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The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors: testing procedures and training interventions
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The Effect of Training Intensity, Age and Diet on Aerobic Capacity and Metabolic Risk Factors

Testing Procedures and Training Interventions

a PhD dissertation in Ecology
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Støa: The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors; testing procedures and training interventions.
Abstract

**Background:** Low cardiorespiratory fitness (CRF) with accompanying metabolic risk factors such as overweight/obesity, reduced blood glucose control, elevated blood pressure, and dyslipidemia, is one of the most important predictors of all-cause mortality. Maximal oxygen uptake (VO\textsubscript{2max}) is considered the single best indicator of CRF. VO\textsubscript{2max} gradually decreases with increasing age, partly because of a reduced activity level, with concurrent increase of body weight (BW), reduced ability of fat oxidation (FatOx) and increased prevalence of T2D. High-intensity aerobic interval training (HAIT) has been found to effectively increase VO\textsubscript{2max}. HAIT may thus have the potential to counteract both age- and inactivity related declines in VO\textsubscript{2max} and concomitant metabolic risk factors. Still, previous studies indicate blunted VO\textsubscript{2max} adaptations among older compared to younger individuals. However, no previous studies have systematically compared the VO\textsubscript{2max} responses after HAIT in different age groups. T2D most often occurs above the age of 30 and is associated with metabolic disturbances in both glucose- and FatOx. T2D is also associated with elevated blood pressure (BP), overweight and impaired blood lipid profile (BLP). Aerobic exercise has been associated with improvements in all these metabolic risk factors, but few studies have investigated the effects of HAIT compared to work matched moderate-intensity continuous training (MIT) on these variables among T2D. When assessing FatOx adaptations to exercise, it is crucial to have a strict diet control since changes in diet will interfere with the FatOx results. Test – retest reliability should also be considered when interpreting FatOx measurements, since day to day variations in FatOx will occur also during equal diet conditions.

**Objectives:** To investigate a) the effects of HAIT on metabolic risk factors among individuals diagnosed with T2D (paper I), b) the effects of age on VO\textsubscript{2max} adaptations (paper II),- and c) to evaluate the impact of diet and testing reliability on FatOx during exercise (paper III).
Methods: Paper I and II are both training interventions, investigating VO$_{2\text{max}}$ adaptations using HAIT; 4 x 4 minutes at an intensity between 85-95% of maximum heart rate (HR$_{\text{max}}$). The main outcome in paper II was to explore if there are differences in VO$_{2\text{max}}$ response between six decade-cohorts from 20-70+ years with a training status typical for their age group. In paper I, the main outcome was to explore VO$_{2\text{max}}$- and glycosylated hemoglobin level (HbA1c) adaptations to HAIT. Responses in lactate threshold (LT), FatOx, BP, body composition and (BLP) was also investigated, and the results were compared to adaptations after work matched MIT (70-75% HR$_{\text{max}}$). 32 and 94 individuals participated in paper I and paper II, respectively, and all exercise sessions were supervised. In paper III, day to day variations in FatOx during equal diet conditions, as well as the influence of either a high-fat diet or high-carbohydrate diet on FatOx during moderate-intensity exercise (60% VO$_{2\text{max}}$) was examined among 9 healthy female adults. Due to the diets influence on FatOx, thorough diet registrations were performed also in paper I. In all papers, an incremental protocol was used to measure VO$_{2\text{max}}$, with the ergo spirometry metabolic test system Sensor Medics Vmax Spectra (Sensor Medics 229, Yorba Linda, Calif., USA). In paper I and III indirect calorimetry based on respiratory exchange ratio was used to estimate FatOx during exercise at 60% VO$_{2\text{max}}$. Three 5-min submaximal work periods with increasing watt or speed, together with the VO$_{2\text{max}}$ measurement represents a linear regression which was used to calculate each persons workload at 60% VO$_{2\text{max}}$.

Results and conclusions: In paper I, 12 weeks of HAIT led to a 21% (ml·kg$^{-1}$·min$^{-1}$) - and 19% (L·min$^{-1}$) increase in VO$_{2\text{max}}$, while no change in VO$_{2\text{max}}$ was found in MIT. Velocity at LT improved in both groups. LT expressed as %VO$_{2\text{max}}$ did not change in either of the groups. A significant improvement was found in HbA1c in HAIT compared to MIT, with a 0.58% points reduction in HbA1c, while no change was found in MIT. A significant correlation was found between change in VO$_{2\text{max}}$ and change in HbA1c ($r=-0.52$, $p<0.01$). There was a tendency towards an improved FatOx at 60% VO$_{2\text{max}}$ in HAIT ($p=0.065$) with a significant difference in change between HAIT and MIT. No change in FatOx was found in MIT. HAIT reduced BW, BMI, %BF, waist circumference and hip circumference. In MIT,
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...there were no changes in BW or BMI, but improvements were discovered in waist circumference, hip circumference and %BF. Only the changes in BW and BMI were significant different between HAIT and MIT, with the greatest improvement in HAIT. HAIT reduced diastolic BP but not systolic BP. No changes were found in any of the BLP measurements in HAIT. In MIT, improvements were found in both systolic BP and diastolic BP, as well as reduced triglycerides and increased HDL. The changes in BP and BLP were not significantly different between the groups. Paper I thus showed HAIT to be an effective strategy to reduce important risk factors associated with T2D, -and a more effective one than MIT in improving VO\textsubscript{2max} and lowering HbA1c.

In paper II, we showed that 8 weeks of HAIT increased VO\textsubscript{2max} with 9-13% with no differences between the six age groups. The percentage improvements were inversely related to baseline training status ($r=0.66$, $p<0.001$). HR\textsubscript{max} was not changed within the respective age cohorts, but the two oldest cohorts exhibited a tendency to increase HR\textsubscript{max} in contrast to a training-induced decrease in the younger cohorts ($p=0.07$). This paper revealed that for people with an aerobic capacity typical for what is observed in the population, the VO\textsubscript{2max} adaptations are likely not affected by age in a short-term training intervention, but may rather be affected by their baseline training status. These results indicate HAIT to be an excellent training strategy to counteract the decrease in VO\textsubscript{2max} associated with aging.

In paper III, the reliability test revealed no differences in FatOx, respiratory exchange ratio (RER), oxygen uptake, carbon dioxide production, heart rate, blood lactate concentration, or blood glucose between 2 habitual diet days with equivalent kilo calories (KCAL) intake and macronutrition composition. However, FatOx was reduced by 31% after the CHO-rich diet compared with the fat-rich diet. FatOx was also decreased after the CHO-rich diet compared with the habitual day 2. No difference was found in FatOx between fat-rich diet and the 2 habitual diet days. The results from this study show the use of RER data to assess FatOx during moderate exercise to be a reliable method as long as the diet is strictly controlled. The importance of diet control is
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emphasized through the finding in this study of a huge change in FatOx after only one day change in macronutrient composition.
List of papers

The dissertation is based on the following papers and will be referred to in the text by their roman numbers.


Støa: The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors; testing procedures and training interventions.
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Abbreviations

ACSM  American College of Sports Science
ADA  American Diabetes Association
AHA  American Heart Association
AMI  Acute myocardial infarction
ANOVA  Analysis of Variance
BF  Body fat
BG  Blood glucose
BMI  Body mass index
BLP  Blood lipid profile
BP  Blood pressure
BW  Body weight
CHO  Carbohydrate
CV  Coefficient of variance
CVD  Cardio vascular disease
CRF  Cardio respiratory fitness
EE  Energy expenditure
FatOx  Fat oxidation
FatOxmax  Maximal fat oxidation
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GLUT4  Insulin-mediated glucose transporter type 4
GI     Glycemic index
HAIT   High-intensity aerobic interval training
HbA1c  Glycosylated hemoglobin
HIIT   High-intensity interval training
HR     Heart rate
HR\text{max}  Maximal heart rate
HR\text{peak}  Peak heart rate
KCAL   Kilo calories
KJ     Kilo joules
[La\text{]}_b  Blood lactate concentration
LBM    Lean body mass
LT     Lactate threshold
MIT    Moderate-intensity continuous training
mRNA   Messenger ribonucleic acid
PA     Physical activity
POX    Protein oxidation
RER    Respiratory exchange ratio
SCD    Sudden cardiac death
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**SEE**  Standard error of estimate

**T2D**  Type 2 diabetes

**TEI**  Total energy intake

**VCO2**  Volume of carbon dioxide

**VO2**  Oxygen uptake

**VO2max**  Maximal oxygen uptake

**WE**  Work economy
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1. Introduction

1.1. The importance of cardiorespiratory fitness on metabolic risk factors

A sedentary life style is increasing worldwide with detrimental consequences on the prevalence of non-communicable diseases such as cancer, cardiovascular disease and diabetes (Warburton et al 2006, WHO 2010). Metabolic risk factors related to these diseases include overweight and obesity, reduced blood glucose control, elevated blood pressure, and dyslipidemia (Twisk et al 1997, Shen et al 2003, Castillo-Garzón et al 2006). These factors increase the risk of developing cardiovascular disease (CVD) and type 2 diabetes (T2D) (Kadota et al 2007). Even though any single one of the metabolic risk factors alone may not be clinically harmful or lethal, when such risk factors are clustered, the potential for serious disease increases exponentially (Roberts et al 2013). The physiological benefits of a physical active lifestyle are well documented (Blair and Morris 2009, Bacon et al 2013), and regular physical activity (PA) protects against coronary heart disease, T2D, several types of cancers, hypertension, obesity, clinical depression, and other chronic disorders (Warburton et al 2006). Cardiorespiratory fitness (CRF) is defined as the ability of the circulatory, respiratory, and muscular systems to supply oxygen during sustained physical activity (Lee et al 2010). Recent research suggest that increasing CRF improves insulin sensitivity, blood lipid profile (BLP), body composition, inflammation and blood pressure (BP) (Lee et al 2010). A low CRF is associated with an increased clustering of metabolic risk factors (Whaley et al 1999), and leads to an increased mortality rate caused by cardiovascular disease (CVD) (Wei et al 1999, Myers et al 2002). An almost linear reduction in mortality has been found as CRF increases (Myers et al 2002), and this applies to both men and women,
and to both healthy persons and persons with cardiovascular disease (Castillo-Garzón et al 2006).

Recent studies have investigated potential mechanisms underlying the counteracting effect from an increased CRF. In short, these studies highlight:

2. Improved glucose control (Lidegaard 2015).
3. Reductions in the inflammatory related proteins adipokines and cytokines (Kullo et al 2007).
4. Neurohormonal adaptations reducing BP such as reduced plasma level of renin, angiotensin and norepinephrine) (Cornelissen and Fagard 2005).
5. Positive adaptations in blood lipid profile (BLP) (Kelley et al 2004).

These positive adaptations constitute the basis for the aerobic exercise recommendations to improve cardiovascular health (Myers 2003).

1.2. **Maximal oxygen uptake**

CRF is a health related measure of physical fitness, as well as a diagnostic and prognostic health indicator for patients in clinical settings (Lee et al 2010). When examining CRF in a population, VO$_{2\max}$ is generally considered the best single indicator (Bassett and Boulay 2000, Thompson 2010). VO$_{2\max}$ is defined as the maximal oxygen uptake or the maximum volume of oxygen that can be utilized in one minute during maximal or exhaustive exercise (Bassett and Howley 2000). VO$_{2\max}$ is determined by the product of maximal cardiac output (L blood · min$^{-1}$) and
arterial-venous oxygen difference (mL O₂ per L blood) (Thompson 2010). VO₂max is measured in L·min⁻¹ or ml·kg⁻¹·min⁻¹. Related to fitness ability, VO₂max is often expressed as milliliters of oxygen used in one minute per kilogram of body weight, or with the body weight raised to a certain power dependent on the type of activity (allometric scaling) (Bergh et al 1991). A person’s VO₂max and VO₂max trainability is partly genetically determined, but may be increased substantially by physical exercise (Bouchard et al 1986, Bacon et al 2013). After a period of aerobic training using whole body exercises such as running or cycling, the improvements found in cardiac output are mainly caused by an increase in stroke volume (Wang et al 2014). VO₂max in whole body exercises is thus mainly determined by the capacity for oxygen delivery (supply), and to a smaller degree by oxygen utilization in muscle (demand) (Di Prampero 1985, Wagner 1991, Bassett and Howley 2000). There are however, still metabolic adaptations in skeletal muscle after aerobic exercise, such as capillarization and increases in mitochondrial density and mitochondrial enzyme activity. These adaptations may lead to higher FatOx rates and lower lactic acid accumulation and thus improve submaximal endurance performance (Holloszy and Coyle 1984).

1.2.1. The importance of exercise intensity to increase VO₂max

Generally, the American College of Sports Medicine (ACSM) and the American heart Association (AHA) recommends a minimum of 150 minutes of moderate-intensity exercise per week, and the exercise may be performed through 30-60 minutes of moderate-intensity exercise (five days per week) or 20-60 minutes of vigorous-intensity exercise (three days per week). Vigorous exercise in this sense is referred to as an intensity above ~ 78% of HRmax (ACSM 2014). In previous training interventions, moderate-intensity continuous training (MIT) is typically referred to as an intensity
between 70 and 85% of HR_{max} (Tjønna et al 2008), while high-intensity aerobic interval training (HAIT), most often refers to an intensity of intervals between 85 and 95% HR_{max} (Østerås et al 2005, Helgerud et al 2007). A numerous of training intervention studies have investigated the effects of various combinations of duration, frequency and intensity of aerobic exercise on VO_{2max}. These investigations show that the duration and frequency of exercise will directly influence the increase in VO_{2max} among inactive persons, and that an intensity representing ~ 50% VO_{2max} is enough to improve VO_{2max} (Midgley et al 2006). These findings form the basis on earlier and many of the current exercise recommendations. However, as VO_{2max} increases after a certain time of training, the VO_{2max} adaptations seems to diminish and will eventually stop improving despite of increasing the volume of exercise (Midgley et al 2006). To evaluate the effects on VO_{2max} from different training intensities, the potential bias of differences in total work may be avoided by matching total energy expenditure in the different training modes (Burke and Franks 1975). The importance of exercise intensity to improve and obtain VO_{2max} levels is not a new research area. In 1975, Burke and Franks showed that exercising at higher intensity was most effective to improve VO_{2max} after comparing the effects in different training intensity groups (85% HR_{max}, 75% HR_{max}, 65% HR_{max}, and control) matched for total energy expenditure. Burke and Franks (1975) also found that a minimum intensity of 75% HR_{max} was necessary to improve VO_{2max} among young males exercising three times per week with an average VO_{2max} of ~ 44 ml·kg^{-1}·min^{-1}. In 1985, Hickson et al emphasized the importance of increasing intensity of work to maintain VO_{2max} if total exercise time was lowered.

During the last two decades, many studies have verified high-intensity aerobic exercise to be more effective than moderate aerobic exercise to improve CRF (Gutin et al 2002, Rognmo et al 2004, O’Donovan 2005, Helgerud et al 2007, Wisløff et al 2007, Bacon et al 2013, Hollekim-Strand et al 2014). HAIT training interventions lasting between 8 and 12 weeks, have typically lead to an increase in VO_{2max} of 0.3-0.7% per session (Rognmo et al 2004, Østerås et al 2005, Wang et al 2014, Hollekim-Strand et al 2014). The level of VO_{2max} adaptations seem to be dependent on initial training status (Wenger and Bell
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1986, Wang et al 2014). Both moderate and high-intensity exercise seems to improve VO$_{2\text{max}}$ in individuals with low VO$_{2\text{max}}$ values (Rognmo et al 2004, Gormley et al 2008, Midgley et al 2006, Tjønna et al 2008, Milanović 2015), but still with the largest improvements after HAIT. However when trained, it seems that exercising at intensities ≥ 85% HR$_{\text{max}}$, is necessary to further improve VO$_{2\text{max}}$ (Midgley et al 2006, Helgerud et al 2007).

1.2.2. Aging and VO$_{2\text{max}}$ trainability

Aging is associated with a progressive decline in physical fitness, and might thus interfere with the capacity for an independent lifestyle in elderly (Paterson et al 2004). Many elderly are reported to have VO$_{2\text{max}}$ values below what is required to perform normal daily activities (Dehn and Bruce 1972, Durstine 2003, Hawkins and Wiswell 2003). This age-related reduction in VO$_{2\text{max}}$ is often accompanied by reductions in muscle mass, strength and joint flexibility (Brown and Miller 1998, Keller and Engelhardt 2013). A physically active lifestyle may counteract the age-related changes in physical fitness, and there is a consensus on the importance of both aerobic exercise and strength training as means to improve physical fitness in elderly (Tanaka and Seals 2008, Bouaziz et al 2016). Improving VO$_{2\text{max}}$ and strength in the elderly can thus contribute to an increased ability to perform everyday tasks.

The age-related decline in VO$_{2\text{max}}$ is first and foremost associated with reductions in maximal stroke volume, age related decline in HR$_{\text{max}}$, and reduced arterio-venous O$_2$ difference, which may partly be due to an observed reduction in lean body mass (LBM) (Ogawa et al 1992, Tanaka and Seals 2008). These changes are partly due to a natural biological age-related decline, but also by a reduction in physical activity level (Hawkins and Wiswell, 2003). According to Hawkins and Wiswell (2003), aerobic training do not seem to affect the HR$_{\text{max}}$ in elderly. Although a small reduction in HR$_{\text{max}}$ has been found
after aerobic training among younger individuals, older individuals do not seem to change HR\textsubscript{max} following short term HAIT (Wang et al 2014). Some studies have found differences in age related VO\textsubscript{2}\textsubscript{max} reductions between men and women (Buskirk and Hodgson 1987). Buskirk and Hodgson (1987) found a 0.40 to 0.50 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} reduction in VO\textsubscript{2}\textsubscript{max} per year among men, while a 0.20-0.35 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} reduction was found among women. However, other studies have found similar age-related VO\textsubscript{2}\textsubscript{max} reductions between men and female after adjusting for differences in body composition (Proctor and Joyner 1997). Several studies have also suggested a slower age related decline rate in VO\textsubscript{2}\textsubscript{max} among trained, -compared to sedentary individuals (Hawkins and Wiswell, 2003). This could be due to a curvilinear reduction in VO\textsubscript{2}\textsubscript{max} over the entire age range, where fit individuals due to their physically active lifestyle have a slower decline in VO\textsubscript{2}\textsubscript{max} until training is reduced (Hawkins and Wiswell, 2003). Accordingly, it could be hypothesized that sedentary individuals reduce their VO\textsubscript{2}\textsubscript{max} more rapidly during younger years, followed by a slower rate of decline as they grow older. It has been suggested that the age-related decline in VO\textsubscript{2}\textsubscript{max} among master athletes who continue to exercise is approximately one-half the rate of reduction among age-matched sedentary subjects (Rogers et al 1990). In addition, findings indicate that aerobic training may reduce the rate decline in HR\textsubscript{max} (Rogers et al 1990). The potential to improve VO\textsubscript{2}\textsubscript{max} in elderly, however, seems to be good. An increase in VO\textsubscript{2}\textsubscript{max} of 20%-30% among older sedentary adults has been reported (Chodzko-Zajko et al 2009). Since HAIT has been found to be an effective training method to increase VO\textsubscript{2}\textsubscript{max}, this training method may thus act as a counteracting strategy to the age-related decline in VO\textsubscript{2}\textsubscript{max}. Few studies have investigated the effects after HAIT among older populations (Østerås et al 2005). However, Østerås et al (2005) found a 13% increase in VO\textsubscript{2}\textsubscript{max} after 10 weeks of HAIT elderly >70 years. Yet, some studies indicate blunted VO\textsubscript{2}\textsubscript{max} adaptations after aerobic exercise among old individuals compared to that of the young (Wang 2014). In Wang (2014), an approximately twice as high increase in VO\textsubscript{2}\textsubscript{max} was found in sedentary young individuals compared to the trained older individuals after HAIT. However, the relative differences in baseline VO\textsubscript{2}\textsubscript{max} level may have affected the different adaptations
after the intervention. Therefore, to thoroughly evaluate HAIT responses on VO$_{2\text{max}}$ in different age groups, age relative pre-intervention fitness levels should probably be taken into consideration. Still, it seems that no studies have investigated the training response to HAIT in different age groups matched for age adjusted VO$_{2\text{max}}$.

1.3. Type 2 diabetes

T2D is a widespread worldwide metabolic disease, with a steadily increasing prevalence (Hawley and Zierath 2008). It is related to premature morbidity and mortality (Hawley and Zierath 2008). Among the most common secondary diseases accompanying T2D are CVD, blindness and kidney disease (Shaw et al 2010). According to lifetime risk estimates, one in three born in 2000 will develop T2D (Narayan et al 2003). The risk of developing T2D increases with age, obesity, and lack of physical activity (ADA 2016). Despite an overwhelming body of evidence showing the importance of physical activity to both prevent and treat T2D, as well as clear encouragements from national and international public health authorities, most people with T2D are not physically active (Morrato et al 2007, Bird and Hawley 2012). Thus, there is an urgent need to develop new strategies and approaches to both prevent and treat the increasing prevalence of T2D.

1.3.1. T2D characteristics and potential exercise adaptations

An HbA1c above 6.5% characterizes T2D, while normal levels are below 5.7 % (ADA 2016). Fasting plasma glycemic index ≥ 126 mg·dL$^{-1}$ (7.0 mmol·L$^{-1}$), and plasma glycemic index ≥ 200 mg·dL$^{-1}$ (11.1 mmol·L$^{-1}$) two hours after taking 75 g of glucose dissolved in water (called oral glucose tolerance test, OGTT) are also used to diagnose T2D (ACSM
T2D is associated with metabolic risk factors such as reduced blood glucose control, overweight/obesity, elevated blood pressure and dyslipidemia (Twisk et al 1997, Shen et al 2003, Castillo-Garzón et al 2006, Ganz et al 2014).

