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# The effect of selected genetic variants, age, sex and training methods on physical activity, capability and trainability

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**Ecology**

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# Preface

My interest for (everything that has to do with) biology and the living world was there from the very beginning. I remember that I, as a little girl, was playing outdoors and found a bit of money in the lawn. I could have run to the shop and bought me some sweets like other children did. But I did not. I asked my parents for a bit more so that I could buy me a children's encyclopaedia about the natural world. And that was definitely not the last that type of book I ever bought. Ever since I learned to read, I slept with a minimum of 3 books under my pillow that I switched between depending on my mood. Things have not changed that much. I do not keep books under my pillow anymore and have not done that for years. Now, I keep them on my nightstand, and, at the moment, there are around 7 I have started reading and switch between. Most of these are popular Science books, and I guess, this just indicates the one thing I crave the most – information!

Knowing this, one could say that it was not too surprising that I now find myself writing this thesis. However, the road to this point was not straight forward. Surprisingly, I chose to study something totally different after finishing secondary school. However, I am extremely glad I found my way back to the natural sciences. I am especially glad about finding campus Bø at USN, and all the amazing colleagues and friends I acquired there. I will always have a special place for Bø in my heart!

# Acknowledgements

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I am also grateful to Karin, Frode and my other colleagues for helping out with lab-logistics, but even more so, for our support and encouragement. Thanks to Andrew Jenkins for asking often about the progress of my PhD and inspiring conversations about microbiology, genetics and more. Ikumi, I thank you for many meaningful conversations about all of the ups and downs we both have experienced during our PhD journeys. And also, I thank Veronica for great guidance of us, PhD fellows, and for starting the nice tradition of "Shut up and write"-sessions. I have enjoyed these a lot!

I am and always will be grateful to my husband, Øyvind, for being so patient and supportive with whatever choices I have made during all these years ever since I applied for my bachelor studies in Bø in 2010. Without your support, I could not have continued through my Master's degree, and later, the PhD. And, our son, Leander, who is making sure I switch my focus away from work whenever we are together. There is no one else out there who can do that like him!

Es vēlos izteikt milzīgu paldies maniem mīļajiem Latvijā! Mammai Silvijai un tētim Sergejam par visu, kas mums tika dots, neskatoties uz, dažreiz, ierobežotām iespējām, un par daudzo grāmatu klātbūtni mājās un daudz ko citu. Mammai par rūpēm, palīdzēšanu ar skolas mājas darbiem un par Džeralda Darela grāmatām un, noteikti, par E.Tompsona-Setona"Koijotito". Tētim par biezajām enciklopēdijām, svētdienu dabas

filmu skatīšanos bērnībā un par interesi par dabu. Santai par būšanu piemēram un iedvesmai, kā arī par lielo atbalstu visa ceļa garumā. Gribu pateikt paldies arī Armandam par to, ka esi iedevis man hobiju, kas ļauj atslēgties no “realitātes” un daudziem foršiem galda spēļu vakariem gadu gaitā kopā ar Paulu. Ir vēl kāds cilvēks, kam es noteikti gribu pateikt paldies. Tā ir mana skolas laika bioloģijas skolotāja Sandra Štrause. Gribu pateikt lielu paldies par atbalstu skolas laikā un, par to, ka Jums vēl joprojām rūp.

# Abstract

The main objectives of this thesis were to investigate the role of selected genetic variants on physical activity (PA), capacity and trainability in homogenous cohorts from South-Eastern Norway. Two of the cohorts (Papers I and II) represented the general population, and the third consisted of well-trained cross-country skiers (Paper III). We wanted also to investigate how age and sex influence the response to maximal strength training (MST) and performance adaptations in cross-country skiing.

In Paper I, three polymorphisms were investigated (ACTN3 R577X, ACE I/D and uVNTR MAOA). Questionnaires were used to divide individuals (n=831) with the mean age (± SD) 55.5 ± 3.8 years (yrs) into groups with either low/medium (LMPA) or high PA (HPA) levels. We investigated the associations between the PA levels and the polymorphisms as well as with several socio-economic variables. We found 10% fewer ACTN3 R577X X allele carriers in the HPA group compared to the LMPA group ($p<0.01$). Education and previous participation in sports or outdoor activities were positively associated with the PA level, and females reported higher PA levels than males ($p<0.01$)

In Paper II, we genotyped three polymorphisms (ACTN3 R577X, ACE I/D and PPARGC1A rs8192678). 49 subjects (males and females aged 20-76 yrs) completed a MST intervention in leg-press. For 8 weeks subjects trained three sessions/week with each session consisting of 4 · 4 repetitions at ~85-90% of one-repetition maximum (1RM) intensity. At pre- and post-tests, 1RM was tested. We found an average 24% increase in 1RM ($p<0.01$) independent of age, sex and, surprisingly, training status. Carriers of the PPARGC1A rs8192678 T allele were 15% stronger at baseline (sex- and age-corrected 1RM) compared to individuals with CC genotype ($p<0.05$). The C allele carriers exhibited 34.2% larger improvements in 1RM (%) than homozygotes for the T allele ($p<0.05$). A trend was observed towards improved response to MST among the individuals with the ACTN3 R577X RR genotype compared to the XX (30% vs. 19%).

In Paper III, seven polymorphisms were investigated (ACTN3 R577X, ACE I/D, ACSL1 rs6552828, IL6 rs1474347, PPARA rs4253778, PPARG rs1801282 and PPARGC1A rs8192678). The study was a 6-month observational study (May to October) based on a cohort of well-trained cross-country skiers (n=29; 16-48 yrs). A number of physiological tests were performed prior (Pre-test), half-way (Post1) and after (Post 2) the study period. Throughout the study, participants maintained and reported their training habits. We found several associations between several of the genetic variants and various physiological/performance variables. For instance, ACTN3 R577X X allele carriers exhibited better DP-VO2max (55.4 vs. 59.4 mL-1·kg·min-1; $p<0.05$) compared to the RR genotype. Individuals with the XX genotype had, however, poorer work economy than the R allele carriers (0.820 vs. 0.765 mL-1·kg-0.67·m; $p<0.05$). In regard to other variables, we found a significant effect of age and sex on TTDP ($p < 0.01$), DP-VO2peak ($p < 0.01$), CDP ($p < 0.05$), MAS ($p < 0.01$), LTv ($p < 0.01$), 1RM half squat ($p < 0.01$) and 1RM pull-down ($p < 0.01$). Sex had also an impact on RUN-VO2max ($p < 0.01$). The total training volume consisted of ~90% low-intensity training and 5% moderate and high-intensity training, each (range: 357.5 - 1056.8 min/week). During the study, there was a significant increase in the total volume and ski-specific training ($p < 0.05$), but the intensity distribution remained the same. We did not observe any improvements in either physiological/performance variables for the whole cohort or training progression/adaptation between age groups or sexes during the 6-month period.

All in all, our results point towards a potential role of the investigated polymorphisms on the complex traits investigated, i.e. PA levels, maximal strength and endurance performance. Also, for all the genes, the allele frequencies were similar to those reported previously in other European populations. Another important observation was that, although age and sex had an effect on both strength and endurance performance, these factors appeared not to affect the adaptability to maximal strength training or endurance training. Training modality was shown to be highly important, as all participants of the leg-press study (Paper II) improved their maximal strength in response to the MST. In the cross-country study (Paper III), however, no significant

changes in endurance parameters were observed during the 6-month study period. Although the participants increased their total training volume, they maintained the same training intensity.

Keywords: Physical activity; Maximal strength; Leg-press; Endurance; Cross-country skiing; Trainability; Age; SNPs; Polymorphisms; ACTN3; ACE; PPARGC1A; PPARA; PPARG; ACSL1; IL6; MAOA

# List of Papers

## Paper 1

Goleva-Fjellet S., Bjurholt A. M., Kure E. H., Larsen I. K., Støren Ø., Sæbø M. Distribution of allele frequencies for genes associated with physical activity and/or physical capacity in a homogenous Norwegian cohort- a cross-sectional study. BMC Genet 21, 8 (2020); https://bmcgenet.biomedcentral.com/articles/10.1186/s12863-020-0813-1

## Paper 2

Hans Torvild Kittilsen*, Sannija Goleva-Fjellet*, Baard I. Freberg, Iver Nicolaisen, Eva M Støa, Solfrid Bratland-Sanda, Jan Helgerud, Eivind Wang, Mona Sæbø, Øyvind Støren. Early responses to maximal strength training were not influenced by age, gender or initial training status

*Shared first authorship

Manuscript; under submission to journal Aging (Albany NY).

## Paper 3

Jan-Michael Johansen, Sannija Goleva-Fjellet, Arnstein Sunde, Lars Erik Gjerløw, Lars Arne Skeimo, Baard I. Freberg, Mona Sæbø, Jan Helgerud, Øyvind Støren. No change – no gain; the effect of age, sex, selected genes and training on physiological and performance adaptations in cross-country skiing. Front. Physiol., 26 October 2020; https://www.frontiersin.org/articles/10.3389/fphys.2020.581339/full

# Abbreviations

ACE - angiotensin-converting enzyme

ACSM - American College of Sports Medicine

ACTN3 - α-actinin-3

DP - double poling

C - work economy/oxygen cost

CMJ (CMJas) - counter-movement jump (counter-movement jump with arm swing)

HR - heart rate

LT- lactate threshold

MAOA - monoamine oxidase A

MAS - maximal aerobic speed

MST- maximal strength training

NCBI - National Center for Biotechnology Information, U.S. National Library of Medicine

PA - physical activity (low/medium PA (LMPA) and high PA (HPA) level)

PAGE - polyacrylamide gel electrophoresis

RM - repetition maximum

SNP - single nucleotide polymorphism

TT - time trial

VO2 (VO2max) - oxygen consumption/uptake (maximal oxygen uptake)

WHO - World Health Organization

# Definitions

Allele - one of at least two genetic versions of the same gene at the same place on a chromosome

Cardiac output - the amount of blood pumped by the heart minute, and depends on the heart rate and stroke volume of the heart

Concentric action - shortening of the muscle while activated

Eccentric action - muscle lengthening while the muscle is activated and force is produced

Genotype - the two alleles an individual possesses for a particular genetic variant

Heritability - an estimate of the degree of variation in a trait in a population that is due to genetic variation

Homozygous/heterozygous - possessing two identical alleles of the same genetic variant/ possessing two different alleles at the same location on the chromosome

Linkage disequilibrium - non-random association of alleles at two or more locations on the chromosome; alleles that are in linkage disequilibrium are physically connected and tend to be inherited together

Myokine - small proteins and peptides that are produced and released by skeletal muscle during muscle contractions

One-repetition maximum (1RM) - the maximal weight that a person can lift with the maximum effort once during strength training

Phenotype - observable traits/characteristics of an organism that often result from individuals' genotype

Physical capacity - ones' ability to perform various activities; it consists of factors like muscle strength, endurance capacity and balance

Polymorphism - the existence of at least two alleles at a specific location on the chromosome

Sarcopenia – age-associated progressive decline in muscle mass, strength power and physical functioning

# Aims of the Study

The overall aim of the study was to investigate the genetic influence on physical activity, capacity and trainability in different cohorts from the same geographical region:

1. To determine allele and genotype frequencies of several widely investigated polymorphisms in two different cohorts representing the general population from South-Eastern Norway, and a cohort of cross-country skiers competing at national level
2. To investigate whether these polymorphisms have an impact on complex traits like physical activity levels and maximal strength trainability in the general population
3. To investigate whether these polymorphisms influence various physiological traits important for the athletic endurance performance in the cross-country skiing cohort
4. To investigate how age, sex and trainability affect the response to maximal strength training in the general public and physiological and performance adaptations in cross-country skiing

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# 1 Introduction

Humans demonstrate large inter-individual variability in physical activity (PA), physical ability, trainability and athletic performance. Genetics has been recognized as one important factor to explain inter-individual differences in these complex phenotypes (Roth and Thomis 2011, Rankinen, Fuku et al. 2016, Jacques, Landen et al. 2019, Van Der Zee and De Geus 2019). The fields of exercise physiology and exercise genetics are both moving towards a better understanding of the impact of training and heredity on PA, ability and trainability, at least in part due to the rapid technological advances (Booth, Kelty et al. 2019, Lightfoot, Hubal et al. 2019). The cost of sequencing has also dramatically dropped since the sequencing of the first human genome was completed in 2003 (Wetterstrand 2020). The field of exercise genomics has provided insights into the genetic factors that influence various exercise and sports-related phenotypes (Lightfoot, Hubal et al. 2019).

It has long been speculated whether and to what extent one could use the individuals' genetic information not only to predict ones athletic ability (Venezia and Roth 2019) and injury risk but also to tailor training programs for the general public and athletes alike (Vlahovich, Hughes et al. 2017). In the last decade, direct-to-consumer genetic tests for sports have become available (Collier 2012). These tests focus on individual genetic variations, mainly single nucleotide polymorphisms (SNPs), the most common genetic variation in humans, where one nucleotide/base is substituted by another (LHNCBC 2020). So far, approximately 155 genetic variants have been associated with elite athletic performance (Ahmetov, Egorova et al. 2016). In 2014, a genetic test screening for a SNP within the *ACTN3* gene and promising to reveal the type of sports/exercise an individual is best suited for, i.e. sprint/power or endurance, became available as an over-the-counter test in Norway (Tjernshaugen 2014). This SNP is one of the most tested genetic variants among the fitness-related genetic testing companies (Williams, Wackerhage et al. 2016). Almost immediately, the test received criticism from various experts for being able to explain only a tiny fraction of the highly complex power and

endurance phenotypes (Pedersen 2014, Kristiansen and Guldteig Larsen 2016, Laustsen 2016). Two years later the test was withdrawn from the Norwegian market supposedly due to poor sales (Laustsen 2016).

In recent years, the numbers of sports-related gene testing companies have increased to more than 60 (Pickering, Kiely et al. 2019) and so have the numbers genetic variations included in these tests (Jones, Kiely et al. 2016, Williams, Wackerhage et al. 2016). Most of them test a number of polymorphisms related to exercise/athletic performance and injury risk (Pickering, Kiely et al. 2019), often in combination with a genetic test for diet/nutrition (Scarr 2019). There are examples of scientific intervention studies that are performed utilizing genetic-test results from these companies. Jones, Kiely et al. (2016) subjected athletes to either a low- or high-intensity resistance program that either matched or mismatched their genetic profile, based on the algorithm of one of the most popular direct-to-consumer fitness genetic test companies. Athletes that trained according to their genetic profile, improved more than those in the mismatched group. Despite these results, the scientific evidence often is exaggerated by the direct-to-consumer test companies to improve sales (Pickering, Kiely et al. 2019). Most of the allele variants included in these tests have not been widely investigated and/or lack sufficient scientific evidence for these tests to be useful in determining the athletic potential or training response at this point of time (Williams, Wackerhage et al. 2016). Furthermore, most of the companies have not made the lists of the polymorphisms they test for available, thus making it difficult to assess the quality of the scientific evidence behind their product (Williams, Wackerhage et al. 2016). Another major problem is the number of ethical challenges associated with this kind of genetic tests, especially regarding talent identification among children (Venezia and Roth 2019).

In 2018, the Chinese Ministry of Science and Technology announced that it would perform genome sequencing on athletes that will represent China at the 2022 Winter Olympics (Lemon 2018, Haff 2019). The sequencing of the athletes representing China should be finalized this year, i.e. 2020, and is aimed to test for speed and endurance,

among other traits (Lemon 2018). This approach might be more informative than looking at known genetic variants, but it also raises a number of ethical questions that goes far beyond the issues raised for SNP analysis.

Very few of more than the 155 genetic variants associated with athletic performance and other aspects of sports have been replicated in other studies (Pickering, Kiely et al. 2019). Furthermore, the genotype frequencies of many polymorphisms vary highly across different populations (Gordish-Dressman and Devaney 2011). Such stratification, if unaddressed, can lead to false results (Marchini, Cardon et al. 2004). Thus, more studies on homogenous cohorts might be necessary to investigate the effect these genetic variants might have on traits like trainability or athletic performance.

## 1.1 Physical activity and its genetic determinants

Physical activity is an umbrella term that covers structured and unstructured forms of leisure, transport, domestic and work-related activities with exercise being a subtype of structured PA (Bangsbo, Blackwell et al. 2019). PA and exercise participation contribute to a range of physical (Hills, Street et al. 2015) and mental health benefits (Mikkelsen, Stojanovska et al. 2017). Increased PA volume and/or intensity has been shown, to improve health variables such as cardiorespiratory/muscular fitness, bone health in addition to reducing the risk of non-communicable diseases and depression in sedentary (Booth, Laye et al. 2008, WHO 2010, Myers, McAuley et al. 2015, Pedersen and Saltin 2015, Mikkelsen, Stojanovska et al. 2017). Consequently, the World Health Organization (WHO) recommends adults to perform a minimum of 150 min of moderate-intensity aerobic activity or a minimum of 75 min of vigorous-intensity aerobic PA or an equivalent combination of these activities every week. In addition, at least 2 days a week with muscle-strengthening activities is recommended (WHO 2010). To boost the health benefits even further, WHO recommends increasing the PA levels to either 300 min or 150 min of moderate or vigorous aerobic activity, respectively. Thus, engaging in regular PA is considered important for maintaining health and normal functioning across the lifespan (Bangsbo, Blackwell et al. 2019).

Despite these recommendations, registered PA levels nowadays appear to be the lowest in human evolutionary history (Myers, McAuley et al. 2015). Physical inactivity has become a global pandemic and is the fourth leading cause of death globally (Kohl, Craig et al. 2012) as it contributes to the development of many non-communicable diseases, including cardiovascular disease, diabetes and cancer (WHO 2010, Lee, Shiroma et al. 2012, Booth, Roberts et al. 2017). In addition, the PA levels tend to decrease with increasing age (Hallal, Andersen et al. 2012, WHO 2019).

Environmental factors, including socio-economic, have long been recognized as important contributors to the PA/physical inactivity phenotype (Bauman, Reis et al. 2012). However, more recently, the importance of genetic factors to PA has been recognized and investigated to a greater extent (Moore-Harrison and Lightfoot 2010). Previously, twin and family study design was often used to calculate the heritability estimates (Jacques, Landen et al. 2019). More recently, gene-finding studies aiming to discover the specific genes/genetic variants influencing the PA have been performed (Van Der Zee and De Geus 2019). In twin studies, heritability estimates vary largely depending on the type of PA measured, age or sex of the individual. For total PA and voluntary exercise phenotypes in adults, it has been estimated that approximately 50% of the inter-individual variability is due to heredity (Van Der Zee and De Geus 2019). When looking at the moderate to vigorous PA phenotype, this estimate falls by approximately 5% points (Van Der Zee and De Geus 2019). PA defined as sports participation has been proposed to be increasingly determined by genetic factors with increasing age from childhood to adulthood (Stubbe, Boomsma et al. 2005) and total PA (Van Der Zee and De Geus 2019).

A large number of genes are likely involved in determining the innate PA levels (Lightfoot, Letsinger et al. 2019). It has been hypothesized that genes involved in motivation/personality traits (Stubbe, Boomsma et al. 2006, Van Der Zee and De Geus 2019), energy intake/expenditure balance and those involved in the ability to perform PA could be targeted to find the “physical activity genes” (Van Der Zee and De Geus

2019). It has also thought that genes favouring fitness and trainability may contribute to higher PA levels through boosting exercise participation (Stubbe, Boomsma et al. 2006). Several candidate genes have been reported to affect PA (Lightfoot 2011, Lightfoot, Letsinger et al. 2019). Some are thought to influence the intrinsic reward system and, thus, the motivation to exercise like *NHLH2* (Lightfoot 2011) and *MAOA* (Good, Li et al. 2015). Other genes have previously been found to have an impact on physical performance, and thus it is hypothesized an indirect impact on PA. The *ACE* and *ACTN3* genes are widely investigated in relation to physical traits like aerobic capacity (Myerson, Montgomery et al. 2001, Deschamps, Connors et al. 2015, Tamburus, Verlengia et al. 2018) and skeletal muscle size and function (Pereira, Costa et al. 2013, Kikuchi, Yoshida et al. 2015). All these phenotypes could potentially contribute to the PA levels. The three candidate genes investigated in relation to PA levels in this thesis (i.e. *ACE* I/D, *ACTN3* R577X and *MAOA* uVNTR) will be described in more detail in the "Candidate genes" section.

