Master's thesis

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

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Aerobic High-Intensity Interval Training Improve VO_{2max} More Than Sprint Interval Training

Master's thesis in Exercise Physiology
Supervisor: Jan Helgerud
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Abstract

Purpose: To compare the effects of sprint interval training (SIT) and high-intensity interval training (HIIT) on endurance performance and endurance performance determinants.

Methods: Twenty-three healthy, trained female subjects were randomly assigned to either 10x30-second SIT (30 sec of running at "all-out" intensity separated by 3.5 min of active recovery) or 4x4-minute HIIT (4 min of running at 90-95% HR_{max} followed by 3 min of active recovery at 70% HR_{max}). Both protocols were performed 3 $d \cdot wk^{-1}$ for 8 wk.

Results: HIIT resulted in significantly larger improvement in absolute VO_{2max}, compared to SIT (3.6% vs -0.5%, respectively). This was accompanied by a larger improvement in vVO_{2max} ($P < 0.01$) and a lower HR at submaximal velocities in HIIT compared to SIT. Only HIIT improved vLT ($P < 0.001$) and O_2 pulse ($P < 0.01$) and tended to improve more than SIT (both $P < 0.06$). Both groups improved RE and 3000m running performance with no difference between groups. Only SIT improved anaerobic capacity (9.5%), had a higher session RPE-score ($P < 0.001$) and improved 300m performance more compared to HIIT (6.2% vs 2.2%, respectively). Of the 17 subjects allocated to SIT, 6 dropped out due to injuries related to the protocol.

Conclusion: 4x4-minute HIIT is significantly more effective to improve VO_{2max} compared to 10x30-second SIT in trained females. As SIT resulted in a high injury rate and a higher perceived exertion, a running SIT-protocol is not recommended unless the goal is to improve anaerobic capacity and sprint performance.

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Abbreviations

ADP: Adenosine diphosphate

ATP: Adenosine triphosphate

a-vO_2diff: Arterio-venous oxygen difference

CO: Cardiac output

CO_{max}: Maximal cardiac output

ET: Continuous endurance training

HIIT: High-intensity interval training

$[H^+]$: Hydrogen ion

HR: Heart rate

HR_{max}: Maximal heart rate

HR_{peak}: Peak heart rate

LT: Lactate threshold

MAOD: Maximal accumulated oxygen deficit

MSS: Maximal sprint speed

O_2: Oxygen

O_2 pulse: Oxygen pulse

PCr: Phosphocreatine

RE: Running economy

R: Respiratory exchange ratio

RPE: Rating of perceived exertion

SD: Standard deviation

SIT: Sprint interval training

SV: Stroke volume

SV_{max}; Maximal stroke volume

TT: Time-trial

TTE: Time-to-exhaustion

vLT: Velocity at lactate threshold

VE: Ventilation

VO_2: Oxygen uptake

VO_{2max}. Maximal oxygen uptake

VO_{2peak}: Peak oxygen uptake

vVO_{2max}: Velocity at maximal oxygen uptake

$[la^-]_b$: Blood lactate concentration

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Introduction

Running is performed to improve sprint performance, endurance performance, health or simply just for enjoyment. There is sufficient evidence that high-intensity interval training (HIIT) can enhance endurance performance and VO_{2max} to a greater extent than continuous endurance training (ET) (Milanovic, Sporis, & Weston, 2015; Rosenblat, Perrotta, & Thomas, 2020). This is of interest as VO_{2max} is an independent predictor of both all-cause and cardiovascular-specific mortality (Keteyian et al., 2008) and endurance performance. (McLaughlin, Howley, Bassett, Thompson, & Fitzhugh, 2010; Saltin & Astrand, 1967).

The outcome of interval training can be manipulated by a number of variables including intensity, duration, work/rest ratio, number of intervals, and frequency between intervals (Buchheit & Laursen, 2013a, 2013b; Laursen, 2010; Milanovic et al., 2015; Wenger & Bell, 1986). Prescribing an optimal training regimen for improving fitness while limiting the development of fatigue or risk of injury in the general community therefore requires knowledge on how the manipulation of these variables influence adaptations in physiological parameters (Rosenblat et al., 2020). Gaps in our understanding of the effects of interval training may remain in part due to the lack of standardization (Viana et al., 2018). Helgerud et al. (2007) showed that HIIT with an emphasis on training at high intensity induced greater effects on VO_{2max} than the same training volume at lower intensity. This is also in line with recent meta-analyses (Bacon, Carter, Ogle, & Joyner, 2013; Milanovic et al., 2015). For the past few years, an interval prescription with supramaximal intensity, low volume and long work/rest ratio, broadly called sprint interval training (SIT), has received the researcher's attention. Thus, several meta-analyses have reported the efficacy of SIT in increasing VO_{2max}, despite its low volume (Gist, Fedewa, Dishman, & Cureton, 2014; Sloth, Sloth, Overgaard, & Dalgas, 2013; Vollaard, Metcalfe, & Williams, 2017; Weston, Taylor, Batterham, & Hopkins, 2014).

The aim of present study is to compare SIT with today's golden standard training method, HIIT, on the effect on endurance performance and the physiological determinants of endurance performance; VO_{2max}, exercise economy, lactate threshold (LT) and anaerobic capacity (Joyner & Coyle, 2008; Pate & Kriska, 1984). Additionally, perceived exhaustion and physiological differences between men and women will be presented, underlining the need for research conducted with female subjects.

VO_{2max}

VO_{2max} is defined as the maximal rate of oxygen uptake and utilization during exhausting exercise (Bassett & Howley, 2000). The Fick equation is an exact physiological definition that describes VO_2, and thus VO_{2max} (Acierno, 2000; Barrett-O'Keefe, Helgerud, Wagner, & Richardson, 2012).

VO_2 = cardiac output (CO) x arterio-venous O_2 difference (a-vO_2diff).

Longitudinal studies have shown that the training-induced increase in VO_{2max} results primarily from an increase in maximal cardiac output (CO_{max}) rather than a-vO_2diff (Bassett & Howley, 2000). CO expresses the amount of blood pumped by the heart to tissues and vital organs during a 1-min period, and computes as follows: *CO = heart rate (HR) x stroke volume (SV)* (McArdle, Katch, & Katch, 2014). Because maximal heart rate (HR_{max}) does not improve with training (Hawkins, Marcell, Jaque, & Wiswell, 2001), while maximal stroke volume (SV_{max}) can (Helgerud et al., 2007), changes in CO_{max} are determined by changes in SV_{max}. SV is determined by the contractility force of the heart, the volume of the heart, and the capacity of refilling the heart (Ferguson, Gledhill, Jamnik, Wiebe, & Payne, 2001; Pollock, 1977; P.-O. Åstrand, Cuddy, Saltin, & Stenberg, 1964)

A-vO_2diff is referred as the difference between the O_2 saturation of arterial blood and mixed venous blood (Tanaka & Seals, 2008). It reflects the capacity of primarily active skeletal muscles and the respiratory muscles to extract and consume oxygen from the blood for production of adenosine triphosphate (ATP), and a low cell PO_2 relative to blood PO_2 is the main driving force of this perfusion (Honig, Connett, & Gayeski, 1992). Maximal a-vO_2diff can be increased with endurance training (Beere, Russell, Morey, Kitzman, & Higginbotham, 1999; Daussin et al., 2007). There are two ways of improving a-vO_2diff. 1) an improved blood distribution to the exercising muscle (Beere et al., 1999; McArdle et al., 2014), and 2) a higher extraction of oxygen from blood to muscle (Wagner, 2006). The main significance of the training-induced increase in capillary density is not to accommodate blood flow but rather to increase muscle-to-blood exchange surface, decrease oxygen diffusion distance, and increase red blood cell mean transit time (Bassett & Howley, 2000; García-Pinillos, Soto-Hermoso, & Latorre-Román, 2017). Also, an increase in muscle mitochondria from endurance training may allow a slightly greater extraction of O_2 from the blood by the working muscles, thus contributing in a minor way to an increased $\dot{V}O_{2max}$ (Bassett & Howley, 2000).

VO_{2max} is a result of an interplay between many factors, depending on both an individual's fitness level and the environmental conditions. It's important to view all the factors possibly limiting VO_{2max} as a whole, since adaptation of one factor may affect other factors (Wagner, 2000, 2006). To illustrate, the CO_{max} difference between endurance athletes vs sedentary is larger compared to their difference in a-vO_2diff (Wagner, 2006). A higher CO_{max} increases blood flow and simultaneously reduces transit time in both lung and muscle capillaries. This worsens diffusion limitation, significantly opposing this convective gain (Dempsey, Hanson, & Henderson, 1984; Wagner, 2006; Zhou et al., 2001). Hence, if increasing to an exceptional CO_{max} without having a matching, exceptional muscle capillary-to-mitochondrial O_2 transport system to permit almost full O_2 extraction from the rapidly flowing blood, this will limit the potential increase in VO_{2max} (Wagner, 2006). Noteworthy, other factors like ventilation, pulmonary diffusion capacity, low blood volume, low haemoglobin concentration, or hypoxia can potentially also be limiting factors to VO_{2max} (Saltin & Astrand, 1967; Sarzynski, Ghosh, & Bouchard, 2017; Wagner, 2000, 2006; Wehrlin & Hallen, 2006).

While the determining factors of VO_{2max} have been shown to be independent of sex (Rossow et al., 2010; Wang, Solli, Nyberg, Hoff, & Helgerud, 2012; Zhou et al., 2001), American Heart Association stated that males (20-29 years) have a higher average VO_{2max} than females at the same age, 43 vs 36 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively (Fletcher et al., 2001). A population study just outside the region where the present study was conducted (n = 193 between 20-29 years) also showed males having a higher average VO_{2max} than females, 54 vs 43 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively (Loe, Steinshamn, & Wisloff, 2014). As subjects with a small m_b generally are overestimated in terms of relative values ($ml \cdot kg^{-1} \cdot min^{-1}$) because the energy cost for movement does not increase in the same rate as m_b (Bergh, Sjödin, Forsberg, & Svedenhag, 1991), there is clearly a sex-difference in initial baseline. This differences is normally attributed to a combination of higher body fat in women and lower red cell mass for a given body weight (Cureton et al., 1986; Joyner, 2017; Schmidt & Prommer, 2010). Females have an average smaller body size compared to males, including dimensions of organs. As healthy, active individuals generally are supply-limited, a small dimension of the heart equals a small SV and thus a lower CO_{max} and VO_{2max} in women compared to men (Joyner & Coyle, 2008; Wang et al., 2012; Zhou et al., 2001). In addition, females are also exposed to hormonal changes (Janse de Jonge, 2003), blood loss throughout menstrual cycles and thus higher risk of iron deficiency (Hallberg, Hogdahl, Nilsson, & Rybo, 1966; Hallberg, Hulthen, & Garby, 2000). However, no change in variables determining running performance has been found during menstrual cycles (Bemben, Salm, & Salm, 1995; De Souza, 2003; De Souza, Maguire, Rubin, & Maresh, 1990).

HIIT vs SIT – effect on VO_{2max}?

Helgerud et al. (2007) found a 7.2% improvement on VO_{2max} in moderately trained men following 8 weeks of 4x4-intervals with an intensity of 90-95% of HR_{max}. In the same study, Helgerud et al. (2007) showed that HIIT with an emphasis on training at high intensity induced greater effects on VO_{2max} than the same training volume at lower intensity. Further, Seiler et al., 2013 found that a larger volume of HIIT, represented by 4x8-minute intervals induced even greater effects on VO_{2max} than a smaller volume of HIIT at maximal sustainable intensity, represented by 4x4-minute intervals. Interestingly, 4x8-minute intervals at an average intensity of 90% of HR_{max} also increased VO_{2max} significantly more than 4x16-minute intervals at 88% of HR_{max}. Therefore, it seems an emphasis on interventions with a large total training volume at an intensity of at least 90% of HR_{max} is needed for an optimal improvement on VO_{2max}, with a greater additional increase for subjects with a lower baseline fitness (Bacon et al., 2013; Helgerud et al., 2007; Milanovic et al., 2015; Seiler, Joranson, Olesen, & Hetlelid, 2013).

Four recent meta-analyses have investigated the effect of SIT on VO_{2max}, with 30s sprints separated by 4min recovery reported as the most commonly used protocol (Gist et al., 2014; Sloth et al., 2013; Vollaard et al., 2017; Weston et al., 2014). Gist et al. (2014) and Weston et al. (2014) analysed 30s and 30-60s SIT protocols with no baseline VO_{2max} exclusion criteria, while Sloth et al. (2013) and Vollaard et al. (2017) included 10-30s SIT protocols with an average baseline VO_{2max} of ≤ 55 $ml \cdot kg^{-1} \cdot min^{-1}$, the latter only including cycling interventions. Although the aforementioned differences in inclusion criteria's, all meta-analyses reported an average VO_{2max} improvement of ~8%. Noteworthy, Milanovic et al. (2015) found a larger size of effect on VO_{2max}, 4.9 $ml \cdot kg^{-1} \cdot min^{-1}$, when including all forms of high-intensity intervals compared with the 3.6 $ml \cdot kg^{-1} \cdot min^{-1}$ (equivalent to 8% increase) reported solely from SIT (Gist et al., 2014).

Similar to HIIT, SIT showed a greater additional increase for subjects with a lower baseline VO_{2max} (Vollaard et al., 2017; Weston et al., 2014). In contrast to HIIT, the SIT meta-analyses found no significant effect on VO_{2max} when analysing for intervention duration (Gist et al., 2014; Vollaard et al., 2017), number of sessions (Vollaard et al., 2017; Weston et al., 2014), or total sprint-duration (Vollaard et al., 2017).

Apart from the results from our own research group (Balto & Helgerud, 2019; Trane & Helgerud, 2018), only four studies have conducted an intervention comparing the effects of HIIT and SIT on VO_{2max} (Esfarjani & Laursen, 2007; Laursen, Shing, Peake, Coombes, & Jenkins, 2002; Lunt et al., 2014; Naves et al., 2018). Although Laursen et al. (2002) found HIIT to be significantly more effective than SIT at improving VO_{2max} (8.1 vs 3.0%) in trained cyclists, no difference between groups has been found following a running intervention (Esfarjani & Laursen, 2007; Lunt et al., 2014). Recently, Trane and Helgerud (2018) found no difference in VO_{2max}-improvement between groups, while Balto and Helgerud (2019) found HIIT to be significantly more effective compared to SIT (7.9 vs 1.6 %) in trained females. However, due to large sample dropout and exclusion, the difference between groups were only based on 5 subjects in each group underlining the need for further research including larger sample sizes. Taken together, the literature suggests that both HIIT and SIT has the potential of increasing VO_{2max}. Still, there are indications that HIIT might have a larger effect on VO_{2max} compared to SIT.