Skeletal muscle is the major source for insulin-stimulated glucose uptake (Hawley and Zierath 2008). Any treatment increasing glucose uptake in this tissue, will thus also improve whole-body glucose homeostasis. Carbohydrate oxidation accounts for 10–15% of total energy production during low intensity aerobic exercise (~30% VO2max), and increases progressively to 70–80% of total energy during exercise of about 85% VO2max (Romijn et al 1993). Exercise has been found to be an effective strategy to improve both insulin-dependent and –independent glucose transport in muscle (Hayashi et al 1997). The two sources of glucose useable to the muscle during exercise are plasma glucose and muscle glycogen (Jensen and Richter 2012). The insulin-independent exercise-mediated glucose uptake from blood into muscle involves complex molecular signaling processes that are different from those activated by insulin (Sylow et al 2016). This acute exercise-induced elevation in glucose uptake are due to increases in both glucose delivery, transport across the muscle membrane, as well as increases in glycolysis and glucose oxidation inside the muscle cell (Sylow et al 2016). Glucose delivery during exercise is enhanced by an increase in blood flow and capillary recruitment (Jensen and Richter 2012, Richter and Hargreaves 2013), as well as an increased hepatic glucose production due to elevated liver glycogenolysis and gluconeogenesis (Kjaer 1998). Simultaneously, exercise increases insulin-mediated glucose transporter type 4 (GLUT4) translocation to the sarcolemma, and thus increases glucose transport across the muscle membrane (Way et al 2016). The underlying mechanisms of elevated GLUT4 translocation and GLUT4 mRNA expression seems to last for approximately 3 to 24 hours (Richter and Hargreaves 2013). In addition, increased intramyocellular oxidative enzyme activity and possibly adaptations in muscle architecture from fast –to slow type muscle fibers also contributes to the enhanced glucose control (Holloszy and Coyle 1984). Regular exercise will thus lead to a persistent enhanced glucose control. In addition to the acute responses to exercise, an increase in whole-body insulin sensitivity
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is shown post-exercise, lasting for up to 72 hours after the last training session (Colberg et al 2010).

The majority of individuals with T2D are overweight or obese (Wilding 2014). BMI classifications are 18.5–24.9 kg/m² (normal), 25–29.9 kg/m² (overweight), 30–34.9 kg/m² (obesity class I) 35–39.9 kg/m² (obesity class II), and ≥40 kg/m² (obesity class III) (Ganz et al 2014). Individuals who are overweight or obese are more likely to develop CVD (Hainer et al 2009) and T2D (Must et al 1999). Previous research has shown a relative mortality risk of 1.5 for overweight adults, 2.5 for adults in obesity class I, 3.6 for adults in obesity class II and 5.1 for obesity class III (Ganz et al 2014). Among individuals with T2D, reductions in BW may improve blood glucose control (Wilding 2014), and weight loss has earlier shown to be associated with decreased cardiovascular risk (Pi-Sunyer et al 2007, Han and Lean 2016). The oversupply of calories leading to obesity might also cause other metabolic disturbances such as increased plasma lipid concentration and accumulation of fat in skeletal muscle (Kiens et al 2011). This fat accumulation may lead to a decrease in insulin sensitivity (Hawley and Zierath 2008).

Reductions in FatOx has been shown to be related to the development of obesity and insulin resistance (Zurlo et al 1990), and FatOx among obese persons have been shown to be reduced compared to lean individuals (Kim et al 2000). Fat metabolism can be regulated both through exercise and diet manipulation (Kiens et al 2011). Aerobic exercise increases the metabolic rate, and will thus increase both CHO and Fat oxidation (Spriet 2014). Aerobic exercise may also induce a more prolonged effect on the capacity of muscle cells to oxidize fat as fuel during sub-maximal aerobic work (Jeukendrup 2002), suggesting that regular exercise could also induce fat loss by increasing FatOx. Thus, interventions aiming to reduce metabolic disturbances through enhancing FatOx has been suggested (Achten and Jeukendrup 2004).

Approximately 60% of individuals with T2D are hypertensive (ADA 1993), and there is a strong association between elevated BP and diabetes related complications, such as myocardial infarction and microvascular complications (Adler et al 2000). The
importance of monitoring and reducing BP to reduce T2D related complications has therefore been addressed in previous studies (Adler et al 2000, El-Shafie 2010). Regular aerobic endurance training has been shown to improve several mechanisms related to cardiovascular health, such as improvements in arterial stiffness and endothelial vasodilator function (Stewart 2004). Physical activity may also improve blood lipid profile among individuals with T2D, although few studies have investigated the effects of aerobic exercise on blood lipid profile among T2D (Sigal et al 2006). The typical diabetic dyslipidemia is characterized with increased level of LDL cholesterol and triglycerides, as well as reduced HDL cholesterol (Solano and Goldberg 2006). Diabetic dyslipidemia is a modifiable risk factor and an essential part of preventing CVD among T2D (Daniel 2011). Aerobic exercise may therefore be an essential part of reducing cardiovascular risk factors among persons with T2D. Some studies indicate a relatively modest effect of exercise on both BP and BLP (Leon et al 2001, Whelton et al 2002), while others have found positive effects after more extensive exercise, in terms of both more volume and higher intensity (Kraus et al 2002). Blood lipid values are also highly related to overweight and obesity (Szczygielska et al 2003).

Due to their associations with CVD, mortality risk, there is an increased focus on exercise interventions aiming to improve VO$_{2\text{max}}$, FatOx, body composition and BLP, and to reduce HbA1c and BP among type 2 diabetics (Blaak et al 2001, Donnelly et al 2009).

1.3.2. Exercise as medicine; HAIT vs MIT as training strategy.

In general, WHO recommends at least 150 min of moderate-intensity aerobic physical activity per week, or at least 75 min of vigorous-intensity aerobic physical activity per week. Further, WHO recommends 300 min of moderate-intensity aerobic physical activity, or 150 min of vigorous-intensity aerobic physical activity per week to get additional health benefits (WHO 2011). For persons with T2D, the ADA (2016) recommends 150 minutes per week of moderate-intensity aerobic (50–70% HR$_{\text{max}}$)
exercise spread over at least 3 days/week with no more than 2 consecutive days without exercise. ACSM however, recommends at least 150 min·wk⁻¹ of “moderate to vigorous” aerobic exercise during a minimum of 3 d during the week, with no more than two consecutive days between bouts of aerobic activity. In addition to aerobic training, it is recommended to do moderate to vigorous resistance training at least 2–3 d·wk⁻¹ (ACSM and ADA 2010). Exercise increase both insulin-independent muscle glucose uptake and insulin sensitivity (Hawley and Zierath 2008). The effect of a single session of aerobic exercise on insulin sensitivity last approximately between 20 and 70 hours depending on duration and intensity of the exercise session (Wallberg-Henriksson et al 1998). It is thus recommended that there should be no longer than 48 hours between exercise sessions among people with T2D. Over the last decade there has been an increased focus on how aerobic exercise affect physiological adaptations such as VO₂max, FatOx, BP, BLP, anthropometrics and HbA1c (DiPietro et al 2006, Kodama et al 2007, Hansen et al 2009, Segerstrøm et al 2010, Hollekim-Strand 2014, Revdal et al 2016). The majority of people with T2D are not physical active, and “lack of time” is one of the most cited reasons for lack of PA (Stutts 2002). It is therefore an increased focus on how to accommodate the barrier toward PA, and on the effect of more intensive but less time consuming training protocols (Gibala et al 2012, Hawley and Gibala 2012, Terada et al 2013, Revdal et al 2016). Several studies and reviews have revealed positive results on one or more of the T2D related risk factors after low volume short duration-exercise and sprint intervals characterized by mainly anaerobic work (Gibala et al 2012, Hawley and Gibala 2012, Terada et al 2013, Revdal et al 2016). However, people with T2D may have contra indications to perform this kind of demanding sprint-intervals (Levinger et al 2015). In addition, longer and predominately aerobic work periods with an intensity between 85 and 95% of HRmax (HAIT), may be more effective to improve CRF (Rognmo et al 2004, Midgley et al 2006, Helgerud et al 2007, Gormley et al 2008, Tjønna et al 2008, Milanović 2015) and should also imply a higher total energy expenditure. Although HAIT has been shown to be effective to increase VO₂max in several populations (Rognmo et al 2004, O’Donovan 2005, Helgerud et al 2007), very few studies have investigated
the effects of HAIT on glucose control among T2D (Hollekim-Strand et al 2014). However, a systematic review and meta-analysis undertaken by Boulé et al. (2001) about HbA1c and body mass adaptations to different training interventions showed that post HbA1c was lower while BW was unchanged in exercise groups compared to control groups. The HbA1c adaptation was independent of any effect on body weight, showing the potential positive HbA1c effects of exercise independently of changes in BW. Boulé et al. (2001) revealed that exercise intensity was a stronger predictor of HbA1c changes than exercise volume, indicating a possible better effect of HAIT than MIT to improve blood glucose control. However, Hansen et al (2009) found improved HbA1c levels among T2D after both low- to moderate (69% HR_{max}), and moderate- to high intensity (85% HR_{max}) continuous exercise. In addition, exercise duration of more than 150 minutes per week was associated with better improvements in HbA1c (-0.89%) than exercise duration of 150 minutes or less per week (-0.36%) in a meta-analysis of Umpierre et al (2011). Regarding the effects of different training intensities on other metabolic risk factors associated with T2D, very few studies have compared the adaptations to HAIT versus MIT among T2D individuals (Hansen et al 2009, Hollekim-Strand et al 2014). Therefore, it is not possible to draw any strong conclusions on what kind of training protocol is the most optimal to improve body composition, FatOx, BP and BLP among T2D. The importance of training intensity is thus still debated, and meta-analyze studies and reviews underpin the uncertainty about whether intensity or volume of exercise are most important to improve glycemic control and other variables related to T2D (Boulé et al 2001, Boulé et al 2003, van Dijk and van Loon 2015).

1.4. Fat Oxidation

Fat and carbohydrates (CHO) are the main sources of energy during both rest and physical activity (Spriet 2014). The effects of manipulating the fat and CHO proportion
in a diet can be reflected in changes in lung gas exchange using respiratory exchange ratio (RER) (McArdle et al 2010). RER is the ratio of CO₂ produced to the O₂ consumed. When only fatty acids are oxidized, 16 CO₂ molecules are produced as 23 O₂ molecules are consumed, giving a RER of ~0.70. For only CHO utilization, RER is ~1.0. If only fatty acids were oxidized, this would imply a 30% lower CO₂ production at a given oxygen consumption compared to CHO oxidation. When fatty acids are oxidized, approximately 6% more O₂ is consumed compared to CHO to generate the same amount of ATP (Sue et al 1989).

In a general population, skeletal muscle accounts for approximately 30%-40% of BW (Blaak 2005). Skeletal muscle metabolism thus plays a central part in both fat and CHO metabolism. During continuous moderate exercise, the substrates needed for energy supply in the working muscles are first and foremost the non-esterified fatty acids (NEFAs) transported into muscle from the circulation, as well as NEFAs from lipolysis of intramuscular triacylglycerol (IMTAG) (van Hall 2015). van Hall (2015) also suggest a possible lipoprotein lipase activity (LPL) generated lipolysis of very-low density lipoproteins in muscle. Aerobic training has been shown to increase the muscles capacity to oxidize fat during aerobic physical activity (Kiens et al. 2011). Individuals with higher VO₂max values seem to have higher total FatOx rates during aerobic exercise than individuals with lower VO₂max (Nordby et al. 2006; Stisen et al. 2006). However, there are great inter-individual variation in maximal FatOx (between 0.18 to 1.01 g·min⁻¹), which are still not fully accounted for in previous research (Venables et al. 2005). It has been suggested that FatOx among women contributes more to oxidative metabolism than among men (Tarnopolsky 2000, Carter et al 2001), but not all studies have found this difference between gender (Roepstorff et al 2002).

Assessment of FatOx during exercise is used to examine possible metabolic disturbances in sedentary individuals (Stisen et al. 2006), as well as to explore FatOx capacity in well trained (Nordby et 2006). An increased FatOx during exercise entail a glycogen sparing effect, thus delaying fatigue during long-lasting strenuous work among athletes.
(McArdle et al. 2010). In a health perspective, an improved FatOx may decrease the risk of developing metabolic-related problems such as obesity and T2D (Blaak 2005), as well as prevent further deterioration of impaired FatOx during exercise among individuals with already impaired glucose tolerance (Mensink et al 2005). The absolute and relative contribution of fat and CHO during exercise is influenced by exercise intensity (Spriet 2014). During low-intensity work, approximately half of the energy is provided by fat, but as the intensity increases, the relative contribution from fats decreases at the same time as the use of CHO increases (Venables et al. 2005). Maximal rates of FatOx have been shown to be at intensities between 59% and 64% of VO$_{2\text{max}}$ among trained individuals and between 47% and 52% of VO$_{2\text{max}}$ among sedentary (Achten and Jeukendrup 2004). The rate of FatOx during exercise is also dependent on diet (Achten and Jeukendrup 2004), fitness level (Nordby et al. 2006), availability of substrates (Philp et al. 2012), type of exercise (Achten and Jeukendrup 2004), gender (Venables et al. 2005) and skeletal muscle fiber type composition (Helge et al. 1999).

1.4.1. FatOx disturbance.

The main source of energy during rest, and low and moderate exercise are long-chain fatty acids (Achten and Jeukendrup 2004). Fatty acids can derive from both muscle triacylglycerol (TAG), fatty acids from adipose tissue and from circulating lipoproteins (Achten and Jeukendrup 2004). FatOx capacity has thus been investigated through the different components of fatty acid metabolism (free fatty acid rate of appearance, plasma-derived fatty acid oxidation, triglyceride-derived fatty oxidation, and total fat oxidation). A reduced uptake and oxidation of fatty acids in skeletal muscle has been found among obese and persons with T2D (Kelley and Simoneau 1994, Colberg et al 1995). Both inheritable factors, environmental factors, and a mix of those (Hawley and Zierath 2008) may cause a reduced FatOx. The reduced FatOx ability may be expressed
as a higher respiratory exchange ratio (RER) during rest and exercise (Ramos-Jiménez et al 2008). A low RER at rest has also been shown to predict a healthy metabolic phenotype in moderately overweight, sedentary men (Rosenkilde et al 2010). However, research on possible disturbed FatOx during exercise among obese and T2D show contradicting results. Some studies indicate FatOx disturbances during exercise among obese and persons with T2D (Blaak et al 2000, Hickner et al 2001, Pérez-Martin et al 2001). Others report that obese individuals do not seem to have decreased capacity to utilize fat during exercise (Goodpaster et al 2002, Larsen et al 2009). The possible FatOx disturbances may be an important factor in the etiology of insulin resistance in liver and skeletal muscle. However, there is no clear evidence for the direction of cause and effect. The evidence of reduced FatOx among obese and T2D patients is thus not conclusive. Nonetheless, for obese individuals with reduced FatOx, data suggest a relatively consistent impairment in the ability of skeletal muscle to oxidize fat with obesity, particularly in extremely obese patients, and even after weight loss (Blaak 2001).

A possible explanation to an impaired FatOx is related to serum insulin concentrations. Insulin is a key hormone intervening with the rate of FatOx. As insulin concentration increases during CHO intake, insulin inhibits hormone sensitive lipase (HSL), which is a rate-limiting enzyme for intramuscular triglyceride lipolysis (Watt 2009). Additionally, insulin also inhibits LPL activity in the muscle cell (Farese et al 1991). Elevated rates of serum insulin concentrations can thus cause higher rate of CHO oxidation and impaired FatOx. Disturbances in fat uptake and utilization can lead to elevated fat storage within both the muscle cell and in adipose tissue, which may in turn induce insulin resistance through interfering with the insulin-signaling pathway within the cell (Mensink et al 2001). In addition, previous research has revealed an increased amount of type 2X fibers in muscle of type 2 diabetic patients (Mogensen et al 2007). Since type II fibers have a lower FatOx capacity, the muscle fiber type distribution also affects the rate of FatOx. In relation to this, reduced mitochondrial function in skeletal muscle has been reported
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among persons with T2D (Joseph et al 2012). A reduced mitochondrial function will consequently affect the cells oxidative capacity (Joseph et al 2012).

1.4.2. Exercise adaptations.

Exercise interventions aiming to improve FatOx disturbances may have positive effects on metabolic risk factors and thus be of great clinical relevance. Metabolic adaptations to chronic exercise is influenced by factors such as exercise specifications (intensity, duration, frequency, and mode), as well as individual characteristics as genetics, age, medical conditions and physical fitness (Sigal et al 2004). Important aerobic exercise adaptations towards increased FatOx are related to improved oxygen delivery- and oxygen utilizing capacity in the cardiovascular system and in skeletal muscle (Melanson et al 2009). Regarding substrate availability, Achten and Jeukendrup (2004) summarizes adipose tissue lipolysis and fatty acid delivery to skeletal muscle not to be affected by aerobic exercise. However, studies indicate an exercise induced increase in fatty acid binding proteins, which may cause an improved fatty acid transport across the muscle membrane. The potential effect on hydrolysis of intramuscular triacylglycerols is still debated, as fatty acid transport across the mitochondrial membranes seems to be affected mainly by higher activity of the FatOx regulating enzyme carnitine palmitoyl transferase (CPT-1) (Achten and Jeukendrup 2004).

Exercise intervention studies investigating the effects of aerobic exercise on FatOx have mainly been performed among healthy younger adults, and most studies show positive effects on FatOx during exercise (Friedlander et al 1998, Horowitz et al 2000, Achten and Jeukendrup 2004, Talanian et al 2007). Both high-intensive (Talanian et al 2007, Perry et al 2008), moderate to vigorous-intensive (Friedlander et al 1998) and low-intensive (Van Aggel-Leijssen et al 2002) exercise have generated improvements in FatOx during exercise. Approximately 80% of type 2 diabetics are obese (Bloomgarden 2000), and it
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It has been hypothesized that overweight or obese individuals with already reduced capacity to mobilize and utilize fat during exercise adapt differently to aerobic exercise than lean individuals (Van Aggel-Leijssen 2002). On the other hand, Mogensen et al (2009) found normal FatOx adaptations after aerobic exercise among type 2 diabetics with normal FatOx_max rates. The same study found that metabolic adaptations were unrelated to changes in insulin sensitivity, and thus suggested that a reduced FatOx capacity is not a major cause of insulin resistance in T2D.

It is still not clear what training intensity is the most effective to improve FatOx during exercise. Only few studies have investigated the effects on FatOx between training regimes with different exercise intensities (Van Aggel-Leijssen et al 2002, Alkahtani et al 2013). Aerobic exercise is a common strategy in clinical interventions addressing the metabolic risk factors often associated with the T2D diagnosis, but studies investigating FatOx adaptations to HAIT compared to MIT among type 2 diabetics seem to be lacking.

1.4.3. Reliability in FatOx testing procedures.

Protocols developed for assessing substrate utilization during exercise are helpful tools to investigate FatOx adaptations after training interventions (Bordenave et al 2007). A number of factors, including the exercise test design, the data analysis procedures as well as pre-test conditions like diet and physical activity (Croci et al 2014), affects the reliability of FatOx measurements during exercise. The accuracy of estimating FatOx during exercise using RER is also limited by the variations in work economy (WE) in addition to the test apparatus accuracy of measuring oxygen consumption (VO₂) and carbon dioxide production (VCO₂).

It has been reported that in obese, T2D and very sedentary patients, a longer time is needed to achieve steady state FatOx rates during submaximal exercise, and thus a longer duration than 3 minutes is needed during FatOx measurements (Bordenave et al 2007). A key regulator of substrate oxidation is exercise intensity. When intensity
increases, the greater glycolytic flux will inhibit long-chain fatty acid transport into the mitochondria and thus reduce long-chain fatty acid oxidation (Coyle et al 1997). The lactate concentration during exercise in a regular cohort will increase gradually until \( \sim 75\% \) of VO\(_{2\text{max}}\), after which there will be a steep increase, and above 85% of VO\(_{2\text{max}}\) the contribution of fat to energy supply is minimal (Achten and Jeukendrup 2003). To attain steady state conditions during FatOx measurements, FatOx during exercise should thus be assessed at a submaximal intensity below 75% VO\(_{2\text{max}}\). Both nutrient status (Gonzalez and Stevenson 2012), and changes in diet (Burke and Hawley 2002; Carey et al. 2001; Helge et al. 2001) may influence FatOx during exercise. Studies aiming to investigate FatOx during exercise should thus ensure a high level of reliability and reproducibility through standardizing and evaluating both testing procedures and nutrition status -and composition of the subjects. In addition, normal day-to-day FatOx variability during equal diet conditions as well as the variability related to the test equipment should be considered.

1.4.4. The influence of diet.

The predominant substrate for metabolism is related to the dietary intake of fat and CHO (Coyle 1995). The nutrient composition of the diet influences the rate of FatOx during exercise (Coyle et al 2001, Patterson and Potteiger 2011), as well as the adaptations during endurance training interventions (Helge et al 1996). A high-fat diet has been suggested as a possible strategy to increase rates of FatOx. Higher rates of FatOx have been found during submaximal exercise (60–70% VO\(_{2\text{max}}\)) after high-fat diets in both sedentary adolescents (Guimaraes Couto 2014) and adults (Burke et al 2016), as well as in well trained (Burke et al 2000). The mechanisms regulating a higher FatOx after a high-fat diet are complex and to some extent still unclear, but decreased CHO availability due to glycogen depletion in muscle and reduced release of liver-derived
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Glucose into blood (Phinney et al. 2003), a larger concentration of plasma free fatty acids and higher plasma fatty acid uptake (Helge et al. 2001), a greater dependence on intramyocellular lipids for oxidation (Spriet 2014), and increased fatty acid transport into mitochondria are common suggested explanations (Achten and Jeukendrup 2004, Spriet 2014).

A reduction in body weight during an intervention indicates a negative energy balance. Long-term energy deficit entails a decrease in whole-body RER, meaning a higher reliance on FatOx for energy supply (Kempen et al. 1998). This may be due to lack of CHO availability (Adam-Perrot 2006), but can also be caused by adaptations in muscle cytosolic fatty acid-binding proteins (Kempen et al. 1998). The dietary fatty acid composition (composition of monounsaturated, -polyunsaturated, -and saturated fatty) acids might also influence FatOx (Krishnan and Cooper 2014). Short term (<6 d) adaptations to a high-fat diet have been shown to reduce submaximal endurance performance among sedentary individuals (Helge 2000), and higher rates of perceived exertion during high-intensity exercise was revealed among competitive endurance athletes after only 3 days of high-fat diet (Stepto et al. 2002). A review by Helge (2002) concludes that although high-intensity training may be tolerated during short-term high-fat diets in both untrained, moderately trained and elite athletes, endurance performance can only be maintained and not improved during long-term high-fat diets.

Due to the seemingly quick metabolic respond and adaptation in substrate utilization after changing nutrition composition, -and/or total energy intake (Achten and Jeukendrup 2004, Patterson and Potteiger 2011), studies investigating FatOx adaptations after exercise should keep track on the participants’ diets to reduce the confounding factor of these changes.
Støa: The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors; testing procedures and training interventions.
2. Rationale and aims of the experiments

2.1. Paper I

Aerobic training is an important treatment strategy to reduce risk factors associated with T2D (Colberg et al 2010). However, the importance of exercise intensity to improve risk factors associated with T2D is still debated (van Dijk and van Loon 2015). HAIT is an effective training strategy to increase VO\textsubscript{2max} (Wisløff et al 2007, Helgerud et al 2007), but few studies have investigated physiological adaptations to HAIT among T2D patients (Hollekim-Strand et al 2014). The aim of this study was thus to investigate if HAIT is an effective training strategy to reduce important risk factors associated with T2D. A secondary purpose was to examine if HAIT is more effective than MIT to improve T2D associated risk factors.

