such as during intense exercise above a threshold of 85% of maximal oxygen consumption ($\dot{V}O_{2max}$; Johnson *et al.*, 1993). However, Illi *et al.* (2012) found a relationship of greater improvements in exercise performance with lower exercise intensity which included tests at levels even below the suggested 85% $\dot{V}O_{2max}$ threshold. Different mechanisms such as, for example, a reduction of the perception of respiratory exertion, even at similar work of breathing (WOB), after RMT may also play an important role in improvements of whole-body exercise performance (Verges *et al.*, 2008).

On the basis of the above, the current work investigated training effects of RMT on leg and respiratory muscle blood oxygenation and development of muscle fatigue (Chapter 6). To elucidate the controversy of mechanisms involved in improving exercise after RMT, i.e. reduced metaboreflex vs. improvement at lower exercise intensity, effects at high and lower exercise intensity were assessed, i.e. changes in fatigue development at high intensity exercise ($\dot{V}O_{2peak}$ > 85%) and changes in performance at lower intensity exercise ($\dot{V}O_{2peak}$ < 85%).

Respiratory muscle performance testing

Respiratory muscle performance is not only important for whole-body exercise performance in healthy individuals but is also important to improve for example the quality of life in different patient populations such as in patients with chronic obstructive pulmonary disease (Gosselink *et al.*, 2011), chronic heart failure (Smart *et al.*, 2013) or spinal cord injury (Sheel *et al.*, 2008). Even though respiratory muscle performance is an important parameter in clinical settings, no standardized testing protocol to assess the function of the respiratory muscles in a comprehensive manner exists today (Sales *et al.*, 2016).

Respiratory muscle performance is commonly assessed by lung function testing and measurements of maximal static inspiratory and expiratory mouth pressures. These measurements are performed in a single, inspiratory or expiratory effort or for a duration of up to 15 s when maximal voluntary ventilation is tested. Thus, these tests do not provide a functional index of respiratory muscle performance. Although different respiratory muscle performance tests have been proposed – such as maximal

sustained ventilator capacity (Leith & Bradley, 1976), constant-load hyperpnoea to exhaustion (Boutellier & Piwko, 1992), sustained inspiratory threshold loading or resistive breathing to exhaustion (Morrison *et al.*, 1989; Hart *et al.*, 2002), or incremental resistive loading (Hlavac *et al.*, 2007) – currently no test combines inspiratory and expiratory muscle endurance (hyperpnoea) and strength (resistive loading) assessment, based on the lack of available devices to allow hyperpnoea with added load in normocapnic conditions. On these grounds, an appropriate respiratory muscle performance test is of great importance to evaluate possible limitations but also training adaptations of respiratory muscles. An ideal test would place a concomitant inspiratory and expiratory load on the entire respiratory system, thus testing respiratory muscle performance in its integrity and address less and more loaded situations.

For this purpose, a novel respiratory muscle rebreathing training and testing device was developed enabling an accurate adjustment of inspiratory and expiratory resistances with dynamic hyperpnoea under normocapnic conditions. This custom-made device allowed the development of a novel incremental respiratory muscle test (IncRMT) combining high inspiratory and expiratory flows and pressures without 'side effects' for the participants such as dizziness during volitional hyperpnoea (due to hyperventilation) or headaches during breathing with high resistance (due to hypoventilation). In short, the IncRMT protocol consists of constant step-wise increases in WOB which are achieved by increasing ventilation (at constant tidal volume with increasing breathing frequency, based on visual and auditory feedback of these variables) and adjustment of breathing resistance accordingly. While feasibility and reproducibility of the IncRMT was tested in several pilot studies, its sensitivity to detect RMT-induced changes in respiratory muscle performance was investigated in the present work (Chapter 5).

Respiratory muscle performance to predict peri-operative complications

Evidence is growing that $\dot{V}O_{2peak,}$ measured during cardiopulmonary exercise testing, is a strong predictor of mortality in healthy individuals (Myers *et al.*, 2002) and correlates with the risk of developing peri-operative complications (Beckles *et al.*, 2003; Smith *et al.*, 2009; Licker *et al.*, 2011). However, cardiopulmonary exercise tests cannot be performed by bedridden patients, denying them

access to a useful tool for risk assessment. Moreover, it has been shown that respiratory muscle function itself affects post-surgical outcomes and may negatively effect on morbidity and mortality (Siafakas *et al.*, 1999). Thus, assessing respiratory muscle performance before operations may be as important the assessment of $\dot{V}O_{2peak}$ and may become a valuable tool for patients who cannot perform whole-body exercise. With the novel IncRMT, a greater demand is imposed on the cardiopulmonary system than with conventional lung function and respiratory muscle strength testing, hence it is possible that the IncRMT may be an indicator of cardiopulmonary fitness. As a first step in this direction, the potential of the newly developed IncRMT to predict whole-body $\dot{V}O_{2peak}$ was analysed in a group of healthy subjects to gain further insights into the its potential to predict peri-operative complications (Chapter 4).

Respiratory muscle training

Analogue to whole-body exercise training, different respiratory muscle training regimens exist to date (Illi *et al.*, 2012): respiratory muscle endurance training (RMET) is characterised by low force production with high ventilation performed as normocapnic hyperpnoea, and respiratory muscle strength training (RMST) is performed with high force and low velocity contraction against an external resistance. While in RMST inspiratory and expiratory muscles can be trained separately or in combination, RMET trains both simultaneously. RMST, similar to whole-body strength training, improves maximal inspiratory and expiratory muscle strength (Chiara *et al.*, 2006; Griffiths & McConnell, 2007), while RMET improves respiratory muscle endurance (Verges *et al.*, 2008). Recently, a new RMT regimen was developed in our laboratory, combining both high flows and high pressures, termed respiratory muscle sprint-interval training (RMSIT). RMSIT is characterized by brief periods of maximal respiratory sprint efforts, interspaced by periods with resting ventilation (Wüthrich *et al.*, 2015). It was shown that a single session of RMSIT can induce similar levels of inspiratory and expiratory muscle fatigue as those seen after 30 min of classical RMET. This suggests that the acute load placed upon respiratory muscles during RMSIT might be large enough to trigger muscular adaptations when chronically applied, i.e. performed over several weeks, while training volume can be reduced drastically. To test whether these adaptations indeed occur and translate into improvements of

respiratory muscle and/or whole-body exercise performance as shown for RMET, the studies presented in Chapter 5 and Chapter 6 were performed.

Respiratory muscle fatigue resistance - sex differences

Over the past two decades, awareness of the importance to distinguish between sexes in health-related research has increased considerably. More specifically, in exercise physiology recent findings have suggested more fatigue resistance in skeletal muscles in females compared to males. Different mechanism for this sex difference have been proposed and are summarized in Figure 3.2.

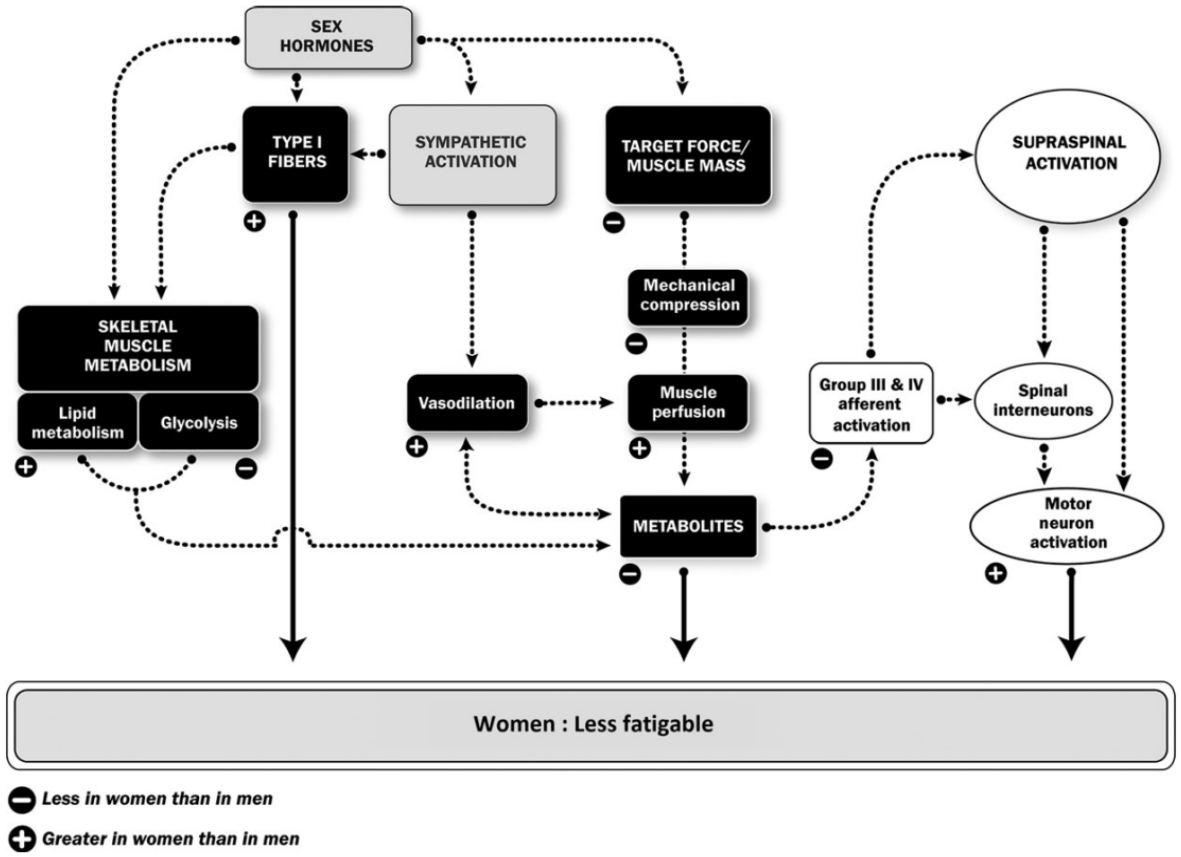

Figure 3.2: Potential physiological mechanisms for the sex difference in muscle fatigability. Black boxes indicate processes within the muscle, white boxes are processes in the nervous system, and the grey are hormonal and sympathetic actions (Hunter, 2014).

Potential sex differences in the development of fatigue are suggested to result from differences in motor neurone activation, contractile function of the activated fibres and the magnitude of metabolites accumulating that interfere with contractile function. For respiratory muscles, first studies suggest that

during high intensity cycling, inspiratory muscles of females are more fatigue resistant than those of males (Guenette et al., 2010), despite increased respiratory mechanical constraints resulting from smaller airways relative to total lung size (Thurlbeck, 1982). The precise mechanism behind more fatigue-resistant respiratory muscles in females may be similar to the one proposed by Hunter (2014) but needs further investigation. If more fatigue resistant respiratory muscles were to attenuate the respiratory muscle metaboreflex, RMT-induced adaptations of whole-body exercise performance would be expected to be smaller in females. Further insights in sex differences of RMT-induced adaptations are crucial for optimal training recommendations in the future. Therefore, potential sex differences in RMT-induced respiratory muscle fatigability, as well as respiratory and whole-body exercise performance in response to different RMT regimens are therefore addressed in Chapter 5 and Chapter 6.

Respiratory muscle oxygen consumption calculation

The measurement of $\dot{V}O_2$ is a well-established tool in exercise physiology to assess aerobic capacity and energy expenditure, the aerobic capacity of individuals, and, as described earlier, even to assess the risk for peri-operative complications (Myers et al., 2002; Smith et al., 2009; Licker et al., 2011). During exercise, $\dot{V}O_2$ is normally assessed with commercially available metabolic carts that measure and calculate the difference between inspired and expired volumes of O_2. Up to date, however, $\dot{V}O_2$ during hyperpnoea using partial breathing and added resistance, has never been assessed due to fast changing conditions of the inspiratory air (gas composition, temperature and humidity) and pressure with the rebreathing devices with added resistance. However, for characterization and comparison of different RMT-training regimens, e.g. RMSIT and RMET, and training-induced adaptations or for the evaluation of the respiratory muscle performance, e.g. in the IncRMT, knowing $\dot{V}O_2$ during normocapnic hyperpnoea would be of great interest. To circumvent the limitations of the commercially available metabolic carts, a custom-made setup was tested in a pilot study to evaluate the potential to measure $\dot{V}O_2$ during normocapnic hyperpnoea with added resistance (Chapter 7).

Given the current gaps in knowledge regarding the different aspects of respiratory muscle function testing and training, main objectives of the present work were:

- To evaluate the potential of the novel IncRMT to predict whole-body $\dot{V}O_{2peak}$ and thus to be a potential tool to assess the risk for peri-operative complications in immobile patients.
- To investigate adaptations in respiratory muscle performance after a period of RMSIT and RMET, measured with the novel IncRMT.
- To assess the effect of a period of RMSIT and RMET on changes related to a modified activity of the respiratory metaboreflex and potential sex-differences thereof.
- To investigate the potential of RMSIT and RMET to improve whole-body exercise performance at a lower intensity where respiratory muscle fatigue is believed to be absent (i.e. metaboreflex not activated) and potential sex-differences thereof.
- To validate a $\dot{V}O_2$ test setup during hyperpnoea and partial rebreathing conditions with added resistance.

4. NOVEL INCREMENTAL RESPIRATORY MUSCLE TEST TO PREDICT WHOLE-BODY $\dot{V}O_{2peak}$

Submitted for publication

ABSTRACT

Peak oxygen consumption ($\dot{V}O_{2peak}$) assessed during incremental cardiopulmonary exercise testing (CPET) is used for risk assessment of perioperative complications. However, for certain patients, whole-body exercise is not possible. Although different strategies for $\dot{V}O_{2peak}$ prediction without exercise were suggested, we hypothesized that adding a functional measurement, such as respiratory muscle performance, could further improve the prediction.

To evaluate the predictive potential of a novel incremental respiratory muscle test (IncRMT), lung function testing, CPET and IncRMT performance were assessed in 36 (18m/18f) healthy participants (age: 26±5 years, $\dot{V}O_{2peak}$: 48±10 $mL \cdot min^{-1} \cdot kg^{-1}$).

A backwards step-wise regression model was performed, including data from anthropometric, lung function and IncRMT performance. The combination of total work of breathing (WOB_{tot}) during the IncRMT and forced vital capacity (FVC) resulted in the best prediction:

$\dot{V}O_{2peak,pred}$ $[mL \cdot min^{-1}]$=105.9·WOB [kJ] + 613.5·FVC [L] - 442.1; R^2=0.79; p<0.001; SEE=436.9 $mL \cdot min^{-1}$. Within subject analysis of residuals between measured and predicted $\dot{V}O_{2peak}$ revealed a 24% reduction in the 95% limits of agreement from the multi-variable model compared to the best single-variable model (FVC).

Thus, implementation of a preoperative IncRMT might be a step forward in perioperative risk profiling.

INTRODUCTION

Maximal aerobic capacity, defined as maximal oxygen consumption and frequently assessed as peak oxygen consumption ($\dot{V}O_{2peak}$) measured during cardiopulmonary exercise testing, is currently the gold standard for the assessment of cardiorespiratory fitness (ATS/ACCP, 2003). In fact, $\dot{V}O_{2peak}$ not only describes cardiorespiratory fitness, it is also a strong predictor of mortality in men (Myers *et al.*, 2002) and correlates with the risk of developing perioperative cardio-pulmonary complications (Beckles *et al.*, 2003; Smith *et al.*, 2009; Licker *et al.*, 2011)

While incremental exercise tests are required to determine $\dot{V}O_{2peak}$, intense whole-body exercise may not be feasible for patients who also suffer from muscular or joint problems of different aetiologies, meaning that a large group of patients likely lacks access to a useful tool to assess their potential risk for perioperative complications. Attempts have been made to predict $\dot{V}O_{2peak}$ based on anthropometric data such as weight, height, sex and vital capacity (VC; Jones *et al.*, 1985) or the ratio between resting heart rate (HR_{rest}) and predicted maximal heart rate (HR_{max}; Uth *et al.*, 2004) thus circumventing the need for exercise. Thus, to improve $\dot{V}O_{2peak}$ prediction, it could be hypothesized that adding a functional measurement not requiring the subjects to exercise with upper or lower limbs might improve the accuracy of $\dot{V}O_{2peak}$ prediction. One such possibility is the assessment of respiratory muscle performance: while on the one hand it is known that the level of respiratory muscle performance is related to the level of physical fitness (Martin & Stager, 1981; Eastwood *et al.*, 2001), on the other hand it has also been shown that reduced respiratory muscle function may increase perioperative morbidity and mortality to a considerable extent (Siafakas *et al.*, 1999). Thus, assessing respiratory muscle performance before surgery may be as important as the assessment of $\dot{V}O_{2peak}$.

Respiratory muscle performance can be assessed in different ways, such as continuous voluntary hyperpnoea or incremental inspiratory loading, as summarized by ATS/ERS (2002). All of these tests, however, focus on a single aspect of the respiratory system, i.e. unloaded, intense hyperpnoea or increased resistive inspiratory load. To our knowledge, no respiratory muscle test exists that increases inspiratory and expiratory muscle load by increasing ventilation as well as resistance, testing a combination of endurance and strength. For this purpose, an incremental respiratory muscle test

(IncRMT) was recently developed in our laboratory consisting of step-wise increases in work of breathing (WOB), achieved by increasing ventilation as well as respiratory resistance.

As a first step towards the goal of verifying the prognostic value of the IncRMT in patients, the present study aimed to investigate whether IncRMT performance combined with anthropometric and non-exercise-related individual characteristics could be used to improve current $\dot{V}O_{2peak}$ predictions in healthy subjects. As respiratory muscle function develops in parallel to the capacity of the cardiovascular system (Coast *et al.*, 1990), we hypothesized a close relationship between respiratory muscle performance and whole-body $\dot{V}O_{2peak}$.

METHODS

Subjects

Thirty-six healthy subjects (18m/18f) of different fitness levels, with normal lung function and normal respiratory muscle strength gave written informed consent to participate in this study. Participants' characteristics are presented in Table 4.1. Subjects were free of any acute or chronic disease, did not take any medication (except contraception), were non-smokers and had normal body weight (body mass index 18.5 – 24.9 $kg \cdot m^{-2}$). Subjects refrained from strenuous physical activity for 48 h prior to each of the three visits, slept for at least 7 h two nights before tests and abstained from caffeinated food and drinks on testing days. The study was approved by the local ethics committee and was performed according to the Declaration of Helsinki 2008.

Table 4.1: Subjects characteristics

Age [years]	26.1 ± 5.3
Height [cm]	172.1 ± 8.2
Weight [kg]	66.0 ± 9.8
$\dot{V}O_{2peak}$ [$mL \cdot min^{-1} \cdot kg^{-1}$]	48.9 ± 10.1
FVC [L]	5.1 ± 1.0
FVC [%pred]	107.1 ± 13.5
FEV_1 [L]	4.1 ± 0.8
FEV_1 [%pred]	104.1 ± 12.5
MVV_{12} [$L \cdot min^{-1}$]	169.9 ± 38.8
MIP [cmH_2O]	130.5 ± 30.0
MIP [%pred]	143.3 ± 40.5
MEP [cmH_2O]	150.5 ± 40.9
MEP [%pred]	169.9 ± 38.8

Values are mean ±SD. $\dot{V}O_{2peak}$, peak oxygen consumption; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; MVV_{12}, maximal voluntary ventilation in 12 s; MEP, maximal expiratory pressure; MIP, maximal inspiratory pressure.

Experimental Overview

On the 1st visit, lung function, maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were measured. After 15 min of rest, subjects performed an incremental cycling test until volitional exhaustion. After another 15-min rest, participants were familiarized with the IncRMT protocol. On the 2nd visit, HR_{rest} was measured and subjects performed an IncRMT to exhaustion for further familiarization. On the third visit, lung function, MIP and MEP were reassessed and after a 15-min rest, the IncRMT was performed.

Lung function and mouth pressure measurements

Lung function was measured according to the American Thoracic Society/European Respiratory Society guidelines (Miller *et al.*, 2005), using a metabolic cart with a calibrated volume sensor (Oxycon Pro; Jaeger, Höchberg, Germany). Maximal mouth pressures were measured using a handheld mouth pressure meter (Micro RPM; Micro Medical Ltd., Rochester, United Kingdom). Participants performed a minimum of three manoeuvres or until values did not improve anymore but they alternated MIP and MEP assessments such that no more than three subsequent MIP or MEP manoeuvers were performed. The larger of the two highest values (differing by ≤ 5%) was selected as the maximum. Variables of pulmonary function and respiratory muscle strength are reported both as absolute and as a percentage of predicted values (Wilson *et al.*, 1984; Quanjer *et al.*, 2013).

Incremental cycling test

Before starting the incremental cycling test, subjects sat on a bicycle ergometer (Ergoline 900, Ergoline, Blitz, Germany) for 5 min, with a nose-clip and mouthpiece in place, connected to the metabolic cart; then, cycling began at 100 W for men and at 70 W for women. Subsequently, the load was increased by 30 W every 2 min until exhaustion. Subjects chose their preferred pedalling frequency (between 70 rpm and 100 rpm) at the beginning of the incremental test, which was then held constant during the remainder of the test. The test was finished when the subject stopped or when the cadence dropped below 70 rpm.

Ventilation and gas exchange were measured breath by breath by the metabolic cart. Heart rate (HR) and oxygen saturation (SpO_2) were recorded as consecutive 4-s averages (Nellcor; Covidien, Mansfield, MA) and aligned with metabolic cart data. $\dot{V}O_{2peak}$ was defined as the highest 30-s $\dot{V}O_2$ average in the cycling test.

Heart rate

Subjects lay still on a stretcher for 12 min and were asked to relax and stay awake. Afterwards, three replicates of 20 s measurements (Complior Analyse, ALAM Medical, France) were performed and averaged to obtain HR_{rest}. The HR_{max} was predicted from age using the formula of Tanaka *et al.* (2001), i.e. HR_{max}=208 - 0.7·age [years].

Incremental respiratory muscle test (IncRMT)

The IncRMT was performed in a sitting position. Participants breathed through a mouthpiece connected to a self-built breathing apparatus while wearing a nose-clip. Tidal volume (V_T) was set at 60% FVC. Breathing frequency (f_B) started at 16 breath·min^{-1} with an increase of 2 breaths·min^{-1} every 2 min. Respiratory resistance started at a customized level (depending on individual FVC and predicted MIP to circumvent the need of MIP assessment in the clinical setting). Every 2 min, breathing resistance was increased to achieve, together with the increase in f_B, a 10%-increase in WOB. Subjects received the following visual and auditory feedback on their respiratory pattern throughout the test: actual and target V_T (with an upper and lower limit of ± 5% V_T) were displayed breath by breath on a screen positioned in front of the subjects and target f_B was given by a digital metronome, Fine Metronome, Fine Software, New Zealand) with inspiration and expiration paced separately in a ratio of 1:1 while the experimenter gave feedback in case of deviation from the target. The test was terminated if subjects stopped voluntarily or after a third warning due to failing to maintain V_T or f_B within target limits.

During the entire test, the apparatus was connected to an in-line capnometer (GOLDWEI, Herndon, VA) with a specific software allowing breath by breath adjustment of inspired fresh air to ensure normocapnia, and to a calibrated metabolic cart (Oxycon Pro; Jaeger, Höchberg, Germany) which provided continuous analog output of flow, O_2 and CO_2 concentrations. Also, mouth pressure was

measured continuously using a calibrated pressure transducer (DPT-100; Utah Medical Products Ltd., Athlone, Ireland). Flow, gas concentrations and pressure were AD-converted by PowerLab (ADInstruments, Castle Hill, Australia) with a sampling rate of 100 Hz, and flow was BTPS corrected. Expired flow was integrated over every breath and expired volume was sent to the screen in front of the subject with a delay of one breath. Mouth pressure, as a surrogate of esophageal pressure, was used to calculate WOB. WOB of each inspiration (WOB_{IN}) and expiration (WOB_{EX}) were calculated separately by analysis of the mouth pressure-volume loop. To obtain WOB_{tot} of each breath, WOB_{IN} and WOB_{EX} were added. Power of breathing (POB) was calculated breath by breath by dividing WOB_{IN} and WOB_{EX} by the duration of the respective inspiration and expiration. POB_{max} was calculated as the highest 30-s average of POB_{IN} and POB_{EX}. WOB and POB were calculated using Matlab (MATLAB 9.1, The MathWorks Inc., Natick, MA).

Statistical analysis

WOB_{tot}, IncRMT duration (T_{lim}), POB_{max}, HR_{rest}, $HR_{rest} \cdot HR_{max}^{-1}$ and FVC were used in a backwards stepwise multiple regression analysis to predict $\dot{V}O_{2peak}$. Based on the results from Jones *et al*. (1985) we chose to use FVC as a substitution of sex, height, weight and age. Since FEV_1, MVV_{12}, MIP and MEP showed collinearity with FVC, they where excluded from the model. In addition, HR_{rest} and $HR_{max} \cdot HR_{rest}^{-1}$ were added based on the study by Uth *et al.* (2004). In addition to the coefficient of determination (R^2), standard error of the estimate (SEE) for the differences between measured and estimated $\dot{V}O_{2peak}$ was calculated.

A Bland-Altman plot (Bland & Altman, 1986) with limits of agreement (LoA) of 95% was created to quantify the agreement (bias and random error) between the actual and predicted $\dot{V}O_{2peak}$. Statistical significance was set at $p<0.05$ for all analyses and results are reported as mean±SD. Statistical analysis was performed with SPSS Statistics 19 (IBM Co., New York, NY).

RESULTS

Incremental respiratory muscle test parameters and resting heart rate

Total work of breathing during IncRMT, time to exhaustion and maximal power reached in the IncRMT are given in Table 4.2, along with HR_{rest}.

Table 4.2: Incremental respiratory muscle test parameters and resting heart rate.