A high trainability of VO_{2max} is unlikely to be achieved if SV_{max} and a-vO_2diff are not optimally increased by exercise training (Sarzynski et al., 2017). The 4x4 HIIT-protocol have shown increased LV mass (+12%) and LV contractility (+13%) in sedentary females (Slordahl et al., 2004) and 10.4% increased SV_{max} in moderately trained males which corresponds to the changes in VO_{2max} following 8 weeks of training (Helgerud et al., 2007). Only a limited number of studies have assessed cardiovascular adaptations following SIT with ≤ 1:3 work/rest ratio, and provided equivocal results (Alguindy & Rognmo, 2019; Macpherson, Hazell, Olver, Paterson, & Lemon, 2011; Trilk, Singhal, Bigelman, & Cureton, 2011). Trilk et al. (2011) showed an increased SV of 11.4% in sedentary obese women at a workload of 50% VO_{2max} following a 4 week SIT-protocol, without significant change in CO or a-vO_2diff. However, short-term SIT did not improve SV and CO despite improved aerobic performance in COPD patients and healthy elderly individuals (Alguindy & Rognmo, 2019), and 6 weeks of SIT showed a 11.5% increase in VO_{2max} in recreationally active males and females with no change in CO_{max} (Macpherson et al., 2011). If subjects are supply-limited, an accompanying CO_{max}-increase to the VO_{2max}-increase should be expected. However, improvements in VO_{2peak} following SIT have been suggested to be more as a result of enhanced oxidative capacity of peripheral muscles (Barnett et al., 2004; Burgomaster et al., 2008; Macpherson et al., 2011). Recent finding supports this notion, as mitochondrial affinity for oxygen after seven sessions of cycling SIT has been shown to directly relate to the increase in VO_{2peak} following SIT in untrained subjects (Larsen et al., 2020). If so, demand-limited subjects would have a higher VO_{2max}-benefit from SIT compared to supply-limited subjects. Therefore, more studies examining VO_{2max} and its determinants in already trained subjects are needed to address this.

Running Economy

Exercise economy is measured as the steady-rate oxygen consumption while exercising at a specific submaximal exercise load below the lactate threshold (Tanaka & Seals, 2008). Running economy (RE) is commonly defined as the steady state VO_2 while running at a standard velocity or as energy cost of running per metre, and it seems not to change at intensities between 60-90 % of VO_{2max} in well-trained runners (Helgerud et al., 2007; Helgerud, Storen, & Hoff, 2010). RE is an important marker and predictor for endurance running performance with better runners generally having lower oxygen consumption at submaximal running speeds (Bransford & Howley, 1977; Conley & Krahenbuhl, 1980; Mayhew, Piper, & Etheridge, 1979; Morgan et al., 1995; Saunders, Pyne, Telford, & Hawley, 2004). Biomechanical factors including vertical displacement and braking probably play a role in RE, as do the elastic properties of muscles and connective tissue and muscle fibre type composition (Joyner & Coyle, 2008). These factors varies with the characteristics of each individual, even when performance is matched (K. R. Barnes, McGuigan, & Kilding, 2014; Morgan et al., 1995; R. Tucker, Santos-Concejero, & Collins, 2013).

When trying a sport for the first time, the novice skill learner will intuitively search for perception-action couplings that require minimal energy expenditure, and the movement economy will rapidly improve or mechanical output increase (Almasbakk, Whiting, & Helgerud, 2001). While a given athlete may be genetically predisposed for having a low energy cost of running (R. Tucker et al., 2013), various acute and chronic interventions have shown to improve an individual's RE. These interventions includes running interventions (Helgerud et al., 2007), resistance training, especially with emphasis on maximal strength, explosive strength and rate of force development (Johnson, Quinn, Kertzer, & Vroman, 1997;

Ronnestad & Mujika, 2014; Storen, Helgerud, Stoa, & Hoff, 2008), altitude exposure (Burtscher, Gatterer, Faulhaber, Gerstgrasser, & Schenk, 2010; Katayama, Matsuo, Ishida, Mori, & Miyamura, 2003), muscle tendon stiffness (Fukunaga, Kawakami, Kubo, & Kanehisa, 2002; G. R. Hunter et al., 2011; G. R. Hunter et al., 2015), and interventions improving the efficient mechanics leading to less energy wasted on braking forces and excessive vertical oscillation (Saunders et al., 2004).

To the authors knowledge, training-induced changes in RE between males and females has not been compared following an intervention-study. The literature's equivocal results in RE between sexes can most likely be explained by the close correlation coefficient between RE and m_b of 0.72 ($P < 0.0001$) found in Bourdin, Pastene, Germain, and Lacour (1993). As mentioned earlier, energy cost for movement does not increase in the same rate as m_b, overestimating the oxygen consumption in relative values of smaller runners (Bergh et al., 1991), hence giving the average smaller females a poorer RE compared to the bigger males. Therefore, scaling for body weight is recommended when presenting RE (Helgerud, 1994; Saunders et al., 2004).

HIIT vs SIT – effect on running economy?

Five 4x4-studies have assessed HIIT's effect on RE. Three studies including 8 weeks of 4x4 HIIT on trained male students or in addition to the training-regime of male soccer players (58.1 - 61 $ml \cdot kg^{-1} \cdot min^{-1}$) increased RE by 3.5-7% (Helgerud, Engen, Wisloff, & Hoff, 2001; Helgerud, Rodas, Kemi, & Hoff, 2011; Trane & Helgerud, 2018). Also, when comparing four different running protocols with similar total workload, a ~10% improved RE was reported, with no significant differences between protocols including ET in moderately trained males (55.5 ± 7.4 $ml \cdot kg^{-1} \cdot min^{-1}$)(Helgerud et al., 2007). The suggestion that RE is not affected by running speed used during training, but perhaps the total amount of work in subjects not accustomed to running, is supported by Kelly et al. (2018) who found no difference in RE following 2 weeks of SIT or ET in soccer players (55.5 ± 3.4 $ml \cdot kg^{-1} \cdot min^{-1}$). Furthermore, Laursen and Jenkins (2002) argues that in already well-trained runners, an additional increase in training of lower intensity does not enhance RE. Trained athletes have a better economy of motion in their sport than a novice skill learner. Hence, the novice skill learner will improve his economy more easily and/or faster than the already skilled learner (Almasbakk et al., 2001; Laursen & Jenkins, 2002). Still, Iaia et al. (2009) were able to show a 5.3-7.2 % lower energy expenditure at 11-16 $km \cdot h^{-1}$ in moderately endurance trained runners with a minimum of 4 year of running experience after only a 4 week SIT-intervention. These results are supported by improvements in RE in moderately- to well-trained runners performing SIT, some when supplemented with training of lower intensity (Bangsbo, Gunnarsson, Wendell, Nybo, & Thomassen, 2009; Skovgaard, Almquist, & Bangsbo, 2018; Skovgaard, Christiansen, et al., 2018). Thus, the improvements were most likely to come from the SIT-induced stimulus (Laursen & Jenkins, 2002).

To the authors knowledge, three studies involving a HIIT- and a SIT protocol has compared their effect on RE. In a variety of uphill interval-training programs, including ~5x5 HIIT at 7% gradient and ~20x12s at 18% gradient and 1:6 work/rest ratio showed that the latter protocol were optimal for improving RE (2.4%) in well-trained runners whereas other aerobic measures were optimal for the 5x5 group (K. Barnes, Hopkins, McGuigan, & Kilding, 2013). In the two recent studies comparing 10x30-second SIT vs 4x4 HIIT on trained males and females, only males conducting HIIT showed significant improvements (Trane & Helgerud, 2018). However, as the study of Balto and Helgerud (2019) had a low sample size, this might explain why no significant differences were reported in females. To summarize, literature is equivocal regarding which of HIIT or SIT has the greater effect on RE. While SIT has been recommended over HIIT for improving RE in trained runners (K. Barnes et al., 2013), the 4x4-protocol has shown greater improvements in RE in trained subjects who were not runners (Trane & Helgerud, 2018). Hence more research is needed to address whether HIIT or SIT are optimal for improving RE.

Lactate Threshold

Lactate threshold (LT) expressed as % of VO_{2max} is defined as the intensity where the blood lactate concentration ($[la^-]_b$) gradually starts to increase during continuous exercise (Davis, 1985). The accumulation of $[la^-]_b$ and hydrogen ions ($[H^+]$) happens at high intensities when pyruvate production by glycolysis exceeds the pyruvate consumption by mitochondria (Balsom, Gaitanos, Ekblom, & Sjodin, 1994; Gladden, 2000). Lactate, however, is not the cause of fatigue and can be oxidized back when oxygen becomes available and used as energy substrate (Turner & Bishop, 2018). Instead, the accumulation of $[H^+]$ decreases intracellular pH. This in turn has been reported to inhibit oxidative phosphorylation and the activity of glycolytic enzymes, as well as the binding of calcium to troponin and thus muscle excitationcontraction coupling (Turner & Bishop, 2018). As a result, high intensity above LT can only be sustained for a limited time due to lactic acid accumulation and limited glycogen stores (P. O. Åstrand, Rodahl, & Strømme, 2003).

There is interindividual variance in LT, and the interactions between the determinants of this variance are extremely complex (Coyle, Coggan, Hopper, & Walters, 1988; Farrell, Wilmore, Coyle, Billing, & Costill, 1993). While VO_{2max} and RE does not seem to have an influence on LT, the oxidative capacity of the skeletal muscle is reckoned to be the most important factor (Joyner & Coyle, 2008). This capacity is one of the factors linked to why LT often is observed to be higher in endurance-trained subjects compared to untrained subjects (Holloszy & Coyle, 1984; Hurley et al., 1984; Joyner & Coyle, 2008; Maughan & Leiper, 1983; Pilegaard, Bangsbo, Richter, & Juel, 1994). Other factors which seem to influence LT are muscle fibre type distribution, lactate transport capacity, carbohydrate availability, and the quantity of muscle mass that the athlete can recruit to share the glycolytic stress from the power production (Hawley, 2002; Hawley & Leckey, 2015; Joyner & Coyle, 2008; Pilegaard et al., 1994; Pilegaard, Terzis, Halestrap, & Juel, 1999; Sjodin, Jacobs, & Svedenhag, 1982).

So far, no studies have reported a change in LT after HIIT or SIT. Still, LT should be reckoned as an important endurance performance determinant, as there is a close link between LT and fractional utilization of VO_{2max} in longer lasting maximal exercise (Helgerud, 1994; Maughan & Leiper, 1983). Moderately trained to elite female runners have been reported to have the same fractional utilization as moderately trained to elite male runners, indicating no sex-difference in LT (Helgerud, 1994; Helgerud, Ingjer, & Strømme, 1990; Helgerud et al., 2010; Maughan & Leiper, 1983). However, males generally have higher velocity at LT (vLT) compared to females. As vLT has been shown to be almost identical with the sum of improvements in the determining factors for endurance performance (Farrell et al., 1993; Hagberg, Mullin, & Nagle, 1978; Helgerud et al., 2001; Storen et al., 2014), males generally have higher VO_{2max} which explains the higher vLT (Bassett & Howley, 2000; Fletcher et al., 2001; Loe et al., 2014).

Anaerobic capacity

Anaerobic capacity can be defined as the maximal amount of ATP that can be formed by the anaerobic processes during exercise (Medbo et al., 1988). The various energy systems collaborate in an overlapping fashion to provide sufficient ATP for the energy demands of exercise (Bogdanis, Nevill, Boobis, & Lakomy, 1996; Gaitanos, Williams, Boobis, & Brooks, 1993; Medbo & Tabata, 1989). The contribution of each energy system is determined by exercise intensity, bout frequency, and the duration of the rest period. The anaerobic formation of ATP can be made by two different energy systems: the phosphagen system and the glycolytic system.

The phosphagen system consists of three reactions, in which the creatine kinase reaction has by far the greater capacity for ATP regeneration. Phosphocreatine (PCr) is stored in muscle at rest and reacts with ADP and $[H^+]$ to create ATP (Baker, Grant, & Robergs, 2010). The importance of the phosphagen system lies in its extremely rapid rates as it requires only one enzymatic reaction at which it can regenerate ATP and is generally accepted to dominate for the first 5-6s of an all-out effort. Thereafter, the energy contribution are gradually offset by an increasing ATP-contribution from glycolysis (Gaitanos et al., 1993; Turner & Bishop, 2018).

The glycolytic system uses 2 ATP-molecules to catabolise glucose to 4 ATP-molecules and pyruvate which normally is sent further towards the mitochondria for an oxygen-dependent ATP-synthesis. However, at high intensities pyruvate production rate by glycolysis exceeds the capacity of mitochondria to take up pyruvate (Balsom et al., 1994; Gladden, 2000). For glycolysis to continue the ATP-production, the excess pyruvate is anaerobically converted to $[la^-]_b$ and $[H^+]$ (Medbo et al., 1988). This ATP-production can be maintained/increased until the $[H^+]$-accumulation changes the metabolic environment, ultimately causing a drop in ATP production rate and accompanied loss in power output (Cheetham, Boobis, Brooks, & Williams, 1986; Gaitanos et al., 1993; Turner & Bishop, 2018).

In repeated sprints the consensus is that a greater quantity of PCr at the start of each sprint would reduce the demand on anaerobic glycolysis (e.g., $[H^+]$) and enhance ATP turnover (Glaister, 2005). Therefore, recovery of PCr between sprints are key to a high average power. Bogdanis et al. (1996) showed that following the first 30s cycling sprint, PCr was resynthesized to 79% of resting values in 4 minutes in recreational athletes, with a natural assumption that a high VO_{2max} will increase recovery rates (Turner & Bishop, 2018).

However, recovery of PCr only happens when the blood supply to the working muscle is not occluded (Harris et al., 1976). Connolly, Brennan, and Lauzon (2003) found that the decrease in sprint peak power and average power was significantly smaller during active vs passive recovery. This suggests that an active recovery expedite PCr resynthesis, in addition to speeding up the removal of $[H^+]$ and increase lactate's use as a fuel source (Turner & Bishop, 2018). If lactate is not fully oxidised and PCr is only partially restored during the recovery phase in repeated sprints, aerobic metabolism will provide a higher % of total energy produced. This aerobic energy supply uses a lower ATP turnover rate and is significantly less than required for repeated sprints (Gaitanos et al., 1993). As such, it would not be able to sustain power output (i.e., repeated sprint performance) (Baker et al., 2010; Gaitanos et al., 1993; Glaister, 2005; McGawley & Bishop, 2015). Thus, VO_{2max} might be a limiting factor to performance in latter sprints (Helgerud et al., 2001; McGawley & Bishop, 2015).

The mechanisms underlying the improvement of anaerobic capacity are complex, probably multifactorial, are most likely affected by the training status of subjects (Iaia & Bangsbo, 2010). Factors such as creatine kinase, phosphofructokinase, lactate dehydrogenase, buffer capacity, alterations in muscle fibre type composition, and ionic transporters in the muscle cells (e.g., Na^+-K^+ pump, K^+-channels, and lactate-H^+ transporters) have been associated with increased anaerobic endurance performance in moderately to endurance-trained males (Abernethy, Thayer, & Taylor, 1990; Bangsbo et al., 2009; Iaia & Bangsbo, 2010; Iaia et al., 2009; MacDougall et al., 1998; Rodas, Ventura, Cadefau, Cussó, & Parra, 2000; Skovgaard, Almquist, et al., 2018). However, these underlying factors and how they may be enhanced with training are beyond the scope of this thesis.

