## Bård Vegard Ytrehauge Balto

High-Intensive Interval Training or Supramaximal Interval Training – What to Choose When the Goal is to Improve VO2max and Aerobic Endurance Performance in Females?

Master's thesis in Exercise Physiology Supervisor: Jan Helgerud

June 2019



Master's thesis

Norwegian University of Science and Technology Faculty of Medicine and Health Sciences Department of Circulation and Medical Imaging

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## Abstract

Aim: The present study aims to compare the effects of high-intensive interval training (HIIT) and all-out supramaximal intensity interval training (SIT) on maximal oxygen uptake (VO<sub>2max</sub>), work economy (WE), lactate threshold (LT), anaerobic capacity (maximal accumulated oxygen deficit (MAOD)), and 300- and 3000-meter running performance in moderately endurance-trained females. Methods: 11 healthy, non-smoking, and recreational active to moderately endurance-trained females were randomized to perform HIIT or SIT three times per week over 8 weeks. HIIT was performed as 4 x 4-minute treadmill running intervals at 90-95 % of maximal heart rate (HR<sub>max</sub>) interspersed with 3-minute active recovery at 70 % of HR<sub>max</sub> between intervals. SIT was performed as 10 x 30-second all-out treadmill running intervals interspersed with 3.5-minute active recovery at 70 % of HR<sub>max</sub> between intervals. **Results**: The VO<sub>2max</sub> and oxygen (O<sub>2</sub>) pulse increased significantly more after HIIT compared to SIT (p < 0.01 and p < 0.05, respectively). VO<sub>2max</sub> increased by 8.9 % after HIIT  $(54.1 \text{ to } 58.9 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})$  and by 3.3 % after SIT (54.1 to 55.8 ml \cdot \text{kg}^{-1} \cdot \text{min}^{-1}). The VO<sub>2max</sub> improvements were closely followed by O<sub>2</sub> pulse improvements of 9.3 % after HIIT and by 3.5 % after SIT. MAOD increased significantly more after SIT compared to HIIT. The SIT group improved MAOD by 32 % (60.6 to 79.8 ml $\cdot$ kg<sup>-1</sup>), while the changes were not significant after HIIT. The 300- and 3000-meter running performance changes were not significantly different between groups. The 3000-meter running performance improved by 5.7 % after HIIT (843 to 794 seconds) and by 5.7 % after SIT (845 to 797 seconds). The 300meter running performance improved by 5.1 % after HIIT (57.5 to 54.6 seconds) and by 5.7 % after SIT (54.3 to 50.8 seconds). Conclusion: HIIT was significantly more effective in improving VO<sub>2max</sub> and O<sub>2</sub> pulse compared to SIT. SIT was significantly more effective in improving MAOD compared to HIIT. There was no significant difference in WE, LT, 300and 3000-meter running performance changes between groups.

**Keywords**: Maximal oxygen uptake, lactate threshold, work economy, anaerobic capacity, maximal accumulated oxygen deficit, high-intensive interval training, supramaximal interval training, endurance performance.

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## Abbreviations

ATP: Adenosine triphosphate a-vO<sub>2diff</sub>: Arterio-venous oxygen difference HIIT: High-intensity interval training HR: Heart rate HR<sub>max</sub>: Maximal heart rate HR<sub>peak</sub>: Peak heart rate LT: Lactate threshold MAOD: Maximal accumulated oxygen deficit MICT: Moderate intensity continuous training O<sub>2</sub>: Oxygen O<sub>2</sub> pulse: Oxygen pulse Q: Cardiac output R: Respiratory exchange ratio SEE: Standard error of estimate SD: Standard deviation SIT: Supramaximal interval training vLT: Velocity at lactate threshold **V<sub>E</sub>: Ventilation** VO<sub>2</sub>: Oxygen uptake VO<sub>2max</sub>. Maximal oxygen uptake VO<sub>2peak</sub>: Peak oxygen uptake vVO<sub>2max</sub>: Velocity at maximal oxygen uptake WE: Work economy [La<sup>-</sup>]<sub>b</sub>: Concentration of blood lactate

## Introduction

Lately, much attention is devoted to exercising at supramaximal intensities. Many studies have reported supramaximal interval training (SIT) to improve endurance performance, and its physiological parameters at the same level of or better than moderate intensity continuous training (MICT) performed at ~70 % of heart rate maximum (HR<sub>max</sub>) in untrained to endurance-trained subjects<sup>[1-7]</sup>. High-intensive interval training (HIIT) is shown to give a better response in maximal oxygen uptake (VO<sub>2max</sub>) than MICT in patient to endurance-trained groups<sup>[8-10]</sup>. Thus, SIT should be compared with HIIT to conclude its effect on physiological parameters and endurance performance.

Most studies involving HIIT and SIT are carried out with males. Females have a lower VO<sub>2max</sub>, stroke volume (SV), hemoglobin levels, and anaerobic capacity compared to males and does explain most of the variation in endurance performance between genders<sup>[11-16]</sup>. The physiological differences between genders are mainly due to differences in body size, muscle mass, and size of the heart<sup>[17]</sup>. Because of these gender differences, it is important to find an optimal training modality to enhance the physiological parameters and endurance performance in females.

## Physiological determinants of endurance performance

Muscle actions require energy in the form of adenosine triphosphate (ATP)<sup>[18]</sup>. Because the ATP stores are limited, a process of continuous regeneration of ATP by the aerobic and anaerobic energy systems is crucial in maintaining muscle actions. The muscle cells can produce energy by regenerating ATP through 1) breakdown of phosphocreatine, 2) glycolysis, and 3) oxidative phosphorylation of carbohydrates and fatty acids. The two first processes are done anaerobically and regenerate ATP faster than the aerobic processes. Performing at higher exercise intensities increases the accumulation of lactic acid, which is associated with skeletal muscle fatigue<sup>[18]</sup>. Hence, the anaerobic energy processes cannot contribute to a quantitatively high level as the duration and energy expenditure increases<sup>[18]</sup>. The oxidative phosphorylation of carbohydrates, fatty acids, and oxygen (O<sub>2</sub>) are done aerobically in the mitochondria. At higher aerobic intensities, the muscle cells prefer using carbohydrates as the main fuel due to faster regeneration of ATP<sup>[18]</sup>.

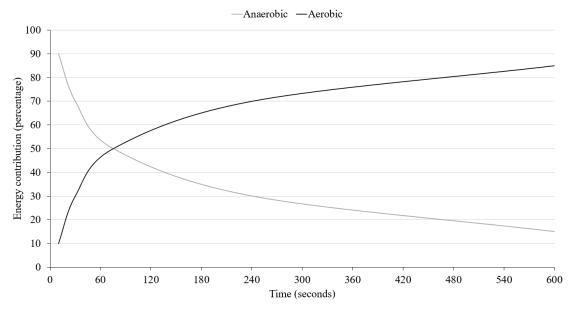


Figure 1. The relative contribution to energy output from aerobic and anaerobic processes in maximal exercise. The figure is based on data from Åstrand et al.<sup>[18]</sup> and Duffield et al.<sup>[19]</sup>.

The relative contribution of the energy systems depends on the intensity and duration of the exercise event. In a 30-second all-out effort exercise, the anaerobic energy contribution is predicted to be more than 70 % of the ATP regeneration<sup>[20]</sup>. The energy demands are somewhat different in a training situation with repeated all-out intensities like SIT. The relative contribution of the aerobic energy system increases and performance decreases with repetitive 30-second all-out sprints due to the depletion of phosphocreatine, reduced glycolytic rate, and accumulation of lactic acid<sup>[21, 22]</sup>. The contribution of both energy systems is equal between 60-90 seconds of all-out effort exercise<sup>[20, 23]</sup>. As the duration of the exercise increase, more of the energy demands derives from the aerobic energy processes. At approximately 10-minute of maximal work, approximately 85 % of the energy demand is predicted to be contributed by the aerobic energy processes<sup>[20]</sup>. The main physiological factors determining the interindividual variation in endurance performance are maximal oxygen uptake (VO<sub>2max</sub>), lactate threshold (LT), work economy (WE), and anaerobic capacity<sup>[24, 25]</sup>.

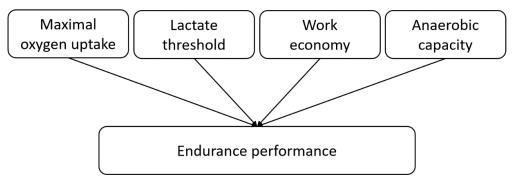


Figure 2. The model is a diagram of the various factors of endurance performance and is modified from the studies of Pate and Kriska<sup>[25]</sup>, and Joyner and Coyle<sup>[24]</sup>.

### VO<sub>2max</sub>

 $VO_{2max}$  reflects the body's ability to supply and utilize  $O_2$  at maximal exercise intensity over an extended period. It is considered to be the most important factor in predicting aerobic endurance performance, independent of gender<sup>[14, 15, 26-29]</sup>.  $VO_{2max}$  is specific to the tested activity-mode and should be tested on the activity-mode the subjects train at regularly<sup>[18, 30]</sup>. Factors determining  $VO_{2max}$  include ventilation, hemoglobin concentration, cardiac output (Q), pulmonary diffusion capacity, and muscle diffusion capacity<sup>[31]</sup>. According to the derivation of the Fick equation ( $VO_{2max} = Q \cdot a \cdot vO_{2diff}$ ),  $VO_{2max}$  changes can be seen in changes of Q or arterio-venous  $O_2$  difference ( $a \cdot vO_{2diff}$ ).

Q is thought to be the major limiting factor of VO<sub>2max</sub> in healthy and fit individuals at sea level<sup>[8, 11, 14, 15, 31, 32]</sup> and is the sum of SV and heart rate (HR). Since HR does not change significantly by training, SV is thought to be the major factor determining Q, independent of gender<sup>[8, 33]</sup>. The contractility force, the volume of the heart, and the capacity of refilling the heart with blood determines  $SV^{[11, 14, 34]}$ . SV is lower in females compared to males, due to smaller body size and thus the size of the heart<sup>[13, 17, 35]</sup>. A lower hemoglobin level in females is the main factor that distinguishes  $VO_{2max}$  between genders<sup>[12, 36]</sup>. Because hemoglobin concentration is independent of body size, and it determines  $O_2$  carrying capacity, females have a disadvantage regardless of Q in terms of  $VO_{2max}$  level compared to males. The a-vO<sub>2diff</sub> reflects mitochondria's ability to extract and utilize  $O_2$ . It is expressed as the difference between the  $O_2$  saturation of arterial blood and mixed venous blood<sup>[18]</sup>. Mitochondrial capacity to utilize  $O_2$  exceeds the  $O_2$  supply during whole-body exercise in healthy and moderately endurance-trained individuals<sup>[37-39]</sup>.