WOB_{tot} [kJ]	5.75 ± 3.53
T_{lim} [min]	13.78 ± 4.84
POB_{max} [W]	12.05 ± 5.21
HR_{rest} [min^{-1}]	64.3 ± 9.6

Values are means ±SD. WOB_{tot}, total work of breathing; T_{lim}, duration; POB_{max}, maximal power of breathing over 30 s; HR_{rest}, resting heart rate.

Multiple regression analysis of $\dot{V}O_{2peak}$ prediction

The backwards stepwise multiple regression resulted in a statistically significant prediction of $\dot{V}O_{2peak}$ with FVC and WOB_{tot} as significant variables. All other parameters did not significantly affect $\dot{V}O_{2peak}$ prediction (T_{lim}; p=0.313, POB_{max}; p=0.988, HR_{rest}; p=0.341, $HR_{max} \cdot HR_{rest}^{-1}$; p=0.385). In the present study, FVC as single variable was a statistically significant predictor of $\dot{V}O_{2peak}$ (R^2=0.634, F(1, 34)=59.010, $p < 0.001$; R^2=0.624) whereas FVC in combination with WOB_{tot} increased R^2 significantly by 0.152 (F (1, 33)=23.627, $p < 0.0001$) compared to FVC alone. The final model using FVC and WOB_{tot} to predict $\dot{V}O_{2peak}$ is given in Table 4.3 and Figure 4.1 shows predicted $\dot{V}O_{2peak}$ plotted against measured $\dot{V}O_{2peak}$.

Table 4.3: Multiple regression analysis to predict $\dot{V}O_{2peak}$ from work of breathing (WOB_{tot}) and forced vital capacity (FVC).

	$\dot{V}O_{2peak}$		
Variable	B	SE	β
Constant	-442.1	387.2	
WOB_{tot}	105.9**	0.0	0.4
FVC	613.5**	79.3	0.7
R^2	0.79		
F	60.94**		
SEE	436.93		

$\dot{V}O_{2peak}$, peak oxygen consumption; B, unstandardized regression coefficient; SE, standard erro; β, standardized regression coefficient; WOB_{tot}, total work of breathing of IncRMT; FVC, forced vital capacity; R^2, coefficient of determination; F, F-test; SEE, standard error of the estimate with a 95% confidence interval. **p<0.001

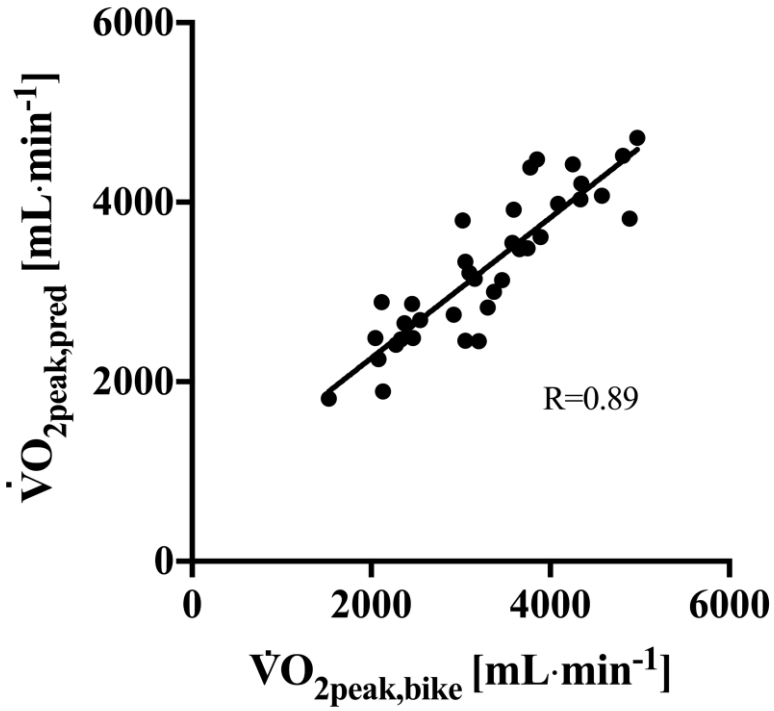

Figure 4.1: Multiple regression analysis to predict $\dot{V}O_{2peak}$ using total work of breathing and forced vital capacity. $\dot{V}O_{2peak,pred}$, predicted peak oxygen consumption; $\dot{V}O_{2peak,bike}$, peak oxygen consumption measured with incremental cycling test; R, pearson correlation coefficient.

Agreement between predicted and measured $\dot{V}O_{2peak}$ using the Bland-Altman plot revealed a bias of -0±546 mL·min^{-1} with 95% LoA of [-1070-1070] mL·min^{-1} for the single-variable model with FVC only (Figure 4.2A) and a bias of -18±417 mL·min^{-1} with 95% LoA of [-835-799] mL·min^{-1} (Figure 4.2B) for the model including FVC and WOB_{tot}.

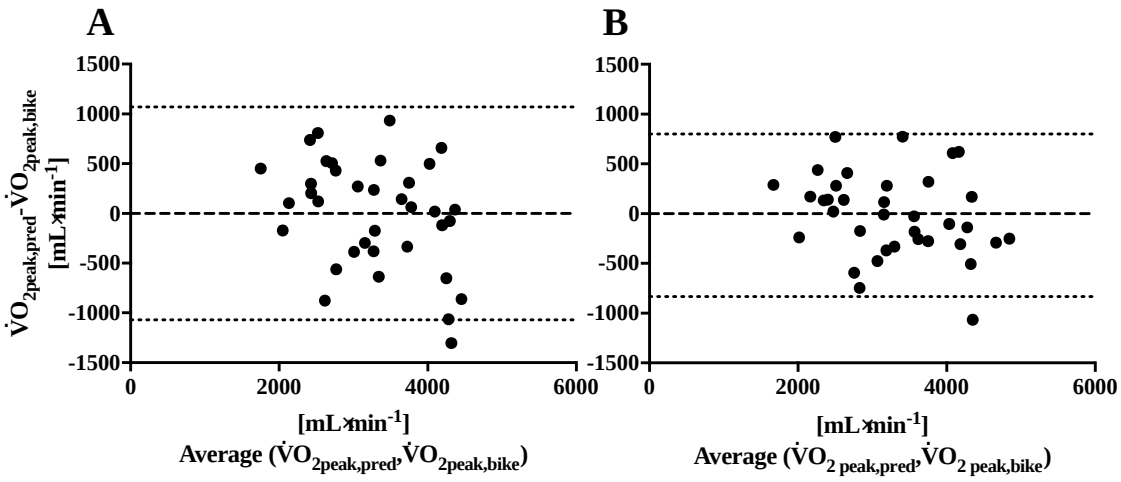

Figure 4.2: Bland-Altman plots using the average versus the difference of measured and predicted peak oxygen consumption ($\dot{V}O_{2peak}$). A: single-variable prediction of $\dot{V}O_{2peak}$ with forced vital capacity, B: multivariable prediction of $\dot{V}O_{2peak}$ with the combination of forced vital capacity and total work of breathing. $\dot{V}O_{2peak,pred}$, predicted peak oxygen consumption; $\dot{V}O_{2peak,bike}$, peak oxygen consumption measured with incremental cycling test.

DISCUSSION

The present study attempted to predict $\dot{V}O_{2peak}$, as measured with a standard incremental exercise test on a cycle ergometer, using a newly developed respiratory muscle performance test in conjunction with additional subject-specific variables. Multiple backwards regression analysis allowed the identification of variables that significantly contributed to predicting $\dot{V}O_{2peak}$, i.e. FVC and WOB_{tot} of the respiratory muscle performance test, while other specific variables assessed during this test, i.e. T_{lim}, POB_{max}, HR_{rest} and $HR_{max} \cdot HR_{rest}^{-1}$ did not significantly contribute to $\dot{V}O_{2peak}$ prediction. Even though FVC as a single-variable also predicted $\dot{V}O_{2peak}$ - in agreement with Jones *et al.* (1985) - adding WOB_{tot} significantly improved the prediction. Thus, adding a variable assessed in a functional measurement of respiratory muscle performance significantly improved the accuracy of $\dot{V}O_{2peak}$ prediction, as evidenced by a 24% reduction in the 95% LoA. This is physiologically in line with studies showing a positive relation of respiratory muscle performance and whole body exercise performance (Martin & Stager, 1981; Coast *et al.*, 1990; Eastwood *et al.*, 2001).

Baseline measures of cardiovascular fitness, quantified with HR_{rest} and $HR_{max} \cdot HR_{rest}^{-1}$ as used by Uth *et al.* (2004), in combination with FVC and WOB_{tot} did not improve $\dot{V}O_{2peak}$ prediction, even though HR_{rest} as a single variable did correlate with $\dot{V}O_{2peak}$. Also, the ratio of $HR_{max} \cdot HR_{rest}^{-1}$ as a single variable was a poor predictor of $\dot{V}O_{2peak}$ (R^2=0.109, F (1,34) =4.169; p=0.049) in the current study in contrast to findings of Uth *et al.* (2004). This difference may result from a different study population as the current study investigated healthy subjects with a large range of fitness levels including both sexes whereas Uth *et al.* (2004) only tested healthy, well trained men. However, selecting only those eight males participants with similar fitness levels as subjects in the study by Uth *et al.* (2004), $\dot{V}O_{2peak}$ was largely underestimated (bias of -929±924 $mL \cdot min^{-1}$ with 95% LoA of [-2740-884] $mL \cdot min^{-1}$). A possible explanation for this discrepancy may result from the different conditions under which HR_{rest} was measured, i.e. 5-min average in the morning with participants still in bed (Uth *et al.*, 2004) versus assessment in the laboratory, at different times of day, after 12 min of rest in supine position with three replicates of 20-s duration. Thus, HR_{rest} was likely somewhat higher in the present study, resulting in a smaller $HR_{max} \cdot HR_{rest}^{-1}$ and thus in an underestimation of $\dot{V}O_{2peak}$ when applying Uth and co-workers' model.

Since in the present study, only healthy individuals were included to test the potential prediction of $\dot{V}O_{2peak}$ including a functional inspiratory and expiratory muscle testing with IncRMT, future studies need to validate this relation, first in the elderly, then in different groups of patients to possibly age-adjust and assess the potential of the IncRMT as a predictor of $\dot{V}O_{2peak}$ in patients planning to undergo surgery.

Clinical implications

Knowing that $\dot{V}O_{2peak}$ is an important predictor of overall postoperative complications and as such is widely used in clinics to identify high-risk patients (Licker *et al.*, 2011; Moran *et al.*, 2016) and that it is also used to determine whether patients should undergo a preoperative physical training – a recent major advance in this area (West *et al.*, 2016) - it is essential to provide equivalent test and training opportunities to subjects not (anymore) able to undergo physical training.

While the IncRMT may provide an excellent functional replacement of a $\dot{V}O_{2peak}$-test, respiratory muscle training was already shown to reduce postoperative pulmonary complications, shorten duration of mechanical ventilation and the length of hospital stay, e.g. in patients undergoing cardiac and upper abdominal surgery (Mans *et al.*, 2015). Interestingly, no data is yet available for the combination of inspiratory and expiratory muscle training and its postoperative effects known to affect morbidity and mortality during surgery (Siafakas *et al.*, 1999).

In the future, testing of respiratory muscle performance with the use of IncRMT can possibly not only be used to predict whole-body $\dot{V}O_{2peak}$ in the preoperative setting, but also to further enable a distinguished assessment of inspiratory or expiratory muscle function and endurance capacity, providing important information for individually optimized preoperative respiratory muscle training protocols.

5. EFFECT OF HIGH INTENSITY INTERVAL AND ENDURANCE RESPIRATORY MUSCLE TRAINING ON RESPIRATORY MUSCLE PERFORMANCE MEASURED WITH A NOVEL INCREMENTAL RESPIRATORY MUSCLE TEST

ABSTRACT

Respiratory muscle training (RMT) has been shown to improve respiratory muscle performance in both healthy individuals and different groups of patients. Recently a novel, time-saving respiratory muscle sprint-interval training (RMSIT) was developed. To test the extent to which RMSIT improves respiratory muscle performance compared to a conventional respiratory muscle endurance training (RMET), a novel incremental respiratory muscle test (IncRMT), loading inspiratory and expiratory muscles, was designed to assess physiological and performance changes associated with RMT.

Healthy, moderately trained males and females (age: 26±5years, $\dot{V}O_{2peak}$: 47±12ml·min^{-1}·kg^{-1}) were randomized and balanced to 3 groups (RMSIT 5m/5f; RMET 6m/6f; SHAM 5m/6f). Lung function, respiratory muscle strength and IncRMT performance were tested before and after one month of RMT. During the IncRMT, muscle activity and muscle deoxygenation were assessed via surface electromyography and near-infrared spectroscopy of sternocleidomastoid (STERNO), intercostal (INTER) and abdominal (ABDO) muscles.

Lung function and respiratory muscle strength did not change differently between groups. Both RMT groups increased work of breathing (WOB) during training sessions to the same extent (RMSIT: +17.4±8.9 kJ, RMET: +26.2±16.1 kJ; p=0.143) with a larger increase in average mouth pressure (RMSIT: +20.0±15.0 cmH_2O, RMET: +3.3±1.5 cmH_2O; p=0.001) and a smaller increase in minute ventilation with RMSIT (RMSIT: +14.4±6.1 L·min^{-1}, RMET: +32.1±10.5 L·min^{-1}, p<0.001). IncRMT duration and total WOB increased significantly in both RMT groups compared to SHAM (Duration: RMSIT: +5.6±2.1 min; p=0.001; RMET: +3.8±4.2 min; p=0.014; SHAM: -0.6±3.7 min; WOB_{IN}: RMSIT: +2.0±0.8 kJ, p<0.001, RMET: +1.4±1.2 kJ; p=0.003, SHAM: -0.2±1.2 kJ); WOB_{EX}: RMSIT: +2.9±1.5 kJ, p<0.001, RMET: +1.5±1.6 kJ; p=0.006, SHAM: -0.8±1.9 kJ). Only INTER

activity during expiration decreased following RMSIT compared to SHAM (RMSIT: -186.3±210.9%; p=0.048, RMET: +36.0±273.1%; p=1.0, SHAM: +134.9±212.0%). No change in deoxyhemoglobin was observed in any muscle or group.

In conclusion, one month of RMSIT and RMET show similar improvements in respiratory muscle performance with similar differences in training intensity and duration.

INTRODUCTION

Respiratory muscle training (RMT) has been shown to improve physical performance in both healthy individuals (Illi *et al.*, 2012; HajGhanbari *et al.*, 2013) and different groups of patients (Gosselink *et al.*, 2011; Smart *et al.*, 2013). Muscle contractions in these existing RMT regimens are either performed at high force and low velocity (i.e. strength training) or at low force and high velocity (i.e. endurance training). Depending on the type of RMT, specific muscular adaptations have been shown, i.e. respiratory muscle strength training improves maximal respiratory muscle force while respiratory muscle endurance training (RMET) improves endurance related parameters (Leith & Bradley, 1976). Similarly, fatigue-related variables are affected differently dependent on RMT regimen (Verges *et al.*, 2009).

We recently developed a new, innovative RMT regimen which combines both moderate to high inspiratory and expiratory pressures as well as high flow, termed respiratory muscle sprint-interval training (RMSIT). RMSIT includes six 30 s bouts of hyperpnoea with added resistance interspaced by 2 min of rest (Wüthrich *et al.*, 2015). A single 11-min session of RMSIT was shown to induce similar levels of inspiratory and expiratory muscle fatigue as those seen after a 30-min RMET session (similar level of ventilation with no breaks and no resistance; Wüthrich *et al.*, 2015), indicating that the acute load placed upon respiratory muscles during RMSIT might be large enough to trigger muscular adaptations when chronically applied. Whether these adaptations indeed occur and how they compare to conventional RMET remains, however, unknown. With the present study, we therefore aimed to test whether respiratory muscle performance is increased to a similar extent with RMSIT and RMET and to investigate whether the different muscles involved in heavy breathing, i.e. M. diaphragmaticus (diaphragm), M. sternocleidomastoideus (STERNO) and M. intercostales externi (external INTER) for inspiration and M. intercostales interni (internal INTER) and M. rectus abdominis (ABDO) for expiration, would adapt to a similar degree. Due to the complex adaptation of muscle recruitment to different loads of the respiratory system (Shadgan *et al.*, 2011), muscular adaptations and changes in recruitment patterns in response to different RMT regimens are not fully predictable. To increase our understanding, we measured deoxygenated haemoglobin (HHb) with near-infrared spectroscopy (NIRS) and muscle activity via surface electromyography (EMG) of STERNO, INTER and ABDO during

incrementally increasing inspiratory and expiratory muscle loading prior to and after the training phase. We designed this new type of assessment, i.e. incrementally increasing work of breathing (WOB) by increasing flow as well as resistance - called incremental respiratory muscle test (IncRMT), such that adaptations in both the inspiratory as well as expiratory muscles could simultaneously be investigated and that strength as well as endurance components could be assessed in one single test. This test was performed to exhaustion prior to and after the training period to test for a change in respiratory muscle performance.

Thus, in the present study, we aimed to compare changes after one month of RMSIT, RMET or sham-training (SHAM). We hypothesized that 1) IncRMT performance would improve to a similar extent after RMSIT and RMET and that 2) muscular adaptations were similar after the two types of training, similar to the previously shown development of muscular fatigue (Wüthrich *et al.*, 2015).

METHODS

Subjects

Thirty-three healthy, moderately trained women and men were matched in groups of three according to their sex, age and aerobic capacity and then members of these groups were randomly assigned to one of three groups, RMSIT, RMET or SHAM (Table 5.1). Subjects refrained from strenuous physical activity for 48h prior to each visit, slept for at least 7 h the night before the visit and abstained from caffeinated beverages on visit days. In addition, all visit days were separated by at least 48h. After a thorough explanation of the study requirements, subjects gave their written informed consent, the study was approved by the local ethics committee and was performed according to the Declaration of Helsinki 2008. Subjects were requested to log their usual training and physical activity and to keep it constant over the study period and to record heart rate during each training (Polar Electro, Kempele, Finland). This information was checked at each visit to ensure compliance.

Table 5.1: Subjects Characteristics

	RMSIT	**RMET**	**SHAM**
Sex [m/f]	5 / 5	6 / 6	5 / 6
Age [yrs]	24 ± 3	27 ± 6	27 ± 6
Height [cm]	172 ± 9	171 ± 8	173 ± 8
Weight [kg]	64 ± 10	66 ± 10	66 ± 10
$\dot{V}O_{2peak}$ [mL·min^{-1}·kg^{-1}]	49 ± 11	47 ± 9	51 ± 11

Values are mean ± SD, m; males participants, f; females participants, $\dot{V}O_{2peak}$; peak oxygen consumption during an incremental cycling test. RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training; SHAM, sham-training.

Overview of study protocol

Subjects reported to the laboratory on a total of five occasions before, during and after one month of training (Figure 5.1). Tests were scheduled at the same time of day in order to avoid any confounding influence by the circadian rhythm (Scheer *et al.*, 2010). On the 1st visit, lung function, maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were measured. Following

approximately 15 min of rest, subjects performed an incremental cycling test until volitional exhaustion, which was used to determine baseline fitness level. Lastly, after another 15 min of rest, participants were familiarized with the IncRMT protocol. On the 2nd visit, participants performed the IncRMT to exhaustion, again for familiarization purposes. On the 3rd visit, pre-training values of lung function and respiratory muscle strength were assessed. Then subjects were equipped with respiratory muscle EMG and NIRS sensors and at least 30 min after the end of respiratory testing, they performed the IncRMT to exhaustion. After another 15 min of rest, participants were familiarized with their personal respiratory training intervention and they performed a first training in the laboratory. For the following one month, subjects trained at home with the middle training supervised in the laboratory (4th visit). The 5th and last visit consisted of the same tests as performed on the 3rd visit.

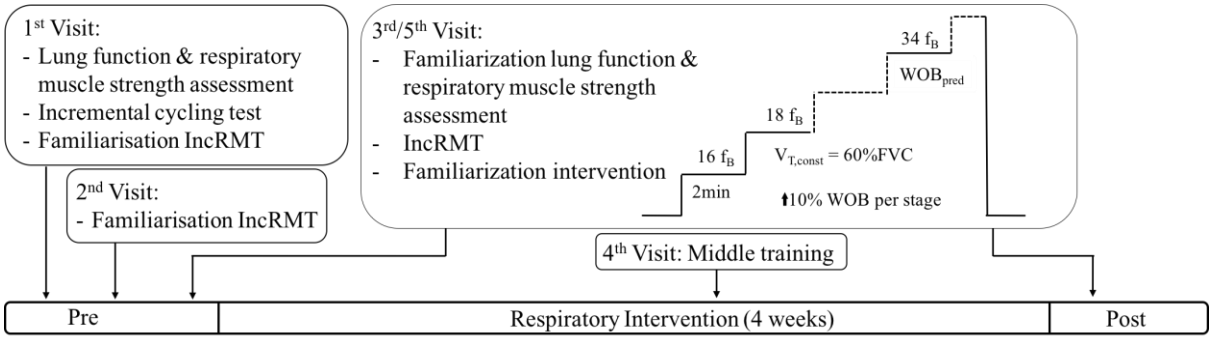

Figure 5.1: Study overview. IncRMT, incremental respiratory muscle test; WOB_{pred}, predicted work of breathing; f_B, breathing frequency; V_T, tidal volume; FVC, forced vital capacity.

Lung function and mouth pressure measurements

Lung function was measured according to the current guidelines (Miller *et al.*, 2005), using a metabolic cart with a calibrated volume sensor (Oxycon Pro; Jaeger, Höchberg, Germany). Maximal mouth pressures were measured using a handheld mouth pressure meter (Micro RPM; Micro Medical Ltd., Rochester, United Kingdom). Participants performed a minimum of three manoeuvres or until values did not improve anymore but they alternated MIP and MEP assessments such that no more than three subsequent MIP or MEP manoeuvers were performed. The larger of the two highest values (differing by ≤ 5%) was selected as the maximum. Variables of pulmonary function and respiratory muscle strength are reported both as absolute and as a percentage of predicted values (Wilson *et al.*, 1984; Quanjer *et al.*, 2013).

Perception of respiratory exertion and breathlessness

Perception of breathlessness (BR) and respiratory exertion (RE) were assessed by means of a visual analog scale ranging from 0 (none) to 10 (maximal breathlessness/exertion). To ensure a proper understanding of the terms, subjects were extensively questioned about their prior experience with different respiratory sensations (Lansing *et al.*, 2000). Thereafter, a definition was given for respiratory exertion ('how hard it is to breathe') and clearly distinguished from breathlessness (the sensation of 'not getting enough air').

Incremental cycling test

Before starting the incremental cycling test, subjects sat on a bicycle ergometer (Ergoline 900, Ergoline, Blitz, Germany) for 5 min, with a nose-clip and mouthpiece in place, connected to the metabolic cart; then, cycling began at 100 W for men and at 70 W for women. Subsequently, the load was increased by 30 W every 2 min until exhaustion. Subjects chose their preferred pedalling frequency (between 70 rpm and 100 rpm) at the beginning of the incremental test, which was then held constant during the remainder of the test. The test was finished when the subject stopped or when the cadence dropped below 70 rpm.

Ventilation and gas exchange were measured breath by breath by the metabolic cart. Heart rate (HR) and oxygen saturation (SpO_2) were recorded as consecutive 4-s averages (Nellcor; Covidien, Mansfield, MA) and aligned with metabolic cart data. $\dot{V}O_{2peak}$ was defined as the highest 30-s $\dot{V}O_2$ average in the cycling test.

Incremental respiratory muscle test (IncRMT)

The IncRMT was performed in a sitting position. Participants breathed through a mouthpiece connected to a self-built breathing apparatus while wearing a nose-clip. Tidal volume (V_T) was set at 60% FVC. Breathing frequency (f_B) started at 16 breath·min^{-1} with an increase of 2 breaths·min^{-1} every 2 min. Respiratory resistance started at a customized level (depending on individual FVC and predicted MIP to circumvent the need of MIP assessment in the clinical setting). Every 2 min, breathing resistance was increased to achieve, together with the increase in f_B, a 10%-increase in WOB. Subjects received the

following visual and auditory feedback on their respiratory pattern throughout the test: actual and target V_T (with an upper and lower limit of ± 5% V_T) were displayed breath by breath on a screen positioned in front of the subjects and target f_B was given by a digital metronome, Fine Metronome, Fine Software, New Zealand) with inspiration and expiration paced separately in a ratio of 1:1 while the experimenter gave feedback in case of deviation from the target. The test was terminated if subjects stopped voluntarily or after a third warning due to failing to maintain V_T or f_B within target limits.

During the entire test, the apparatus was connected to an in-line capnometer (GOLDWEI, Herndon, VA) with a specific software allowing breath by breath adjustment of inspired fresh air to ensure normocapnia, and to a calibrated metabolic cart (Oxycon Pro; Jaeger, Höchberg, Germany) which provided continuous analog output of flow, O_2 and CO_2 concentrations. Also, mouth pressure was measured continuously using a calibrated pressure transducer (DPT-100; Utah Medical Products Ltd., Athlone, Ireland). Flow, gas concentrations and pressure were AD-converted by PowerLab (ADInstruments, Castle Hill, Australia) with a sampling rate of 100 Hz, and flow was BTPS corrected. Expired flow was integrated over every breath and expired volume was sent to the screen in front of the subject with a delay of one breath. Mouth pressure, as a surrogate of esophageal pressure, was used to calculate WOB. WOB of each inspiration (WOB_{IN}) and expiration (WOB_{EX}) were calculated separately by analysis of the mouth pressure-volume loop. To obtain WOB_{tot} of each breath, WOB_{IN} and WOB_{EX} were added. WOB was calculated using Matlab (MATLAB 9.1, The MathWorks Inc., Natick, MA).

Surface electromyography (EMG) of respiratory muscles

Bipolar surface electrodes (TELEmyo DTS sensors; Noraxon, Scottsdale, AZ, USA) were placed on the skin above the muscle belly of STERNO, INTER externi and interni, and ABDO on the right side of the thorax according to SENIAM recommendations.