To date, there are no methods that directly measures anaerobic capacity. Medbo et al. (1988) proposed that the best method for measuring anaerobic capacity is through the difference between accumulated O_2 demand and VO_2, known as maximum accumulated oxygen deficit (MAOD). This method is performed as an all-out effort at an intensity of 120 ± 10 % of VO_{2max} which subjects normally can sustain for 2-3min, and has the lowest statistical error on calculating MAOD (Medbo et al., 1988; Medbo & Tabata, 1989). In addition, Poole and Jones (2017) argue that a verification test of VO_{2max} at a constant workload at ~110 % of VO_{2max} should be included, as not all subjects reach VO_{2max} during today's protocol. As the MAOD test is close to their recommendations, and VO_{2max} can be reached within 1-3 minutes (Caputo & Denadai, 2008; P. O. Åstrand et al., 2003), the MAOD test can be used as verification test.

HIIT vs SIT - sex-differences in anaerobic capacity

Intense sprint exercise results in a rapid increase in energy turnover from both aerobic and anaerobic metabolism (Barnett et al., 2004). When aiming for anaerobic improvements, intensity is typically higher than during ET and HIIT, and intervals at a supramaximal intensity has been shown to improve anaerobic capacity (Balto & Helgerud, 2019; Medbo & Burgers, 1990; Tabata et al., 1996). However, as small amounts of lactate accumulate in between breaks in the 4x4-protocol (Storen et al., 2017), it is plausible that the small anaerobic contribution from HIIT may also improve anaerobic capacity. To date two recent studies have compared 8 weeks of 4x4 HIIT vs 10x30-second SIT on anaerobic capacity measured as MAOD (Balto & Helgerud, 2019; Trane & Helgerud, 2018). While Trane and Helgerud (2018) found no significant difference in trained males, Balto and Helgerud (2019) showed a significantly larger increase in MAOD following SIT than HIIT expressed as both L and $ml \cdot kg^{-1}$ in trained females. However, as mentioned earlier, the results of Balto and Helgerud (2019) were only based on 5 subjects in each group underlining the need for further research including larger sample sizes of females. Still, when comparing the baseline differences in anaerobic capacity between the trained males and females in Balto and Helgerud (2019) and Trane and Helgerud (2018), this supports the 17% lower anaerobic capacity for females reported in Medbo and Burgers (1990). This could be due to less type II muscle fibres and smaller muscle fibre size in females compared to males (Shephard, 2000), as type II fibres has greater glycolytic capacity, motor unit recruitment, discharge rate, and ATP resynthesis compared to type I fibres (Essen, Jansson, Henriksson, Taylor, & Saltin, 1975; McArdle et al., 2014). In summary, there is not much research on anaerobic capacity measured through MAOD with females as subjects. The existing literature suggests that females generally have a lower anaerobic capacity compared to males but have at least the same potential of improving it. Furthermore, literature suggests that SIT induce greater changes in anaerobic capacity compared to HIIT, at least in female subjects.

Endurance running performance

Endurance training programs should be optimized to improve athletic performance while limiting the development of fatigue or risk of injury (Rosenblat et al., 2020). Endurance performance reflects the performance of whole-body at high-intensity for extended periods and is determined by VO_{2max}, running economy (RE), lactate threshold (LT), and anaerobic capacity (Joyner & Coyle, 2008; Pate & Kriska, 1984). Of the mentioned determinants VO_{2max} is considered the single most important factor when predicting endurance performance, especially in heterogenous groups (McLaughlin et al., 2010; Saltin & Astrand, 1967; Stratton et al., 2009). However, in more homogenous groups, the inclusion of the other factors is increasingly important at predicting endurance performance. To illustrate, within an elite cluster of finishers, 65.4% of the variation observed in race performance on the 10 km run could be explained by variation in running economy, while individual differences in LT resulted in up to 10% faster 1hr TT-performance in cycling athletes when matched for VO_{2max} (Conley & Krahenbuhl, 1980; Coyle et al., 1991). Worth mentioning, the velocity at VO_{2max} (vVO_{2max}) and vLT has both been shown to be the strongest related factors to a 3000m and 5000m performance in both untrained, trained, collegiate runners and elite athlete men and women (Grant, Craig, Wilson, & Aitchison, 1997; Slattery, Wallace, Murphy, & Coutts, 2006; Yoshida, Udo, Iwai, & Yamaguchi, 1993). vVO_{2max} and vLT are determined by the sum of determinants for endurance performance, and can therefore explain differences in aerobic endurance better than VO_{2max} alone (Alves Pasqua et al., 2018; Bassett & Howley, 2000; Farrell et al., 1993; Nummela & Rusko, 1995). Lacour, Padilla-Magunacelaya, Barthelemy, and Dormois (1990) indicated that the relative importance of vVO_{2max} (and thus vLT) to performance increased as race distance increased, while at shorter distances performance is dependent on an increasing contribution from anaerobic capacity (Brandon, 1995).

As there has been shown a significant relationship between maximal sprint speed (MSS) and middle-distance running performance, scientists are arguing that MSS could affect race pace in middle-distance running (Bachero-Mena et al., 2017; Bundle, Hoyt, & Weyand, 2003; G. Sandford & T. Stellingwerff, 2019; Sandford, Kilding, Ross, & Laursen, 2019). It is argued that this could be due to a lower imposed physiological strain when running at a lower proportion of the anaerobic sprint reserve (MSS and velocity at VO_{2max} combined) (Buchheit, Hader, & Mendez-Villanueva, 2012; Sandford et al., 2019). Also, runners with a high MSS have the opportunity to run faster relaxed race paces compared to a slower athlete assuming similar aerobic capability, improving their running performance (Sandford et al., 2019).

Sex-differences in endurance performance

The general running performance among females have rapidly improved from the mass participation beginning in the 1970s. Women's distance running records and top times in comparable cohorts of elite athletes are now typically 10-12% slower than men (S. K. Hunter, Stevens, Magennis, Skelton, & Fauth, 2011). At puberty, differences in hormonal activity results in physical and physiological differences between genders (Shephard, 2000; Thomas & French, 1985). As mentioned, females have a smaller body size including muscle mass, dimensions of organs, and skeletal size, in addition to a higher fat percentage and lower red cell mass for a given body weight compared to males (Cureton et al., 1986; Joyner, 2017; Pate & O'Neill, 2007; Schmidt & Prommer, 2010; Shephard, 2000). These factors influence aerobic power, anaerobic power, and muscle power which all are believed to influence running performance (Brandon, 1995).

Studies concerning HIIT and SIT are mainly conducted with males as subjects. Recently a few SIT-studies have included both sexes and reported equivocal findings regarding if a sex-difference in adaptation to SIT exists. In short, studies has shown equivocal findings regarding if SIT induce different effects between sexes on mitochondrial biogenesis, a factor influencing endurance performance (Bagley et al., 2018; Burgomaster, Heigenhauser, & Gibala, 2006; Gibala et al., 2006; Gibala & McGee, 2008; Scalzo et al., 2014; Skelly et al., 2017). Regardless, a meta-analysis showed strong evidence that SIT improves both aerobic and anaerobic performance in healthy, sedentary, or recreationally active men and women (Sloth et al., 2013), with the only study to report sex-difference in performance found females to have larger improvement than males in 3000m TT (Cicioni-Kolsky, Lorenzen, Williams, & Kemp, 2013). Furthermore, SIT-interventions involving both male and female inactive, recreationally active, moderately trained, and trained runners did not report any improvement-differences between sex in 50m, avg 30s sprint speed, 2000m, 3000m and 10000m TT running performance (Koral, Oranchuk, Herrera, & Millet, 2017; Macpherson et al., 2011; Skovgaard, Christiansen, et al., 2018; Sokmen, Witchey, Adams, & Beam, 2018; Willoughby, Thomas, Schmale, Copeland, & Hazell, 2016). Still, more studies concerning HIIT and SIT should include females as subjects.

HIIT vs SIT – effect on endurance running performance?

One of the most important variables to consider when prescribing exercise is the intensity at which an athlete trains as this metric strongly influences physiological and performance adaptations (Helgerud et al., 2007). A meta-analysis comparing HIIT- vs SIT-interventions on time-trial (TT) endurance performance in at least moderately trained males and females showed no difference in performance improvements between the two types of intervals. Further, when conducting a subgroup analysis differentiating short-HIIT and long-HIIT with long-HIIT being ≥ 4 min interval duration, the authors found indications for a 2% greater improvement following work-bouts between 4-6 min in duration with 2-4 min of recovery when compared to SIT (Rosenblat et al., 2020). However, as SIT is a relatively new field of research, this subgroup analysis was only based on 3 studies focusing solely on a HIIT vs SIT protocol. When the sample size is this low, and with large subject heterogeneity between studies it may skew the results by increasing the variability and risk of error (Rosenblat et al., 2020).

The aerobic energy contribution to an athlete's 3000m is about 85-90 % (Billat, 2001; Duffield, Dawson, & Goodman, 2005b). To date, a total of 6 studies have involved a 3000m TT-test following a SIT-running intervention in physical active - trained runners (47.9 - 63 $ml \cdot kg^{-1} \cdot min^{-1}$). All studies involved 2-3 trainings per week, 30s intervals with 3-4 minutes of recovery between intervals and showed a 3000m-improvement of 2.3-8.8%, respectively (Balto & Helgerud, 2019; Bangsbo et al., 2009; Cicioni-Kolsky et al., 2013; Esfarjani & Laursen, 2007; Koral et al., 2017; Trane & Helgerud, 2018). 4 of the 6 mentioned studies also involved a group conducting HIIT with 4-8 bouts of intervals with intensities at either 90-95% of HR_{max} or vVO_{2max}, 3.5-4 minutes duration and 3.5-4 minutes of active recovery (Balto & Helgerud, 2019; Cicioni-Kolsky et al., 2013; Esfarjani & Laursen, 2007; Trane & Helgerud, 2018). While none of the above mentioned studies showed that HIIT resulted in a less % improvement in 3000m TT-performance compared to SIT, only Trane and Helgerud (2018) showed a significantly larger 3000m TT improvement following a 4x4 HIIT compared to 30s SIT protocol. To summarize, it seems both 30s SIT- and 4x4 HIIT-protocols have an enhancing effect on endurance performance. Therefore, future SIT vs HIIT investigations on endurance performance should include larger sample sizes, not involve multiple training protocols within groups and include long-HIIT when feasible (Rosenblat et al., 2020).

SIT vs HIIT – effect on sprint-endurance performance?

Nine SIT-studies involving a 30s SIT running protocol have included a sprint performance test. These have shown significant improvements in inactive to trained runners in 40m (Cicioni-Kolsky et al., 2013), 50m (Sokmen et al., 2018), 30s sprint test (Bangsbo et al., 2009; McKie et al., 2018), MSS (Hazell, Hamilton, Olver, & Lemon, 2014; McKie et al., 2018; Willoughby et al., 2016), and 300m (Balto & Helgerud, 2019; Trane & Helgerud, 2018). The latter mentioned studies showing 300m-improvement in trained students are the first to also show sprint TT improvement following a 4x4 HIIT-protocol without supplemented strength, as three other studies reported no significant improvements in sprint on soccer players (Ferrari Bravo et al., 2008; Helgerud et al., 2001; McMillan, Helgerud, Macdonald, & Hoff, 2005) except from a 20% increase in distance covered and 100% increase in the number of sprints during a match was found (Helgerud et al., 2001). Of the mentioned studies involving both male and female participants no sex-difference were reported on sprint performance (Cicioni-Kolsky et al., 2013; McKie et al., 2018). However, larger improvement in both repeated sprint ability, 40m, and 300m TT has been found following a SIT- compared to HIIT-intervention (Balto & Helgerud, 2019; Cicioni-Kolsky et al., 2013).

Perceived exertion

A rating of perceived exertion (RPE) is the degree of heaviness and strain experienced in physical work (G. Borg, 1998), and are often used for estimating training load (Seiler & Sylta, 2017). When comparing different RPE-scales, Grant et al. (1999) found that for sensitivity, the Borg RPE scale had the highest ratio estimate for measuring general fatigue. Furthermore, the Borg RPE scale is the most commonly used scale for estimating breathlessness and general fatigue during physical work. Two main advantages of the Borg RPE scale are firstly that it is unique because of its special use of verbal anchors to permit level determinations. Secondly, the given ratings grow linearly with exercise intensity, HR, and VO_2 (G. Borg, 1998). This makes the ratings easy to use and compare. Still, RPE is a debatable assumption as perceptions are subjective phenomena. Factors such as experience enduring exhaustion and difficulties understanding the scale affect the validity and reliability of the results. The test leader should identify exactly the dimensions of variables to be tested and give well-planed, identical instructions to enhance the intersubjective agreement (G. Borg, 1998).

HIIT vs SIT – effect on perceived exertion

Follador et al. (2018) examined the effect of three running protocols thereof 4x4 HIIT and three cycling protocols thereof 4x30s SIT on RPE. Results showed that the HIIT-protocols thereof 4x4 HIIT elicited a significantly lower RPE than the other protocols including SIT. Further RPE has been shown to increase with the amount of both 30s SIT- and 4min HIIT interval bouts (Rowley, Espinoza, Akers, Wenos, & Edwards, 2017; Seiler & Hetlelid, 2005), and decrease when recovery is prolonged from 2 to 4 minutes in HIIT (Seiler & Hetlelid, 2005). Although 30s SIT has not been compared to long-HIIT in the same training modality or in an intervention period, strong evidence suggests 30s SIT to elicit higher perceived exertion compared to 4x4 HIIT.

Aim and hypothesis

The aim of the present study is to compare the effects of 4x4-minute HIIT and 10x30-second SIT on VO_{2max}, RE, LT, anaerobic capacity, running performance and RPE. Even though male and female physiology have some dissimilarities, both sexes seem to respond similarly to training. However, because trained females generally seem underrepresented in interval training research, the author find it beneficial to use trained females as subjects. It is hypothesised that HIIT will improve VO_{2max} and 3000m more than SIT, and that SIT will increase anaerobic capacity and 300m performance more than HIIT.

Methods

Subjects

Thirty-three healthy, non-smoking, trained females volunteered for the study, which was performed according to the ethical standards established by the Helsinki Declaration of 1975. The Institutional Review Board of NTNU approved the protocol and all participants signed an informed consent prior to participation. Because no subjects were younger than 18 years, parental or guardian consent was not collected.

Inclusion criteria to participate were VO_{2max} between 45-60 ml·kg^{-1}·min^{-1} at baseline and all subjects had to be engaged in endurance training at least once per week, or other recreational activities for at least 3 times per week. A history of cardiovascular, coronary or chronic lung disease were set as exclusion criteria. In addition, all subjects had to complete at least 20 out of 24 supervised training sessions over the 8-week intervention period to be included in the post-testing. The endurance-trained females were randomly assigned and matched into groups based on their VO_{2max}. The subjects were asked to avoid other high-intensity activities while participating in the study. Ten subjects did not complete the study.