#### HIIT and SIT-induced adaptations on VO<sub>2max</sub>

VO<sub>2max</sub> changes are directly related to intensity, duration, and frequency of training<sup>[34]</sup>. However, intensity is the most important factor on improving VO<sub>2max</sub>. In patient to endurancetrained populations, HIIT performed at 90-95 % of HR<sub>max</sub> is shown to give a better response in VO<sub>2max</sub> compared to energy matched (total oxygen consumption (VO<sub>2</sub>)) MICT performed at ~70 % of HR<sub>max</sub> (p < 0.001)<sup>[8, 10, 40, 41]</sup>. SV increases with workloads up to VO<sub>2max</sub> in moderately to endurance-trained, independent of gender<sup>[15, 42]</sup>. Exercising at intensities close to VO<sub>2max</sub> and maximal SV is thought to be an important factor of morphological adaptation as it overloads the diastolic stretch and ventricular emptying of the heart due to increased afterload of blood to the heart<sup>[43]</sup>. The improvements in VO<sub>2max</sub> were closely followed by an increase in SV<sup>[8, 10]</sup>. HIIT is also reported to have a great effect on improving VO<sub>2max</sub> in females. VO<sub>2max</sub> is reported to improve by 13 % followed by an 11.4 % increase in O<sub>2</sub> pulse  $(\frac{VO_2 (ml \cdot min^{-1})}{HR (beats \cdot min^{-1})})$  performed at ~85 % of VO<sub>2max</sub> in untrained females (36.3 ± 3.7 ml·kg<sup>-1</sup>·min<sup>-1</sup>) after 7 sessions of HIIT in 2 weeks<sup>[44]</sup>. O<sub>2</sub> pulse is shown to represent an acceptable and reliable non-invasive measurement of SV in untrained (42.3 ± 6.5 ml·kg<sup>-1</sup>·min<sup>-1</sup>, r = 0.78-0.84) and endurance-trained subjects (62.2 ± 8.5 ml·kg<sup>-1</sup>·min<sup>-1</sup>, r = 0.71)<sup>[45-47]</sup>. In recreationally active females (42.6 ± 2.9 ml·kg<sup>-1</sup>·min<sup>-1</sup>), VO<sub>2max</sub> is reported to improve by 18 % (p < 0.001) followed by an 13 % increase (p < 0.001) in atrioventricular plane displacement during exercise might indicate an improved SV as it shows changes in ventricular systolic function<sup>[48]</sup>. Considering SV being the major factor distinguishing VO<sub>2max</sub> in moderately endurance-trained and elite endurance female athletes<sup>[14, 15]</sup>, HIIT is thought to increase VO<sub>2max</sub> and SV in moderately endurance-trained females.

VO<sub>2max</sub> is reported to improve by 5-19 % in untrained to recreationally active male and females ( $< 47 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) after 2-12 weeks of SIT (4-6 x 30-second SIT)<sup>[2, 6, 7, 49-51]</sup>. In these studies the training and testing was performed on an ergometer bike. VO<sub>2max</sub> values are on average ~10 % lower in cycling compared to running if the subjects are not trained cyclists <sup>[18]</sup>. Hence, some of the changes in VO<sub>2max</sub> may be due to familiarization of cycling. Cardiovascular measurements after SIT are only done on an untrained to recreationally active population. The only study done exclusively with females reported a 12 % increase in VO<sub>2max</sub> followed by an 11 % in SV (CO<sub>2</sub> breathing) after 4 weeks of SIT in untrained females ((36.3  $\pm$  3.7 ml·kg<sup>-1</sup>·min<sup>-1</sup>)<sup>[50]</sup>. In recreational active male and females (43.6  $\pm$  5.4 ml·kg<sup>-1</sup>·min<sup>-1</sup>), SIT is reported to increase VO<sub>2max</sub> and O<sub>2</sub> pulse (at VO<sub>2max</sub>) by 6 % after 6 sessions of cycling SIT over 2 weeks<sup>[49]</sup>. This is in contrast to active recreational males and females population (46.8  $\pm$  1.6 ml·kg<sup>-1</sup>·min<sup>-1</sup>) that have been reported to increase VO<sub>2max</sub> by 12 % followed by a 7 % increase in a-vO<sub>2diff</sub><sup>[7]</sup>. The changes in SV (acetylene ( $C_2H_2$ ) non-breathing procedure) were not significant after six weeks of SIT<sup>[7]</sup>. The different methods of examining the cardiovascular adaptations and gender differences may influence the differences in results between the studies. In moderately endurance-trained males (~51 ml·kg<sup>-1</sup>·min<sup>-1</sup>), VO<sub>2max</sub> is reported to improve by 6-7 % after both cycling and running SIT (10 x 30-second SIT)<sup>[4, 22]</sup>. The results of VO<sub>2max</sub> changes in SIT studies of moderately to endurance-trained males (> 55 ml·kg<sup>-1</sup>·min<sup>-1</sup>) are equivocal. Two studies have reported no significant change in VO<sub>2max</sub> after

4-9 weeks of SIT after reducing the training volume (km per week) by 34-64 % (p < 0.05) in moderately to endurance-trained male runners (55-61 ml·kg<sup>-1</sup>·min<sup>-1</sup>)<sup>[1, 52]</sup>. SIT is also shown not to change VO<sub>2max</sub> significantly when added in the regular training of endurance-trained males (63.6 ± 5.2 ml·kg<sup>-1</sup>·min<sup>-1</sup>)<sup>[53]</sup>. In contrast, VO<sub>2max</sub> is reported to improve by 3 % (p < 0.05) after 4 weeks of SIT in endurance-trained triathletes and cyclists (62.6 ± 4.1 ml·kg<sup>-1</sup>·min<sup>-1</sup>). VO<sub>2max</sub> is also reported to improve by ~2 % improvement (p < 0.05) after 10 all-out SIT sessions in 40-days in moderately endurance-trained runners (59.3 ± 3.2 ml·kg<sup>-1</sup>·min<sup>-1</sup>)<sup>[54]</sup>. However, the results should be interpreted with caution as the VO<sub>2max</sub> changes are within a day-to-day variation of VO<sub>2max</sub> (± 3 %)<sup>[18]</sup>. Considering it takes 1-2 minutes to adjust to an intensity<sup>[18]</sup>, SIT may only have a minor effect on VO<sub>2max</sub> and SV in moderately to endurance-trained subjects are needed due to the equivocal findings. This especially applies to the female population, as no studies have examined the effect of SIT on VO<sub>2max</sub> in moderately to endurance-trained females.

#### HIIT vs. SIT

Several studies have reported SIT to improve VO<sub>2max</sub> similarly to or significantly more than MICT in unfit to moderately endurance-trained subjects ( $< 52 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )<sup>[2, 4, 6, 7]</sup>. Esfarjani and Laursen<sup>[4]</sup> compared SIT with HIIT and reported no significant difference in VO<sub>2max</sub> changes between groups in moderately endurance-trained male runners ( $51.6 \pm 2.7$ ml·kg<sup>-1</sup>·min<sup>-1</sup>). However, the subjects worked with a fixed and constant workload that was calculated from the pre-VO<sub>2max</sub> test (SIT: 130 % of <sub>V</sub>VO<sub>2max</sub>; HIIT: <sub>V</sub>VO<sub>2max</sub>). As subjects improve VO<sub>2max</sub> during the training period, the relative intensity at the same absolute workload decreases. This applies especially for the HIIT group, as the training intensity is more likely close to LT at the end of the training intervention. Exercising at the intensity of LT is reported to not change VO<sub>2max</sub> significantly in moderately endurance-trained subjects  $(59.6 \pm 7.6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1})^{[8]}$ . By adjusting the fixed and constant intensity halfway through an intervention with mid-VO<sub>2max</sub> values, HIIT improved VO<sub>2max</sub> significantly more compared to SIT (8 vs. 3 %, p < 0.05) after a 4-week intervention in well-trained triathletes and cyclists  $(64.5 \pm 5.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})^{[55]}$ . However, this study also used a fixed and constant workload from pre- and mid-VO<sub>2max</sub> values (SIT: 130 % of vVO<sub>2max</sub>; HIIT: power output at VO<sub>2max</sub>). A more practical and time-efficient method to control and adjust intensity during a training intervention would be to use HR, as HR increases proportionally to aerobic exercise intensity<sup>[18]</sup>. Studies doing a meta-analysis of randomized controlled groups revealed that

HIIT ( $\geq 15$  minutes of HIIT performed at 90-95 % of HR<sub>max</sub> per session) is more effective in improving VO<sub>2max</sub> compared to low volume SIT ( $\leq 5$  minutes of SIT per session) performed as  $\leq 30$ -seconds per interval<sup>[56, 57]</sup>. Based on these findings, HIIT may represent a more optimal training modality on improving VO<sub>2max</sub> in subjects of different fitness level compared to SIT. No studies have examined the effects of HIIT and SIT on VO<sub>2max</sub> and SV and compared the training modalities in moderately endurance-trained females.

### Lactate threshold

LT describes the intensity where the concentration of blood lactate [La<sup>-</sup>]<sub>b</sub> starts to accumulate during continuous exercise<sup>[27]</sup>, expressed as the % of VO<sub>2max</sub> it occurs<sup>[8]</sup>. Accumulation of [La<sup>-</sup> ]<sub>b</sub> is a result of pyruvate production by glycolysis exceeding the pyruvate consumption by mitochondria<sup>[58]</sup>. Performing at intensities at or above LT can only be sustained for a limited time due to limited glycogen stores and accumulation of lactic acid<sup>[18]</sup>. Several factors may determine LT, but they are complex and not fully understood. The oxidative capacity of mitochondria seems to play an important role<sup>[59]</sup>, as it may determine the relative intensity (% of  $VO_{2max}$ ) that can be sustained over an extended duration. Muscle fiber type composition may determine LT, as type I muscle fibers have a greater amount and size of mitochondria and aerobic enzyme activity compared to type II muscle fibers<sup>[60]</sup>. Nevertheless, all three fiber types can improve mitochondria density and enzyme activity through training<sup>[61]</sup>. So far, no studies have reported a change in LT expressed as % of VO<sub>2max</sub> after HIIT<sup>[8, 62]</sup> or SIT<sup>[53, 63]</sup>. However, workload at LT has been reported to change significantly after HIIT<sup>[8, 53, 62]</sup> and SIT<sup>[53, 63]</sup>. Improved performance at LT is due to enhanced VO<sub>2max</sub> or WE, or both<sup>[8]</sup>. So far, no studies have examined HIIT and SIT's effect on LT with moderately to endurance-trained females.

#### Work economy

WE refers to the O<sub>2</sub> cost at a given workload, commonly expressed as the steady-state VO<sub>2</sub> or O<sub>2</sub> cost per meter at a given workload<sup>[8]</sup>. There are interindividual variation in O<sub>2</sub> cost at given velocities, independent of gender<sup>[13, 64-67]</sup>. WE can be a strong predictor for endurance performance in homogeneous samples (r = 0.79-0.82, p < 0.01)<sup>[13, 66-68]</sup>. Several factors may determine WE, including mitochondria's ability to extract and utilize O<sub>2</sub> and muscle fiber distribution<sup>[69]</sup>, motor unit recruitment pattern<sup>[70, 71]</sup>, ability to store and release elastic energy by increasing stiffness of the muscles and tendons, and anatomical trait<sup>[25, 69]</sup>.

#### HIIT and SIT-induced adaptations on WE

By increasing training volume, independent of intensity at submaximal workloads, WE have been reported to improve by 5-11 % (p < 0.05) in moderately endurance-trained subjects (56- $60 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})^{[8]}$ . It has also been reported to increase by 4-7 % (p < 0.05) by supplementing HIIT to the regular training in soccer players<sup>[62, 72]</sup>. The VO<sub>2max</sub> changes were not significant in a control group only performing the regular soccer training<sup>[62]</sup>. In performance matched marathoners, the females performed at the same level as the males despite having 10 % lower VO<sub>2max</sub> (203 ± 1.1 vs.  $180 \pm 3.9 \text{ ml} \cdot \text{kg}^{-0.75} \cdot \text{min}^{-1}$ , p < 0.05)<sup>[13]</sup>. However, the females compensated with a greater WE  $(p < 0.05)^{[13]}$ . The higher WE in females were likely due to the females higher training volume of running compared to the males, as stated by the author. WE have been reported to improve by 3-7 % (p < 0.05) after 4-9 weeks of SIT, despite reducing the training volume (p < 0.05) in moderately to endurancetrained male runners<sup>[1, 52, 54]</sup>. It indicates that WE can improve significantly despite reducing training volume, as long as some of the training is kept at supramaximal intensity. Only one study has compared the HIIT and SIT's effect on WE and found no significant difference in change on WE in endurance-trained males<sup>[53]</sup>. No studies have examined the HIIT and SITinduced effect on WE or compared them in moderately endurance-trained females.