EMG signals were recorded at a sampling frequency of 1500 Hz. The electrocardiogram was filtered according to an in-built filter of the Noraxon system, then the signal was band-pass-filtered (10-500Hz, Clancy *et al.*, 2002), rectified and smoothened with root mean square of 100 ms using the inbuilt

function of the Noraxon software. EMG signals were normalized to the average EMG signal of the first 20% of the IncRMT duration of the shorter of the two tests (i.e. same duration for both tests). Mouth pressure signals were aligned to EMG activity using the highest cross-correlation between the STERNO activity and the inspiratory mouth pressure during the last 2 min of the test. After visual inspection of the alignment throughout the entire test duration, the distinction between inspiratory (RMS_{IN}) and expiratory (RMS_{EX}) muscle activity could be made.

Near-infrared spectroscopy (NIRS) of respiratory muscles

In order to measure changes in respiratory muscle HHb, NIRS optodes (OxyMon MK III; Artinis Medical Systems B.V., Zetten, Netherlands) were used. A transmitter and a receiver optode, interspaced by 4 cm, were attached to the skin above the muscle belly of STERNO, INTER and ABDO, as a mirror image of the EMG placement. The transmitter optodes emitted impulses of infrared light at the wavelengths of 761 nm and 855 to 858 nm. Signals were sampled at 10 Hz. Only signals at wavelengths 761 nm were analysed for HHb measurements which represents muscle oxygen extraction (Ferreira *et al.*, 2007). Similar to the EMG-analysis, the average signal of the first 20% of the IncRMT duration was used as reference. This average NIRS signal was set to zero.

Respiratory muscle training/sham-training

Subjects of all three training interventions recorded each training sessions in their logbook. Subjects in the RMT groups measured heart rate with a portable heart rate monitor (Polar Electro, Kempele, Finland) and were asked to rate their perceived breathlessness and respiratory exertion at the end of the training session on a scale from 0 to 10 where 0 was defined as “none” and 10 is “maximal” breathlessness or respiratory exertion. WOB and average mouth pressures (P_m) of the first and last training of the RMT groups were estimated based on reported ventilations and previous flow-pressure characteristics of the training device setup used in these training sessions.

RMSIT

Subjects in the RMSIT group completed 12 training sessions, each lasting 11 min, over one month.

They performed three training sessions per week with at least 48 h rest between any 2 training sessions. Each training consisted of 6 cycles of 1 min respiratory sprint with 1 min break in between 2 cycles. During the sprints, subjects breathed through the SpiroTiger® device (idiag, Fehraltorf, Switzerland) with an added resistance introduced by adding an orifice with reduced diameter between the mouthpiece and device to maximize respiratory muscle work. The smallest diameter with which subjects were able to sustain a f_B of 30 breaths·min^{-1} with a V_T of 60% FVC was used in the first training session. If respiratory exertion was ≤ 8 points after a sprint (on a 0-10 scale), subjects were instructed to increase f_B of the next sprint by 1 breath·min^{-1}. If respiratory exertion was 9 or 10, subjects were instructed to keep f_B constant. If subjects were not able to hold the frequency over the 1-min sprint, they were instructed to reduce f_B by 1 breath·min^{-1} for the next sprint while keeping V_T constant. If subjects reached an f_B of 35 breaths·min^{-1}, an orifice with a smaller diameter was inserted and f_B was reduced to 30 breaths·min^{-1}.

RMET

Subjects of the RMET group completed 20 training sessions, each lasting 30 min, over 30 days. Two consecutive days of training were followed by a day of rest, as was shown to be effective in previous studies (Verges *et al.*, 2007). Subjects performed volitional, normocapnic hyperpnoea using the SpiroTiger® device (idiag, Fehraltorf, Switzerland). The target ventilation of the first training session was set to 60% of the individual's maximal voluntary ventilation (MVV_{12}). The duty cycle was set to 0.5 and V_T was set to 60% of FVC with the breathing frequency (f_B) adjusted accordingly. Subjects were instructed to sustain this ventilation for 30 min. If after 25 min of breathing subjects felt that they would not be exhausted after 30 min, f_B was increased by 2 breaths·min-^{1} and the next training session began with this increased f_B. If subjects could just manage to finish the 30 min with the starting f_B, f_B of the next training session was increased by 1 breath·min^{-1}. If after 25 min, subjects felt that they could not finish the training session with this frequency, f_B was decreased by 2 breaths·min^{-1} and the next training session began with the starting f_B of the previous training session.

SHAM

Subjects of the SHAM group completed a sham training, 3-4 times a week, for 30 days using a mock asthma inhaler, HandiHaler® (Boehringer Ingelheim, Ingelheim, Germany) filled with lactose powder. Subjects were instructed to inhale the powder once and to then perform five full inspirations to total lung capacity using a self-constructed tubing system, including a small resistance.

Data analysis and statistics

Since not all of the subjects reached pre-training IncRMT duration, iso-time was defined as the duration of the shorter of each individual's two tests. WOB_{IN} and WOB_{EX} represents the sum of WOB of every breath until iso-time and until exhaustion. HR was averaged over the last 30 s before iso-time and exhaustion. For analysis of blood lactate, BR and RE end-values of the last completed stage before iso-time and at test termination were considered. EMG and NIRS data were averaged over 20% packages of the IncRMT iso-time duration and over the last 30 s of each IncRMT. Due to technical problems one HR and two blood lactate measurements are missing ([RMSIT, RMET, SHAM], HR [9, 12, 11], Lactate [10, 11, 10]). One data set of BR and RE is missing because of coordination difficulties of one subject during the IncRMT (BR and RE [10, 12, 10]). Due to an intense movement of the rib cage during the IncRMT, some EMG and NIRS sensors moved in relation to the muscle, e.g. over the rib, as described earlier (Shadgan *et al.*, 2011), rendering the signals implausible. This resulted in a lower sample sizes for STERNO (RMS_{IN} [10, 12, 9], HHb [8, 10, 9]), for INTER (RMS_{IN} [10, 11, 6], RMS_{EX} [10, 12, 6], HHb [9, 9, 9]) and ABDO (RMS_{EX} [10, 12, 10], HHb [9, 11, 11]).

IncRMT durations of all subjects before the training started (independent of their group assignment) were compared between sexes with an unpaired t-Test while absolute changes in test duration (pre- vs. post-training) were compared with a two-way ANOVA with main factors sex and group. Since no between-sex difference in test duration prior to training and pre-post training was detected, sex was no longer considered as a factor for further analyses.

Subjects characteristics pre-training values and absolute changes from pre-to-post training were compared between groups with a one-way ANOVA and if differences were detected, a post hoc test with Bonferroni correction was performed. Within each group, pre-to-post differences were assessed

using paired t-tests after testing for normal distribution (Shapiro-Wilk test). For data without normal distribution, a Wilcoxon signed rank test was applied. Statistical analyses were performed with SPSS Statistics 23 (IBM Company, New York, NY). The level of significance was set at $p < 0.05$ for all statistical comparisons.

RESULTS

Subjects characteristics

Before training, the three groups did not differ in age, height, weight and $\dot{V}O_{2peak}$ (Table 5.1).

Training characteristics RMSIT and RMET

At the first training session $\dot{V}_E$ and total WOB were similar in both RMT-groups whereas P_m was significantly higher in RMSIT. With both training regimens, WOB increased significantly throughout the training period with no difference between WOB changes within groups (RMSIT: +17.4±8.9 kJ vs. RMET: +26.2±16.1 kJ; p=0.143). In the last (12^{th}) training session of RMSIT, subjects had increased average P_m to a greater extent than subjects did in the last (20^{th}) training sessions of RMET compared to the first (RMSIT: +20.0±15.0 cmH_2O vs. RMET: +3.3±1.5 cmH_2O; p=0.001), while subjects in the RMSIT group increased $\dot{V}_E$ significantly less than subjects in the RMET group (RMSIT: +14.4±6.1 $L \cdot min^{-1}$ vs. RMET: +32.1±10.5 $L \cdot min^{-1}$, p<0.001; Figure 5.2). In the middle RMSIT session average in-/expiratory P_m expressed in percentage of baseline maximal in-/expiratory mouth pressure (inspiratory P_m: 46±15% MIP; expiratory P_m: 27±8% MEP) reached similar inspiratory P_m but lower expiratory P_m as during traditional strength training (Griffiths & McConnell, 2007).

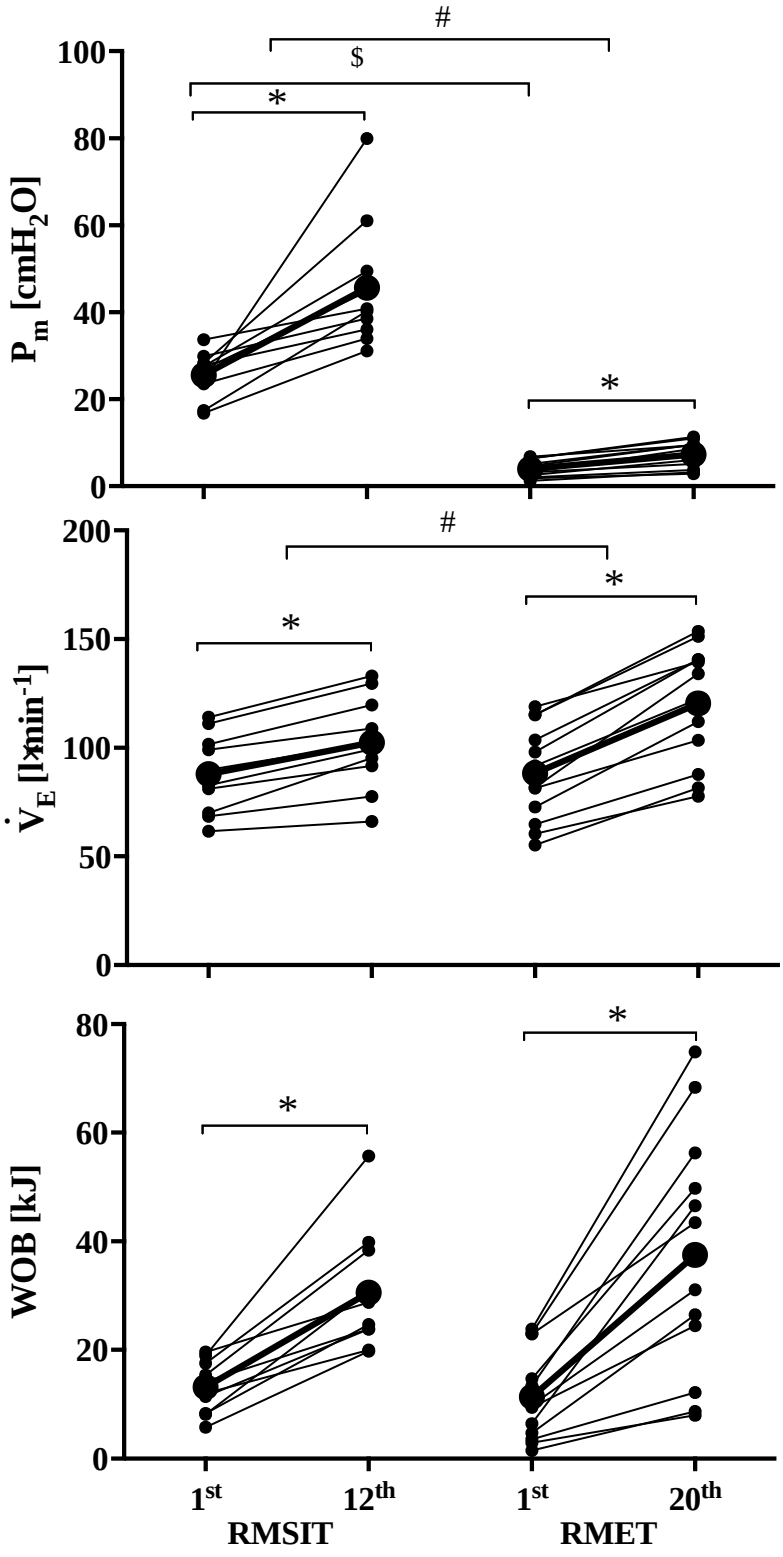

Figure 5.2: Respiratory muscle training parameters first vs. last training. P_m, average mouth pressure; $\dot{V}_E$, average minute ventilation; WOB, total work of breathing; RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training. Group means are represented in bold, *$p<0.05$ first vs. last training session within groups, $^{\$}p<0.05$ first training session between groups, $^{\#}p<0.05$ changes between groups.

Lung function and respiratory muscle strength

Lung function and respiratory muscle strength did not differ between groups before training nor were pre-post training changes different between groups (Table 5.2). Within groups FVC, forced expiratory volume within 1 s (FEV_1), MIP and MEP did not change significantly. MVV_{12} increased significantly with RMET (+9.1±7.0 $L \cdot min^{-1}$; p=0.001) and showed a similar trend in the RMSIT group (+9.6±15.9 $L \cdot min^{-1}$; p=0.09). Pre-post training, MEP showed a trend towards increase after RMSIT (+11.9±18.4 cmH_2O; p=0.07).

Table 5.2: Lung function and respiratory muscle strength.

	RMSIT		**RMET**		**SHAM**	
	Pre	Post	Pre	Post	Pre	Post
FVC [L]	5.0 ± 1.0	5.1 ± 1.0	5.1 ± 1.0	5.2 ± 1.0	5.1 ± 1.0	5.0 ± 1.1
FEV_1 [L]	4.2 ± 0.9	4.3 ± 0.9	4.1 ± 0.9	4.6 ± 0.3	4.1 ± 0.9	4.0 ± 0.3
MVV_{12} [$L \cdot min^{-1}$]	174.0 ± 40.1	183.5 ± 37.6	166.3 ± 30.7	175.4 ± 30.0*	168.6 ± 49.0	171.9 ± 16.2
MIP [cmH_2O]	128.8 ± 27.3	134.5 ± 29.1	134.7 ± 32.9	144.3 ± 32.4	122.8 ± 27.9	128.8 ± 31.2
MEP [cmH_2O]	169.2 ± 30.6	181.1 ± 31.8	186.9 ± 41.7	188.3 ± 51.0	181.2 ± 52.2	183.6 ± 49.3

Values are mean ± SD. FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; MVV_{12}, maximal voluntary ventilation in 12 s; MIP, maximal inspiratory pressure; MEP, maximal expiratory pressure; RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training; SHAM, sham-training. *p<0.05 pre vs. post within groups.

Incremental respiratory muscle test performance measurement

Pre training all parameters measured during the IncRMT were similar between groups and no difference between RMT group was found. As shown in Figure 5.3, duration of the IncRMT improved significantly in both RMT groups compared to SHAM with a more consistent response in RMSIT than RMET (RMSIT: +5.6±2.1 min vs. SHAM: -0.6±3.7 min; p=0.001; RMET: +3.8±4.2 min vs. SHAM p=0.014). At iso-time no difference in changes between groups in any cardio-respiratory parameter or perception was found, as shown in Table 5.3. Additionally, WOB_{IN} at iso-time increased compared to SHAM independent of the intervention (RMSIT: +0.4±0.4 kJ vs. SHAM: +0.2±0.3 kJ; p=1.00; RMET: +0.3±0.4 kJ vs. SHAM; p=1.00) whereas WOB_{EX} did not change (RMSIT: +0.1±0.0.6 kJ vs. SHAM:

+0.2±0.7 kJ; p=0.9; RMET: +0.0±0.5 kJ vs. SHAM; p=1.00). Moreover, RE decreased significantly in both RMT groups but changes were not statistically different to SHAM (RMSIT: -1.1±1.9 point vs. SHAM -0.8±2.4 point; p=1.00; RMET: -2.0±2.3 point vs. SHAM; p=0.609). At the point of exhaustion, changes in WOB_{IN} (RMSIT: +2.0±0.8 kJ vs. SHAM: -0.2±1.2 kJ, p<0.001; RMET: +1.4±1.2 kJ vs. SHAM; p=0.003) and WOB_{EX} (RMSIT: +2.9±1.5 kJ vs. SHAM: -0.8±1.9 kJ, p<0.001; RMET: +1.5±1.6 kJ vs. SHAM; p=0.006) were significantly higher for both RMT regimens compared to SHAM. Changes in HR and perception of RE did not differ between groups but within the RMET group, HR increased (RMET: +16.4±18.5 bmp; p=0.011) and BR decreased (RMET: -1.9±2.6 points; p=0.009) at the point of exhaustion. Blood lactate concentration increased at the point of exhaustion in both RMT groups but the pre-post change was different only for RMSIT compared to SHAM (RMSIT: +1.1±0.7 $mmol \cdot L^{-1}$ vs. SHAM: -0.1±0.7 $mmol \cdot L^{-1}$; p=0.001; RMET: +0.5±0.5 $mmol \cdot L^{-1}$ vs. SHAM; p=0.130).

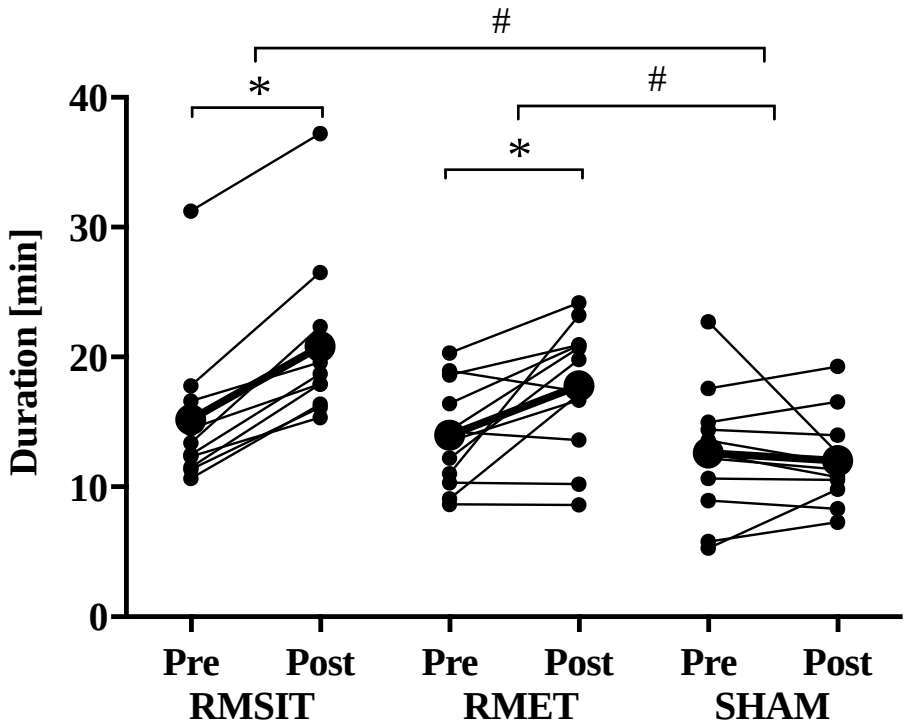

Figure 5.3: Incremental respiratory muscle test duration pre vs. post respiratory muscle training. RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training; SHAM, sham-training. Group means are represented in bold, *p<0.05 pre vs. post within groups, #p<0.05 changes between groups.

Table 5.3: Incremental respiratory muscle test parameters pre and post one month of respiratory muscle training at iso-time and exhaustion

	RMSIT		RMET		SHAM	
at iso-time	Pre	Post	Pre	Post	Pre	Post
WOB_{IN} [kJ]§	2.5 ± 1.6	2.9 ± 1.8*	2.3 ± 1.2	2.58 ± 1.4*	1.9 ± 1.2	2.1 ± 1.3*
WOB_{EX} [kJ] §	3.5 ± 2.3	3.7 ± 2.3	3.2 ± 2.1	3.2 ± 2.0	2.7 ± 1.6	2.5 ± 1.3
HR [bpm]	110.2 ± 21.2	111.5 ± 24.9	96.6 ± 23.5	102.3 ± 26.5	95.6 ± 15.3	96.6 ± 20.3
Lactate [mmol· L^{-1}]	1.1 ± 0.3	1.2 ± 0.5	0.9 ± 0.3	1.0 ± 0.3	0.9 ± 0.3	1.1 ± 0.3
RE [point]	3.8 ± 1.5	2.7 ± 1.2*	3.6 ± 1.2	2.7 ± 1.5*	3.3 ± 1.5	3.0 ± 1.3
BR [point]	0.8 ± 0.9	0.4 ± 0.6	1.3 ± 1.0	0.6 ± 0.7*	1.2 ± 1.5	0.9 ± 1.1
at exhaustion						
WOB_{IN} [kJ] §	2.5 ± 1.6	4.5 ± 2.2*#	2.3 ± 1.3	3.7 ± 1.8*#	2.5 ± 1.9	2.7 ± 1.3
WOB_{EX} [kJ] §	3.5 ± 2.3	6.4 ± 3.1*#	3.2 ± 2.1	4.7 ± 2.3*#	3.5 ± 2.3	2.2 ± 1.4
HR [bpm]	114.0 ± 17.6	120.2 ± 24.9	99.4 ± 24.0	114.6 ± 31.5*	99.1 ± 17.1	100.8 ± 22.9
Lactate [mmol· L^{-1}]	1.8 ± 0.6	2.9 ± 1.1*#	1.5 ± 0.7	2.0 ± 0.8*	1.8 ± 0.9	1.7 ± 0.8
RE [point]	7.9 ± 1.8	9.1 ± 1.4	7.9 ± 1.9	8.1 ± 2.1	7.5 ± 2.9	7.4 ± 2.2
BR [point]	2.7 ± 3.1	2.0 ± 3.0	3.1 ± 3.0	1.2 ± 1.9*	2.8 ± 3.7	2.7 ± 2.8

Values are mean ± SD. RMSIT, respiratory muscle spring interval training; RMET, respiratory muscle endurance training; SHAM, sham-training; WOB_{IN}, total inspiratory work of breathing until iso-time and until exhaustion; WOB_{EX}, total expiratory work of breathing until iso-time and until exhaustion; HR, average heart rate last 30 s before iso-time and at exhaustion; Lactate, blood lactate last completed stage at iso-time and at exhaustion; RE, respiratory exertion last completed stage at iso-time and at exhaustion; BR, breathlessness last completed stage at iso-time and at exhaustion. §calculations from start until iso-time/exhaustion, *$p<0.05$ pre vs. post within groups, #$p<0.05$ changes compared to SHAM.

Training adaptations in muscular activity and oxygen extraction

As shown in Figure 5.4, inspiratory STERNO activity decreased following RMSIT compared to SHAM at 80% and 100% of iso-time duration of the IncRMT with no significant change within the RMSIT group (averaged decreases of 80% and 100%; RMSIT: -240±643% vs. SHAM +121±513%; p=0.042; RMET: -44±558% vs. SHAM; p=1.000). Inspiratory INTER activity did not change significantly different between groups. Expiratory INTER activity decreased at 20% iso-time duration in the IncRMT compared to RMET and SHAM but changes were very small. At 80% and 100% of iso-time duration, expiratory INTER activity decreased after RMSIT compared to SHAM which was also significant within the RMSIT group (averaged decreases of 80% and 100%; RMSIT: -186.3±210.9% vs. SHAM: +134.9±212.0%; p=0.048; RMET: +36.0±273.1% vs. SHAM; p=1). Pre-post changes of ABDO activity during expiration were not significantly different between groups. However, within the RMSIT group, an increased ABDO activity was observed at exhaustion (+1411±1586%; p=0.020).

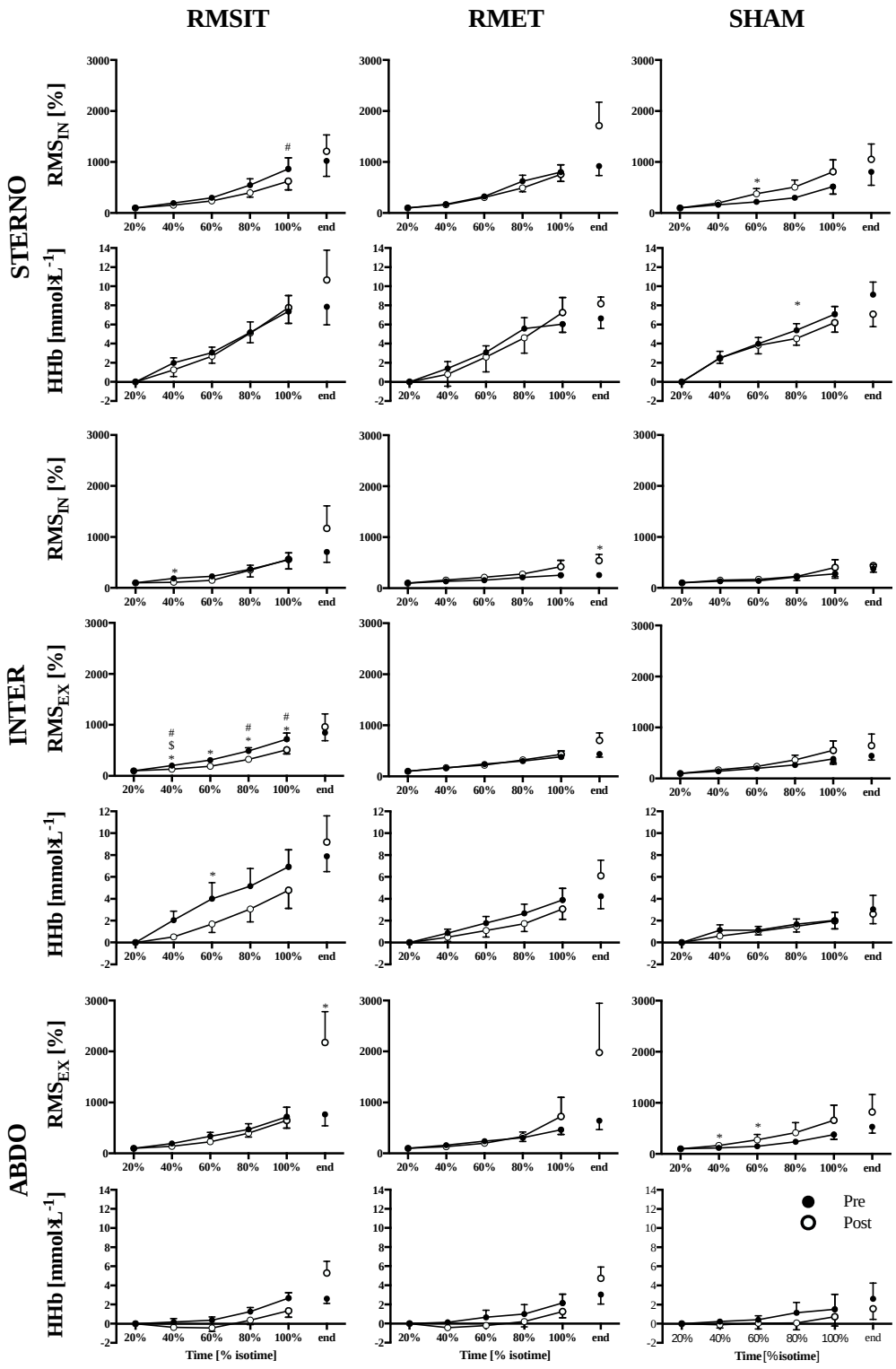

Figure 5.4: Respiratory muscle activity and oxygen extraction during incremental respiratory muscle test. Values are mean ± SEM. RMS_{IN}, electromyography root mean square during inspiration; RMS_{EX}, electromyography root mean square during expiration; HHb, deoxygenated haemoglobin; RMSIT, Respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training; SHAM, sham-training; STERNO, sternocleidomastoid, INTER, intercostales; ABDO, abdominal muscle. *$p<0.05$ pre vs. post within groups, $^{\#}p<0.05$ changes between groups.