## 1.2 Muscle strength trainability and its genetic determinants

Muscular strength is influenced by many variables, including muscle cross-sectional area and neuromuscular function (Maughan 2005). While muscle cross-sectional areal gives the potential for the muscles' force production, the neuro-muscular function determines the ability to utilize this potential (Behm 1995, Campos, Luecke et al. 2002, Erskine, Fletcher et al. 2014, Psilander, Eftestøl et al. 2019). Low muscle strength has been associated with increased all-cause mortality risk in the elderly (Li, Xia et al. 2018) as well as increased dysfunctionality (Mendonca, Pezarat-Correia et al. 2017). Muscle function and/or strength is also associated with PA levels (Rojer, Reijnierse et al. 2018, Tomás, Galán-Mercant et al. 2018). Maximal strength or power in large muscle groups, especially the knee- or the hip extensors, has been associated with physical functioning (Reid and Fielding 2012), and cognitive ability (Fiatarone Singh, Gates et al. 2014, Steves, Mehta et al. 2016). Strength training activities have therefore been recommended by the WHO (WHO 2010).

Advancing age from approximately 50 yrs, has been associated with an exponential decline in muscular strength and power (Unhjem, Lundestad et al. 2015, Mendonca, Pezarat-Correia et al. 2017), with some differences between the sexes (Dey, Bosaeus et al. 2009, Wu, Delahunt et al. 2016, Francis, Lyons et al. 2017). The decline in muscle strength appears to be larger in the lower limbs than in the upper body, making older individuals even more vulnerable to e.g. falls (Mendonca, Pezarat-Correia et al. 2017). Both mechanical and neuro-muscular declines likely contribute to the decline in muscle strength and function associated with advancing age (Cartee, Hepple et al. 2016, Wu, Delahunt et al. 2016). However, the decline in muscle strength has in several studies been shown to be counteracted by strength training (Raymond, Bramley-Tzerefos et al. 2013, Wang, Nyberg et al. 2017, Distefano and Goodpaster 2018).

Conventional strength training is usually performed at a low/moderate intensity with a range of an 8–12 repetition maximum (RM) employing loads at 60–70 % of 1RM (Ratamess, Alvar et al. 2009, Heggelund, Fimland et al. 2013), i.e. the individual manages to perform 8-12 repetitions with a correct technique and the specific load. This type of training typically targets an increase in cross-sectional area, i.e. muscle hypertrophy (Campos, Luecke et al. 2002). Maximal strength training (MST), on the other hand, employs heavy loads (85–90% of 1RM) and few repetitions (up to 5 RM) (Heggelund, Fimland et al. 2013). This type of training typically targets neuromuscular function, although it will also affect hypertrophy to some extent (Behm 1995, Campos, Luecke et al. 2002, Mangine, Hoffman et al. 2015, Jenkins, Miramonti et al. 2017, Schoenfeld, Grgic et al. 2017). Thus, MST has been shown to be a more efficient way of improving maximal muscle strength compared to the conventional strength training (Campos, Luecke et al. 2002, Heggelund, Fimland et al. 2013, Mangine, Hoffman et al. 2015, Schoenfeld, Grgic et al. 2017, Lasevicius, Ugrinowitsch et al. 2018), with similar improvements in males and females (Lewis, Kamon et al. 1986, Tracy, Ivey et al. 1999, Lemmer, Hurlbut et al. 2000). MST has also been shown to improve bone mineral density (Mosti, Carlsen et al. 2014), rate of force development and work efficiency (Heggelund, Fimland et al. 2013, Wang, Nyberg et al. 2017).

Healthy older subjects, in general, respond well to resistance exercise (Hagerman, Walsh et al. 2000, Hakkinen, Pakarinen et al. 2000, Lemmer, Hurlbut et al. 2000), including MST (Tracy, Ivey et al. 1999, Raymond, Bramley-Tzerefos et al. 2013, Wang, Nyberg et al. 2017, Berg, Kwon et al. 2018). MST is considered to be an effective (Berg, Kwon et al. 2018) and safe (Raymond, Bramley-Tzerefos et al. 2013) exercise mode for elderly to improve muscle strength (Raymond, Bramley-Tzerefos et al. 2013, Wang, Nyberg et al. 2017) and muscle volume (Tracy, Ivey et al. 1999, Berg, Kwon et al. 2018), among other variables, and is even able to reverse the effects of ageing on skeletal muscle (Wang, Nyberg et al. 2017, Berg, Kwon et al. 2018). Whether elderly are able to improve muscle strength to the same extent as their younger counterparts is not yet clear as the results vary (Hagerman, Walsh et al. 2000, Hakkinen, Pakarinen et al. 2000, Lemmer, Hurlbut et al. 2000, Petrella, Kim et al. 2005). However, the elderly appear to maintain the gains in muscle strength equally well when the strength training has ceased (Lemmer, Hurlbut et al. 2000).

Although all individuals may experience a decline in muscle mass and/or strength with increasing age, not all are equally susceptible (Carmelli, Kelly-Hayes et al. 2000). Also, large heterogeneity between individuals in different muscle strength-related phenotypes, both at baseline and in response to exercise, have been observed. For instance, Bamman, Petrella et al. (2007) observed that some individuals exhibited a large hypertrophic response, while some did not exhibit any response following a standardized strength training program. Hubal, Gordish-Dressman et al. (2005) found changes in 1RM to range from 0% to 250% in response to a 12-week training program targeting the elbow flexor muscles. Such variations are influenced by a vast number of factors, including genetics and environment (e.g. age, sex, training status) (Barberio, Pistilli et al. 2019, Thomis 2019), as well as an interaction between both (Hubal, Urso et al. 2011). Also, ethnicity appears to play a role in the variability of age-related decline (Francis, Lyons et al. 2017). The evidence for the genetic component behind muscle strength-related phenotypes is strong (Roth 2012), but not fully understood.

Heritability studies point towards a similar proportion of the muscle strength-related phenotypes being attributed to genetic (~52%) and environmental factors (~48%) (Thomis, Beunen et al. 1998). Some studies have shown that heritability estimates for strength gain responding to strength training programs could be up to 85% (Hubal, Urso et al. 2011). The role of the genetic factors appears to be highest among adolescents and seems to decrease after 40 yrs of age when the environmental factors play a larger role (Zempo, Miyamoto-Mikami et al. 2017). Also, the reported heritability estimates for the phenotypic response to training interventions are highly variable, depending on the measured strength phenotypes such as explosive power, grip strength or baseline lean mass, etc., and depending on the type of strength training applied (Arden and Spector 1997, Calvo, Rodas et al. 2002, Campos, Luecke et al. 2002, Thomis 2019).

There have been indications of specific sets of genes determining different strength phenotypes (Thomis 2019), and that genes affecting baseline strength might be different from those influencing the training response (Thomis, Beunen et al. 1998). Furthermore, there appears to be a strong genetic component to the longitudinal changes in lower limb muscle strength with a heritability estimate of 64% (Zhai, Ding et al. 2005). Traditionally, the genes investigated in relation to baseline muscle strength/size as well as trainability are those potentially affecting muscle structure (neuromuscular pathways) as well as growth and inflammatory factors (Hubal, Urso et al. 2011, Barberio, Pistilli et al. 2019). A number of genetic variants, mostly SNPs, have been studied in relation to muscle strength-related phenotype (Pescatello, Devaney et al. 2013) in athletes, general public and elderly. Some of the most extensively studied polymorphisms, associated with various aspects of exercise genetics, are *ACE* I/D, *ACTN3* R577X and *PPARGC1A* rs8192678 (Ahmetov and Fedotovskaya 2015). These will be described in more detail in the “Candidate genes” section.

## 1.3 Endurance performance and its genetic determinants

Humans are known to possess a great endurance capacity, especially at moderate intensities (Bramble and Lieberman 2004, Raichlen, Webber et al. 2019). Raichlen,

Webber et al. (2019) even describes humans as the “endurance primate”, due to the superior ability of heat regulation in humans. Aerobic endurance capacity, also referred to as aerobic capacity or cardiorespiratory fitness, is one of the main predictors of all-cause mortality (Strasser and Burtscher 2018). Endurance capacity has been shown to have a large impact not only on the physical capacity/function (Tomás, Galán-Mercant et al. 2018) but also on cognition (Zettel-Watson, Suen et al. 2017), especially in the elderly.

Aerobic endurance performance is determined by maximal oxygen uptake ($VO_{2max}$), lactate threshold (LT) and work economy (C) (Pate and Kriska 1984, Bassett and Howley 2000, Tanaka and Seals 2008, Joyner 2019), and may be evaluated by the following equation based on Pate and Kriska (1984) and di Prampero (2003):

$$\text{Aerobic endurance performance} = (\text{LT or fractional utilization}) \cdot (VO_{2max}/C)$$

In this equation, the last parenthesis denotes the maximal aerobic speed (MAS), while the first parenthesis denotes the percentage of MAS that can be performed for a given distance or duration, or at LT (Støren, Rønnestad et al. 2014).

$VO_{2max}$ is regarded as the golden standard measure of cardiorespiratory fitness (Bouchard, Sarzynski et al. 2011). It is defined as the maximal rate of oxygen consumption (Raichlen, Webber et al. 2019) and reflects the highest rate at which the oxygen can be utilized by an individual under strenuous/maximal exercise (Bassett and Howley 2000). $VO_{2max}$ is not only highly associated with a range of health-related outcomes in the general public (Pedersen and Saltin 2015, Strasser and Burtscher 2018), but is considered to be the most important physiological determinant associated with the elite endurance performance (Pate and Kriska 1984, di Prampero 2003). $VO_{2max}$ measured for the same individual can differ depending on the type of activity performed (Helgerud, Høydal et al. 2007). Therefore, especially in athletes, testing should be performed using the type of athletes’ sports-specific activity (Stromme, Ingjer et al. 1977). Of the endurance sports, cross-country skiing is among the most demanding ones

(Sandbakk and Holmberg 2017), and the cross-country skiers possess some of the highest $VO_{2max}$ measured (Ingjer 1991, Sandbakk and Holmberg 2014, Holmberg 2015).

LT also referred to as anaerobic threshold, is the workload (and/or $VO_{2max}$) at which the blood lactate concentration $[La^-]_b$ starts to increase (Davis 1985, Faude, Kindermann et al. 2009). At this point, the balance in $[La^-]_b$ production and removal (Davis 1985) is disturbed, and the production rate exceeds the removal rate (Faude, Kindermann et al. 2009). The LT can be used to assess the endurance capacity as changes in LT may indicate changes in aerobic capacity (Faude, Kindermann et al. 2009).

Last but not least, C is a ratio between work output and oxygen cost (Helgerud, Høydal et al. 2007), and is also referred to as oxygen cost (consumption) at a given velocity/workload (Barnes and Kilding 2015). C is a complex endurance performance determinant, influenced by an array of extrinsic and intrinsic factors (Daniels 1985, Saunders, Pyne et al. 2004), including metabolic, cardiorespiratory, biomechanical (Barnes and Kilding 2015) and anthropometric factors (Daniels 1985). Work economy varies among individuals, including highly trained athletes (Losnegard, Schäfer et al. 2014). Athletes that use less oxygen, and therefore also less energy, at the same steady-state velocity possess a better work economy compared to those using more (Saunders, Pyne et al. 2004, Barnes and Kilding 2015). C can be improved by e.g. maximal strength training (Hoff, Helgerud et al. 1999, Støren, Helgerud et al. 2008, Sunde, Storen et al. 2010).

Endurance capacity, including determinants like $VO_{2max}$, in the sedentary state, is known to vary highly among individuals (Bouchard, Lesage et al. 1986, Bouchard, Daw et al. 1998, Pérusse, Gagnon et al. 2001). $VO_{2max}$ can be improved by exercise, and the trainability of the $VO_{2max}$ depends on training frequency, intensity and duration (Pollock 1977), among other things. However, it appears that training does not necessarily minimize the inter-individual differences in $VO_{2max}$ (Schutte, Nederend et al. 2016), as individuals respond differently to the same training program (Prud'homme, Bouchard et al. 1984, Bouchard, An et al. 1999, Skinner, Wilmore et al. 2000, Bratland-Sanda,

Pedersen et al. 2020). For instance, in a study by Bouchard (1999), following a 20-week endurance training program, participants' improvements ranged from no changes to ~1000 mL·min$^{-1}$, with the mean increase being ~400 mL·min$^{-1}$ (Bouchard, An et al. 1999). Evidence from both animal (Avila, Courtney et al. 2019) and human (Barber and Sarzynski 2019) studies point towards a considerable genetic component both to the endurance capacity as well as its trainability (Bouchard, Sarzynski et al. 2011, Rankinen, Fuku et al. 2016).

While it appears that few, if any, studies have investigated the heritability of LT and C, several studies have focused on the heritability/genetics of the $VO_{2max}$, although, almost exclusively at lower to moderate intensities (Bouchard 2019). Bouchard, Daw et al. (1998) investigated the familial resemblance in baseline $VO_{2max}$ and calculated the heritability estimates to be at least 50% (adjusted for age, sex, body weight and body composition). However, this estimate was likely inflated by non-genetic factors, therefore the authors cautioned that the real estimates are lower. The authors did, also, report that ~30% of the inheritance could be attributed to the maternal heritability. The relatively large maternal contribution is likely due children inheriting the mitochondria from the mother and/or mediated through epigenetics during the fetal development (Barber and Sarzynski 2019).

The heritability estimates for the trainability of the various aspects of the aerobic capacity tend to be lower than for the baseline $VO_{2max}$ (Pérusse, Gagnon et al. 2001). For instance, maximal heritability estimates of submaximal oxygen consumption in response to a 20-week training program ranged from 23% to 57% depending on the power output (Pérusse, Gagnon et al. 2001), and an overall maximal heritability estimate of 47% for the $VO_{2max}$ response (Bouchard, An et al. 1999). In childhood and adolescence, genetics might even play a greater role in determining the $VO_{2max}$. Heritability estimates from meta-analysis that included 8 studies were as high as 59% and 72% for $VO_{2max}$ measured as mL·min$^{-1}$ and mL·kg$^{-1}$·min$^{-1}$, respectively (Schutte, Nederend et al. 2016). Overall, based on 15 studies, the weighted mean heritability for

the submaximal endurance phenotype was 49%, and for the endurance performance-53%, (Miyamoto-Mikami, Zempo et al. 2018). Despite these moderate to high heritability estimates, it is important to note that they vary greatly between the studies (Miyamoto-Mikami, Zempo et al. 2018) which can, in part, be explained by sex differences (Barber and Sarzynski 2019).

Results from these and other studies on the endurance capacity and training-induced adaptability, despite using different training doses, points towards a strong genetic contribution (Rankinen, Fuku et al. 2016, Bouchard 2019). Yet, the role of specific genes and gene variants is not fully understood due to the highly complex nature of traits like endurance capacity and performance (Bouchard 2019). There is a large number of SNPs and other genetic variants that have been investigated in relation to the baseline $VO_{2max}$ and/or its trainability and other determinants of the endurance performance either through candidate gene studies or genome-wide association studies. Of the candidate genes, *ACE*, *ACTN3* and *PPARGC1A* are among those that have received a lot of attention in recent years (Rankinen, Fuku et al. 2016). Others, such as *ACSL1* (Bouchard, Sarzynski et al. 2011) and *IL6* (Harvey, Voisin et al. 2020) have also been looked at as promising candidates. These particular genes will be described in more detail in the "Candidate genes" section.

## 1.4 Candidate genes

### 1.4.1 ACTN3

*ACTN3*, which has commonly been referred to as "gene for speed", is among the most studied genes in association with sports and exercise (Seto, Garton et al. 2019). The gene codes for the α-actinin-3 protein, a member of the α actin-binding protein family, and is mainly expressed in type 2 (fast-twitch) muscle fibres (North and Beggs 1996). There, it plays a role as a structural component in the contractile apparatus (North, Yang et al. 1999, Seto, Garton et al. 2019). Located within the gene, a common SNP rs1815739, commonly referred to as R577X, leads to a premature stop codon (X). Individuals

carrying two copies of the X allele (i.e. XX genotype) are deficient of the α-actinin-3 protein, which is the case for around 18% of the global population (Mills, Yang et al. (2001), MacArthur and North (2004), Seto, Garton et al. (2019)). The frequency of the X allele appears to covary with the latitude (Friedlander, Herrmann et al. 2013), thus, leading to large variations among different ethnicities. The X allele is most common in Europeans and Asians (Mills, Yang et al. 2001, Head, Chan et al. 2015). The polymorphism is one of only two known loss-of-function polymorphisms in humans that have a selective advantage (Lee, Houweling et al. 2016).

The R577X polymorphism has a range of effects on various muscle phenotypes (Pickering and Kiely 2017), not only in athletes (Moran, Yang et al. 2007, Yang, Garton et al. 2009, Eynon, Hanson et al. 2013) but also in the general population of various ages (Pereira, Costa et al. 2013, Del Coso, Hiam et al. 2018, Houweling, Papadimitriou et al. 2018, Pickering and Kiely 2018)). The presence of the α-actinin-3 (especially the RR genotype) is thought to be advantageous for performing forceful/powerful muscle contractions (Del Coso, Hiam et al. 2018). Furthermore, it appears to influence the exercise adaptations (Delmonico, Kostek et al. (2007); Pereira, Costa et al. (2013), Silva, Bolani et al. (2015), Pickering and Kiely (2018), Norman, Esbjörnsson et al. (2009)), and, in most cases, in favour of the RR genotype. Also, with the ageing population in mind, the RR genotype has been associated with greater bone mineral density, reduced risk of falls and sarcopenia (Pickering and Kiely 2018), i.e. age-related loss of skeletal muscle strength, mass, power and physical functioning (Roth 2012).

The more common RR genotype is more frequently found among elite sprint/power athletes than among the general population (Yang, Garton et al. 2009, Ma, Yang et al. 2013, Ahmetov, Egorova et al. 2016). This finding has been replicated repeatedly and indicates that the absence of α-actinin-3 protein impacts sprint and power performance negatively (Tharabenjasin, Pabalan et al. 2019). Furthermore, the genotype frequencies in sprint/power and endurance athlete groups differ in opposite directions (Seto, Garton et al. 2019). The R577X polymorphism is thought to explain around 2% of the differences

in speed/power phenotype (Moran, Yang et al. 2007, Pickering, Kiely et al. 2019). Although the number does not seem high, possessing the optimal sprint-genotype, i.e. the R allele, might prove to be crucial at the elite level (Papadimitriou, Lucia et al. 2016). The effects of α-actinin-3 deficiency on athletic performance may differ between males and females, at least partly due to the hormonal differences (Yang, MacArthur et al. 2003, Clarkson, Devaney et al. 2005, Delmonico, Kostek et al. 2007). In the general population, *ACTN3* R577X polymorphism has been shown to influence traits like baseline maximal strength/power (Erskine, Williams et al. 2014) and strength trainability (Pickering and Kiely 2017, Romero-Blanco, Artiga-González et al. 2020). A previous study of MST intervention on elderly women found that R allele was advantageous in increased maximal dynamic strength (Pereira, Costa et al. 2013). This indicates that the R allele may be advantageous not only for sprint/power athlete status (Weyerstraß, Stewart et al. 2018) but also for adaptations to MST among the general public.