## **Anaerobic capacity**

Anaerobic capacity reflects the capacity of regenerating ATP via phosphocreatine and carbohydrates through glycolysis<sup>[18]</sup>. Exercising at supramaximal intensity leads to an accumulation of lactate acid and increased hydrogen concentration (decreased pH) in the working muscles<sup>[73-75]</sup>. These factors limit the working skeletal muscles ability to maintain a relatively high force production and rate of force development. The ATP regeneration is higher in type II muscle fibers compared to type I muscle fibers<sup>[60]</sup>, making the ability to produce force and rate of force development better in type II muscle fibers due to higher motor unit recruitment and discharge rate<sup>[18]</sup>. Because females have fewer type II muscle fibers and less muscle mass in general compared to males, females have a lower capacity to regenerate ATP through the anaerobic energy processes<sup>[76]</sup>.

To this date, there are no methods that directly measure and determine anaerobic capacity. Calculating maximum accumulated oxygen deficit (MAOD) with a supramaximal intensity lasting 2-3 minutes has been proposed to be the best method of determining anaerobic capacity<sup>[77]</sup>. MAOD is calculated as the difference between theoretical accumulated O<sub>2</sub> demand and the actual  $O_2$  uptake expressed as an equivalent in absolute (L) and relative (ml·kg<sup>-1</sup>) values throughout an exhaustive MAOD-test.

### HIIT and SIT-induced adaptations in anaerobic capacity

MAOD is reported to be 30 % higher in sprint runners (200-400 meter) and middle distance runners (800-1500 meter) compared to endurance-trained and untrained male and females (p < 0.001)<sup>[78, 79]</sup>. Few studies have examined the effect of HIIT on MAOD. HIIT is not reported to improve MAOD significantly in endurance-trained males  $(61.0 \pm 5.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})^{[53]}$ . In the same study, all-out SIT did neither change MAODsignificantly in endurance-trained males  $(63.6 \pm 5.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})^{[53]}$ . The nonsignificant changes in MAOD after SIT may be due to the initial values of MAOD were high in the subjects. However, SIT is shown to improve MAOD by 17 % in two different SIT protocols (8 x 20-second all-out sprints interspersed with 5-minute rest and 3 x 2-minute supramaximal intensity interval interspersed with 8-minute rest) in recreationally active males. A SIT protocol of shorter recovery periods (7-8 x 20-second intervals interspersed with 10-second passive recovery between intervals) is also reported to improve MAOD (ml·kg<sup>-1</sup>) by 14.5 % (p < 0.05) in endurance-trained males  $(62.9 \pm 5.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})^{[63]}$ . Thus, supramaximal intensity is required to improve MAOD. The only study including females did not report a significant change in MAOD after SIT in recreationally active females<sup>[79]</sup>. Few female participant in the study does give a weaker statistical power<sup>[80]</sup> and more training intervention studies must be done with females. No studies have compared the effect of HIIT and SIT on MAOD in moderately endurance-trained females.

## **Endurance performance**

 $VO_{2max}$  is considered as the most important factor in predicting aerobic endurance performance<sup>[26-28]</sup>. It distinguishes the different level of endurance performances in events as short as ~45 seconds<sup>[29]</sup>. WE is a good predictor of endurance performance in groups of similar  $VO_{2max}$ <sup>[13, 66-68]</sup>. The fractional utilization of  $VO_{2max}$ , or LT, is suggested to be of less importance in endurance events lasting shorter than 20 minutes<sup>[81]</sup>. In shorter lasting exercise events up to 2-3 minutes, a higher maximal anaerobic power, anaerobic capacity, and WE at supramaximal intensity may be of greater importance<sup>[23, 29, 66, 78, 82]</sup>.

#### Aerobic endurance events

HIIT is reported to improve 3000-meter running performance (~10-13 minute effort time) by 5-7 % (p < 0.05) in moderately to endurance-trained males (52-61 ml·kg<sup>-1</sup>·min<sup>-1</sup>)<sup>[3, 4, 53]</sup>. The changes are similar in 40 km cycling time-trial performance (6 %, p < 0.05, ~58 minute effort time) after HIIT in endurance-trained triathletes and cyclists<sup>[55]</sup>. The only study including females have shown a 5 % improvement (p < 0.001) in 3000-meter running time-trial performance (18.5 minute effort time) after HIIT in recreationally active females<sup>[3]</sup>. SIT is reported to improve 3000-meter running performance (~10-13 minute effort time) by 2-3.5 % (p < 0.05) in moderately and endurance-trained males (52-63 ml·kg<sup>-1</sup>·min<sup>-1</sup>)<sup>[1, 3, 4, 53]</sup>. The changes are similar in 10 km running performance (3 %, p < 0.05, ~36-42 minute effort time) and 40 km cycling time-trial (6 %, p < 0.05, ~57 minutes of exhaustive effort) in moderately and endurance-trained males [1, 54, 55]. The only study including females have shown a 5 % improvement (p < 0.001) in 3000-meter running time-trial performance (~18 minute effort time) after SIT in recreationally active females<sup>[3]</sup>.

Both HIIT and SIT may lead to improved endurance performance in recreationally active male and females to endurance-trained males. Few studies have compared the effect of HIIT and SIT on endurance-performance and the findings are equivocal. The performance changes in 3000-meter running time-trial and 40 km cycling time-trial has not been significantly different between HIIT and SIT in moderately to endurance-trained males<sup>[4, 55]</sup>. The only study including females did not report a significant difference in 3000-meter running performance changes between HIIT and SIT in recreationally active females<sup>[53]</sup>. However, a study of endurance-trained males reported HIIT being superior (p < 0.05) to SIT in changing the 3000-meter running performance<sup>[53]</sup>. Considering the high aerobic energy contribution in a 10-15 minute maximal running event<sup>[18, 19]</sup> and VO<sub>2max</sub> being an important factor in endurance performance<sup>[26-29]</sup>, HIIT may improve endurance performance more than SIT. No studies have examined the effect of HIIT and SIT and compared them on endurance performance in moderately endurance-trained females.

#### Anaerobic endurance events

HIIT is reported to improve 300-meter running performance (~46 second effort time) by 1.3 % (p < 0.05) in endurance-trained males ( $61.0 \pm 5.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )<sup>[53]</sup>. HIIT is also reported to improve repeated sprint ability (6 x 40-meter interspersed with 24-second rest between each repeated sprint) by 3 % (p < 0.05) in moderately trained males (~36-second of total effort time) and by 5 % (p < 0.001) in recreationally active females (~45-second of total effort time)<sup>[3]</sup>. SIT is reported to improve 300-meter running performance (~45 second effort time) by 3.3 % (p < 0.01) in endurance-trained males<sup>[53]</sup>. SIT is also reported to improve covered distance in 30-second running performance by 5-7 % (p < 0.05) in moderately trained male runners (59-63 ml·kg<sup>-1</sup>·min<sup>-1</sup>) after 4-9 weeks of SIT<sup>[1, 54]</sup>. The repeated sprint ability is reported to improve by 2.7 % (p < 0.001) in moderately trained males (~45 second effort time) and by 2.1 % (p < 0.001) in recreationally active females (~44 second effort time) after SIT<sup>[3]</sup>.

Few studies have compared the effect of HIIT and SIT on shorter-lasting endurance events, and the results are equivocal. The performance changes in 300-meter running time-trial have not been significantly different between HIIT and SIT in endurance-trained males<sup>[53]</sup>. However, SIT is reported to improve repeated sprint ability significantly more (p < 0.01) than HIIT in recreationally active females and moderately endurance-trained males<sup>[3]</sup>. Differences in testing protocols make it challenging to compare the results between the studies. However, no studies have examined the effect of and compared HIIT and SIT in moderately to endurance-trained females. Considering the energy contribution of aerobic and anaerobic energy processes being equal (40-50 % aerobic vs. 50-60 % anaerobic energy contribution) in a maximal exercise event of 50-60 seconds<sup>[18, 19]</sup>, changes in anaerobic endurance performance may not be significant between HIIT and SIT.

## Aim and hypothesis

The present study aims to compare the effect of 10 x 30-second all-out SIT and 4 x 4-minute HIIT performed at 90-95 % of  $HR_{max}$  on  $VO_{2max}$ , WE, LT, anaerobic capacity, and endurance performance in moderately endurance-trained females. The hypothesis proposed that HIIT improves  $VO_{2max}$  and 3000-meter running performance significantly more than SIT, while SIT improves anaerobic capacity significantly more than HIIT.

## Method

## **Subjects**

22 healthy, non-smoking, recreationally active, and moderately endurance-trained female university students volunteered to participate in the present study. Inclusion criteria to participate were  $VO_{2max}$  level exceeding 45 and less than 58 ml·kg<sup>-1</sup>·min<sup>-1</sup> at baseline. In addition, the participants had to do endurance training at least once per week, or other recreational activities for at least 3 times per week before the study. During the study, the participants had to perform at least 20 out of 24 supervised training sessions over the 8-week training intervention. The participants were excluded if they had a history of cardiovascular diseases, musculoskeletal injuries, use of medication that could affect the physiological responses to the training, and if they were out of training for over a week due to illness or other reasons that could affect the training responses. The study was approved by The Institutional Review Board of NTNU and was carried out in accordance with the recommendation of the Norwegian Data Protection Center and the Declaration of Helsinki. All subjects had to sign a written informed consent to participate in the study.

Table 1. Descriptive data of the subjects.

	HIIT $(n = 5)$	SIT (n = 6)	
Age (year)	$24.6 \pm 2.3$	$21.0 \pm 1.7^{\$}$	
Height (cm)	$167 \pm 6$	$173 \pm 4$	
Body mass (kg)	$59.5 \pm 5.4$	$66.3 \pm 9.9$	
VO <sub>2max</sub>			
VO <sub>2</sub> (L·min <sup>-1</sup> )	$3.22 \pm 0.38$	$3.60 \pm 0.65$	
VO <sub>2</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	$54.1 \pm 1.9$	$54.1 \pm 2.4$	
VO <sub>2</sub> (ml·kg <sup>-0.75</sup> ·min <sup>-1</sup> )	$150.2 \pm 8.3$	$154.1 \pm 11.6$	

Data are presented as mean  $\pm$  standard deviation (SD). VO<sub>2max</sub>, maximal oxygen uptake. <sup>§</sup>Significant difference between groups (p < 0.05) at baseline.

## **Testing and training procedures**

The subjects performed 2 physiological and 1 performance test at 3 separate days both before and after the training intervention. All subjects were told to avoid strenuous activity for the last 24 hours before a test and got at least 48 hours of recovery between each test. The subjects were randomized to either 4 x 4-minute HIIT performed at 90-95 % of HR<sub>max</sub> or 10 x 30-second all-out SIT, performed 3 times per week over 8 weeks.