Table 1. Descriptive data of the subjects.

	HIIT (n = 13)	SIT (n = 10)
Age (year)	22.4 ± 1.7	22.3 ± 1.7
Height (cm)	170 ± 5	166 ± 6
Body mass (kg)	65.2 ± 4.7	65.7 ± 8.4
VO_{2max}		
VO_2 (L · min^{-1})	3.35 ± 0.29	3.36 ± 0.37
VO_2 (ml · kg$^{-0.75}$ · min^{-1})	147.0 ± 10.5	146.4 ± 9.1
VO_2 (ml · kg^{-1} · min^{-1})	51.6 ± 4.3	51.4 ± 3.7

Data are presented as means ± standard deviation (SD). VO_{2max}, maximal oxygen uptake.

Testing

The subjects performed 2 days of testing at the lab and 1 performance test on separate days both before and after the 8-week intervention period. All 3 post-intervention tests were carried out within 14 days of the last training session. Subjects were told to avoid strenuous activity for the last 24 hours before a test and had at least 48 hours of recovery between tests. The lab-tests were conducted on a motorized treadmill (Woodway PPS 55 Sport, Waukesha, Germany). Cortex Metamax II portable test-system (Cortex Biophysik GmbH, Leipzig, Germany) were used for all measurements of pulmonary oxygen uptake, and this system has been validated against the Douglas bag method (Larsson, Wadell, Jakobsson, Burlin, & Henriksson-Larsen, 2004). $[La^-]_b$ were analysed from 20 μL hemolyzed blood from fingertip using a Biosen C-line lactate analyzer (EKF-diagnostic GmbH, Leipzig, Germany). For HR measurements during both testing and training, Polar heart rate monitors and watches (Polar F11, polar Electro Oy, Kempele, Finland) were used.

Test day 1 - VO_{2max}, RE and LT

VO_{2max}, RE and LT was measured in the lab using a motorised treadmill with 5.3% inclination. LT was defined as the VO_2, HR or velocity were $[La^-]_b$ was measured 1.5 $mmol \cdot L^{-1}$ above the lowest measured value. The test started with a 10-minute warmup with a following lactate measurement. Further, the speed increased by 1 $km \cdot h^{-1}$ each 5 minutes, separated by a small break taking a lactate sample, until the lowest $[La^-]_b$ value (typically after warmup) + 1.5 $mmol \cdot L^{-1}$ was reached (Helgerud et al., 1990). The advantage of using such a model based on individual warmup values compared with a fixed 4 $mmol \cdot L^{-1}$ limit, is the less vulnerability to day-to-day variations in subjects (Storen et al., 2014). For all subjects, this included a 5-minute step at 7 $km \cdot h^{-1}$ for the determination of RE at this standardized workload. Notably, the starting speed was set modestly, as all subjects had to complete at least 3x5-minute stages of the LT-protocol in order to calculate MAS for the MAOD test described below.

Following the LT and RE procedure, subjects had a break of approximately 5 minutes depending on their $[La^-]_b$ build-up on their last 5-minute step which was over LT, and the subject were encouraged to walk during the break. The VO_{2max} protocol started at the same incline and intensity as the final intensity in the LT protocol and increased by 1 $km \cdot h^{-1}$ every minute till exhaustion. As the highest 30 second average VO_2 was used as the subjects VO_{2max}, the subjects were in advance given the possibility to stay at the highest speed instead of increasing if they thought they couldn't endure 20 seconds at the next intensity. The VO_{2max} protocol had a duration of 4-7 minutes, and strong verbal encouragement was given in both pre- and posttest. Achievement of VO_{2max} was accepted when VO_2 levelled off despite increased velocity combined with either R > 1.05 and/or $[La^-]_b > 8$ (Helgerud et al., 2007; Helgerud et al., 2010). The HR_{peak} during the last minute of the VO_{2max} protocol was used as HR_{max}. O_2 pulse was used as a non-invasive measurement of SV and was calculated from VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$) multiplied by m_b (kg) and then divided by HR_{max} (bpm) (Crisafulli et al., 2007; Whipp, Higgenbotham, & Cobb, 1996).

Test day 2 - Anaerobic capacity

The second day of testing consisted of a MAOD-test on a motorized treadmill with 5.3% incline. The protocol started with a 15-minute warmup interspersed with 2 increases in speed from warmup-velocity to 5 seconds at the set velocity for the MAOD test. Following warmup was a 10-minute passive recovery with the intention to refill all anaerobic energy storages. During this period $[La^-]_b$ was controlled to be approximately at baseline values, and subjects received verbal instructions to run an all-out effort till exhaustion without revealing the target duration. If subjects ran ± 15 seconds outside the target duration of 2-3 minutes, the test had to be repeated with a minimum of 48 hours in between tests. HR_{peak} and $[La^-]_b$ was measured following the run till exhaustion.

MAOD for a given exercise bout at a constant supramaximal intensity is defined as the accumulated O_2 demand ($ml \cdot kg^{-1}$) minus the measured accumulated O_2 uptake ($ml \cdot kg^{-1}$) (Medbo et al., 1988). The accumulated O_2 demand is given as a product of the estimated O_2 demand ($ml \cdot kg^{-1}$) at a given velocity ($km \cdot h^{-1}$), and the total duration of the exercise (min^{-1}). vVO_{2max} was calculated from the VO_{2max} value, from the relationship between VO_2 and velocity at different submaximal VO_2-measurements in the LT-test, and from adding a simplified Y-intercept value of 5 $ml \cdot kg^{-1} \cdot min^{-1}$ at rest to extrapolate a linear regression line for each subject. The intensity corresponding to 120 ± 10 % of vVO_{2max} was set with the purpose of exhausting the subjects after 2-3 minutes, a method which has been demonstrated to gives the highest accumulated O_2 deficit, with a precision of 3 $ml \cdot kg^{-1}$ or 4% (Medbo et al., 1988). Including the 5 $ml \cdot kg^{-1} \cdot min^{-1}$ at rest to extrapolate a linear regression line for each subject is a simplified procedure described in Medbo et al. (1988) which did not give significantly different results from the original procedure involving 10 submaximal bouts. Since VO_{2max} can be reached within 1-3 minutes (Caputo & Denadai, 2008; P. O. Åstrand et al., 2003)**,** the MAOD test fits the recommendations as a verification test for VO_{2max} (Poole & Jones, 2017). If subjects achieved a higher 30 second average VO_2 during MAOD-test than achieved during the incremental VO_{2max}-test, this replaced the lower VO_{2max}..

Test day 3 - 300 meter and 3000 meter time-trial

The third day of testing consisted of a 300m- and a 3000m running time-trial performance test. The tests were conducted indoor on a banked 200m indoor running track, which secured similar environmental conditions at pre- and posttest. Subjects had a non-controlled warmup for 10 minutes and were encouraged to do some speed-increases at the end of warmup.

Identical information and verbal encouragement for maximal effort were given to all subjects before the 300m individual start and the 3000m mass start. The two tests were separated by a 20-minute passive break and 10-minute re-warmup. A 105-decibel whistle and a simultaneous arm swing were used as starting mechanism, and a manual stopwatch was used to measure time, given in seconds. These were managed by the same physiologists at pre- and posttest to minimize the error of measurement. The validity and reliability of this kind of time trial test have been established by Denham, Feros, and O'Brien (2015) with an intraclass correlation coefficient = 0.99 and a 3.4% coefficient of variation.

Training

Both interventions consisted of three weekly supervised sessions for 8 weeks, and all running sessions were supervised on motor driven Gymsport TX200 treadmills (Trondheim, Norway) at 5.5% inclination**.** RPE was recorded 2 minutes after completion of each interval session by using Borg scale, ranging from “6 -No exertion at all” to “20- Maximal exertion” (G. Borg, 1998). Subjects were told to disregard any one factor such as leg pain or shortness of breath but to try to focus on the whole feeling of exertion post-workout. Each interval session started with a 10-minute warm-up and ended with a 10-minute cooldown. The HIIT group started off with 4 intervals of 4 minutes duration with each interval separated by 3 minutes of active recovery at 70% HR_{max} (Fig. 1). The target intensity of this workout was 90 - 95% of HR_{max}. This typically meant having an approximately steady pacing with an increasing HR throughout the session. HR was controlled every 3.30 into each interval, and the workload was adjusted the next interval if HR did not reach the target intensity.

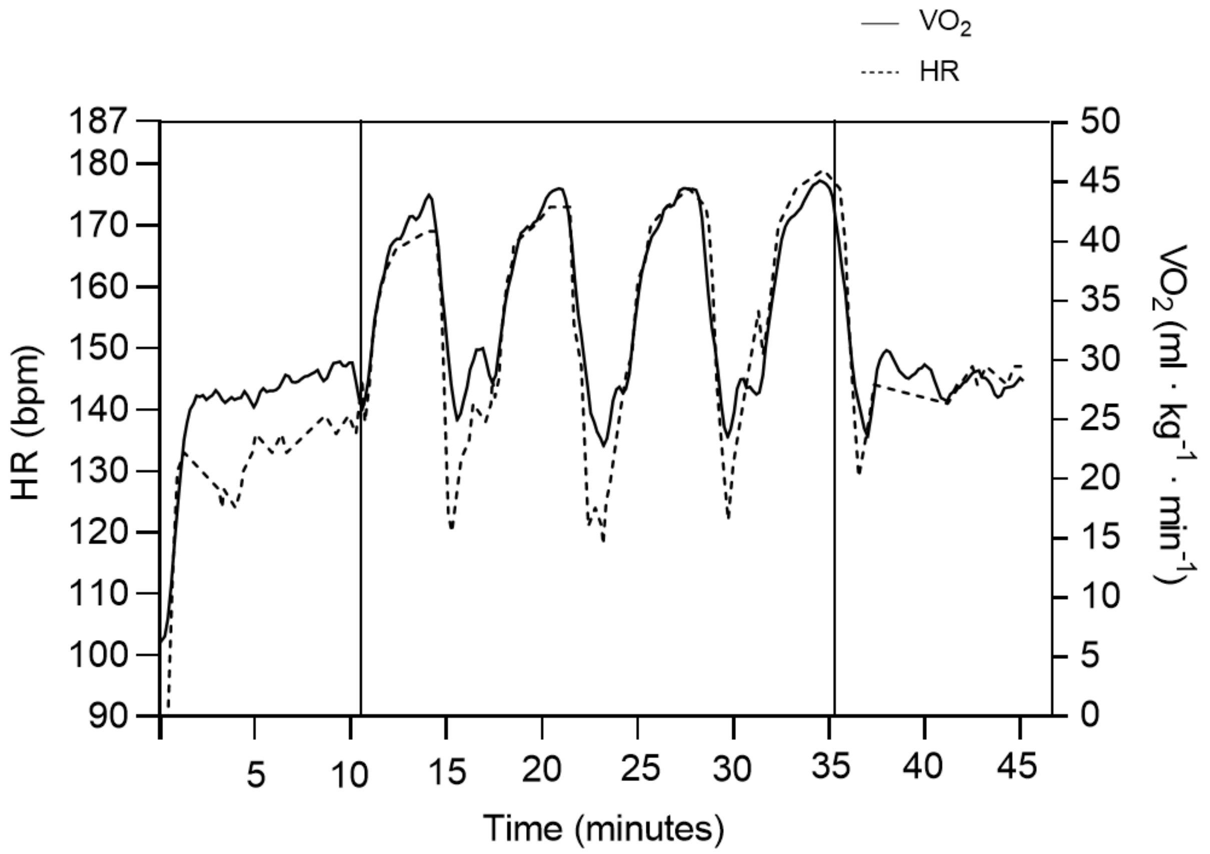

Figure 1. An example of a 4x4-minute HIIT session performed at 90-95 % of HR_{max} interspersed with 3min active recovery at ~70 % of HR_{max} and includes HR and VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) responses. Each session started with a 10min warm-up and ended with a 10min cooldown. In this example, the subject HR_{max} was 187 $beats \cdot min^{-1}$ and a VO_{2max} at 52 $ml \cdot kg^{-1} \cdot min^{-1}$. The velocity of all intervals presented was 9.6 $km \cdot h^{-1}$ and 5.5% incline on the treadmill.

The SIT group were encouraged to do some speed-increases at the end of warmup. Accordingly, the subjects performed 10 intervals of 30 seconds separated by 3.5 minutes of active recovery (Fig. 2). All 10 intervals were performed with an all-out intensity, while verbal encouragement was given for motivation. The velocity at each interval was simply the fastest possible speed the subject could maintain for approximately 30 seconds. If the subject failed to complete 28 seconds at the set treadmill-velocity due to skeletal muscular fatigue, the speed was adjusted down by 0.5 $km \cdot h^{-1}$. The first three sessions were used as familiarization sessions with increasing speed, and the intervals were not performed at all-out intensities. Also, the first and last session only consisted of 6 instead of 10 intervals.