DISCUSSION

The main findings of this study are that IncRMT performance improved to a similar extent after RMSIT and RMET compared to SHAM with no differences in changes in lung function and respiratory muscle strength between groups. Also with both RMSIT and RMET, subjects similarly increased WOB from the 1st to the last training despite of the smaller number and reduced duration of RMSIT trainings, i.e. reduced training volume compared to RMET.

Respiratory muscle performance

In both RMT groups, IncRMT duration, WOB_{IN} and WOB_{EX} at exhaustion improved significantly compared to SHAM with a more consistent response in RMSIT. Interestingly, WOB_{IN} at iso-time increased in all groups to the same extent while WOB_{EX} and T_{in}/T_{tot} (data not shown) remained unchanged. This difference between changes in WOB_{IN} and WOB_{EX} may result from subjects adopting a different muscle recruitment pattern towards increased recruitment of inspiratory muscles after training. This is supported by the fact that motor cortical representation of respiration is changed already after 2 weeks of volitional breathing (Mehiri *et al.*, 2006) and that SHAM - consisting of 5 slow, volitional and maximal inspirations only - lead to a similar change, possibly suggesting a potential shift towards increased lung volume, increasing the elastic recoil of the thorax thus increasing WOB_{IN} while decreasing WOB_{EX}. In fact, we have previously seen changes in spontaneous breathing pattern after one month of using incentive spirometry as a sham training modality (unpublished data).

Overall, after both RMTs, an increase in respiratory muscle performance with a more pronounced improvement in inspiratory muscle activity was seen. This increase in respiratory muscle performance is in line with previously reported improvements in respiratory muscle endurance after RMET measured via constant-load normocapnic hyperpnoea (Sales *et al.*, 2016). In addition, Verges *et al.* (2009) showed that inspiratory muscle training (IMT) also improved respiratory muscle endurance to a lesser extent than RMET under similar study conditions. The similar increase in IncRMT performance after RMSIT and RMET suggests that the combination of high pressures with high flows applied to inspiratory and expiratory muscles with RMSIT, lasting as little as 12x 6 min in total has similar effects on respiratory muscle endurance as RMET, with high flows and low pressures, performed 20x 30 min. These results

show for the first time similarities of respiratory muscle performance to improvements in whole-body exercise performance after either high-intensity interval training or whole-body endurance training (Jones & Carter, 2000).

Contrary to the IncRMT performance, changes in MVV_{12} were not different between groups with only a significant improvement within the RMET group (+5±2%), which is in line with previous studies showing -2%-15% changes in MVV after RMET (Sales *et al.*, 2016). RMSIT increased MVV_{12} to the same extent (+5±7%; p=0.09) as RMET in the current study however did not reach a significant change. Respiratory muscle strength did not change in any of the groups but a trend towards increased MEP after RMSIT (p=0.07) was observed. This data is in accordance with training specificity of respiratory muscles (Leith and Bradley 1976) as it was previously observed that RMET does not improve respiratory muscle strength in healthy individuals (Verges *et al.*, 2009) whereas IMT and expiratory muscle training (EMT) are known to improve MIP and MEP, respectively (Chiara *et al.*, 2006; Griffiths & McConnell, 2007). Surprisingly, despite high average inspiratory P_m (46±15 %MIP) and expiratory P_m (27±8 %MEP) during RMSIT sessions, similar to classical respiratory muscle strength training, and with a similar or higher number of breaths per training (Romer & McConnell, 2003) respiratory muscle strength did not increase to a similar degree (Volianitis *et al.*, 2001). Possible explanations for this phenomenon could be i) a lower weekly number of breaths (540-630 breaths per week) compared to IMT (1350 breaths per week; Witt *et al.*, 2007), ii) a different range of lung volume used during training (Hostettler *et al.*, 2011); classical strength training is performed over 100% FVC (Romer *et al.*, 2002) compared to 60% FVC during RMSIT, thus not training respiratory muscles over the entire range of motion. During MIP and MEP manoeuvers peak pressures are performed isometric at fully filled or emptied lungs, respectively, thus at a respiratory muscle length not trained during RMSIT. Alternatively, iii) it was shown that time under tension (TUT) affects the response of mitochondrial and sarcoplasmic protein synthesis (Burd *et al.*, 2012). TUT in traditional IMT and EMT is of 1-2 s whereas RMSIT with a f_B of 30-35 breaths·min^{-1} and a duty-cycle of 50% results in an inspiratory and expiratory TUT of 0.8-1 s which could be too short a stimulus to improve respiratory muscle strength.

Altogether, RMSIT and RMET similarly increased measures of respiratory muscle performance. Thus, it will be important to test whether exercise performance will also be affected in a similar way or

whether the subtle differences in training outcomes might provide a larger improvement with RMSIT, as suggested by the review of Illi *et al.* (2012). Also, in the context of cardio-respiratory or neuro-muscular disease, it will be important to test whether improvements in respiratory muscle performance might be higher with RMSIT than with RMET in patients with weak respiratory muscles and whether exercise performance and quality of life will be affected to a similar or larger extent with RMSIT compared to those known from RMET (Holm *et al.*, 2004; Verges *et al.*, 2007, 2008; Lemaitre *et al.*, 2013).

Physiological training adaptations

Contrary to exercise-related adaptations observed after whole-body endurance training of similar load (Jones & Carter, 2000), HR was not significantly decreased during the IncRMT after both types of RMT. This also contrasts previous observations where HR was reduced during volitional hyperpnoea after RMET (Verges *et al.*, 2008) and during resistive breathing after inspiratory resistive training (Witt *et al.*, 2007), the latter, however, having a much larger resistive training load than RMSIT in the present study. Also blood lactate accumulation remained unchanged during the IncRMT which also contrasts the lower levels observed after RMET and IMT during hyperpnoea (Spengler *et al.*, 1999; Brown *et al.*, 2008; Verges *et al.*, 2008, 2009). These findings suggest a smaller cardio-metabolic training effect than was previously observed.

Inspiratory STERNO activity and expiratory INTER activity were decreased during the IncRMT after RMSIT compared to SHAM, although without a change of expiratory ABDO activity and inspiratory INTER activity, indicating an increase in fatigue resistance and/or neuro-muscular adaptations in some of the respiratory accessory muscles (Decorte *et al.*, 2012; Sales *et al.*, 2016). However, no change was observed in the RMET group. Also, reduced muscle activity is expected to result in a change in metabolism, either lower oxygen extraction or reduced anaerobic metabolism, which both was not seen in STERNO and INTER of the RMSIT group. This discrepancy might possibly be explained by either i) the small change in muscle activity resulting in an even smaller change in oxygen extraction that would not be detectable with NIRS or by ii) the fact that NIRS signals were averaged over entire breaths reducing the sensitivity towards changes specifically related to inspiratory or expiratory muscle activity

while EMG activity was analysed separately for inspiration and expiration. A separation between inspiration and expiration for NIRS signals was unfortunately not possible because no clear distinction between inspiration and expiration was visible as it was with EMG signals.

Furthermore, RE was decreased after both RMSIT and RMET but these changes were not different to SHAM. This may suggest, in face of similar HR and blood lactate concentration at iso-time, central or neuro-muscular adaptation being involved in the increased tolerance of metabolic disturbance after RMT, as suggested previously (Verges *et al.*, 2008).

Limitations

Unfortunately, we were not able to assess diaphragmatic muscle activity, to measure local blood flow by the indocyanin green method or to calculate oxygen consumption during the IncRMT with the current technology available in our laboratory. These additional variables would have given further insight into changes in muscle activity, blood flow and metabolism of the respiratory muscles.

Conclusion and outlook

In summary, RMSIT, a time-saving respiratory muscle training, combining inspiratory and expiratory muscle loading with high flows and high pressures, was shown to induce adaptations similar to RMET. Thus, further studies are warranted to test whether RMSIT may improve whole-body exercise performance in healthy subjects (Illi *et al.*, 2012) as well as different groups of patients (Gosselink *et al.*, 2011; Smart *et al.*, 2013) to a similar extent as established RMT regimens. Furthermore, this combined training may be advantageous also to improve cough in elderly (Kim *et al.*, 2009) and groups of patient with weak expiratory muscles (Chiara *et al.*, 2006; Pitts *et al.*, 2009) that were shown to benefit from expiratory muscle training.

Moreover, the IncRMT protocol showed respiratory muscle performance adaptations reflecting the increase in performance during RMT sessions, i.e. the increase in WOB, while IncRMT- improvements were not related to changes in brief, maximal manoeuvres such as MVV_{12}, MIP and MEP. This fact underlines that performance in the IncRMT includes endurance and strength components thus better reflects respiratory muscle performance, as suggested by Sales *et al.* (2016). Since the IncRMT-load is

personalized based on FVC and predicted MIP (age, height, sex), a global assessment of the respiratory muscles with standardized test conditions and a relatively narrow range of test duration is possible. It will be important to investigate feasibility and discriminative capacity of this test in different groups of patients, e.g. patients with obstructive (flow-limited) lung disease or with respiratory muscle weakness (strength limited), and possibly systematic ways to test training adaptations will need to be developed.

6. EFFECTS OF HIGH INTENSITY INTERVAL AND ENDURANCE RESPIRATORY MUSCLE TRAINING ON METABOREFLEX AND WHOLE-BODY EXERCISE PERFORMANCE IN MALES AND FEMALES

ABSTRACT

Respiratory muscle training (RMT) was shown to improve physical performance. However, the mechanisms of action are not yet fully understood. A reduction in respiratory metaboreflex is suggested. Here, we tested whether this reflex was changed after one month of respiratory muscle sprint-interval training (RMSIT) and respiratory muscle endurance training (RMET) compared to a sham training (SHAM) and to which degree the change was related to changes in physical performance.

Thirty-three healthy, moderately trained subjects (50% female; age: 26±5years; $\dot{V}O_{2peak}$: 49±10ml·min^{-1}·kg^{-1}) were randomized to 3 groups. Assessments of lung function, respiratory muscle strength, respiratory and leg muscle fatigue in a work-matched constant-load cycling test at 90% peak power (CLT) and 12-km time trial (TT) performance were done before and after one month of RMT. During the CLT, muscle activity via surface electromyography and deoxyhemoglobin via near-infrared spectroscopy were assessed of sternocleidomastoid (STERNO), intercostal (INTER) and vastus lateralis (VAST) muscles. Inspiratory mouth twitch pressure (ΔP_m) and quadriceps twitch torque (ΔQ_{TW}) were assessed prior to and after the CLT.

Changes in lung function, respiratory muscle strength, muscle activity, deoxyhemoglobin and ΔP_m were not different between groups. RMSIT showed a reduction in ΔQ_{TW} compared to RMET with RMSIT males showing a difference to RMET and SHAM (RMSIT: -7.9±8.7% vs. RMET: +6.4±13.2; p=0.002, vs. SHAM: +7.0±10.8%; p=0.002) while ΔQ_{TW} did not differ between groups in females. RMET males increased TT performance (-1.5±1.4 min; p=0.049) compared to SHAM (+1.5±1.2 min; p=0.440) but it was not different from RMSIT males (-0.1±0.5 min; p=0.727) while no changes were observed in females.

In conclusion, one month of RMSIT and RMET show training- and sex-specific adaptations in whole-body exercise performance. The mechanism behind these distinct training adaptations remain inconclusive.

INTRODUCTION

Respiratory muscle training (RMT) has been shown to improve physical performance in both healthy individuals (Illi *et al.*, 2012; HajGhanbari *et al.*, 2013) and different groups of patients (Gosselink *et al.*, 2011; Smart *et al.*, 2013). The mechanisms behind the improved physical performance following RMT, however, are not yet fully understood. One of the most accepted models connecting respiratory muscle performance to whole-body exercise performance is the so-called respiratory muscle metaboreflex which states that during intense exercise fatiguing respiratory muscles elicit metaboreflex feedback leading to sympathetically mediated vasoconstriction (St Croix *et al.*, 2000; Sheel *et al.*, 2001) also in locomotor muscles, limiting oxygen supply and exacerbating contractile fatigue (Romer *et al.*, 2006; Wüthrich *et al.*, 2013). In support of this model, loading of the respiratory muscles has been shown to result in increased deoxygenation (Turner *et al.*, 2013) – a proxy for O_2 extraction – of the respiratory and leg muscles, while respiratory muscle unloading translated into improved exercise performance (Harms *et al.*, 2000). Interestingly, a sex difference in the development of respiratory muscle fatigue was observed with inspiratory muscles of females being more fatigue resistant than those of males during high intensity cycling (Guenette *et al.*, 2010), but no study systematically compared changes in leg muscle oxygenation during the course of heavy exercise. The current hypothesis is therefore that respiratory muscle endurance training (RMET) increases resistance of respiratory muscles to fatigue during high intensity cycling (Verges *et al.*, 2007), delaying or reducing the respiratory metaboreflex, consequently delaying or reducing vasoconstriction and thus reducing perfusion of leg muscles, thereby delaying leg muscle fatigue (Wüthrich *et al.*, 2013) and thus improving cycling performance.

However, the respiratory metaboreflex may not be the only mechanism to improve exercise performance after RMT. While Johnson *et al.* (1993) suggested that respiratory muscles develop fatigue and thus trigger the metaboreflex only during high intensive cycling above a threshold of 85% peak oxygen consumption ($\dot{V}O_{2peak}$), Illi *et al.* (2012) observed in a systematic review of all available RMT studies that improvements in exercise performance are larger with lower exercise intensity, even below the suggested 85% $\dot{V}O_{2peak}$ threshold. One possible factor contributing to the improvement could be a reduction of respiratory sensation during similar work of breathing after RMT (Gething *et al.*, 2004; Verges *et al.*, 2007; Mickleborough *et al.*, 2010).

As with whole-body exercise training, different RMT modalities exist, i.e. inspiratory only, expiratory only or combined. RMT is either performed at high force and low velocity, similar to strength training, or at low force and high velocity, similar to endurance training. It is known, also for respiratory muscles, that training-specific muscular adaptations can be expected (Leith & Bradley, 1976), i.e. inspiratory respiratory muscle strength training (IMT) was shown to improve specifically maximal respiratory muscle force (Griffiths & McConnell, 2007) while respiratory muscle endurance training (RMET) improves endurance related parameters (Verges *et al.*, 2009). To which degree a combination of both, resistive and endurance training of inspiratory and expiratory muscles, would provide an even larger stimulus and thus improvement in exercise performance, as suggested by Illi *et al.* (2012), had not been systematically assessed in comparison to an established protocol.

Recently, we have developed a new RMT regimen combining both moderate to high flows and high pressures, termed respiratory muscle sprint-interval training (RMSIT). RMSIT is characterized by 6x 1 min intervals of maximal respiratory sprint efforts against a resistance, interspaced by 1 min of rest (adapted from Wüthrich et al., 2015), and is therefore much less time consuming than RMET (11 min vs. 30 min for RMET). We could show that one month of RMSIT improved respiratory muscle performance in a test where inspiratory and expiratory muscles were loaded with high flow and increasingly high resistance (Chapter 5). The improvement was similar to that seen after one month of RMET.

Thus, in the current study we aimed to test whether the improvement in respiratory muscle performance after RMSIT translate into improved whole-body exercise performance to a similar degree as seen with conventional RMET. To better understand the effects of one month of RMT on the metaboreflex during high intensity cycling, a constant-load, work-matched cycling test at 90% maximal power output (90% W_{max} CLT) before and after either a period of RMSIT, RMET or placebo training (SHAM) was performed. Surface electromyography (EMG) and near infrared spectroscopy (NIRS) and were used to investigate muscle activity and muscle deoxygenation (HHb) of M. sternocleidomastoideus (STERNO), Mm. intercostales externi and interni (INTER) and M. vastus lateralis (VAST). Further, exercise-induced inspiratory muscle and quadriceps fatigue were assessed before and after the training period via direct nerve stimulation evoking a twitch muscle contraction. To investigate potential differences

in whole-body performance at lower exercise intensity in response to RMSIT, RMET and SHAM and potential sex differences, a 12-km time trial (TT) was performed. We hypothesize that one month of RMSIT and RMET would reduce the development of respiratory muscle fatigue and thus i) decrease leg and accessory respiratory muscle deoxygenation, ii) decrease inspiratory and leg muscle fatigue after a work-matched CLT and iii) increase TT performance to the same extent. In addition, we hypothesized that females show iv) less inspiratory muscle fatigue at the point of exhaustion before training and therefore v) improve TT performance to a lesser degree than males.

METHODS

Subjects

Thirty-three healthy, moderately trained women and men were matched in groups of three according to their sex, age and aerobic capacity and then members of these groups were randomly assigned to one of three groups, RMSIT, RMET or SHAM (Table 6.1). Subjects refrained from strenuous physical activity for 48 h prior to each visit, slept for at least 7 h the night before the visit and abstained from caffeinated beverages on visit days. In addition, all visit days were separated by at least 48 h. After a thorough explanation of the study requirements, subjects gave their written informed consent, the study was approved by the local ethics committee and was performed according to the Declaration of Helsinki 2008. Subjects were requested to log their usual training and physical activity and to keep it constant over the study period and to record heart rate during each training (Polar Electro, Kempele, Finland). This information was checked at each visit to ensure compliance.

Table 6.1: Subjects Characteristics

	RMSIT		**RMET**		**SHAM**	
	males	females	males	females	males	females
n	5	5	6	6	5	6
Age [yrs]	24 ± 3	24 ± 3	29 ± 8	24 ± 2	27 ± 6	27 ± 7
Height [cm]	177 ± 7	167 ± 8	177 ± 4	164 ± 2	178 ± 7	168 ± 6
Weight [kg]	71 ± 11	58 ± 3	74 ± 6	58 ± 6	72 ± 12	61 ± 4
$\dot{V}O_{2peak}$ [mL·min^{-1}·kg^{-1}]	55 ± 12	44 ± 8	53 ± 8	43 ± 8	58 ± 12	45 ± 7
W_{max} [W]	303 ± 79	204 ± 37	295 ± 51	202 ± 56	300 ± 54	207 ± 43

Values are mean ± SD. n, number of subjects; $\dot{V}O_{2peak}$; peak oxygen consumption during incremental cycling test; W_{max}, maximal power during incremental cycling. RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training; SHAM, sham-training.

Overview of study protocol

Subjects reported to the laboratory on a total of 7 occasions. On the 1st visit, lung function, MIP and MEP were measured. 15 min following the assessments of respiratory muscle strength, subjects performed an incremental cycling test until volitional exhaustion. Lastly, after 15 min of rest,

participants were familiarized with femoral nerve stimulation (FNS) and cervical magnetic stimulation (CMS) protocols. On the 2nd visit, participants performed a 90% W_{max} CLT until volitional exhaustion and after 30 min of recovery performed the TT for familiarization purpose. The 3rd visit contained the same exercise test as the 2nd with additional fatigue measurements. At baseline and 2 min after the CLT, subjects were subject to FNS and 10 min after cycling, CMS was applied. During the CLT, STERNO, INTER and VAST activity and HHb were measured. TT was started 30 min after the end of the CLT. On the 4th visit pre-training values of lung function and respiratory muscle strength were assessed. In addition, participants were familiarized with their personal respiratory training intervention and they performed a first training in the laboratory. For the following one month, subjects trained at home with the middle training supervised in the laboratory (5th visit). The 6th visit consisted of the same lung function and respiratory muscle strength assessments as on the 4th visit. The 7th visit consisted of the same tests as performed on the 3rd visit with the only difference that CLT was stopped after the individual maximal cycling duration of the 3rd visit (iso-time).

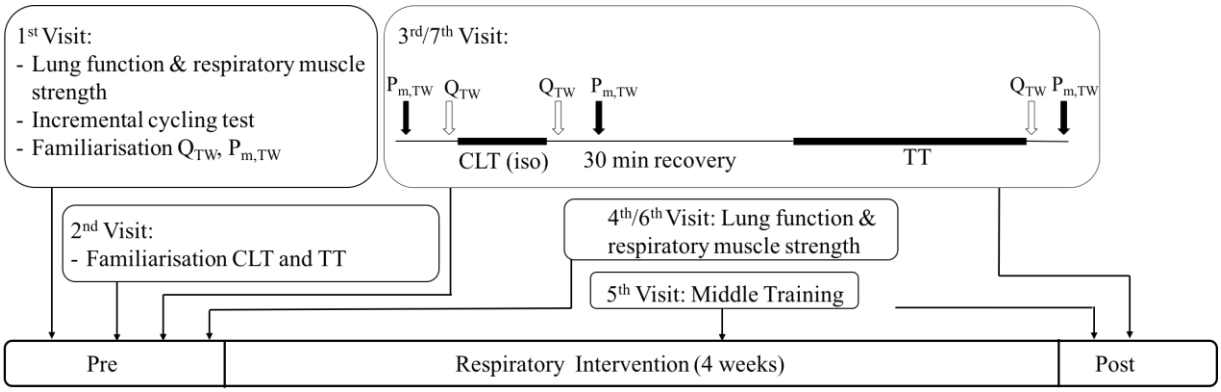

Figure 6.1: Study overview. Q_{TW}, Quadriceps twitch; $P_{m,TW}$, mouth pressure twitch; CLT, 90% W_{max} constant load cycling test; TT, 12-km time trial cycling test.

Lung function and mouth pressure measurements

Lung function was measured according to the current guidelines (Miller *et al.*, 2005), using a metabolic cart with a calibrated volume sensor (Oxycon Pro; Jaeger, Höchberg, Germany). Maximal mouth pressures were measured using a handheld mouth pressure meter (Micro RPM; Micro Medical Ltd., Rochester, United Kingdom). Participants performed a minimum of three manoeuvres or until values did not improve anymore but they alternated MIP and MEP assessments such that no more than three

subsequent MIP or MEP manoeuvers were performed. The larger of the two highest values (differing by ≤ 5%) was selected as the maximum. Variables of pulmonary function and respiratory muscle strength are reported both as absolute and as a percentage of predicted values (Wilson *et al.*, 1984; Quanjer *et al.*, 2013).

Perception of respiratory exertion and breathlessness

Perception of breathlessness (BR) and respiratory exertion (RE) were assessed by means of a visual analog scale ranging from 0 (none) to 10 (maximal breathlessness/exertion). To ensure a proper understanding of the terms, subjects were extensively questioned about their prior experience with different respiratory sensations (Lansing *et al.*, 2000). Thereafter, a definition was given for respiratory exertion ('how hard it is to breathe') and clearly distinguished from breathlessness (the sensation of 'not getting enough air').

Incremental cycling test

Before starting the incremental cycling test, subjects sat on a bicycle ergometer (Ergoline 900, Ergoline, Blitz, Germany) for 5 min, with a nose-clip and mouthpiece in place, connected to the metabolic cart; then, cycling began at 100 W for men and at 70 W for women. Subsequently, the load was increased by 30 W every 2 min until exhaustion. Subjects chose their preferred pedalling frequency (between 70 rpm and 100 rpm) at the beginning of the incremental test, which was then held constant during the remainder of the test. The test was finished when the subject stopped or when the cadence dropped below 70 rpm.

Ventilation and gas exchange were measured breath by breath by the metabolic cart. Heart rate (HR) and oxygen saturation (SpO_2) were recorded as consecutive 4-s averages (Nellcor; Covidien, Mansfield, MA) and aligned with metabolic cart data. $\dot{V}O_{2peak}$ was defined as the highest 30-s $\dot{V}O_2$ average in the cycling test.