2.2. Paper II

VO\textsubscript{2max} decreases with age (Fleg et al 2005). VO\textsubscript{2max} is a strong predictor of CVD and mortality (Myers 2002, Carnethon 2005), and high VO\textsubscript{2max} values among older individuals are associated with physical independence and improved quality of life (Hawkins and Wiswell 2003). Many studies have shown HAIT to be an effective training method to improve VO\textsubscript{2max} (Rognmo et al 2004, Østerås et al 2005, Helgerud et al 2007, Tjønna et al 2008). The age-related VO\textsubscript{2max} decline may thus be counteracted with HAIT. Previous research indicate that VO\textsubscript{2max} responses to HAIT may differ between young and old individuals when they are matched for the same absolute baseline VO\textsubscript{2max} (Wang et al 2014). However, no studies have compared VO\textsubscript{2max} adaptations to HAIT between different age groups matched for pretest VO\textsubscript{2max} relative to age mean. The scope of this
study was therefore to investigate the effects of HAIT on VO$_{2\text{max}}$ in different cohorts from 20- to 70+ year old males and females.

### 2.3. Paper III

To measure FatOx during exercise may give important information on metabolic conditions as well as substrate utilization in a performance perspective (Brandou et al. 2003, Stisen et al. 2006, Venables and Jeukendrup 2008). Indirect calorimetry is a non-invasive test procedure, where RER is used to calculate FatOx during sub maximal exercise (Battezzati and Viganò 2001, McArdle et al. 2010). However, to give accurate information on substrate utilization capacity, the test procedures need to be reliable. Nutrient status (Gonzalez and Stevenson 2012), and changes in the diets macronutrient composition (Burke and Hawley 2002; Carey et al. 2001; Helge et al. 2001) are two key factors influencing the rate of FatOx during exercise. The accuracy of estimating FatOx during exercise is also affected by the variations in work economy (WE) as well as by the accuracy of measuring oxygen consumption (VO$_2$) and carbon dioxide production (VCO$_2$). The aim of this study was therefore to explore day-to-day variations in FatOx during equal test conditions with strictly controlled diets, and to investigate the effect of a short-term (1 day) diet manipulation on FatOx during exercise among healthy adults.
3. Methods

3.1. Participants

This thesis presents data from three experimental studies, where paper I and II are exercise intervention studies, and paper III is a FatOx reliability study. The Regional Committee for Medical and Health Research Ethics in Southern Norway and the institutional review board at Telemark University College and Norwegian University of Science and Technology approved the studies. All studies were conducted in accordance with ethical principles of the Helsinki Declaration.

141 subjects (87 males and 54 females) participated in the studies, and the subjects characteristics are presented in table 1. All participants signed an informed consent form prior to participation. In addition, the subjects in paper I underwent a medical examination due to their T2D diagnosis. The general inclusion criteria for study participation were a minimum age of 18 years and no medical contra indications for testing and training according to the ACSM guidelines. The participants were also generally excluded if they were sick or injured for a longer period of time (>1 week) the last month prior to testing, if they were ill during the last week prior to physical testing, or if they did not follow the established diet recommendations and standardization (in paper III).

An evaluation of use of medications that could affect the reliability of testing and the responsiveness of aerobic endurance training were made in each study. In the two training intervention studies (paper I, and II), the subjects were excluded if they were injured or sick more than one week during the intervention period. They were also excluded if they completed less than 75% (paper I) and 80% (paper II) of the training sessions. General preparation procedures included no strenuous exercise the last 1-2
days before testing and they could only ingest water the last 2 h before testing. All participants agreed to maintain their habitual physical activities during the study period. In accordance with the study primary goals in paper I and III, thorough diet registrations were conducted using 1 g accurate food scales (Wilfa, KW-4, Hagan, Norway) and food registration forms.

Table 1 Subject characteristics

<table>
<thead>
<tr>
<th>Paper Group</th>
<th>I Type 2 diabetes</th>
<th>II 20 to 80 yrs old</th>
<th>III Healthy adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>38</td>
<td>94</td>
<td>9</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>58.7±10.5</td>
<td>48.6±18.1</td>
<td>22.4±1.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171±6</td>
<td>178±7</td>
<td>165±6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>92.7±15.2</td>
<td>83.3±14.9</td>
<td>62.1±7.2</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>31.7±4.5</td>
<td>26.1±4.0</td>
<td>23.0±1.1</td>
</tr>
<tr>
<td>Maximal oxygen uptake (mL·kg⁻¹·min⁻¹)</td>
<td>25.6±5.9</td>
<td>41.4±12.8</td>
<td>43.8±4.3</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation. n, number of participants. yrs, years. cm, centimeter. kg, kilogram. mL, milliliters. L, liters. min, minutes.

3.2. Training protocols

Paper I and paper II were training interventions where HAIT was performed three sessions per week in addition to habitual physical activity for 12 and for 8 weeks respectively. HAIT included 4 · 4 minutes at an intensity between 85-95% HRmax. The interval periods were separated by 3-min active recovery periods. In paper I, a second exercise group conducted continuously moderate work at 70-75% HRmax for a duration designed to match the total work of the 4 · 4 session. All exercise sessions were supervised and monitored. In paper I, the training sessions were carried out as walking or running in an outdoor environment. In paper II, the training was performed on either a treadmill at a 5% inclination, or using a cycling ergometer. All subjects learned how to
use a polar heart rate monitor to ensure the right training intensity. They were also given thorough instructions about how to register duration, average heart rate, and time in their specific individual intensity zones.

### 3.3. Testing protocols

The testing was carried out at the University College of Southeast Norway (USN) and Norwegian University of Science and Technology (NTNU). All subjects were thoroughly familiarized with testing procedures and equipment before test start. VO$_{2\text{max}}$, LT and FatOx data were obtained using ergo-spirometrical measurements from the Sensor Medics Vmax Spectra (Sensor Medics 229, Yorba Linda, California, USA) system (USN) and the Metamax II (Coretex, Leipzig, Germany) system (NTNU). Blood lactate (venous whole blood) was measured using a Lactate Pro Analyzer (Arcray Inc. Kyoto, Japan), and HR during testing and exercise was measured using Polar heart rate watches (Polar Kempele, Finland). The physical tests were performed on either a cycle ergometer (Lode Excalibur Sport; Lode, Groningen, Netherlands) or a treadmill (Woodway PPS 55 Sport, Waukesha, Germany). The cycling sitting position on the ergometer was accurately fitted to each subject and registered for the next test.

#### 3.3.1. Anthropometrics

Body weight was measured on calibrated body scales and the participants’ heights were measured with a wall-mounted measuring tape. %BF was calculated based on five-site skinfolds (triceps, chest, abdomen, suprailiac and thigh) with a Harpenden skinfold caliper (Saehan Medical Skinfold Caliper, SH5020, Korea). Waist- and hip circumferences were measured using a measuring tape. The same test leader performed the
anthropometric measurements at pre- and post-test to avoid potential bias by different measuring techniques. BMI was calculated as weight in kilograms divided by height in squared meters (kg·m\(^{-2}\)).

3.3.2. Maximal oxygen uptake and lactate threshold

\(\text{VO}_{2\text{max}}\) was tested by use of an incremental protocol in all three studies. The participants started at a speed or watt representing approximately their individual LT intensity. In paper I, all participants walked or ran on a treadmill with an incline of 3-4\%, and at a speed between 3 and 6 km·h\(^{-1}\) adjusted to each individual’s physical fitness. Every 30 seconds, the speed, - and/or the incline was increased by 0.5 km·h\(^{-1}\) and 1\% respectively according to a subjective evaluation of the VO\(_2\) curve assessed by experienced test leaders. In paper II, velocity or watt was progressively increased every minute with 1 km·h\(^{-1}\) (3\% treadmill) and 25W (bicycle), respectively. In paper III, the brake power was increased every 30 s by 10 or 20W depending on the individual VO\(_2\) curve and the subjective evaluation of the test leader. The durations of the \(\text{VO}_{2\text{max}}\) tests ranged between 4 and 12 minutes. The \(\text{VO}_{2\text{max}}\) tests ended at voluntary exhaustion, and the average of the two highest continuous VO\(_2\) measurements (USN), or the three highest continuous VO\(_2\) measurements (NTNU), was set as \(\text{VO}_{2\text{max}}\). The highest HR + 5 beats per minute at the last stages of the \(\text{VO}_{2\text{max}}\) test was registered as HR\(_{\text{max}}\). In all three papers, the following criteria used to determine if \(\text{VO}_{2\text{max}}\) was accomplished: 1. a possible levelling of the VO\(_2\) curve. 2. RER ≥ 1.05 (≥ 1.10 in paper II). 3. HR\(_{\text{max}}\) ≥ 95% of expected HR\(_{\text{max}}\). 4. blood lactate [La\(^-\)] \(\geq 8\) mmol·L\(^{-1}\) (Støren et al 2008, Sunde et al 2010, Helgerud et al 2010).

LT was only measured in paper III. Three or four 5 minutes submaximal workloads at 3\% incline were performed with increasing workloads. The first workload corresponded to \(~60\%\) \(\text{VO}_{2\text{max}}\). Venous whole blood [La\(^-\)] was measured after each period using a Lactate
Pro Analyzer (Arcray Inc. Kyoto, Japan). The workload was increased at each 5 minutes period, until the intensity exceeded LT defined as the warm up [La\(^{-}\)]\(_{b}\) + 2.3 mmol·L\(^{-1}\). This protocol is in accordance with the one used in Støren et al (2008). If the LT was not reached during the third workload, a fourth workload was performed.

### 3.3.3. Rate of fat oxidation

In paper I and III, FatOx was measured using a 10-minutes protocol at a workload representing 60% VO\(_{2\text{max}}\). VO\(_{2}\) and RER values were registered every 20 seconds and averaged between 4 and 10 minutes. To establish the workload (treadmill velocity in paper I and brake power in paper III), three submaximal 5 minutes bouts at increasing workloads, and a VO\(_{2\text{max}}\) test was performed. The participants started at an intensity of approximately 50% VO\(_{2\text{max}}\), and during the next two 5 minutes bouts, the intensity was increased to reach an intensity of approximately 75% and 85% VO\(_{2\text{max}}\). Each individual workload representing 60% VO\(_{2\text{max}}\) was calculated based on the measurements during these three work periods and a VO\(_{2\text{max}}\) test, based on the linear regression for VO\(_{2\text{max}}\) and workload (example in figure 1).
Fig. 1 Assessment of FatOx velocity. The linear regression equation in Fig. 1 is: $y=3.314x + 0.911$, $r=0.98$. $VO_{2\text{max}}$ for this participant is 32.1 ml·kg$^{-1}$·min$^{-1}$. 60% $VO_{2\text{max}}$ for this participant is 19.3 ml·kg$^{-1}$·min$^{-1}$. From the equation, the corresponding velocity is 5.5 km·h$^{-1}$. The test was performed with 3% treadmill incline.

3.3.4. Blood pressure, blood lipids, HbA1c and insulin resistance

BP, BLP, HbA1c and IR were only measured in paper I. BP was measured manually by the projects’ physician using stethoscope and a blood pressure cuff (Welcyallyn SK, Germany and Tycos 2006z, USA). The subject was sitting still for 5 minutes before measurement. Blood lipids and blood glucose measurements were performed by blood tests with requisitions from the project physician. The test results were evaluated by the project physician. The homeostasis model of assessment for insulin resistance index (HOMA-IR) was used to assess IR (The Oxford University HOMA2-IR online calculator; http://www.dtu.ox.ac.uk/homacalculator/, accessed June 7, 2015).
3.4. Statistics

Statistics were performed using the Statistical Package for Social Sciences (SPSS) version 19 (Chicago, USA). The Shapiro-Wilk test and normal Q-Q plot were used to test for normality. In all tests, significance was accepted at p<0.05. Experimental data are presented as mean ± standard deviation, as well as delta values (Δ) and coefficient of variance (CV) in percent (in paper I).

In paper I, a Pearson bivariate correlational test was performed to determine possible relationships between baseline values and between changes in the physiological variables from baseline to post-test. Paired student t-test was used to explore differences between baseline and post-test within each intervention group. To investigate significant differences in changes between the two exercise groups, independent t-tests were performed. HbA1c were not normally distributed, therefore non-parametric tests were used when analyzing these data. Further, a Wilcoxon signed rank test was used to identify pre- to post changes in HbA1c within each group, and a Mann-Whitney U test was used to assess between group differences in changes.

In paper II, unpaired t-tests were used to determine between group differences at baseline and potential across groups gender differences. Paired t-tests were used to detect within group differences following training. Two-way repeated measures ANOVAs (age · time) were used to explore differences between groups pre- to post-training, followed by a Tukey post hoc analysis. Pearson`s bivariate correlation test was used to investigate correlations between variables. A linear regression analysis was used to assess standard error of estimate (SEE) in correlations.

In paper III, a general linear model with Tukey’s post hoc test was performed to assess potential differences in VO₂, VCO₂, RER, FatOx, HR, blood lactate concentration, BG, body weight, and BMI between different test days. The general linear model (ANOVA), with Tukey’s post hoc tests (ANCOVA) were used to account for the 4 different steps
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(test days 1–4) in which FatOx was measured. CV was used to assess variability between subject at the different test days and variability in difference between test days. A Bland–Altman plot was used to assess whether or not the size of FatOx would have an impact on the test–retest variability between the 2 test days with identical diet (test days 1–2). Intraclass correlation coefficient tests were performed to further evaluate the reliability. A Pearson bivariate correlational test was used to determine a possible relationship between VO₂max and FatOx at baseline.
4. Summary of interventions


Paper I investigated physiological adaptations after supervised high-intensive aerobic interval training (HAIT) on risk factors associated with T2D. The results were compared to results from a moderate-intensity continuous (MIT) training group. Both groups exercised for 12 weeks, and the two different training modes were matched for total work. There were 38 participants in this study, with 23 males and 15 females. VO\textsubscript{2max} and HbA1c were the main outcomes. In addition, anthropometrics, FatOx, LT, BP and BLP were also tested. All participants maintained their normal diet. The intervention was a supplement to usual physical activity habits. The main results were increased VO\textsubscript{2max} (21%) and decreased HbA1c (-0.58% points) in HAIT compared with MIT. Changes in BW and BMI were also significantly different from MIT, with the greatest improvement in HAIT. There was a tendency towards an improved FatOx in HAIT, and the pre-to post difference was significantly different from MIT. In conclusion, HAIT was found to be an effective exercise strategy to improve aerobic fitness and reduce risk factors associated with T2D.
4.2. **Paper II: The Impact of Age on the VO\(_{2\text{max}}\) Response to High-Intensity Interval Training**

Paper II investigated the effects of supervised high-intensive aerobic interval training on VO\(_{2\text{max}}\) responses in six decade-cohorts from 20 to 70+ year old males and females. 94 subjects participated in the study, and the training intervention lasted for 8 weeks. The main result from the study was that there was no significant differences in training response in VO\(_{2\text{max}}\) between the different age groups. In addition, the results revealed no differences in the training responses between males and females. As expected, the percentage improvements in VO\(_{2\text{max}}\) after training were inversely associated with baseline training status (r=0.66; p<0.001). HR\(_{\text{max}}\) was not changed as a consequence of the training program in any of the age groups. However, there was a tendency (p=0.07) to a difference in HR\(_{\text{max}}\) response when the two oldest age cohorts were combined (60-70+ years) and contrasted to a cluster of the younger cohorts (20-59 years). In conclusion, all age groups have a great potential for VO\(_{2\text{max}}\) improvement, and high-intensity aerobic interval training is an effective method of training to improve cardiovascular health across different age groups.

4.3. **Paper III: Day to day variability in fat oxidation and the effect after only 1 day of change in diet composition**

The aim of this study was to investigate day-to-day variations in FatOx during moderate exercise given the same diet and 2 different isoenergetic diets. Nine healthy females participated in the study. The FatOx tests were performed at a relative workload of 60% VO\(_{2\text{max}}\). FatOx was measured pre- and post normal diet conditions, as well as pre- and
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post one day of diet manipulations (high fat vs high CHO diets). Additionally, VO$_{2\text{max}}$ was tested prior to the remaining protocol. The day-to-day variability tests revealed no differences in FatOx, respiratory exchange ratio (RER), oxygen uptake, carbon dioxide production, heart rate, blood lactate concentration, or blood glucose between the 2 habitual diet days. Results after the CHO-rich diet revealed that FatOx was reduced by 31% compared to the fat-rich diet, and a significant reduction in FatOx (from $0.42 \pm 0.15$ to $0.29 \pm 0.13$ g·min$^{-1}$, p < 0.05) was found after the CHO-rich diet compared with the habitual day 2. In conclusion, using RER data is a reliable method to estimate FatOx as long as the diet is strictly controlled. Further, the results from this study showed that even a 1-day change in the diets macronutrient composition most likely affects FatOx results.
Støa: The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors; testing procedures and training interventions.
5. Discussion

5.1. Maximal oxygen uptake

$VO_{2\text{max}}$ was assessed in all three studies. The participants baseline $VO_{2\text{max}}$ values were below age and gender-related average in paper I (25.6±5.9 mL·kg$^{-1}$·min$^{-1}$), on age and gender-related average in paper II (41.4±12.8 mL·kg$^{-1}$·min$^{-1}$), and above age and gender-related average in paper III (43.8±4.3 mL·kg$^{-1}$·min$^{-1}$) (Heyward 1998, Astrand 1960). The average pre $VO_{2\text{max}}$ level of 27.7±7.3 ml·kg$^{-1}$·min$^{-1}$ among the male participants in paper I is lower than in age matched healthy non-athlete males (Wilmore and Costill 2005). The female participants in paper I with an average $VO_{2\text{max}}$ of 24.4±4.2 ml·kg$^{-1}$·min$^{-1}$ are also in the lower part of the scale compared to age matched healthy non-athlete females (Wilmore and Costill 2005). This is in accordance with other studies reporting lower cardio respiratory fitness (CRF) among T2D compared to healthy age matched individuals (Regensteiner et al 1995, Kunitomi et al 2000, Hollekim-Strand et al 2014).

Paper I and paper II both studied $VO_{2\text{max}}$ adaptations after a HAIT intervention. The positive HAIT-induced $VO_{2\text{max}}$ adaptations in paper I (approximately 20%) and II (approximately 10%) are in line with several previous studies (Østerås et al 2005, Helgerud et al 2007, Wisløff et al 2007, Wang et al 2014). Neither paper I nor paper II showed any differences in $VO_{2\text{max}}$ responses between genders. The longer duration of the training period in paper I will influence the results, as well as the lower baseline values. The impact of baseline values on training induced $VO_{2\text{max}}$ adaptations (ml·kg$^{-1}$·min$^{-1}$) was shown in paper II ($r=-0.33$, $p<0.01$). Although a previous study by Wang et al (2014) found a blunted response to HAIT $VO_{2\text{max}}$ adaptations in old compared to younger people, paper II found no differences in $VO_{2\text{max}}$ responses between age groups. This difference in results is probably due to a difference in matching young and old in the two studies. While in Wang et al (2014), the participants were matched for total
VO₂max, the participants in paper II were matched for VO₂max relative to age mean (Heyward 1998). This implies a more physically active old cohort in Wang et al (2014), compared with a less physical active old cohort in paper II.

The average age in paper I was 59±10 years. When comparing the results from paper I with the results from the two age cohorts representing the same average age in paper II, the average increase in VO₂max is 0.7% -and a 0.5% per training session in paper I and II respectively. The higher increase in paper I is probably due to the lower baseline VO₂max level. The influence of training status on total % improvement in VO₂max was shown in figure 3 in paper II. The average VO₂max level of 25.6±5.9 ml·kg⁻¹·min⁻¹ in paper I corresponds to approximately 75% of age mean VO₂max (Heyward 1998). When calculating the relationship between training status and % improvement per session in paper II, the regression formula can be used to calculate the VO₂max increase per session when starting at 75% of mean age VO₂max (figure 3 in paper II). The calculation shows that those starting at similar baseline level in paper II as paper I, increase their VO₂max by 0.83% per session. It could be speculated that the often-accompanying metabolic disturbances and higher use of medications after several years with T2D diagnosis may reduce the potential to improve VO₂max. However, there was no relationship between duration of T2D diagnosis and VO₂max adaptations. On the contrary, the relatively similar improvements found in the above calculations show the great- and even similar potential of improving CRF among sedentary persons with T2D as healthy individuals.

Still, the surprisingly lack of VO₂max improvement in MIT in paper I as well as in Hollekim-Strand (2014) could indicate that a higher minimum intensity level is needed to improve CRF among individuals with T2D compared to healthy individuals. However, possible differences in training induced VO₂max adaptations at moderate training intensities between T2D and healthy individuals need further investigations.

The tendency towards a slightly increased HRmax in the two oldest groups may indicate a possible HAIT induced response on HRmax, and it is interesting that this tendency is pointing in the opposite direction as the slightly reduced HRmax younger groups. This
indication of different autonomic and non-autonomic training responses in the heart between young and old has also been addressed in other studies (Zavorsky 2000, Wang et al 2014). The HRmax pre- to post measurements in paper I and II cannot be compared since the participants in paper I are more homogenous in age than paper II.

The increased VO$_{2\text{max}}$ led to increased work capacity in both paper I and II. This was shown as an increased treadmill velocity at LT in paper I and as an increased workload (watt and velocity) during the VO$_{2\text{max}}$ test in paper II.