A motor-driven Woodway treadmill (PPS 55 Sport, Waukesha, Germany) and motor-driven Gymsport TX200 treadmills (Trondheim, Norway) were calibrated at 5.3 % and 5.5 % and

used during the tests and training, respectively. The measurements of VO<sub>2max</sub>, WE, LT, MAOD, ventilatory parameters, and gas exchange were carried out using a Cortex Metamax II portable metabolic test system (Cortex Biophysik GmbH, Leipzig, Germany). The Cortex Metamax II is tested against the Douglas bag method and found to be valid for metabolic gas measurements up to 250 Watts<sup>[83]</sup>, equaling to running velocities up to13 km·h<sup>-1 [18]</sup>. [La<sup>-</sup>]<sub>b</sub> was measured by drawing hemolyzed blood samples from the fingertips and analyzed using a Biosen C-line lactate analyzer (EKF-diagnostic GmbH, Leipzig, Germany). HR was recorded during testing and training by an HR monitor (Polar F11, Polar Electro Oy, Kempele, Finland).

#### Test day 1

The first day of testing consisted of a VO<sub>2max</sub>, WE, and LT-tests. It started with a 10-minute warmup at 50-60 % of predicted VO<sub>2max</sub>. A blood sample was drawn to establish a baseline value of [La]<sub>b</sub> after the warmup. LT was determined through a minimum of three 5-minute intervals at increasing intensities between 60-95 % of VO<sub>2max</sub>, separated by a short recovery period to draw blood samples to determine [La<sup>-</sup>]<sub>b</sub> at each intensity. All subjects performed a 5-minute interval at 7 km $\cdot$ h<sup>-1</sup> to determine WE at a standardized workload. WE is expressed as allometrically scaled values (ml·kg<sup>-0.75</sup>·min<sup>-1</sup>) as it decreases the standard deviation (SD) of WE<sup>[84]</sup>. The average 30-second value of recorded VO<sub>2</sub> and HR within the last minute of each interval was used to determine the intensity at each velocity. The VO<sub>2</sub>, % of VO<sub>2max</sub>, HR, and velocity at LT was calculated to correspond  $[La^-]_b$  of 1.5 mmol·L<sup>-1</sup> above the baseline value<sup>[85]</sup>. The subjects continued directly to the  $VO_{2max}$ -testing protocol as they finished the WE and LT testing protocol. The VO<sub>2max</sub> test started at a velocity exceeding vLT and increased by 1 km·h<sup>-1</sup> each minute until exhaustion. The subjects reached exhaustion within 4-6 minutes. VO<sub>2max</sub> was accepted when achieving two out of three following criteria; 1) a plateau in VO<sub>2</sub> despite an increase in workload, 2) [La<sup>-</sup>]<sub>b</sub>  $\ge$  8.0 mmol·L, and 3) a respiratory exchange ratio (R) of  $\geq 1.05^{[8, 84]}$ . The highest average VO<sub>2</sub> measured over 30 seconds defined VO<sub>2max</sub>. The highest recorded HR defined HR<sub>max</sub>, and O<sub>2</sub> pulse was calculated and used as a non-invasive measurement of SV<sup>[18, 46, 47]</sup>.

### Test day 2

The second day of testing consisted of a MAOD-test. It started with a 15-minute warmup at 50-60 % of VO<sub>2max</sub><sup>[77]</sup>. The subjects performed two bouts of 10 seconds at the velocity of MAOD-test during the warmup. A 10-minute passive recovery was followed after the warm-up<sup>[77]</sup>. A blood sample was drawn at the end of the 10-minute recovery period to make sure that each subject started the test at baseline values of  $[La^-]_b$  from the first day of testing. The MAOD-test was performed at a constant supramaximal intensity of  $120 \pm 10$  % of VO<sub>2max</sub>, expecting to last between 2-3 minutes. The subjects received strong verbal encouragement to run until exhaustion.  $[La^-]_b$  was measured and analyzed immediately after the test. The MAOD-test was used to verify VO<sub>2max</sub>, expressed as VO<sub>2peak</sub>. It is argued that many subjects never reach a plateau in VO<sub>2</sub> at an incremental VO<sub>2max</sub> test, and a verifying phase should be performed at a constant supramaximal intensity to verify VO<sub>2max</sub> from the incremental test<sup>[86]</sup>.

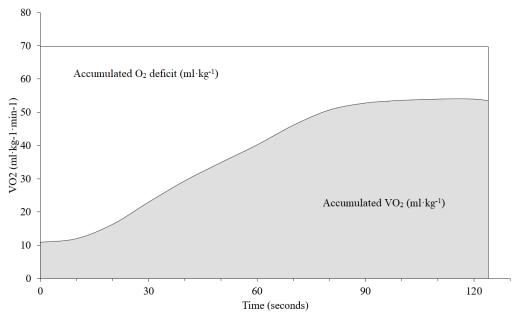


Figure 3. MAOD is the sum of the difference between the estimated accumulated  $O_2$  deficit and the actual  $O_2$  uptake through the whole test, presented as an equivalent value (L or ml·kg<sup>-1</sup>)<sup>[77]</sup>. In this example, the subject ran at 120 % of VO<sub>2max</sub>. The intensity corresponded to a velocity of 14.7 km·h<sup>-1</sup>, which equated to an estimated accumulated  $O_2$  deficit of 69.7 ml·kg<sup>-1</sup>·min<sup>-1</sup> (upper horizontal line) for the subject. The MAOD value of this example was 68.1 ml·kg<sup>-1</sup>.

MAOD is calculated as the difference between the estimated accumulated O<sub>2</sub> deficit and the actual VO<sub>2</sub> of the entire test<sup>[77]</sup>. Performing an all-out exercise within 2-3 minutes is suggested to give the highest accumulated O<sub>2</sub> deficit and the lowest SD ( $\pm 4 \%$ )<sup>[77]</sup>. The highest workload that can be sustained for 2-3 minutes is estimated to equal the intensity of 120  $\pm$  10 % of VO<sub>2max</sub><sup>[77]</sup>. The velocity at the MAOD test was calculated by using the steady-state VO<sub>2</sub> values from the intervals of WE and LT test from the first day of testing, VO<sub>2max</sub> values, and adding a simplified Y-intercept value of 5 ml·kg<sup>-1</sup>·min<sup>-1</sup> at rest to extrapolate a linear

regression line for each subject. With this procedure, the velocity at VO<sub>2max</sub> (vVO<sub>2max</sub>) was also calculated. Two assumptions must be met to calculate MAOD; 1) O<sub>2</sub> is constant at the given workload during the test, and 2) O<sub>2</sub> demand increases linearly to increased workload<sup>[77]</sup>. However, VO<sub>2</sub> is reported to increase non-linearly at workloads above 90 % of VO<sub>2max</sub><sup>[84, 87]</sup>. Hence, calculating MAOD with the assumption of a linear increase in VO<sub>2</sub> with increased workload could underestimate the anaerobic contribution and the anaerobic capacity. The motivation and effort of the subjects, duration of the test, and slope of the linear regression line are other sources of error in the MAOD protocol, according to Medbø et al.<sup>[77]</sup>. The simplified procedure of using a Y-intercept of 5 mL·kg<sup>-1</sup>·min<sup>-1</sup> at rest, and few submaximal intervals to calculate O<sub>2</sub> demand and MAOD in the present study may also decrease the precision of the MAOD-results<sup>[77]</sup>.

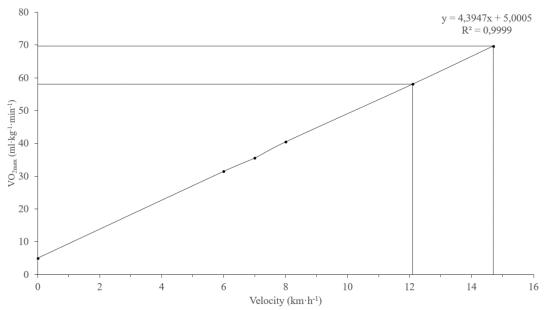


Figure 4. The assumption of a linear increase in VO<sub>2</sub> with an increased workload (velocity) must be met and assumed to calculate the velocity at supramaximal intensities<sup>[77]</sup>. The linear regression is calculated with three submaximal velocities at 7, 8, and 9 km·h<sup>-1</sup>, a simplified Y-intercept of 5 ml·kg<sup>-1</sup>·min<sup>-1</sup> at 0 km·h<sup>-1</sup>, and VO<sub>2max</sub> value of 58.1 ml·kg<sup>-1</sup>·min<sup>-1</sup> at 12.1 km·h<sup>-1</sup> (lower vertical and horizontal line). In this example, the velocity at MAOD (upper vertical and horizontal line) was calculated to be 14.7 km·h<sup>-1</sup> and represented an estimated accumulated O<sub>2</sub> deficit of 69.7 ml·kg<sup>-1</sup>·min<sup>-1</sup> at 120 % of VO<sub>2max</sub>.

#### Test day 3

The third day of testing consisted of a 300- and a 3000-meter running time-trial in a 200meter indoor running track (Ranheim, Trondheim). It started with a 10-minute warmup at a low intensity, including two bouts of 5-10 seconds at a higher intensity. The test started with a 300-meter running time-trial (1.5 x 200-meter) and was performed individually. After the 300-meter running time-trial, the subjects had 30 minutes to recover and to do a re-warmup before the 3000-meter running time-trial (15 x 200-meter). The subjects performed the 3000meter running time-trials in groups not exceeding more than ten. During both 300- and 3000meter running time trial, the subjects received verbal encouragement to run as fast as possible during the tests. A stopwatch was used to measure time, given as seconds.

#### 4 x 4-minute high-intensive interval running

The 4 x 4-minute HIIT-protocol was performed as described by Helgerud et al.<sup>[8]</sup>. Each session started with a 10-minute warmup and ended with a 3-minute cooldown at ~70 % of HR<sub>max</sub>. During the intervals, the subjects were told to reach the target intensity (90-95 % of HR<sub>max</sub>) within the first two minutes of each interval. HR was controlled every third minute of each interval, and the workload was consistently adjusted if HR did not reach the target intensity within the three first minutes of the first interval. During the 3-minute active recovery period, the velocity was reduced to reach the target intensity of ~70 % of HR<sub>max</sub>. The total duration of each HIIT-session was 38 minutes.

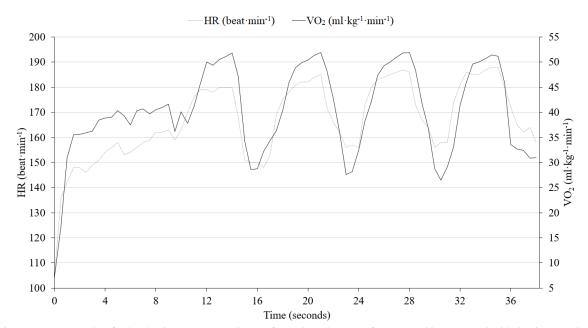


Figure 5. An example of a 4 x 4-minute HIIT session performed at 90-95 % of HR<sub>max</sub> and interspersed with 3-minute active recovery at ~70 % of HR<sub>max</sub>, and includes VO<sub>2</sub> (ml·kg<sup>-1</sup>·min<sup>-1</sup>) responses. Each session started with a 10-minute warm-up and ended with a 3-minute cooldown performed at ~70 % of HR<sub>max</sub> for a subject with an HR<sub>max</sub> of 199 beats·min<sup>-1</sup> and a VO<sub>2max</sub> of 55.7 ml·kg<sup>-1</sup>·min<sup>-1</sup>. The average velocity of the intervals was at 10.2 km·h<sup>-1</sup> and 5.5 % incline on the treadmill.