Constant-load cycling test

Subjects performed a CLT on the same bike ergometer as the incremental test. Before starting the test, subjects sat quietly for 5 min in order to determine baseline values. Subsequently, subjects cycled at 40% W_{max} for 2 min, followed by 2 min at 60% W_{max}, and then at 90% W_{max} to volitional exhaustion. Subjects were instructed to maintain the same pedalling frequency as for the incremental bicycle test. If a subject's pedalling frequency fell below 70 rpm, the test was terminated (for two female subjects, termination frequency was adapted to 60 rpm since their preferred pedalling frequency was at 63 rpm and 65 rpm). Muscle activity was measured via EMG. Bipolar surface electrodes were placed on the skin above the muscle belly of the right STERNO, INTER and VAST according to SENIAM recommendations (Surface Electromyography for the Non-Invasive Assessment of Muscles). EMG signals were recorded at a sampling frequency of 1500 Hz using TELEmyo DTS sensors (Noraxon, Scottsdale, AZ, USA). The electrocardiogram was filtered with a bandpass filter of 10-500 Hz, rectified and smoothened with root mean square (RMS) of 100 ms using the inbuilt function of the Noraxon software. For VAST RMS signals (RMS_{VAST}) active and inactive periods during cycling were distinguished with a threshold of 0.02 µV and a minimal inactive duration 0.2 s and only active periods were used for further analysis. EMG signals were normalized to the activity of the first 20% of the CLT. In order to measure changes in HHb of the STERNO, INTER and VAST, NIRS optodes (OxyMon MK III; Artinis Medical Systems B.V., Zetten, Netherlands) were used. A transmitter and a receiver optode, interspaced by 4 cm, were attached to the skin above the muscle belly of the STERNO, INTER and VAST as a mirror image of the EMG placement. The transmitter optodes emitted impulses of infrared light at the wavelength of 761 nm and 855 to 858 nm however only signals at wavelengths 761 nm were analysed for HHb measurements which represents muscle oxygen extraction (Ferreira *et al.*, 2007). NIRS values are expressed as changes relative to the first 20% of the CLT. Ventilation and gas exchange were measured breath by breath by the metabolic cart (Oxycon Pro; Jaeger, Höchberg, Germany). During rest, every 2 min during the test and at exhaustion, subjects were asked to rate their perception of BR, RE, and LE. Right after, 20 µl of arterialized capillary blood was taken from an earlobe for blood lactate analysis (BiosenC-line Sports, EKF-diagnostics, Barleben, Germany). After

the training period, subjects performed an identical CLT but they were stopped at iso-time, i.e. after the duration of the individual pre-training CLT.

Time trial

A 12-km time trial (TT) was performed on a road bicycle equipped with a calibrated SRM system (Power MTB, Schoberer Rad Messtechnik SRM, Jülich, Germany) placed on an indoor cycle-trainer (Tacx Basic Cycle Force; Tacx, Wassenaar, Nederlands). Subjects were allowed to freely adjust their cycling power and they had a visual feedback of the kilometres cycled throughout the test. Ventilation and gas exchange were measured breath by breath by a metabolic cart (Oxycon Pro; Jaeger, Höchberg, Germany). At baseline and every 2 km, subjects were verbally encouraged and were asked to rate their perception of BR, RE and LE. Right after, 20 µl of arterialized capillary blood was taken from an earlobe for blood lactate analysis.

Femoral nerve stimulation

Quadriceps muscle force was determined with electrical FNS with simultaneous assessment of quadriceps twitch torque (Q_{TW}). After 5 min of cycling at 70 W to warm up, subjects were seated in a semi-supine position with knees flexed at 90°, fixed with a waist strap and the ankle of the dominant leg fixed with non-elastic strap to a force transducer (Strain gauge LC4102-K060; A&D CO, Tokyo, Japan). The femoral nerve was stimulated via a Grass S48 Stimulator of 0.5 cm diameter (GRASS Instruments, West Warwick, RI, USA), placed on the femoral triangle and repositioned systematically to determine the position resulting in the larges Q_{TW}, with a receiving electrode placed at the lower end the M. gluteus maximus. This position was marked and used throughout the visit. Electrical current was increased until Q_{TW} was maximal and a plateau was reached with increasing current. To ensure supramaximal stimulations, the intensity was set at 115% of the value where the force plateau was reached. First, subjects performed three 5-s voluntary contractions at intensities perceived as 40%, 60% and 80% to slowly approach their maximal voluntary contraction (MVC). Subsequently, subjects performed three 5-s MVCs with 5-s breaks in between to ensure quadriceps was potentiated, since potentiated twitches are shown to be a more sensitive index of contractile fatigue compared to un-

potentiated twitches (Kufel et al., 2002). In the relaxed state, after the MVCs, the femoral nerve was stimulated by double stimulations (doublets) at 100 Hz and 10 Hz and with a single stimulation. This was followed by two blocks consisting of a single MVC followed by three stimulations, one 100 Hz doublet, one 10 Hz doublet and one single stimulation. During the entire protocol, subjects were instructed to keep their arms crossed over their chest to prevent them pushing with their arms. After the CLT, subjects only performed the FNS protocol. Mean torque response was obtained for each type of stimulation, i.e. the average of three 100 Hz doublets (Q_{TW100}), three 10 Hz doublets (Q_{TW10}), and three single stimulations (Q_{TW1}). Quadriceps muscle fatigue (ΔQ_{TW}) was calculated as the percent-change from baseline to post-exercise and compared pre to post training. Sarcolemma membrane excitability of the quadriceps for each stimulation was determined by means of peak-to-peak amplitudes of the compound muscle action potential (M-wave) measured with bipolar surface electrodes (Noraxon TeleMyo DTS; Noraxon, Scottsdale, USA) placed on the muscle belly. Prior to electrode placement, the skin was shaved, treated with abrasive paste (OneStep AbrasivPlus; H+H Medizinprodukte, Münster, Germany) and degreased with alcohol. Signals were recorded at a sampling frequency of 1500 Hz and DD-converted by PowerLab (ADInstruments, Castle Hill, Australia) and M-wave analysed using Matlab (MATLAB 9.1, The MathWorks Inc., Natick, MA).

Cervical magnetic stimulation

Inspiratory muscle contractility was assessed by means of CMS of the phrenic nerve using a circular 90 mm coil powered by a magnetic stimulator (MagStim 200 stimulator, Whitland, UK). Subjects were seated on a chair without backrest with a nose-clip in place and the centre of the coil was positioned at the 7th cervical vertebra. The subject's position on the chair and the coil position on the neck were marked and continuously monitored throughout the experiment. Subjects were breathing through a flow head (Pneumotach 3813, Hans Rudolph inc., Shawnee, OK, USA) attached to a differential pressure transducer (DP45-34, Validyne, Northridge, CA, USA). Flow and pressure signals were amplified (CD 19A, Validyne, North- ridge, CA, USA), A/D converted and recorded in LabChart (ADInstruments, Bella Vista, Australia) with 4 kHz sampling frequency. Before any stimulation or maximal contraction, an airway occlusion was created by an automated shutter system (Zan, Oberthulba, Germany). Prior to

baseline mouth twitch pressure measurements ($P_{m,TW}$), three volitional submaximal inspiratory contractions at functional residual capacity (FRC) against occluded airway at a perceived intensity of 40%, 60% and 80% of the maximal possible effort were performed. Subsequently, subjects performed three 5-s maximal inspirations at FRC with 5-s breaks in between to ensure potentiation, since potentiated twitches are shown to be a more sensitive index of contractile fatigue compared to un-potentiated twitches (Kufel *et al.*, 2002). In the relaxed state, after the maximal inspirations, three cervical nerve stimulation were performed. Prior to each manoeuvre, subjects inhaled until total lung capacity and then exhaled passively to FRC to ensure similar lung volume prior to each stimulation which is known to influence $P_{m,TW}$ magnitude (Hamnegard *et al.*, 1995). As soon as participants reached the point of zero flow, the shutter occluding the airway closed and subjects inhaled gently until 3 cmH_2O was reached (Windisch *et al.*, 2005). Once 3 cmH_2O was reached a single magnetic stimulation was automatically delivered. A second investigator continuously monitored the expiratory volume from total lung capacity until FRC (V_T). In case of a large deviation of expired air a fourth stimulation was applied. For each baseline (9 stimulations) the 3 highest $P_{m,TW}$ within 10% and a V_T within 15% were averaged and for post-exercise the 3 highest $P_{m,TW}$ within 10% and a V_T within 15% of baseline V_T were averaged. If the criteria were not matched the 2 highest $P_{m,TW}$ within 10% and a V_T within 15% of baseline or the 2 highest $P_{m,TW}$ within 10% with the higher twitch having V_T within 15% of baseline were chosen. Inspiratory muscle fatigue ($\Delta P_{m,TW}$) was calculated as the percent-difference between baseline to post-exercise and compared pre to post intervention. Sarcolemma membrane excitability of the diaphragm for each stimulation was determined by means of peak-to-peak amplitudes of the compound muscle action potential (M-wave) measured with bipolar silver surface electrodes (Nihon Kohden, Bad Homburg, Germany) placed in the 7^{th} or 8^{th} intercostal space on the right side of the body at the midclavicular line. Prior to electrode placement, the skin was shaved, treated with abrasive paste (OneStep AbrasivPlus; H+H Medizinprodukte, Münster, Germany) and degreased with alcohol. Signals were recorded at 4'000 Hz, pre-amplified (gain=1'000), band- pass filtered (20-1'000 Hz; Nihon Kohden, Bad Homburg, Germany; common mode rejection ratio ≥94 db), A/D converted (MacLab interface, ADInstruments, Castle Hill, Australia) and M-wave analysed using Matlab (MATLAB 9.1, The MathWorks Inc., Natick, MA).

Respiratory muscle training/sham-training

Subjects of all three training interventions recorded each training sessions in their logbook. Subjects in the RMT groups measured heart rate with a portable heart rate monitor (Polar Electro, Kempele, Finland) and were asked to rate their perceived breathlessness and respiratory exertion at the end of the training session on a scale from 0 to 10 where 0 was defined as "none" and 10 is "maximal" breathlessness or respiratory exertion. WOB and average mouth pressures (P_m) of the first and last training of the RMT groups were estimated based on reported ventilations and previous flow-pressure characteristics of the training device setup used in these training sessions.

RMSIT

Subjects in the RMSIT group completed 12 training sessions, each lasting 11 min, over one month. They performed three training sessions per week with at least 48 h rest between any 2 training sessions. Each training consisted of 6 cycles of 1 min respiratory sprint with 1 min break in between 2 cycles. During the sprints, subjects breathed through the SpiroTiger® device (idiag, Fehraltorf, Switzerland) with an added resistance introduced by adding an orifice with reduced diameter between the mouthpiece and device to maximize respiratory muscle work. The smallest diameter with which subjects were able to sustain a f_B of 30 breaths·min^{-1} with a V_T of 60% FVC was used in the first training session. If respiratory exertion was ≤ 8 points after a sprint (on a 0-10 scale), subjects were instructed to increase f_B of the next sprint by 1 breath·min^{-1}. If respiratory exertion was 9 or 10, subjects were instructed to keep f_B constant. If subjects were not able to hold the frequency over the 1-min sprint, they were instructed to reduce f_B by 1 breath·min^{-1} for the next sprint while keeping V_T constant. If subjects reached an f_B of 35 breaths·min^{-1}, an orifice with a smaller diameter was inserted and f_B was reduced to 30 breaths·min^{-1}.

RMET

Subjects of the RMET group completed 20 training sessions, each lasting 30 min, over 30 days. Two consecutive days of training were followed by a day of rest, as was shown to be effective in previous studies (Verges *et al.*, 2007). Subjects performed volitional, normocapnic hyperpnoea using the

SpiroTiger® device (idiag, Fehraltorf, Switzerland). The target ventilation of the first training session was set to 60% of the individual's maximal voluntary ventilation (MVV_{12}). The duty cycle was set to 0.5 and V_T was set to 60% of FVC with the breathing frequency (f_B) adjusted accordingly. Subjects were instructed to sustain this ventilation for 30 min. If after 25 min of breathing subjects felt that they would not be exhausted after 30 min, f_B was increased by 2 breaths·min^{-1} and the next training session began with this increased f_B. If subjects could just manage to finish the 30 min with the starting f_B, f_B of the next training session was increased by 1 breath·min^{-1}. If after 25 min, subjects felt that they could not finish the training session with this frequency, f_B was decreased by 2 breaths·min^{-1} and the next training session began with the starting f_B of the previous training session.

SHAM

Subjects of the SHAM group completed a sham training, 3-4 times a week, for 30 days using a mock asthma inhaler, HandiHaler® (Boehringer Ingelheim, Ingelheim, Germany) filled with lactose powder. Subjects were instructed to inhale the powder once and to then perform five full inspirations to total lung capacity using a self-constructed tubing system, including a small resistance.

Data analysis and statistics

For analysis of the CLT two blood lactate samples and one HR measurement are missing due to participant's intolerance to blood draw at the earlobe (RMET female), contamination of blood samples with sweat (SHAM female) and technical problems during HR monitoring. Due to intense movement of the rib cage during the CLT, some EMG and NIRS sensors moved in relation to the muscle, e.g. over the rib, as described earlier (Shadgan *et al.*, 2011), rendering the signals implausible. This resulted in a smaller number of subjects of whom EMG and NIRS recordings could be used (STERNO: RMS [RMSIT: 8, RMET: 10, SHAM, 6], HHb [8,11,8], INTER: RMS [7,11,10], HHb: [9,11,11], VAST: RMS [10,12,10], HHb [10,12,11]). Difficulties in performing the manoeuvre and/or intolerance to CMS resulted in a reduced sample of respiratory muscle twitch data (RMSIT [4f, 4m], RMET [3f, 4m], SHAM [4m, 1f]). In the TT, blood lactate data is missing in 4 subjects due to intolerance to blood draw

at the ear lobe (RMET female) or sweat contamination of blood samples (RMSIT male, RMET female, SHAM male) and HR data of one RMET female is missing due to technical problems.

Between-group comparisons of subjects' characteristics and baseline values before training were assessed with one-way ANOVA and if differences were detected, a post hoc test with Bonferroni correction was performed. Sex differences before training were compared with an independent t-test with assumed equal variance. Within each group, pre-to-post differences were assessed using paired t-tests after testing for normal distribution (Shapiro-Wilk test). For data without normal distribution, a Wilcoxon signed rank test was applied. Absolute changes from before to after training were assessed between group and sex using a two-way ANOVA. Interactions of group*sex were tested with post hoc interaction contrast that compared the difference between groups separated by sex with Bonferroni correction. Statistical analyses were performed with SPSS Statistics 23 (IBM Company, New York, NY). All data are shown as mean±SD with exception of NIRS and EMG data shown as mean ± SEM. The level of significance was set at $p < 0.05$.

RESULTS

Subjects Characteristics

Before training, the three groups did not differ in age, height, weight and $\dot{V}O_{2peak}$ (Table 6.1).

Lung function and respiratory muscle strength

Lung function and respiratory muscle strength did not differ between groups before training nor were pre-post training changes different between groups separated by sex and no group*sex effect was found Figure 6.2. Within groups, FVC, FEV_1, MIP and MEP did not change significantly. MVV_{12} increased significantly with RMET males (+7.9±6.8 $L \cdot min^{-1}$) and RMSIT females (+13.1±5.0 $L \cdot min^{-1}$). Pre-post training, MEP showed a trend towards an increase after RMSIT males and females combined (+11.9±18.4 cmH_2O; p=0.07).

Table 6.2: Lung function and respiratory muscle strength.

	RMSIT				RMET				SHAM			
	males		females		males		females		males		females	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
FVC [L]	5.6 ± 0.7	5.7 ± 0.8	4.3 ± 0.9	4.5 ± 0.8	5.9 ± 0.3	6.1 ± 0.5	4.2 ± 0.9	4.2 ± 0.6	5.9 ± 0.8	6.0 ± 0.8	4.4 ± 0.3	4.3 ± 0.5
FEV_1 [L]	4.7 ± 0.5	4.8 ± 0.5	3.7 ± 0.9	3.8 ± 0.9	4.6 ± 0.2	4.7 ± 0.2	3.5 ± 0.6	3.5 ± 0.5	4.8 ± 0.7	4.9 ± 0.6	3.4 ± 0.3	3.3 ± 0.3
MVV_{12} [$L \cdot min^{-1}$]	201.4 ± 31.8	207.3 ± 27.9	146.5 ± 31.7	159.7 ± 31.4*	195.0 ± 7.7	202.9 ± 7.7*	132.5 ± 14.7	141.6 ± 22.4	214.9 ± 28.9	223.8 ± 31.2	129.9 ± 13.7	128.7 ± 6.9
MIP [cmH_2O]	128.0 ± 11.4	139.6 ± 26.1	129.6 ± 39.3	129.4 ± 34.1	153.2 ± 31.2	160.3 ± 40.6	112.3 ± 25.4	121.3 ± 16.9	139.8 ± 27.2	143.8 ± 14.0	108.7 ± 21.0	116.3 ± 18.2
MEP [cmH_2O]	179.6 ± 31.0	194.6 ± 20.0	158.8 ± 29.7	167.6 ± 37.7	203.3 ± 36.0	194.7 ± 52.6	150.5 ± 39.6	172.8 ± 57.6	206.6 ± 58.5	211.6 ± 54.9	160.0 ± 39.1	160.3 ± 32.0

Values are mean ± SD. RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training; SHAM, sham-training; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; MVV_{12}, maximal voluntary ventilation in 12 s; MIP, maximal inspiratory pressure; MEP, maximal expiratory pressure; *$p<0.05$ pre vs. post within groups.

Constant load cycling test

After the training period, two females in the RMET group and 3 males in the SHAM group did not reach iso-time (Δtime: RMET: -1.4±0.5 min, SHAM: -2.0±0.3 min). For these participants, pre vs. post comparisons were made in that for the pre-test, only data up to the time of exhaustion in the post-test was analysed, i.e. post-tests defined iso-time duration.

Before training, neither the entire groups nor sex-specific subgroups (data not shown) differed in average ventilation, gas exchange, HR, blood lactate concentration, subjective ratings and workload during cycling. CLT intensity was different before training in RMSIT and RMET compared to SHAM (RMSIT: 96.3±5.4% $\dot{V}O_{2peak}$ vs. SHAM: 88.6±4.8% $\dot{V}O_{2peak}$; p=0.003, RMET: 93.9±4.6% $\dot{V}O_{2peak}$ vs. SHAM; p=0.045). No differences in training-specific changes were detected for gas exchange, HR and BR. However, $\dot{V}_E$ showed a group*sex effect (p=0.013) with a significant decrease of $\dot{V}_E$ after training in RMSIT males (-8.2±9.5 $L \cdot min^{-1}$) compared to SHAM males (+12.0±10.6 $L \cdot min^{-1}$; p=0.003) while the change in RMET males (+3.5±9.5 $L \cdot min^{-1}$) was not different from SHAM (p=0.642).-Changes in subjective ratings were not different between groups despite of RE and LE decreasing significantly within the RMSIT group, independent of sex. Changes in blood lactate concentration did not differ between groups but in the RMET group, a sex-independent small but significant reduction in blood lactate concentration (-0.9±1.2 $mmol \cdot L^{-1}$; p=0.043) was detected, associated with a significant within-group decrease in V_T in the RMET group (0.1±0.2 L; p=0.031) and the SHAM group (0.1±0.1 L; p=0.032). Overall, RMS and HHb did not change with training in any of the groups, independent of sex during the CLT, as shown in Figure 6.2.

Table 6.3: Ventilation, gas exchange, heart rate, blood lactate concentration and subjective ratings during constant-load cycling.

	RMSIT		RMET		SHAM	
CLT	Pre	Post	Pre	Post	Pre	Post
$\dot{V}_E$ [L·min^{-1}]	104.0 ± 26.6	100.0 ± 21.9	97.5 ± 23.3	100.6 ± 26.4	92.9 ± 13.7	96.3 ± 21.2
V_T [L]	2.6 ± 0.2	2.6 ± 0.2	2.7 ± 0.7	2.8 ± 0.8*	2.6 ± 0.6	2.7 ± 0.7*
f_B [min^{-1}]	41.6 ± 6.2	38.7 ± 7.2	36.8 ± 6.9	36.4 ± 6.7	36.7 ± 5.6	36.4 ± 4.6
$\dot{V}O_2$ [mL·min^{-1}]	3075.3 ± 943.8	3031.2 ± 892.5	2984.4 ± 842.0	2977.4 ± 868.1	2955.8 ± 636.2	2955.7 ± 685.3
$\dot{V}CO_2$ [mL·min^{-1}]	3393.9 ± 993.9	3325.9 ± 931.0	3334.9 ± 878.5	3322.5 ± 886.7	3303.2 ± 689.6	3424.1 ± 866.3
$P_{ET}CO_2$ [mmHg]	36.1 ± 2.9	36.4 ± 4.8	37.3 ± 2.0	36.3 ± 3.2	38.6 ± 4.2	38.6 ± 1.0
HR [min^{-1}]	174.6 ± 4.9	178.8 ± 6.9	168.1 ± 9.3	169.6 ± 9.3	172.1 ± 9.3	174.2 ± 7.4
Lactate [mmol· L^{-}	6.3 ± 1.1	6.0 ± 1.2	6.3 ± 1.0	5.5 ± 1.2*	7.0 ± 2.6	6.2 ± 1.6
BR [point]	0.8 ± 0.8	0.7 ± 1.2	0.8 ± 1.2	0.4 ± 0.6	1.7 ± 1.5	1.3 ± 1.5
RE [point]	3.2 ± 1.3	2.4 ± 1.7*	2.6 ± 1.3	2.2 ± 1.3	2.7 ± 1.3	2.3 ± 1.3
LE [point]	4.7 ± 0.9	4.2 ± 1.1*	3.8 ± 0.7	3.6 ± 1.0	4.3 ± 1.0	3.4 ± 1.1*

Values are mean ± SD. RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training, SHAM, SHAM-training; $\dot{V}_E$, ventilation; V_T, tidal volume; f_B, breathing frequency; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide elimination; $P_{ET,CO2}$, End-tidal carbon dioxide pressure; HR, heart rate; BR, breathlessness; RE, respiratory exertion; LE, leg exertion. *p<0.05 pre vs. post within groups.

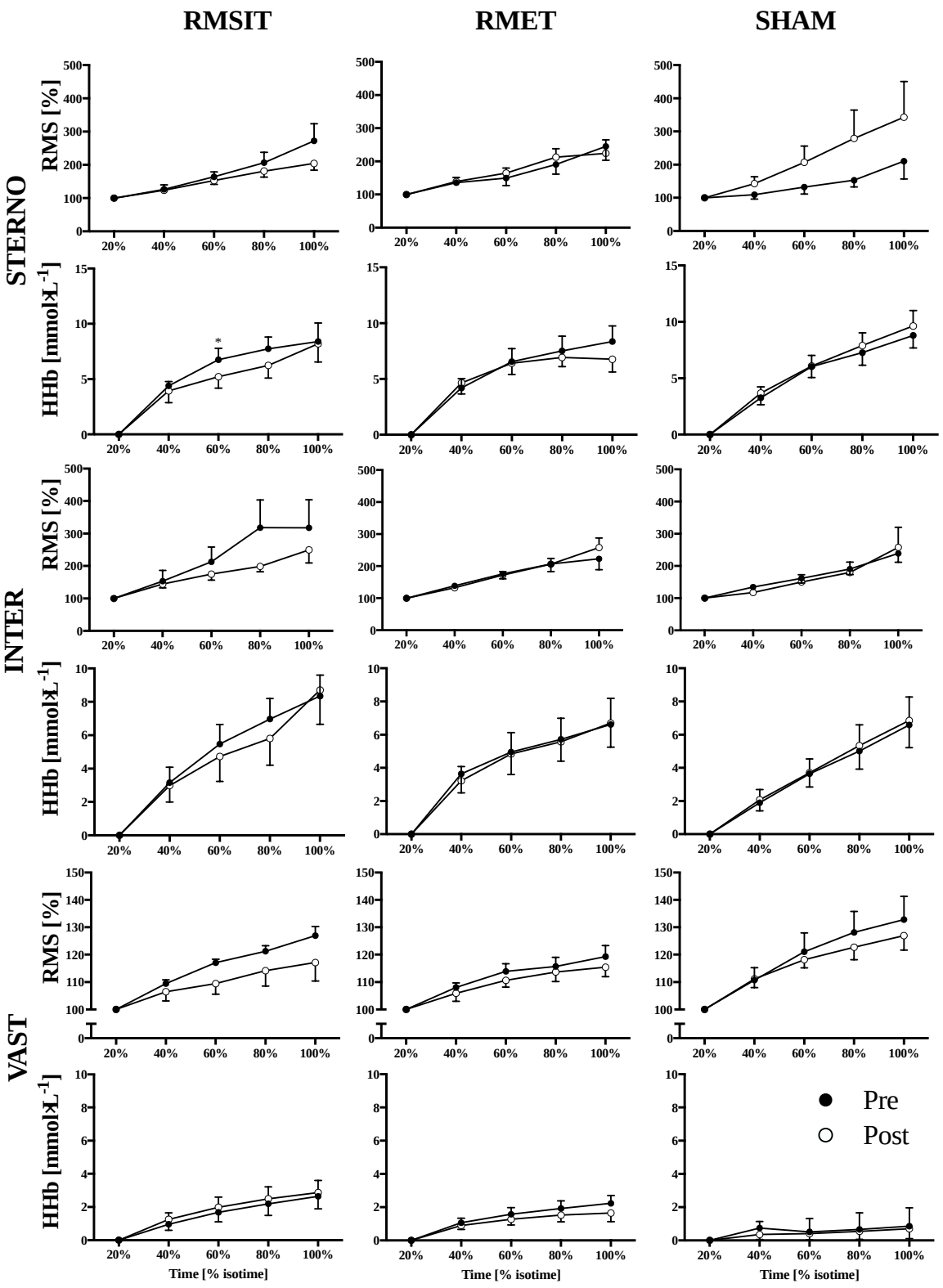

Figure 6.2: Muscular activity and deoxygenation time-course during constant load cycling test. Values are mean ± SEM. RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training; SHAM, sham-training; RMS, electromyography root mean square; HHb, deoxygenated haemoglobin. *p<0.05 pre vs. post within groups.

Individual data of leg and inspiratory muscle fatigue are presented in Figure 6.3. Before training, no difference was present between sex, neither for any of the three ΔQ_{TW} nor for $\Delta P_{m,TW}$ (females: -13.6±8.1%, males: -15.3±13.8%; p=0.736).