5.2. HbA1c

In paper I, a 0.58% point reduction (from 7.78±1.39 to 7.19±1.10 %) was found in HAIT, while no difference was found in MIT. Few studies have investigated the effects of HAIT among individuals with T2D. However, Hollekim-Strand (2014) used a very similar training protocol as paper I. There was a similar number of participants, and the training intervention was performed as HAIT; 4· 4 min interval, 90-95% HR$_{\text{max}}$ 3 times per week versus MIT; home-based moderate exercise, 12 weeks duration). The results from HAIT in Hollekim-Strand et al (2014) are in accordance with the results in paper I, with HbA1c reduced with 0.4% points (from 7.0±1.2 to 6.6±0.9 %). However on the contrary to paper I, the HbA1c changes were not significantly different between the two exercise groups in Hollekim-Strand et al (2014). The larger baseline HbA1c levels may have caused the somewhat larger effect in paper I (7.78±1.39 % vs 7.0±1.2 %). The lack of improvement in both VO$_{2\text{max}}$ and HbA1c in MIT in paper I is in accordance with Hollekim-Strand et al (2014) who also found no changes in VO$_{2\text{max}}$ or HbA1c after moderate exercise in T2D. It should be noted that MIT in Hollekim-Strand et al (2014) was home-based and not supervised as in paper I.
A limitation to paper I was the lower baseline HbA1c in MIT compared to HAIT, which could, at least partly explain the differences HbA1c change between HAIT and MIT. It is likely that higher pre HbA1c values would lead to a greater decrease in HbA1c (Krook et al 2003, Snowling and Hopkins 2006, Revdal et al 2016), and in accordance to this, paper I showed a correlation between pre HbA1c levels and changes in HbA1c after exercise (R= 0.652, P<0.01). However, due to the significant difference in pre HbA1c levels between the two groups in paper I, a post hoc analyze was conducted where the HbA1c data was corrected for skewness. After this correction, there was no baseline difference in HbA1c between the two training groups. The corrected reduction of 0.47 % in HAIT in paper I is similar to the reduction found in HAIT after 12 weeks of exercise in Hollekim-Strand (2014) and after 6 months of continuous exercise at 85% HR\(_{\text{max}}\) in Hansen et al (2009).

Increasing VO\(_{2\max}\) will not necessarily reduce HbA1c among persons with T2D (Revdal et al 2016). A 10% increase in VO\(_{2\max}\) in Revdal et al (2016) did not reduce HbA1c levels after sprint-interval training of either 20 or 60 seconds bouts. Neither of the two groups reduced their HbA1c after 12 weeks of exercise despite of a 0.23 and 0.13 L·min\(^{-1}\) increase respectively in VO\(_{2\max}\). The greater increase in VO\(_{2\max}\) in HAIT in paper I (0.45 L·min\(^{-1}\)) compared to Revdal et al (2016) could partly explain the lack of HbA1c reductions in Revdal et al (2016) compared to paper I. Although paper I showed a relationship between improvement in VO\(_{2\max}\) and reduction in HbA1c (R= -0.52 p<0.01), SEE revealed that an increase of 0.44 L·min\(^{-1}\) was needed to predict reductions in HbA1c, and only 25% of the reductions in HbA1c could be related to increased VO\(_{2\max}\). Also, Hansen et al (2009) found 0.1% points and 0.2% points reduction after 2 months of exercise, and 0.2% points and 0.5% points after 6 months of low- to moderate (69% HR\(_{\text{max}}\)), and moderate- to high intensity (85% HR\(_{\text{max}}\)) continuous exercise, respectively. After 2 and 6 months VO\(_{2\max}\) were increased with 0.16 and 0.19 L·min\(^{-1}\) in low intensity, and 0.32 and 0.33 L·min\(^{-1}\) in moderate to high intensity, respectively. Although there were no significant differences in change between the two exercise groups in either
VO2\textsubscript{max} or HbA1c in Hansen et al (2009), it should still be noted that the improvements were almost twice as high in the moderate to high intensity group.

The positive adaptations after high-intensity exercise from these studies, as well as paper I, are in line with a meta-analysis by Boulé et al (2003) where exercise intensities during training ranged from \(\sim 50\% \text{VO}_2\text{max}\) to \(> 75\% \text{VO}_2\text{max}\) and exercise volume ranged between 8.75–24.75 MET-hours/week. The meta-analysis revealed an average of 11.8\% increase in \text{VO}_2\text{max}, and the exercise intensity predicted post intervention weighted mean difference in HbA1c (\(R = -0.91, P = 0.002\)) to a greater extent than exercise volume (\(R = -0.46, P = 0.26\)). Skeletal muscle plays a major role in whole-body glucose disposal. HAIT causes a larger degree of muscle fiber recruitment than MIT (Vøllestad and Blom 1985), with the potential following metabolic adaptations as mitochondrial biogenesis and increased GLUT4 content (Roberts et al 2013). Together with the faster depletion of the muscle glycogen stores during HAIT (Vøllestad and Blom 1985), these may be possible explanations to why HAIT seems to be more effective than MIT to improve glucose control.

The National Health and Nutrition Examination Survey showed that only 37\% of persons diagnosed with T2D achieved the treatment goal of < 7\% HbA1c (Saydah et al 2004). A recent study from Norway (Mouland 2014) revealed that 55\% achieved the treatment goal of < 7\% HbA1c. The 0.58\% points reduction in HbA1c in HAIT after only 12 weeks of exercise in paper I is thus a strong reduction. The HbA1c reduction in paper I also appears to be very similar to the effects found after long-term (> 12 weeks) medication (drug or insulin) treatment only (0.6-0.8\% points) (UKPDS 1998, Snowling and Hopkins 2006). This indicates the potential of HAIT to be an effective additive, or even a substitute treatment to medication to reduce T2D risk factors, highlighting the potential of HAIT to reduce the use of medications. However, a substantial increase in VO2\textsubscript{max} seems to be needed to improve HbA1c values, and the cause-effect relationship is not conclusive.
5.3. **FatOx; adaptations and testing reliability**

In paper I, no change was found in FatOx in MIT and there was only a tendency towards an improved FatOx in HAIT (from 0.368 to 0.420 g·min\(^{-1}\), \(p=0.065\)). However, the delta values in HAIT was significantly different from those in MIT. Research investigating the importance of CRF on FatOx during exercise differ in methodologies. Whether the studies are cross-sectional or longitudinal intervention studies, and whether FatOx has been measured at absolute or relative intensity, will influence the interpretation of the results. In addition, energy balance and the diets ´macronutrient composition could also influence FatOx during exercise (Coyle et al 2001, Patterson and Potteiger 2011). A decreased BW after an intervention indicates a negative energy balance and imply difficulty to draw strong conclusions on which training method is the most effective to improve FatOx. However, energy intake and expenditure was found to be balanced in both papers I and III. In paper III, only one day of diet manipulation revealed a large change in FatOx during exercise (31% lower FatOx after CHO rich diet compared to fat rich diet). This shows the importance of thorough diet control and registrations to reduce the confounding factor of change in diet composition. It should be noted that the manipulations were not extreme. Consuming extreme diets with a very high proportion of fat or CHO, is not realistic in a practical sense, and is probably not well tolerated for the participants. Therefore, we found it more realistic to explore RER differences between isocaloric diets containing high, yet realistic proportions of fat and CHO to contrast the FatOx responds to more normal dietary changes. To assess FatOx adaptations to diet manipulation, a longer standardization period (>2 days prior to testing) has been suggested to reduce intra-individual variability in FatOx (Croci et al 2014). However, although rigid pre-test standardizations may cause a higher internal validity, it may also be less realistic and translatable in a practical setting, and thus reduce the external validity. Thus, a shorter manipulation duration was chosen to achieve realistic variations in diet composition.
Several studies have found persons with higher VO$_{2\text{max}}$ to rely more on fat utilization when exercising at the same submaximal relative workload, than those with lower VO$_{2\text{max}}$ (Jansson and Kaijser 1987, van Loon et al 1999, Nordby et al. 2006; Stisen et al. 2006). Many of these studies are typical cross-sectional studies comparing well-trained endurance athletes with more sedentary individuals (Jansson and Kaijser 1987, van Loon et al 1999, Nordby et al 2006, Stisen et al 2006). This does not necessarily imply causal relationship between an increase in VO$_{2\text{max}}$ and increased FatOx. In addition, among the participants in paper III, no correlation was found between VO$_{2\text{max}}$ and FatOx. This could be due to the homogenous VO$_{2\text{max}}$ values (CV 9.8%), as well as a low number of subjects. A feasible explanation to the lack of significant improvement in FatOx in paper I could be the relatively short exercise duration compared to studies comparing well-trained individuals. Although VO$_{2\text{max}}$ increased with 21% in HAIT, it is still possible that the training duration was not sufficient to improve fat metabolism during exercise in paper I. Since previous studies have found higher FatOx during submaximal exercise among well-trained compared to untrained individuals in cross-sectional studies (Jansson and Kaijser 1987, van Loon et al 1999, Nordby et al. 2006; Stisen et al. 2006), it could be expected to find a correlation between VO$_{2\text{max}}$ and FatOx in a group of subjects with heterogeneous VO$_{2\text{max}}$ values. However, although pre VO$_{2\text{max}}$ values in paper I ranged between 14.6 to 38.9 ml·kg$^{-1}$·min$^{-1}$ (CV 23%), no correlation was found between pre VO$_{2\text{max}}$ and pre FatOx. This could be due to the sedentary characteristics of the subjects compared to the long training adaption period among well-trained individuals. Therefore, a correlation analysis between VO$_{2\text{max}}$ post (ml·kg$^{-1}$·min$^{-1}$) and FatOx post was conducted. The rationale for doing this correlation is that the sedentary characteristics of the participants in paper I shown as low CRF values will make other factors than training status of more importance to rate FatOx during exercise compared to more physically active individuals. It will thus be of interest to explore how moving from a sedentary group towards being a “physically active group” influences FatOx. A significant correlation between post VO$_{2\text{max}}$ and post FatOx was found (ml·kg$^{-1}$·min$^{-1}$; R=0.48, p<0.01, L·min$^{-1}$; R=0.69, p<0.01) in paper I, indicating a positive training
adaptation to FatOx. The difference in FatOx from pre to post also correlated with the difference in VO₂max from pre to post (ml·kg⁻¹·min⁻¹; R=0.42, p<0.01, L·min⁻¹; R=0.45, p<0.01). A correlation between VO₂max level and FatOx during exercise was also found in Suk et al (2015) in 12 Korean women. Yet, the interpretation of the correlation in paper I should be interpreted with caution since only a tendency to increased FatOx was found in HAIT, and since R² indicates that only 23% of FatOx variations in paper I can be explained by VO₂max. In addition, the reliability test in paper III revealed that significant FatOx adaptations to training or diet interventions must exceed a 5% difference if the stimulus are to be regarded effective on FatOx. The tendency of improved FatOx after HAIT in paper I equals a 14% improvement, indicating that 9% of the improvement could be due to the exercise intervention.

Regarding longitudinal studies exploring FatOx adaptations after training interventions, these studies also differ in testing protocols. Many studies have tested FatOx at the same absolute workload pre and post an exercise intervention (Hurley et al 1986, Talanian et al. 2007; Perry et al. 2008), while other studies have tested at the same relative workload (Friedlander 1998, Alkahtani et al 2013), and a few have tested at both absolute- and relative intensity (Carter et al 2001). An exercise-induced increase in VO₂max will consequently mean a decreased %VO₂max, a lower RER and thus increased FatOx (g·min⁻¹) at post-test when measuring FatOx at the same absolute intensity pre- and post training intervention. Circulating hormone levels might also differ when assessing FatOx at the same absolute workload pre- and post-exercise (Deuster et al. 1989). In accordance with paper I, a lack of significant improvement in FatOx have also been found in other studies, which similar to the FatOx protocols in paper I and III have tested FatOx at relative intensity (Friedlander et al 1998, Carter et al 2001). However, Alkahtani et al (2013) found an increased FatOx measured at a relative intensity (45% VO₂max) after both moderate and high-intensity training. Yet, unlike paper I, there was no significant difference between the two exercise groups. Similar to paper I, Alkahtani et al. (2013) investigated the effects of interval training ≥ 90% HRpeak (30 sec at 90%VO₂max and 30 sec rest x 45) compared to moderate training (5 min cycling stages at
45%VO$_{2\text{max}}$). Noteworthy, the participants were overweight/obese men, and had low FatOx rates (between 0.10 ±0.10 and 0.13 ±0.07 g·min$^{-1}$ at 45 % VO$_{2\text{max}}$). Thus, it cannot be excluded that a possible higher potential to increase FatOx may explain the difference in FatOx adaptations.

The rate of FatOx reaches a peak at 50-60% of VO$_{2\text{max}}$ after which the contribution of fat is reduced in both relative and absolute terms (Sahlin et al 2008). Thus, lower exercise intensity that achieves FatOx$_{\text{max}}$ has been suggested for improving FatOx (Suk et al 2015). Bordenave et al (2008) found increased FatOx after exercising at an intensity close to FatOx$_{\text{max}}$ among 11 individuals diagnosed with T2D. In contrary to Bordenave et al (2008), the MIT group in paper I did not improve FatOx although exercising at an intensity closer to FatOx$_{\text{max}}$ (~56% VO$_{2\text{max}}$). It should be noticed that Bordenave et al (2008) did not measure VO$_{2\text{max}}$, and the FatOx measurements were performed at different percentages of watt max during cycling.

Another plausible explanation to the lack of improvement in FatOx in paper I could be already established metabolic disturbances in substrate utilization. Yet, the level of FatOx during exercise in paper I was not different from that found among the healthy adults in paper III. It must be noted that while the exercise mode in paper III was cycling, the exercise mode in paper I was walking or running. According to Achten and Jeukendrup (2004), FatOx is higher in walking and running than in cycling. Additionally, the participants in paper III had lower BW compared to the participants in paper I as well as a higher VO$_{2\text{max}}$. All the participants in paper III were females. The FatOx level in paper III was not different from the FatOx level in paper I either when comparing with only the female participants (0.392±0.083 vs 0.350±0.072 g·min$^{-1}$, p>0.2) in paper I, or with all the participants in paper I (0.392±0.083 vs 0.355±0.089 g·min$^{-1}$, p>0.2). CV were also similar between paper I and III (21% and 20%, respectively). The participants in paper I were slightly obese (BMI; 31.7±4.5). Earlier research have found reduced FatOx capacity among obese individuals with or without T2D (Blaak et al 2000, Hickner et al 2001, Pérez-Martin et al 2001). Recent studies also indicate that FatOx during exercise differ
between obese and non-obese T2D patients (Blaak et al 2000, Borghouts et al 2002) and between T2D patients and healthy controls (Suk et al 2015). Mogensen et al (2009) found similar FatOx capacity during exercise between obese persons with T2D and obese controls. However, in contrast to paper I, Mogensen et al (2009) found a high capacity for increasing lipid oxidation in response to aerobic training in both groups. After 8 weeks of moderate aerobic training (63±1% of VO$_{2\text{max}}$), the magnitude of FatOx$_{\text{max}}$ in T2D (0.43 g·min$^{-1}$) and obese controls (0.45 g·min$^{-1}$) were similar to that observed in endurance-trained, healthy young subjects (0.46 g·min$^{-1}$) (Nordby et al 2006). The pre FatOx$_{\text{max}}$ level (0.28 g·min$^{-1}$) in Mogensen et al (2009) were lower than pre FatOx at 65% VO$_{2\text{max}}$ in paper I (0.36 g·min$^{-1}$).

In paper I, no difference in RER measurements from pre to post-test in either of the exercise groups were found during FatOx measurements, meaning that the relative contribution of FatOx to total energy expenditure was unchanged. Thus, the increased VO$_{2\text{max}}$ and consequently the higher absolute workload (increased treadmill speed) at post-test is a likely explanation for the tendency towards an increased absolute FatOx in HAIT. Mitochondrial adaptations and changes in gene expression of FA transporters might also have influenced the results (Achten and Jeukendrup 2004, Bordenave et al 2008, Melanson et al 2009) but this was not measured.

Previous researches have suggested that FatOx among women contributes more to oxidative metabolism than among men (Tarnopolsky 2000, Carter et al 2001). However, paper I showed no difference in either RER values or FatOx between the male and female participants (0.361±0.113 and 0.350±0.072 g·min$^{-1}$, 0.82±0.04 and 0.82±0.11 in male and female respectively). These results are thus in accordance with Roepstorff et al (2002) where similar RER and leg respiratory quotient were found between females and males exercising at 58% VO$_{2\text{max}}$. 

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5.4. Anthropometrics

In paper I, both HAIT and MIT showed small improvements in body composition, although only HAIT reduced BW and BMI significantly (-1.7 kg and -0.6 kg·m⁻², respectively) and different from MIT. Both HAIT and MIT showed small reductions in BF, waist circumference and hip circumference, and with no differences between the groups. The changes in body composition in both groups in paper I are similar to other exercise interventions with no diet restrictions (Giannopoulou 2005, Hansen et al 2009, Hollekim-Strand et al 2014, Revdal et al 2016). Persons with T2D who are overweight or obese are recommended to accomplish a weight loss of at least 5% (Helsedirektoratet 2009). The relatively small changes in body composition in HAIT may thus still be of clinical importance in a longer perspective to increase metabolic health among individuals with T2D. The results from HAIT are thus in accordance with other studies which have found similar or greater body composition improvements after high-intensive training compared to energy cost matched moderate-intensive training (Tjønna et al 2008, Karstoft et al 2013), indicating that at a given energy cost of exercise, high-intensity training is at least as effective as moderate-intensive exercise to improve body composition.

In paper II, only BW and BMI were measured, and no changes were found from pre to post in any of the age groups. It is well known that restrictions in energy intake is needed to give effective fat loss (Verheggen et al 2016), and since the participants in both paper I and II maintained their normal diet habits, the minor improvements in body compositions were expected. The BMI values were also different between paper I and II (31.7±4.5 versus 26.1±4.0 kg·m⁻²), which implies a better potential to reduce BMI in paper I compared to paper II.

Studies comparing effects on BF after different training intensities are inconclusive, as some studies indicate better improvements in body composition after high-intensive
exercise (Tremblay et al 1994, Trapp et al 2008, Karstoft et al 2013), while others have found better effects after moderate exercise (Keating et al 2014), similar effects (Tjønna et al 2008) and no effects in any of the programs (Alkahtani et al 2013). Adaptations after different training protocols may be influenced by different energy cost of the two regimes. Like in paper I, Tjønna et al (2008), Karstoft et al (2013), Keating et al (2014), and Alkahtani et al (2013) matched the energy cost of exercise in the two different exercise protocols. Interestingly, Tremblay et al (1994) showed that 15 weeks of high-intensity exercise gave a greater decrease in %BF measured as the sum of 6 skinfolds compared to 20 weeks of continuous moderate aerobic exercise, although the total energy cost of the moderate program was more than twice than that of the HIIT (120.4 vs 57.9 MJ). When corrected for the energy cost of training, the decrease in the sum of six subcutaneous skinfolds induced by the high-intensive training program was nine fold greater than the moderate-intensive program. Possible explanations to the positive adaptations after HAIT may be positive intra-muscular enzymatic responses (Brooks and Mercier 1994), increased post-exercise energy expenditure and increased FatOx the hours after exercise (Pillard et al. 2010). However, these factors were not measured in paper I.

5.5. Blood pressure

Only paper I measured BP and BLP. MIT reduced both systolic (-12 mmHg) and diastolic BP (-8 mmHg), while HAIT reduced diastolic BP (-6 mmHg). The BP reductions in paper I is in accordance with Fagard (2001) who found slightly hypertensive to reduce BP by -7.4/-5.8 mmHg systolic and diastolic respectively. Physical inactivity as a single factor is believed to be responsible for 5% to 13% of hypertension (Samadian et al 2016). Review articles and meta-analyzes evaluating the effects of physical exercise on blood pressure underpin the importance to reduce BP to decrease metabolic risk factors among
The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors; testing procedures and training interventions.

Hypertensive individuals (Cornelissen and Smart 2013; Pescatello et al 2004). A 10 mmHg reduction in systolic BP was in Adler et al (2000) associated with a 15% reduction for deaths related to diabetes, 11% reduction for myocardial infarction, and a 13% reduction for microvascular complications. The reduction in BP in paper I has thus likely reduced the risk of common T2D complications. As BP is determined by the cardiac output (stroke volume multiplied by the heart rate) and the total peripheral resistance of the blood vessels, exercise may act by improving endotel function of the blood vessels (Sabbahi et al 2016). There are no strong conclusions on whether the intensity of aerobic exercise is of importance to reduce BP (Whelton et al 2002). In paper I, only MIT revealed significant improvements in both systolic and diastolic BP, but there was no significant difference in change between MIT and HAIT. This is in accordance with a meta-analysis of RCTs investigating the effects of aerobic exercise on BP (Whelton et al 2002). Whelton et al (2002) found that the decrease in BP after aerobic exercise did not significantly differ between different training modes regarding frequency or intensity of exercise. Although many studies have shown positive BP adaptation after aerobic exercise (Whelton et al 2002), the effectiveness of aerobic exercise to reduce systolic and diastolic BP among T2D is still debated as exercise interventions show contradictive results (Dobrosielski 2012, Cornelissen and Smart 2013, Colberg et al 2010). Recent statements from the ACSM and ADA (2010) summarize that exercise may lead to a reduction in systolic BP, while decreases in diastolic BP are less common among T2D (Colberg et al 2010). However, paper I found reductions in diastolic BP in both MIT and HAIT.


Paper I showed improvements in BLP only in the MIT group, shown as an increase in HDL (from 1.24±0.38 to 1.33±0.38 mmol·L⁻¹, p< 0.05) and a decrease in triglycerides.
Diabetic dyslipidemia is a modifiable risk factor and an essential part of preventing CVD among T2D (Wilson et al 1998, Maron 2000, Daniel 2011). Maron et al (2000) suggests that in a group with already established metabolic disturbances, every 0.026 mmol ·L⁻¹ increase in HDL is associated with 2-3% reduction in risk of CVD (Maron 2000).

The changes in BLP were not significantly different between the groups, and neither MIT nor HAIT led to any changes in LDL. In paper I, both groups had baseline LDL levels slightly above the recommended 2.5 mmol·L⁻¹ treatment goal (Daniel 2011), but none of the groups changed their LDL level. This finding is in accordance with other studies (Trejo-Gutierrez and Fletcher 2007) and may be due to the lack of diet restrictions.

Earlier research have suggested a minimum of exercise duration per session to be needed to find an effect on HDL after exercise (Durstine et al 2001, Kodama et al 2007). In a meta-analysis by Kodama et al (2007) investigating the effect of aerobic exercise on HDL, exercise duration per session was more important than frequency and intensity. The lower total exercise volume in HAIT compared to MIT could thus have influenced the lack of HDL change in HAIT. However, research have also found positive effects on BLP after more extensive exercise, in terms of both more volume and higher intensity (Kraus et al 2002), while other studies indicate a relatively modest effect of exercise on both BP and BLP (Leon et al 2001, Whelton et al 2002). In Kodama et al (2007), exercise was also more effective among the individuals with initially lower body mass index. In paper I, there was a tendency to a negative relationship between initial BMI and HDL changes (R=-0.30, p=0.07) and a negative correlation between baseline BF% and change in triglycerides (R=-0.37, p<0.05). Although speculative, this might indicate that it is more difficult for obese subjects to improve BLP by exercise only, and that weight loss by diet restrictions is needed to effectively improve BLP.