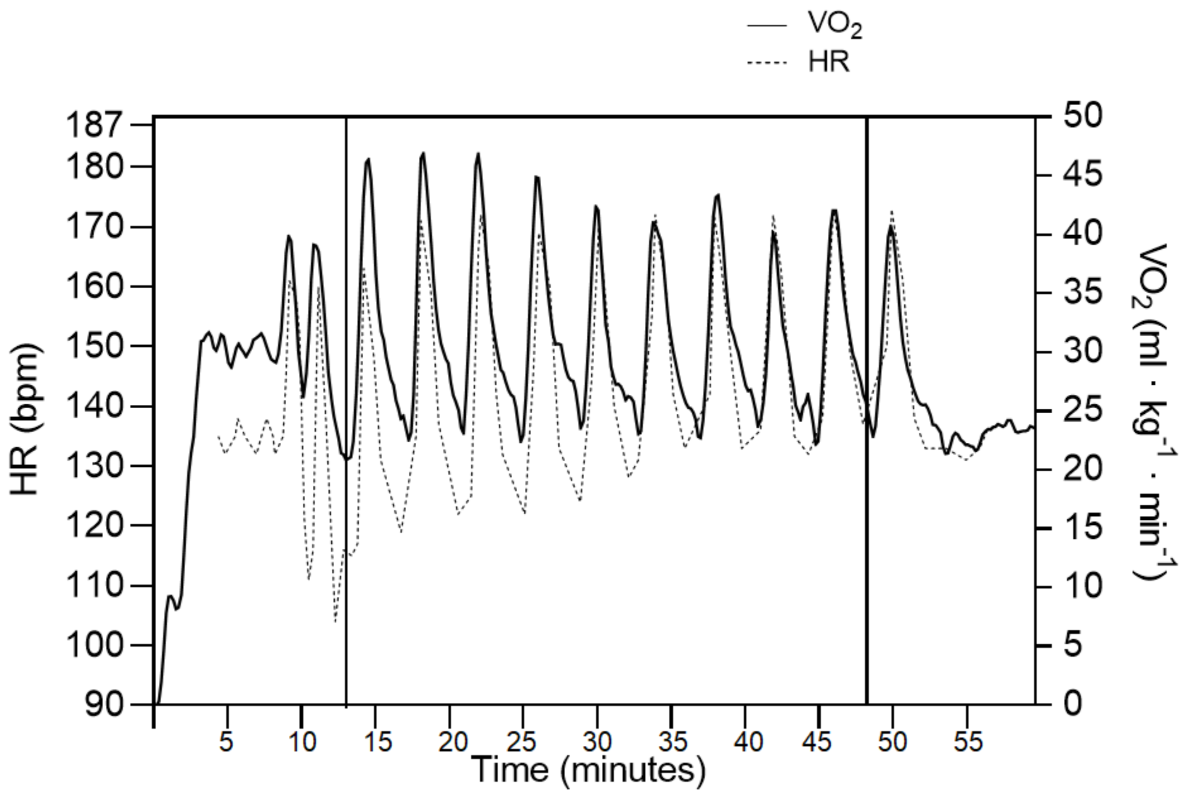

Figure 2. An example of a 10x30-second all-out SIT session including HR and VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) responses. Each session started with a 10min warmup and ended with a 10min cooldown performed at ~70 % of HR_{max}. In this example, the subjects HR_{max} was 187 beats$\cdot min^{-1}$ and VO_{2max} at 52 $ml \cdot kg^{-1} \cdot min^{-1}$. The velocity at the first and last interval presented were 22 $km \cdot h^{-1}$ and 20 $km \cdot h^{-1}$, respectively, at a treadmill with 5.5% incline

Statistical analysis

Statistical analysis was performed using the software program IBM SPSS Statistics 25 (Armonk, NY: IBM Corp.). Due to normally distributed data paired samples T-tests and ANOVA repeated measures were used to analyse significance levels within and between groups. Correlations was calculated using linear regression analysis. In all cases, $P < 0.05$ are presented as the level of significance. Mean ± standard deviation (SD) are presented in text and tables to facilitate comparison with other studies, and graphs are presented as mean ± SE.

Results

Of the 33 subjects randomized to either HIIT or SIT, 23 were included in the analysis (Fig. 3). Among the 17 subjects allocated to the SIT-intervention 6 dropped out due to injury related to the study and 1 due to protocol intensity. While 13 of 17 subjects allocated to SIT reported adverse effects during the intervention, no adverse effects were reported among the subjects allocated to HIIT. Among these 16 subjects 2 subjects dropped out not completing ≥ 20 training sessions and one subject dropped out because of an injury not related to the study. Later, two subjects from HIIT were excluded from the MAOD data material, one due to inaccurate supramaximal O_2 demand values obtained from the extrapolation of individual regression lines, and one due to unreliable VO_2 measurements.

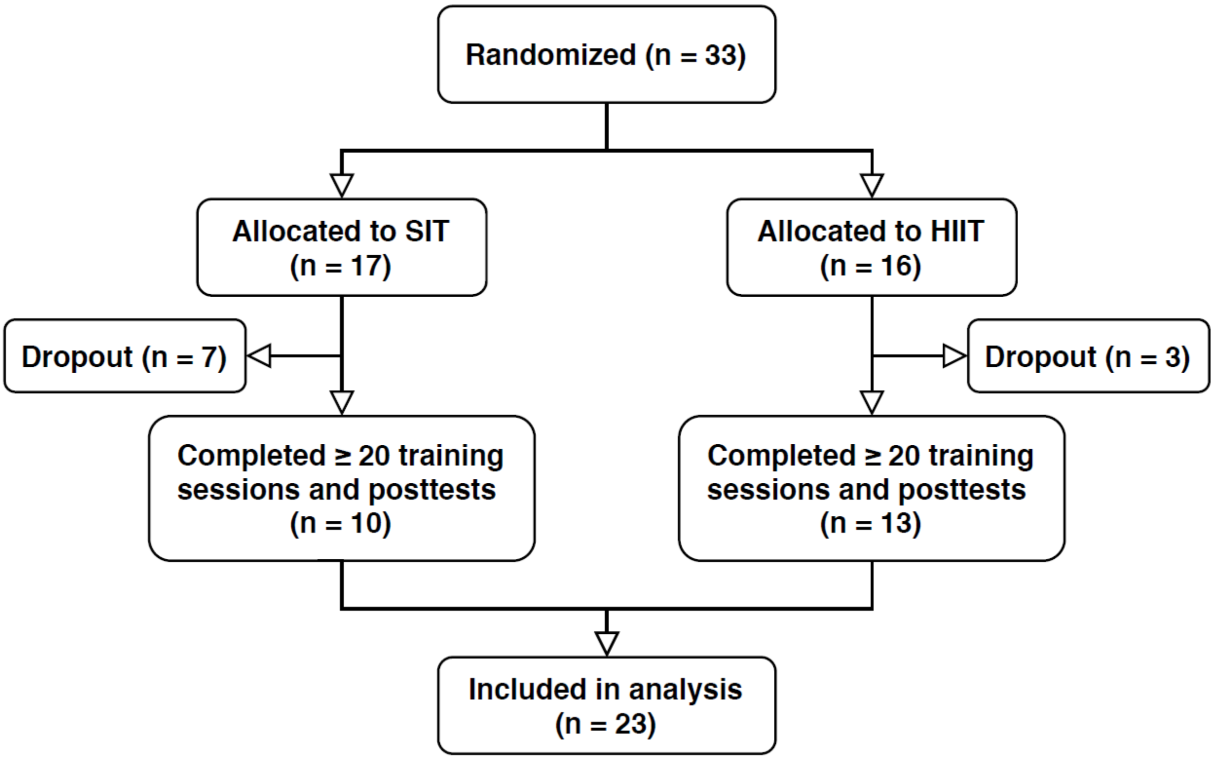

Figure 3. Flow diagram of study design. HIIT, high-intensity interval training; SIT, sprint interval training.

Table 2. Interval sessions.

	HIIT (n = 13)	SIT (n = 10)
Number of training sessions	22.7 ± 1.4	21.4 ± 1.2
Session RPE	14.8 ± 0.3	17.2 ± 1.7[c]
Velocity ($km \cdot h^{-1}$) first interval of workout	9.76 ± 8.30	20.1 ± 1.4[c]
Velocity ($km \cdot h^{-1}$) last interval of workout	9.84 ± 8.41	18.8 ± 1.4[c]
% HR_{max} after 3.30 min each interval	92.7 ± 1.8	
% HR_{max} after 3.30 min last interval	93.5 ± 1.4	

Data are presented as means ± SD. All sessions were carried out running on treadmill at 5.5% inclination. RPE, Rating of perceived exertion; % HR_{max}, percent of maximal heart rate measured from VO_{2max}-test. [c] Significant difference ($P < 0.001$) between groups.

HIIT- and SIT sessions were conducted according to the protocol, and there was no significant difference in compliance between groups (Table 2). At baseline, no parameter was significantly different between groups (Table 1, Table 3, and Table 4). During the intervention period SIT ran at a significantly higher velocity ($P < 0.001$) and reported a higher session RPE ($P < 0.001$) than HIIT (Table 2).

VO_{2max}, RE and LT

The HIIT group increased absolute VO_{2max} ($L \cdot min^{-1}$) significantly more than SIT. VO_{2max} increased 3.6% from pre- to posttest following HIIT, but no change was apparent in the SIT group (-0.5%) (Table 3, Fig. 4). O_2 pulse improved following HIIT and tended to increase more in HIIT compared to SIT ($P = 0.06$). Only HIIT improved vVO_{2max} and increased significantly more compared to SIT. RE expressed in $L \cdot min^{-1}$ improved significantly in both HIIT and SIT, by 4.0% and 3.7%, respectively. However, when expressed in $ml \cdot kg^{-1} \cdot min^{-1}$, $ml \cdot kg^{-0.75} \cdot min^{-1}$, and $ml \cdot kg^{-0.75} \cdot m^{-1}$, only HIIT showed significant improvements. LT did not change for any group when expressed as $\%VO_{2max}$ (Table 3). The vLT was, however, significantly improved only in HIIT by an average of 7.6% % as a consequence of changes in running economy and VO_{2max} and tended to improve more than SIT (2.8%) ($P = 0.06$).

Table 3. Changes in endurance performance and endurance performance determinants.

	HIIT (4 x 4 min) (n = 13)		SIT (10 x 30 sec) (n = 10)	
	Pretest	Posttest	Pretest	Posttest
VO_{2max}				
VO_2 ($L \cdot min^{-1}$)	3.35 ± 0.29	3.46 ± 0.25**a	3.37 ± 0.39	3.35 ± 0.35a
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	51.4 ± 4.6	53.5 ± 4.7**	51.5 ± 3.6	51.9 ± 3.4
VO_2 ($ml \cdot kg^{-0.75} \cdot min^{-1}$)	146.2 ± 11.9	152.2 ± 11.3**	146.4 ± 9.1	147.0 ± 8.9
V_E ($L \cdot min^{-1}$)	108.5 ± 9.3	111.5 ± 11.0*	105.1 ± 15.0	110.0 ± 13.6*
R	1.10 ± 0.04	1.12 ± 0.04*	1.12 ± 0.04	1.13 ± 0.05
$[La^-]_b$ ($mmol \cdot L^{-1}$)	12.5 ± 1.7	13.36 ± 1.5**	10.84 ± 2.63	11.62 ± 1.90
HR_{max} (bpm)	197 ± 4	196 ± 5*	193 ± 9	192 ± 9
O_2 pulse ($ml \cdot beat^{-1}$)	17.0 ± 1.5	17.6 ± 1.4**	17.6 ± 2.5	17.5 ± 2.1
$v\dot{V}O_{2max}$ ($km \cdot h^{-1}$)	9.8 ± 1.0	10.9 ± 1.1***b	10.6 ± 1.3	10.8 ± 1.2b
Running Economy				
VO_2 ($L \cdot min^{-1}$)	2.47 ± 0.23	2.37 ± 0.19*	2.38 ± 0.39	2.28 ± 0.33*
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	38.0 ± 3.1	36.6 ± 3.3*	36.0 ± 2.9	35.2 ± 2.5
VO_2 ($ml \cdot kg^{-0.75} \cdot min^{-1}$)	107.8 ± 8.6	104.0 ± 8.4*	102.7 ± 9.5	99.8 ± 8.1
VO_2 ($ml \cdot kg^{-0.75} \cdot m^{-1}$)	0.92 ± 0.07	0.89 ± 0.07*	0.88 ± 0.08	0.86 ± 0.07
HR (bpm)	177 ± 10	170 ± 9*a	167 ± 15	167 ± 9a
Lactate Threshold				
VO_2 ($L \cdot min^{-1}$)	2.60 ± 0.26	2.61 ± 0.20	2.61 ± 0.39	2.56 ± 0.29
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	40.0 ± 3.8	40.6 ± 3.9	39.8 ± 3.7	39.7 ± 3.8
VO_2 ($ml \cdot kg^{-0.75} \cdot min^{-1}$)	113.5 ± 10.1	115.0 ± 10.0	113.2 ± 10.6	112.5 ± 9.8
% VO_{2max}	77.8 ± 2.9	75.9 ± 1.9	77.4 ± 4.9	76.6 ± 6.4
% HR_{max}	91.8 ± 2.2	91.5 ± 1.6	92.3 ± 1.7	92.8 ± 1.7
vLT ($km \cdot h^{-1}$)	7.5 ± 0.8	8.0 ± 0.7***	7.9 ± 1.0	8.1 ± 0.8
$[La^-]_b$ ($mmol \cdot L^{-1}$)	3.03 ± 0.35	2.99 ± 0.40	3.12 ± 0.76	3.22 ± 0.75
Running performance				
300 meter (sec)	56.4 ± 3.9	55.1 ± 3.8***c	59.5 ± 5.3	55.5 ± 4.9***c
3000 meter (sec)	911 ± 69	870 ± 68***	899 ± 83	861 ± 63**
Body mass (kg)	65.2 ± 4.7	64.6 ± 4.5	65.7 ± 8.4	64.6 ± 6.9

Data are presented as means ± SD. The VO_{2max}-test were carried out running on treadmill at 5.3% inclination. Performance tests were carried out on a 200m indoor track. VO_2, oxygen uptake; HR_{max}, maximal heart rate; V_E, pulmonary ventilation; $[La^-]_b$, blood lactate concentration; RER, respiratory exchange ratio; O_2 pulse, maximal oxygen pulse; vVO_{2max}, maximal velocity at VO_{2max}-test; vLT, velocity at lactate threshold. * Significant differences ($P < 0.05$) within groups from pre- to posttraining; ** significant difference ($P < 0.01$) within groups from pre- to posttraining; *** significant difference within groups ($P < 0.001$) from pre- to posttraining; [a] significant difference ($P < 0.05$) between groups from pre- to posttraining; [b] significant difference ($P < 0.01$) between groups from pre- to posttraining; [c] significant difference ($P < 0.001$) between groups from pre- to posttraining.

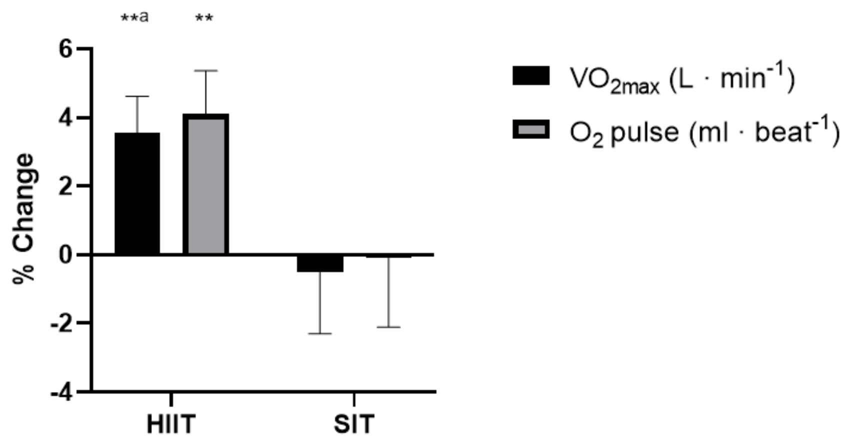

Figure 4. Percentage change in $\dot{V}O_{2max}$ and O_2 pulse from pre-to post-training, presented as mean ± SE. ** Significant difference within group ($P < 0.01$) from pre-to post-training; [a] significant difference between groups ($P < 0.05$) from pre-to post-training.

Running performance

The 3000-meter running performance improved by an average 41 seconds after HIIT and by 38 seconds after SIT with no significant difference between groups (Table 3, Fig. 5). SIT improved 300m running performance significantly more than HIIT ($P < 0.001$). Subjects from the HIIT-group improved their 300-meter performance by an average 1.3 seconds and SIT by 4.0 seconds, both significant ($P < 0.001$).