No difference within groups and no difference in changes between groups were found in vastus and diaphragm M-waves. Changes in ΔQ_{TW} and $\Delta P_{m,TW}$ did not differ significantly after training, and no systematic group*sex effect was detected. However, within the RMSIT group, ΔQ_{TW10} decreased significantly (-8.1±7.9 %; p=0.011), independent of sex and a group*sex effect was detected for ΔQ_{TW1} (p=0.048) and ΔQ_{TW100} (p=0.042). An analysis separated by sex showed a tendency towards a smaller degree of leg fatigue in RMSIT males (ΔQ_{TW1}: -9.4±10.1%; ΔQ_{TW100}: -7.8±9.8 %) compared to the other groups (ΔQ_{TW1}: vs. RMET +7.2±11.9%; p=0.052; vs. SHAM +8.8±9.2%; p=0.093; ΔQ_{TW100}: vs. RMET +2.0±14.3 %; p=0.386, vs. SHAM +7.6±9.0%; p=0.077). To test whether the sample size was too small despite power analysis, we pooled data of all three stimulation frequencies. This resulted in a significant decrease in $\Delta Q_{TW\ all}$ (-5.2±9.1%) in the RMSIT group which was significantly different from the decrease in RMET (+1.8±13.5%; p=0.047) but not SHAM (-2.4±14.7; p=0.995), however, with a group*sex effect. Sex-specific subgroup analyses showed the $\Delta Q_{TW\ all}$ decrease in RMSIT males (-7.9±8.7%) being significantly different from changes in both RMET (+6.4±13.2; p=0.002) and SHAM (+7.0±10.8%; p=0.002) while no difference was seen in females.

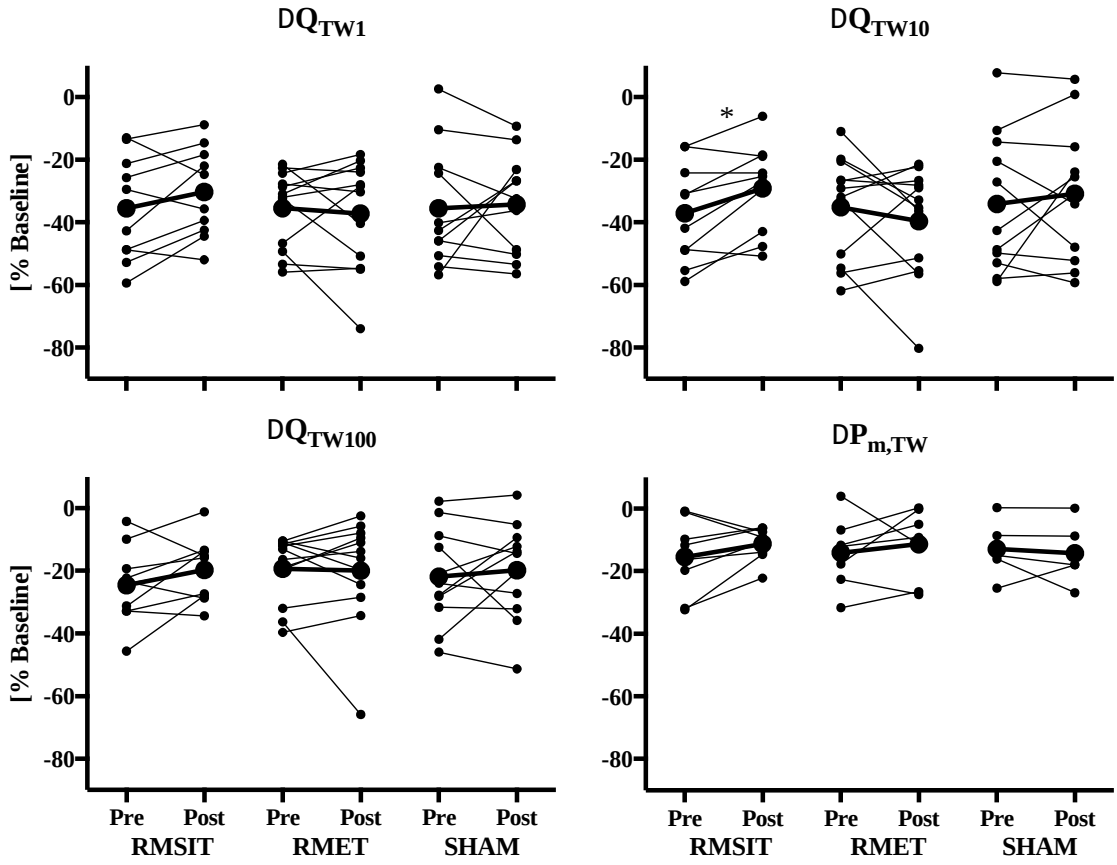

Figure 6.3: Quadriceps and inspiratory muscle fatigue after the constant-load cycling test. RMSIT; respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training; SHAM, SHAM-training; ΔQ_{TW1}, change in quadriceps twitch torque in response to a single stimulation; ΔQ_{TW10}, change in twitch torque in response to a doublet stimulation at 10 Hz; ΔQ_{TW100}, change in twitch torque in response to a 100 Hz doublet stimulation; $\Delta P_{m,TW}$, change in twitch mouth pressure in response to a single cervical magnetic stimulus. Group means are represented by a thicker line, *p<0.05 pre vs. post, within groups

Time trial cycling test

TT intensity pre training did not differ between groups (RMSIT 84.0±12.0% $\dot{V}O_{2peak}$, RMET 75.4±16.1% $\dot{V}O_{2peak}$, SHAM 76.2±7.9% $\dot{V}O_{2peak}$). Neither TT duration, ventilation and gas exchange, HR, blood lactate concentration nor ratings of BR, RE and LE differed between groups prior to training when both sexes were pooled (Table 6.4) or separately analysed (data not shown). Changes in TT duration did not differ between groups but a group*sex interaction was found (p=0.018) and the improvement was located in the male subgroup of RMET. RMET males decreased TT-duration significantly (-1.5±1.4 min; p=0.049), the decrease being significantly different from SHAM males

(+1.5±1.2 min; p=0.440) but it was not different from RMSIT males (-0.1±0.5 min; p=0.727). Individual data given by sex are provided in Figure 6.4. Also for the other variables, changes did not differ between groups despite significant increases in HR (+2.9±2.7 min^{-1}), $\dot{V}O_2$ (+225.8±340.7 $mL \cdot min^{-1}$), $\dot{V}CO_2$ (+220.4±336.8 $mL \cdot min^{-1}$) and blood lactate concentration (+0.9±0.7 $mmol \cdot L^{-1}$) within RMET, independent of sex. However, a group*sex effect was detected for blood lactate concentration (p=0.007) and a significant decrease was located after training in RMSIT males (-1.7±1.7 $mmol \cdot L^{-1}$) compared to RMET males (+0.9±0.7 $mmol \cdot L^{-1}$; p=0.056) and SHAM males (+1.5±2.1 $mmol \cdot L^{-1}$; p=0.027). In females, no difference was found between groups.

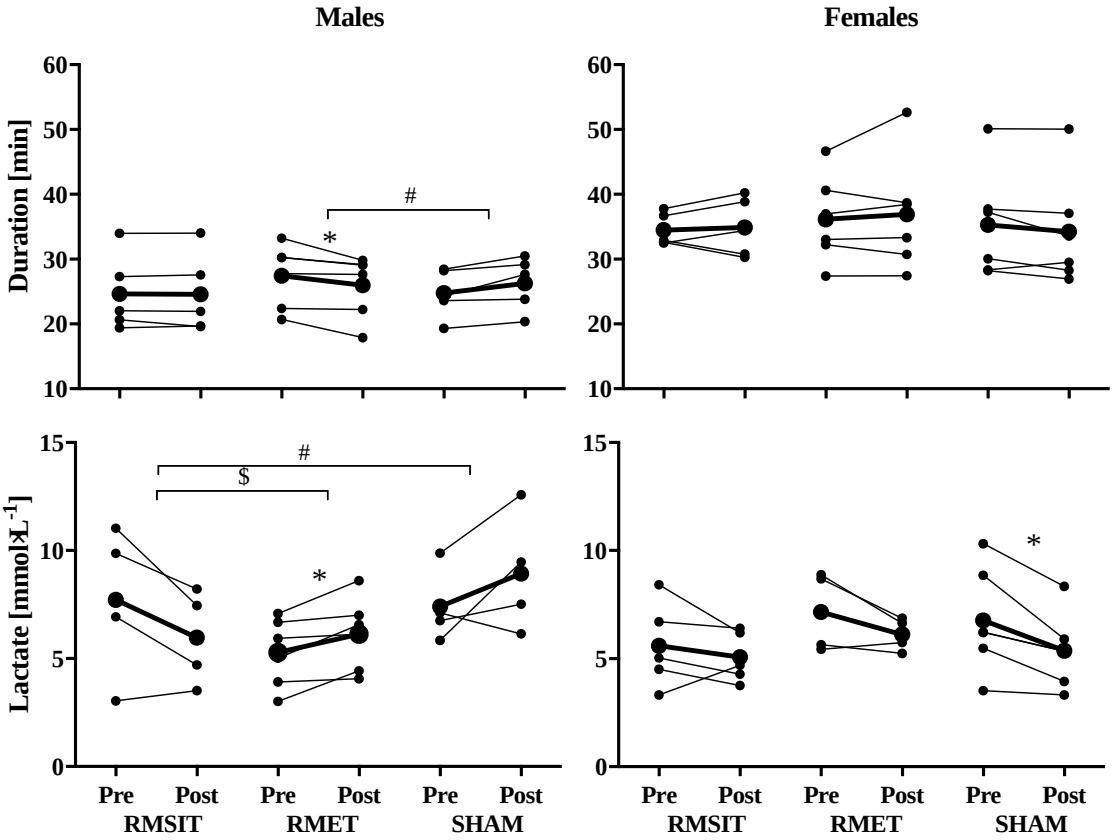

Figure 6.4: Duration and blood lactate concentration of the 12-km time trial for males and females separately. RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training; SHAM, SHAM-training. *p<0.05 pre vs. post within groups, #p<0.05 changes compared to SHAM, $p<0.05 changes compared to RMET.

Table 6.4: 12-km time trial duration -, cardiovascular - and perception of effort parameters

	RMSIT		RMET		SHAM	
TT	Pre	Post	Pre	Post	Pre	Post
Duration [min]	29.6 ± 6.8	29.7 ± 7.5	31.8 ± 7.3	31.4 ± 8.9	30.5 ± 8.4	30.6 ± 7.8
$\dot{V}_E$ [L· min^{-1}]	80.1 ± 27.8	78.7 ± 30.0	67.6 ± 19.4	78.9 ± 25.5	72.5 ± 18.6	71.8 ± 20.1
V_T [L]	2.1 ± 0.6	2.2 ± 0.5	2.0 ± 0.7	2.1 ± 0.6	2.2 ± 0.5	2.1 ± 0.6
f_B [min^{-1}]	38.7 ± 3.9	35.4 ± 4.9	34.7 ± 6.3	37.9 ± 8.9*	34.0 ± 7.8	34.8 ± 6.3
$\dot{V}O_2$ [mL· min^{-1}]	2685.6 ± 942.6	2656.5 ± 945.8	2350.0 ± 705.8	2568.5 ± 773.4*	2545.5 ± 631.8	2446.5 ± 530.1
$\dot{V}CO_2$ [mL· min^{-1}]	2573.7 ± 948.0	2531.5 ± 953.0	2200.9 ± 652.3	2417.7 ± 750.0*	2486.3 ± 575.9	2416.7 ± 542.0
$P_{ET,CO2}$ [mmHg]	34.7 ± 3.1	34.9 ± 4.5	35.5 ± 3.0	33.9 ± 4.1	35.7 ± 4.6	35.5 ± 4.1
HR [min^{-1}]	168.5 ± 5.3	169.0 ± 8.4	161.2 ± 11.9	165.3 ± 10.8*	166.2 ± 8.0	169.6 ± 5.8
Lactate [mmol· L^{-1}]	6.5 ± 2.8	5.5 ± 1.7	6.0 ± 1.9	6.1 ± 1.3	7.0 ± 2.1	6.8 ± 2.7
BR [point]	0.7 ± 0.8	0.5 ± 1.1	1.0 ± 1.3	0.5 ± 0.7	1.9 ± 2.6	1.9 ± 2.1
RE [point]	3.2 ± 1.2	2.5 ± 2.0	2.8 ± 1.4	2.6 ± 1.3	2.6 ± 2.2	2.8 ± 1.4
LE [point]	5.2 ± 1.0	5.2 ± 1.4	4.4 ± 1.3	4.4 ± 1.0	4.3 ± 1.6	4.1 ± 0.9

Values are mean ± SD. RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training; SHAM, sham-training $\dot{V}_E$, ventilation; V_T, tidal volume; f_B, breathing frequency; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide elimination; $P_{ET,CO2}$, end-tidal carbon dioxide pressure; HR, heart rate; BR, breathlessness; RE, respiratory exertion; LE, leg exertion. $^{\#}p<0.05$ changes compared to SHAM, $^{\$}p<0.05$ changes compared to RMET; *$p<0.05$ pre vs. post within groups.

DISCUSSION

In the present study, RMSIT and RMET were not associated with a generally smaller degree of respiratory and leg muscle fatigue in an intense, work-matched CLT at 90% W_{max} (93.0±5.7% $\dot{V}O_{2peak}$) and no systematic sex difference was observed. However, in males of the RMSIT group, a trend towards a smaller degree of leg muscle fatigue was observed, a potential indication of a reduced respiratory muscle metaboreflex. No changes were seen in STERNO, INTER and VAST activity and deoxygenation during CLT, which indicates that effects of RMSIT and RMET, if present, were too small to be detected by the EMG- and NIRS-technology. Performance testing at 78.3±12.8% $\dot{V}O_{2peak}$, i.e. below the postulated 85% $\dot{V}O_{2peak}$ triggering the respiratory muscle metaboreflex, showed an improvement only in males of the RMET group, while females' performance was not affected.

Respiratory muscle metaboreflex

Contrary to our hypothesis, EMG activity and muscle deoxygenation of STERNO, INTER and VAST did not change during the CLT after RMT. We had assumed that RMSIT and RMET might (in part) have similar effects on leg muscle perfusion as did respiratory muscle unloading during exercise (Harms *et al.*, 1997), i.e. increase leg blood flow and as a consequence, we postulated that leg muscle deoxygenation might be reduced since respiratory muscle loading was shown to increase leg muscle deoxygenation (Turner *et al.*, 2013). We postulated that the decrease in deoxygenation would show an increased oxygen supply to leg muscles resulting from the reduced need of oxygen extraction and as a consequence, the degree of exercise-induced leg muscle fatigue would be smaller. Similarly, we postulated that respiratory muscle deoxygenation would be decreased due to more efficient respiratory muscles since increased respiratory muscle loading during exercise was also shown to increase deoxygenation of respiratory muscles (Turner *et al.*, 2013). However, the effect was possibly beyond detection of the NIRS technique. In fact, Harms *et al.* (1997) showed that inspiratory unloading increased leg blood flow to a smaller extent than respiratory muscle loading, i.e. -63% WOB increased leg blood flow by only +0.8±0.3 $L \cdot min^{-1}$ while +28% WOB decreased leg blood flow by -1.3±0.2 $L \cdot min^{-1}$. This is in line with recent findings of Turner *et al.* (2016) showing no change in HHb in INTER and VAST when subjects were cycling with added moderate inspiratory resistive loading

after 6-weeks of IMT while heavy inspiratory resistive loading, thus massively increased WOB, showed a decreased deoxygenation in locomotor and respiratory muscles. Likewise, a possible effect that RMT may have had on the leg O_2 delivery was not sufficient to elicit a detectable change in VAST EMG activity, consistent with the lack of change in ΔQ_{TW}. Interestingly, when data of the three stimulation frequencies were pooled, the decrease in the RMSIT group was significantly different from the one in RMET with a group*sex effect showing the decrease in RMSIT males being significantly different from both, RMET and SHAM groups while no difference was seen in females. In the separate analysis by stimulation frequency, a within-group reduction in RMSIT males was observed. The change was, however, not seen in the EMG, which may be due to the high standard deviation of EMG compared to FNS or due to the smaller sample size. However, the lack of clearly significant changes in ΔQ_{TW} after training might possibly also be explained by the suggested time-course of development of peripheral fatigue during exercise. It is suggested that peripheral fatigue of respiratory and leg muscles develop early during a fatiguing exercise and then stays almost constant while the central drive is increased to sustain the work output (Decorte *et al.*, 2012; Göhl *et al.*, 2016; Hureau *et al.*, 2016). This explanation would be in line with current data, showing a much larger increase in VAST RMS between 20% and 60% of the iso-time duration (+17.7±13.4%) than from 60% to 100% (+9.1±9.7%; p=0.003) in pooled data of all subjects prior to training. Thus, a larger difference in ΔQ_{TW} pre vs. post training might be expected earlier in the test, being too small to be detected at the point of exhaustion. This would be supported by data of Wüthrich *et al.* (2013) who found no difference in ΔQ_{TW} and $\Delta P_{m,TW}$ in a 85% W_{max} cycling test when stopped 1.7±2.3 min before exhaustion compared to the measurement at exhaustion.

Interestingly, before the training phase, no sex differences were found in $\Delta P_{m,TW}$, contrary to our hypothesis that females would develop a smaller degree of respiratory muscle fatigue than their male counterparts also under present exercise conditions, as previously shown by Guenette *et al.* (2010). However, this discrepancy my result from our reduced sample size and/or the different measurement technique [mouth twitch pressure vs. transdiaphragmatic twitch pressure ($P_{di,TW}$)]. $P_{m,TW}$ is in fact known to be more variable than $P_{di,TW}$ (Kabitz *et al.*, 2007).

A small but significant reduction in average blood lactate concentration within the RMET group was detected during the CLT which is in line with previous findings (Verges *et al.*, 2008). However, this reduction was not different from changes in the other groups. Lactate production by the diaphragm has been identified as a potential mediator of the respiratory muscle metaboreflex (Rodman *et al.*, 2003), thus a reduction of the blood lactate concentration with RMT might reduce the respiratory muscle metaboreflex. However, blood lactate concentration did not correlate with ΔQ_{TW}, VAST HHb and VAST RMS in the present study (data not shown).

Finally, perception of both RE and LE decreased within the RMSIT group although this decrease was not different from the changes of RMET and SHAM. However, the reduced perception of exertion would fit with the decrease in pooled ΔQ_{TWall}, indicating a change in the respiratory metaboreflex. The lack in decreased perception of RE in the RMET group, however, is in contrast to previous studies showing a reduction in RE after RMET (Verges *et al.*, 2007, 2008). This discrepancy may possibly result from the different relative intensity of the CLT, as Verges *et al.* (2008) used 70% W_{max} for untrained and 85% W_{max} for trained subjects whereas in the current study 90% W_{max} was chosen. Changes in respiratory sensations were shown to result form i) changes in force production of the respiratory muscles relative to their maximal force (Redline *et al.*, 1991) and/or ii) increased fatigue resistance of respiratory muscles during exercise performance (Verges *et al.*, 2007; Wüthrich *et al.*, 2013). However, in the current study no correlation was found between absolut changes of RE and MIP, MEP or $\Delta P_{m,TW}$ (data not shown).

Time trial performance

Surprisingly, only TT performance in RMET males improved although changes in leg muscle fatigue suggest no change in the respiratory metaboreflex in this group. However, the improvement is in line with the systematic review by Illi *et al.* (2012) on studies mainly including males, showing that improvements in exercise performance after RMT are larger with lower intensity exercise, reaching intensities below the postulated threshold of 85% $\dot{V}O_{2peak}$ needed to trigger the respiratory muscle metaboreflex (Johnson *et al.*, 1993). Illi *et al.* (2012) suggested that a combination of reduced respiratory muscle fatigue and reduced RE may lead to improved exercise performance. However, in

the current study, respiratory muscle fatigue and RE assessed at 90% W_{max} in the CLT did not differ. Similarly, RE was not different in RMET males during the TT but a change in RE was possibly masked by a trend towards higher $\dot{V}_E$ (+29.0±39.9%; p=0.09) resulting in an increased WOB both associated with the increased performance.

Surprisingly, and in contrast to our hypothesis, RMSIT did not improve TT performance, independent of sex. However, this result should be interpreted with caution since the change in blood lactate concentration during the TT was significantly different to changes in RMET and SHAM, with a tendency towards lower concentrations. This may reflect an increased aerobic metabolism and/or higher efficiency of the respiratory and/or leg muscles at similar speed in agreement with changes observed in the CLT. Nevertheless, this potential change in metaboreflex did not result in an improved TT performance in the RMSIT group, independent of sex.

A possible explanation for the difference in TT performance between RMSIT males and RMET males may be the difference in training volume, while RMSIT participants performed only 12 training sessions of 11 min, participants in the RMET group performed 20 training sessions of 30 min each, over the duration of one month. This is in line with Fairbarn *et al.* (1991) and Verges *et al.* (2008) who reported increases in respiratory muscle endurance without a change in cycling performance with reduced training volume of either only 16 sessions of intermitted RMET (3x 8 min isocapnic hyperpnoea training alternated with 8 min breaks) or twenty 15-min training sessions. On the other hand, many studies using 2x 30 inspirations daily with added resistance, i.e. 420 inspirations/week showed improved exercise performance after one month of training (Romer *et al.*, 2002; Bailey *et al.*, 2010). Possibly, RMSIT trained daily might yield similar results.

The general lack of improvement in females is in agreement with Guenette *et al.* (2010) who showed inspiratory muscles of females to be less fatigable than those of males during high intensity cycling despite increased respiratory mechanical constraints resulting from smaller airways relative to total lung size (Thurlbeck, 1982). A possible explanation might be the repetitive exposure to high levels of respiratory muscle work during exercise in females, resulting in increased resistance to fatigue. Thus respiratory muscles may already be more trained in females than in males and therefore be less important as a limiting factor for exercise performance. If this was the case, on the other hand, it would

be expected that females would also show less $\Delta P_{m,TW}$ after exercise compared with males, which was not the case in the current study.

Limitations of the study

Difficulties of some subjects with performance of $P_{m,TW}$ manoeuvres let to a very small sample size which compromised the power to locate sex-specific differences in $\Delta P_{m,TW}$. Furthermore, because of Q_{TW} measurements proceeding $P_{m,TW}$ assessments and the need for calm, controlled breathing, $P_{m,TW}$ assessments were performed after 10 min of recovery after CLT. However, many previous studies, though assessing diaphragmatic fatigue by means of balloon catheters, showed respiratory muscle fatigue after 10 and more minutes (Johnson *et al.*, 1993; Walker *et al.*, 2011). No invasive measures to assess blood flow like thermodilution for leg blood flow or indocyanin green for local muscle blood flow were used that would have given more direct insight into blood flow redistribution.

Conclusion

In the present study, RMSIT but not RMET was associated with a generally smaller degree of leg muscle fatigue in an intense, work-matched CLT at 90% W_{max}, indicating a diminished metaboreflex. Potentially associated physiological changes due to a reduced respiratory muscle metaboreflex on a muscular level were inconclusive. RMET but not RMSIT improved TT performance, a lower intensity exercise test. Possibly, the training load of RMSIT, adapted from whole-body, high-intensity interval training, was too small to affect TT performance. Overall, mainly males showed selected improvements while females did not change.

7. VALIDATION OF RESPIRATORY MUSCLE OXYGEN CONSUMPTION CALCULATIONS DURING NORMOCAPNIC HYPERPNOEA – PILOT STUDY

ABSTRACT

Measurement of oxygen consumption ($\dot{V}O_2$) is a well-established method in exercise physiology to evaluate aerobic metabolism and exercise capacity. However, with partial rebreathing of expired air and added resistance during loaded normocapnic hyperpnoea, causing very fast changing gas composition and pressures during breathing, correct calculation of $\dot{V}O_2$ are not possible with commercially available devices.

Therefore, the purpose of this pilot study was to develop a test setup to evaluate $\dot{V}O_2$ during normocapnic hyperpnoea with added resistance and verify this with the gold standard, i.e. the Douglas bag method. Sensors to assess humidity, temperature, pressure, gas fraction and flow were placed in series before a rebreathing device that was connected to a Douglas bag. Five healthy young males (age: 28±3 y) performed 4-5 different breathing tasks with constant tidal volume, different breathing frequencies, with or without added resistance. During 2 min of each breathing task, exhaled air was collected in the Douglas bag and analysed for volume and oxygen fraction. $\dot{V}O_2$ was calculated based on the continuous measurements of the sensors ($\dot{V}O_{2,Calc}$) and compared to the Douglas bag $\dot{V}O_2$ ($\dot{V}O_{2,Douglas}$).

Bland-Altman plots between $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Douglas}$ revealed overall good 95% limits of agreement (LoA) and small bias with a slight underestimation of $\dot{V}O_{2,Calc}$ during breathing tasks with added resistance compared to without added resistance (with resistance bias=-22.4 mL·min^{-1}, LoA=[-149.2-104.4] mL·min^{-1}, without resistance bias=-0.9 mL·min^{-1}, LoA=[-107.8-106.0] mL·min^{-1}). Linear regression analysis showed a high correlation between $\dot{V}O_{2,Douglas}$ and $\dot{V}O_{2,Calc}$ with no statistical difference between the two measurements (with resistance R^2=0.92, SEE=47.39 mL·min^{-1}, p=0.78, without resistance R^2=0.83, SEE=51.50 mL·min^{-1}, p=0.83, with and without resistance R^2=0.89, SEE=49.22 mL·min^{-1}, p=0.80).

In conclusion, the newly developed test setup is a valid tool to evaluate $\dot{V}O_2$ during normocapnic hyperpnoea with a collection period of 2 min.