According to the ACSM position stand on exercise and T2D (2010), many studies have failed to find any effects of exercise on either cholesterol or triglycerides from exercise alone. However, as emphasized in Kelley et al (2004), many factors might influence the
exercise effects on BLP as well as other risk factors measured, and many of these are underreported in previous studies. Variables such as control of diet, ethnicity, use of medications, the effect of pre values, changes in body weight and fitness, as well as the feasibility of the participants to perform the exercise protocol may all be of importance, and were all accounted for in paper I.

5.7. Potential risks in performing HAIT

Recommendations of high-intensity aerobic exercise should be evaluated against the potential increased risk of adverse responses, which may be relevant for individuals with cardio metabolic diseases (Levinger et al 2015). A five times higher rate of cardiac arrest has been found during high-intensive exercise compared to moderate-intensive exercise (Rognmo et al 2012). It should be noticed that the event rates during exercise interventions still are generally very low (1 per 23 182 hours during or after high-intensity exercise), and most of the events are mild in nature (Wisløff et al 2007, Freyssin et al 2012, Levinger et al 2015). However, a systematic review and meta-analysis of Dahabreh et al (2011), found that episodic physical activity was associated with a substantial increased risk of acute myocardial infarction (AMI) and sudden cardiac death (SCD) among physically inactive individuals. In contrast, the same study also revealed that for every additional exercise bout per week, there was 30% reduction in the risk of SCD and 45% reduction in the risk of AMI during physical activity (Dahabreh et al 2011).

Individuals with T2D have a higher risk of CVD, and should therefore undergo a medical screening before starting with HAIT. Exercise sessions should also be, at least in the beginning, supervised by experienced exercise physiologists. It has also been recommended that inactive persons start with a period of moderate exercise before starting with HAIT (Levinger et al 2015). Indisputably, the success of an exercise
intervention depends on adherence to exercise also after the intervention has ended. Few studies have investigated long-term effects after training interventions. The long-term adherence to exercise thus also relies on the enjoyment of the activity being prescribed. More studies on the safety, sustainability and adherence to HAIT in a non-supervised, homebased environment are needed, as well long-term investigations.
6. Limitations

The participants in paper I were matched in physical fitness, age, BMI and duration of diagnosis. However, baseline HbA1c values turned out to be significantly lower in MIT, which probably has affected the results. Some of the age groups in paper II had a small sample size (n). Paper III also had a quite small sample size. In addition, paper I and II did not have non-exercising control groups. The study design of paper I, II and III may thus be repeated with; a) similar HbA1c values in the two exercise groups in paper I b) with additional control groups and c) greater sample size in the oldest age group in paper II, as well a higher n in total in paper III.

A change in daily activity patterns during the intervention could have influenced the adaptations to a specific exercise regime. However, the participants in our studies registered their exercise during the interventions and were told to maintain their pre intervention habitual physical activity. However, we cannot be sure that there were not any differences between groups or changes within each group in daily activity patterns since they did not use e.g. accelerometers.

An accurate diet control was particularly important in paper I and III due to its relevance and influence on FatOx assessment reliability and the potential exercise adaptations on FatOx, HbA1c, body composition and BLP. Due to practical reasons, we chose to register energy intake by using 1g accurate food scales and focused on as equal diets as possible the two days before- and during test-days. Some studies have prepared individually standardized meals given directly to the participants prior- and/or during test-days, which may increase the internal reliability but potentially decrease the validity of a habitual diet.

The FatOx measurements in paper I and III are whole body measurements that do not reflect metabolism only in the skeletal muscle. Local measurements of FatOx in the active muscle during exercise can give more accurate information about substrate
Støa: The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors; testing procedures and training interventions.

utilization during exercise, but this is a more time, cost, - and equipment demanding study design.
7. Conclusions and future perspectives

The result from the papers in this dissertation show a) HAIT to be an excellent exercise method to improve CRF and reduce risk factors associated with T2D b) the great potential of HAIT as a training strategy to improve CRF independently of age, and c) that measuring FatOx using indirect calorimetry is a reliable method as long as the diet is strictly controlled for.

Metabolic health is a multifactorial construct including variables like CRF (VO$_{2\text{max}}$), CHO and fat metabolism, BP, BLP, body composition, chronic disease morbidity and all-cause mortality (Pate 1995). What kind of exercise needed to improve each of these specific health benefits among T2D are still debated, with intervention studies still showing, at least to some extent, contradictory results about the importance of intensity. Although paper I showed HAIT to be an effective strategy to improve other metabolic risk factors in addition to VO$_{2\text{max}}$, more research is required in this area. No studies have earlier compared the VO$_{2\text{max}}$ adaptations after HAIT between different age groups matched for physical fitness. Therefore, more studies are needed to further investigate and confirm the results from paper II. However, the VO$_{2\text{max}}$ results in paper I and paper II are in accordance with the increasing number of studies revealing aerobic capacity to be more important than volume of exercise to reduce risk factors associated with cardio vascular diseases (CVD). Since CRF per se seems to be a stronger determinant of CVD than physical activity level, and low CRF is an independent predictor of all-cause mortality among T2D (Wei et al 1999), this should be taken more into consideration in the future when designing exercise prescriptions and establishing physical activity guidelines.
Støa: The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors; testing procedures and training interventions.
8. Clinical and Practical implications

Despite the increasing amount of public information on the benefits of exercise training, exercise as a strategy to prevent and treat metabolic risk factors associated with T2D, this strategy still seems to be underused. Up to 77% of adult individuals with T2D do not meet the physical activity recommendations (Speight 2012). Earlier research has emphasized the importance of giving specific instructions about type, amounts and intensity of exercise needed for health benefits rather than general exercise recommendations (Stewart 2004). Through education about specific training adaptations and by transferring the effective training principles into a person’s everyday life and personal environment, the results from the present studies contribute to the knowledge about how to provide a more individualized and effective exercise prescription. This perspective of specific training recommendations may provide a higher level of long-term training compliance and a more effective reduction of metabolic risk factors.

The most cited barrier to exercise is “lack of time” (Stutts 2002), and adherence to exercise seems to be low (Robison 1994). The shorter bursts of activity characterizing HAIT may be a more enticing option than continuous exercise for an extended period of time (Weston et al 2014). The potential of a reduced training time compensated with an increase in exercise intensity among T2D have thus recently been investigated with very good results on metabolic risk factors (Gibala et al 2012, Hawley and Gibala 2012, Terada et al 2013, Revdal et al 2016). Most of these studies have used training interventions with low volume short duration-, and sprint intervals characterized by mainly anaerobic work (HIIT). It should be noted, that sprint intervals are physically and mentally demanding, and it can be challenging to assess the right intensity. It may also be less safe among T2D to perform supramaximal bouts of exercise (Levinger et al 2015). Also, the distinctive character of a lower total energy expenditure during typical sprint sessions may not be favorable among overweight T2D. Although HAIT has longer interval
durations (~4 min), the intensity of 85% HR_{max} might be easier to accomplish and is still time-efficient. These intervals can also easily be performed during e.g. walking, running, bicycling or cross-country skiing. High-intensity interval training interventions are most often defined HIIT independently of the duration and intensity of the work bouts. To make it clearer whether the intervals are characterized by mainly aerobic or anaerobic work, the term HAIT was suggested in paper I to include the word “aerobic” in the term. Due to the very different characteristics between sprint intervals and aerobic intervals, future studies should be more precise about the use of proper terms.

Paper I and II indicate that the most sedentary individuals may exhibit the largest gain in CRF after HAIT. Notably, the VO_{2max} adaptations found in paper I and II in this dissertation are in accordance with several recent studies investigating the effects of high-intensity interval training among both healthy individuals and persons with different chronic diseases (Wang et al 2014, Weston et al 2014, Levinger et al 2015, Ramos et al 2015). Taken together, improving VO_{2max} seem to prevent chronic lifestyle diseases, improve the prognosis of persons with already established chronic diseases, as well as improving functionality among older due to its multiple effects on cardiovascular health. If participants are adequately screened and the program is prescribed and supervised by appropriately trained and qualified personals, then HAIT should be an achievable and recommended training option among most individuals.
9. References


Støa: The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors; testing procedures and training interventions.


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Article I

High-intensity aerobic interval training improves aerobic fitness and HbA1c among persons diagnosed with type 2 diabetes

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Abstract

Purpose  It remains to be established how high-intensity aerobic interval training (HAIT) affects risk factors associated with type 2 diabetes (T2D). This study investigated effects of HAIT on maximal oxygen uptake (VO$_{2\text{max}}$), glycated Hemoglobin type A1C (HbA1c), insulin resistance (IR), fat oxidation (FatOx), body weight (BW), percent body fat (%BF), lactate threshold (LT), blood pressure (BP), and blood lipid profile (BLP) among persons with T2D. Results were compared to the effects after a moderate-intensity training (MIT) program.

Methods  Thirty-eight individuals with T2D completed 12 weeks of supervised training. HAIT consisted of 4×4 min of walking or running uphill at 85–95% of maximal heart rate, and MIT consisted of continuous walking at 70–75% of maximal heart rate.

Results  A 21% increase in VO$_{2\text{max}}$ (from 25.6 to 30.9 ml kg$^{-1}$ min$^{-1}$, $p<0.001$), and a reduction in HbA1c by −0.58% points (from 7.78 to 7.20%, $p<0.001$) was found in HAIT. BW and body mass index (BMI) was reduced by 1.9% ($p<0.01$). There was a tendency towards an improved FatOx at 60% VO$_{2\text{max}}$ (14%, $p=0.065$). These improvements were significant different from MIT. Both HAIT and MIT increased velocity at LT, and reduced %BF, waist circumference, hip circumference, and BP, with no significant differences between the two groups. Correlations were found between change in VO$_{2\text{max}}$ and change in HbA1c when the two intervention groups were combined ($R=-0.52$, $p<0.01$).

Conclusion  HAIT is an effective exercise strategy to improve aerobic fitness and reduce risk factors associated with T2D.

Keywords  Exercise intensity · Interval training · Maximal oxygen uptake · HbA1c · Fat oxidation

Abbreviations

%BF  Percent body fat
BG  Blood glucose
BMI  Body mass index
BP  Blood pressure
BLP  Blood lipid profile
BW  Body weight
CHO  Carbohydrate
CRF  Cardiorespiratory fitness
CV  Coefficient of variance
CVD  Cardiovascular disease
FatOx  Fat oxidation
GI  Glycemic index
HAIT  High-intensity aerobic interval training
HbA1c  Glycated hemoglobin type A1C
HOMA-IR  Homeostasis model of assessment for insulin resistance index
HR$_{\text{max}}$  Maximal heart rate
HR$_{\text{peak}}$  Peak heart rate
KCAL  Kilo calories
KJ  Kilo joules
[La−]b  Blood lactate concentration
MIT  Moderate-intensity continuous training
POX  Protein oxidation
RER  Respiratory exchange ratio
T2D  Type 2 diabetes
TEI  Total energy intake
VCO2  Volume of carbon dioxide
VO2  Oxygen uptake
VO2max  Maximal oxygen uptake

Introduction

Type 2 diabetes (T2D) is recognized as a cause of premature mortality, and is related to several severe medical conditions, such as cardiovascular disease (CVD), neuropathy, retinopathy, and kidney disease (American Diabetes Association 2016). Exercise is one of the cornerstones of both treatment and prevention of T2D (Carroll and Duffield 2004; Colberg et al. 2010). A reduced cardiorespiratory fitness (CRF) expressed as a low maximal oxygen uptake (VO2max) is related to a higher risk of developing CVD, obesity, and reduced glycemic control (McMurray et al. 1998; Bertoli et al. 2003; Solomon et al. 2015). Individuals with T2D have a reduced aerobic exercise capacity compared to healthy age-matched controls (Regensteiner et al. 1995; Kunitomi et al. 2000). Aerobic exercise has been well established as an intervention to improve aerobic capacity among persons with T2D (Boulé et al. 2003), and an increased VO2max can thus be beneficial to reduce T2D-associated risk factors.

Aerobic exercise has traditionally been used and prescribed as an effective and suitable mode of exercise to prevent and treat T2D (Pedersen and Saltin 2006; Colberg et al. 2010). The American Diabetes Association (2016) recommends 150 min per week of moderate-intensity aerobic (50–70% HRmax) exercise spread over at least 3 days/week with no more than 2 consecutive days without exercise. In training interventions, moderate-intensity training (MIT) is typically referred to as an intensity between 70 and 85% of maximal heart rate (HRmax) (Tjønna et al. 2008), while high-intensity aerobic interval training (HAIT), most often refers to intensities between 85 and 95% HRmax (Helgerud et al. 2007). Previous research have shown that HAIT results in greater increases in VO2max than aerobic exercise at lower intensities among healthy young individuals (Helgerud et al. 2007), patients with heart failure (Wisløff et al. 2007), individuals with metabolic syndrome (Tjønna et al. 2008), and persons with T2D (Hollekim-Strand et al. 2014). The increase in VO2max after HAIT is typically 0.3–0.7% per training session in interventions with a duration of 8–12 weeks (Rognmo et al. 2004; Østerås et al. 2005; Helgerud et al. 2007; Hollekim-Strand et al. 2014; Wang et al. 2014; Støren et al. 2016). Although HAIT has been shown to give large improvements in VO2max (Rognmo et al. 2004; Helgerud et al. 2011; Wisløff et al. 2007; Støren et al. 2016), few studies have explored the physiological adaptations after HAIT among T2D patients (Hollekim-Strand et al. 2014).

Over the last decade, there has been an increased focus on how different training modes affect physiological adaptations such as VO2max, fat oxidation (FatOx), blood pressure (BP), blood lipid profile (BLP), anthropometrics, and glycated hemoglobin type A1c (HbA1c) (DiPietro et al. 2006; Kodama et al. 2007; Hansen et al. 2009; Segerstrøm et al. 2010; Hollekim-Strand et al. 2014; Revdal et al. 2016). Most of these studies have investigated the effects after moderate-intensity exercise. Several studies and reviews have also revealed positive results on one or more of these T2D-related risk factors after low-volume sprint intervals characterized by mainly anaerobic work (Gibala et al. 2012; Hawley and Gibala 2012; Terada et al. 2013; Revdal et al. 2016). The importance of training intensity is still debated, and reviews underpin the uncertainty about whether intensity and/or volume of exercise are most important to improve glycemic control and other variables related to T2D (Boulé et al. 2003; van Dijk and van Loon 2015).

The aim of this study was to investigate if HAIT is a more effective training strategy than moderate-intensity exercise to reduce important risk factors among individuals with T2D.

Methods

Participants

Thirty-eight sedentary overweight individuals (23 females, 15 males) diagnosed with T2D completed the 12 weeks training intervention. Average VO2max values for men and women were 27.7±7.3 and 24.4±4.2 ml kg−1 min−1, respectively. Subject characteristics in the two exercise groups are shown in Table 1.

Eligible volunteers for study participation were individuals diagnosed with T2D, aged between 20 and 70 years, and no medical contra-indications for testing and training. The exclusion criteria were medical contradictions to physical testing and exercise according to the ACSM guidelines, sickness for a minimum of 2 consecutive weeks the last month prior to testing, illness during the last week prior to physical testing, diseases or injuries lasting more than 1 week during the 12 weeks intervention period, change in diet habits, and less than 75% of the training sessions
Table 1 Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>MIT (N=19)</th>
<th>HAIT (N=19)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis (years)</td>
<td>6 ± 5</td>
<td>9 ± 7</td>
<td>0.073</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59 ± 10</td>
<td>59 ± 11</td>
<td>0.745</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170 ± 6</td>
<td>172 ± 6</td>
<td>0.146</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>89.1 ± 15.6</td>
<td>95.0 ± 15.3</td>
<td>0.250</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>31.1 ± 4.5</td>
<td>32.0 ± 4.7</td>
<td>0.564</td>
</tr>
<tr>
<td>BF (%)</td>
<td>33.2 ± 7.6</td>
<td>33.1 ± 7.6</td>
<td>0.962</td>
</tr>
<tr>
<td>VO₂max (ml kg⁻¹ min⁻¹)</td>
<td>25.8 ± 5.5</td>
<td>25.6 ± 6.2</td>
<td>0.934</td>
</tr>
<tr>
<td>VO₂max (L min⁻¹)</td>
<td>2.29 ± 0.61</td>
<td>2.39 ± 0.55</td>
<td>0.571</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.84 ± 0.88</td>
<td>7.78 ± 1.39</td>
<td>0.020*</td>
</tr>
<tr>
<td>HOMA2-IR##</td>
<td>1.83 ± 0.73</td>
<td>1.76 ± 0.94</td>
<td>0.825</td>
</tr>
<tr>
<td>FatOx (g min⁻¹)</td>
<td>0.341 ± 0.083</td>
<td>0.368 ± 0.095</td>
<td>0.371</td>
</tr>
<tr>
<td>SystBP (mmHg)</td>
<td>160 ± 20</td>
<td>160 ± 22</td>
<td>0.984</td>
</tr>
<tr>
<td>DiastBP (mmHg)</td>
<td>86 ± 12</td>
<td>87 ± 9</td>
<td>0.621</td>
</tr>
<tr>
<td>Triglycerides (mmol L⁻¹)</td>
<td>1.58 ± 0.78</td>
<td>1.66 ± 0.79</td>
<td>0.822</td>
</tr>
<tr>
<td>HDL (mmol L⁻¹)</td>
<td>1.24 ± 0.38</td>
<td>1.09 ± 0.34</td>
<td>0.611</td>
</tr>
<tr>
<td>LDL (mmol L⁻¹)</td>
<td>3.05 ± 0.64</td>
<td>2.98 ± 0.72</td>
<td>0.777</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation

MIT moderate training intensity group, HAIT high-intensity aerobic interval training group, BW body weight, BF body fat percentage, BMI body mass index, VO₂max maximal oxygen consumption, HbA1c glycated hemoglobin type A1C, HOMA2-IR homeostatic model assessment of insulin resistance, FatOx fat oxidation, Syst. BP systolic blood pressure, Diast. BP diastolic blood pressure, LDL low-density lipoproteins, HOMA-IR The homeostatic model assessment of quantifying insulin resistance

*p < 0.05 different from MIT, **p < 0.01 different from pre-value.

The participants were recruited from the local community through regional newspaper advertisement, local medical offices and hospitals, local rehabilitation centers, and information folders at public places. The study is a non-randomized study, as the two different training interventions started at two different time points with 5 months in between. However, the participants did not know which training protocol they were recruited to before volunteering. The specific training intensity was given after the subjects volunteered to the study. Thorough oral and written information about the purpose and possible risks of the intervention were given to the subjects before they volunteered to participate. The two groups were matched in age and physical activity level (Table 2).

Fifty-nine volunteered for the study and 43 were included (Fig. 1). The subjects underwent medical examination by a physician including an electrocardiogram examination prior to inclusion. All participants refrained from exercise for at least 24 h before blood samples were taken. All the participants were Norwegians with Scandinavian origin, except from two individuals who were of African origin (one in HAIT and one in MIT). A medical consult with the same physician was performed during both assessment days. The subjects gave a written informed consent to participate in the study. The Regional Committees for Medical Research Ethics—South East Norway approved the study (2010/3016), and all the procedures undertaken in the study is in accordance with the principles outlined in the Declaration of Helsinki. The study is registered in the clinical trial registry ISRCTN.
Training protocol

The HAIT group conducted high-intensity aerobic interval training of 4 × 4 min at an intensity between 85–95% HR_{peak}. The MIT group conducted continuously moderate work at 70–75% HR_{peak}. All exercise sessions were supervised and carried out as walking or running in an outdoor environment. Both HAIT and MIT trained three times per week, and all training sessions were monitored. The HAIT and MIT training protocols were matched for total work, and the equations made to calculate %HR_{peak} to %VO_{2max} are based on the formula:

\[ \%HR_{peak} = 0.6463 \times \%VO_{2max} + 37.182 \] (Swain et al. 1994).

The matching of energy cost during exercise was calculated using the participants’ mean VO_{2max} values. Mean VO_{2max} was 2.34 L min⁻¹. The HAIT session started with ~15 min warm up at ~52% VO_{2max} (70% HR_{peak}), followed by 4 × 4 min at ~82% VO_{2max} (90% HR_{peak}) with 3-min recovery between intervals at ~52% VO_{2max}, and ~12 min cool down at ~52% VO_{2max}. This amounts 36 min at 52% VO_{2max} and 16 min at 82% VO_{2max}. 82% VO_{2max} would imply a mean O₂ expenditure of 1.92 L min⁻¹, a RER of ~0.88, and an energy cost of ~4.89 kcal L⁻¹ O₂ consumption (McArdle et al. 2010). 52% VO_{2max} means an average O₂ expenditure of 1.22 L min⁻¹, an RER of ~0.80, and an energy cost of ~4.80 kcal L⁻¹ O₂ consumption. The equation would therefore be 1.92 L × 4.89 kcal L⁻¹ × 16 min = 150 kcal, and 1.22 L × 4.80 kcal L⁻¹ × 36 min = 211 kcal. This means 361 kcal for each HAIT session lasting ~52 min. The MIT training consisted of continuous work at ~56% VO_{2max} (73% HR_{peak}, RER ~0.83, 4.84 kcal L⁻¹ O₂ consumption). To find the same energy cost as for HAIT, the duration was calculated as \( x \) min × 0.56 × 2.34 L × 4.84 kcal L⁻¹ O₂ consumption = 361 kcal, giving an \( x \) of ~57 min. The MIT group exercised for ~60 min, since a few minutes were added in order to gradually reach steady-state heart rate representing 70–75% HR_{peak} and to ensure that energy cost in HAIT did not exceed the energy cost in MIT. The training protocol is similar to other studies (Helgerud et al. 2007; Tjønna et al. 2008).

Before the training started, all subjects learned how to use a polar heart rate monitor to ensure the right training intensity. They were also given thorough instructions about how to register duration, average heart rate, and time in their specific individual intensity zones of either 85–95% HR_{peak} or 70–75% HR_{peak}.

Anthropometric measurements

Height measurements were collected using a wall-mounted measuring tape. Body weight was measured on a Tefal Sensitive Computer scale (Pp 6010, France) that was calibrated before the test period. The participants’ heights were measured with a wall-mounted measuring tape. Their body weights were measured on a Tefal Sensitive Computer scale (Pp 6010, France) that was calibrated before the test period. %BF was calculated based on five-site skinfolds (triceps, chest, abdomen, suprailiac, and thigh) with a Harpenden skinfold caliper (Saehan Medical Skinfold Caliper, SH5020, Korea). Waist and hip circumferences were measured using a measuring tape. Waist circumference was measured between iliac crest and the lowest rib, while hip measurements were taken at the widest point around the hip. The same experienced investigator made all the anthropometric measurements at all time points to avoid different individual measuring techniques. BMI was calculated as weight in kilograms divided by height in squared meters (kg m⁻²).
Blood pressure

The same physician performed the BP measurements during the medical consult on both pre- and post-test days. BP was measured manually using stethoscope and a blood pressure cuff (Welcallyn SK, Germany and Tycos 2006z, USA). The subject was sitting still for 5 min before measurement.