#### 10 x 30-second all-out supramaximal interval running

The 10 x 30-second all-out SIT-protocol was performed as described by Skovgaard et al.<sup>[54]</sup>. Each session started with a 10-minute warmup and ended with a 3-minute cooldown at ~70 % of HR<sub>max</sub>. The first three sessions were used as familiarization sessions and the intervals were not performed at all-out intensities. The workload in the first interval at the first session was calculated to be at the same velocity as the average 300-meter time-trial velocity from the pretest. The velocity of each interval was adjusted consistently within each session due to

skeletal muscular fatigue. Verbal encouragement was given to the subjects during each interval to keep an all-out intensity on every interval in all sessions, except the three first sessions. The total duration of each SIT-session was 49 minutes. However, the first and last session consisted only of six all-out intervals, and the total duration for the sessions was 33.5 minutes.

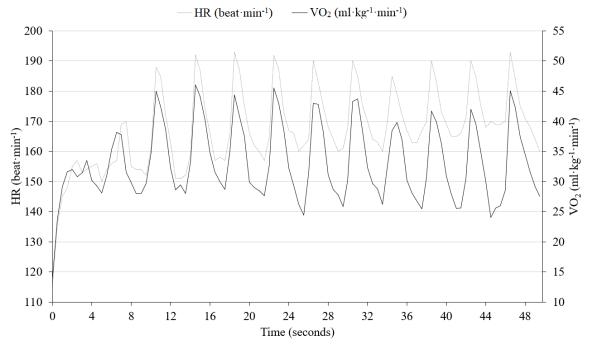


Figure 6. An example of a 10 x 30-second all-out SIT session with HR and VO<sub>2</sub> (ml·kg<sup>-1</sup>·min<sup>-1</sup>) responses. Each session started with a 10-minute warm-up and ended with a 3-minute cool down performed at ~70 % of HR<sub>max</sub>. In this example, the subjects HR<sub>max</sub> was 197 beats·min<sup>-1</sup> and VO<sub>2max</sub> at 51.9 ml·kg<sup>-1</sup>·min<sup>-1</sup>. The session was performed at a laboratory treadmill (Woodway PPS 55 Sport, Waukesha, Germany) with a maximal velocity of 20 km·h<sup>-1</sup>. The average velocity of the intervals was at 20.0 km·h<sup>-1</sup>, and the incline started at 9 % and gradually decreased to 5 % due to muscle skeletal fatigue.

## **Statistical analysis**

The software program IBM SPSS, version 25.0 (Statistical Package for Social Science, Chicago, IL) was used for the statistical analysis, and Microsoft Excel version 16.0 was used to make the figures. Non-parametric tests were used to analyze the data as it is hard to predict if the data are normally distributed or not due to small sample size. A Wilcoxon signed-ranks was used to analyze the differences between pre- and posttests withing a group, and the Mann-Whitney U test was used to analyze the differences between pre- and posttests between groups. Correlations were analyzed with the Spearman's rank order correlation test. The level of significance was set to p < 0.05 in all cases. The results are presented as mean  $\pm$  standard deviation (SD) in the text and tables for descriptive purposes and to be able to compare the results with other studies. The figures are presented as mean  $\pm$  standard error of the mean.

## Results

11 of 22 subjects fulfilled all inclusion criteria for the data analysis in the present study. 7 subjects dropped out of the study. 2 of the subjects in each group dropped out of the study due to medical reasons. Another 2 of the subjects in the SIT group dropped out due to injuries related to the exercise in the present study, and 1 subject dropped out due to difficulties committing the SIT-protocol. 4 subjects were excluded despite completing a minimum of 20 sessions and posttests due to unreliable VO<sub>2</sub> measurements and illness during the endurance performance tests.

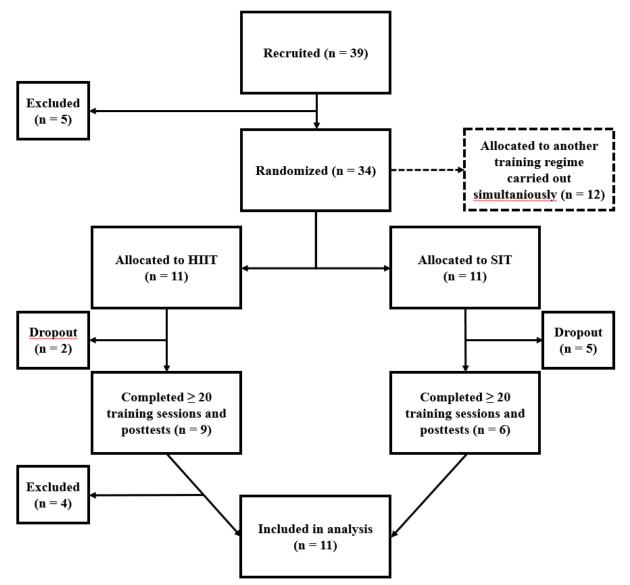


Figure 7. Flow diagram of the study design. HIIT, high-intensity interval training; SIT, supramaximal interval training

The HIIT group carried out significantly more sessions than the SIT group did (p < 0.05). The HIIT group completed  $23 \pm 1$  sessions, while the SIT group completed  $21 \pm 1$  sessions.

Table 2. Changes in physiological factors of endurance performance.

	HIIT	HIIT $(n = 5)$		SIT $(n = 6)$	
	Pretest	Posttest	Pretest	Posttest	
VO <sub>2max</sub>					
VO <sub>2</sub> (L·min <sup>-1</sup> )	$3.22 \pm 0.38$	$3.47 \pm 0.38^{*a}$	$3.60 \pm 0.65$	$3.65 \pm 0.65^{*a}$	
VO <sub>2</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	$54.1 \pm 1.9$	$58.9 \pm 3.3^{*a}$	$54.1 \pm 2.4$	$55.8 \pm 1.8^{*a}$	
VO <sub>2</sub> (ml·kg <sup>-0.75</sup> ·min <sup>-1</sup> )	$150.2 \pm 8.3$	$163.2 \pm 11.1^{*a}$	$154.1 \pm 11.6$	$158.4 \pm 10.8^{*a}$	
VE (L $\cdot$ min <sup>-1</sup> )	$97.8 \hspace{0.2cm} \pm \hspace{0.2cm} 20.5$	$101.5 \pm 21.6^*$	$108.4 \pm 14.9$	$109.7 \pm 17.2$	
R	$1.08 \pm 0.04$	$1.16 \pm 0.05*$	$1.11 \pm 0.04$	$1.09 \pm 0.07$	
$[La^{-}]_{b} (mmol \cdot L)$	$10.2 \pm 1.6$	$11.2 \pm 2.0*$	$11.3 \pm 1.5$	$11.2 \pm 2.0$	
HR <sub>max</sub> (bpm)	$192 \pm 17$	$190 \pm 16$	$201 \pm 8$	$197 \pm 7$	
$O_2$ pulse (ml·beat <sup>-1</sup> )	$16.8 \pm 2.0$	$18.3 \pm 1.6^{*b}$	$17.9 \pm 2.9$	$18.5 \pm 3.1^{*b}$	
$vVO_{2max}$ (km·h <sup>-1</sup> )	$10.9 \pm 0.9$	$12.2 \pm 0.5^{*b}$	$11.4 \pm 0.5$	$11.7 \pm 0.4^{b}$	
Work economy					
$VO_2$ (L·min <sup>-1</sup> )	$2.18 ~\pm~ 0.28$	$2.12 \pm 0.17$	$2.38 \pm 0.52$	$2.32 \pm 0.40$	
VO <sub>2</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	$36.7 \pm 3.1$	$35.9 \pm 1.7$	$35.7 \pm 2.8$	$35.6 \pm 1.2$	
VO <sub>2</sub> (ml·kg <sup>-0.75</sup> ·min <sup>-1</sup> )	$101.8 \pm 8.9$	$99.5 \pm 5.0$	$101.7 \pm 11.3$	$101.1 \pm 6.3$	
$VO_2 (ml \cdot kg^{-0.75} \cdot m^{-1})$	$0.87 \pm 0.08$	$0.85 \pm 0.04$	$0.87 \pm 0.10$	$0.87 \pm 0.06$	
HR (beat · min <sup>-1</sup> )	$160 \pm 20$	$148 \pm 17*$	$170 \pm 9$	$162 \pm 7^*$	
Lactate Threshold					
VO <sub>2</sub> (L·min <sup>-1</sup> )	$2.53 \pm 0.36$	$2.68 \pm 0.27$	$2.70 \pm 0.51$	$2.84 \pm 0.47$	
VO <sub>2</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	$42.4 \pm 3.2$	$45.4 \pm 2.1$	$40.7 \pm 3.0$	$43.5 \pm 1.8^*$	
VO <sub>2</sub> (ml·kg <sup>-0.75</sup> ·min <sup>-1</sup> )	$117.9 \pm 10.3$	$125.8 \pm 7.3$	$116.1 \pm 10.6$	$123.4 \pm 7.3^*$	
% VO <sub>2max</sub>	$78.5 \pm 4.9$	$77.3 \pm 4.9$	$75.4 \pm 5.5$	$78.0 \pm 3.6$	
% HR <sub>max</sub>	$90.8 \pm 2.3$	$89.7 \pm 1.8$	91.1 ± 4.3	92.1 ± 3.6	
vLT (km $\cdot$ h <sup>-1</sup> )	$8.3 \pm 0.5$	$9.1 \pm 0.8^{*}$	$8.3 \pm 0.6$	$8.9 \pm 0.4^{*}$	
$[La^{-}]_{b}$ (mmol·L)	$3.0 \pm 0.5$	$2.7 \pm 0.4^{*b}$	$2.9 \pm 0.4$	$3.1 \pm 0.3^{b}$	
Body mass (kg)	$59.5 \pm 5.4$	$58.8 \pm 3.7$	$66.3 \pm 9.9$	$65.2 \pm 9.9$	

Data are presented as mean  $\pm$  SD. VO<sub>2max</sub>-, WE-, and LT-tests were performed as treadmill running at 5.3 % inclination. HIIT, high-intensive interval training; SIT, supramaximal interval training; VO<sub>2max</sub>, maximal oxygen uptake; V<sub>E</sub>, ventilation; R, respiratory exchange ration; [La<sup>-</sup>]<sub>b</sub>, blood lactate concentration after VO<sub>2max</sub>-test and at lactate threshold; HR<sub>max</sub>, maximal heart rate; O<sub>2</sub> pulse, oxygen pulse; vVO<sub>2max</sub>, velocity at VO<sub>2max</sub>; HR, heart rate at work economy tests; VO<sub>2</sub>, oxygen uptake; vLT, velocity at lactate threshold. \*Significant difference within group (p < 0.05) from pre- to posttest; <sup>a</sup>Significant difference in changes between groups (p < 0.05) from pre- to posttest.

## VO<sub>2max</sub>

 $VO_{2max}$  values increased significantly more after HIIT compared to SIT. The absolute  $VO_{2max}$  (L·min<sup>-1</sup>) values increased by 7.9 ± 2.8 % after HIIT, and by 1.6 ± 1.5 % after SIT (Table 2, Figure 8). The O<sub>2</sub> pulse improved significantly more after HIIT compared to SIT. It increased by 9.3 ± 4.4 % after HIIT and by 3.5 ± 1.8 % after SIT (Table 2, Figure 8). There was a strong correlation between the changes in  $VO_{2max}$  and  $O_2$  pulse (r = 0.91, p < 0.001). The <sub>V</sub>VO<sub>2max</sub> increased significantly more after HIIT compared to SIT. It increased by 11.7 ± 5.6 % after HIIT, while the changes were not significant after SIT (Table 2).