INTRODUCTION

The measurement of oxygen consumption ($\dot{V}O_2$) is well-established in exercise physiology to assess aerobic capacity and energy expenditure. Traditionally, $\dot{V}O_2$ was assessed with the Douglas bag method, which is still considered the gold standard today (Shephard, 2017). The Douglas bag method consists of expiratory gas collection into a gas impermeable bag followed by the analysis of volume and oxygen fraction of the collected air. $\dot{V}O_2$ is then calculated as follows:

$$\dot{V}O_2 = (F_IO_2 \cdot V_I - F_EO_2 \cdot V_E) / \Delta t$$

where F_IO_2 and F_EO_2 are oxygen fractions of the average inspired and expired dry gas, and V_I and V_E are the inspired and expired volumes of air under standard condition (STPD; standard temperature, 0 °C;, pressure, 101.3 kPa; dry) and Δt the collection period. The Douglas bag method has its disadvantages, as for example, the impossibility of breath-by-breath assessment, and thus it is not sensitive to track rapid changes in $\dot{V}O_2$. With improving technology, these disadvantages were circumvented by metabolic carts providing fully automated, breath-by-breath analyses. Most commercially available metabolic carts today assess inspiratory and expiratory air flow via a flow or volume sensor, while O_2 fraction - (FO_2) and CO_2 fraction (FCO_2) are calculated via O_2 and CO_2 gas analysers. For gas analyses, the air is first dried in a drying tube to eliminate water vapour before gas fractions are quantified. To minimize errors in the calculation of $\dot{V}O_2$, and $\dot{V}CO_2$, in most cases, only V_E are used for further calculations while V_I are calculated based on the inert gas equation. Based on the Haldane transformation (Wilmore & Costill, 1973), it is assumed that the volumes of inspired and expired inert gases, mostly N_2, are equal over several breaths:

$$V_I \cdot F_IN_2 = V_E \cdot F_EN_2 \quad \Leftrightarrow \quad V_I = (F_EN_2 / F_IN_2) \cdot V_E$$

where F_IN_2 and F_EN_2 are the inspired and expired dry N_2 (plus minimal amounts of other inert gases) fraction.

Because of:

$$F_IN_2 + F_IO_2 + F_ICO_2 = 1 \text{ and } F_EN_2 + F_EO_2 + F_ECO_2 = 1$$

It follows:

$$V_I = (1 - F_EO_2 - F_ECO_2) / (1 – F_IO_2 – F_ICO_2) \cdot V_E$$

Hence V_I can be substituted in the initial equation:

$$\dot{V}O_2 = (F_IO_2 \cdot (1 - F_EO_2 - F_ECO_2) / (1 – F_IO_2 – F_ICO_2) \cdot V_E - F_EO_2 \cdot V_E) / \Delta t$$

and thus reduce potential measurement errors of $\dot{V}O_2$ due to intra-breath differences of inspiratory and expiratory volumes (Wilmore & Costill, 1973).

On a breath-by-breath basis, conventional metabolic carts calculate time averages of F_IO_2, F_EO_2, F_ICO_2, F_ECO_2 and expiratory volumes (V_E) and use an adaptation of the equation above, i.e.

$$\dot{V}O_2 = (F_IO_2 \cdot (1 - F_EO_2 - F_ECO_2) / (1 – F_IO_2 – F_ICO_2) \cdot V_E - F_EO_2 \cdot V_E) \cdot f_B$$

where f_B is the breathing frequency of the current breath.

This substitution of time-averaged gas fractions, however, is only valid if gas fractions are approximately constant over the entire inspiration and expiration, which is not the case during inspiration with partial rebreathing of the expired air to achieve normocapnia during volitional hyperpnoea with rebreathing devices as the one used in Chapter 4 and 5. In short, a partial rebreathing device collects most of the expiratory volume in a rebreathing bag to prevent hyperventilation, i.e. loss expired CO_2 and consequently dizziness and more severe effects, during intense, volitional hyperpnoea. Thus, during inspiration the entire volume of the bag will be inhaled together with a smaller fraction of fresh air. The percentage of fresh air and bag ‘air’ varies during the course of one single inspiration,

making gas fractions not only time but also flow dependent. Hence, F_IO_2, F_EO_2, F_ICO_2, F_ECO_2 need to be volume averaged based on the corresponding flow, and therefore not only the expiratory flow but also the inspiratory flow needs to be taken into account. Also, inspiratory air conditions (humidity and temperature) change rapidly depending on the proportion of fresh air to bag 'air', requiring continuous measurement of humidity and temperature with fast responding sensors. In addition, with added resistance in the breathing tube (e.g. in order to increase respiratory muscle work as for experiments described in Chapter 4 and 5), flow measurements are performed in the presence a wide range of pressures, from very high negative to very high positive pressures. Thus, in case of high negative pressures, the measured flow overestimates the number of gas particles passed (if not corrected for pressure) and vice versa with high positive pressures. Consequently, in order to calculate correct gas volumes in STPD conditions, temperature, pressure and humidity need to be measured in real time, data commercially available metabolic carts do not provide.

Therefore, the purpose of the current pilot study was to develop a system enabling calculation of $\dot{V}O_2$ and $\dot{V}CO_2$ (STPD) during normocapnic hyperpnoea using a rebreathing bag, by measuring flow/volume, gas concentrations, temperature, pressure and humidity to correct gas fractions during each inspiration and expiration and to validate these calculations against the gold standard method, the Douglas bag. To potentially facilitate future test setups, multi-breath averages, rather than continuous measurements, of inspiratory and expiratory values of temperature and humidity were calculated and used for $\dot{V}O_2$ calculations. These calculations were compared to those using continuous data to evaluate the importance of instantaneous temperature and humidity measurements.

METHODS

Experimental overview

Five young healthy male participants (age: 28±3 y) with experience in performing normocapnic hyperpnoea, performed 10 different normocapnic breathing tasks of 2 min duration, interspaced by 10 min of rest. The test setup is shown in Figure 7.1.

Normocapnic hyperpnoea breathing tasks

The breathing tasks consisted of 2 min warm-up with a hand held device (SpiroTiger®; idiag, Fehraltorf, Switzerland) directly followed by approximately 2 min of gas collection through the test setup. Each breathing task was performed sitting up straight with a nose clip in place. The mouthpiece was connected to a bacterial filter before being connected to the setup shown in Figure 7.1. Subjects were breathing at constant tidal volume (approximately 3.6 L) and constant breathing frequency (f_B). Tidal volume and f_B were different between tasks. In total, 10 different breathing tasks were performed with f_B of 20, 25, 30, 35, 40 breaths·min^{-1}. The task with each of the 5 f_B was performed with and without additional breathing resistance. The resistance consisted of a plastic ring which reduced the diameter of the breathing tube from 18 mm to 8 mm.

Gas collection started and ended at end-expiration. Subjects started with inspiring fresh air and ended by expiring only into the Douglas bag while the SpiroTiger® bag was manually closed, so that all expired gas from the task was collected into the Douglas bag. Thereafter, for Douglas bag analysis, the direction of the two-way Y-valve was changed such that flow head, gas analyser, temperature -, humidity - and pressure sensor were directly connected to the two-way Y-valve. The Douglas bag was then emptied with a 3-L syringe until completely empty.

Test setup

The experimental setup (Figure 7.1) consisted of a mouthpiece, filter (MicorGard® II, CareFusion, San Diego, USA), humidity sensor (DS 0: F-TUPTN.41, U.P.S.I, Champigny Sur Marne, France), pressure sensor (DP45-34, Validyne, Northridge, USA), temperature sensor (MLT1402 with T-Type Pod, ADInstruments, Castle Hill, Australia), metabolic cart (Oxycon Pro®; Jaeger, Höchberg, Germany) with

tube for gas withdrawal (for FO_2, FCO_2 analysis) included in the flow head (turbine), SpiroTiger® including valve, ± resistance and rebreathing bag, two-way Y-Valve (2730 large, Hans Rudolph Inc., Shawnee, USA), manual directional control valve (three way t-shape stopcock-type 2130, Hans Rudolph Inc., Shawnee, USA) and at the end a non-diffusing gas collection bag (6170, Hans Rudolph Inc., Shawnee, USA). All sensors were positioned in series as shown in Figure 7.1.

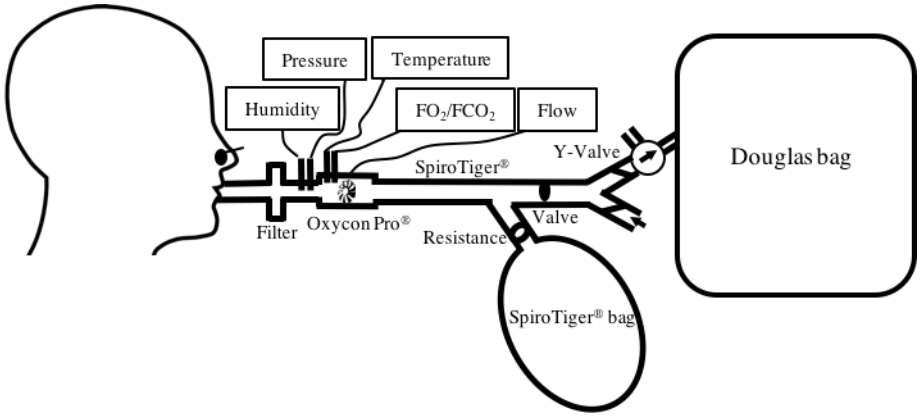

Figure 7.1: Test setup for the validation of $\dot{V}O_2$ measurements during normocapnic hyperpnoea.

Data recording and calculations

Raw signals of humidity, temperature and pressure were sampled with a sampling frequency of 10 kHz, A/D converted with Powerlab (ADInstruments, Castle Hill, Australia) and recorded in LabChart (ADInstruments, Castle Hill, Australia). Raw volume, FO_2 and FCO_2 signals were sampled with a sampling frequency of 100 Hz, D/A converted in the Oxycon Pro, A/D converted with Powerlab and recorded simultaneously in LabChart. Signals were synchronized based on visual inspection and time-adjustments were kept constant between subjects. All following calculations were performed online in LabChart and shown in real-time on the screen. Continuous flow signals (from flow head) were converted to STPD with the corresponding measured humidity, temperature and pressure values at each point in time:

$$\dot{V}\,(STPD) = \dot{V}\cdot(273 / (273 + T))\cdot(P_m + P_{baro} - (e^{(20.386-5132 / (273 + T))}\cdot 1.333\cdot RH)) / 1013$$

where $\dot{V}$ is air flow, T is temperature, P_m is mouth pressure (equal to pressure in the tubing system), P_{baro} is the barometric pressure and RH the relative humidity at temperature T and pressure (P_{baro} + P_m). STPD-corrected flow was integrated in a cyclic manner over every in - and expiration to obtain V_I and V_E volume for every breath. STPD-corrected flow was then multiplied by FO_2 and FCO_2, respectively, and both signals were integrated in the same manner as mentioned above to obtain V_IO_2, V_EO_2, V_ICO_2, and V_ECO_2. To obtain a volume-dependent F_IO_2, F_EO_2, F_ICO_2 and F_ECO_2 for every breath, V_IO_2, and V_ICO_2 were divided by V_I, and V_EO_2 and V_ECO_2 were divided by V_E resulting in the following calculation of $\dot{V}O_{2\,Calc}$:

$$\dot{V}O_{2,Calc} = (V_IO_2 / V_I \cdot (1 - V_EO_2 / V_E - V_ECO_2 / V_E) / (1 - V_IO_2 / V_I - V_ICO_2 / V_I) \cdot V_EO_2 / V_E \cdot V_E) \cdot f_B$$

During the emptying of the Douglas bag, flow, FO_2, humidity and temperature were recorded in the same way as during the breathing task and manually analysed in LabChart. The Douglas bag volume ($V_{Douglas}$) was STPD corrected as described before. FO_2 was corrected with the number of breaths taken during the recording and the dead space between the gas analyser and the Y-valve as follows:

$$FO_{2\,corr} = (V_{Douglas} \cdot FO_{2\,meas} - N_{breaths} \cdot dead\ space \cdot FO_{2\,ambient}) / (V_{Douglas} - N_{breaths} \cdot dead\ space)$$

where $FO_{2\,corr}$ is the corrected FO_2, $FO_{2\,meas}$ the measured FO_2 inside the Douglas bag, $N_{breaths}$ is number of breaths, dead space is the sum of dead spaces of Y-valve, SpiroTiger® tubing and volume sensor (measured to be 300 mL) and $FO_{2\,ambient}$ is the FO_2 of the ambient air.

$V_{Douglas}$ was then multiplied with $FO_{2\,corr}$ to obtain VO_2 of the Douglas bag and divided by the exact collection time to obtain $\dot{V}O_{2,Douglas}$.

To facilitate potential future measurements average humidity and temperature off all breathing task combined were used as substitution of continuous humidity and temperature measurements to calculate $\dot{V}O_2$ ($\dot{V}O_{2,Calc\ average}$) and compared to $\dot{V}O_{2,Calc}$.

Statistical analysis

A Bland-Altman plots (Bland & Altman, 1986) with limits of agreement (LoA) of 95% were created to quantify the agreement (bias and random error) between $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Douglas}$ and $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Calc\ average}$. With a linear regression model, the coefficient of determination (R^2) and standard error of the estimate (SEE) between $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Douglas}$ and $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Calc\ average}$ were calculated. To test for a significant difference between the Douglas bag method and the test setup, a paired t-tests between $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Douglas}$ and $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Calc\ average}$ were performed for the breathing tasks with resistance, without resistance and both combined. Statistical significance was set at $p<0.05$ for all analyses and results are reported as mean±SD. Statistical analysis was performed with Prism 6 (GraphPad, La Jolla, USA).

RESULTS

Signal quality

Figure 7.2 shows a representative, synchronized data set of raw signals of humidity, temperature, pressure, FO_2, FCO_2 and flow. Signals delay times were not affected by pressure changes in the range of [-53-67 cmH_2O]. However, for mouth pressures more negative than -53 cmH_2O, the Oxycon system was unable to overcome the negative pressure and to draw gas samples ('occlusion' was reported), resulting in missing gas fraction values. This occurred for an average of 4 breaths / 2 min in the breathing task at 40 breaths·min^{-1} with added resistance. These breaths were not taken into consideration.

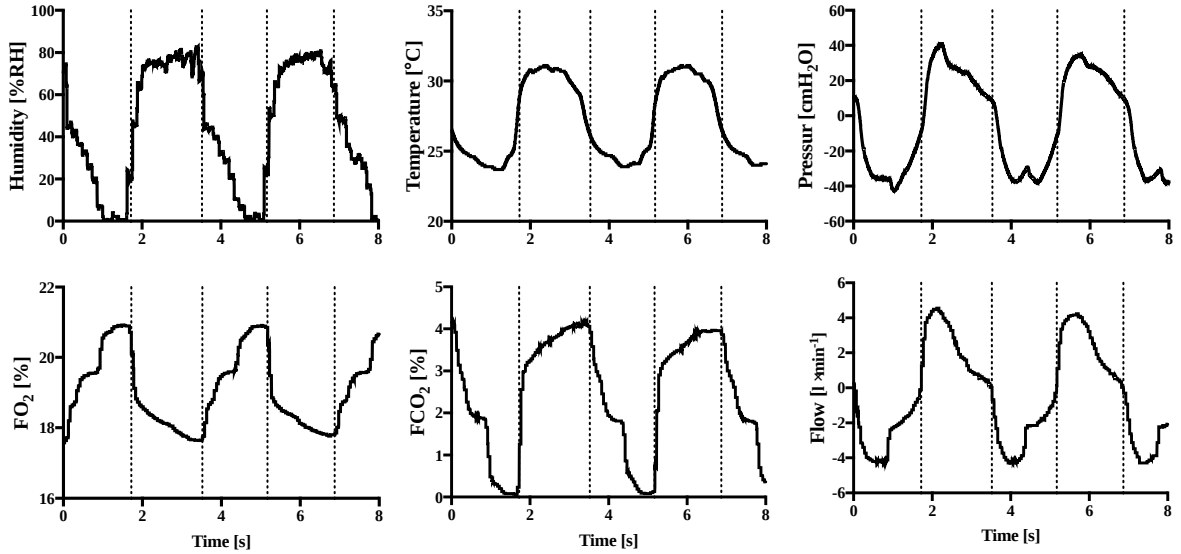

Figure 7.2: Raw signals of humidity, temperature, pressure, oxygen fraction (FO_2), carbon dioxide fraction (FCO_2) and flow.

Calculated $\dot{V}O_2$ versus Douglas bag $\dot{V}O_2$

Bland-Altman plot and linear regressions of $\dot{V}O_{2,Calc}$ vs. $\dot{V}O_{2,Douglas}$ are shown in Figure 7.3, separated by breathing tasks with and without resistance and in combination. The LoA and bias (Figure 7.3A) with resistance was slightly larger than without resistance (with resistance: bias=-22.4 mL·min^{-1}, LoA=[-149.2-104.4] mL·min^{-1}; without resistance: bias=-0.9 mL·min^{-1}, LoA=[-107.8-106.0] mL·min^{-1}). In both cases, $\dot{V}O_{2,Calc}$ crossed the identity line, indicating a trend towards underestimating high $\dot{V}O_2$ values. This effect was smaller when no resistance was applied (slope with resistance=0.83,

slope without resistance=0.77). The SEE and R^2, however, were smaller in the presence of a resistance compared to without, as shown in Figure 7.3B. No significant difference between $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Douglas}$ for breathing tasks with and without resistance and in combination was measured (with resistance p =0.78, without resistance p =0.99, with and without resistance p=0.80).

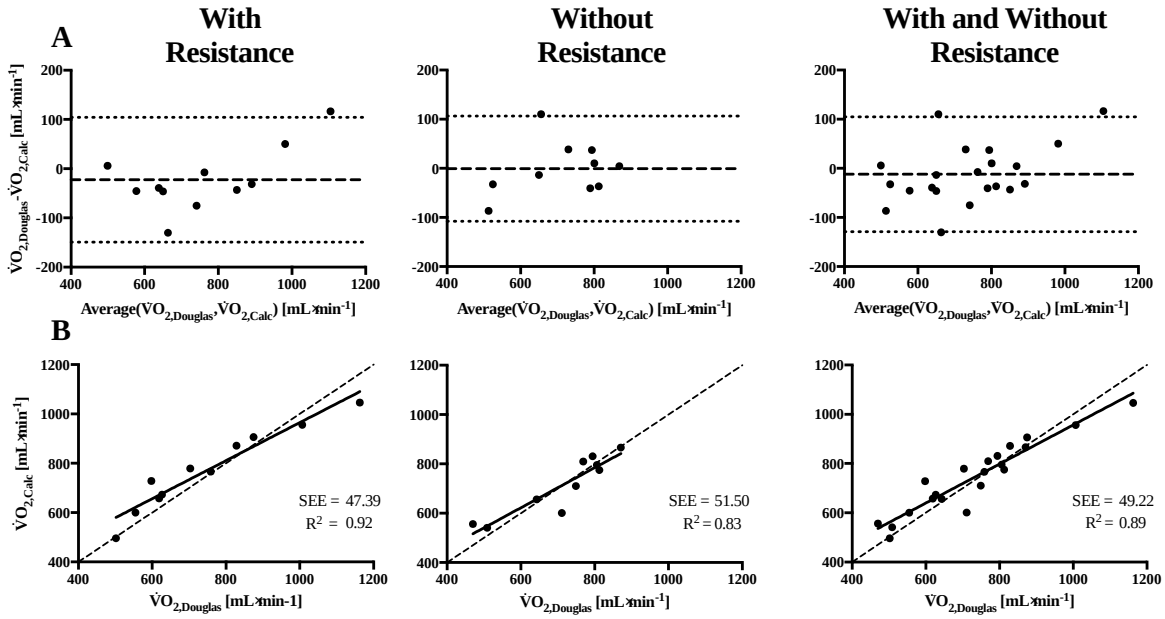

Figure 7.3: A: Bland-Altman plots using the average versus the difference of calculated oxygen consumption ($\dot{V}O_{2,Calc}$) and measured oxygen consumption with the Douglas bag ($\dot{V}O_{2,Douglas}$). B: linear correlation between $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Douglas}$. Data are separated by breathing tasks with resistance, without resistance and both combined. R^2, coefficient of determination; SEE, standard error of the estimate.

Average versus continuous humidity and temperature

A Bland-Altman plot and linear correlation plot of the average humidity (inspiratory=27% RH, expiratory=78% RH) and temperature (inspiratory=26 °C, expiratory=31 °C) calculations ($\dot{V}O_{2,Calc\ average}$) versus $\dot{V}O_{2,Calc}$ are shown in Figure 7.4. When using average humidity and temperature, $\dot{V}O_2$ is slightly overestimated compared to $\dot{V}O_{2,Calc}$ (bias=12.7 mL·min^{-1}, LoA=[-16.9-42.3] mL·min^{-1}). However, SEE and R^2 show a very good correlation, very close to the line of identity, as shown in Figure 7.4.

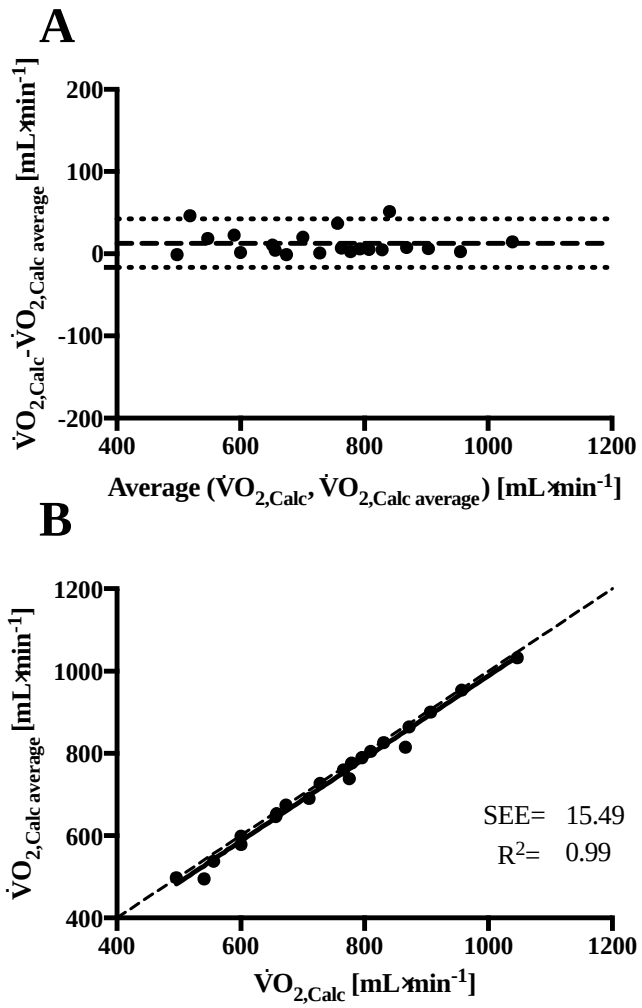

Figure 7.4: A: Bland-Altman plots using the average versus the difference of continuous calculated oxygen consumption ($\dot{V}O_{2,Calc}$) and average calculated oxygen consumption ($\dot{V}O_{2,Calc\ average}$). B: linear correlation between $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Calc\ average}$. Data are separated by breathing tasks with resistance, without resistance and both combined. R^2, coefficient of determination; SEE, standard error of the estimate.

DISCUSSION

This pilot study showed that it is possible to calculate $\dot{V}O_2$ with good accuracy compared to gold standard, in challenging conditions such as variable gas concentrations and pressures within inspiration and expiration based on volume-averaged gas fractions and STPD-corrected flow with real-time measurements of humidity, temperature and pressure during normocapnic hyperpnoea. Using the of average humidity and temperature measured during all breathing tasks combined for STPD-correction of flow resulted in a very small but physiologically irrelevant difference to the values calculated with continuous humidity and temperature measurements.

Comparison of $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Douglas}$ in the presence of a resistance, and with continuous measurement of temperature and humidity included in calculations, showed good agreement with a small underestimation at low $\dot{V}O_2$ (low P_m) and overestimation at high $\dot{V}O_2$ (high P_m). However, the largest deviation between $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Douglas}$ was -130 $mL \cdot min^{-1}$ which is in the range of previous studies comparing different commercially available metabolic carts (Carter & Jeukendrup, 2002) or custom made breath-by-breath measurement methods with the Douglas bag method (Bassett *et al.*, 2001) The breathing tasks without resistance showed an even greater agreement between $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Douglas}$. In addition, no significant difference was detected between $\dot{V}O_{2,Calc}$ and $\dot{V}O_{2,Douglas}$ when values with or without resistance, or combined were compared, suggesting the test setup to be a valid method for $\dot{V}O_2$ measurements during normocapnic hyperpnoea using partial rebreathing. However, the test setup used was rather complex in face of the many sensors in line, increasing dead space before the gas sampling line and thereby compromising the accuracy of further values as for example end-tidal CO_2 partial pressure. In addition, the setup is too complex for regular use e.g. in clinics. To circumvent the complexity of the initial setup, average humidity and temperature were calculated from all of the performed breathing tasks and used for STPD volume correction, replacing the continuous humidity and temperature data used for $\dot{V}O_{2,Calc}$. This resulted in a very small, physiologically not relevant deviation in $\dot{V}O_{2,Calc\ Average}$ compared to $\dot{V}O_{2,Calc}$. Therefore, if average humidity and temperature are once evaluated for a specific rebreathing setup, humidity and temperature sensors are not needed for future experiments with the same setup, minimizing dead space and simplifying the overall setup.

Technical considerations

Synchronized raw data are represented in Figure 7.2, showing a good synchronization between signals and fast response times of all sensors. With negative pressures that exceeded -53 cmH_2O, at which point the metabolic cart was unable to withdraw gas, the time-delay between sensors stayed constant and could be directly used for STPD-correction of flow in real-time. The response time of the different sensors were of great importance due to the very fast changing air conditions (bag air versus fresh air). From this point of few, the slowest and technically most difficult measurement is humidity. Due to condensation, most humidity sensors have a very low response time (> 10 s), however, the sensor used in the current study contains a very recently developed technology with a heated polymer (40 °C) that prevents condensation to a great extent and thus decreases response time to 0.1 s. As shown in Figure 7.2, the sensor is fast enough to detect changes between fresh air and bag 'air' thus it can be used for real-time STPD-correction of flow.

Limitations

Only a limited range of $\dot{V}O_2$ (470-1163 $mL \cdot min^{-1}$) could be tested with the current setup because of the limited tolerance for negative pressures of the metabolic cart. To obtain higher $\dot{V}O_2$ from breathing only, increasing resistance and/or f_B would have needed to be achieved, resulting in even more negative pressures. This problem could only be overcome with a more powerful pump of the metabolic cart which is currently not available.