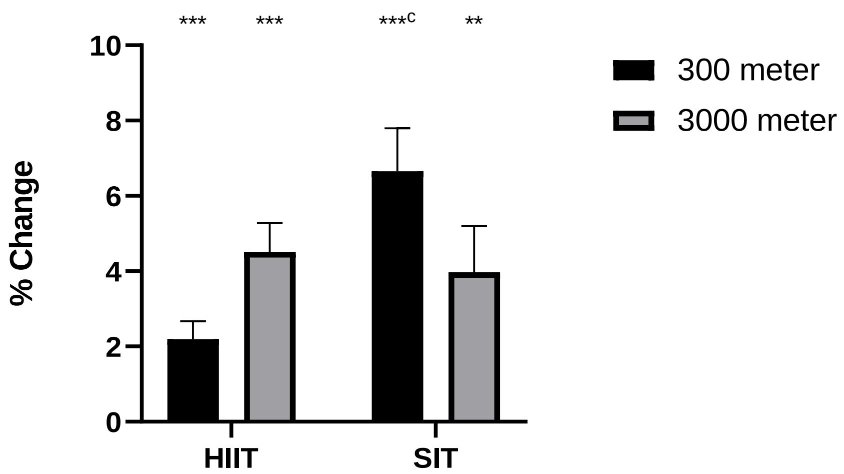

Figure 5. % change in 300- and 3000-meter running time-trial performance. Data are presented as mean ± SE. **Significant difference within group ($P < 0.01$) from pre- to posttraining; ***significant difference within group ($P < 0.001$) from pre- to posttraining; [c] significant difference between groups ($P < 0.001$) from pre- to posttraining.

Anaerobic capacity – MAOD

The SIT group improved MAOD ($ml \cdot kg^{-1}$) significantly more than HIIT. MAOD increased 9.5% from pre- to posttest following SIT, but no change was apparent in the HIIT group (2.5%) (Table 4). Both HIIT and SIT increased velocity at the MAOD-test significantly ($P < 0.001$), by 5.8% and 3.7%, respectively, with no difference between groups. HIIT significantly decreased in both velocity expressed as % of MAS, and % of VO_{2max} reached from pre- to posttest. This happened without any drop in HR expressed as % of HR_{max}. For HIIT and SIT combined, the average VO_{2max} ($L \cdot min^{-1}$) from all VO_{2max}-tests were 5.7% larger than VO_{2peak} from MAOD-test ($P < 0.001$), with no significant difference between groups.

Table 4. Changes in anaerobic capacity (maximal accumulated oxygen deficit).

	HIIT (4 x 4 min) (n = 11)		**SIT (10 x 30 sec) (n = 10)**	
	Pretest	Posttest	Pretest	Posttest
MAOD				
L	4.56 ± 0.68	4.71 ± 0.67	4.15 ± 0.84	4.36 ± 0.56
$(ml \cdot kg^{-1})$	70.5 ± 13.3	72.2 ± 13.5	62.9 ± 10.0	68.2 ± 7.4*
Velocity % of vVO_{2max}	124.5 ± 6.0	119.6 ± 5.5*[a]	118.6 ± 9.0	120.8 ± 9.1[a]
Velocity $(km \cdot h^{-1})$	12.6 ± 1.1	13.3 ± 1.1***	12.7 ± 1.2	13.2 ± 1.2***
Time to exhaustion (sec)	151 ± 24	153 ± 26	139 ± 21	140 ± 15
VO_{2peak}				
VO_2 $(L \cdot min^{-1})$	3.29 ± 0.20	3.24 ± 0.20	3.25 ± 0.37	3.17 ± 0.30
VO_2 $(ml \cdot kg^{-1} \cdot min^{-1})$	50.5 ± 4.6	49.8 ± 4.4	49.8 ± 4.6	49.0 ± 2.33
VO_2 $(ml \cdot kg^{-0.75} \cdot min^{-1})$	143.1 ± 10.6	141.8 ± 10.0	141.2 ± 11.9	139.1 ± 5.5
R	1.12 ± 0.07	1.14 ± 0.05	1.15 ± 0.05	1.12 ± 0.06
HR_{peak} (bpm)	190 ± 5	188 ± 5	184 ± 9	182 ± 10
VE	106.5 ± 10.2	111.5 ± 9 **	104.4 ± 14.8	108.1 ± 14.2
$[La^-]_b$ $(mmol \cdot L^{-1})$	11.37 ± 1.87	11.48 ± 2.87	11.42 ± 2.21	10.96 ± 1.37
% of VO_{2max} reached $(L \cdot min^{-1})$	96.6 ± 4.6	91.7 ± 5.2*	99.5 ± 10.1	97.2 ± 9.3[a]
% of HR_{max} reached	96.8 ± 1.3	96.7 ± 1.3	95.9 ± 2.0	94.3 ± 2.9

Data are presented as means ± SD. The MAOD-test were carried out running on treadmill at 5.3% inclination. MAOD, maximal accumulated oxygen deficit; VO_{2peak}, highest 10s peak oxygen uptake during MAOD; RER, respiratory exchange ratio; HR_{peak}, peak heart rate; V_E, pulmonary ventilation; $[La^-]_b$, blood lactate concentration; % of VO_{2max}, highest 10s VO_{2peak} from MAOD compared to highest 30 sec from VO_{2max}-test. * Significant differences ($P < 0.05$) within groups from pre- to posttraining; ** significant difference ($P < 0.01$) within groups from pre- to posttraining; *** significant difference within groups ($P < 0.001$) from pre- to posttraining; [a] significant difference between groups ($P < 0.05$) from pre-to post-training.

Discussion

The main finding of the present study is that 8 weeks of 4x4 HIIT performed at 90-95% of HR_{max} improved VO_{2max} significantly more than 10x30-second all-out SIT in trained females. The increased O_2 pulse and a lower HR at a submaximal velocity seen in the HIIT-group may indicate that the VO_{2max} improvements were due to enhanced SV. Further, SIT increased MAOD significantly (9.5%), while changes were not significantly different following HIIT. This may indicate that when running, a supramaximal intensity is required to enhance anaerobic capacity. Performance in 300m and 3000m was significantly improved in both groups with SIT improving 300m significantly more than HIIT.

VO_{2max}

The absolute VO_{2max} in $L \cdot min^{-1}$ increased significantly more following HIIT (3.6%) compared to SIT (-0.5%) (Fig. 3). The present VO_{2max}-improvement of 3.6% following 4x4 HIIT is the smallest reported following a 4x4-intervention. In comparison, it is only half of the improvements reported in moderately trained men (55.5 $ml \cdot kg^{-1} \cdot min^{-1}$) (Helgerud et al., 2007), and less than one third of the improvements reported young healthy subjects (50.5 $ml \cdot kg^{-1} \cdot min^{-1}$) (Wang et al., 2014). When comparing results, it is important to consider the subjects' baseline fitness. Earlier meta-analyses show there are likely a moderate greater additional increase in VO_{2max} following an intervention for subjects with a lower baseline fitness (Milanovic et al., 2015; Vollaard et al., 2017; Weston et al., 2014). As females 20-29 years generally have about 10 $ml \cdot kg^{-1} \cdot min^{-1}$ lower VO_{2max} compared to males at the same fitness relative to their sex (Fletcher et al., 2001; Loe et al., 2014), the endurance trained females (51.4 $ml \cdot kg^{-1} \cdot min^{-1}$) have an initial higher baseline VO_{2max} compared to the males reported in the above mentioned 4x4-protocols (Helgerud et al., 2007; Wang et al., 2014). Interestingly, a recent study investigating an identical protocol on endurance trained males (61.0 $ml \cdot kg^{-1} \cdot min^{-1}$) found that absolute VO_{2max} increased 4.1%, not far from the present 3.6% (Trane & Helgerud, 2018). Another study investigating the same protocol with only 5 endurance trained females (54.1 $ml \cdot kg^{-1} \cdot min^{-1}$) reported 7.9% improvement in absolute VO_{2max}, an improvement only seen in 2 of our 13 subjects despite slightly lower baseline (Balto & Helgerud, 2019; Hov & Helgerud, 2019). Therefore, the modest increase in present compared to earlier investigations can only partly be explained by the subjects' baseline fitness.

At intensities up to the level of maximum aerobic velocity, the intensity of training has been shown as a key determinant on the training response (Helgerud et al., 2007). Thus, the lower VO_{2max}-improvement seen in present study could be due to the training intensity. Hov and Helgerud (2019) reported a 94% avg HR_{max} 3min into each interval when reporting the 7.9% VO_{2max}-improvement in trained female students, an improvement only bypassed in 2 of our 13 subjects. Interestingly these 2 subjects had the highest average % of HR_{max} in training, both with an average HR of between 94-95 % measured 3.30min into each interval, compared to the subject average of 92.7% of HR_{max}. As HR were measured 30 seconds later compared to Hov and Helgerud (2019), these extra 30s most likely resulted in an even larger difference in avg % of HR_{max} per interval since HR increase with interval duration from a combination of a longer build-up of lactic acid and cardiovascular drift. Furthermore, despite present study using 90-95% of HR_{max} like earlier 4x4-interventions, it came to the author's awareness that 4x4-protocols conducted by researchers Helgerud and Wang adds 4 heart beats to the HR found in the incremental VO_{2max}-test when deciding HR_{max}. This has not been practised earlier by our research group (Balto & Helgerud, 2019; Hemmingsen & Helgerud, 2018; Hov & Helgerud, 2019; Trane & Helgerud, 2018; Yi & Helgerud, 2018) as HR_{peak} from the incremental VO_{2max}-test has been used as HR_{max}. Thus, if adding 4 heart beats to subjects HR_{max}, the avg % of HR_{max} per interval declines from 92.7% to 90.9%. While this is still within the desired 90-95% intensity, three subjects would have an avg % of HR_{max} per interval beneath 90%. Although no significant correlation was found between VO_{2max}-improvements and avg % of HR_{max} during intervals in present study, there are indications that intensity during 4x4-intervals were lower than optimal, and that an average intensity of 94-95% of HR_{max} (decided from an incremental VO_{2max}-test) could be optimal for improving VO_{2max} in already trained females.

Following 30s SIT, no change in VO_{2max} was observed (Fig. 3). This is somewhat surprising as all four meta-analyses conducted on SIT reported SIT to increase VO_{2max} by ~8% in less trained subjects (Gist et al., 2014; Sloth et al., 2013; Vollaard et al., 2017; Weston et al., 2014). However, the present results are in line with earlier 30s SIT interventions conducted on endurance trained male and female runners (55-63 $ml \cdot kg^{-1} \cdot min^{-1}$), showing no increase in VO_{2max} (Bangsbo et al., 2009; Iaia & Bangsbo, 2010; Skovgaard, Almquist, et al., 2018). As mentioned, meta-analyses conducted explicitly on SIT showing a moderate greater additional increase in VO_{2max} following an intervention for subjects with a lower baseline fitness (Vollaard et al., 2017; Weston et al., 2014). As the subjects in present study were already trained females who ran at least once a week or trained 3 times a week, with similarities to the endurance trained male and female runners (Bangsbo et al., 2009; Iaia & Bangsbo, 2010; Skovgaard, Almquist, et al., 2018; Trane & Helgerud, 2018), the initially high baseline VO_{2max} could be an explanation for the lack of VO_{2max}-improvements seen following SIT.

Although meta-analyses conclude that HIIT should emphasis on interventions of longer duration and longer repetitions above a certain intensity induce great effects on VO_{2max}, HIIT of shorter repetitions like 15-15- and 30-15 intervals have also shown great effects on VO_{2max} when work-matched to longer intervals (Helgerud et al., 2007; B. Rønnestad, Hansen, Nygaard, & Lundby, 2020; B. R. Rønnestad, Hansen, Vegge, Tønnessen, & Slettaløkken, 2015). Still, 30s with longer recovery, like SIT in present study were not work-matched, HR declined between bouts and resulted in an unchanged VO_{2max}. Thus, there are compelling evidence that 10x30-second SIT does not have the same effect on VO_{2max} as 4x4 HIIT.

Cardiovascular adaptations

The 3.6% VO_{2max}-increase in HIIT combined with a significant decrease in HR_{max} after HIIT were followed by a 4.1% increase in O_2 pulse, a non-invasive measure of SV (Table 3). This is in line with earlier studies showing significant improvements in O_2 pulse following 4x4 HIIT (Balto & Helgerud, 2019; Trane & Helgerud, 2018). As both HR_{max} and HR at $7 km \cdot h^{-1}$ decreased significantly following HIIT, while VO_{2max} increased, this argues that the improvements in VO_{2max} are most likely due to an enhanced SV_{max} and/or enhanced a-vO_2diff. Longitudinal studies have shown that the training-induced increase in $\dot{V}O_{2max}$ results primarily from an increase in SV_{max} rather than a-vO_2diff (Bassett & Howley, 2000). Further, time spent at an intensity associated with SV_{max} is reckoned as important for improving maximal cardiac function (Cooper, 1997; Lepretre, Koralsztein, & Billat, 2004; Stanley & Buchheit, 2014). As 4x4 HIIT are conducted at approximately this intensity and earlier has been shown to improve SV in both males and females (Helgerud et al., 2007; Slordahl et al., 2004; Wang et al., 2014), this gives a strong indication that the improvements in VO_{2max} were due to increased SV. Other underlying factors determining VO_{2max} such as blood volume and Hb have earlier been reported unchanged following 4x4 HIIT (Helgerud et al., 2007; Wang et al., 2014; Wisloff et al., 2007), Thus, these factors probably did not affect the VO_{2max}-improvement seen in present study.

As VO_{2max} and O_2 pulse remained unchanged (-0.5% and -0.1%) following SIT, we can assume that all variables in the Fick equation ($VO_{2max}/HR_{max} = SV_{max} \cdot$ a-vO_2diff) remained unaltered in the SIT-group. This matches the results of other 30s SIT studies investigating O_2 pulse or SV at maximal intensity (Alguindy & Rognmo, 2019; Bækkerud et al., 2016; Litleskare, 2011; Macpherson et al., 2011; Trane & Helgerud, 2018). Notably, while Balto and Helgerud (2019) are the only study reporting significant increase in O_2 pulse following SIT, they are also the only study reporting HIIT to improve O_2 pulse more than SIT, in line with the tendency shown from present intervention (P = 0.06). A weakness with the use of O_2 pulse is the inability to differentiate changes to SV_{max} and a-vO_2diff relationship when no measure of a-vO_2diff is included (Crisafulli et al., 2007). As the earlier improvements in VO_{2peak} following SIT has been speculated to come from enhanced oxidative capacity of peripheral muscles (Barnett et al., 2004; Burgomaster et al., 2008; Larsen et al., 2020; Macpherson et al., 2011), theoretically a right-shift towards lower SV_{max} and improved a-vO_2diff following SIT could have happened. If so, SIT could be a more effective short term way to enhance VO_{2max} in a sedentary and untrained population, as their VO_{2max} has been shown to be more demand-limited, compared to an active population, who are supply-limited (Richardson, Harms, Grassi, & Hepple, 2000; Wagner, 2000). However, when comparing 12 weeks of continuous walking, 4min HIIT and 30s SIT in overweight and obese subjects, HIIT but not SIT showed VO_{2max} to increase significantly more compared to walking (Lunt et al., 2014), arguing that 4min intervals of HIIT should be the recommended protocol for improving VO_{2max}.