Despite lacking the α-actinin-3 protein, individuals with the XX genotype do not appear to be affected by a disease or a pathology (North, Yang et al. 1999). However, the absence of the protein alters the muscle function (Lee, Houweling et al. 2016) leading to more efficient muscle metabolism due to changes in calcium handling (Head, Chan et al. 2015). The polymorphism also alters structural, metabolic and signalling pathways (Lee, Houweling et al. 2016). Studies have demonstrated that XX individuals tend to have a higher percentage of type I muscle fibres (Yang, MacArthur et al. 2003), and also the metabolism of the type II fibres is altered (Pasqua, Bueno et al. 2016). It has been shown that individuals with XX genotype reach the ventilatory threshold at higher speeds compared to the RR genotype (Pasqua, Bueno et al. 2016), appear to have smaller muscle volume (Erskine, Williams et al. 2014) and strength (Clarkson, Devaney et al. 2005, Erskine, Williams et al. 2014). They may also be more prone to muscle damage following exercise as well as an increased risk of injuries (Pickering and Kiely 2018). In older individuals, thus, the effect of possessing the XX genotype might have clinical importance (Roth 2012).

It is still not clear how possessing two different alleles, as in the heterozygous RX genotype, affects various muscle-related phenotypes. The α-actinin-3 appear to function in a dose-dependent fashion (Hogarth, Garton et al. 2016), however, the heterozygotes (RX) tend to generate variable outcomes (Garton and North 2016, Seto, Garton et al. 2019). A short summary of the relevant information regarding the *ACTN3* R577X is presented in Table 1.

### 1.4.2 ACE

The *ACE* I/D polymorphism was the first genetic variation to be investigated in relation to physical performance phenotypes (Baumert, Lake et al. 2016). It has since been widely investigated and replicated in sports and exercise genomics (Jacques, Landen et al. 2019). The *ACE* gene codes for a protein called the angiotensin I-converting enzyme, that plays a role in the regulation of blood pressure, fluid-electrolyte balance and is also thought to affect the muscle function (Puthucheary, Skipworth et al. 2011, Pescatello, Corso et al. 2019) and metabolism (Jones and Woods 2003). Through these mechanisms, it might be able to influence the aerobic capacity (Goh, Chew et al. 2009, Tamburus, Verlengia et al. 2018) and other exercise-induced adaptations (Pescatello, Corso et al. 2019). I/D stands for insertion/deletion polymorphism, where insertion is the presence of a 287-bp Alu repeat, and deletion is the absence of the repeat (Rigat, Hubert et al. 1992). The I and D alleles have been shown to influence the blood enzyme levels in opposite directions in subjects of European descent (Rigat, Hubert et al. 1990).

Traditionally, the I allele has been regarded as the endurance allele (Ma, Yang et al. 2013, Pescatello, Corso et al. 2019), however, this may depend on the cohort investigated (Gineviciene, Jakaitiene et al. 2016). The D allele, on the other hand, has been regarded as the sprint/power allele (Myerson, Hemingway et al. 1999, Weyerstraß, Stewart et al. 2018), however, this association has not been as clear (Pescatello, Corso et al. 2019). The *ACE* I/D polymorphisms might also lead to differences in muscle fibre type distribution between the genotypes. The I allele carriers possessed a larger percentage of type I (slow-twitch) fibres compared to the DD genotype, with the ID

heterozygotes being intermediates (Zhang, Tanaka et al. 2003). Also, in an untrained state, I allele carriers appear to possess a better aerobic performance compared to the DD genotype. Repeated endurance exercise could, in part, override the genotype effects (Valdivieso, Vaughan et al. 2017).

The *ACE* I/D genotype may have an impact on the physical function of older adults (Yoshihara, Tobina et al. 2009, Wilson, Mavros et al. 2019). It has been also shown to modulate the response of physical function variables to exercise training and/or PA, with the D allele being advantageous (Buford, Hsu et al. 2014, Wilson, Mavros et al. 2019). Wilson, Mavros et al. (2019) suggested that the D allele carriers responded better to PA and/or exercise interventions, based on the results of several studies. Furthermore, the D allele alone (Williams, Day et al. 2005) and/or in combination with the optimal *ACTN3* R-allele (Erskine, Williams et al. 2014), may be associated with increased baseline muscle strength (Williams, Day et al. 2005, Erskine, Williams et al. 2014). It is not clear, however, if the I/D polymorphism influences the muscle strength response to resistance training as the results are inconsistent (Folland, Leach et al. 2000, Williams, Day et al. 2005, Pescatello, Kostek et al. 2006). On the other hand, the I allele might provide a cardioprotective effect in response to resistance exercise (Montrezol, Marinho et al. 2019), but also increase the susceptibility to muscle damage (Yamin, Amir et al. 2007). In response to intense endurance exercise, I allele may lead to more beneficial improvements in mitochondrial metabolism despite poorer baseline measures in $VO_{2max}$ and capillary density (Vaughan, Huber-Abel et al. 2013). Studies have also indicated that the I/D polymorphism might be associated with PA levels, however, the results are inconclusive (Fuentes, Perola et al. 2002, Winnicki, Accurso et al. 2004, Maestu, Latt et al. 2013).

All in all, *ACE* I/D is a plausible candidate gene to influence the endurance performance and trainability, however, the results are contradictory or inconclusive, and the potential effect the polymorphism plays is likely small (Rankinen, Perusse et al. 2000, Woods, World et al. 2002, Defoor, Vanhees et al. 2006, Pescatello, Corso et al. 2019). A

short summary of the relevant information regarding the *ACE* I/D is presented in Table 1.

### 1.4.3 PPARs and their co-activators

The peroxisome proliferator-activated receptor (PPAR) signalling pathway is likely highly important for the trainability of the aerobic capacity (Ghosh, Vivar et al. 2013, Alvarez-Romero, Voisin et al. 2020). Genes within the pathway, including peroxisome proliferator-activated receptor genes (*PPARA, PPARD* and *PPARG* (Phua, Wong et al. 2018)), and their co-activators (e.g. *PPARGC1A*; Correia, Ferreira et al. (2015)) play a master-regulator role at the gene transcription level. PPARs act like fatty acid sensors (Lamichane, Dahal Lamichane et al. 2018) and are essential players in a range of physiological processes. They regulate energy and nutrient metabolism (Correia, Ferreira et al. 2015, Lamichane, Dahal Lamichane et al. 2018, Hong, Pan et al. 2019), including the glucose and lipid homeostasis (Phua, Wong et al. 2018, Hong, Pan et al. 2019) both locally and systemically (Correia, Ferreira et al. 2015, Phua, Wong et al. 2018). The genes have different expression patterns, and they are especially actively expressed in metabolic tissues, including adipose tissue, heart and skeletal muscle (Dillon, Rebelo et al. 2012, Correia, Ferreira et al. 2015, Lamichane, Dahal Lamichane et al. 2018, Phua, Wong et al. 2018). Because of the role this group of genes play in energy metabolism, among other things, several of them have been investigated in relation to various aspects of $VO_{2max}$ (Ghosh, Vivar et al. 2013, Alvarez-Romero, Voisin et al. 2020) and muscle strength/power phenotypes (Ahmetov and Fedotovskaya 2015).

Among the PPAR pathway genes, *PPARGC1A* might be among the most investigated (Petr, Maciejewska-Skrendo et al. 2019). The gene codes for the peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) which is a transcriptional co-activator that, together with transcription factors, up-/down-regulate expression of other genes (Quindry and Roberts 2019). PGC-1α has a range of functions, including regulation of mitochondrial biogenesis and metabolism (Correia, Ferreira et al. 2015, Quindry and Roberts 2019), angiogenesis and muscle fibre type distribution

(Arany 2008, Ahmetov and Fedotovskaya 2015), just to name a few. PGC-1α role in exercise-induced adaptations have been recognized (Lira, Benton et al. 2010, Ruas, White et al. 2012), and endurance exercise leads to upregulation of the protein (Quindry and Roberts 2019). Ageing, on the other hand, leads to downregulation of the PGC-1α which have a range of negative effects in both cardiac and skeletal muscle tissues (Dillon, Rebelo et al. 2012). The *PPARGC1A* gene, depending on the tissue type and the physiological stimuli, can be transcribed and/or spliced into several isoforms with different biological functions (Martinez-Redondo, Pettersson et al. 2015, Martinez-Redondo, Jannig et al. 2016). Stimuli like different training modalities, i.e. endurance vs. strength training, appear to lead to differential isoform formation (Martinez-Redondo, Jannig et al. 2016). Probably the most investigated SNPs within the *PPARGC1A* gene is the rs8192678 (Chen, Wang et al. 2019). The SNP is more widely known as the Gly482Ser polymorphism, where the amino acid glycine (Gly) is substituted by serine (Ser), with around 35% of Caucasians being carriers of the Ser-allele (NCBI 2020f). The SNP has been associated with exercise trainability (Lira, Benton et al. 2010, Ruas, White et al. 2012, Steinbacher, Feichtinger et al. 2015, Petr, Stastny et al. 2018), sports performance and athletic ability (Gineviciene, Jakaitiene et al. 2016, Chen, Wang et al. 2019, Petr, Maciejewska-Skrendo et al. 2019, Tharabenjasin, Pabalan et al. 2019). Homozygotes of the common Gly allele possess a superior aerobic capacity (Ahmetov and Fedotovskaya 2015) and more efficient aerobic metabolism (Petr, Maciejewska-Skrendo et al. 2019), in both general public and athletes (Ahmetov and Fedotovskaya 2015). They also respond better to aerobic exercise (Petr, Stastny et al. 2018). On the other hand, homozygotes of the minor Ser allele might be underrepresented among endurance athletes in some cohorts (Eynon, Meckel et al. 2010, Ahmetov and Fedotovskaya 2015), and could potentially be non-responders to aerobic exercise programs (Petr, Stastny et al. 2018). The Ser/Ser genotype might, however, be advantageous to power/strength athletes (Gineviciene, Jakaitiene et al. 2016).

*PPARA* gene codes for peroxisome proliferator-activated receptor alpha (PPARα), and is expressed in metabolically active tissues, such as skeletal muscle, heart and adipose

tissue (Hong, Pan et al. 2019). The protein plays a major role in lipid metabolism (Hong, Pan et al. 2019) as it regulates the expression of genes involved in fatty acid uptake and oxidation (Lamichane, Dahal Lamichane et al. 2018), among other things. The PPARα role changes depending on the nutritional state (Hong, Pan et al. 2019). The rs4253778 polymorphism, where G has been substituted by C (Jamshidi, Montgomery et al. 2002), has been associated with resistance trainability (Alvarez-Romero, Voisin et al. 2020) as well as heart hypertrophy response to training (Jamshidi, Montgomery et al. 2002). GG homozygotes have been demonstrated to possess a larger proportion of slow-twitch fibres compared to their CC counterparts (Ahmetov, Mozhayskaya et al. 2006). The G allele might be associated with endurance athlete status (Eynon, Meckel et al. 2010, Tural, Kara et al. 2014, Lopez-Leon, Tuvblad et al. 2016, Petr, Maciejewska-Skrendo et al. 2019). Furthermore, there are indications towards an advantage of the C allele in power/strength-oriented sports as they may possess a more efficient anaerobic metabolism (Petr, Stastny et al. 2014, Stastny, Lehnert et al. 2019).

*PPARG* codes for peroxisome proliferator-activated receptor gamma (PPARγ), a protein mainly expressed in adipose tissue (Phua, Wong et al. 2018). PPARγ is crucial for glucose metabolism (Hong, Pan et al. 2019), and promotes glucose uptake in skeletal muscle (Phua, Wong et al. 2018). *PPARG* Pro12Ala (rs1801282) polymorphism is a common SNP where C is substituted with G leading to an amino acid substitution (Stumvoll and Häring 2002) modifying the activity of the protein (Maciejewska-Karłowska 2013). The minor G (Ala) allele is found in around 11% of the Caucasians (Stumvoll and Häring 2002, NCBI 2020b). The SNP appears to affect the trainability. The carriers of the Ala allele, which also possess a lower transcriptional activity (Maciejewska-Karłowska 2013), demonstrate improved insulin response in response to aerobic exercise compared to Pro allele homozygotes (Petr, Stastny et al. 2018, Blond, Schnurr et al. 2019). Furthermore, the Ala allele has been associated with power/strength athlete status at an elite level (Maciejewska-Karlowska, Sawczuk et al. 2013, Petr, Maciejewska-Skrendo et al. 2019). A short summary of the relevant information regarding the *PPARGC1A* rs8192678, *PPARA* rs4253778 and *PPARG* rs1801282 is presented in Table 1.

### 1.4.4 ACSL1

*ACSL1* gene codes for acyl-CoA synthase long-chain member 1 protein that plays a role in the activation and transport of long-chain fatty acids into mitochondria (Ghosh, Vivar et al. 2013). A SNP within the *ACSL1* gene (rs6552828) was previously found to explain as much as 6% of the $VO_{2max}$ trainability in response to a standardized endurance training program. Carriers of the more common G allele demonstrated a larger $VO_{2max}$-increase than homozygotes of the minor A allele (Bouchard, Sarzynski et al. 2011). This has later been validated by others (Ghosh, Vivar et al. 2013), thus, making the rs6552828 a robust candidate (Ghosh, Vivar et al. 2013). Despite the robust association between the SNP and training response in untrained/sedentary individuals, the rs6552828 does not appear to be associated with elite endurance athlete status (Yvert, He et al. 2012). A short summary of the relevant information regarding the *ACSL1* rs6552828 is presented in Table 1.

### 1.4.5 IL-6

Interleukin-6 (*IL-6*) gene codes for a peptide with the same name that skeletal muscle (and other tissues) release into circulation during and after exercise (Schnyder and Handschin 2015, Lee and Jun 2019). The peptide is the most studied and one of the first to be recognized as a myokine, a molecule that is released by skeletal muscle during muscle contractions (Hoffmann and Weigert 2017). It plays a role in inflammation (Schnyder and Handschin 2015), fatty acid oxidation, and is also an important player in glucose metabolism (Lee and Jun 2019). Furthermore, IL-6 contributes to hypertrophic muscle growth (Serrano, Baeza-Raja et al. 2008) and myogenesis (Muñoz-Cánoves, Scheele et al. 2013), and have been investigated in relation to muscle strength phenotypes (Garatachea and Lucia 2013). Recently, an association between a SNP (rs1474347) and trainability of $VO_{2max}$ in moderately and well-trained individuals was reported (Harvey, Voisin et al. 2020). This SNP is in strong linkage disequilibrium with other *IL-6* SNPs (e.g. rs1800795; Harvey, Voisin et al. (2020)) that also have been

explored in association with traits like power athlete status (Ruiz, Buxens et al. 2010, Eynon, Ruiz et al. 2011, Ahmetov and Fedotovskaya 2015) and sprint performance (Pickering, Suraci et al. 2019). A short summary of the relevant information regarding the *IL-6* rs1474347 is presented in Table 1.

*Table 1 Summary of candidate genes/polymorphisms associated with physical performance, trainability and/or athletic performance*

| Gene (polymorphism) | Phenotype | Beneficial genotype/allele |
|---|---|---|
| *ACTN3* | | |
| R577X | sprint/power performance[1] and elite power athlete status[14] | R allele |
| | endurance capacity[1] | XX |
| | risk of falls and sarcopenia[2] | RR |
| | baseline maximal strength/power[3] | RR |
| | resistance trainability[1,4,5] | R allele |
| *ACE* | | |
| I/D | sprint performance[8] | DD |
| | exercise-induced physiological changes[7] | I allele |
| | muscle strength[6] | D allele |
| | resistance trainability[5] | D allele |
| *PPARGC1A* | | |
| rs8192678 (Gly482Ser) | strength/power athlete status[12] | Ser/Ser (TT) |
| | athletic ability and sports performance[13] | Gly allele (C) |
| | aerobic capacity[9] and efficient aerobic metabolism[10] | Gly allele (C) |
| | aerobic trainability[5, 11] | Gly allele (C) |
| *PPARA* | | |
| rs4253778 | ability of endurance sports[15] and endurance athlete status[10] | G allele |
| | slow-twitch fibre proportion[16] | GG |
| | strength/power[17] | CC |
| | resistance trainability[5,15] | C allele |
| *PPARG* | | |
| rs1801282 (Pro12Ala) | strength/power elite athlete status[10] | Ala allele (G) |
| | exercise-induced insulin response[11] | Ala allele (G) |
| *ACSL1* | | |
| rs6552828 | $VO_{2max}$ trainability in sedentary individuals[5,18] | G allele |

| | | |
|---|---|---|
| *IL-6* | | |
| rs1474347 | $VO_{2max}$ trainability in moderately and highly trained individuals[19] | A allele |
| | sprint performance*[20] | G allele* |
| | power athlete status*[9] | G allele* |

* Associations for the rs1800795 (rs1474347 is in strong linkage disequilibrium with); rs1474347 A allele is linked with the rs1800795 G allele; rs1474347 C allele is linked with rs1800795 C allele; [1]Seto, Garton et al. (2019); [2]Pickering and Kiely (2018); [3]Erskine, Williams et al. (2014); [4]Pickering and Kiely (2017); [5]Alvarez-Romero, Voisin et al. (2020); [6]Wilson, Mavros et al. (2019); [7]Pescatello, Corso et al. (2019); [8]Papadimitriou, Lucia et al. (2016); [9]Ahmetov and Fedotovskaya (2015); [10]Petr, Maciejewska-Skrendo et al. (2019); [11]Petr, Stastny et al. (2018); [12]Gineviciene, Jakaitiene et al. (2016); [13]Tharabenjasin, Pabalan et al. (2019)a; [14]Tharabenjasin, Pabalan et al. (2019)b; [15]Lopez-Leon, Tuvblad et al. (2016); [16]Ahmetov, Mozhayskaya et al. (2006); [17]Stastny, Lehnert et al. (2019); [18]Bouchard, Sarzynski et al. (2011); [19]Harvey, Voisin et al. (2020); [20]Pickering, Suraci et al. (2019)

### 1.4.6 MAOA

All of the genes described above can potentially affect physical capability/function, trainability and/or athletic ability/performance. In addition to being physically capable, possessing the motivation to do something is another important factor necessary to perform voluntary behaviour, such as PA (Rhodes 2019). A large proportion of the observed differences in PA levels is likely due to the motivation, and not as much the physical capability (Rhodes, Gammie et al. 2005, Good, Li et al. 2015, Rhodes 2019). Thus, it is likely that genes that alter the PA motivation (Good, Li et al. 2015) by e.g. producing a strong reward signal in response to exercise (Rhodes 2019) may play a role in determining the PA level (Good, Li et al. 2015). One of the pathways thought to be involved in determining the voluntary PA levels is the dopaminergic system (Rhodes, Gammie et al. 2005, Rhodes 2019). It has been hypothesized that genetic variants involved in this pathway could lead to differences in PA behaviour (Van Der Mee, Fedko et al. 2018, Rhodes 2019).