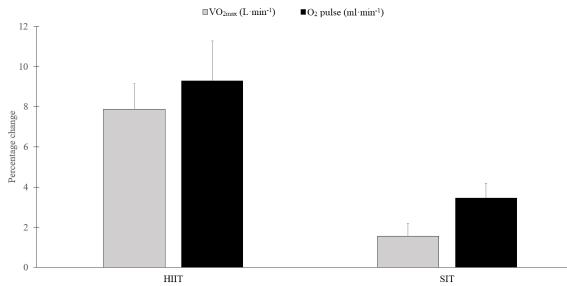


Figure 8. Percentage change in VO<sub>2max</sub> (L·min<sup>-1</sup>) and O<sub>2</sub> pulse (ml·beat<sup>-1</sup>) after HIIT and SIT. The data are presented as mean  $\pm$  SE. \*Significant different within group (p < 0.05) from pre- to posttest; \*Significant difference in change between groups (p < 0.01) from pre- to posttest; bSignificant difference in change between groups (p < 0.05) from pre- to posttest.

## Lactate threshold

LT expressed as % of VO<sub>2max</sub> did not change significantly between or within groups (Table 2). The vLT changes were not significantly different between groups but increased by 9.4  $\pm$  6.5 % after HIIT and by 7.4  $\pm$  6.6 % after SIT (Table 2). The allometrically VO<sub>2</sub> at LT (ml·kg<sup>-0.75</sup>·min<sup>-1</sup>) was not significantly different between groups. It increased by 6.7  $\pm$  6.5 % after SIT, while the changes were not significant after HIIT (Table 2). The [La<sup>-</sup>]<sub>b</sub> at LT decreased significantly more after HIIT compared to SIT.

#### Work economy

WE changes were not significantly different between or within groups. HR at WE changes were not significantly different between groups but decreased by  $7.3 \pm 1.7$  % after HIIT and by  $4.6 \pm 3.8$  % after SIT (Table 2).

### Anaerobic capacity

Table 3. Changes	in anaerobic capac	ty (maximal accu	umulated oxygen deficit)

	HIIT $(n = 5)$		SIT (n = 5)	
	Pretest	Posttest	Pretest	Posttest
MAOD				
L	$4.07 \hspace{0.2cm} \pm \hspace{0.2cm} 1.01$	$4.16~\pm~0.89^a$	$3.85 \pm 0.66$	$4.98 \pm 0.83^{*a}$
ml⋅kg <sup>-1</sup>	$68.1 \hspace{0.2cm} \pm \hspace{0.2cm} 13.0$	$70.3 \pm 12.7^{a}$	$60.6 \pm 5.8$	$79.8 \pm 6.8^{*a}$
% of VO <sub>2max</sub>	$120.2 \pm 6.3$	$118.8 \pm 2.8^{b}$	$116.4 \pm 6.0$	$124.2 \pm 5.5^{*b}$
Time to exhaustion (sec)	$132 \pm 28$	$124 \pm 21$	$169 \pm 56$	$153 \pm 28$
VO <sub>2peak</sub>				
$VO_2$ (L·min <sup>-1</sup> )	$3.07 \pm 0.36$	$3.25 \pm 0.28*$	$3.20 \pm 0.37$	$3.40 \pm 0.44*$
VO <sub>2</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	$51.4 \pm 3.0$	$55.1 \pm 3.7*$	$50.6 \pm 0.4$	$54.5 \pm 1.2^*$
VO <sub>2</sub> (ml·kg <sup>-0.75</sup> ·min <sup>-1</sup> )	$142.9 \pm 9.6$	$152.5 \pm 10.3^*$	$142.5 ~\pm~ 4.2$	$153.0 \pm 7.2^{*}$
% of VO <sub>2max</sub> reached	$95.2 \pm 4.1$	$93.7 \pm 5.3$	$94.8 \pm 3.5$	$98.5 \pm 1.5^*$
R	$1.09 \pm 0.05$	$1.14 \pm 0.12$	$1.12 \pm 0.04$	$1.10 \pm 0.11$
HR <sub>peak</sub> (beat · min <sup>-1</sup> )	$186 \pm 14$	$184 \pm 18$	$191 \pm 6$	$188 \pm 5$
$[La]_b (mmol \cdot L)$	$10.8 \pm 2.0$	$11.6 \pm 2.2$	$11.3 \pm 0.9$	$11.9 \pm 0.9$

Data are presented as mean  $\pm$  SD. The MAOD-tests were performed as treadmill running at 5.3 % inclination. HIIT, highintensity interval training; SIT, supramaximal interval training; MAOD, maximal accumulated oxygen deficit; VO<sub>2peak</sub>, peak oxygen update during MAOD testing; HR<sub>peak</sub>, peak heart rate reached during MAOD testing; [La<sup>-</sup>]<sub>b</sub>, blood lactate concentration after MAOD testing. \*\*\*Significant difference within group (p < 0.001) from pre- to post-tests; \*\*Significant difference within group (p < 0.01) from pre- to post-tests; \*Significant difference within group (p < 0.05) from pre- to posttests; aSignificant difference in changes between groups (p < 0.01) from pre- to posttest; bSignificant difference in changes between groups (p < 0.05) from pre- to posttest.

One subject of the SIT group was excluded from the analysis due to unreliable VO<sub>2</sub> measurements. MAOD increased significantly more after SIT compared to HIIT. MAOD (ml·kg<sup>-1</sup>) improved by  $32.0 \pm 9.0$  % after SIT, while the changes were not significant after HIIT (Table 3, Figure 9). The relative intensity at MAOD increased significantly more after SIT compared to HIIT. It increased by  $6.2 \pm 3.8$  % after SIT, while the changes were not significant after HIIT (Table 3).

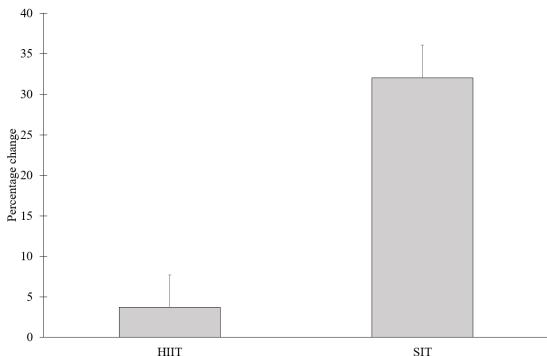


Figure 9. Percentage change in MAOD (ml·kg<sup>-1</sup>) after HIIT and SIT. The data are presented as mean  $\pm$  SE. \*Significant different within group (p < 0.005) from pre- to posttest; a Significant difference in change between groups (p < 0.001) from pre- to posttest.

The VO<sub>2peak</sub> (L·min<sup>-1</sup>) changes were not significantly different between groups but increased by 7.9  $\pm$  2.8 % after HIIT and by 5.9  $\pm$  2.5 % after SIT (Table 3). The % of VO<sub>2max</sub> reached during the MAOD test were not significantly different between groups at pre- or posttest. It increased by 4.2  $\pm$  3.2 % after SIT, while the changes were not significant after HIIT (Table 3).

#### Performance

Table 4. Changes in endurance performance events.

	HIIT	HIIT $(n = 5)$		SIT $(n = 6)$	
	Pretest	Posttest	Pretest	Posttest	
300-meter (sec)	$57.5 \pm 3.1$	$54.6 \pm 2.6^{*\#}$	$54.3 \pm 2.4$	$50.8 \pm 1.3^{*\#}$	
3000-meter (sec)	$843 \pm 38$	$794 \pm 38*$	$845 \pm 31$	$797 \pm 27*$	

Data are presented as mean  $\pm$  SD. The endurance performance tests were carried out as running in an indoor running track. HIIT, high-intensity interval training; SIT, supramaximal interval training. \*Significant difference within group (p < 0.05) from pre- to posttest; #Significant difference between groups (p < 0.05) at posttest.

The 3000-meter running performance changes were not significantly different between groups. It improved by  $5.7 \pm 1.5$  % after HIIT and by  $5.7 \pm 2.1$  % after SIT (Table 4). The 300-meter running performance changes were not significantly different between groups. It improved by  $5.1 \pm 0.9$  % after HIIT and by  $6.4 \pm 2.2$  % after SIT (Table 4). The SIT group ran the 300-meter running performance test significantly faster than the HIIT group at posttest.

## Correlations

3000-meter running performance was significantly correlated with VO<sub>2max</sub> (L·min<sup>-1</sup>) (r = -0.49, p < 0.05), and <sub>v</sub>LT (r = -0.61, p < 0.01). The standard error of estimate (SEE) between 3000-meter running performance and VO<sub>2max</sub> was 4.2 % (34.6 seconds) and 3.9 % (32.1 seconds) between 3000-meter running performance and <sub>v</sub>LT of the average 3000-meter running performance time. 300-meter running performance was significantly correlated with MAOD (ml·kg<sup>-1</sup>) (r = -0.63, p < 0.01). The SEE between 300-meter running performance and MAOD was 5.0 % (2.7 seconds) of the average 3000-meter running performance time.

## Discussion

The main findings of the present study were that 8 weeks of 4 x 4-minute HIIT performed at 90-95 % of HR<sub>max</sub> improved VO<sub>2max</sub> significantly more than 10 x 30-second all-out SIT in moderately endurance-trained females. However, both HIIT and SIT improved VO<sub>2max</sub> significantly. The significant increase in VO<sub>2max</sub> corresponded with the changes in O<sub>2</sub> pulse. The increased O<sub>2</sub> pulse may indicate that the VO<sub>2max</sub> improvements were due to enhanced SV. SIT improved MAOD significantly more than HIIT. SIT increased MAOD significantly, while the changes were not significantly after HIIT. This may indicate that endurance training must be performed at supramaximal intensities to enhance anaerobic capacity. The 300- and 3000-meter running performance changes were not significantly different between groups. Both groups improved 300- and 3000-meter running performance were likely due to improved VO<sub>2max</sub> after HIIT and improved MAOD after SIT.

## VO<sub>2max</sub>

The present study is the first to examine the effects of HIIT and SIT on VO<sub>2max</sub> and O<sub>2</sub> pulse in moderately endurance-trained females. VO<sub>2max</sub> is considered to be the most important factor in predicting endurance performance, independent of gender<sup>[14, 15, 26-29]</sup>. Thus, an optimal training program should aim to improve VO<sub>2max</sub>. HIIT was significantly more effective in improving VO<sub>2max</sub> compared to SIT. HIIT is also reported to be superior to SIT on improving VO2max in endurance-trained triathletes and cyclists<sup>[55]</sup>. The findings are also consistent with a meta-analysis showing that a higher volume of HIIT gives a better response than a lower volume of SIT on improving VO<sub>2max</sub> in patient to endurance-trained population groups<sup>[56, 57]</sup>.

The 7.9 % improvement in VO<sub>2max</sub> (L·min<sup>-1</sup>) after HIIT in the present study agree with the VO<sub>2max</sub> improvements seen in similar studies of moderately trained and endurance-trained males (56-61 ml·kg<sup>-1</sup>·min<sup>-1</sup>)<sup>[8, 53, 62, 72]</sup>. Two studies of untrained and recreationally active females have shown a greater magnitude of change in VO<sub>2max</sub> after HIIT<sup>[44, 48]</sup>. The magnitude of change in VO<sub>2max</sub> depends on the initial level of the subjects<sup>[9]</sup>. Hence, females do respond similarly to HIIT as males in unfit to moderately endurance-trained population.