VO_{2max} verification using the MAOD-protocol

As proposed by Poole and Jones (2017), the present study included a constant work rate at supramaximal intensity, the MAOD-protocol of Medbo et al. (1988), to verify the VO_{2max}-values obtained during the incremental VO_{2max}-protocol. 3 subjects from each group reached a higher 30s average VO_2-measurement in the MAOD-protocol, which were interpreted as their new VO_{2max}. Thus, from a practical perspective the MAOD-protocol was useful when adding to the existing incremental VO_{2max}-protocol used in present investigation.

The average 10s VO_{2peak} during the MAOD-protocol was significantly lower when compared to the average VO_{2max} reached during the incremental test, in line with Trane and Helgerud (2018). This can most likely be explained by a lower $[La^-]_b$ and HR especially seen at post

MAOD-test compared to the incremental protocol. Furthermore, contrary to earlier findings (Balto & Helgerud, 2019; Trane & Helgerud, 2018), measures of VO_{2peak} following HIIT measured in the MAOD-test did not increase from pre- to posttest (-1.5%) even though HIIT in the incremental protocol significantly increased in absolute VO_{2max} (3.6%). Thus, it seems the MAOD-protocol of Medbo et al. (1988) is not suited to replace the incremental VO_{2max}-protocol used in present study.

Running economy

RE expressed in absolute values ($L \cdot min^{-1}$) improved significantly in both HIIT and SIT by 4% and 3.7%, respectively, with no difference between groups (Table 3). However, as there were less subjects completing SIT and those subjects losing twice as much m_b compared to HIIT, this partly explains why HIIT but not SIT showed significant improvements when expressed as $ml \cdot kg^{-0.75} \cdot m^{-1}$ (3.7% vs 2.7%), in line with the results of Trane and Helgerud (2018). Training status is important when assessing changes in RE, as trained runners have better RE than untrained runners (Bransford & Howley, 1977; Conley & Krahenbuhl, 1980; Mayhew et al., 1979; Morgan et al., 1995; Saunders et al., 2004). Although the initial endurance training status was relatively high in the present study, the subjects could not be characterised as trained runners. Some were not accustomed to the relatively high amount of running carried out in the HIIT-protocol, and thus, the significant improvement in RE following HIIT was expected. When comparing findings with earlier 4x4 RE-investigations at 5.3-5.5% incline, the 3.7% ($ml \cdot kg^{-0.75} \cdot m^{-1}$) improvement in RE is in the lower part of the earlier shown improvements of between 3.5-10% (Helgerud et al., 2001; Helgerud et al., 2007; Helgerud et al., 2011; Trane & Helgerud, 2018). However, the increase in RE is slightly larger compared to the non-significant findings of Balto and Helgerud (2019) on female students. This makes sense as their subjects had slightly better training status. Notably, comparison of values across studies should be interpreted with caution since methodological differences likely exists (e.g. actual velocity, actual inclination, and softness of the treadmill belt).

Although RE following SIT were only significant in absolute values ($L \cdot min^{-1}$), the non-significant 2.7% improvement expressed as O_2 cost per metre ($ml \cdot kg^{-0.75} \cdot m^{-1}$) are in accordance with the 2-3% improvements found in trained runners (Bangsbo et al., 2009; K. Barnes et al., 2013; Skovgaard, Almquist, et al., 2018; Skovgaard, Christiansen, et al., 2018).

This RE-improvement after SIT could like HIIT come from an increase in training volume of running from what the subjects were used to before the study, as subjects ran with a low volume in SIT but including a 10-minute warmup and cooldown three times a week. However, earlier SIT-investigations have found improved RE despite decreasing training volume in runners (Bangsbo et al., 2009; K. Barnes et al., 2013; Iaia et al., 2009; Skovgaard, Almquist, et al., 2018; Skovgaard, Christiansen, et al., 2018). As the factors determining RE were not measured, reasons for the RE-improvement in present investigation can only be speculated. An earlier HIIT vs SIT investigation reported that SIT improved biomechanical measures including stride rate and muscle power measures including muscle stiffness and rate of force development more than long-HIIT in runners (K. Barnes et al., 2013). This indicates that HIIT and SIT could induce different adaptations to the factors affecting RE. Regardless, HIIT but not 30s SIT improved RE expressed as O_2 cost per metre ($ml \cdot kg^{-0.75} \cdot m^{-1}$), as also shown in Trane and Helgerud (2018). This argues that 4x4 HIIT seems to be a preferred protocol for improving RE in trained males and females, at least for those who are not running at a regular basis.

Lactate threshold

The present study is one of few studies examining the effects of SIT on LT expressed as % of VO_{2max}. In present investigation using a graded protocol where LT was reached when 1.5 $mmol \cdot L^{-1}$ $[la^-]_b$ higher than warmup-$[la^-]_b$, there was no changes within or between groups in the measures of LT. This is expected as to the authors knowledge no previous HIIT or SIT intervention have reported a change in LT expressed as % of VO_{2max}. Noteworthy, the Biosen C-line lactate analyser used in this study analysed hemolysed instead of non-hemolysed blood. As hemolysed blood samples have shown a significant increase in lactate dehydrogenase due to the breakdown of red blood cells (Medbo, Mamen, Holt Olsen, & Evertsen, 2000; Rako, Mlinaric, Dozelencic, Juros, & Rogic, 2018; Yücel & Dalva, 2019), LT in the present investigation should have been defined as warmup $[la^-]_b$ + 2.3 $mmol \cdot L^{-1}$ (Helgerud et al., 1990; Storen et al., 2014; Sunde et al., 2010). Theoretically, defining LT as warmup + 2.3 $mmol \cdot L^{-1}$ instead of 1.5 $mmol \cdot L^{-1}$ would have given slightly higher values at both pre- and posttest in VO_2, % of VO_{2max}, % of HR_{max}, vLT and 0.8 higher $[la^-]_b$ in both groups. Still, results are presented because this is the final continuation of a larger study, which all have used an identical protocol including the Biosen C-line lactate analyser with 1.5

mmol·L^{-1} [la^{-}]$_b$ for defining LT (Balto & Helgerud, 2019; Hemmingsen & Helgerud, 2018; Hov & Helgerud, 2019; Trane & Helgerud, 2018; Yi & Helgerud, 2018).

vLT has been reported to be almost identical with the sum of improvements in the determining factors for endurance performance (Farrell et al., 1993; Hagberg et al., 1978; Helgerud et al., 2001; Storen et al., 2014). Thus, significant improvements in vLT were only found in HIIT (7.6%) and tended to improve more in HIIT compared to SIT ($P = 0.06$). This can be explained by the increase in VO_{2max} only seen following HIIT, and the fact that only HIIT improved RE when expression included m_b. The 7.6% increase in vLT following 4x4 HIIT is in line with earlier investigations (Balto & Helgerud, 2019; Helgerud et al., 2001; Helgerud et al., 2007; Trane & Helgerud, 2018). However, the non-significant vLT-increase of 2.8% following SIT differ from earlier 30s SIT investigations, who found 4.2-7.4% improvement in vLT in endurance trained males and females; (Balto & Helgerud, 2019; Esfarjani & Laursen, 2007; Trane & Helgerud, 2018), although the 4.7% improvement found in Esfarjani and Laursen (2007) were not significant ($P = 0.07$). In summary, the significant findings of vLT found only in HIIT indicates that HIIT had a larger sum of improvements in the determining factors for aerobic endurance performance compared to SIT.

Anaerobic capacity

The present study demonstrated that MAOD expressed as ml·kg^{-1} was significantly increased by 9.5% following 8 weeks of 30s SIT, while remaining unchanged after 4x4 HIIT. This is in line with the 10% increase in anaerobic capacity following 6 weeks of SIT (Medbo & Burgers, 1990). The result of the present study indicates that when running, a supramaximal intensity is needed to change the anaerobic capacity in trained subjects. To date, only two studies have compared the effects of HIIT and SIT on anaerobic capacity, both conducting 4x4 HIIT vs 10x30s SIT on trained males and females, with equivocal findings. While Trane and Helgerud (2018) found HIIT and SIT to have no effect on anaerobic capacity in males, Balto and Helgerud (2019) found a remarkable 32% MAOD-increase in females following SIT, significantly higher than the unchanged MAOD in HIIT. This is a surprisingly high MAOD-increase, as no female in present study increased to this extent despite running the exact same protocol and MAOD-calculation method. Noteworthy, Tabata et al. (1996) demonstrated a 28% increase in MAOD following 6 weeks of 8x20s SIT with 10s recovery in moderately trained male subjects (53 ml·kg^{-1}·min^{-1}). The study of Tabata et al. (1996)

therefore suggests that a 32% increase could be plausible following an 8-week intervention. Further, it supports earlier research stating that anaerobic MAOD-improvements may not be sex-dependent (Medbo & Burgers, 1990). The mechanisms underlying the improvement in anaerobic capacity are complex and beyond the scope of this study.

Following the 8-week training intervention vVO_{2max} increased significantly more in HIIT compared to SIT. As the intensity at MAOD-test were within the range of 120% ± 10 of vVO_{2max}, HIIT were expected to run either faster or longer at the MAOD-test. Interestingly, no significant differences between groups were seen as both HIIT and SIT improved their velocity at MAOD by 5.7% and 3.7%, respectively. As anaerobic energy contribution in women is about 30% in a 2-3 minute all-out effort (Duffield, Dawson, & Goodman, 2005a), this improvement can, at least partly, be explained by the improved anaerobic capacity seen only following SIT. As the trained exercise physiologist decide the set velocity on each individual's MAOD-test, the factor velocity can easily be manipulated. However, if incorrectly manipulated this would be recognised by a changed time to exhaustion, and/or changed $[La^-]_b$ and HR_{peak} from pre-to posttest. In present investigation time to exhaustion were identical in both groups from pre- to posttest with neither group changed their $[La^-]_b$ and HR_{peak}. Arguably, the MAOD-test in present investigation can serve as a kind of TTE-test which reflects velocity as performance improvements at 2-3 minutes, while simultaneously measuring energy distribution from aerobic and anaerobic energy systems.

Only a handful studies have measured % of vVO_{2max} and VO_2 at an intended fixed duration with "all-out" intensity before and after an intervention. To the authors knowledge, this is the second study showing a significant between-group difference in velocity as % of vVO_{2max} reached during an all-out TTE-test following a HIIT and SIT-intervention. In present study HIIT reached a 4.9% lower velocity expressed as % of vVO_{2nax} at post-MAOD test, while SIT, although not significant, increased 2.2%. The 4.9% lower % of vVO_{2max} reached following HIIT is in line with Hemmingsen and Helgerud (2018) also reporting a non-significant 3.8% lower velocity as % of vVO_{2max} following 4x4 HIIT. Interestingly, the other study reporting significant between-group difference reported a significantly increased velocity as % of vVO_{2max} in SIT compared to HIIT (Balto & Helgerud, 2019).

The reduced velocity as % of vVO_{2max} were accompanied by a lower % of VO_{2max} reached from pre- to post- MAOD-test in HIIT (-4.9%), while remaining unchanged after SIT (-2.6%).

This is somewhat in line with earlier investigations, as Balto and Helgerud (2019) reported the difference in % of VO_{2max} reached to be twice as large between HIIT and SIT as present study. They however found a 3.7% increase following SIT, while HIIT remained unchanged (-1.5%). Unfortunately, % of VO_{2max} were not reported following 4x4 HIIT and 30s SIT in 19 endurance trained males (Trane & Helgerud, 2018). Hence, further studies are required to investigate whether the reported HIIT vs SIT- difference in % of VO_{2max} and velocity as % of vVO_{2max} reached after 2-3 minutes can be seen in males and at longer distances.

Endurance running performance

Among the factors predicting endurance running performance, VO_{2max}, vVO_{2max}, and vLT has been considered the most important (Grant et al., 1997; Slattery et al., 2006; Yoshida et al., 1993). In present study VO_{2max} and vVO_{2max} improved significantly more, and vLT tended (P=0.06) to improve more in HIIT compared to SIT. Although anaerobic capacity only improved following SIT, 85-90% of the energy requirements following a 3000m are reported to come from the aerobic energy system (Billat, 2001; Duffield et al., 2005b). Hence, the effect of an increased energy contribution from an improved anaerobic capacity to a 3000m are minor compared to the contribution from the aerobic energy system. Therefore, the author argues that in present study the sum of determinants predicting 3000m performance overall increased more following HIIT compared to SIT. Nevertheless, both SIT (4%) and HIIT (4.5%) improved 3000m performance, with no significant difference between groups (Table 3, Fig. 5). While Trane and Helgerud (2018) found significant between-group difference in 3000m improvement following HIIT or SIT, present study adds to the range of studies showing no difference in 3000m-improvement between the two types of intervals (Balto & Helgerud, 2019; Cicioni-Kolsky et al., 2013; Esfarjani & Laursen, 2007).

The 4.5% improvement in 3000m performance following HIIT can be explained by significant increases in VO_{2max} and RE, which resulted in improved vVO_{2max} and vLT. However, the 4.5% improvement is less than the 5.5-7.4% previously reported following 4 minute HIIT-protocols in physical active to endurance trained subjects (51.6 - 61 $ml \cdot kg^{-1} \cdot min^{-1}$) (Balto & Helgerud, 2019; Cicioni-Kolsky et al., 2013; Esfarjani & Laursen, 2007; Trane & Helgerud, 2018). Naturally, this can be explained by an even larger increase in VO_{2max} and RE seen in all the aforementioned studies reporting these determinants.

The remarkable 4% improvement in 3000m performance following SIT is actually in agreement with previous 30s SIT-research demonstrating 3000m improvements in the range of 2.3-8.8% in groups of physical active - trained runners as subjects (47.9 - 63 $ml \cdot kg^{-1} \cdot min^{-1}$) (Balto & Helgerud, 2019; Bangsbo et al., 2009; Cicioni-Kolsky et al., 2013; Esfarjani & Laursen, 2007; Koral et al., 2017; Trane & Helgerud, 2018). However, unlike present investigation, most endurance-running TT-improvements following SIT-interventions are reported to come from an improved VO_{2max} (Balto & Helgerud, 2019; Denham et al., 2015; Esfarjani & Laursen, 2007; Koral et al., 2017; Macpherson et al., 2011; McKie et al., 2018; Skovgaard, Almquist, et al., 2018; Willoughby et al., 2016). To explain what caused the 4% improvement in present investigation is somewhat challenging as other studies reporting TT-performance improvements without an improved VO_{2max}, only reported a 2-3.2% improvement (Bangsbo et al., 2009; K. Barnes et al., 2013; Skovgaard, Christiansen, et al., 2018; Trane & Helgerud, 2018). At least part of the improvement can be explained by a 9.5% and 3.7% increase in anaerobic capacity and RE expressed as $L \cdot min^{-1}$.