For proof of concept, the sampling period was set at 2 min in the present study since only about 10% of every breath was collected in the Douglas bag due to the rebreathing system and the period had to be long enough for a relevant sample in the Douglas bag. However, testing accuracy with a shorter time period would be of interest which would, however, need a different rebreathing system.

Conclusion

This pilot study showed the feasibility to calculate $\dot{V}O_2$ with good accuracy compared to gold standard, also in challenging conditions such as within-breath variability of gas concentrations and pressures

when using volume-averaged gas fractions and STPD-corrected flow with real-time measurements of humidity, temperature and pressure during normocapnic hyperpnoea.

8. NEW INSIGHTS AND GENERAL DISCUSSION

The present work provides new insights into testing and training of respiratory muscles and the relation of respiratory muscle work to whole-body exercise capacity and performance. A new robust functional respiratory muscle performance test was established which also allows the prediction of $\dot{V}O_{2peak}$ in healthy individuals, circumventing the need for whole-body exercise testing. Furthermore, new mechanistic insights on the effects of different RMT regimens (sprint-interval vs. endurance) on respiratory muscle and whole-body exercise performance including sex- and training regimen - specific adaptations were gained, providing new insights for the future development of optimized RMT training and testing protocols.

Respiratory muscle performance and cardio-metabolic fitness

$\dot{V}O_{2peak}$ is a predictor of peri-operative complications in clinical settings and is widely used to identify high-risk patients (Licker *et al.*, 2011; Moran *et al.*, 2016) and to determine whether pre-operative physical training shall be recommended (West *et al.*, 2016). Therefore, for patients who are not able to perform whole-body exercise, an alternative to $\dot{V}O_{2peak}$ measurements on a bicycle ergometer, and pre-operative physical training, is essential. Results from Chapter 4 showed a strong prediction of $\dot{V}O_{2peak}$ using respiratory muscle performance measurements assessed in the IncRMT (FVC and WOB) in healthy individuals. The IncRMT is a potential functional replacement to evaluate the risk of peri-operative complications and it may provide important information to develop personalized pre-operative RMT protocols. Since RMT was shown to reduce postoperative pulmonary complications, shorten duration of mechanical ventilation and the length of hospital stay (Mans *et al.*, 2015), RMT may be a good alternative also to whole-body exercise training specifically in immobile patients. As the current findings are based on healthy individuals from 18-45 yrs, future studies are required to validate the potential of the IncRMT to predict $\dot{V}O_{2peak}$ in the elderly and also in different groups of patients.

Analyses in Chapter 4 were restricted to performance measurements such as WOB, to evaluate IncRMT intensity and predict $\dot{V}O_{2peak}$ because $\dot{V}O_2$ measurements during the IncRMT ($\dot{V}O_{2peak,IncRMT}$) were not

possible from a technical point of view. With the setup developed and validated in Chapter 7, however, $\dot{V}O_{2peak,IncRMT}$ could now be assessed.

For this purpose, average humidity and temperature during the IncRMT were measured in 2 subjects (inspiratory humidity=18%RH, expiratory humidity=78% RH, inspiratory temperature=26 °C, expiratory temperature=31 °C) and used to calculate $\dot{V}O_{2peak,IncRMT}$ during the last 30 s of the IncRMT ($\dot{V}O_{2peak,IncRMT}$) for all tests performed in the study reported in Chapter 4.

To estimate the cardio-metabolic stress of the IncRMT in relation to whole-body exercise, $\dot{V}O_{2peak,IncRMT}$ was calculated as a percentage of $\dot{V}O_{2peak,bike}$ which came out to be 25±6% corresponding to 12.4 $mL \cdot min^{-1} \cdot kg^{-1}$ of O_2 or 3.6 MET (metabolic equivalent defined as 1 $kcal \cdot kg^{-1} \cdot h^{-1}$. This energy expenditure is equivalent to moderate intensity exercise (WHO, 2010) such as walking at a pace of 5.6 $km \cdot h^{-1}$ (Ainsworth *et al.*, 2000) and is significantly higher than the $\dot{V}O_2$ of respiratory muscles during whole-body exercise, which has been calculated to amount to ~ 10±7% (Aaron *et al.*, 1992) of whole-body $\dot{V}O_{2peak}$ when peak pulmonary ventilation is achieved during exercise. As shown in Figure 8.1, $\dot{V}O_{2peak,IncRMT,}$ as single variable, significantly correlates with $\dot{V}O_{2peak,bike}$ ($p<0.001$), suggesting a strong relationship between maximal respiratory muscle $\dot{V}O_2$ and maximal cycling $\dot{V}O_2$.

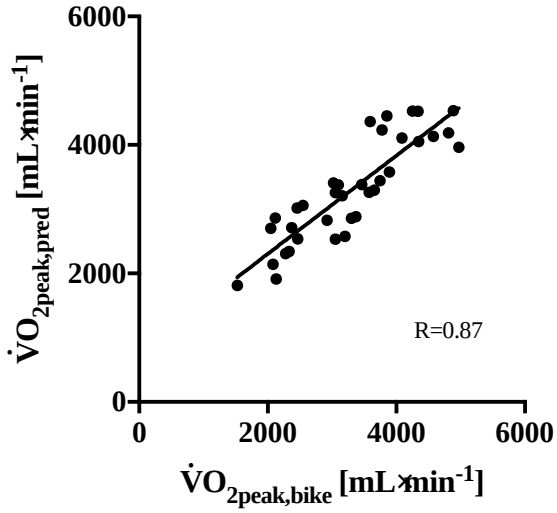

Figure 8.1: Correlation between peak oxygen consumption during the incremental respiratory muscle test and incremental cycling test. $\dot{V}O_{2peak,IncRMT}$, peak oxygen consumption measured during an incremental respiratory muscle test; $\dot{V}O_{2peak,bike}$, peak oxygen consumption measured during an incremental cycling test; R, pearson correlation coefficient.

By subsituting WOB by $\dot{V}O_{2peak,IncRMT}$ in the multi-variable linear regression model optained in Chapter 4, a similar prediction is found as with WOB, shown in Table 8.1 and Figure 8.2.

Table 8.1: Multiple regression analysis to predict peak oxygen consumption from maximal oxygen consumption during the IncRMT and forced vital capacity or total work of breathing of the IncRMT and forced vital capacity.

	$\dot{V}O_{2peak,IncRMT}$ and FVC			WOB_{tot} and FVC		
Variable	B	SE	β	B	SE	β
Constant	-536.7	406.9		-442.1	387.2	
$\dot{V}O_{2peak,IncRMT}$	1.4**	0.3	0.4	105.9**	0.0	0.4
FVC	531.2**	92.6	0.6	613.5**	79.3	0.7
R^2	0.77			0.79		
F	53.68**			60.94**		
SEE	450.83			436.93		

$\dot{V}O_{2peak}$, peak oxygen consumption; B, unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient; $\dot{V}O_{2peak,IncRMT}$, maximal oxygen consumption during the IncRMT; FVC, forced vital capacity; WOB_{tot}, total work of breathing during IncRMT; R^2, coefficient of determination; F, F-test; SEE, standard error of estimate with a 95% confidence interval. **p<0.001.

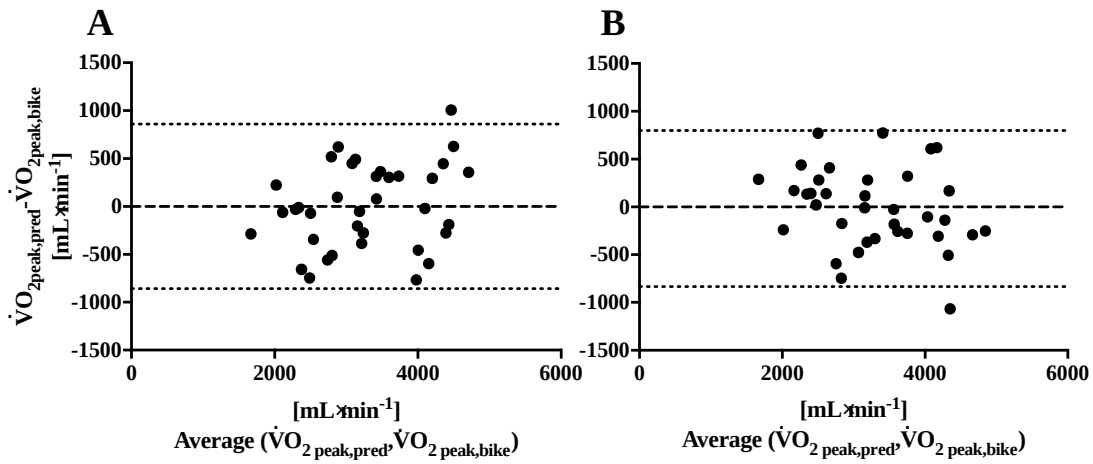

Figure 8.2: Bland-Altman plots using the average versus the difference of measured and predicted peak oxygen consumption ($\dot{V}O_{2peak}$) using multivariable prediction with the combination of (A) forced vital capacity and maxixmal oxygen consumption during the IncRMT or (B) forced vital capacity and total work of breathing during the IncRMT. $\dot{V}O_{2peak,pred}$; predicted peak oxygen consumption; $\dot{V}O_{2peak,bike}$, peak oxygen consumption measured with incremental cycling test.

When using $\dot{V}O_{2peak,IncRMT}$ instead of WOB, the bias and LoA of the Bland-Altman plot were reduced from ~18 mL · min^{-1} to ~0 mL · min^{-1} with an increase of only 5% of LoA thus should be the preferred parameter in future prediction of $\dot{V}O_{2peak}$. The correlation between $\dot{V}O_{2peak,IncRMT}$ and $\dot{V}O_{2peak,bike}$ and the considerable stress placed on the cardio-metabolic system during the IncRMT support the potential of the IncRMT as functional measurement to replace whole-body $\dot{V}O_{2peak}$ assessment of immobile patients in a clinical setting.

Respiratory muscle performance changes after respiratory muscle sprint-interval – and respiratory muscle endurance training

The findings of the study described in Chapter 5 showed that one month of RMSIT and RMET will improve IncRMT performance compared to SHAM, with no statistical difference between RMSIT and RMET. This suggests that the combination of high flows with high pressures applied on inspiration and expiration used in RMSIT have similar effects on respiratory muscle endurance as RMET, with high flows and low pressures. This similar improvement in IncRMT performance between the two training groups is further supported by the similar increase of WOB from the first to the last training session, as shown in Chapter 5. It is important to note, however, that the total training volume was much lower during RMSIT compared to RMET (12 sessions of 11 min vs. 20 sessions of 30 min) thus making RMSIT more efficient in improving respiratory muscle performance than RMET. These findings are in accordance with studies on whole-body exercise adaptations after high-intensity sprint-interval training compared to traditional endurance training (Jones & Carter, 2000; Weston *et al.*, 2014).

In the current project, the potential physiological training adaptations explaining this increase in respiratory muscle performance with RMT could only be partially elucidated. The lack of clear evidence is mainly due to the small magnitude of the physiological changes, the high variability of the measurement associated with some methods used, and the very complex interaction of the difficult to access respiratory muscles. However, with the knowledge obtained from Chapter 7, one additional physiological parameter, $\dot{V}O_{2peak,IncRMT}$, can now be assessed. Thus, calculation of $\dot{V}O_{2peak,IncRMT}$ was performed on data described in Chapter 5 and the same statistics and representation as for the other

physiological parameters of Chapter 5 were applied. Figure 8.3 shows the time course of $\dot{V}O_2$ during the IncRMT before and after one month of RMT. Three subjects (2 RMET, 1 SHAM) showed a very distinct breathing pattern after the training period, at the start of the IncRMT, when f_B was very slow; these subjects inspired rather fast and waited for the signal of the next expiration. This resulted in an overestiamtion of $\dot{V}O_2$, likely due to the inertia of the flow-measuring turbine, drastically overestimating inspired volumes. If inspiration is directly followed by a expiration – as is normally the case – the direction of the flow and thus turn direction of the turbine is immediately changed, preventing the overestimation. This phenomenon explains the difference in $\dot{V}O_2$ of RMET in the first 40% of the iso-time period of the IncRMT. At 100% iso-time, RMSIT showed a decrease in $\dot{V}O_2$ compared to before training which was significantly different from RMET and SHAM (RMSIT vs. RMET p=0.040, RMSIT vs. SHAM p=0.008) with no group*sex interaction. This finding suggests a lower $\dot{V}O_2$ for similar or even higher WOB at iso-time, implying more efficient breathing after RMSIT compared to RMET and SHAM. In conclusion, RMSIT showed a greater physiological training adapation than RMET even though training volume was reduced to a considarable extent.

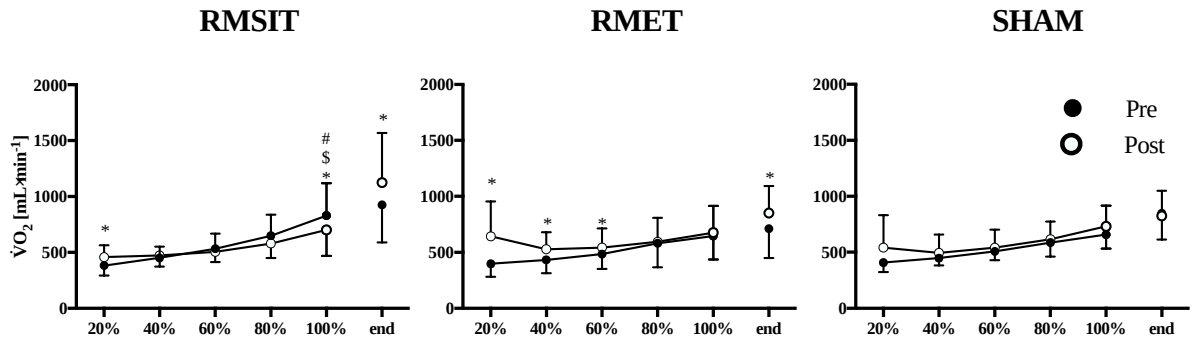

Figure 8.3: Oxygen consumption during the incremental respiratory muscle test pre and post respiratory muscle intervention. Values are mean ± SEM. $\dot{V}O_2$, oxygen consumption; RMSIT, respiratory muscle sprint-interval training; RMET; respiratory muscle endurance training; SHAM, sham-training. *p<0.05 pre vs. post within groups, #p<0.05 changes compared to SHAM, $p<0.05 changes compared to RMET.

Respiratory muscle metaboreflex

According to the theory of a reduced respiratory muscle metaboreflex being responsible for improvements in exercise performance, improvements in respiratory muscle efficiency as seen in the study of Chapter 5 would reduce respiratory muscle fatigue and therefore lead to reduced locomotor

fatigue during work-matched exercise. Indeed, results from Chapter 6 suggest – after RMSIT - a lower degree of leg muscle fatigue with no measurable change in respiratory muscle fatigue (ΔP_m) after a work-matched CLT. However, no correlation was detected between changes in leg muscle fatigue, respiratory muscle fatigue, deoxygenated haemoglobin and muscular activity of the respiratory and leg muscles in any RMT group, RMT groups pooled or separated by sex (all p>0.2). If absolute changes of ΔQ_{TW10} were correlated to changes in oxygen consumption at 100% iso-time of the IncRMT ($\dot{V}O_{2\ iso,IncRMT}$) of subjects participating in both studies, no significant correlation was found for any parameter when analysing sex and RMT groups separately (all p > 0.100). When pooling RMSIT and RMET, males showed a significant positive correlation between ΔQ_{TW10} and $\dot{V}O_{2\ iso,IncRMT}$ (p=0.020, R^2=0.47) but females showed no significant correlation (p=0.445, R^2=0.07), as shown in Figure 8.4. Importantly, one RMSIT male improved $\dot{V}O_{2\ iso,IncRMT}$ to a much greater extent than all the other participants, with only little improvement in ΔQ_{TW10}; if this participant is considered an outlier and excluded from the analysis, the strength of the correlation between ΔQ_{TW10} and $\dot{V}O_{2\ iso,IncRMT}$ increases significantly (p=0.0008, R^2=0.77).

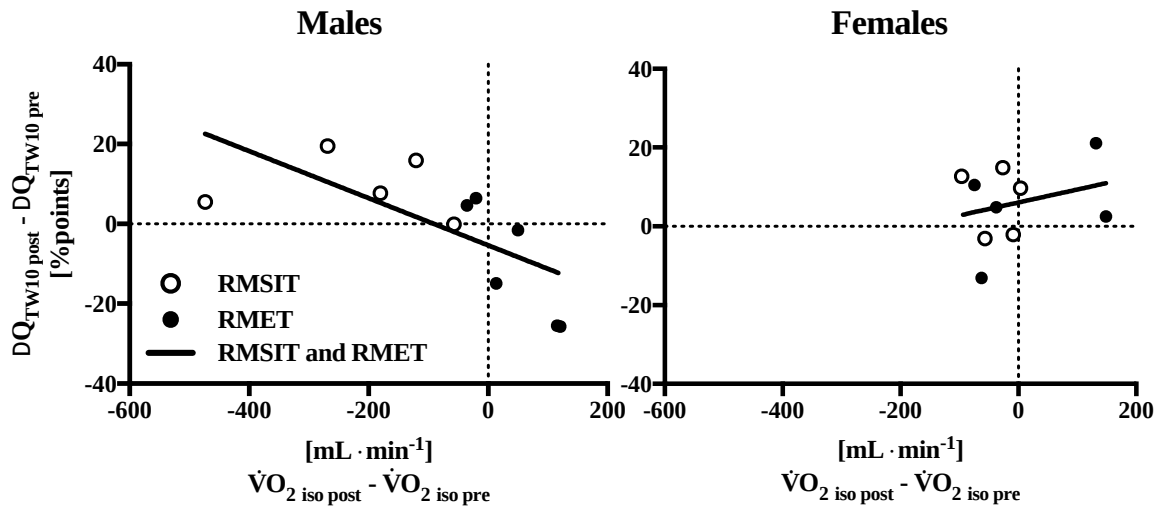

Figure 8.4: Correlations between absolute changes in reduction of quadriceps 10 Hz twitch force (ΔQ_{TW10}) and changes in oxygen consumption at iso-time of the IncRMT ($\dot{V}O_{2\ iso,IncRMT}$). RMSIT, respiratory muscle sprint-interval training; RMET, respiratory muscle endurance training. Data are expected in the grey quadrants if improvements in respiratory muscle efficacy correlates with improvements in locomotor fatigue during exercise.

The fact that training groups separated by sex consisting of only 5-6 subjects may explain the lack of differences in variables known to show high variability. On these grounds, it is perhaps not surprising to find no significant correlation between ΔQ_{TW10} and $\Delta \dot{V}O_{2\ iso,IncRMT}$ when training groups are separated but a significant correlation when sample size is increased by pooling the RMT groups. This correlation is in agreement with the theory of the respiratory muscle metaboreflex described earlier (Chapters 3 and 6). Improved respiratory muscle efficiency is hypothesized to result in more fatigue resistant respiratory muscles thus leading, through the blunted respiratory muscle metaboreflex, to less locomotor muscle fatigue for work matched exercise. This decreased or delayed respiratory muscle metaboreflex after RMT would also potentially lead to a correlation between ΔQ_{TW10}, ΔP_m, ΔHHb_{vastus} and ΔRMS_{vastus}, which, as mentioned before, was not seen. This discrepancy may be due to the high variability and small expected changes in the measured parameters of Chapter 6, while respiratory muscle performance and efficiency measurements with the IncRMT may be more sensitive to training adaptations of the respiratory system.

No correlation between ΔQ_{TW10} and $\Delta \dot{V}O_{2\ iso,IncRMT}$ was found in females after either RMT program. As discussed in Chapter 6, the inspiratory muscles of females were shown to be more fatigue resistant than those of males during high intensity cycling (Guenette et al., 2010), thus female respiratory muscles may be less limiting during exercise performance due to a reduced respiratory muscle metaboreflex. Therefore, in females, improvement in respiratory muscle performance and efficiency after RMT might not necessarily translate into a smaller ΔQ_{TW10} or improvement in whole-body exercise performance.

In conclusion, if RMT increases respiratory muscle efficiency in males it translates into decreased locomotor muscle fatigue for work-matched exercise, which can explain at least in part the improved whole-body exercise performance after RMT found in the meta-analysis by Illi *et al.* (2012). Furthermore, the different response to RMT in males and females underline the importance to separate participants by sex and take these potential differences into account when developing and describing RMT for different sport and patient groups.

Summary of findings and outlook

- With a newly developed functional respiratory muscle test, IncRMT, whole-body $\dot{V}O_{2peak}$ could be predicted in healthy subjects. Thus, implementation of a pre-operative IncRMT might be a step forward in peri-operative risk profiling in immobile patients. Further studies should focus on the validation of the $\dot{V}O_{2peak}$ prediction with the IncRMT in different age and patient groups.
- Respiratory muscle performance, measured by the IncRMT and WOB during RMT sessions, was shown to improve after one month of RMSIT and RMET to the same extent despite lower training volume during RMSIT compared with RMET. Both training regimens improved $\dot{V}O_{2peak}$ during the IncRMT but only RMSIT improved respiratory muscle efficiency. However, physiological training adaptation on the muscular level were inconclusive and remain to be established.
- RMSIT lead to a reduced development of leg muscle fatigue in a work-matched, high-intensity CLT, a potential indication of a reduced respiratory muscle metaboreflex. Potentially associated physiological changes due to a reduced respiratory muscle metaboreflex on a muscular level were inconclusive and should be further examined in future studies. RMET improved TT performance, a lower intensity exercise test, in males.
- Changes in respiratory muscle efficiency after RMSIT and RMET correlated with changes in leg muscle fatigue after a work-matched CLT in males but not in females. This further suggests a reduced respiratory muscle metaboreflex due to more fatigue resistant respiratory muscles.
- Females improved respiratory muscle performance after RMSIT and RMET but this improvement did not translate into gains in whole-body exercise performance, suggesting less limiting respiratory muscles, despite no significant difference in the level of fatigue assessed after CLT.
- A test setup was successfully developed to asses $\dot{V}O_2$ during volitional hyperpnoea with partial rebreathing and added resistance providing STPD-corrected flow using corrections based on real-time measurements of humidity, temperature and pressure or based on average humidity, temperature and real-time pressure measurements. In a pilot study, $\dot{V}O_2$ calculation were

validated against the gold standard method with Douglas bag for 2-min periods. Future studies should focus on the validation of shorter gas collection periods, possibly even breath-by-breath analysis.

9. LITERATURE

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10. ABBREVIATIONS

ABDOM	M. rectus abdominis
BMI	body mass index
BR	breathlessness
CLT	constant load cycling test
EMG	electromyography
EMT	expiratory muscle training
f_B	breathing frequency
FCO_2	fractional carbon dioxide content
FEV_1	forced expiratory volume in 1 s
FN_2	fractional nitrogen
FO_2	fractional oxygen content
FVC	forced vital capacity
HHb	deoxygenated haemoglobin
HR	heart rate
HR_{max}	maximal heart rate
$HR_{max} \cdot HR_{rest}^{-1}$	ratio of maximal heart rate over resting heart rate
IncRMT	incremental respiratory muscle test
IMT	inspiratory muscle training
INTER	M. intercostales
Lactate	blood lactate concentration
LE	leg exertion
MEP	maximal expiratory pressure
MET	metabolic equivalent
MIP	maximal inspiratory pressure
MVV	maximal voluntary ventilation
MVV_{12}	maximal voluntary ventilation in 12 s

NIRS	near infrared spectroscopy
$P_{ET}CO_2$	partial pressure of end-tidal CO_2
P_m	mouth pressure
POB_{EX}	expiratory power of breathing
POB_{IN}	inspiratory power of breathing
POB_{max}	maximal power of breathing
$P_{m,TW}$	twitch mouth pressure
Q_{TW}	quadriceps twitch force
RE	respiratory exertion
RMET	respiratory muscle endurance training
RMS	root mean square
RMS_{EX}	root mean square during expiration
RMS_{IN}	root mean square during inspiration
RMSIT	respiratory muscle sprint-interval training
RMT	respiratory muscle training
RPM	revolution per minute
STERNO	M. sternocleidomastoideus
STPD	standard temperature pressure dry
T_{lim}	incremental respiratory muscle test duration
TT	12-km time trial
TUT	time under tension
VAS	visual analog scale
VAST	M. vastus lateralis
VC	vital capacity
$V_{Douglas}$	collected volume in the Douglas bag
V_E	expiratory volume
V_I	inspiratory volume
$\dot{V}$	air flow

$\dot{V}_E$	expiratory ventilation
$\dot{V}_I$	inspiratory ventilation
$\dot{V}O_2$	oxygen consumption
$\dot{V}O_{2\ Calc}$	calculated oxygen consumption
$\dot{V}O_{2\ Calc\ esti}$	calculated oxygen consumption with estimated humidity and temperature
$\dot{V}O_{2\ Douglas}$	oxygen consumption measured with the Douglas bag
$\dot{V}O_{2\ iso}$	oxygen consumption at iso-time of IncRMT
$\dot{V}O_{2peak}$	peak oxygen consumption
$\dot{V}O_{2peak,bike}$	measured peak oxygen consumption during cycling
$\dot{V}O_{2peak,IncRMT}$	peak oxygen consumption during IncRMT
$\dot{V}O_{2peak,pred}$	predicted peak oxygen consumption
$\dot{V}CO_2$	carbon-dioxide elimination
V_T	tidal volume
W_{max}	maximal work load
WOB	work of breathing
WOB_{EX}	expiratory work of breathing
WOB_{IN}	inspiratory work of breathing
WOB_{tot}	total work of breathing
$\Delta P_{m,TW}$	change of twitch mouth pressure
ΔQ_{TWall}	change of quadriceps twitch force, single, 10 Hz and 100 Hz combined
ΔQ_{TW1}	change of quadriceps single twitch force
ΔQ_{TW10}	change of quadriceps 10 Hz doublet force
ΔQ_{TW100}	change of quadriceps 100 Hz doublet force

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12.CURRICULUM VITAE

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