The significant improvement in  $VO_{2max}$  (L·min<sup>-1</sup>) by 1.6 % after SIT in the present study was unexpected. However, the  $VO_{2max}$  changes after SIT are in line with a previous study of

endurance-trained triathletes and cyclists, when SIT is added to the subjects regular training regime<sup>[55]</sup> but in contrast to a similar study of endurance-trained males<sup>[53]</sup>. The results should be interpreted with caution in the present study, as VO<sub>2max</sub> changes are within the standard error ( $\pm$  3 % in SD) of the average pre-VO<sub>2max</sub> values<sup>[18]</sup>. The changes may be due to day-to-day variation in VO<sub>2max</sub>. It is also important to note that only six subjects are part of the analysis in the SIT group, and thus the statistical power is low<sup>[80]</sup>. Accordingly, more research examining the effect of SIT on VO<sub>2max</sub> is needed with moderately to endurance-trained female population.

#### **VO<sub>2max</sub> verification phase**

Poole and Jones<sup>[86]</sup> proposed using a constant workload at a supramaximal intensity (~110 % of VO<sub>2max</sub>) to verify the VO<sub>2max</sub> from the incremental test. The reason has been to verify the VO<sub>2max</sub> of the subjects who do not reach a plateau in VO<sub>2</sub> during the incremental test. The MAOD-protocol by Medbø et al.<sup>[77]</sup> using a supramaximal intensity (120  $\pm$  10 % of VO<sub>2max</sub>) of short duration (~2-3 minutes of exhaustive work) was used in the present study to verify VO<sub>2max</sub> from the incremental test. The subjects reached a plateau in VO<sub>2</sub> in 19 out of 22 incremental VO<sub>2max</sub> tests, and all the subjects achieved their VO<sub>2max</sub> in the incremental tests. The average VO<sub>2peak</sub> values from the MAOD-tests at both pre- and posttests were not significantly different from the VO<sub>2max</sub> values of the incremental test of the present study. It may suggest that the MAOD-protocol to verify VO<sub>2max</sub> are needed to conclude this.

#### **Cardiovascular adaptation**

According to the Fick equation, changes in VO<sub>2max</sub> are due to changes in Q or a-vO<sub>2diff</sub>. The O<sub>2</sub> supply is the most limiting factor of VO<sub>2max</sub> in healthy subjects performing at a maximal intensity during whole-body exercise<sup>[31, 37]</sup>. O<sub>2</sub> pulse was used as a non-invasive measure of SV in the present study. The present study is the first to examine the cardiovascular effect of HIIT and SIT in moderately endurance-trained females. O<sub>2</sub> pulse increased significantly more after HIIT compared to SIT, with no significant change in HR<sub>max</sub> in neither of the groups. Thus, changes in VO<sub>2max</sub> may be due to enhanced SV. Exercising at intensities close to VO<sub>2max</sub> and maximal SV overloads the ventricular stretch and emptying due to increased afterload of blood to the heart, which are important factors of morphological adaptation<sup>[8, 43]</sup>. Considering it takes 1-2 minutes to adjust to an exercise intensity<sup>[18]</sup>, a higher volume per

interval and total volume of HIIT challenges the cardiovascular system more than a lower volume of SIT<sup>[48, 56]</sup>.

The 9.3 % improvement in O<sub>2</sub> pulse after HIIT agree with changes in O<sub>2</sub> pulse and SV (single-breath method of acetylene uptake) in a similar study of endurance-trained males<sup>[8, 53]</sup>. The results of the present study also agree with changes in O<sub>2</sub> pulse with untrained females<sup>[44]</sup> and atrioventricular plane displacement in recreationally active females<sup>[48]</sup>. It indicates that untrained to moderately endurance-trained females respond similarly to HIIT in VO<sub>2max</sub> and SV changes as untrained to endurance-trained males.

The 3.5 % improvements in O<sub>2</sub> pulse is in contrast to a similar SIT study of endurance-trained males<sup>[53]</sup>. However, it is in line with other SIT-studies with subjects of lower initial VO<sub>2max</sub> levels (< 44 ml·kg<sup>-1</sup>·min<sup>-1</sup>)<sup>[49, 50]</sup>. The changes must be interpreted with caution, as specified in the VO<sub>2max</sub> discussion. More research replicating the cardiovascular measurement-methods of previous SIT studies and the present study is necessary before concluding the effect of SIT on cardiovascular adaptation in moderately endurance-trained females.

In addition to SV, the  $O_2$  carrying capacity of the blood is an essential factor in the  $O_2$  supply<sup>[32]</sup>. No studies have reported an significant change in blood volume or hemoglobin concentration after HIIT or SIT in moderately to endurance-trained males<sup>[8, 53]</sup>. Blood volume is only reported to change significantly in the early stages of endurance training in untrained males<sup>[88]</sup>. Findings from previous reseach may indicate that it is less likely that the VO<sub>2max</sub> changes were due to changes in the O<sub>2</sub> carrying capacity in the present study. There are none or few studies that have examined the effect of HIIT and SIT on O<sub>2</sub> carrying factors in moderately to endurance-trained females.

## Lactate threshold

LT describes the intensity where  $[La^-]_b$  starts to accumulate during continuous exercise<sup>[27]</sup>. No studies have examined the effect of HIIT and SIT and compared them on LT in females. The present study is one of few studies examining the effects of SIT on LT expressed as % of VO<sub>2max</sub>. Neither HIIT or SIT changed LT significantly between or within groups. The results are in line with similar studies of moderately to endurance-trained males<sup>[8, 53, 62, 72]</sup>.

Changes in performance workload (VO<sub>2</sub> or velocity) at LT is the effect of changes in VO<sub>2max</sub> or WE, or both<sup>[8]</sup>. The changes in vLT and VO<sub>2</sub> at LT were not significantly different between

groups. The 9.4 % improvement in vLT after HIIT is in line with similar HIIT studies of moderately to endurance-trained males<sup>[8, 62, 72]</sup>. The 7.4 % improvement in vLT after SIT is in line with a similar study of endurance-trained males<sup>[53]</sup>. The 6.7 % increase in VO<sub>2</sub> at LT after SIT is likely a consequence of increased velocity at LT, as VO<sub>2</sub> increases with an increased workload<sup>[84]</sup>.

[La<sup>-</sup>]<sub>b</sub> at LT changes were significantly different between groups. It decreased significantly after HIIT, whilevthe changes were not significant after SIT. However, the [La<sup>-</sup>]<sub>b</sub> at LT was not significantly different between groups. The [La<sup>-</sup>]<sub>b</sub> at LT is set to be 1.5 mmol·L<sup>-1</sup> above the warm-up/baseline value<sup>[85]</sup>. Hence, the changes in [La<sup>-</sup>]<sub>b</sub> at LT are due to lower baseline values after the warmup after HIIT. The relative intensity at the standardized velocity of WE at 7 km·h<sup>-1</sup> decreased significantly (from 68.8 to 61.1 % of VO<sub>2max</sub>, p < 0.05) after HIIT. This may result in a greater reliance on fat oxidation during the warm-up, which can result in less lactate production during a workload of a given intensity<sup>[59]</sup>. However, the respiratory exchange values were not significantly different from pre- to posttest within groups. Another reason could be that the relative intensity at the warmup was reduced after HIIT, and could result in a smaller disturbance of homeostasis and thus produce less lactate acid during the warmup<sup>[59]</sup>.

#### Work economy

WE refers to the  $O_2$  cost at a given submaximal workload<sup>[8]</sup>. The WE workload in the present study was set at 7 km·h<sup>-1</sup> with 5.3 % inclination<sup>[8]</sup>. WE changes were not significantly different between or within groups. The nonsignificant change in WE between HIIT and SIT is in line with a similar study of endurance-trained males, <sup>[53]</sup>. However, the HR decreased significantly in both groups, but the changes were not significantly different between groups. The decreased HR at WE may be due to enhanced SV.

The subjects of the present study are considered as moderately endurance-trained but not as runners, and the results are somewhat surprising. In earlier studies, increasing the training volume on the specific activity mode has improved WE<sup>[8, 13, 53, 62, 72]</sup>. The lack of change in WE after HIIT and SIT in the present study may be due to no difference in training volume of running during the intervention from what the subjects were used to before the study. However, SIT is reported to improve WE by 3-7 % despite a decrease in training volume<sup>[1, 52, 54]</sup>. Supramaximal intensive exercises close to peak power may be sufficient to activate type

IIa and IIx muscle fibers<sup>[89]</sup>. At these intensities, the type IIa and IIx muscle fibers may increase the oxidative enzyme activity<sup>[60, 61]</sup> and may also cause a shift in muscle fiber type<sup>[90]</sup>. The training was carried out on a running track in these studies. Although not measured, it is likely that the subjects of these studies ran at velocities closer to their peak power at the beginning of an interval and gradually decreased during the interval as described by other studies<sup>[7, 21]</sup>. Peak power output has been reported to increase by 5-10 % in cycling SIT studies<sup>[2, 49]</sup> and by 5 % in a running SIT study<sup>[7]</sup> in 30-second all-out anaerobic tests in untrained males and recreationally active females (44-47 ml·kg<sup>-1</sup>·min<sup>-1</sup>). Improvements in peak power output may affect the force production or rate of force development, and thus motor recruitment pattern. Improving one or both of the factors could lead to a relatively less force production and rate of force development per stride at a given workload. Increased rate of force development can also shorten the muscle contraction and improve the O<sub>2</sub> delivery through increased blood perfusion due to the longer duration between each stride and muscle contraction. This may affect the O<sub>2</sub> demand of the working muscles and decrease VO<sub>2</sub> and blood flow to the working muscles, which was the case after eight weeks of maximal strength training in moderately endurance-trained cyclists  $(58.7 \pm 2.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})^{[70]}$ . Because the subjects of the SIT group in the present study ran at the average velocity throughout an interval, the subjects were likely not close to their peak power output in any of the intervals during the intervention. Thus, the motorized treadmill SIT-protocol may not have challenged the neuromuscular system sufficiently.

## Anaerobic capacity

Anaerobic capacity reflects the capacity of regenerating ATP via phosphocreatine and carbohydrates through glycolysis<sup>[18]</sup>. A MAOD-protocol by Medbø et al.<sup>[77]</sup> is used to measure the anaerobic capacity in the present study. The present study is the first study examining the effect of HIIT on MAOD in females, and the first study to compare the effect of HIIT and SIT on MAOD in females. SIT improved MAOD significantly more than HIIT. These findings are in contrast to a similar study with endurance-trained males<sup>[53]</sup>. The initial level of MAOD was relatively high in subjects of the SIT group and may be the cause of the nonsignificant changes in MAOD<sup>[53]</sup>. The results of the present study do agree with other studies showing that training at supramaximal intensity is needed to change the anaerobic capacity significantly in moderate to endurance-trained subjects<sup>[63, 79]</sup>.