Physical testing procedures

The testing procedures were equal at each time point and consisted of 2 consecutive test days. The participants were not allowed to do any strenuous physical activity the last 2 days prior to testing. All food and fluid intake the 2 days before and during the test days were thoroughly monitored using a 1 g accurate food scale in addition to recording in food registration forms.

Only water was allowed the last 2 h before test-start. At day 1, anthropometric measurements, lactate threshold (LT), work economy (WE), and VO2max were measured. In addition, the participants were interviewed and answered questionnaires about physical activity habits (IPAQ questionnaire), vitality (Subjective Vitality Scale), and depression (Beck Depression Inventory—II). At day 2, a FatOx test was completed at 60% VO2max. All physical tests were carried out using a Woodway PPS 55 sport (Waukesha, Germany) treadmill calibrated for inclination and speed. Heart rate was registered continuously during all physical tests using a Polar rs100 (Polar Kempele, Finland).

The first test consisted of three or four submaximal workloads each lasting 5 min. The velocity during the first warm up period corresponded to ~60% VO2max. The tests were conducted at an incline of 3%. This incline was set to maintain a normal walking speed during all periods. Blood lactate ([La−]b) was measured after each period using a Lactate Pro Analyzer (Arcray Inc). The speed was increased at each period, until the subjects elicited an intensity above the medical consult on both pre- and post-test days. Blood lactate ([La−]b) value +2.3 mmol L−1. If the LT was not reached during the third period, a fourth period was completed. An incremental VO2max was performed where the test started at an incline of 3–4%, and at a speed between 3 and 6 km h−1 according to each individual’s physical fitness. The incline was increased with 1% and/or the speed was increased by 0.5 km h−1 every 30 s depending on the individual VO2 curve and the subjective evaluation of the test leader. The test ended at voluntary exhaustion, and verbal encouragement was given towards the end of the test. The criteria used to determine if VO2max was accomplished were flattening of the VO2 curve, RER ≥1.05, HRpeak ≥95% of expected HRmax, and concentration of blood lactate above 8 mmol L−1. These criteria have been used in earlier studies (Støren et al. 2008; Sunde et al. 2010; Helgerud et al. 2010). The average of the two highest continuous VO2 measurements was set as VO2max, and the highest heart rate at the end of the VO2max test was recorded as peak heart rate (HRpeak).

Measurements of insulin resistance

The homeostasis model of assessment for insulin resistance index (HOMA-IR) was used to assess IR. The IR was calculated by the Oxford University HOMA2-IR online calculator (http://www.dtu.ox.ac.uk/homacalculator/, accessed June 7, 2015), based on measurements from c-peptide and fasting blood glucose as described in Wallace et al. (2004). HOMA-IR is the reciprocal of HOMA %S, where HOMA %S (insulin sensitivity) represents values of 100% in normal adults. The validity and accuracy of measuring IR by HOMA-IR has been evaluated several times and has a high correlation with the glucose clamp test (Matthews et al. 1985; Okita et al. 2013).

FatOx calculations

The fat oxidation tests were performed at approximately the same time during the day (±3 h). The FatOx test was a 10-min protocol where VO2 and VCO2 were used to calculate respiration exchange ratio (RER) values. Values were measured every 20 s. FatOx was calculated from the average RER values between 4 and 8 min, using the a formula from Frayn (1983): FatOx (g min−1) = (VO2 × 1.67) − (VCO2 × 1.67) × 0.307 × (POX), where POX (g min−1) is the protein oxidation rate, assumed to be (KJ min−1) × (0.12 g J)/17.74 KJ. The 10-min duration of the FatOx test is in line with Bordenave et al.’s (2007) who recommended a work duration of longer than 3 min to ensure a stable level gas exchange when using indirect calorimetry to assess FatOx during exercise.

The FatOx test was performed at the relative workload of 60% VO2max. VO2max and the 5 min submaximal workloads were used to establish the linear regression for VO2max and velocity (Helgerud et al. 2010). This linear regression was used to determine the velocity at 60% VO2max. The calculation of the specific individual velocity representing 60% VO2max is based on the physiological principle that oxygen consumption is linearly related to the work intensity (Størøe et al. 2008; Sunde et al. 2010; Helgerud et al. 2010). Sunde et al. (2010) showed that the linearity from this regression averaged an $R^2 = 0.992 ± 0.005, p < 0.0001$.

Ergo spirometry equipment

Sensor Medics Vmax Spectra (Sensor Medics 229, Yorba Linda, California, USA) was used during all physical tests. Gas exchange was measured with 20 s intervals. The
A flow sensor was calibrated against a 3.0 L syringe (Hans Rudolph, Kansas City, MO, USA). \( \text{O}_2 \) and \( \text{CO}_2 \) sensors were calibrated against known gases (26% \( \text{O}_2 \) and 16% \( \text{O}_2 \)) before each test. According to the manufacturer, the Sensor Medics Vmax Spectra has a \( \text{VO}_{2\text{max}} \) accuracy within a range of ±3%. However, test-to-test variations in our laboratory have shown to be less than ±1%.

**Diet registrations**

The participants agreed to continue with their habitual diets through the participation period, but were encouraged to increase energy intake equivalent to the energy expended during exercise. They registered their diets 2 days prior to testing, during both test days (Table 3), and also during 3 consecutive days in the middle of the 12 weeks intervention, to achieve an extra control of habitual diet patterns. To ensure accurate diet registration, all food was weighed on a 1-g accurate food scale (Wilfa, KW-4, Hagan, Norway) and recorded in food registration forms.

**Use of medications**

A physician examined the use of medications in both groups (Table 4), which were similar between the two groups.

**Statistics**

Experimental data are presented as mean ± standard deviation, as well as delta values (Δ) and coefficient of variance (CV) in percent. A Pearson bivariate correlational test was used to determine possible relationships between baseline values and between changes in the physiological variables from baseline to post-test. Paired student \( t \) test was performed to discover differences between baseline and post-test within each intervention group. Independent \( t \) tests were used to discover significant differences in changes between the two exercise groups. The Shapiro–Wilk test and normal \( Q-Q \) plot were used to test for normality. The HbA1c values were not normally distributed. Therefore, non-parametric tests were used when analyzing the HbA1c data. A Wilcoxon signed rank test was used to assess pre- to post-changes within each group, and a Mann–Whitney \( U \) test was used to explore between-group differences in changes. Due to a significant difference in pre-HbA1c levels between the two groups, a post hoc analysis was conducted. In the post hoc analysis, the HbA1c data were corrected for skewness (1.7 ± 0.4), which normalized the distribution and excluded the two extreme outliers in the HAIT group. An independent \( t \) test was then used to explore whether the differences in HbA1c adaptations between the groups were caused by these outliers. The results from this analysis are only presented within the “Results” section, and not in the main result table.

In all tests, significance was accepted at \( p < 0.05 \). Analyses were performed using Statistical Package for the IBM SPSS (version 22; IBM Corp., Armonk, NY, USA).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Diet registrations at pre- and post-tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIT (N=17)</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
</tr>
<tr>
<td>TEI (Kcal)</td>
<td>2166±445</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>215±71</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>100±26</td>
</tr>
<tr>
<td>Prot (g)</td>
<td>101±23</td>
</tr>
<tr>
<td>%CHO</td>
<td>38.7±6.5</td>
</tr>
<tr>
<td>%Fat</td>
<td>42.0±7.3</td>
</tr>
<tr>
<td>%Prot</td>
<td>19.4±4.0</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation, delta values (Δ), and coefficient of variance (CV) in per cent. Kcal kilo calories, g gram, TEI total energy intake, CHO carbohydrate, Prot protein. \( p < 0.05 \) different from pre-values.
Results

Results are presented in Table 5. The HAIT group increased their relative VO_{2max} by 21% (25.6 to 30.9 ml kg^{-1} min^{-1}; p < 0.001) in ml kg^{-1} min^{-1} and absolute VO_{2max} by 19% (2.39 ± 0.55 to 2.84 ± 0.66 L min^{-1}; p < 0.001), respectively. There was no change in VO_{2max} in MIT. Velocity at lactate threshold improved in both groups (HAIT; from 5.5 ± 1.0 to 6.2 ± 1.2 km h^{-1}, p < 0.001, MIT; from 5.7 ± 0.4 to 6.1 ± 0.6 km h^{-1}, p < 0.001). LT expressed as % VO_{2max} did not change in either of the groups from pre- to post-measurements, and there was no difference in change between the groups.

A significant improvement was found in HbA1c in HAIT compared to MIT (p < 0.01), with a 0.58% reduction in HbA1c (from 7.78 to 7.20%, p < 0.001) in HAIT, while no change was found in MIT (Fig. 2). A post hoc analysis of HbA1c corrected for skewness still revealed significant difference in change between HAIT and MIT (p < 0.01) with a significant reduction of 0.47% (from 7.36 to 6.89%, p < 0.01) in HAIT, and no reduction in MIT (p = 0.804).

Significant correlations were found between change in absolute VO_{2max} and change in HbA1c when the two intervention groups were combined (L min^{-1}; R = −0.524, p < 0.01, SEE = 0.44), see Fig. 3.

There were no within-group changes or between-group differences in change in HOMA-IR. There was a tendency towards an improved FatOx at 60% VO_{2max} (from 0.368 to 0.420 g min^{-1}) in HAIT (p = 0.065). No change was found in MIT.

**Table 5** Physiological adaptations after 12 weeks of exercise

<table>
<thead>
<tr>
<th>Anthropometrics</th>
<th>Moderate (N=19)</th>
<th>HAIT (N=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>89.1 ± 15.6</td>
<td>88.6 ± 15.4</td>
</tr>
<tr>
<td>BMI (kg m^{-2})</td>
<td>31.1 ± 4.5</td>
<td>31.2 ± 4.1</td>
</tr>
<tr>
<td>BF (%)</td>
<td>33.2 ± 7.6</td>
<td>31.4 ± 7.3</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>108 ± 11</td>
<td>106 ± 12</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>108 ± 11</td>
<td>107 ± 11</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.84 ± 0.88</td>
<td>6.83 ± 0.84</td>
</tr>
<tr>
<td>HOMA-IR (%)</td>
<td>1.83 ± 0.73</td>
<td>1.79 ± 0.77</td>
</tr>
<tr>
<td>VO_{2max} (L min^{-1})</td>
<td>2.29 ± 0.61</td>
<td>2.25 ± 0.58</td>
</tr>
<tr>
<td>VO_{2max} (mL kg^{-1} min^{-1})</td>
<td>25.8 ± 5.5</td>
<td>25.6 ± 5.4</td>
</tr>
<tr>
<td>FatOx (g min^{-1})</td>
<td>0.34 ± 0.083</td>
<td>0.32 ± 0.087</td>
</tr>
<tr>
<td>RER FatOx (VCO2/VO2)</td>
<td>0.81 ± 0.04</td>
<td>0.82 ± 0.05</td>
</tr>
<tr>
<td>VO_{2FatOx}</td>
<td>1.37 ± 0.58</td>
<td>1.27 ± 0.51</td>
</tr>
<tr>
<td>Velocity FatOx (km h^{-1})</td>
<td>3.9 ± 1.4</td>
<td>3.9 ± 1.4</td>
</tr>
<tr>
<td>LT (% VO_{2max})</td>
<td>77.5 ± 11.1</td>
<td>80.2 ± 9.3</td>
</tr>
<tr>
<td>LT at 3% incline (km h^{-1})</td>
<td>5.7 ± 0.4</td>
<td>6.1 ± 0.6</td>
</tr>
</tbody>
</table>

^a^N MIT = 16, N HAIT= 16; Reduced N in HbA1c due to change in medications during intervention period. ^b^N MIT = 16, N HAIT = 14; Reduced N in IR due to change in medications during intervention period and two missing c peptide values in HAIT. Values are mean ± standard deviation. Moderate, moderate training intensity group

HAIT high intensity aerobic interval training group, BW body weight, BMI body mass index, BF body fat percentage, Waist waist circumference, Hip hip circumference, VO_{2max} maximal oxygen consumption, mL milliliters, L liters, HbA1c glycated hemoglobin type A1C, FatOx fat oxidation, RER respiratory exchange ratio, Syst. BP systolic blood pressure, Diast. BP diastolic blood pressure, HDL high-density lipoprotein, LDL low-density lipoprotein, La- lactate, Mmol L^{-1} millimoles per litre, HR heart rate

*p < 0.05 different from pre value, **p < 0.01 different from pre value, §p < 0.05 different from change in MIT. §§ p < 0.01 different from change in MIT.
post-test within each exercise group were not significant, an independent \( t \) test revealed a significant difference in change between HAIT and MIT \((p < 0.05)\).

In HAIT, reductions were found in BW (95.0 ± 15.3 to 93.3 ± 15.1 kg; \( p < 0.01 \)), BMI (32.0 ± 4.7 to 31.4 ± 4.7 kg; \( p < 0.01 \)), %BF (33.1 ± 7.6 to 31.4 ± 7.6 kg; \( p < 0.001 \)), waist circumference (110 ± 11 to 108 ± 10 cm; \( p < 0.01 \)), and hip circumference (107 ± 9 to 106 ± 9 cm; \( p < 0.01 \)). In MIT, there were no changes in BW or BMI, but waist circumference (108 ± 11 to 106 ± 12 kg; \( p < 0.001 \)), hip circumference (108 ± 11 to 107 ± 11 kg; \( p < 0.01 \)), and %BF (33.2 ± 7.6 to 31.4 ± 7.3 kg; \( p < 0.001 \)) were all reduced. Only the changes in BW and BMI were significant different between HAIT and MIT \((p < 0.05)\), with the greatest improvement in HAIT (−1.7 versus −0.6 kg, respectively). The changes in BP and BLP were not significantly different between the groups.

Discussion

The main findings in this study were increased \( VO_{2\text{max}} \) and decreased HbA1c in HAIT compared with MIT. The changes in BW, BMI, and FatOx were also significantly different from MIT.

Maximal oxygen uptake and lactate threshold

The 21% (ml kg\(^{-1}\) min\(^{-1}\))—and 19% (L min\(^{-1}\)) increase in \( VO_{2\text{max}} \) after HAIT, imply an average increase of 0.7% per training session, which is somewhat higher than the results from other studies with different adult populations (Rognmo et al. 2004; Østerås et al. 2005; Hollekim-Strand et al. 2014; Wang et al. 2014; Støren et al. 2016). To our knowledge, very few studies have investigated the importance of exercise intensity among T2D, using a HAIT protocol. However, a pilot study by Hollekim-Strand et al. (2014) found an increase of 13 and 11% in relative and absolute \( VO_{2\text{max}} \) in ml kg\(^{-1}\) min\(^{-1}\) and L min\(^{-1}\), respectively, after 12 weeks of exercise with a similar training protocol. The larger improvements in the present study could be due to the lower baseline values. Hansen et al. (2009) also investigated the clinical benefits of continuous exercise at either low-to-moderate (50% \( VO_{\text{peak}} \)) intensity or at moderate-to-high (75% \( VO_{\text{peak}} \)) intensity among T2D patients. Improvements of 8% (50% \( VO_{\text{peak}} \)) and 16% (75% \( VO_{\text{peak}} \)) were found in \( VO_{2\text{max}} \) after 8 weeks of exercise (Hansen et al. 2009). The intensity at 50% and 75% \( VO_{\text{peak}} \) corresponds to 69% and 85% \( HR_{\text{max}} \), respectively (Swain et al. 1994). This is slightly lower than the two different intensity levels in the present study, and may explain the difference in improvements.

A low pre-\( VO_{2\text{max}} \) level might influence the exercise response since persons with lower CRF have a greater potential to improve their physical fitness (Støren et al. 2016). It has been hypothesized that the lower baseline \( VO_{2\text{max}} \) level found among T2D may be caused by higher BG levels, low capillary density, reduced oxygen delivery capacity, increased blood viscosity, and presence of vascular and neuropathic complications typically found in T2D (Reusch et al. 2013). The positive effects of HAIT on improving \( VO_{2\text{max}} \) in the present study are also shown in studies with different subject characteristics, such as young healthy (Helgerud et al. 2007), old healthy sedentary (Østerås et al. 2005), and different patient populations (Rognmo et al. 2004; Wisløff et al. 2007; Helgerud et al. 2011). These compliant results reveal the great potential of HAIT to be an effective strategy to improve cardiovascular health in all age groups (Støren et al. 2016), counteracting the metabolic disturbances accompanied with T2D (Fletcher et al. 2002; Hawley and Zierath 2008).
The lack of VO\textsubscript{2max} improvement in MIT in the present study was somewhat surprising. MIT was expected to increase VO\textsubscript{2max}, although not to the same extent as HAIT. The effectiveness of MIT to increase VO\textsubscript{2max} among T2D is still inconclusive. Hollekim-Strand et al. (2014) failed to discover changes in VO\textsubscript{2max} after moderate exercise, while others have revealed positive effects (Hansen et al. 2009; Giannopoulou et al. 2005). It should be noted that baseline VO\textsubscript{2max} in both Hansen et al. (2009) and Giannopoulou et al. (2005) were lower compared to the present study. In Hollekim-Strand et al. (2014), only relative VO\textsubscript{2max} increased when measured as ml kg\textsuperscript{-1} min\textsuperscript{-1} and not when measured as L min\textsuperscript{-1}, thus suggesting a weight reduction effect.

The velocity at LT increased in both groups although not significantly different between groups. The lack of change in LT expressed as % VO\textsubscript{2max} in both groups supports the findings from other studies among more well-trained individuals, which have revealed minor or no effects on this variable after aerobic exercise (Helgerud et al. 2007; Støren et al. 2014).

### HbA1c

The 0.58% point reduction in HbA1c in HAIT (from 7.78 ± 1.39 to 7.19 ± 1.10%) represents 8% improvement. This implies an important reduction in risk of CVD, as earlier studies have shown a 15–20% reduction in CVD events when HbA1c is reduced by 1% point (Stratton et al. 2000; Selvin et al. 2004). For HAIT, this would mean a risk reduction of approximately 8–10% after only 12 weeks of exercise. Although few studies have investigated the effects of HAIT and HbA1c among T2D, Hollekim-Strand et al. (2014) also found a similar positive effect on HbA1c after 12 weeks of HAIT. A reduction in HbA1c was observed (7.0 ± 1.2 to 6.6 ± 0.9%) as a result of HAIT. The greater effect in the present study may be due to higher baseline HbA1c levels (7.78 ± 1.39 vs 7.0 ± 1.2%). Similar to our study, Hollekim-Strand et al. (2014) found no changes in HOMA-IR.

There was no change in HbA1c in MIT. The lack of VO\textsubscript{2max} improvement in MIT may have influenced the lack of HbA1c improvements since the present study showed a relationship between improvement in VO\textsubscript{2max} and reduction in HbA1c (R = −0.52, p < 0.01) meaning that approximately 25% of the reductions in HbA1c could be related to increased VO\textsubscript{2max}. However, since SEE was 0.44 L min\textsuperscript{-1}, a quite high increase in VO\textsubscript{2max} is needed to predict reductions in HbA1c. It may of course not be causality between the improvements in VO\textsubscript{2max} and HbA1c. However, our study’s results are consistent with a meta-analysis conducted by Boulé et al. (2003) which concluded that exercise intensity was a better predictor of weighted mean difference in HbA1c than exercise volume.

Similar to the results in MIT in the present study, Hollekim-Strand et al. (2014) found no changes in either VO\textsubscript{max} or HbA1c after moderate exercise in T2D. The lower baseline HbA1c in MIT compared to HAIT in our study is also one plausible explanation behind the HbA1c results. It could be expected that higher pre-HbA1c values would lead to a greater decrease in HbA1c (Krook et al. 2003). In contrast, Revdal et al. (2016) examined the physiological adaptations comparing high-intensity short-interval training (HIIE; 27 min per bout; 10 min at 90% of HR\textsubscript{max}) and extremely low-volume sprint interval exercise (SIE; 10 min per bout; 2 × 20 s at maximum achievable intensity). None of the groups found a significant change in HbA1c, despite a significant difference in baseline HbA1c (pre-values were 6.53 ± 0.96 versus 7.87 ± 1.21 in HIIE and SIE, respectively) and improvement in CRF (HIIE 10%; SIE 4.3%). Hollekim-Strand et al. (2014) also had baseline levels of HbA1c below 7.0%. In contrary to MIT in our study, Hansen et al. (2009) found improved HbA1c levels in T2D after both low- to moderate-intensity (69% HR\textsubscript{max}) and moderate- to high-intensity (85% HR\textsubscript{max}) continuous exercise. After 8 weeks, HbA1c were reduced by 0.1% points from 7.4 ± 0.3 to 7.3 ± 0.3% in LI and by 0.2% points from 7.1 ± 0.2 to 6.9 ± 0.2 in HI. Unlike MIT in our study, 69% HR\textsubscript{max} in Hansen et al. (2009) improved VO\textsubscript{2max}, and the training effects accompanying improvements in VO\textsubscript{2max} might have influenced their HbA1c results.

Due to the significant difference in pre-HbA1c levels between the two groups in the present study, a post hoc analysis was conducted where the HbA1c data were corrected for skewness (1.7 ± 0.4%). This correction normalized the distribution and excluded the two outliers in the HAIT group. After the correction for skewness, there was no significant difference between MIT and HAIT baseline HbA1c values. The post hoc analysis still revealed a significant difference in improvement between HAIT and MIT (p < 0.01) with a significant reduction of 0.47% (from 7.36 to 6.89%, p < 0.01) in HAIT, and no reduction in MIT (p = 0.804). The “corrected” reduction of 0.47% in HAIT is similar to the reduction found in Hollekim-Strand et al. (2014).

The National Health and Nutrition Examination Survey showed that only 37% of persons diagnosed with T2D achieved the treatment goal of <7% HbA1c (Saydah et al. 2004). A recent study from Norway (Moulard 2014) revealed that 55% achieved the treatment goal of <7% HbA1c. The −0.58% points reduction in HbA1c in HAIT after only 12 weeks of exercise in the present study is very similar to the effects found after long-term (>12 weeks) medication (drug or insulin) treatment only (0.6–0.8% points) (UKPDS 1998). This indicates the potential of
HAIT to be an effective additive or even a substitute treatment to medication to reduce T2D risk factors, highlighting the potential of HAIT to reduce the use of medications.