Monoamine oxidase A (*MAOA*), a gene located on the X chromosome, is involved in the dopaminergic pathway (Sabol, Hu et al. 1998). The gene codes for an enzyme that plays a role in the oxidation of several types of neurotransmitters, including serotonin, norepinephrine and dopamine (Shih and Thompson 1999). It is also one of several genes that have been investigated in association with voluntary PA and sedentary behaviour (Morishima, Harada et al. 2006, Good, Li et al. 2015, Van Der Mee, Fedko et al. 2018,

Goleva-Fjellet, Bjurholt et al. 2020). A genetic variant within the promoter region of the gene, a 30-bp repeated sequence called the variable number of tandem repeat (uVNTR), exists as several different alleles, depending on the number of the 30-bp repeats (Sabol, Hu et al. 1998, Goleva-Fjellet, Bjurholt et al. 2020). The uVNTR affects the transcriptional activity of the gene (Sabol, Hu et al. 1998), with the 3-repeat allele leading to lower transcriptional activity than the 3.5 and 4-repeat alleles (Sabol, Hu et al. 1998, Deckert, Catalano et al. 1999). This genetic variant has been demonstrated to affect the reward dependence (Shiraishi, Suzuki et al. 2006) and voluntary PA behaviour (Good, Li et al. 2015). According to the hypothesis, individuals with higher transcriptional activity alleles (i.e. 3.5 and 4-repeats) degrade neurotransmitters more rapidly thus may possess lower motivation for PA behaviour (Good, Li et al. 2015).

### 1.4.7 Differences in allele frequencies across populations

Allele frequencies for many genetic variants vary across different populations (Gordish-Dressman and Devaney 2011). To demonstrate this, genotype frequencies for the *ACTN3* R577X and *ACE* I/D polymorphisms in different populations are presented in Figure 1. For instance, it has been hypothesized that the *ACTN3* R577X vary with the latitudinal gradient due to a potential evolutionary advantage of the XX genotype in more scarce environments (Friedlander, Herrmann et al. 2013). The X allele frequency varies from around 13% in some African populations, 44% in Europeans, to 66% in the Hawaiian population (NCBI 2020c). Similarly, the *ACE* alleles vary highly (Figure 1b). In Caucasian populations, both the D and I alleles are equally common, i.e. 50% frequency each. In other populations, however, the D allele frequencies range from 9% in Samoans to 60% in Jamaicans (Scott, Irving et al. 2010). For the other candidate polymorphisms, the minor allele frequencies range as follows: from 6% to 54% for the *PPARGC1A* rs8192678 (NCBI 2020f); 0% - 100% for the *PPARA* rs4253778 (NCBI 2020d); 0%-16% fo the *PPARG* rs1801282 (NCBI 2020b); 22% - 68% for the *ACSL1* rs6552828 (NCBI 2020e); 1% - 50% fo the *IL*-6 rs1474347 (NCBI 2020a). *MAOA* uVNTR exists as several alleles. Some of the alleles are rare, and two alleles are common, i.e. the 3- and 4- repeat alleles

(Sabol, Hu et al. 1998, Goleva-Fjellet, Bjurholt et al. 2020). Depending on the population, the frequencies for the two common alleles range from 30% to 60%, and from 36% to 71%, for the 3- and 4-allele, respectively (Sabol, Hu et al. 1998).

If unaccounted for, the variations in allele frequencies can have unwanted consequences as even small amounts of admixture have the potential to lead to either false positive or negative results in association studies (Marchini, Cardon et al. 2004). This is even more true for studies with large sample sizes (Marchini, Cardon et al. 2004). To avoid this, ethnically homogenous samples must be included in the study (Mathew, Basheeruddin et al. 2001) or one must control for the stratification (Marchini, Cardon et al. 2004, Gordish-Dressman and Devaney 2011).

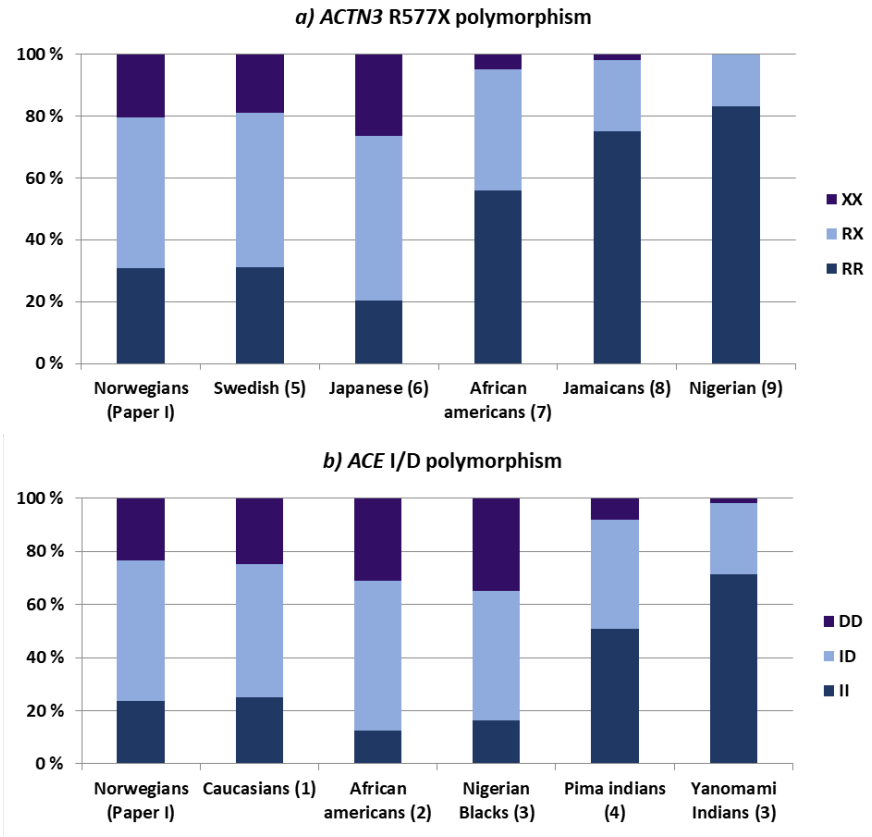


*Figure 1 a) ACTN3 R577X and b) ACE I/D polymorphism genotype frequencies (%) across different populations*

*[1]Jones and Woods (2003); [2]Mathew, Basheeruddin et al. (2001); [3]Barley, Blackwood et al. (1994); [4]Foy, McCormack et al. (1996); [5]Norman, Esbjörnsson et al. (2009); [6]Mikami, Fuku et al. (2014); [7]Roth, Walsh et al. (2008); [8]Scott, Irving et al. (2010); [9]Yang, Macarthur et al. (2007)*

# 2 Material and methods

## 2.1 Ethical considerations

For all papers included, informed written consent was obtained from all participants. In Paper III, for participants under 18 years of age, parental written consent was obtained. All three studies were conducted following the Declaration of Helsinki and were in accordance with the ethical standards of the institutional and/or national research committee. Studies covered in Paper I and Paper III were evaluated and approved by the regional ethics committee of Southeast Norway (REK 3087 (paper I); REK 2017/2522 (Paper III)), and Paper II was approved by the Norwegian Centre for Research Data (NSD, reg. 45185/3/AH). Furthermore, studies covered in papers I and II were registered in Clinical Trials with identifiers NCT00119912 and NCT02589990, respectively. Participants included in Paper II and III were assessed by a physician before inclusion in the study.

## 2.2 Cohort description

The three papers included in this thesis were each based on a different cohort. Two of these represented the general population (Papers I and II), while the third represented a well-trained cohort (Paper III). Subject characteristics are displayed in Table 2.

*Table 2 Overview of the subject characteristics for the three cohorts included in the thesis*

| | | Paper I | Paper II | Paper III |
|---|---|---|---|---|
| | n | 831 | 49 | 29 |
| Age (yrs) (Mean ± SD) | | 55.5 ± 3.8 | 45.4 ± 16.0 | 22.1 ± 8.4 |
| Sex (Males Females) | + | 415 + 416 | 22 + 27 | 17 + 12 |
| Weight (kg) | | 78.5 ± 13.9 | 76.3 ± 12.2 | 69.4 ± 9.3 |
| BMI (kg/m$^2$) | | 26.1 ± 3.8 | 25.5 ± 3.0 | 22.3 ± 2.0 |
| Training level | | untrained to moderately trained | untrained to well-trained | well trained |
| METs* | | 4.8 ± 2.4 | - | 14.4 ± 3.4 |

n- number of subjects; yrs- years; BMI- Body Mass Index; METs- metabolic equivalents; *mean METS per minute during physical activity

### 2.2.1 The KAM cohort (Paper I)

A total of 831 individuals from the county of Telemark (Norway) were included in the study described in Paper I. These individuals were from the cohort "Kolorektal cancer, Arv og Miljø (KAM)", a molecular epidemiological study based partly on the screening group of the Norwegian Colorectal Cancer Prevention Study (The NORCCAP study; Bretthauer, Gondal et al. 2002, Skjelbred, Sæbø et al. 2006). Blood samples and questionnaires covering topics like self-reported PA data and socioeconomic data were available for each participant. Anthropometric measures for the cohort are presented in Table 3.

*Table 3 Anthropometric data for the KAM cohort*

| Variable | All | Females | Males |
|---|---|---|---|
| n | 831 | 416 | 415 |
| Age (yrs) | 55.5 ± 3.8 | 55.4 ± 3.8 | 55.6 ± 3.7 |
| Weight (kg) | 78.5 ± 13.9 | 72.1 ± 12.6 | 84.9 ± 12.2 |
| BMI (kg/m$^2$) | 26.1 ± 3.8 | 25.9 ± 4.3 | 26.4 ± 3.3 |

Data are presented as mean ± SD. n- number of subjects; yrs- years; PA- physical activity; BMI- Body Mass Index.

### 2.2.2 The leg-press study cohort (Paper II)

Participants from Telemark County (Norway) were invited to participate in the study through announcements on social media/posters placed at the university campus and other locations. In total, 76 healthy adults (33 males and 43 females) were included in the study investigating the effects of age, gender and polymorphisms on the MST in leg-press (Paper II). Age of the participants ranged from 20 to 76 years, and the participants were divided into five age-based groups (Table 4). The subjects in the groups were matched based on the baseline 1 repetition maximum (RM) in the leg-press, corrected for age, sex and body mass. Based on the physicians' assessment, participants that were of general good health status with no contra-indications for MST and testing were approved for participation in the study. Exclusion criteria were any injury and/or illness that could prevent subjects from performing MST or testing. Compliance of at least 80% of all training sessions was required for inclusion in the final data analysis. Thus, those subjects with compliance of under 80% were excluded from the study.

*Table 4 Anthropometric data for the Leg-press cohort across age groups*

| Variable | All | Age group 1 | Age group 2 | Age group 3 | Age group 4 | Age group 5 |
|---|---|---|---|---|---|---|
| n | 49 | 10 | 9 | 12 | 8 | 10 |
| Age (yrs) | 45.4 ± 16.0 | 25.6 ± 2.8 | 33.9 ± 2.8 | 44.2 ± 3.2 | 53.5 ± 3.0 | 70.3 ± 4.3 |
| $Weight_{pre}$ (kg) | 76.3 ± 12.2 | 74.4 ± 8.9 | 83.5 ± 11.8 | 74.4 ± 12.5 | 80.8 ± 15.1 | 70.3 ± 10.2 |
| $BMI_{pre}$ ($kg/m^2$) | 25.5 ± 3.0 | 24.9 ± 3.2 | 27.6 ± 3.0 | 25.6 ± 2.5 | 25.1 ± 2.1 | 24.6 ± 3.6 |

Data are presented as mean ± SD. n- number of subjects; yrs- years; BMI- Body Mass Index; pre- baseline values

### 2.2.3 The cross-country skiing cohort (Paper III)

Subjects from the high-schools for skiers in South-eastern Norway or regional cross country ski teams were invited to participate in the cross-country skiing project. In total, 46 well-trained cross-country skiers (30 males and 16 females) with a broad age (16 - 48 years) and performance-level range (mid-junior level to top national level) were recruited. Of these, 17 had to be excluded as they could not comply with the three testing sessions during the 6-month study period or because of insufficient training habit reporting. Therefore, the final study cohort included data from 29 skiers of both genders (Table 5), and these were divided into two age groups (16-18 years and ≥19 years).

*Table 5 Anthropometric data for the Cross-country skiing cohort*

| Variable | All | Males | Females | 16 – 18 yr | 19+ yr |
|---|---|---|---|---|---|
| n | 29 | 17 | 12 | 16 | 13 |
| Age (yrs) | 22.1 ± 8.4 | 24.1 ± 10.2 | 19.3 ± 4.1 | 17.3 ± 0.8 | 28.0 ± 9.8 |
| Weight (kg) | 69.4 ± 9.3 | 73.2 ± 8.6 | 64.0 ± 7.8 | 64.4 ± 6.7 | 75.5 ± 8.5 |
| BMI ($kg/m^2$) | 22.3 ± 2.0 | 22.3 ± 2.0 | 22.3 ± 2.0 | 21.3± 1.8 | 23.5 ± 1.5 |

Data are presented as mean ± SD. n- number of subjects; yrs- years; BMI- Body Mass Index

## 2.3 Sample collection and DNA extraction

For all three studies, venous blood was drawn by trained professionals and collected in BD Vacutainer® EDTA Tubes. Samples were stored at -20 °C until the DNA was extracted.

### 2.3.1 Salting-out method (Paper I)

To extract the genomic DNA, a salting-out procedure was used (Miller, Dykes et al. 1988) with previously described modifications (Skjelbred, Sæbø et al. 2006). In short, to thawed blood samples, lysis buffer (155 mM $NH_4Cl$, 10 mM $KHCO_3$, 1mM EDTA, pH 7.4) was added roughly three times the sample volume. Then, the samples were subjected to incubation at 3°C for 30-60 min followed by centrifugation. The pellet now contained nucleated cells and was re-suspended in 10 ml SE buffer (75 mM NaCl, 24 mM EDTA, pH 8.0). Then, 500 µl 20% SDS and 50 µl protease K were added to each sample. These were incubated overnight at 40°C. On the following day, 2.0 ml 6M NaCl was added to each sample followed by vortexing for 15 seconds. Then, to create a protein pellet, the lysate was centrifuged at 5000rpm for 15 min at 3°C followed by the addition of two volumes of cold absolute ethanol to the supernatant to precipitate the DNA. The DNA was first washed in 3 ml 70% ethanol, then air-dried and placed in a 2.0 ml tube containing 200-

1000 µl TE-buffer (10 mM Tris, 0.1 mM EDTA, pH 7.5). Finally, the DNA was dissolved by shaking at ambient temperature for 24 hours at 800 rpm and stored at 4$^{o}$C.

### 2.3.2 Kit based DNA extraction (Papers II and III)

For Papers II and III, the DNA was extracted from 100 µl of blood using the DNeasy Blood & Tissue Kit (Qiagen, MD, USA) according to the manufacturer's instructions. In short, 20 µl of proteinase K (provided by the manufacturer) was added into a microcentrifuge tube followed by 100 µl of anticoagulated blood and 100 µl PBS. 200 µl of lysis buffer (provided) was added, then mixed by vortexing and incubated at 56$^{o}$C for 10 min. After incubation, 200 µl 96-100% ethanol was added and vortexed. The solution was then transferred to a DNeasy Mini spin column and centrifuged at 8000rpm for 1min. The flow-through was discarded together with the collection tube and the spin column was again placed into a clean tube. Two washing steps followed where 500 µl of the provided washing buffers 1 and 2, respectively, were added to the spin column. The columns were centrifuged at 8000 rpm for 1 min and 14000 rpm for 3 min at the first and second washing step, respectively. At both steps, the flow-through was discarded. Finally, the spin column was placed in a clean microcentrifuge tube and 200 µl of the provided elution buffer was added followed by a 1 min incubation, and then centrifuged for 1 min at 8000 rpm. The flow-through elute now contained the DNA.

## 2.4 Genotyping

### 2.4.1 PCR (Paper I)

To genotype the *ACE* I/D polymorphism, Eppendorf Mastercycler Gradient (Eppendorf AG, Germany) was used. The reaction mixture of 25.5 µl for each sample contained 2% DMSO, 1 x PCR buffer, 0.2 mM dNTP, 2 mM MgCl, 0.2 pmol/µl of each primer, 0.5 U/µl Taq polymerase, and 1 µl of DNA (~ 100 ng). Following forward and reverse primers were used: 5'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'-GATGTGGCCAT-CACATTCGTCAGAT-3', respectively, as described previously (Rigat, Hubert et al. 1992). The PCR program

consisted of these steps: initial denaturation at 95°C for 3 min followed by denaturation (95°C; 15 s), hybridization (53°C; 45 s) and extension (72°C; 30 s) for 30 cycles in total, finished by the final elongation (72 °C; 5 min) step. The PCR products, stored at 4°C, were separated for 30 min at 150 V by 6% polyacrylamide gel electrophoresis (PAGE). There were three possible outcomes: DD, ID and II. Importantly, the heterozygotes can often be mistyped as the I allele is often weakly amplified, and thus is invisible on the gel, in individuals with the ID genotype. Therefore, samples that yielded the DD genotype were re-analysed with slightly modified PCR reaction: an insertion specific forward primer 5'-TTTGAGACGGAGTCTCGCTC-3' and the abovementioned standard reverse primer (Shanmugam, Sell et al. 1993). The reaction mix in each 25.0 µl-sample: contained 12.5 µl AmpliTaq Gold® PCR Master Mix (Thermo Fisher Scientific, Inc.; MA, USA), 5% DMSO, 0.2 pmol/µl of each primer, and ~ 100 ng template DNA. Following PCR program was used: initial denaturation at 95°C for 3 min followed by 30 cycles of denaturation (92°C), hybridization (61°C) and extension (72°C) for 1 min each, and then finishing with the final elongation for 7 min at 72°C. The products were then stored at 4°C. When the PCR products were visualized by using 6% PAGE, two outcomes were possible: either a 408 bp long band in carriers of the I-allele or no band in case of the DD genotype. Likely due to the presence of an inhibitor, 215 (25.8%) samples yielded no results ever after repeated PCR runs for the *ACE* I/D polymorphism.

To genotype the *MAOA* promoter polymorphism, it was first amplified by PCR with the following conditions: initial denaturation (95°C; 2 min) was followed by denaturation (95°C; 1 min), annealing (55.5°C; 1 min) and elongation (72°C; 2 min) for 35 cycles in total, and a final elongation (72°C; 5 min). The 15 µl-reaction mixture of the contained: 5% DMSO, 1x PCR buffer, 0.2 mM dNTP, 2.5 mM MgCl, 0.4 mM of each primer, 1 U/µl Taq polymerase and 1 µl of DNA. The PCR products were then separated by capillary electrophoresis on an Applied Biosystems 3130xl genetic analyzer using GeneMapper® (Applied Biosystems®, CA, USA) Software 5. Primers used were as follows: a FAM labelled forward primer 5'-ACAGCCTGACCGTGGAGAAG-3' and a reverse primer 5'-

GAACGGACGCTCCATTCGGA-3' (Sabol, Hu et al. 1998). Of the 831 samples, 110 (13.2%) yielded no results even after a repeated PCR run.

In addition to the samples where the genotyping was inconclusive, around 10% of all samples were re-analysed for both *ACE* I/D and *MAOA* uVNTR polymorphisms. The samples that, despite repeated testing, did not yield results or were still inconclusive, were excluded further analysis.

### 2.4.2 Real-time PCR (Paper I, II and III)

The *ACTN3* R577X polymorphism in the Paper I, and all genotyping in the Papers II and III was performed on StepOnePlus™ Real-Time PCR System (Applied Biosystems®, CA, USA) using the TaqMan® SNP Genotyping Assay (Thermo Fisher Scientific, MA, USA). Genotype calling was performed by StepOne Software v2.0. All assay IDs are displayed in Table 6. In paper II, *ACE* I/D, *ACTN3* R577X and *PPARGC1A* rs8192678 were genotyped. In paper III, in addition to the aforementioned three polymorphisms, *ACSL1* rs6552828, *IL6* rs1474347, *PPARA* rs4253778 and *PPARG* rs1801282 were also genotyped. To genotype the *ACE* I/D polymorphism, the assay of one of the best proxies to the I/D polymorphism was run (rs4343 (Abdollahi, Huang et al. 2008)),

For each of these, the 16.7-µl reaction genotyping mixture contained 8.44 µl Genotyping Master Mix, 0.42 µl Assay mix (40x), 6.33 µl distilled $H_20$ and ~ 1.5 µl of DNA template. Reaction conditions were following: 30 s at 60°C, followed by initial denaturation for 10 min at 95°C, then denaturation at 95 °C for 15 s and annealing at 60 °C for 1 min in cycling stage (40 cycles in total). Finally, the post-read temperature was kept at 60 °C for 30 s. A minimum of 10% of samples was re-run in addition to those where the genotype was unclear after the first run.