For the first time, MAOD is shown to increase by training in females. MAOD ( $ml \cdot kg^{-1}$ ) increased by 32 % after SIT, and are in contrast to another study showing no change in MAOD in two different SIT protocols in recreationally active females<sup>[79]</sup>. However, due to few females in the two SIT protocols (3 and 4 in each group), the results have a low statistical power<sup>[80]</sup> and should be interpreted with caution. The results of the present study are also in contrast to a similar study, showing a nonsignificant change in MAOD of endurance-trained males<sup>[53]</sup>. However, the MAOD changes in the present study are in line with studies of different SIT protocols than the SIT protocol in the present study, in moderately to endurancetrained males<sup>[63, 79]</sup>. It is uncertain what may have caused the changes in MAOD in the present study. Changes in MAOD have been followed by changes in creatine kinase in endurancetrained males<sup>[63]</sup>. Other studies have shown an increase in creatine kinase, phosphofructokinase, lactate dehydrogenase, or ionic transporters in the muscle cells (e.g., Na<sup>+</sup>-K<sup>+</sup> pump, K<sup>+</sup>-channels, and lactate-H<sup>+</sup> transporters) that has been associated with improved anaerobic endurance performance in moderately to endurance-trained males<sup>[1, 22, 52, 52, 52]</sup> <sup>54, 90]</sup>. Increased buffer capacity (bicarbonate concentration) have also shown to improve anaerobic endurance performance when used as supplements<sup>[91]</sup>, but have so far not been reported to improve after training in moderately to endurance-trained males<sup>[53, 54, 63]</sup>. Future research should include more females examining the causes of change in MAOD.

### **Endurance performance**

### **3000-meter running performance**

The 3000-meter running performance changes were not significantly different between groups. The results are in contrast to other studies comparing the two training modalities in endurance-trained males<sup>[53, 55]</sup>. The nonsignificant difference in changes between groups could be due to the homogeneous sample in VO<sub>2max</sub>. Another reason could be that the subjects were not familiarized with 3000-meter running time-trial. Allowing subjects to get familiarized with a performance test can improve the reliability of pacing strategy in the given test<sup>[92]</sup>. Because of the quantitative higher contribution of aerobic energy compared to the anaerobic energy processes during a 3000-meter run<sup>[18, 19]</sup> and HIIT being superior to SIT on improving VO<sub>2max</sub>, HIIT was thought to improve 3000-meter running performance more than SIT. However, approximately 15 % of the energy contribution comes from the anaerobic energy system in 12-15 minutes of maximal exercise<sup>[18]</sup>. The significant improvements in MAOD after SIT may have contributed to the similar improvements in 3000-meter running

performance as HIIT. Increasing anaerobic capacity may increase the ability to resist changes in intracellular pH<sup>80, 81]</sup> and may increase the ability to push the limits further. The subjects of the SIT group increased the relative intensity at MAOD significantly more than the HIIT group. It increased by  $6.2 \pm 3.8$  % without significant change in time to exhaustion after SIT, while the changes were not significant after HIIT. In another study, SIT is reported to improve the time to exhaustion at 130 % of VO<sub>2max</sub> by 36 % (p < 0.001) in endurance-trained male runners<sup>[1]</sup>. Hence, the increased anaerobic capacity may have allowed the subjects to run within the same duration at higher relative supramaximal intensities or longer duration at the same relative supramaximal intensity before exhaustion.

Both HIIT and SIT improved 3000-meter running performance significantly. The 3000-meter running performance improved by 5.7 % (48 seconds) after HIIT, and the results are similar to previous HIIT studies<sup>[4, 53]</sup>. The performance improvements may be due to VO<sub>2max</sub> improvements, as the other physiological parameters did not change significantly after HIIT. The SIT group also improved the 3000-meter running performance by 5.7 % (48 seconds). The improvement is in close agreement with previous SIT studies using 3000-meter running performance tests in moderately to endurance-trained males<sup>[1, 4, 53]</sup>. It also agrees with other SIT studies improving 10 km running performance<sup>[1, 54]</sup> and 40 km cycling time-trial<sup>[55]</sup> in moderately to endurance-trained males. The significant improvements in 3000-meter running performance after SIT may mainly be due to enhanced anaerobic capacity and partly due to VO<sub>2max</sub> improvements, as WE and LT did not change significantly.

#### **300-meter running performance**

The 300-meter running performance changes were not significantly different between HIIT and SIT. The results are in line with a similar study of endurance-trained males<sup>[53]</sup>. In a running event lasting 50-60 seconds, the energy release between the aerobic and anaerobic are similar<sup>[23, 93]</sup>. Thus, the nonsignificant performance changes may be due to the significant improvement in VO<sub>2max</sub> after HIIT and the significant increase in MAOD after SIT in the present study.

Both groups improved 300-meter running performance significantly. The 300-meter running performance improved by 5.1 % after HIIT and is somewhat higher but in line with a similar study of endurance-trained males<sup>[53]</sup>. The difference in 300-meter running performance improvements between the studies may be due to higher VO<sub>2max</sub> improvements after HIIT in the present study. The changes in 300-meter running performance may be attributed to the

significant changes in VO<sub>2max</sub> after HIIT. The SIT group improved 300-meter running performance by 6.4 % and is somewhat higher but in agreement with a similar study of endurance-trained males<sup>[53]</sup>. Similar SIT protocols have also improved covered distance during a 30-second running performance tests in moderately to endurance-trained male runners <sup>[1, 52, 54]</sup>. The changes in 300-meter running performance in the present study are likely due to the significant changes in MAOD and VO<sub>2max</sub>. However, the VO<sub>2max</sub> improvements are within the SD of the day-to-day variation of VO<sub>2max</sub> in the SIT group<sup>[18]</sup>. Thus, it may only play a minor role in the 300-meter running performance changes after SIT.

#### Correlations

The 3000-meter running performance was significantly correlated with VO<sub>2max</sub> (ml·kg<sup>-</sup> <sup>0.75</sup>·min<sup>-1</sup>) (r = -0.51, p < 0.05), vLT (r = -0.61, p < 0.01). The significant correlation between VO<sub>2max</sub> and 3000-meter running performance supports the notion of VO<sub>2max</sub> being an important factor in predicting endurance performance<sup>[26-29]</sup>. However, the moderate correlation coefficient may be due to the homogeneous sample. The standard error of estimate (SEE) between 3000-meter running performance and VO<sub>2max</sub> was 34.6 seconds, equal to 4.2 % of the average 3000-meter running performance time. The wide range of performance level at similar VO<sub>2max</sub> values reveals that other factors are also affecting the 3000-meter running performance in the present study. The significant correlation between vLT and running performance is less but in agreement with other studies<sup>[94]</sup>. vLT is the sum of VO<sub>2max</sub> and WE<sup>[8]</sup>. Combining two physiological factors should be a stronger predictor of performance than VO<sub>2max</sub> or WE alone. The SEE between 3000-meter running performance at vLT is 32.1 seconds, equal to 3.9 % of the average 3000-meter running performance time in the present study. The wide range of performance level at similar vLT shows that there are other factors also affecting the running performance of the females in the present study.

The 300-meter running performance correlated significantly with MAOD (r = -0.59, p < 0.01) in the present study. This agrees with another study where MAOD correlated with 300-meter performance (35-39 seconds of exhaustive effort) (r = -0.76, p < 0.01) in sprinters to long-distance runners (60-71 ml·kg<sup>-1</sup>·min<sup>-1</sup>)<sup>[78]</sup>. In the same study, there was no significant correlation between MAOD and 400-meter running performance (48-52 seconds of exhaustive effort)<sup>[78]</sup>. However, the 400-meter running performance correlated with 300-meter running performance (r = -0.86, p < 0.001), which may show the importance of anaerobic

energy release in ~50 seconds of exhaustive effort. Anaerobic capacity is considered an important factor in distinguishing performance level in homogeneous groups<sup>[29]</sup>.

## **Training considerations**

The HIIT group carried out significantly more sessions than the SIT group  $(23 \pm 1 \text{ vs. } 21 \pm 1 \text{ sessions}, p < 0.05)$ . The differences may be due to the strenuous nature of all-out SIT. On six occasions, the subjects in the SIT group had to end a session due to muscular issues or nausea, due to the strenuous nature of all-out SIT exercises. The subjects also experienced nausea after the sessions, which could last up to 30-minutes before they were able to get back home from the training sessions. Two subjects dropped out due to shin splint and one because of motivational problems with completing the intervention due to the strenuous nature of all-out SIT. Furthermore, two more subjects experienced discomforts like pain in hamstring and shin splints, but it did not keep them from training. A similar SIT study has also reported injuries and discomforts related to all-out SIT<sup>[95]</sup>. There were no occasions of subjects ending a session or having trouble completing the intervention due to injury-related and motivational problems in the HIIT group.

If the goal is to maintain or improve health and endurance performance and its determinants, implementing HIIT in a training program is a safe and effective training modality to choose<sup>[8, 48, 53]</sup>. However, it is necessary to exercise at supramaximal intensity if the goal is to improve anaerobic capacity. When  $VO_{2max}$  is maximized in an athlete, SIT could be used to further improve endurance performance or to peak performance.

#### **Study limitations**

Some issues might have affected the results in the present study. The low number of subjects decrease the statistical power of the results<sup>[80]</sup>. It is also preferable to have more subjects due to the strenuous nature of all-out SIT, as there were many dropouts in the present study. Having more subjects is also preferable if one must exclude subjects to the end analysis, as the case was with the HIIT group in the present study. O<sub>2</sub> pulse is not an ideal method of measuring cardiovascular adaptation, and future studies should aim toward more accurate methods of evaluating cardiovascular adaptations after HIIT and SIT (e.g., the single-breath acetylene uptake method). The present study did not include hematological or muscular biopsy measurements, and these measurements could give answers on what may be the

reasons of changes in anaerobic capacity and if the  $O_2$  carrier capacity is partly responsible for the VO<sub>2max</sub> changes. The present study did not control for menstrual cycle, which may affect the physiological responses to training<sup>[76]</sup>. However, performance has not been reported to change during menstrual cycles<sup>[96]</sup>. The simplified Y-intercept of 5 ml·kg<sup>-1</sup>·min<sup>-1</sup> at rest and few submaximal measures used in the present study increases the standard deviation and thus increase the likelihood of errors in the MAOD results. The present study had limited economic resources and time constraints due to lab hour restrictions. Because of that, it was impossible to use the necessary equipment and measurements to get the most reliable results as wanted in the present study.

#### **Future research**

Future research should aim to have more females completing a similar study to gain a stronger statistical power. It should also aim to clarify the cause of changes in  $VO_{2max}$  and anaerobic capacity in females after HIIT and SIT. This can be done by using a more direct method of measuring SV and including hematological and muscle biopsy measures and analysis.

### Conclusion

The present study demonstrates that 4 x 4-minute HIIT performed at 90-95 % of HR<sub>max</sub> is more effective than 10 x 30-second all-out SIT in improving VO<sub>2max</sub> and O<sub>2</sub> pulse. The increased O<sub>2</sub> pulse might indicate that the VO<sub>2max</sub> improvements were due to enhanced SV. There was no significant difference in WE and LT changes between groups. SIT was significantly more effective in improving MAOD compared to HIIT. The study revealed that supramaximal intensities are needed to change anaerobic capacity significantly. The 300- and 3000-meter running performance changes were not significantly different between groups. The endurance performance improvements were due to improved VO<sub>2max</sub> after HIIT and mainly by improved MAOD after SIT.

The present study revealed some issues regarding the subjects ability to complete the SITprotocol, as many subjects dropped out due to the strenuous nature of all-out SIT. Increasing the intensity from high to supramaximal at the expense of training volume does not give a better response to most of the physiological parameters if the goal is to maintain or improve health and endurance performance. Implementing a 4 x 4-minute HIIT in a training program may be a safer method in terms of training-related injuries and may make it easier to continue this type of training in the long run. These findings may also be of clinical value, as it confirms with other studies that HIIT gives a better response on  $VO_{2max}$  than SIT and may do so with less risk of getting training-related injuries. However, exercising at supramaximal intensity is required if the goal is to improve anaerobic capacity in moderately to endurancetrained subjects.

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