Fat oxidation

There was a tendency towards an improved FatOx in HAIT in the present study ($p=0.065$). A longer duration of the exercise intervention might have led to a significant improvement. Although the changes from pre- to post-test within each exercise group were not significant, an independent $t$ test revealed a significant difference in change between HAIT and MIT ($p<0.05$). Energy status and nutrition composition can have profound effects on metabolism and therefore act as a potential confounding factor on “exercise effects” (Støa et al. 2016). A strict control of energy status and nutrition composition was therefore emphasized to ensure a high internal validity. Although both groups reduced their BW during the 12 weeks of exercise, the BW reductions were only minor ($-1.7$ versus $-0.6$ kg in HAIT and MIT, respectively), and nutrition composition were not changed from pre- to post-test. The results in the present study are thus most likely not biased by the participants’ diets. It has been suggested that individuals with T2D should exercise at lower intensities closer to FatMax (Suk et al. 2015) since FatOx during the FatMax intensity has been suggested to be twofold greater than at any other exercise intensity (Sahlin et al. 2008). Although exercising at an intensity closer to FatMax ($-56\%$ VO$_{2\text{max}}$), MIT did not improve FatOx after 12 weeks of exercise. Generally, FatOx effects after exercise are influenced by the responses in muscle oxidative capacity by the adaptations in mitochondrial density, and mitochondrial enzyme content and activity, and oxygen delivery to muscle (Melanson et al. 2009). As described in Achten and Jeukendrup (2004), it is also suggested that aerobic exercise increases the gene expression and protein content of several FA transporters, which may improve the uptake and delivery of FA to mitochondria. Other studies with training protocols of typical high-intensity, shorter duration, and more sprint-like intervals have revealed positive effects on these factors as thoroughly summarized in other studies (Gibala and McGee 2008; Bird and Hawley 2012). Although mitochondrial measurements were not conducted in the present study, these factors have likely influenced the FatOx adaptations.

Anthropometrics

Both HAIT and MIT led to positive adaptations in body composition. The small reduction in BW ($-1.7$ versus $-0.6$ kg in HAIT and MIT, respectively), and also the relatively minor changes in BMI, %BF, waist circumference, and hip circumference, was expected since the intervention did not include a diet restriction program. The improvements in body composition in both groups are similar to other exercise interventions with no diet restrictions (Giannopoulou et al. 2005; Hollekim-Strand et al. 2014), which is in accordance with the well-known importance of a combination of exercise and diet to obtain an effective loss of fat mass (Wing 2002). Although minor changes, the body composition adaptations found in the present study are still of great importance in a longer perspective to increase glucose control among T2D, since a reduction in fat mass is associated by improved insulin sensitivity (Racette et al. 2006), and any increase in muscle mass itself will lead to increased blood glucose uptake without changing the muscle’s intrinsic ability to react to insulin. It may be noteworthy to mention that despite the instruction to the participants to increase their caloric intake after the training sessions to match the increase in energy expenditure, both groups had a slight but not significant decrease in caloric intake of about 2–5%. This could be due to a change in appetite, but appetite was not measured in the present study.

There may be a possible link between the improvements in VO$_{2\text{max}}$, FatOx, HbA1c, and body composition. The improvements in VO$_{2\text{max}}$ could be due to an increased ability to utilize oxygen in the exercising muscles (Hellerud et al. 2007). Since an increased FatOx may rely on an increased O$_2$ supply (Nordby et al. 2006), the improvements in VO$_{2\text{max}}$ may partly explain the tendency towards an increased FatOx in HAIT, in addition to the peripheral musculature adaptations. On the other hand, the actual training sessions during HAIT reveal higher RER values than MIT training sessions. This means a higher stimulation of muscle glycogenolysis and glucose uptake (Romijn et al. 1993). Adaptations to this stress may partly explain the lowering of HbA1c in HAIT. An improved VO$_{2\text{max}}$ also improves the ability of energy production at any given submaximal intensity (McArdle et al. 2010). These improvements taken together may thus explain the reduced %BF.

Blood pressure

The decreases in systolic BP and diastolic BP ($-12$ and $-7$ mmHg, respectively) in MIT and diastolic BP ($-6$ mmHg) in HAIT in the present study are similar to the results in Cornelissen and Smart (2013). Although there seems to be an agreement on the effectiveness of exercise to reduce BP among healthy individuals (Pescatello et al. 2004), the effectiveness of aerobic exercise to reduce systolic and diastolic BP among T2D is still debated as exercise interventions show contradictory results (Dobrosielski 2012; Cornelissen and Smart 2013; Colberg et al. 2010). In the 2010 joint position statement from the American College of Sports Medicine (ACSM)/American Diabetes Association (ADA), the authors conclude that exercise may lead
to a reduction in systolic BP, while decreases in diastolic BP are less common among T2D (Colberg et al. 2010). The positive adaptations in diastolic BP in both MIT and HAIT could partly be due to the high baseline BP levels in the present study.

**Blood lipids**

Improvements in BLP were only found in MIT, where triglycerides decreased (from 1.58 ± 0.78 to 1.37 ± 0.81 mmol L⁻¹, p < 0.05) and HDL increased (from 1.24 ± 0.38 to 1.33 ± 0.38 mmol L⁻¹, p < 0.05). However, the changes in BLP were not significantly different between the groups. The improvements in both triglyceride level and HDL are still of importance due to the BLP association with CVD (Wilson et al. 1998). The increase in HDL in MIT is of clinical relevance since every 0.026 mmol L⁻¹ increase in HDL is associated with 2–3% reduction in risk of CVD (Maron 2000). None of the groups changed their LDL level although both groups had LDL levels above the recommended 2.5 mmol L⁻¹ treatment goal among persons with T2D (Daniel 2011). This finding is in accordance with other studies (Trejo-Gutierrez and Fletcher 2007) and may be due to the lack of diet restrictions.

**Practical implications**

In the present study, we demonstrated that HAIT is an effective exercise strategy to improve cardiovascular risk factors. The public health message remains to focus on “increasing physical activity.” However, the present study demonstrated an additional effect on CRF and HbA1c with higher aerobic intensity. CRF is an independent prognostic marker for death among persons with T2D (Wei et al. 2000), and should thus be taken into consideration when designing exercise prescriptions and establishing physical activity guidelines. The ~20% increase in VO₂max would also imply ~20% increase in energy expenditure during exercise at a given exercise intensity (%VO₂max). This implies a beneficial consequence of HAIT in a weight reduction perspective.

Two out of three individuals with T2D do not exercise regularly (Thomas et al. 2004), and “lack of time” is one of the most common explanations for inactivity (Stutts 2002). These are the arguments behind the increased investigation on alternative training models that are less time consuming, yet effective to improve cardiovascular health among T2D. The training protocol in the present study can be accomplished in ~30–40 min three times per week. Different exercise methods (such as walking, running, bicycling, cross-country skiing, and rowing) can be used in this training protocol as long as it involves large muscle mass to give optimal stress on the cardiovascular system.

Intervals may advantageously be conducted in hills or with treadmill inclination, since this makes it easier to obtain the right intensity zone, and is also less stressful on the joints. The training response to HAIT seems not to be affected by age (Støren et al. 2016). With increasing age however, individuals may have different physical restrictions. Therefore, a personalized training regime should be developed to each individual based on potential physically restrictions, personal experiences with different training methods, and practical feasibility.

**Conclusion**

High-intensity aerobic interval training (85–95% HR_max) is an effective strategy to improve important risk factors associated with T2D, and more effective than moderate continuous exercise in improving VO₂max and lowering HbA1c.

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**Compliance with ethical standards**

**Conflict of interest** There is no conflict of interest.

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Article II

Støren Ø, Helgerud J, Sæbø M, Støa EM, Bratland-Sanda S, Unhjem RJ, Hoff J, Wang E.

The Effect of Age on the $\dot{V}O_{2\text{max}}$ Response to High-Intensity Interval Training

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ABSTRACT

STOREN, Ø., J. HELGERUD, M. SÆBO, E. M. STØA, S. BRATLAND-SANDA, R. J. UNHJEM, J. HOFF, and E. WANG. The Effect of Age on the $\dot{V}O_{2\text{max}}$ Response to High-Intensity Interval Training. Med. Sci. Sports Exerc., Vol. 49, No. 1, pp. 78–85, 2017. Purpose: High-intensity interval training (HIIT) is documented to yield effective improvements in the cardiovascular system and be an excellent strategy for healthy aging. However, it is not determined how age may affect the training response of key components of aerobic endurance. Methods: We recruited 72 males (mean ± SD, weight = 84.9 ± 12.9 kg, height = 180.4 ± 5.8 cm) and 22 females (weight = 76.0 ± 17.2 kg, height = 171.2 ± 6.7 cm) from 20 to 70+ yr with a training status typical for their age group and divided them into six decade cohorts. The participants followed supervised training with a targeted intensity of 90%–95% of maximal HR (HR max) three times a week for 8 wk. Results: After HIIT, all age groups increased ($P < 0.001–P = 0.004$) maximal oxygen consumption ($\dot{V}O_{2\text{max}}$) with $0.39 ± 0.20$ (20–29 yr), $0.28 ± 0.21$ (30–39 yr), $0.36 ± 0.08$ (40–49 yr), $0.34 ± 0.27$ (50–59 yr), $0.33 ± 0.23$ (60–69 yr), and $0.34 ± 0.14$ (70+ yr) L·min⁻¹, respectively. These 9%–13% improvements were not significantly different between the age groups. In contrast to age, the percentage improvements after HIIT were inversely associated with baseline training status ($r = 0.66, P < 0.001$). HH max was not altered within the respective age cohorts, but the two oldest cohorts exhibited a tendency ($P = 0.07$) to increase HR max in contrast to a training-induced decrease in the younger cohorts. Conclusion: In healthy individuals with an aerobic capacity typical for what is observed in the population, the training response is likely not affected by age in a short-term training intervention but may rather be affected by the initial training status. These findings imply that individuals across age all have a great potential for cardiovascular improvements, and that HIIT may be used as an excellent strategy for healthy aging. Key Words: AGING, $\dot{V}O_{2\text{max}}$, HEART RATE, TRAINING STATUS, ENDURANCE TRAINING, HIIT

Maximal oxygen consumption ($\dot{V}O_{2\text{max}}$) is one of the strongest predictors for cardiovascular health and mortality (8,27) and is observed to decrease with ~1% per year until old age where the decline may accelerate (12,16). As a consequence, individuals suffer an elevated risk of cardiovascular disease and premature death with increasing age. Recognizing that the age-related decline in $\dot{V}O_{2\text{max}}$ indeed has multifactorial causes (9); some of it may be explained by reductions in the physical activity level as this also is shown to decrease with age (7). Thus, countering inactivity with effective aerobic endurance training to attenuate some of the $\dot{V}O_{2\text{max}}$ decline may be an excellent strategy for healthy aging.

There is strong evidence that supervised aerobic exercise training can improve $\dot{V}O_{2\text{max}}$ in healthy middle-age and older adults (35,37). However, a sufficient training intensity (≥60% of pretraining $\dot{V}O_{2\text{max}}$), frequency (more than three times a week), and duration (≥16 wk) may be required (10). Indeed, higher aerobic intensity has been heralded as a key factor to induce large improvements in $\dot{V}O_{2\text{max}}$ (15,19). Whole-body high-intensity interval training (HIIT), performed with a targeted intensity of 85%–95% of maximal HR (HR max), has been shown to induce large improvements in $\dot{V}O_{2\text{max}}$ across initial training status in a vast number of studies with healthy young individuals (18,19,38), healthy old individuals (30,40), and several patient populations (13,20,21,32,36,42). In fact, longer intervals, typically 3–5 min, combined with...
high-intensity continuous training are argued to induce marked \( \text{VO}_2\text{max} \) improvements in most relatively young adults (3). The improvements are large and typically yield a response of 0.3%–0.7% per training session in a 2- to 3-month training intervention (19,30,32,40), suggesting that even shorter (<16 wk) exercise training programs are also effective for \( \text{VO}_2\text{max} \) enhancement. The commonly observed age-related decline in \( \text{VO}_2\text{max} \) may thus be counteracted with HIIT. This has been demonstrated in a study with >70-yr-old adults, where a 13% improvement was observed after 10 wk of training (30). In addition to reducing the risk of cardiovascular events and premature death, the \( \text{VO}_2\text{max} \) improvements are also associated with improved endurance performance (6,39) and will likely lead to an improved physical independence and quality of life at old age (16). Importantly, it is the aerobic system that should be targeted after high-intensity endurance training, especially the heart, because a close relationship between cardiac output and \( \text{VO}_2\text{max} \) improvements has been demonstrated (11,25,33).

Recently, it was shown that training-induced \( \text{VO}_2\text{max} \) responses after HIIT were large but blunted compared with young individuals (40). From a similar baseline, the \( \text{VO}_2\text{max} \) training response in young individuals was approximately twofold compared with that of the old. By experimental design, the young and old subjects in the study of Wang et al. (40) were matched for baseline \( \text{VO}_2\text{max} \), implying that the 60-yr-old men were moderately active and that the young men had a training status typical for their age, but with both groups having a great potential for \( \text{VO}_2\text{max} \) improvements. This observation indicates that a smaller training-induced plasticity may be expected with age and is in accordance with previous observations showing that even if training status is taken into account, there are still differences between age groups (16). It has been argued that \( \text{VO}_2\text{max} \) improvements may be predominantly due to an increased cardiac output (19,33,34). In turn, the cardiac output improvements are predominantly explained by an increased stroke volume of the heart (19,40). Although HR\(_{\text{max}}\) is largely contributing to the attenuation in cardiac output with age, training appears not to influence the HR\(_{\text{max}}\) decline (16). Old adults are also demonstrated not to change HR\(_{\text{max}}\) after short-term HIIT (40). Interestingly, by contrast, young subjects are often shown to exhibit a small HR\(_{\text{max}}\) decline as a consequence of endurance training (40,43). Although these training-induced alterations in HR\(_{\text{max}}\) are small, they may have important implications for appropriate training intensity administration. This is important in the high end of the intensity scale, where effective training of the heart is a fine balance between too high-lactate accumulation from anaerobic metabolism and the intention of stressing the oxygen taxing organs maximally. Noteworthy are also possible training-induced differences in HR\(_{\text{max}}\) between old and young individuals, as they may provide important indications of how plasticity of both autonomic and nonautonomic factors of the heart may change with age.

HIIT is documented to be an excellent strategy for improving \( \text{VO}_2\text{max} \). Although training-induced improvements previously have been reported in specific groups of old, it is unclear if these improvements are representative for what is typically observed in the population. To our knowledge, no previous study has systematically investigated the training response to HIIT in age groups ranging from 20 to 70+ yr of age. This may provide an important insight into expected effects if HIIT is used to improve \( \text{VO}_2\text{max} \) in the average population in the worldwide war against inactivity. HIIT may not only be of vital importance for the individual but may also be a cost-effective socioeconomic enterprise for public health. Thus, in the current study, our aim was to examine supervised HIIT-induced \( \text{VO}_2\text{max} \) responses in six decade cohorts from 20- to 70+-yr-old males and females. Our hypothesis was that all of the cohorts would exhibit significant improvements in \( \text{VO}_2\text{max} \), but that improvements would be significantly smaller with advancing age.

METHODS

Subjects. A total of 94 healthy male (\( n = 72 \)) and female (\( n = 22 \)) volunteers with an age ranging from 20 to 83 yr (48.6 ± 18.1 yr) participated in this study. Subjects’ characteristics are given in Table 1. The participants were matched for pretest \( \text{VO}_2\text{max} \) relative to age mean \( \text{VO}_2\text{max} \) (22) to represent what is observed in the population and typically meant that they engaged in weekly activities with low to moderate aerobic intensity from 0 to 2 h wk\(^{-1}\). The subjects were divided in six decade age cohorts of 20–29, 30–39, 40–49, 50–59, 60–69, and 70+ yr, respectively. The subjects were recruited from advertisement, and by invitation to workplaces or other arenas in the local communities where we expected to find participants of the right age. The exclusion criteria were history of cardiorespiratory or musculoskeletal diseases or use of medications that could affect the responsiveness of aerobic endurance training. The subjects were also excluded if they had experienced any kind of disease or injuries lasting more than a week, during the last month before the intervention. The limit for inclusion to the data material was a compliance of 80% of all training sessions. Informed consent was obtained from all subjects, and the study was approved by the ethical committee of the University College of Southeast Norway and the institutional review board of Telemark University College. The study was performed in accordance with the declaration of Helsinki. All invited subjects who met the inclusion criteria for participation and compliance, and with a \( \text{VO}_2\text{max} \) representative for their age group, were included in the data material, resulting in differences in the number of participants in each age group.

Study timeline. The subjects performed pretesting 1–2 d before the 8-wk HIIT training intervention and posttesting 2–5 d after the last training session. Testing protocols were identical and conducted at the Telemark University College and the Norwegian University of Science and Technology. Subjects were instructed to not exercise, or only perform light exercise the last 24 h before the test days, and not to eat. 
within 2–4 h before the tests, and only to drink water for the last 2 h.

**Testing.** Before the training intervention, subjects performed a whole-body VO$_{2}$max test using a cycle ergometer (Lode Excalibur Sport, Lode, Groningen, Netherlands; Ergomedic 839, Monark Exercise, Sweden) applying a cadence of ~1 Hz, or a treadmill (Woodway PPS 55 Sport, Waukesha, Germany) starting at 5% inclination. At inclusion, to minimize the dropout rate from the study, the participants chose one of the two whole-body testing and training modes, based on what they thought would be most motivating and/or involve the least orthopedic restriction to carry out. Pulmonary oxygen uptake was measured with the ergospirometry test systems SensorMedics Vmax Spectra (Sensor Medic, Yorba Linda, CA) and Metamax II (Cortex, Leipzig, Germany). The two different systems had been validated against each other for the range of measures that included all of the participants, and the different systems had been validated against each other for the range of measures that included all of the participants, and the metabolic system was used for the same individuals at pre- and posttest. HR was continuously measured throughout the test (Polar RS 100, Polar Electro Oy, Finland). After 10 min of warm-up, the incremental VO$_{2}$max test started at an intensity corresponding to ~70%–80% of HR$_{max}$. The work was then progressively increased every minute with 1 km h$^{-1}$ (treadmill) and 25 W (bicycle), respectively. The length of the test ranged between 4 and 10 min, until exhaustion. Subjects that cycled were encouraged to take standing position toward the end of the testing protocol, and verbal encouragement was given from the tester. Together with voluntary exhaustion, the following criteria were used to determine whether VO$_{2}$max was reached: a plateau in VO$_2$ despite increase in workload, RER $\leq$ 1.10, within 5 bpm of the subject’s HR$_{max}$ (if HR$_{max}$ was known) (41). VO$_{2}$max was recorded as the highest 30-s average during the test. HR$_{max}$ was measured at the same time and calculated as the highest observed HR + 5 bpm. Because VO$_{2}$max comparisons between individuals, groups or males and females with different body mass will overestimate subjects with a large body mass in terms of absolute values (L min$^{-1}$) and underestimate subjects with a small body mass in terms of relative values (mL kg$^{-1}$ min$^{-1}$), it has been suggested that to compare VO$_{2}$max comparisons relative to body mass raised to the power of 0.75 (5). Thus, in the current study, mL kg$^{-0.75}$ min$^{-1}$ was used as an additional term to take possible body weight differences between genders and age cohorts into account.

**Training intervention.** The training was conducted three times a week for 8 wk, and all the training sessions were supervised, with the HR continuously monitored, to ensure that the targeted aerobic intensity was met. The training sessions started with a 10-min warm-up and ended with a 5-min cooldown at an intensity corresponding to 70% of HR$_{max}$. After the warm-up, the participants performed 4 × 4 min work intervals of HIIT with an intensity corresponding to 90%–95% of HR$_{max}$. The intensive work periods were separated by 3-min active recovery periods at 70% of HR$_{max}$. The absolute intensity was thus adjusted throughout the training period to correspond to the same relative intensity. The targeted intensity during the HIIT intervals had to be met in every training session. Using the treadmill, the training was performed at an inclination $\geq$5%, and using the cycling ergometer, cycling was performed with a cadence of 60–80 rpm. Participants were instructed to carry out their regular physical activities as usual.

**Statistics.** Statistics were performed using the Statistical Package for Social Sciences (SPSS) version 19 (Chicago, IL). Figures were made by GraphPad Prism 5. VO$_{2}$max values were evaluated for normal distribution by using a QQ-plot and were found to be normally distributed. HIIT-induced improvements are presented as mean percentage changes. Two-way repeated-measures ANOVAs (age × time) were used to identify differences between groups pre- to posttraining and

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**TABLE 1. Subject characteristics.**

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>All (n = 94)</th>
<th>20–29 (n = 26)</th>
<th>30–39 (n = 9)</th>
<th>40–49 (n = 8)</th>
<th>50–59 (n = 15)</th>
<th>60–69 (n = 29)</th>
<th>70+ (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (%)</td>
<td>23</td>
<td>14</td>
<td>38</td>
<td>29</td>
<td>27</td>
<td>28</td>
<td>14</td>
</tr>
<tr>
<td>Cycling (%)</td>
<td>40</td>
<td>55</td>
<td>44</td>
<td>62</td>
<td>53</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178 ± 7</td>
<td>179 ± 8</td>
<td>180 ± 6</td>
<td>181 ± 6</td>
<td>178 ± 8</td>
<td>178 ± 7</td>
<td>178 ± 6</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>83.3 ± 14.9</td>
<td>78.3 ± 12.9</td>
<td>88.8 ± 21.1**</td>
<td>89.0 ± 17.2**</td>
<td>80.5 ± 13.1</td>
<td>86.7 ± 14.8</td>
<td>81.9 ± 13.4</td>
</tr>
<tr>
<td>BMI (kg m$^{-2}$)</td>
<td>26.1 ± 4.0</td>
<td>24.4 ± 3.2</td>
<td>27.5 ± 6.3</td>
<td>27.3 ± 5.7</td>
<td>25.4 ± 3.2</td>
<td>27.4 ± 3.8</td>
<td>25.8 ± 2.9</td>
</tr>
<tr>
<td>VO$_{2}$max pre L min$^{-1}$</td>
<td>3.96 ± 0.97****</td>
<td>3.99 ± 0.81*****</td>
<td>3.99 ± 1.16***</td>
<td>3.42 ± 0.95</td>
<td>3.26 ± 0.81</td>
<td>2.80 ± 0.72******</td>
<td>2.74 ± 0.91***</td>
</tr>
<tr>
<td>mL kg$^{-1}$ min$^{-1}$</td>
<td>41.4 ± 12.8***</td>
<td>51.3 ± 8.3***</td>
<td>46.7 ± 15.8***</td>
<td>40.1 ± 14.1*****</td>
<td>41.3 ± 10.8*****</td>
<td>33.3 ± 10.5***</td>
<td>33.3 ± 8.9***</td>
</tr>
<tr>
<td>VO$_{2}$max post L min$^{-1}$</td>
<td>3.70 ± 0.96******</td>
<td>4.38 ± 0.76******</td>
<td>4.26 ± 1.06***</td>
<td>3.78 ± 0.97</td>
<td>3.61 ± 0.87</td>
<td>3.13 ± 0.68*****</td>
<td>3.06 ± 0.91***</td>
</tr>
<tr>
<td>mL kg$^{-1}$ min$^{-1}$</td>
<td>45.5 ± 12.6***</td>
<td>56.5 ± 7.4***</td>
<td>49.9 ± 14.5***</td>
<td>43.9 ± 14.1*****</td>
<td>45.4 ± 10.3*****</td>
<td>37.2 ± 9.7**</td>
<td>36.0 ± 8.3***</td>
</tr>
<tr>
<td>HR$_{max}$ (bpm)</td>
<td>176.3 ± 21.8*****</td>
<td>198.0 ± 8.0*****</td>
<td>182.1 ± 12.7***</td>
<td>182.1 ± 7.8***</td>
<td>172.7 ± 11.7**</td>
<td>161.2 ± 20.2***</td>
<td>149.7 ± 22.2***</td>
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<tr>
<td>RER</td>
<td>1.10 ± 0.08</td>
<td>1.17 ± 0.09</td>
<td>1.07 ± 0.05</td>
<td>1.11 ± 0.07</td>
<td>1.10 ± 0.06</td>
<td>1.06 ± 0.06</td>
<td>1.05 ± 0.07**</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. BMI, body mass index.