*Table 6 TaqMan™ SNP Genotyping Assay IDs*

| Gene | Polymorphism | Assay ID |
|---|---|---|
| *ACE* | rs4343 (I/D)* | C__11942562_20 |
| *ACTN3* | R577X | C____590093_1 |
| *ACSL1* | rs6552828 | C__30469648_10 |
| *IL6* | rs1474347 | C___1839698_20 |
| *PPARA* | rs4253778 | C___2985251_20 |
| *PPARG* | rs1801282 | C___1129864_10 |
| *PPARGC1A* | rs8192678 | C___1643192_20 |

*rs4343 is considered to be among the best proxies to the I/D polymorphism (Abdollahi, Huang et al. 2008)

## 2.5 Characterization of the PA levels (Paper I)

The PA level estimates are based on the KAM study questionnaire described previously (Skjelbred, Sæbø et al. 2006). The questions covering the PA habits and answer options that the participants reported on are displayed in Table 7. The rationale behind the PA estimates is as follows: the different activities covered in the questionnaire were scaled as the energy demand differs among them (Ainsworth, Haskell et al. 1993, Ainsworth, Haskell et al. 2011). The scaling was as follows: hiking = 1 (reference; represents moderate to vigorous intensity), walking/bicycling = 0.5 (low to moderate intensity), exercise = 1.5 (vigorous intensity). Then the activity score for each participant was calculated by summing the weekly frequencies of the scaled activities. The recommendations of the American College of Sports Medicine (ACSM) were used to determine whether a participant had low/moderate PA levels (LMPA) or high PA levels (HPA). ACSM recommend either a minimum of 30 min moderate-intensity cardiorespiratory exercise five times per week, a minimum of 20 min vigorous exercise three times per week or a combination of these (Garber, Blissmer et al. 2011). The weekly activity score of 3 had to be achieved to comply with the ACSM recommendations and was considered to have a sufficiently high PA level (i.e. HPA). On the other hand, participants with PA score under 3, were considered as having insufficient PA level (LMPA). As an example, a person exercising for 45 min two times per week, or hiking three times per week, or performing walking or cycling six times per

week, would reach the activity score 3. These two PA level groups (LMPA and HPA) were assessed for any associations with the *ACE* I/D, *ACTN3* R577X and *MAOA* uVNTR genotypes. Furthermore, the associations between the PA level group and socioeconomic variables were investigated.

***Table 7 Questions and response options covering the participants' physical activity habits in the KAM study that are used as a base for PA estimates in Paper I***

| Question | Response options |
|---|---|
| Have you cycled or walked to work in the past five years? | No |
| | Once a week |
| | Several times a week |
| | Daily |
| Do you hike (cross country)? | No |
| | Once a week |
| | Several times a week |
| How often do you exercise for at least 20 minutes? | Never |
| | Less than once a week |
| | Once a week |
| | Several times a week |
| | Daily |
| If you exercise, do you perspire? | No |
| | Yes |
| Were you participating in sports or outdoor activities at a younger age? | No |
| | Yes |

## 2.6 Experimental set-up and physiological testing (Papers II and III)

### 2.6.1 The leg-press study (Paper II)

The leg-press study was an intervention study, where participants of different age groups volunteered to perform an 8-week MST program with three MST sessions/week with a minimum of one day of rest between the sessions. Participants were instructed to continue with their usual training habits and to log both the MST training and the habitual PA. Training sessions consisted of a general 10 min moderate-intensity warm-up that was performed either as walking, running or cycling based on the subjects' preferences. Following the general warm-up, a leg-press specific warm-up was performed using the leg-press machine (OPS161 interchangeable leg press, Vertex USA). This consisted of three 10-repetition sets with increasing load (30-70% 1RM). The MST training included four sets of 4RM in the leg-press machine ($90^{o}$ degrees between femur and tibia) separated by 3-min rest. When the participant was able to perform five repetitions during one set, 2.5-5kg were added to the next set. Throughout the training period, guidance and instructions were given to all subjects.

### 2.6.2 The cross-country skiing study (Paper III)

The main purpose of the cross-country skiing study was to assess whether six months of training lead to changes in physiological/ performance variables. Baseline values and training-induced changes were assessed based on sex, age (younger vs. older) and genotypes of the seven different polymorphisms. To do this, skiers were instructed to maintain their normal training habits/program and to log and report the training for the duration of the six-month study period. On each of the two consecutive testing days (before (PRE), mid-way (POST1) and after the study period (POST2)), several physiological/performance/strength variables were tested. The test battery was as follows: $VO_{2max}$ in running (RUN-$VO_{2max}$), $VO_{2peak}$ in double poling (DP-$VO_{2peak}$), time to exhaustion, performance in a 5.64 km double poling time trial ($TT_{DP}$), work economy in

DP ($C_{DP}$), lactate threshold (LT), 1RM and maximal power tests (half squat and pull-down). For genotyping purposes, blood samples were taken at the pre-test. On day 1 of testing, three maximal jump height tests, an incremental running test for determining RUN-$VO_{2max}$, and a $TT_{DP}$ were performed. On Day2, the sub-maximal $VO_2$ and $[La-]_b$ measurements in DP (to determine $C_{DP}$ and LT), followed by RAMP protocol to exhaustion (to determine DP-$VO_{2peak}$) and maximal strength testing. PRE tests were performed in April/May, POST1 - in July/August, and POST2 - in October/November. At all three occasions, testing procedures were the same.

Participants were instructed not to eat/drink nutritious drinks the last hour and only to conduct light training 24 hours before the first test. They were allowed to consume light, energy-rich meals/drinks between the different tests. These meals/drinks, as well as the last meal prior to testing, were registered, and they were instructed to consume the same food/drinks at the POST1 and POST2. To avoid the impact of the circadian rhythm, all tests were also conducted at the same time of day (± 2 h).

## 2.7 Physiological testing procedures (Papers II and III)

### 2.7.1 Maximal strength (Paper II and III)

Paper II

Pre-tests were performed 2-4 days before the intervention period, and post-tests 2-5 days after the last training session. Participants were instructed to refrain from exercising at least 24 h before the pre and post-tests, not to eat 2-4 h and only to drink water for the last 2 h before the pre/post-tests. Importantly, pre and post-tests were identical and were attempted to be carried out at the same time of day (±1 h).

Prior to testing, a general 10 min moderate-intensity warm-up was performed (walking, running or cycling). The subject conducted the same mode of warm-up at both tests. Following the general warm-up, a specific warm-up was performed on the leg-press machine. The leg-press specific warm-up consisted of 10, 5 and 3-repetition sets at ~ 50

%, 60 %, and 70 % of 1RM, respectively, with a 3 min rest between the sets. Then, the 1RM was assessed by one repetition at ~ 80% 1RM followed by another lift with a 5-15 kg increase in load compared to the previous lift, followed by a 3-min rest, and a new attempt with increased loads until the 1RM was reached. The lifts were performed with a controlled slow eccentric phase, a ~1 s stop of movement at the lowest position (90$^{o}$ between femur and tibia), followed by a maximal mobilization of force in the concentric phase, as described previously (Støren, Helgerud et al. 2008, Sunde, Storen et al. 2010). MuscleLab system (Ergotest Innovation A.S., Porsgrunn, Norway) was used to measure lifting time and distance to control the work distance.

Paper III

Maximal strength testing was performed 60-minutes after the DP tests on day two of testing. Støren, Helgerud et al. (2008) had previously shown that 30 min of rest after aerobic tests is sufficient not to influence 1RM half-squat results. 1RM and maximal power in half-squat were performed using Smith-machine (PreCore, Woodinville, WA, USA) and pull-down using Gym 2000 (Vikersund, Norway). The protocol has been described in detail previously (Sunde, Johansen et al. 2019), and was as follows: after 10 reps at ~ 50% of 1RM for both tests, the consecutive three sets were performed at ~ 60% (5 reps), 70% (3 reps) and 80% (2 reps), separated by 3 min rest. Finally, the subjects performed a minimum of 1 rep of their estimated 1RM. Then, load increments of 2.5 – 10 kg were added to the subsequent lifts until the 1RM was reached. Similarly to the leg-press protocol, all repetitions were performed with a slow eccentric phase; then a complete stop of movement for ~ 1 s in the lowest position (half-squat)/highest position (pull-down) followed by a maximal mobilization in the concentric phase. Also here, the MuscleLab system was used for power output measurements.

### 2.7.2 Jump height tests (Paper III)

Jump height tests were the first test on day 1 of testing. Before the jump tests, subjects performed a minimum of 10 min self-conducted warm-up, which was registered and

repeated on the consecutive tests (POST 1 and 2). Then, squat jump, counter-movement jump (CMJ) and counter-movement with arm swing (CMJas) were performed. At each of these three separate tests, the best of at least three attempts was registered. Between the three tests, a minimum of 3 min rest was given to ensure restitution. The same testing leader controlled the knee-angle to be 90° for squat jump tests, and no counter-movements were allowed for this particular test. For the other two, i.e. CMJ and CMJas, there were no counter-movement restrictions. To measure the jump height for all three tests, a force platform (Ergotest Innovation, Porsgrunn, Norway) was used, and the platform was calibrated based on the manufacturers' instructions.

### 2.7.3 VO2max (Paper III)

On day 1, before an incremental $VO_{2max}$ test in running and at least 20 min after the jump tests, another 10 min warm-up was conducted. As previously, the warm-up was registered and repeated at POST 1 and 2. The incremental $VO_{2max}$ test has been described previously (Sunde, Johansen et al. 2019). In short, the test started at 6% inclination and speed of 7-8 $km \cdot h^{-1}$ for females and 9-10 $km \cdot h^{-1}$ for males. Then, every 30s, the inclination was increased by 1% until 8% inclination was reached. At this point, only speed was increased by 0.5 $km \cdot h^{-1}$ every 30 s. Participants continued to run until they reached voluntary fatigue. The three highest subsequent $VO_2$ measurements were used to calculate the average $VO_{2max}$-RUN. To evaluate whether or not the $VO_{2max}$ was reached, measurements of HR (≥ 98% of $HR_{max}$), respiratory exchange ratio (≥ 1.05), blood lactate concentration ($[La^-]_b$) ≥ 8.0 $mmol \cdot L^{-1}$, rate of perceived exertion (Borg scale 6-20) ≥ 17, and flattening of the $VO_2$ was used. All $VO_2$ measurements were taken every 10 s by the MetaLyzer II Cortex (Biophysic GmbH, Leipzig, Germany) metabolic test system. The $O_2$-analyser was calibrated with ambient air and certified calibration gasses (16% $O_2$/ 4% $CO_2$). Before each test, 3-L calibration syringe (Biophysic GmbH, Leipzig, Germany) was used to calibrate the flow sensors. The test was performed on a Woodway PPS 55 sport (Waukesha, WI, USA) treadmill which has been calibrated for

speed and incline. To measure HR, participants used their HR monitors or Polar s610 HR monitors (Kempele, Finland).

### 2.7.4 Time trial test (Paper III)

The last test of day 1, was a 5.64 km $TT_{DP}$ performance test in a paved roller ski course track of 940 m, and it was conducted at least 1 h after the incremental $VO_{2max}$ test. Throughout the $TT_{DP}$ test, only the DP technique was allowed. The TT was organized as individual starts with 30 s starting intervals. No drafting was allowed. The participants used their poles and roller-skis for classic skiing with wheel type 2 for this particular test at all three tests (PRE, POST1 and 2). As differences in temperature and humidity may influence the rolling resistance of the roller skis, the measurements were corrected. The TT procedures and correction factor calculations for temperature and humidity have been described previously in Sunde et al. (2019).

### 2.7.5 $VO_{2peak}$ (Paper III)

On day 2, DP tests were performed on a motorized treadmill specialized for cross-country skiing (Rodby RL 2700E, Rodby Innovation, Vänge, Sweden). To get familiarized with the DP treadmill, each subject performed one 30-minutes workout before testing. For all DP tests (PRE, POST1 and 2), all participants used the same pair of roller skis (Swenor Fiberglass, Sarpsborg, Norway) and the same binding system (NNN, Rottefella, Klokkarstua, Norway). Poles and additional skiing equipment was participants' own and were the same at all DP tests. To avoid falls and injuries during testing, participants were attached to a safety harness connected to the roof. Testing consisted of three to six 4-min work periods. To calculate LT and $C_{DP}$, $VO_2$ and HR measurements were registered during the last minute of the work periods. Work periods were separated by a 1-min break to measure [La-]b. Whole blood lactate values were measured by a Lactate Scout+ (SensLab GmbH, Leipzig, ray Inc., Kyoto, Japan). The first work period begun at a work intensity assumed to be 50-70% of their DP-$VO_{2peak}$. For all subjects, the inclination was set at 4%, but the initial speed was set at 10-11.5 $km \cdot h^{-1}$ for males and 6-8 $km \cdot h^{-1}$ for

females. For each following work period, the speed was increased by 1-3 km·h$^{-1}$, until the measured [La-]$_b$ levels exceeded the subjects' LT and the testing was terminated. To define LT, warm-up lactate value (i.e., the lowest measured lactate value) + 2.3 mmol·L$^{-1}$ was used (based on the protocol of Helgerud, Ingjer et al. (1990) which is described in detail in Støren, Rønnestad et al. (2014) and Sunde, Johansen et al. (2019)).

Following the DP-test and a 5-min active rest, the RAMP protocol to exhaustion was conducted for determining DP-$VO_{2peak}$. For all participants, the inclination was set to 6% inclination throughout the test. The initial speed was set at 7 km·h$^{-1}$ for all participants and the speed increased by 1 km·h$^{-1}$ every 60 s. The test was stopped when the skiers slowly moved backwards and reached a pre-defined mark 1 m behind their starting position on the treadmill despite intense motivational feedback. When the testing was terminated, time to exhaustion was registered. DP-$VO_{2peak}$ was defined as the mean of the two highest subsequent $VO_2$-measurements. Maximal aerobic speed (MAS) in DP was calculated as described by Sunde et al. (2019) and Johansen et al. (2020), i.e. DP-$VO_{2peak}/C_{DP}$.

## 2.8 Statistical analysis

Most of the statistical tests were performed on various versions of the SPSS software (IBM, Chicago, IL, USA), except the Pearson's Chi-square test ($\chi 2$) comparing genotype frequencies between different studies/populations, and testing for the Hardy-Weinberg equilibrium (HWE). In this case, the R Commander package and R software were used. Where appropriate, Kolmogorov-Smirnov test and Q-Q plots were used to test variables for normality. All main variables were found to be normally distributed, and thus, parametric tests were used to analyse the associations. To account for multiple testing, either Bonferroni (Paper I) or Tukey (Papers II and III) Post-Hoc tests were used. The significance level was set at $p<0.05$ for two-tailed tests.

### 2.8.1 Paper I

Independent sample t-test was used to investigate associations between the BMI and PA level groups. To test for the differences in categorical variables across the PA level groups, Pearson's Chi-square test ($\chi 2$) was used. Furthermore, binomial logistic regression was applied to investigate the contribution of socioeconomic factors (i.e. gender, age, BMI, education, participation in sports/outdoor activities earlier in life) in addition to the polymorphisms (*ACTN3* R577X, *ACE* I/D and *MAOA* uVNTR genotypes) to the PA level. In this case, two models were analysed: model 1 included socioeconomic factors only; model 2 included the socioeconomic together with the genotype data. For the significant associations from the two regression models, odds ratios (OR) were calculated. Results were presented as mean ± SD.

Importantly, to analyse the *MAOA* genotypes, they were divided into groups based on their transcriptional activity (Sabol, Hu et al. 1998, Deckert, Catalano et al. 1999). Therefore, males carrying the 3-repeat allele and homozygous females for the 3-repeat allele were considered to have low transcriptional activity. On the other hand, males with either 3.5- or 4-repeat allele were considered to have a high TA. Also, females homozygous for either 3.5 or 4-repeat-alleles and those heterozygous for 3.5 or 4-repeat-alleles were grouped into high transcriptional activity group. As only females can be heterozygotes, those that were carrying one 3-repeat and either 3.5- or 4-repeat allele were grouped into the heterozygous group. Those participants that carried rare alleles were excluded from the data analysis.

### 2.8.2 Paper II

To analyse the 1RM and Δ1RM across the age groups, a general linear model was used. The overall differences in the 1RM and Δ1RM between the genders and baseline strength (corrected for age, sex and body weight; 1RMcorr) and Δ1RM (%) between the alleles of the three polymorphisms were analysed by the independent sample t-tests. The gender differences across the age groups were not investigated due to the low sample size per group. To test the associations between the genotypes and the

continuous variables, one-way ANOVA was used. Pearson correlation test was applied for correlation analyses. Pearson's Chi-square test ($\chi 2$) was used to test the differences in categorical variables. For a genetic association study, the sample size is relatively small. Therefore, the authors chose to calculate Cohen's d effect size by using Microsoft® Excel® (Redmond, WA, USA) for 1RMcorr and Δ1RM (%) across phenotypes. Effect sizes had a following interpretation: d= <0.35 (trivial), d= 0.35-0.80 (small); d= 0.80-1.50 (moderate); d= >1.50 (large effect size), as described by Rhea (2004) specifically for resistance training.

### 2.8.3 Paper III

A GLM Univariate test was used to evaluate the changes across the whole study period for the whole cohort, as well as for both sexes and both age groups. The differences between the age groups and sexes in physiological response and training characteristics during the study period GLM Univariate with pairwise comparisons and independent sample t-tests were used. For correlations (baseline and Δ), correlation coefficients r were used from Pearson's bivariate tests. The correlation coefficients were evaluated according to Hopkins (2016), with a detailed presentation previously by Sunde, Johansen et al. (2019). Partial correlations were also conducted corrected for sex and age. To evaluate the practical implications of the r values, standard error of the estimates were obtained from the regression analyses. Values were expressed as mean ± SD together with the coefficient of variance (CV).

For the genetic analysis, one-way ANOVA was used to investigate the associations between the genotypes and physiological/performance variables. The effects of the alleles on the variables of interest were assessed by a two-tailed independent sample t-test. To analyse the effects of different genotypes on physiological parameters, female physiological test values were multiplied by the average gender difference between males and females in Paper III. This was performed to avoid bias effects of uneven distribution of the sexes across the different candidate genes/genotypes.

Similar to Paper II, Cohen's d was calculated using Excel® for the gender corrected variables across the genotypes. To evaluate the magnitude of the effect, Cohen's d was interpreted as follows: below 0.50 - small effect, 0.5 and above - moderate effect, 0.8 and above - large effect (Cohen 1988).

# 3 Discussion

The project aimed at investigating the distribution and possibly influence of selected common polymorphisms on complex human traits such as PA level, maximal muscle strength and endurance capacity/performance. Factors like age and/or sex of the participants were also evaluated to see if they played a role in PA, trainability of maximal strength and/or endurance performance. The cohorts included in this thesis were all of the Scandinavian descent and could, thus, be regarded as ethnically homogenous. Two of the cohorts represent the general public (the KAM and the Leg-press cohort). The Cross-country skiing cohort, on the other hand, were highly trained athletes.