Some studies indicate that the best runners have the ability to utilize 100% of their VO_{2max} (Davies & Thompson, 1979; Lacour et al., 1990). However, Støa, Støren, Enoksen, and Ingjer (2010) found no correlation between percent utilisation of VO_{2max} and 5-km performance in eight well-trained runners. According to Bassett and Howley (2000) and Joyner and Coyle (2008), fractional utilization is still an important factor influencing running performance irrespective of distance and time spent in competition. In opposition to this, it is argued that fractional utilization is merely a consequence of time spent in competitions up to 5km (Coetzer et al., 1993; Davies & Thompson, 1979; Støa et al., 2010). In a group of runners, those with the best aerobic determinants for endurance performance will complete a given middle/long-distance at a shorter time. Consequently, they will be able to run at the higher individual intensity and thus show a higher average fractional utilization of VO_{2max} compared to the slower runners. When conducting a TTE-test like the MAOD-test in present study, the runners complete a given distance in approximately the same time. Consequently, these runners should normally show approximately the same fractional utilization of VO_{2max}, as shown in Støa et al. (2010). In present study, although the time to exhaustion were identical from pre- to posttest, velocity expressed as % of vVO_{2max} were significantly different between groups, and % of VO_{2max} declined significantly only following HIIT. Hypothetically, if the trend of a higher % of VO_{2max} reached during SIT after 2-3 minutes also could be seen in a

3000m performance, this could partly explain why SIT improved by 4% without a significant improvement in VO_{2max} and RE expressed as $ml \cdot kg^{-0.75} \cdot m^{-1}$.

A possibly increased maximal sprint speed (MSS) in the SIT-group, indicated by improved anaerobic capacity, 300m performance and earlier SIT-findings (Hazell et al., 2014; McKie et al., 2018; Willoughby et al., 2016), could have influenced the 3000m performance, as scientists are arguing that maximal sprint speed (MSS) affects middle-distance running performance (Bachero-Mena et al., 2017; Bundle et al., 2003; Sandford et al., 2019; G. N. Sandford & T. Stellingwerff, 2019). Although 3000m is not within the range of middle distance (800-1500m) (G. Sandford & T. Stellingwerff, 2019), it is close to, and therefore plausible that a higher MSS following SIT could have benefited the improvement in 3000m performance.

Another factor possibly influencing the 3000m performance were the lack of familiarization with a running 3000m TT-protocol. The validity and reliability of this kind of time trial test have been established by Denham et al. (2015) with an intraclass correlation coefficient = 0.99 and a 3.4% coefficient of variation. Allowing subjects to get familiarized with a performance test can improve the reliability, as an pacing strategy can be challenging in a 3000m (D. N. Borg et al., 2018; Laursen, Francis, Abbiss, Newton, & Nosaka, 2007; Laursen, Shing, & Jenkins, 2003), Noteworthy, although all subjects received the exact same instructions, one subject from the SIT-group misunderstood the concept of a 3000m TT and self-reported during the training intervention that her pre-test result was conducted with a submaximal effort. Consequently, she improved 3000m TT by 8% although her VO_{2max} decreased by 3 $ml \cdot kg^{-1} \cdot min^{-1}$. If excluding her from the data material, SIT would have had a 3.5% improvement, still not significantly different from HIIT.

Sprint performance

Both HIIT and SIT improved 300m performance by 2.2% and 6.7%, respectively. This is the first study showing that SIT improved 300m running performance significantly more than HIIT. Anaerobic capacity is generally considered one of the most important determinants of anaerobic performance. Notably, in a running event lasting 50-60 seconds, approximately 60% of the energy required has been reported to come from anaerobic energy systems (Duffield et al., 2005a; Hill, 1999). Thus, a substantial amount of the required energy for a

300m comes from aerobic sources. As HIIT-interventions earlier has failed in showing improvement in single sprint performance (Ferrari Bravo et al., 2008; Helgerud et al., 2001; McMillan et al., 2005), and anaerobic capacity were unaltered, the significant performance changes may be attributed to an increased aerobic capacity improving the 40% aerobic energy contribution. The results are in line with earlier 300m performance improvement seen in endurance trained males following 4x4 HIIT (Trane & Helgerud, 2018). However, it is somewhat less than the 5.1% improvement seen earlier in trained females following 4x4 HIIT, which can be explained by their larger improvement in aerobic determinants compared to present study (Balto & Helgerud, 2019).

The significantly larger improvement in 300-meter running performance seen following 30s SIT can mainly be explained by an improved anaerobic capacity, as no improvements in VO_{2max} were seen. Another factor that might have influenced is improved RE at 300m time-trial pace due to 300m specificity during SIT. As RE can only be measured reliable at intensities beneath lactate threshold, it does not necessarily reflect RE-improvements at supramaximal velocities (Daniels & Daniels, 1992; Sandford et al., 2019). Almasbakk et al. (2001) reported that a novice skill learner intuitively searches for a technique that requires minimal energy expenditure and/or increased mechanical output, with a novice skill learner improving faster than an already skilled learner. Also, training adaptations are specific to the stimulus applied, involving movement patterns and force-velocity characteristics (Haugen, Seiler, Sandbakk, & Tønnessen, 2019). In present investigation, subjects were not used to sprinting and SIT were conducted at an average velocity of 20.1 $km \cdot h^{-1}$, 2 $km \cdot h^{-1}$ above the average 18.1 $km \cdot h^{-1}$ during their 300m pretest. Based on these considerations, it is reasonable to assume that their specificity during training enhanced their 300m performance.

The 6.7% improved 300m performance following 30s SIT in the present study is in line with the results seen in trained females, (Balto & Helgerud, 2019), and somewhat higher than the 3.3% increase seen in endurance trained males (Trane & Helgerud, 2018). Noteworthy, the similar improvement in 300m performance seen in Balto and Helgerud (2019) were explained by a 32% and 3.5% improvement in anaerobic capacity and VO_{2max}, while only a 9.5% increase in anaerobic capacity were seen in present investigation. However, their subjects were also 5 seconds faster at baseline (54 vs 59 seconds), which could explain why a similar improvement in 300m performance were seen.

Perceived exhaustion

In present study, 10x30-second SIT (17.2 ± 1.7) showed higher RPE than 4x4 HIIT (14.8 ± 0.3) ($P \leq 0.001$), in accordance with Follador et al. (2018). The significant difference between protocols are presumably a result of the supramaximal intensity with accompanying lactic acid build-up following SIT. 10x30-second SIT could not be completed at a constant pace as the 3.5min recovery was not sufficient to both remove the lactic acid build-up and refill PCr for the following sprint (Table 1)(Gaitanos et al., 1993; Glaister, 2005; Harris et al., 1976; Sahlin & Ren, 1989). In contrast, 4x4 with 3min active recovery has been reported to remove the lactic acid build-up between intervals (Storen et al., 2017), leading to a continuous/slightly increasing pace throughout the intervals. Although RPE has been examined earlier in 4x(4 or 5)-protocols, most of these studies have used "maximal sustainable intensity" resulting in a 17.6-18.5 RPE-score (Ronnestad & Mujika, 2014; B. Rønnestad et al., 2020; Seiler et al., 2013) far from the 14.8 RPE-score in this investigation. This illustrates just how different a protocol including a maximal sustainable intensity is compared to an intensity of 90-95% of HR_{max}. Although the Borg scale has been reported to be highly reproducible both after 1 week and after 40 week (Wilson & Jones, 1991), RPE are subjective phenomena, and earlier experience enduring exhaustion and difficulties understanding the scale affects the validity and reliability of the results (G. Borg, 1998). The inaccuracy of this method was reflected by one individual's RPE-score of 14.2 following SIT. Her score, far from the SIT-average of 17.2, were most likely due to difficulties in understanding the requests to respond according to the Borg RPE which has been reported to happen for 5-10% of the population (G. Borg, 1998).

Training considerations

The goal for physicians is to prescribe an optimal training regimen for improving fitness while limiting the development of fatigue or risk of injury in the general community (Rosenblat et al., 2020). A meta-analysis stated that SIT is well tolerated with only few (non-serious) adverse events and low dropout rates reported (Sloth et al., 2013). However, of the 21 SIT-studies included in the meta-analysis, 20 were conducted with a cycling modality. Still, running has been recommended as an effective alternative to cycling when performing SIT in healthy individuals (Kavaliauskas, Jakeman, & Babraj, 2018). The non-weight-bearing nature of cycling, coupled with minimal eccentric contraction of leg muscles compared to running, mitigates the risk of injury and discomfort. Gist et al. (2014) therefore requested studies to investigate whether SIT in various modalities is safe and can be sustained for longer periods. In present study, conducted on motorised treadmill at 5.5% incline, a total 13 of 17 subjects allocated to SIT reported adverse events like light-headedness, nausea, vomiting, shin splints, Achilles tendon inflammation, hamstring issues and/or groin issues during the intervention. Of these, 6 dropped out due to injury related to the study and 1 due to the intense nature of the protocol as she experienced nausea and/or vomiting post-workout. Subjects who developed shin splints or hamstring issues were allowed to sprint at 8% inclination as this is reckoned as less strenuous. Injuries and/or adverse effects have also been reported in other running SIT studies (Balto & Helgerud, 2019; Hazell et al., 2014; Lunt et al., 2014; Rowley et al., 2017; Skovgaard, Almquist, & Bangsbo, 2017; W. J. Tucker, Angadi, & Gaesser, 2016; Willoughby et al., 2016). Of them, only the elderly group in Willoughby et al. (2016) showed similar rates of injuries following SIT as in present study. In comparison, Bækkerud et al. (2016) is the only 4x4-study reporting an exercise related injury, hip pain in an overweight and obese subject. However, Lunt et al. (2014) had 3 injuries following only 3 reps of SIT in overweight and obese subjects with no injuries following 4min HIIT, in line with present and other studies showing no adverse effects following a 4x4 HIIT-intervention (Balto & Helgerud, 2019; Cicioni-Kolsky et al., 2013; Helgerud et al., 2001; Helgerud et al., 2007; Lunt et al., 2014; Naves et al., 2018; Storen et al., 2017; Tjønna et al., 2013; Trane & Helgerud, 2018; Wang et al., 2014; Wisloff et al., 2007). As previous running experience and weekly physical activity involvement are inversely related to injury risk (Schneider, Seither, Tönges, & Schmitt, 2006; Taunton et al.), and injuries still were seen in trained students and runners conducting a 30s SIT-intervention (Balto & Helgerud, 2019; Skovgaard et al., 2017) along with the present findings, shows that running SIT induce a high risk of injuries.

VO_{2max} is an independent predictor of both all-cause and cardiovascular-specific mortality (Keteyian et al., 2008). As only HIIT improved VO_{2max}, the author recommends 4x4 HIIT when the goal is to improve health. Clearly SIT is poorly tolerated with side events, risk of injuries, a high drop-out rate and a higher RPE. Gillen et al. (2016) clearly state that the use of SIT requires a very high level of motivation and that it is definitely not suited for everyone. Therefore, caution is needed before such training is advocated to the general community. Notably, the author experienced that girls in 45-60 ml/kg/min had no problem comprehending to the three 4x4 HIIT sessions a week. No adverse effects were reported throughout the intervention, most likely because the intensity was not accumulating lactic acid throughout the session and treadmill inclination limited the strenuous load. As the fundamental role of sports coaches is to enhance athleticism and decrease the risk of sports injuries, HIIT and not SIT should be interpreted when the goal is to improve endurance performance. However, at shorter distances performance is dependent on an increasing contribution from anaerobic capacity (Brandon, 1995). As HIIT did not improve anaerobic capacity and improved less than SIT at 300m, SIT can be effective when used as a supplementation for highly motivated athletes who compete in sports with high anaerobic demands.

Limitations

The author is well aware that O_2 pulse is not an optimal method of measuring SV. Hence, future investigations of cardiovascular adaptations to SIT and HIIT should aim towards more accurate methods (e.g. the single-breath acetylene uptake method) to evaluate changes in SV and CO. In addition, the present study did not include haematological or muscular biopsy measurements. If included, it would have strengthened the study as one could investigate what may be the reasons of changes in aerobic and anaerobic capacity e.g. blood volume, Hb concentration, aerobic and anaerobic enzyme activity. Another limitation is the determination method of LT as one should use warmup $[la^-]_b$ + 2.3 when analysing hemolyzed blood, and not +1.5 which used in current study (Helgerud et al., 1990; Storen et al., 2014; Sunde et al., 2010). However, as the same method were used on both groups at pre- and posttest, arguably it still gives valuable information about the adaptations following HIIT and SIT. Furthermore, most subjects were unfamiliar with the performance tests despite their well-developed fitness level. Even though time trials in general are more reliable than time to exhaustion tests (Laursen et al., 2007), a single familiarization session would probably improve the reliability of the performance tests. Also, participants' training background and dietary intake were not controlled, but the randomization process should limit these factors' influence on current findings. Finally, the simplified Y-intercept of 5 $ml \cdot kg^{-1} \cdot min^{-1}$ at rest and few submaximal measures used in the present study have been reported to increase the standard deviation and thus increase the likelihood of errors in the MAOD results. If including an additional lab-test to retrieve stable VO_2 values at 10 different submaximal velocities, the MAOD calculations would be more accurate.

Conclusion

The present study has revealed that trained females performing 4x4-minute intervals at high intensity (i.e., 90-95% HR_{max}) increased VO_{2max} by 3.6% and significantly more than the SIT-group performing 10x30-second intervals at supramaximal intensity (-0.5%). The increased O_2 pulse and a lower HR at a submaximal velocity seen in the HIIT-group suggest that the VO_{2max} improvements were due to enhanced SV. Further, vVO_{2max} improved more, and vLT tended to improve more in HIIT compared to SIT. Still, there were no difference in 3000m performance between groups. As the % utilisation of VO_{2max} declined significantly only in the HIIT group during the 2-3-minute MAOD-test, future investigation needs to address if this could have affected their 3000m performance. Furthermore, 300m performance improved more in SIT compared to HIIT. The 300m and 3000m- performance improvements seen in both groups were due to improved VO_{2max} and RE following HIIT and by improved anaerobic capacity and RE following SIT.

An optimal training regime should improve fitness while limiting the development of fatigue or risk of injury. Present study reports a high injury rate and high RPE in trained females conducting a running SIT-protocol. Implementing a 4x4-minute HIIT in a training program is clearly safer in terms of training-related injuries and thus easier to commit in the long run. Based on present findings, supramaximal intensity seems to be required for improving anaerobic capacity in trained subjects and can be implemented with careful consideration if the goal is to improve anaerobic capacity and sprint performance. However, if the goal is to improve health, aerobic training adaptations, and/or endurance running performance, interval training with longer aerobic intervals like 4x4-minute training administered in this experiment is recommended.

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