*P < 0.05 different from all other age groups.

**P < 0.05 different from the 20- to 29-yr age group.

***P < 0.05 different from the 60- to 69-yr and the 70+ yr age groups.

****P < 0.01 increasing with increasing age.

*****P < 0.01 decreasing with increasing age.

******P < 0.01 different from the 20- to 29-yr age group.

*******P < 0.01 different from the 60- to 69-yr and the 70+ yr age groups.
were followed up with a Tukey post hoc analysis. Unpaired t-tests were used to determine between group differences at baseline and potential across groups gender differences. Paired t-tests were used to detect within-group differences after training. Pearson’s bivariate correlation test was used to investigate correlations between variables. A linear regression was used to assess standard error of estimate (SEE) in correlations. The level of significance was set as \( P < 0.05 \) for all variables, and data are presented as mean ± SD unless stated otherwise.

RESULTS

Mean compliance with the 8-wk HIIT intervention was 92% ± 4%, with no significant differences between age groups or gender. No adverse effects of the HIIT training were reported among the participants. Neither body mass nor BMI changed in any of the groups after the training period. As expected, ANOVA analyzes displayed both a reduced \( \dot{V}_\text{O}_2 \text{max} \) improvement with the 10.0% increase. Expressed relative to body weight, the results after training. Pearson regression was used to assess standard error of estimate (SEE) in all variables, and data are presented as mean ± SD unless otherwise.

As for the different age groups, there were no differences in the HIIT responses between males and females. When combing all the age cohorts the males exhibited a \( \dot{V}_\text{O}_2 \text{max} \) improvement of 0.35 ± 0.22 L·min\(^{-1}\) (\( P < 0.001 \)), which represented a 10.8% ± 8.1% increase. The females improved \( \dot{V}_\text{O}_2 \text{max} \) by 0.29 ± 0.20 L·min\(^{-1}\), which represented a 13.8% ± 10.0% increase. Expressed relative to body weight, the results confirmed the similar \( \dot{V}_\text{O}_2 \text{max} \) improvements with the males exhibiting a 4.1 ± 2.5 mL·kg\(^{-1}\)·min\(^{-1}\) increase (\( P < 0.001 \)) and the females exhibiting a 4.2 ± 2.5 mL·kg\(^{-1}\)·min\(^{-1}\) increase (\( P < 0.001 \)). No differences in HIIT-induced \( \dot{V}_\text{O}_2 \text{max} \) improvement were evident between the genders (\( P = 0.30 \)). Also, work performance enhancements were similar for males and females, revealing that males increased external work by 24.5% ± 34.4% (\( P < 0.001 \)) and females increased external work by 23.8% ± 43.7% (\( P < 0.001 \)). There was no significant difference between genders (\( P = 0.98 \)). The participants using the treadmill as a training and testing modality (\( n = 52 \)) improved \( \dot{V}_\text{O}_2 \text{max} \) by 0.35 ± 0.22 L·min\(^{-1}\) (\( P < 0.001 \)). Participants using the cycling as training and testing modality (\( n = 42 \)) improved \( \dot{V}_\text{O}_2 \text{max} \) by 0.31 ± 0.21 L·min\(^{-1}\) (\( P < 0.001 \)). There was no significant difference between the two modalities (\( P = 0.50 \)). HIIT-induced improvements in work performance were in accordance with the \( \dot{V}_\text{O}_2 \text{max} \) increase, showing participants that trained at the treadmill to increase external work by 35.1 ± 36.2 W (\( P < 0.001 \)). In a practical terms, the training-induced improvement after HIIT typically resulted in an increase of ~1 km·h\(^{-1}\) if speed was adjusted when running (e.g., 12 km·h\(^{-1}\) (pre)–13 km·h\(^{-1}\) (post)) and an increase of ~4% if inclination was adjusted when walking (e.g., 10% (pre)–14% (post)). The participants that performed cycling improved external work by 37.8 ± 21.5 W (\( P < 0.001 \)) after HIIT. Again, in work improvement, there was no significant difference between the two modalities (\( P = 0.67 \)). HR\(_\text{max} \) was not altered as a consequence of HIIT in any of the age groups. However, if the two oldest age cohorts were combined (60–70+ yr) and contrasted to a cluster of the younger cohorts (20–59 yr), a tendency (\( P = 0.07 \)) to a difference in HR\(_\text{max} \) response was observed after training (Fig. 2). Although all subjects improved their aerobic capacity, the percentage improvement was dependent on the participants’ initial training status. Subjects that were more sedentary had the greatest \( \dot{V}_\text{O}_2 \text{max} \) training response (Fig. 3).

DISCUSSION

HIIT has been shown to effectively improve \( \dot{V}_\text{O}_2 \text{max} \) and, thus, be an advantageous strategy for reducing the risk for cardiovascular disease and premature death, especially with advancing age. However, it is unclear how age may affect the magnitude of the training response to HIIT and the quantification of the expected benefits. We therefore sought to investigate training responses in 94 males and females from 20 to 70+ yr, with a \( \dot{V}_\text{O}_2 \text{max} \) corresponding to what is

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### TABLE 2. Physiological responses (Δ) to 8 wk of HIIT.

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>All (n = 94)</th>
<th>20–29 (n = 26)</th>
<th>30–39 (n = 9)</th>
<th>40–49 (n = 8)</th>
<th>50–59 (n = 15)</th>
<th>60–69 (n = 29)</th>
<th>70+ (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta \dot{V}_\text{O}_2 \text{max} )</td>
<td>0.34 ± 0.22**</td>
<td>0.39 ± 0.20**</td>
<td>0.28 ± 0.21**</td>
<td>0.36 ± 0.08**</td>
<td>0.34 ± 0.27**</td>
<td>0.33 ± 0.23**</td>
<td>0.34 ± 0.14**</td>
</tr>
<tr>
<td>L·min(^{-1})</td>
<td>4.2 ± 2.5**</td>
<td>5.2 ± 2.5**</td>
<td>3.3 ± 2.2**</td>
<td>3.8 ± 1.1**</td>
<td>4.2 ± 2.7**</td>
<td>3.9 ± 2.7**</td>
<td>2.7 ± 1.8**</td>
</tr>
<tr>
<td>mL·kg(^{-1})·min(^{-1})</td>
<td>12.4 ± 7.4**</td>
<td>15.3 ± 7.3*</td>
<td>9.8 ± 6.8**</td>
<td>11.9 ± 8.0*</td>
<td>12.6 ± 8.1**</td>
<td>11.7 ± 8.0**</td>
<td>8.3 ± 5.0**</td>
</tr>
<tr>
<td>Pct.</td>
<td>11.6 ± 8.6**</td>
<td>10.8 ± 6.8*</td>
<td>8.8 ± 7.3*</td>
<td>10.8 ± 5.0*</td>
<td>11.5 ± 9.1**</td>
<td>13.0 ± 10.9**</td>
<td>9.2 ± 5.6**</td>
</tr>
<tr>
<td>( \Delta \text{HR}_\text{max} ) (bpm)</td>
<td>−1.3 ± 1.9</td>
<td>−2.6 ± 3.9</td>
<td>−1.4 ± 3.4</td>
<td>−1.9 ± 7.7</td>
<td>−2.1 ± 5.9</td>
<td>0 ± 5.5</td>
<td>0.6 ± 2.8</td>
</tr>
<tr>
<td>Watts</td>
<td>36.3 ± 30.5**</td>
<td>40.2 ± 21.4**</td>
<td>19.5 ± 22.7**</td>
<td>27.4 ± 15.1**</td>
<td>39.6 ± 38.9**</td>
<td>38.1 ± 38.0**</td>
<td>35.8 ± 20.8**</td>
</tr>
<tr>
<td>Pct.</td>
<td>24.3 ± 36.6**</td>
<td>17.2 ± 36.6**</td>
<td>7.2 ± 7.2**</td>
<td>13.8 ± 9.8**</td>
<td>19.8 ± 24.2**</td>
<td>37.1 ± 53.5**</td>
<td>39.3 ± 58.5**</td>
</tr>
<tr>
<td>( \Delta \text{Body weight} ) (kg)</td>
<td>−0.4 ± 2.7</td>
<td>−0.4 ± 2.3</td>
<td>−0.6 ± 0.9</td>
<td>0.2 ± 1.9</td>
<td>−0.4 ± 3.4</td>
<td>−0.5 ± 1.8</td>
<td>0.1 ± 1.5</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD.
**P < 0.01 different from pretest.
typically observed in the population. The main findings were as follows: 1) All age groups exhibited similar absolute and relative VO$_{2\text{max}}$ improvements and yielded 9%–13% improvements after the 8-wk HIIT intervention. 2) In contrast to age, initial training status was associated with the VO$_{2\text{max}}$ response, meaning that the most sedentary subjects exhibited the largest improvement. 3) HR$_{\text{max}}$ tended to decrease after HIIT in the young, in contrast to old, where it exhibited a tendency to increase, implying that autonomic and nonautonomic training responses in the heart may be different with age. Our results imply that a short-term HIIT intervention results in similar effective and large VO$_{2\text{max}}$ improvements with age, and that the most sedentary individuals may exhibit the largest gain in cardiovascular health and risk-reduction of premature death.

**VO$_{2\text{max}}$ response, age, and training status.** HIIT-induced VO$_{2\text{max}}$ responses were not different between the age groups in the current study, but all of the groups exhibited a VO$_{2\text{max}}$ increase from pre- to posttest. Previously, only a limited number of studies have investigated physiological adaptations from HIIT with an aerobic intensity as high as 90%–95% of HR$_{\text{max}}$ in healthy individuals older than 60 yr (30,40). The results from the current study are in accordance with the study of Osterås et al. (30) (13% VO$_{2\text{max}}$ improvement), showing that even moderately old individuals, with a VO$_{2\text{max}}$ typical for what is observed in the population, have a great potential for improving VO$_{2\text{max}}$. In fact, the VO$_{2\text{max}}$
improvements in the two oldest age cohorts in our study correspond to a $\dot{V}O_{2\text{max}}$ decline that is commonly observed with ~10 yr of aging (12,16). Thus, a training intervention of relatively short duration may yield important benefits, and the magnitude of the beneficial effects seems not to be reduced up to 80 yr of age. The $\dot{V}O_{2\text{max}}$ improvements (9%–13%) in the old subjects in the current study are somewhat larger than the 6% increase that was observed in the 60-yr-old participants in the study of Wang et al. (40) where an identical HIIT intervention was applied. However, this should be expected considering the effect of initial training status. Although the subjects in the current study had a $\dot{V}O_{2\text{max}}$ representative for what is typically observed in the population (22), the subjects in the study of Wang et al. (40) were moderately active and participated in activities such as running, orienteering, and cross-country skiing. In combination, our study and the study of Wang et al. (40) are in line with regard to the notion of a reduction in $\dot{V}O_{2\text{max}}$ increases with improvements in training status.

Aerobic training intensity has been argued to be a key factor for $\dot{V}O_{2\text{max}}$ improvements (19,32,36,42), and it is likely that the 90%–95% of $HR_{\text{max}}$ intensity applied in our study played a key role in the large improvement. Thus, it is somehow surprising that a recent meta-analysis that investigated the magnitude of $\dot{V}O_{2\text{max}}$ alterations after endurance training in sedentary old adults reported that exercising at intensities higher than 75%–80% of HR reserve (HRR) (i.e., between 80% and 90% of $HR_{\text{max}}$) did not lead to greater enhancement of $\dot{V}O_{2\text{max}}$ improvements compared with exercising at 66%–73% of HRR (23). However, the meta-analysis of Huang et al. (23) included only a limited number of studies (2,4,24,34) that applied an intensity between 75% and 80% of HRR and no studies with an intensity corresponding to 90%–95% of $HR_{\text{max}}$. This may explain the discrepancies between our study and the meta-analysis of Huang et al. (23). Indeed, compared with another study with a similar duration (4), applying a fairly high (75% of HRR) yet lower intensity than ours, the participants in our study had an almost twofold $\dot{V}O_{2\text{max}}$ improvement (12% vs. 7%). The ~70-yr-old subjects in the Bellman and Gaesser (4) study were also more sedentary (24 mL·kg$^{-1}$·min$^{-1}$) than ours, implying that they both physiologically and mathematically should have a higher potential for percentage improvement. In combination, these results indicate that a training intensity higher than 90% of $HR_{\text{max}}$ may be important for the largest training-induced improvements in $\dot{V}O_{2\text{max}}$ with age.

Although individuals may reach a slightly higher $\dot{V}O_{2\text{max}}$ during running compared with cycling (1), the aerobic intensity during the intervals in the current study was high, but not maximal. The difference between cycling and running was also reduced because cyclists were able to carry out training in a weight-bearing standing position. Thus, despite that cycling, walking, and running were all used as training modalities, all the participants across age and gender were able to reach the intended intensity training zone for effectively taxing the oxygen transporting organs, yielding a similar training stimulus for $\dot{V}O_{2\text{max}}$ improvements. However, the cycle test may have resulted in somewhat lower absolute measurements both before and after training, and this may have contributed to a slight underestimation of $\dot{V}O_{2\text{max}}$ for the respective age cohorts.

A low baseline $\dot{V}O_{2\text{max}}$ has been suggested to imply a higher risk of illness or injury during high-intensive exercise and potentially have harmful cardiovascular effects (29,31). However, there was no adverse incident due to the training regime in the present study. HIIT has previously also been applied as a strategy for improved cardiovascular health in several groups of patients with various medical conditions. Interestingly, the magnitude of $\dot{V}O_{2\text{max}}$ improvements in our study is also in accordance with studies that have used an identical HIIT protocol on a wide range of patients with a variety of medical conditions. Some of these studies involve patients with coronary artery disease (21,32), peripheral arterial disease (21,26,36), heart failure (42), chronic stroke (14), schizophrenia (17), and substance use disorder (13), respectively. Taken together, all these report that HIIT should be recommended for cardiovascular health. Notably, many of the patients in these studies have a similar age as the two oldest cohorts in the present study. Despite experiencing the various medical conditions, most of the patients share the characteristic that they are untrained, and, consequently, respond similarly to a strong and effective cardiovascular stimulus. Thus, it is not surprising that they exhibit $\dot{V}O_{2\text{max}}$ improvements of 9%–18%, also similar to what is presented in the current study, after an HIIT intervention. However, the HIIT study with heart failure patients (42) contrasts the other studies because it reported a $\dot{V}O_{2\text{max}}$ improvement of 46% (13.0–19.0 mL·kg$^{-1}$·min$^{-1}$) after a 12-wk HIIT intervention. The longer training intervention and the extremely poor training status for these patients may explain the large percentage improvement.

**Maximal HR and age.** As expected, our results confirmed an $HR_{\text{max}}$ decline with age, evident as a 6% decline per decade of increased age. The most marked difference was observed from the 60- to 69-yr-old cohort to the 70+-yr-old cohort (9%). Although the apparently inevitable age-related decline in $HR_{\text{max}}$ is likely a major contributor to the $\dot{V}O_{2\text{max}}$ reduction, it is noteworthy that the decline in $\dot{V}O_{2\text{max}}$ per decade was higher (8.5%) in our study. This is in line with previous observations (6,16) that have showed up to a two-fold decline in $\dot{V}O_{2\text{max}}$ compared with $HR_{\text{max}}$. Indeed, both maximal stroke volume and maximal arteriovenous oxygen difference are also suggested to be important contributors, in addition to $HR_{\text{max}}$ to the decline in $\dot{V}O_{2\text{max}}$ with age (28). Among the proposed mechanisms for the age-related decline in $HR_{\text{max}}$ are attenuated electrophysiology of the sinoatrial node, β-adrenergic function, and intrinsic HR (39).

As a consequence of HIIT, $HR_{\text{max}}$ remained unaltered within the respective age cohorts in the current study. However, there was a tendency ($P = 0.07$) to a difference in training-induced $HR_{\text{max}}$ response when the two oldest cohorts were contrasted to the youngest cohorts, with the young and...
old exhibiting a lower and higher HR_{max}, respectively, after HIIT. Although this indication should be interpreted with caution, an HR_{max} decrease after endurance training is not uncommon in young subjects and has previously been reported in several studies (43).

A decline in the HR_{max} was also observed in young (~20 yr), but not old (~60 yr), subjects after an identical HIIT intervention (40). Although training-induced changes in HR_{max}, if documented, typically are small (1–8 bpm), they may be very important for correct training intensity administration, especially in the high end of the intensity scale.

**Practical implications.** The HIIT was supervised in the current study. Although the training is relatively easy to carry out, careful intensity administration involves an HR_{max} test and fairly strenuous exercise. Thus, it may be advantageous with guidance from an educated instructor, at least in the beginning of an HIIT program. Importantly, HIIT intervals are not to be performed with a maximal training intensity. The targeted 90%–95% of HR_{max} intensity typically means that the individuals are able to continue another 1–2 min after the termination of the 4-min intervals. The intention is to stress the cardiovascular system optimally and to minimize the involvement of the anaerobic system and too high-lactate accumulation. Of course, a certain lactate build up is inevitable, making it necessary with at least 3 min active recovery periods between the intervals to ensure sufficient lactate removal. If a treadmill is applied, the inclination should be set to ≥5% inclination and intensity adjustment may be performed by increasing either speed or inclination. For minimal stress on joints, the intervals may be performed by walking with adjustment of treadmill inclination only or cycling. Alternating between standing and sitting position during cycling can make it easier to maintain the right intensity during the high-intensity aerobic intervals. With age, individuals may experience various situations or orthopedic restrictions that could limit participation in certain training modalities (e.g., running/walking). However, if a sufficient taxation of oxygen transporting organs is attained during training, cardiovascular health benefits will be achieved. Not only could cycling or walking/running be used as in this study, but also other whole-body modalities such as, e.g., cross-country skiing or rowing may be applied. Thus, the tailored training regime for the individual should be based on both what is feasible and motivating, as long as large muscle groups are involved in the training.

Although the observations in the current study clearly suggest that the training response in individuals with an aerobic capacity typical of what is observed in the population is likely not affected by age, the results have some limitations. Of notice is the small number of participants in some of the age cohorts. Also, the unequal proportion of male and female participants across age may bias the assessment of relative VO_{2max} because the body composition between genders differs. Thus, the implications should be interpreted with caution with regard to these limitations.

**CONCLUSION**

Against our hypothesis, the current study revealed similar improvements in VO_{2max} in participants from 20 to 70+ yr of age and implies that the training response to short-term HIIT is not affected up to moderate age in individuals with a VO_{2max} representative for what is typically observed in the population. By contrast, our results showed that the magnitude of VO_{2max} improvement was affected by the initial training status. Our results advocate that HIIT can be used as an effective strategy to improve VO_{2max} in the aging population, and given the close association between VO_{2max} and physical health, this may be beneficial for the quality of life of the individual and serve as a cost-effective socioeconomic enterprise for public health.

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33. Saltin B, Calbet JA. Point: in health and in a normoxic environment, VO_{2max} is limited primarily by cardiac output and locomotor muscle blood flow. *J Appl Physiol*. 2006;100:744–8.


Støa: The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors; testing procedures and training interventions.

Article III


Paper omitted from online edition due to publisher restrictions
Endringer

Paper I er lagt inn i publiseringsform.
Skrivefeil er rettet opp i referanseliste.
Side V linje 7; ordet increased er lagt inn foran HDL.
Side VII; Fullstendig publiserings informasjon er lagt inn under paper I i «List of papers».
Side IX, Abbreviations; Stor I er lagt inn for «Insulin-mediated...», i tillegg er følgende forkortelser lagt til; AMI, SCD og HIIT
Side 4; Burke et al (1975) har blitt rettet til Burke and Franks (1975).
Side 11, linje 13; mellomrom er lagt til foran «The».
Side 15, linje 12; ordet «contradictory» har blitt fjernet.
Side 27, første linje under 3.3.3; «a» har blitt skrevet inn foran «workload».
Side 27, linje 4 under 3.3.3; paper II er byttet ut med paper III
Side 32, linje 12; ordet «all» er fjernet foran «have a great potential...».
Side 32, linje 13; «method of» er satt inn foran «training».
Side 33, første linje; stor H er erstattet med liten h.
Side 38, linje 19; ordet «in» er satt inn foran «paper»
Side 39, linje 1; VO2max er endret til VO\textsubscript{2max}
Side 48, linje 6 nedenfra; ordet «HDL» er fjernet etter «exercise».
Side 50, linje 2 nedenfra; «The study design of paper I, II and III...»
Side 51, linje 11, ordet “the” er fjernet foran “practical reasons..”
The effect of training intensity, age and diet on aerobic capacity and metabolic risk factors: testing procedures and training interventions

Dissertation for the degree of Ph.D

Eva Maria Støa

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