## 3.1 Summary of papers

### 3.1.1 Paper I

*Distribution of allele frequencies for genes associated with physical activity and/or physical capacity in a homogenous Norwegian cohort- a cross-sectional study. Goleva-Fjellet S., Bjurholt A. M., Kure E. H., Larsen I. K., Støren Ø., Sæbø M.; BMC Genet 21, 8 (2020).*

Physical activity (PA) is a highly complex trait with well-described health effects and is influenced by a large number of factors, including genetics. This paper reports the genotype frequencies of three polymorphisms located in genes associated with PA and/or physical capacity, namely *ACTN3* R577X, *ACE* I/D and *MAOA* uVNTR in a homogenous cohort of middle-aged Norwegians (n = 831). The genotype distributions of the three polymorphisms were similar to other populations of European descent. When the genotype distributions were compared between the groups reporting high and low/medium PA levels, a significant 10% difference was found for the X allele of the *ACTN3* R577X polymorphism. More individuals with lower/medium PA levels were carriers of the X allele compared to individuals with higher PA levels. The questionnaires used to determine the PA levels allowed to assess the importance of other variables on

the PA levels. The study showed also that education and previous participation in sports or outdoor activities were positively associated with the PA levels. This was the first study to find an association between *ACTN3* R577X genotype and PA level in middle-aged Scandinavians, yet, it is likely that the contribution of a single polymorphism to PA level trait, is small.

https://bmcgenet.biomedcentral.com/articles/10.1186/s12863-020-0813-1

### 3.1.2 Paper II

*Early responses to maximal strength training were not influenced by age, gender or initial training status. Kittilsen, H.T.*, Goleva-Fjellet, S.*, Freberg, B.I., Nicolaisen, I., Støa, E.M., Bratland-Sanda, S., Helgerud, J., Wang, E., Sæbø, M., Støren, Ø. (Manuscript; under submission)*

**shared first authorship*

Maximal muscle strength is important for everyday functionality in all age groups, but it does decline with age. The genotypes of interest were *ACTN3* R577X, ACE I/D and *PPARGC1A* Gly482Ser. We wanted to investigate how and to what extent selected genetic variants, age, sex, and initial training status impact maximal strength training (MST) adaptations in response to a standardized maximal training program. For this reason, 49 subjects (22 males, 27 females) aged 20-76 years were divided into five age groups. They completed an 8-week MST intervention in leg-press with three sessions per week. Each session consisted of 4·4 repetitions at ~85-90% of 1RM intensity in leg-press. 1RM was tested before and after the 8-week study period. Blood samples were taken for genotyping purposes. We found that all age groups increased 1RM ($p<0.01$) by ~24% on average. We found no significant differences in gains across the five age groups. The improvements were also independent of sex. Surprisingly, initial training status appeared to be unrelated to 1RM improvements. We found a significant association with one of the three investigated genotypes. *PPARGC1A* Gly482Ser T allele carriers had a 15% higher age- and gender corrected baseline 1RM than the CC genotype ($p<0.05$).

On the other hand, C allele carriers improved 1RM(%) by 34.2% more than homozygotes for the T allele ($p<0.05$). Although insignificant, there was a trend towards better response to MST for the *ACTN3* R577X RR genotype compared to the XX (30% vs. 19%).

### 3.1.3 Paper III

*No Change – No Gain; The Effect of Age, Sex, Selected Genes and Training on Physiological and Performance Adaptations in Cross-Country Skiing. Johansen, J.M., Goleva-Fjellet, S., Sunde, A., Gjerløw, L.E., Skeimo, L.A., Freberg, B.I., Sæbø, M., Helgerud, J. and Støren, Ø. Frontiers in Physiology, 26 October 2020*

Cross-country skiing is among the most demanding aerobic endurance sports. The study aimed to examine how factors like sex, age, training and selected genetic variants affect different physiological and performance variables in a cohort of well-trained national-level cross-country skiers. The study was an observational study, where the participants were tested before (pre-test), half-way (post-1) and after (post-2) the 6-month study period from May to October. During the study period, participants maintained their training habits and reported all training based on HR measures. A range of tests was performed, including an outdoor double poling time trial ($TT_{DP}$), peak oxygen uptake in double poling (DP-$VO_{2peak}$), maximal oxygen uptake in running (RUN-$VO_{2max}$), lactate threshold (LT), the oxygen cost of double poling ($C_{DP}$), maximal strength (1RM) and jump height. Following polymorphisms were genotyped using venous blood samples: *ACE* I/D*, ACSL1* rs6552828, *ACTN3* R577X, *IL6* rs1474347, *PPARA* rs4253778, *PPARG* rs1801282 and *PPARGC1A* rs8192678.

In total, 29 participants were tested at all three occasions and had reported sufficient training data to be included in the study. Sex and age had a significant effect on $TT_{DP}$ ($p < 0.01$), DP-$VO_{2peak}$ ($p < 0.01$), $C_{DP}$ ($p < 0.05$), MAS ($p < 0.01$), $LT_v$ ($p < 0.01$), 1RM half squat ($p < 0.01$) and 1RM pull-down ($p < 0.01$) at pre-test. Sex had also an effect on RUN-$VO_{2max}$ ($p < 0.01$). Only some minor effects of some of the investigated polymorphisms on physiological and/or performance variables were found. Total reported training

volume per week ranged widely among skiers (from 357.5 to 1056.8 min). Training consisted mostly of low-intensity training (90%), while moderate and high-intensity training accounted for 5% of the total training volume each. While the intensity distribution remained the same during the study period, the volume and ski-specific training increased significantly ($p < 0.05$). There were no improvements during the 6-month study period in either physiological/performance variables for the whole cohort or training progression/adaptation between age groups or sexes.

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## 3.2 Genotype and allele distribution

Frequency distributions for the gene polymorphisms genotyped across all three studies are displayed in Table 8. Around 19% of the human population possess the *ACTN3* R577X XX genotype leading to the absence of the α-actinin-3 within the type-2 muscle fibres (MacArthur and North 2004). This frequency is in agreement with the findings in this study for all three cohorts. The Cross-country skiing cohort appears to have a slightly higher, but insignificantly, frequency of the XX genotype compared to the two other cohorts representing the general public. The D and I alleles of the ACE polymorphism are equally distributed (i.e. 50% each) in populations of European descent (Jones and Woods 2003), which is similar to what was found in the three cohorts. Although the D allele was slightly more frequent in the Cross-country cohort, the difference was not significant. MAOA uVNTR polymorphism was also genotyped across the three cohorts. The genotype frequency was similar, and the common 3- and 4-repeat allele frequencies were around 39% and 61%. The observed heterozygosity for the two common alleles in females was 42.5%. Allele frequencies corresponded also well with other reports (Sabol, Hu et al. 1998, Deckert, Catalano et al. 1999). Overall, genotype/allele distributions for the *ACTN3* R577X, *ACE* I/D and *MAOA* uVNTR were similar across the three Scandinavian cohorts investigated.

*Table 8 ACTN3 R577X and ACE I/D genotype and allele frequencies across the three cohorts (KAM, Leg-press and Cross-country)*

| Genotypes | Cohort | | |
|---|---|---|---|
| | KAM | Leg-press | Cross-country |
| n | 831 | 49 | 29 |
| *ACTN3* R577X | | | |
| RR | 254 (31%) | 13 (27%) | 7 (24%) |
| RX | 415 (50%) | 25 (51%) | 13 (45%) |
| XX | 155 (19%) | 11 (22%) | 9 (31%) |
| | | | |
| R allele | 462 (56%) | 26 (52%) | 14 (47%) |
| X allele | 363 (44%) | 24 (48%) | 16 (53%) |
| | | | |
| *ACE* I/D | | | |
| DD | 151 (24%) | 12 (26%) | 9 (31%) |
| ID | 325 (53%) | 21 (45%) | 16 (55%) |
| II | 142 (23%) | 14 (30%) | 4 (14%) |
| | | | |
| D allele | 314 (51%) | 23 (48%) | 17 (59%) |
| I allele | 305 (49%) | 25 (52%) | 12 (41%) |

n- number of subjects (percentage of total)

PPARGC1A Gly482Ser (rs8192678) polymorphism genotyped was investigated in two of the cohorts, the Leg-press and the Cross country-skiing cohorts. Minor T (Ser) allele frequency reported in Europeans is ~35% (NCBI 2020f), which is similar to what was found in both cohorts (Table 9). Although the allele frequency did not differ significantly across the cohorts, the genotype frequencies did ($p<0.05$), and this will be discussed in more detail in the next section.

*Table 9 PPARGC1A rs8192678 (Gly482Ser) genotype and allele frequencies in the Leg-press and Cross-country skiing cohorts*

| Gene (polymorphism) | Cohort | |
|---|---|---|
| | Leg-press | Cross-country |
| n | 49 | 29 |
| *PPARGC1A Gly482Ser* | | |
| CC | 20 (41%) | 8 (28%) |
| CT | 19 (39%) | 20 (69%) |
| TT | 10 (20%) | 1 (3%) |
| | | |
| C allele | 30 (60%) | 18 (62%) |
| T allele | 20 (40%) | 11 (38%) |

N- number of subjects (percentage of total)

The following polymorphisms were only genotyped in the Cross-country cohort: *PPARA* rs4253778, *PPARG* rs1801282, *ACSL1* rs6552828, and IL-6 rs1474347. Their respective minor allele frequencies were ~20% (C allele), 9% (C allele), 40% (A allele) and 41% (C allele). These frequencies are similar to those reported in Europeans in the NCBI SNP database (NCBI 2020a, NCBI 2020b, NCBI 2020d, NCBI 2020e).

## 3.3 The effect of genetic variants

### 3.3.1 Genetic variants and self-reported PA levels

Significant effects for some of the investigated polymorphisms were found in all three studies. In Paper I, we found that the allele distribution of the *ACTN3* R577X polymorphism differed significantly between the group with low/medium PA levels and the group with high PA levels. Carriers of the R allele were more likely to have higher self-reported PA level. One could speculate that this may be due to the more protective role of the R allele, and the RR genotype in particular, to incident disability (Wilson, Mavros et al. 2019), sarcopenia (Pickering and Kiely 2018) and a greater muscular and functional adaptations to exercise intervention compared to the X allele (Pickering and Kiely 2017, Wilson, Mavros et al. 2019). Variables like sarcopenia, muscle strength and function may be important correlates of PA (Leblanc, Taylor et al. 2015, Viken, Aspvik et

al. 2016, Gomes, Figueiredo et al. 2017, Rojer, Reijnierse et al. 2018). None of the two other investigated polymorphisms, i.e. *ACE* I/D and *MAOA* uVNTR, in relation to self-reported PA levels yielded significant associations. The significant association between the PA level and R577X genotype, when analysed by sex, was significant only in male participants. Previous studies on athletic ability have indicated that the R577X genotype might influence males and females differently due to hormonal differences (Yang, MacArthur et al. 2003, Clarkson, Devaney et al. 2005).

### 3.3.2 Genetic variants and maximal strength and/or power

In the leg-press study (Paper II), we found a significant association between the *PPARGC1A* rs8192678 (Gly482Ser) and both the 1RM (corrected for age, sex and body weight; 1RMcorr) and the improvements in 1RM (Δ1RM%). Individuals with the heterozygote CT (Gly/Ser) genotype and the carriers of the T (Ser) allele were ~18% and ~15% stronger, respectively, at baseline when compared to the CC (Gly/Gly; $p<0.05$). The T allele, the TT genotype more specifically, has been associated with power athlete status (Gineviciene, Jakaitiene et al. 2016). Based on Cohen's d effect size measures defined for strength training in particular (Rhea 2004), there was a moderate effect of possessing the beneficial CT genotype on the baseline maximal strength. The association remained statistically significant ($p<0.05$) when all the individuals with baseline data were included in the analysis (N=72). However, the C allele carriers responded significantly better to the MST intervention compared to the TT genotype, with 25.7% vs 18.2% improvements, respectively. This could, in part, be due to the lower baseline strength among the C allele carriers, thus having a larger potential for improvements. However, the baseline 1RM and gains in 1RM(%) did not correlate in Paper II.

In Paper II, neither *ACTN3* R577X nor *ACE* I/D were associated with the corrected baseline 1RM, also when all 72 individuals with the baseline data were included. However, although insignificant, there was a 46.5% difference with a moderate effect size in the Δ1RM%. Individuals with the RR genotype improved the 1RM by 30%, on average, compared to the ~19% improvement among individuals with the XX genotype.

This association was likely insignificant due to the large individual variations across the R577X genotypes, especially among the individuals with the RR genotype. Larger improvements in maximal strength among RR individuals in response to the strength training program is in line with some previous reports (Pereira, Costa et al. 2013). Greater improvements in CMJ, functional performance (Pereira, Costa et al. 2013), peak torque (Norman, Esbjörnsson et al. 2009) and power (Delmonico, Kostek et al. 2007) among the RR genotype have also reported.

Studies on human and mice models have provided insights on some potential mechanisms differentially affecting the RR and XX genotypes (Seto, Quinlan et al. 2013, Lee, Houweling et al. 2016, Del Coso, Hiam et al. 2018). It appears that the type II fibres required for forceful contractions do not function optimally in individuals lacking the α-actinin 3, as in the XX genotype (Lee, Houweling et al. 2016). There are, however, studies reporting that women with the XX genotype (and/or X allele carriers) may be experiencing larger improvements in various strength-related phenotypes (Clarkson, Devaney et al. 2005, Romero-Blanco, Artiga-González et al. 2020). Differences in results could be, in part, due to differences in training programs. Our Leg-press cohort was subjected to an 8-week MST intervention, while Clarkson, Devaney et al. (2005) applied more conventional exercise protocol over 12 weeks, and Romero-Blanco, Artiga-González et al. (2020) used mixed training program consisting of endurance, balance/mobility and strength training for 12 months.

In Paper III, *ACTN3* R577X RR genotype demonstrated higher 1RM (pull-down) and power (half squat and pull-down) compared to the RX and/or XX genotype. However, these associations were not statistically significant. It has been replicated repeatedly that individuals with XX genotype, lacking the α-actinin-3 protein within the type II muscle fibres, possess lower muscle force and power (Seto, Garton et al. 2019). As to *ACE* I/D polymorphism, individuals with the DD genotype exhibited a moderately higher 1RM in pull-down compared to the I allele carriers (104.3 vs 95.9 kg; N=40; d=0.67; $P<0.05$). This could, in part, explain the differences in the work economy in double poling

(Hoff, Helgerud et al. 1999, Støren, Helgerud et al. 2008, Sunde, Storen et al. 2010). The heterozygotes (ID) demonstrated intermediate results at both the work economy and 1RM in the pull-down. *PPARGC1A* rs8192678 TT carriers had significantly higher 1RM in half squat compared to carriers of the C allele (165.0 vs 129.5 kg; d=1.73; P<0.05). Thus, it appears that TT genotype is beneficial for maximal strength among athletes. The TT (Ser/Ser) genotype has previously been shown to be overrepresented in some strength/power athlete cohorts (Gineviciene, Jakaitiene et al. 2016). Among the skiers investigated in Paper III, there were no homozygotes for the minor allele (GG) of the *PPARG* rs1801282. However, those carrying the G allele (CG) demonstrated higher power in squat and pull-down compared to the CC genotype (1105.5 vs. 892.6 W and 685.8 vs. 538.9 W, respectively; N=40; large effect size), and, thus, are in line with the notion that the G allele may be favourable for power performance. The minor G (Ala) allele of the is generally associated with power/strength athlete status (Maciejewska-Karlowska, Sawczuk et al. 2013, Petr, Maciejewska-Skrendo et al. 2019).

All in all, several of the investigated polymorphisms appear to affect either maximal strength or power among the general public (*PPARGC1A* rs8192678) and well-trained athletes (*ACE* I/D, *PPARGC1A* rs8192678 and *PPARG* rs1801282).

### 3.3.3 Genetic variants and endurance phenotypes

Sports performance is a highly complex trait and, thus, many genes play a role (Jacques, Landen et al. 2019). The optimal genetic profile for athletic performance, however, is sport specific (Pickering, Kiely et al. 2019). In cross-country skiing, for instance, genetic variants favouring a greater aerobic capacity as well as response to endurance training may influence the athletic performance (Quindry and Roberts 2019). However, it has also been shown that traits like maximal strength can affect performance in double poling (Sunde, Johansen et al. 2019). Thus, it can be speculated that genetic variants influencing muscle strength/power and their trainability may play a role in determining the athletic ability of a cross-country skier. Strength/power related associations were described in the previous section. Following polymorphisms were investigated in

relation with various endurance phenotypes: *ACE* I/D*, ACSL1* rs6552828, *ACTN3* R577X, *IL6* rs1474347, *PPARA* rs4253778, *PPARG* rs1801282 and *PPARGC1A* rs8192678. We found associations between several of the SNPs and various physiological variables. All the following associations are reported for the gender corrected data.

Previously, the heritability has been estimated to determine ~66% of the athlete status in females (De Moor, Spector et al. 2007). Although not consistently confirmed, the *ACTN3* XX genotype and/or the X allele have thought to be advantageous for the endurance performance, due to more efficient aerobic metabolism (Head, Chan et al. 2015, Seto, Garton et al. 2019). Also, the genotype frequencies tend to vary in opposite directions among endurance and strength/power athletes, with the XX genotype being the most common among endurance athletes (Yang, MacArthur et al. 2003). In our study on cross-country skiers, a moderate to large positive effect of the X allele on several endurance-related traits was observed. Compared to the RR genotype, the X allele of the R577X polymorphism had a large positive effect (Cohen's d=0.94) on the $VO_{2max}$ in double poling (DP-$VO_{2max}$; 55.4 vs. 59.4 $mL^{-1}{\cdot}kg{\cdot}min^{-1}$; $p<0.05$). However, XX genotype carriers possessed a significantly poorer economy in double-poling ($C_{DP}$) compared to the R allele carriers (0.820 vs. 0.765 $mL^{-1}{\cdot}kg^{-0.67}{\cdot}m$; $p<0.05$). Interestingly, individuals with the heterozygous RX genotype demonstrated the highest maximal aerobic speed compared to both RR and XX genotypes (330.3 vs. 294.9 and 290.4 $m{\cdot}min^{-1}$, respectively; $p<0.05$). The effects of being heterozygote (RX) have varied a lot in studies with some reporting RX having similar phenotype as the RR genotype, while others have reported an intermediate effect (Garton and North 2016). These associations remained significant also when all 40 participants were included. This corresponds well to findings of some of the studies (Eynon, Ruiz et al. 2012, Pimenta, Coelho et al. 2013), however, others, have not confirmed that the XX genotype is beneficial for endurance performance (Döring, Onur et al. 2010, Papadimitriou, Lockey et al. 2018). To the best of our knowledge, there is only one study investigating the effects of *ACTN3* R577X on skiing performance. Mägi, Unt et al. (2016) reported that males with the XX genotype

exhibited larger gains in $VO_{2peak}$ over the 5-year follow-up period. In our study, however, no changes in the oxygen uptake were observed during the 6-month study period.

The traditional view on the I allele of the *ACE* I/D polymorphism have been that it is associated with the endurance performance (Ma, Yang et al. 2013). Since then, the highly investigated polymorphism has yielded rather differing results (Pescatello, Corso et al. 2019). In the cross-country study (Paper III), the II genotype of the I/D polymorphism appears to have a large positive effect on the RUN-$VO_{2max}$ compared to the D allele carriers (73.0 vs 66.5 $mL^{-1}{\cdot}kg{\cdot}min^{-1}$; d=1.14; P<0.05). On the other hand, the I allele carriers possessed a poorer work economy compared to the DD genotype (0.197 vs 0.176 $mL^{-1}{\cdot}kg{\cdot}m$; p<0.01; large effect size) meaning that they spend more oxygen per meter distance covered. These associations remained also when all 40 participants were included in the study (medium effect size). The observed differences in 1RM in the pull-down, described previously, between the DD and I allele carriers, could, in part, explain the differences in the work economy in double poling (Hoff, Helgerud et al. 1999, Støren, Helgerud et al. 2008, Sunde, Storen et al. 2010).

We were able to find two studies investigating the I/D polymorphisms on skiing athletic performance. Mägi, Unt et al. (2016) found the ID allele to be more common among male cross-country skiing athletes compared to controls. In female athletes, the ID genotype was also associated with larger increases in peak $VO_2$ uptake after a 5-year follow-up. On the other hand, Orysiak, Zmijewski et al. (2013) did not find any association between the *ACE* polymorphism and aerobic capacity variables in a mixed winter endurance sports cohort. Taken together, these and other studies have produced highly varying results, and thus, at this point, it is not possible to confirm or reject that *ACE* I/D polymorphism plays a role in athletic endurance performance (Pescatello, Corso et al. 2019).

The Gly allele of the PPARGC1A Gly482Ser (rs8192678) is regarded as the most favourable for both the response to endurance exercise as well as for the endurance athletic ability (Petr, Stastny et al. 2018, Petr, Maciejewska-Skrendo et al. 2019).

However, among the cross-country skiers investigated, the 2 individuals, both males, with the TT (Ser/Ser) genotype, regarded as detrimental to endurance performance (Petr, Maciejewska-Skrendo et al. 2019), possessed the highest RUN-$VO_{2max}$ (78.6 $mL^{-1}{\cdot}kg{\cdot}min^{-1}$) compared to the C (Gly) allele carriers (67.0 $mL^{-1}{\cdot}kg{\cdot}min^{-1}$; d=1.77; N=40; $p<0.05$).

An interesting pattern emerged when the genotype frequencies for this SNP in the Cross-country skiing cohort were compared to those of the Leg-press cohort. Although the TT genotype presented with the highest RUN-$VO_{2max}$, the genotype was underrepresented among the endurance athletes, compared to the Leg-press cohort representing the general public (3% vs. 20%). These results are in line with previous studies (Eynon, Meckel et al. 2010, Ahmetov and Fedotovskaya 2015). Despite the differences in genotypes, allele frequencies did not differ due to the high proportion of the heterozygotes among the skiers. It remained true when the genotyping results from all individuals in both cohorts were included (N= 72 and N=40 for the Leg-press and Cross country-skiing cohorts, respectively). This might indicate that, despite the TT genotype, in general, being detrimental to endurance performance, the T allele provides some benefit for the skiers investigated, possibly through the beneficial effects on strength/power (Gineviciene, Jakaitiene et al. 2016), among other things.

The G allele of the *PPARA* rs4253778 has been associated with endurance athlete status (Eynon, Meckel et al. 2010, Tural, Kara et al. 2014, Lopez-Leon, Tuvblad et al. 2016, Petr, Maciejewska-Skrendo et al. 2019), and a higher percentage of type I muscle fibres (Ahmetov, Mozhayskaya et al. 2006). Among the cross-country skiers, the G allele was associated with higher RUN-$VO_{2max}$ and DP-$VO_{2peak}$ compared to the CC genotype with a large effect size (68.1 vs 58.1 $mL^{-1}{\cdot}kg{\cdot}min^{-1}$; 4.4 vs. 3.5 $mL^{-1}{\cdot}kg^{-0.67}{\cdot}min^{-1}$; N=40; $P<0.05$). No significant associations were found for either of the following polymorphisms in Paper III when corrected for gender: *IL6* rs1474347 and *ACSL1* rs6552828.

To conclude, we found several associations between some of the polymorphisms of interest and different performance variables in the cross-country skiing study. Most of

the findings are in line with previous studies (Eynon, Meckel et al. 2010, Eynon, Ruiz et al. 2012, Ma, Yang et al. 2013, Pimenta, Coelho et al. 2013, Tural, Kara et al. 2014, Ahmetov and Fedotovskaya 2015, Lopez-Leon, Tuvblad et al. 2016, Petr, Maciejewska-Skrendo et al. 2019), thus, indicating that these commonly investigated polymorphisms may influence the athletic performance of national-level/well-trained cross-country skiers. Of these, only the *ACTN3* R577X have been replicated across different populations with most of the studies being in agreement that the RR genotype is associated with muscle strength/power, and, possibly, the XX genotype - with endurance. Findings regarding the other polymorphisms are less consistent (Jacques, Landen et al. 2019). It is clear that genetics influence many aspects of athletic performance, including but not limited to, physiological traits, anthropometric variables as well as psychological traits (Pickering, Kiely et al. 2019). However, it is unlikely that any single polymorphism, of those investigated, alone has a large effect on any one particular physiological trait investigated, and, thus on the athletic ability and performance (Jacques, Landen et al. 2019, Joyner 2019). Furthermore, the more genetic variants influencing any of the traits determining the endurance performance are being discovered, the less likely it is that an individual will possess the optimal genotype for all of these polymorphisms (Williams and Folland 2008).

## 3.4 The effect of age

### 3.4.1 The effect of age on PA levels

The study design in Paper I did not allow us to investigate the effects of age on PA levels directly. Rather, it allowed us to determine the PA levels in that particular cohort with a relatively narrow age span, i.e. 50 to 65 (mean age 55.5 ± 3.8 yrs). The nature of the questionnaire allowed us also to investigate various socioeconomic correlates of PA, including PA at a younger age. Results from Paper I showed a strong association between the present PA level and PA at younger ages ($P < 0.01$). PA levels have been shown to decrease with age, and are highly dependent on the geographical region investigated

(Gerovasili, Agaku et al. 2015, Gomes, Figueiredo et al. 2017). In our Scandinavian cohort, as many as 74% reported high PA levels. Typically, cohorts of various ages from Northern Europe demonstrate higher PA levels than other European cohorts (Gerovasili, Agaku et al. 2015, Gomes, Figueiredo et al. 2017, Lübs, Peplies et al. 2018). Gomes, Figueiredo et al. (2017) reported low levels of inactivity in a Swedish cohort (~5%) of 55 yrs and older compared to ~30% in Portugal. Maintaining a physically active lifestyle at older age leads to a range of physical and cognitive benefits, including improved quality of life and physical functioning (Bangsbo, Blackwell et al. 2019).

### 3.4.2 The effect of age on maximal strength

In the leg-press study (paper II), we tested 49 individuals (20-70+ years of age) divided into five different age groups spanning 10 years each except for the oldest age group with the mean age of 70.3. (60-70+yrs). Second age group (30-39yrs) presented the highest, and the oldest age group (60-70+) - the lowest absolute maximal strength values (362.2±135.3 vs. 191.0±50.8, respectively; $P<0.01$)). Thus, in line with previous studies (Lindle, Metter et al. 1997, Lambert and Evans 2002, Petrella, Kim et al. 2005), we observed an average decline of 1.3% in baseline maximal strength (1RM (kg)) per year.

Muscle strength normally peaks at 25-35 years of age (Mendonca, Pezarat-Correia et al. 2017). In our cohort, the highest absolute muscle strength was observed in the second age group (30-40 yrs). The first age group (20-30 yrs) possessed significantly lower 1RM (kg) compared to the second group (224.5±53.3 vs. 362.2±135.3, respectively). This may be explained by a higher percentage of female participants in the 20-29 than in the 30-39 age group (70% vs 22%). When correcting female 1RM (kg) for gender, the difference was still in favour of the oldest of the two groups (323.8 vs. 397.4 kg; $p>0.05$).

The decline was obvious already at the age of 40-49 yrs compared to the previous age group (30-39yrs). Unlike some other studies (Lindle, Metter et al. 1997, Lambert and Evans 2002, Petrella, Kim et al. 2005), we did not observe a sharper decline from around

50 years of age in maximal strength. Instead, the decline in maximal muscle strength was even across the age groups. To account for initial training status, the baseline 1RM (kg) values were corrected for age, gender and body weight. No statistically significant differences in the corrected 1RM were observed between the age groups.

In response to the 8-week MST, participants across all age groups improved their maximal strength by an average of 24%. This is similar to findings of other studies on MST, reporting average gains of 23-33% (Støren, Helgerud et al. 2008, Sunde, Storen et al. 2010, Barrett-O'Keefe, Helgerud et al. 2012). The findings in paper II suggest that maximal strength can be improved by about the same relative amount in any age-group between 20 and 70+ years of age, as demonstrated in Figure 2. Thus, at any age, it is possible to improve muscle strength by a MST program. This means that muscle strength will be maintained at a higher level, despite the continuous age-associated decline. This can lead to various health benefits as poorer muscle strength has been associated with e.g. decreased physical function (Mendonca, Pezarat-Correia et al. 2017) and increased all-cause mortality risk (Li, Xia et al. 2018). Thus, MST can serve as a tool to counteract and/or slow down such unwanted effects on skeletal muscle (Raymond, Bramley-Tzerefos et al. 2013, Wang, Nyberg et al. 2017).

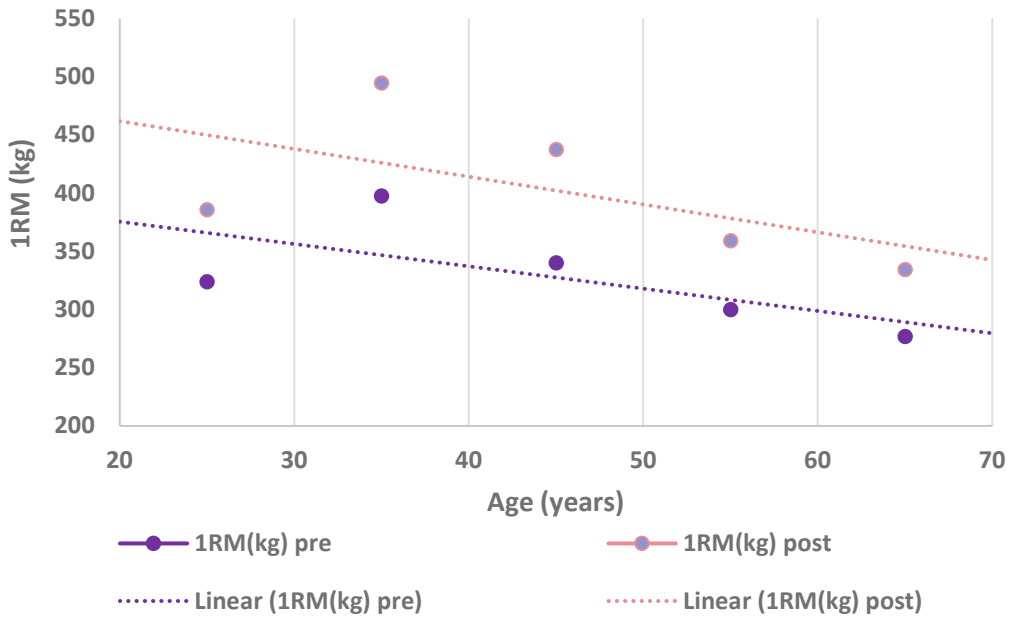


*Figure 2 Maximal strength training-induced changes in gender corrected one repetition maximum (1RM) in kilograms (kg) across different ages (Paper II)*

These results strongly suggest that MST is an effective method to improve maximal strength which confirms what has previously been shown by e.g. Campos, Luecke et al. (2002), comparing MST with strength training with a higher number of repetitions. The superiority of MST in improving maximal strength may be due to the maximal neuromuscular activation in this type of training (Jenkins, Miramonti et al. 2017, Wang, Nyberg et al. 2017). Maximal neuromuscular activation seems to be a necessary stimulus for neural adaptations including both selective activation of motor units, increased recruitments of motor units, increase of motor unit discharge rate, motor unit synchronization and co-contraction regulation of antagonists (Behm 1995). Although MST most likely led to some hypertrophy also in Paper II, the hypertrophy responses were not large enough to induce any changes in body weight. It would have been interesting to measure the cross-sectional area in the leg-press study (Paper II), but this was not possible to conduct. Thus, based on the mean 24% improvements in maximal strength, and the lack of changes in body weight, it may be suggested that the adaptations in the group as a whole were first and foremost neural ones.

No significant differences in the training response were observed between the different age groups. The initial training status did not appear to influence the improvements in maximal strength. This is somewhat surprising as a study by Støren, Helgerud et al. (2017) on the trainability of $VO_{2max}$ across different ages found that the initial training status had a great influence on the extent of training-induced changes. A similar effect in the gains in muscle strength should be expected, especially when taking into the consideration the rapid neural adaptations during the first weeks of strength training (Hakkinen, Pakarinen et al. 2000, Sunde, Storen et al. 2010, Unhjem, Lundestad et al. 2015, Unhjem, Nygard et al. 2016, Wang, Nyberg et al. 2017). Regrettably, no previous studies on MST have to my knowledge reported correlations or lack of correlations between baseline status and MST improvements.

Several previous studies have reported a large inter-individual variation in the response to a standardized training program with both non-, medium- and high-responders (Hubal, Gordish-Dressman et al. 2005, Bamman, Petrella et al. 2007, Erskine, Jones et al. 2010, Ahtiainen, Walker et al. 2016). This may be due to many factors, including genetics and various environmental factors, such as age, training status and sex (Barberio, Pistilli et al. 2019, Thomis 2019). In the leg-press study, no non-responders to maximal strength increase were detected, but the increase in 1RM(%) ranged from 8.5 to 82.9%, thus, confirming a large variability in trainability maximal strength among individuals. Furthermore, the response range was relatively similar across the age groups, as observed by others (Ahtiainen, Walker et al. 2016). As described previously, neither training status, nor age appeared to influence the training response in Paper II. Taken together, this indicates that MST is an effective exercise mode to increase muscle strength across all age groups, including the elderly.

### 3.4.3 The effect of age on training characteristics on endurance performance

Across different age groups, relative (%) responses to endurance training in $VO_{2max}$ (Støren, Helgerud et al. 2017) were the same as the responses to MST in 1RM (Paper II)

although approximately of half the size (means of 24% vs. 12% in Paper II and Støren, Helgerud et al. (2017), respectively). In both studies, the relative adaptations to training were found to be independent of age. These findings do not imply that physical capacity is not deteriorating with ageing. In previous studies, advancing age has been found to lead to an impairment of cardiorespiratory function, including a decrease in $VO_{2max}$/$VO_{2peak}$ (Wilson, O'Hanlon et al. 2010, Mendonca, Pezarat-Correia et al. 2017). This deterioration appears in both sedentary as well as in well-trained individuals, however, the declines are slower and athletes are able to maintain higher $VO_{2max}$ than their age-matched sedentary counterparts (Wilson, O'Hanlon et al. 2010). Thus, it seems possible to improve $VO_{2max}$ by about the same relative amount in any age-group between 20 and 70+ years of age, as illustrated in figure 3.

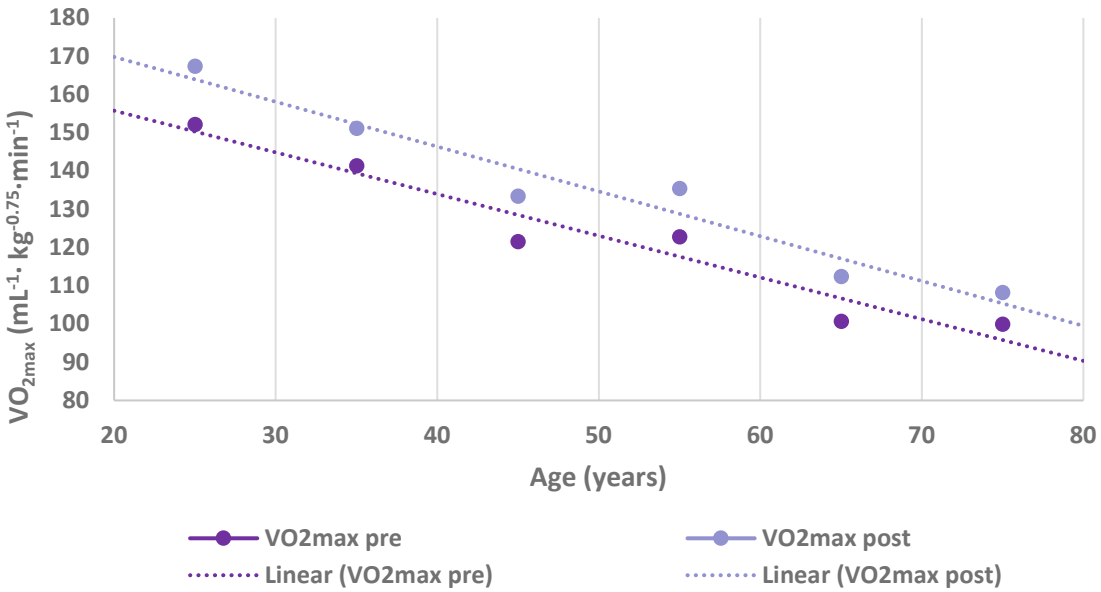


*Figure 3 High-intensity interval training (HIIT) induced changes in $VO_{2max}$ across different ages (adapted from Støren, Helgerud et al. (2017))*

As shown in Figure 3, at any age it is possible to jump from the lower dotted line to the upper dotted line by a HIIT intervention. Similar to the results from paper II on maximal strength training, the age decline will continue – but at a higher level.

As to Paper III, we were not aware of other studies that had looked at the impacts of age (16 – 18 yrs old/junior vs. ≥ 19 yrs old/ senior athletes) on the training characteristics and training adaptations. Both age groups had approximately the same total training volume 3 months before the pre-test. Similarly, during the season preparation period from May to October, the total training volume as well as light- and high-intensity training, ski-specific training, running strength and speed/jump training volumes were approximately the same. However, junior athletes trained ~20 min/week less at moderate training intensity compared to senior athletes. Training intensity distribution in the junior athlete group was in line with what has been reported in previous studies regarding the same age-group, except slightly lower volumes of moderate and high intensity in the Paper III (Sandbakk, Holmberg et al. 2011). The senior athlete group in Paper III had lower training volumes than world-class cross-country skiers, but approximately the same relative intensity distribution (Losnegard, Myklebust et al. 2013, Tonnessen, Sylta et al. 2014, Sandbakk, Hegge et al. 2016, Solli, Tønnessen et al. 2017).

During the 6-month season preparation, the skiers in both age groups had very little training progression, meaning that they continued with approximately the same volume and intensity distribution. Not surprisingly, this lack of progression led to no changes in any of the measured performance- or physiological variables This training pattern was similar to what has been reported previously during the season preparation period among elite cross country skiers (Losnegard, Myklebust et al. 2013, Sandbakk, Hegge et al. 2016).

When it comes to the differences in physiological variables between junior and senior athletes, the older skiers weighed 15% more than the younger group at baseline. Also, the senior athletes exhibited ~18% higher MAS and performed ~11% better at a time trial in double poling ($TT_{DP}$). Nearly the same difference as for MAS was observed for the velocity at LT. This in support of MAS being the main predictor of the velocity at LT. The observed age difference in MAS can be explained by the differences in DP-$VO_{2peak}$ and

$C_{DP}$ (~11% and ~8%, respectively), and could partly be due to the cardiac system and muscle mass being still under development in the junior athletes (Ingjer 1992, Rusko 1992, Armstrong and Welsman 2019). Another potential explanation could be fewer total years of training in the youngest athlete group. However- if the training lacks progression, more years of training would not in itself lead to improved performance (Bompa and Haff 2009, McNicol, O'Brien et al. 2009, McArdle, Katch et al. 2015, Booth, Orr et al. 2017). We also observed a 21% difference in muscle strength, with the senior athletes being stronger than their younger counterparts. Also here, the age differences may be partly due to the junior athletes still being under development regarding muscle mass and hormonal levels (Handelsman, Hirschberg et al. 2018, Armstrong and Welsman 2019). Muscle strength has been shown to be important in determining skiing performance in DP (Sunde, Johansen et al. 2019).

Despite finding significant age-related differences between the junior and senior athletes, these differences might be partly due to the unequal sex distribution in the two groups. When analysing the age differences in males and females separately, some of the associations were no longer significant. In males, on the other hand, only 1RM in pull-down and DP-$VO_{2peak}$ were still significant when comparing the two age groups. In females, the previously described differences between junior and senior athletes were still significant except for the $C_{DP}$ and muscle strength. Another factor that could, in part, play a role in explaining these differences, is that the senior female athletes competed at a national level, while the female junior athletes competed at the regional level, and also possessed poorer endurance capacity.

## 3.5 The effect of sex

### 3.5.1 The effect of sex on PA levels

In Paper I, sex played a significant role in the self-reported PA levels with females demonstrating higher PA levels compared to males ($P < 0.01$). This is despite the fact that males reported participating in sports or outdoor activities earlier in life at a higher

frequency than females (~59% vs. 44%, respectively), and this factor was positively associated with currently reported PA ($P < 0.01$). Females being more PA than males are in line with findings reported by Viken, Aspvik et al. (2016), which was a study that included objectively measured PA levels on older Norwegian subjects. However, this is likely not a universal finding, but may be specific to this geographic region, as in more general terms, females are usually less physically active than males (Kaplan, Newsom et al. 2001, Lightfoot 2011, Gerovasili, Agaku et al. 2015).

### 3.5.2 The effect of sex on maximal strength training

In paper II, males had a higher maximal strength in leg-press than females. We found a 56% difference in 1RM(kg) between males and females (~315 vs ~203 kg, respectively). Also in paper III, males were stronger than females in both 1RM half-squat and pulldown (131 kg vs. 108 kg (19%) and 97 vs. 74 kg (27%), respectively). These findings are in line with previous studies finding that males generally have ~40-60% higher muscle strength than females (Bishop, Cureton et al. 1987, Miller, MacDougall et al. 1993, Petrella, Kim et al. 2005, Reynolds, Gordon et al. 2006), at least in part due to larger muscle size (Bishop, Cureton et al. 1987). The differences observed among highly trained cross-country skiers are similar to those observed by others (Sandbakk, Ettema et al. 2014, Sandbakk, Solli et al. 2018, Sunde, Johansen et al. 2019).

In Paper II, in response to the MST intervention, both sexes experienced relatively similar improvements, i.e. 26.2% in males compared to 22.6% in females. This is in line with other reports not finding significant sex differences in responses to strength training programs (Lewis, Kamon et al. 1986, Tracy, Ivey et al. 1999, Hakkinen, Pakarinen et al. 2000, Lemmer, Hurlbut et al. 2000, Støren, Helgerud et al. 2008, Sunde, Storen et al. 2010, Kanegusuku, Queiroz et al. 2015).

Among cross-country skiers in paper III, we observed a significant increase in strength training volume in the second half of the 6-month training period (August-October) compared to the first half (May-July). The increase in strength training volume was larger

among females compared to males (28% vs. 19%, respectively). During this period, females improved their maximal strength to a greater extent than males, i.e. 10.1% vs. 6.9% in half squat and 5.4% vs. 1.6% in the pull-down. However, these differences were not statistically significant.

### 3.5.3 The effect of sex on training characteristics and endurance performance

Before the 6-month study period, females had a lower training volume compared to male skiers, but these differences were no longer present during the study period. Previously, elite male cross-country skiers have been reported to have a larger total training volume during a one-year period (Solli, Kocbach et al. 2018). We did, however, observe that females had four times higher amounts of speed and jump training compared to males.

When it comes to the performance variables at baseline, males exhibited 19% higher RUN-$VO_{2max}$ and DP-$VO_{2peak}$, 32% higher MAS, 9% and 15% better $C_{DP}$ and $TT_{DP}$, respectively. These findings are similar to what has been found previously (Sandbakk, Ettema et al. 2014, Andersson, Govus et al. 2019, Sunde, Johansen et al. 2019). Sex differences observed in MAS corresponded well with the sum of DP-$VO_{2peak}$ and $C_{DP}$, which was also expected as MAS is the product of these two variables. Also, the sex difference in MAS appeared to explain the difference observed in $TT_{DP}$. Furthermore, these two variables were correlated at baseline (r = -0.58; gender corrected).

Previously, it has been shown that sex differences increase in DP-$VO_{2peak}$ due to larger contribution of the upper-body strength compared to RUN-$VO_{2max}$ (Sandbakk, Ettema et al. 2014, Hegge, Bucher et al. 2016, Sandbakk, Solli et al. 2018). In our study, the differences were the same for these two variables, although the sex differences in 1RM pull-down were larger than in 1RM half-squat (30% vs 21%).

Most of the observed sex differences, i.e. $TT_{DP}$, RUN-$VO_{2max}$ and DP-$VO_{2peak}$, remained during the 6-month study period. Thus, pointing towards similar adaptations to a similar

training regimen in males and females, as reported earlier (Astorino, Allen et al. 2011, Støren, Helgerud et al. 2017, Varley-Campbell, Cooper et al. 2018). We did, however, observe a significant improvement in females' $C_{DP}$ values from pre- to post1 tests (May to July) possibly due to the increase in roller-ski specific training volume compared to the period before the study, and to a volume similar to males'. Thus, the sex difference in $C_{DP}$ became smaller as males maintained their $C_{DP}$. There were no further improvements in females' $C_{DP}$ during the second half of the study period (August to October) when the roller-skiing volume was held constant. This, again, underlines the need for progression in training load (Bompa and Calcina 1994, McNicol, O'Brien et al. 2009, McArdle, Katch et al. 2015, Booth, Orr et al. 2017) to achieve further improvements.

## 3.6 Limitations

The largest limitation, especially in the leg-press (paper II) and cross-country studies (paper III), is the relatively low sample size regarding the genetic analysis. In these two studies, the sample size was typical for a physiological training intervention study. Candidate gene studies with small sample size and individual polymorphisms with only a small effect on the measured phenotype are generally prone to type I statistic error (Wang, Padmanabhan et al. 2013), i.e. there is a risk of false-positive results. Thus, the results of papers II and III have to be treated with caution/as preliminary. On the other hand, in all three studies, we included highly homogenous cohorts from the same geographical region, thus, increasing statistical power (Marchini, Cardon et al. 2004).

In intervention studies drop-out and/or insufficient data due to different reasons is common. This leads to even lower final sample size. In the leg-press study, 76 participants were initially recruited with 72 of these consenting to the genetic analysis. 49 participants completed the 8-week intervention and could be included in the final data analysis, which is a drop of 35.5% in sample size. There were several reasons for dropping out, and the most common being the inability to complete a minimum of the required 80% of the training sessions. Although some participants reported muscle/joint

pain during the study, previous reports indicate that lower-limb MST interventions have similar drop-out rates and injury rates as conventional strength training (Raymond, Bramley-Tzerefos et al. 2013). Furthermore, the drop-out rated in our study was similar across all age groups. Thus, we can still recommend MST 2-3 times/week to improve muscle strength, and, potentially delay age-associated decline in muscle function. Among the cross-country skiers, of the 46 initially recruited skiers, 17 (37%) were excluded either due to insufficient reporting of the training habits, not participating in all three testing sessions (pre, post1 and post2) or sickness/injuries. Genetic data in combination with pre-test data were available for 40 participants. The final sample size was 29 well-trained cross-country skiers.

Another limitation, specific for Paper I, is that the PA levels are calculated based on questionnaires. Questionnaires can both over- and underestimate the real PA levels (Prince, Adamo et al. 2008) when compared to directly measured PA. This can, in part, explain the large proportion (74%) of the cohort reporting high PA levels. However, questionnaires are widely used to map PA behaviour, including in genetic studies, as it is more cost-efficient, thus, allowing larger sample sizes to be included in the study (Bray, Fulton et al. 2011). Another questionnaire-based study mapping the physical inactivity levels across many European countries (N= 19298) have reported lower inactivity rates in Scandinavia (~6%) compared to Southern-Europe (e.g. 29% in Portugal; Gomes, Figueiredo et al. (2017)). Another similar study (N= 19978) found that around 72% of Swedish participants could be characterized as highly active compared to ~40% in Portugal (Gerovasili, Agaku et al. 2015). Thus, although the real PA levels might be overestimated, our results are in line with these findings. Furthermore, we found a small, but significant difference in BMI in favour of the group with the higher PA levels, indicating that there might be a real difference in PA levels between the two groups. BMI is considered to be negatively correlated with PA levels is several other studies (Viken, Aspvik et al. 2016, Ekelund, Kolle et al. 2017, Lübs, Peplies et al. 2018), and increased BMI leads to lower PA rather than lower PA leading to an increase in BMI

(Ekelund, Brage et al. 2008, Metcalf, Hosking et al. 2011, Viken, Aspvik et al. 2016, Ekelund, Kolle et al. 2017).

# 4 Conclusions and future perspectives

Age and sex had, unsurprisingly, an impact on both maximal strength and endurance performance, in favour of young adults and males. Regarding trainability, however, age or gender had no impact on the adaptability to maximal strength training or endurance training. The environmental factor training modality was thus shown to be crucial. All individuals from the Leg-press cohort responded to the maximal strength training regimen that they were subjected to, independently of their initial training status or single candidate gene status, leading to significant increases in the muscle strength. In the Cross-country skiing cohort, on the other hand, we did not observe any significant changes in any of the endurance parameters studied during the 6-month season preparation. During this period, they increased the training volume without increasing the exercise intensity. Throughout the study period, participants trained at similar intensities, i.e. 90% at low intensity, and 5% moderate and high-intensities, each.

Significant associations between *ACTN3* R577X and PA, *PPARGC1A* Gly482Ser and maximal strength trainability, and *ACTN3* R577X, *ACE* I/D, *PPARGC1A* Gly482Ser, *PPARA* rs4253778 and *PPARG* rs1801282 and endurance performance were found in Paper I, II and III, respectively. Furthermore, the associations were found both among the general population (paper I and II) as well as in cross-country skiers competing at a national level (paper III). However, the effects of the polymorphisms investigated individually are likely small. Both in papers II and III, training modality seemed to have a much larger impact on the strength or endurance performance than the candidate genes. Papers II and III, however, are based on sample sizes typical for training intervention studies and are considered small in genetic association studies. Thus, the results of papers II and III have to be treated with caution. To achieve sufficiently high sample size, a multi-centre study approach may be more appropriate. Thus, in addition to a large number of common and rare genetic variants, epigenetic and transcriptomic approaches may be needed to gain further insights into the complex nature of physical performance and trainability.

# 5 References

Abdollahi, M. R., S. Huang, S. Rodriguez, P. A. Guthrie, G. D. Smith, S. Ebrahim, D. A. Lawlor, I. N. Day and T. R. Gaunt (2008). "Homogeneous assay of rs4343, an ACE I/D proxy, and an analysis in the British Women's Heart and Health Study (BWHHS)." Dis Markers **24**(1): 11-17.
Ahmetov, II, E. S. Egorova, L. J. Gabdrakhmanova and O. N. Fedotovskaya (2016). "Genes and Athletic Performance: An Update." Med Sport Sci **61**: 41-54.
Ahmetov, II and O. N. Fedotovskaya (2015). "Current Progress in Sports Genomics." Adv Clin Chem **70**: 247-314.
Ahmetov, II, I. A. Mozhayskaya, D. M. Flavell, I. V. Astratenkova, A. I. Komkova, E. V. Lyubaeva, P. P. Tarakin, B. S. Shenkman, A. B. Vdovina, A. I. Netreba, D. V. Popov, O. L. Vinogradova, H. E. Montgomery and V. A. Rogozkin (2006). "PPARalpha gene variation and physical performance in Russian athletes." Eur J Appl Physiol **97**(1): 103-108.
Ahtiainen, J. P., S. Walker, H. Peltonen, J. Holviala, E. Sillanpää, L. Karavirta, J. Sallinen, J. Mikkola, H. Valkeinen, A. Mero, J. J. Hulmi and K. Häkkinen (2016). "Heterogeneity in resistance training-induced muscle strength and mass responses in men and women of different ages." AGE **38**(1).
Ainsworth, B. E., W. L. Haskell, S. D. Herrmann, N. Meckes, D. R. Bassett, Jr., C. Tudor-Locke, J. L. Greer, J. Vezina, M. C. Whitt-Glover and A. S. Leon (2011). "2011 Compendium of Physical Activities: a second update of codes and MET values." Med Sci Sports Exerc **43**(8): 1575-1581.
Ainsworth, B. E., W. L. Haskell, A. S. Leon, D. R. Jacobs, Jr., H. J. Montoye, J. F. Sallis and R. S. Paffenbarger, Jr. (1993). "Compendium of physical activities: classification of energy costs of human physical activities." Med Sci Sports Exerc **25**(1): 71-80.
Alvarez-Romero, J., S. Voisin, N. Eynon and D. Hiam (2020). "Mapping Robust Genetic Variants Associated with Exercise Responses." Int J Sports Med(EFirst).
Andersson, E. P., A. Govus, O. M. Shannon and K. McGawley (2019). "Sex Differences in Performance and Pacing Strategies During Sprint Skiing." Front Physiol **10**: 295.
Arany, Z. (2008). "PGC-1 coactivators and skeletal muscle adaptations in health and disease." Curr Opin Genet Dev **18**(5): 426-434.
Arden, N. K. and T. D. Spector (1997). "Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study." J Bone Miner Res **12**(12): 2076-2081.
Armstrong, N. and J. Welsman (2019). "Youth cardiorespiratory fitness: evidence, myths and misconceptions." Bull World Health Organ **97**(11): 777-782.
Astorino, T. A., R. P. Allen, D. W. Roberson, M. Jurancich, R. Lewis, K. McCarthy and E. Trost (2011). "Adaptations to high-intensity training are independent of gender." Eur J Appl Physiol **111**(7): 1279-1286.
Avila, J. J., S. M. Courtney and M. P. Massett (2019). Heritability of Endurance Traits from Animal Research Models. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 164-177.
Bamman, M. M., J. K. Petrella, J. S. Kim, D. L. Mayhew and J. M. Cross (2007). "Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans." J Appl Physiol (1985) **102**(6): 2232-2239.
Bangsbo, J., J. Blackwell, C. J. Boraxbekk, P. Caserotti, F. Dela, A. B. Evans, A. P. Jespersen, L. Gliemann, A. F. Kramer, J. Lundbye-Jensen, E. L. Mortensen, A. J. Lassen, A. J. Gow, S. D. R. Harridge, Y. Hellsten, M. Kjaer, U. M. Kujala, R. E. Rhodes, E. C. J. Pike, T. Skinner, T. Skovgaard, J. Troelsen, E. Tulle, M. A. Tully, J. G. Z. van Uffelen and J. Viña (2019). "Copenhagen Consensus statement 2019: physical activity and ageing." Br J Sports Med **53**(14): 856-858.

Barber, J. L. and M. A. Sarzynski (2019). Heritability of Endurance Traits from Human Research Models. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 178-186.
Barberio, M. D., E. E. Pistilli and M. J. Hubal (2019). Genetic Contributions to Muscle Strength. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 264-276.
Barley, J., A. Blackwood, N. D. Carter, D. E. Crews, J. K. Cruickshank, S. Jeffery, A. O. Ogunlesi and G. A. Sagnella (1994). "Angiotensin converting enzyme insertion/deletion polymorphism: association with ethnic origin." J Hypertens **12**(8): 955-957.
Barnes, K. R. and A. E. Kilding (2015). "Running economy: measurement, norms, and determining factors." Sports medicine - open **1**(1): 8-8.
Barrett-O'Keefe, Z., J. Helgerud, P. D. Wagner and R. S. Richardson (2012). "Maximal strength training and increased work efficiency: contribution from the trained muscle bed." J Appl Physiol (1985) **113**(12): 1846-1851.
Bassett, D. R., Jr. and E. T. Howley (2000). "Limiting factors for maximum oxygen uptake and determinants of endurance performance." Med Sci Sports Exerc **32**(1): 70-84.
Bauman, A. E., R. S. Reis, J. F. Sallis, J. C. Wells, R. J. Loos and B. W. Martin (2012). "Correlates of physical activity: why are some people physically active and others not?" Lancet **380**(9838): 258-271.
Baumert, P., M. J. Lake, C. E. Stewart, B. Drust and R. M. Erskine (2016). "Genetic variation and exercise-induced muscle damage: implications for athletic performance, injury and ageing." Eur J Appl Physiol **116**(9): 1595-1625.
Behm, D. G. (1995). "Neuromuscular implications and applications of resistance training." Journal of Strength and Conditioning Research **9**: 264-274.
Berg, O. K., O. S. Kwon, T. J. Hureau, H. L. Clifton, T. Thurston, Y. Le Fur, E.-K. Jeong, M. Amann, R. S. Richardson, J. D. Trinity, E. Wang and G. Layec (2018). "Maximal strength training increases muscle force generating capacity and the anaerobic ATP synthesis flux without altering the cost of contraction in elderly." Experimental Gerontology **111**: 154-161.
Bishop, P., K. Cureton and M. Collins (1987). "Sex difference in muscular strength in equally-trained men and women." Ergonomics **30**(4): 675-687.
Blond, M. B., T. M. Schnurr, M. Rosenkilde, J. S. Quist, A. S. Gram, M. H. Reichkendler, P. L. Auerbach, P. Nordby, L. T. Skovgaard and R. Ribel-Madsen (2019). "PPARG Pro12Ala Ala carriers exhibit greater improvements in peripheral insulin sensitivity in response to 12 weeks of aerobic exercise training." Physiological genomics **51**(6): 254-260.
Bompa, T. O. and O. Calcina (1994). Theory and methodology of training : the key to athletic performance. Dubuque, Iowa, Kendall/Hunt.
Bompa, T. O. and G. G. Haff (2009). Periodization: theory and methodology of training. Champaign, Ill, Human Kinetics**:** 31-55.
Booth, F. W., T. J. Kelty, K. B. Grigsby and G. N. Ruegsegger (2019). Why Study the Systems Genetics of Sport and Exercise? Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 7-15.
Booth, F. W., M. J. Laye, S. J. Lees, R. S. Rector and J. P. Thyfault (2008). "Reduced physical activity and risk of chronic disease: the biology behind the consequences." Eur J Appl Physiol **102**(4): 381-390.
Booth, F. W., C. K. Roberts, J. P. Thyfault, G. N. Ruegsegger and R. G. Toedebusch (2017). "Role of Inactivity in Chronic Diseases: Evolutionary Insight and Pathophysiological Mechanisms." Physiol Rev **97**(4): 1351-1402.
Booth, M., R. Orr and S. Cobley (2017). "Call for coordinated and systematic training load measurement (and progression) in athlete development: a conceptual model with practical steps." British Journal of Sports Medicine **51**(7): 559-560.

Bouchard, C. (2019). "DNA Sequence Variations Contribute to Variability in Fitness and Trainability." Med Sci Sports Exerc **51**(8): 1781-1785.
Bouchard, C., P. An, T. Rice, J. S. Skinner, J. H. Wilmore, J. Gagnon, L. Pérusse, A. S. Leon and D. C. Rao (1999). "Familial aggregation of VO(2max) response to exercise training: results from the HERITAGE Family Study." J Appl Physiol (1985) **87**(3): 1003-1008.
Bouchard, C., E. W. Daw, T. Rice, L. Pérusse, J. Gagnon, M. A. Province, A. S. Leon, D. C. Rao, J. S. Skinner and J. H. Wilmore (1998). "Familial resemblance for VO2max in the sedentary state: the HERITAGE family study." Med Sci Sports Exerc **30**(2): 252-258.
Bouchard, C., R. Lesage, G. Lortie, J. A. Simoneau, P. Hamel, M. R. Boulay, L. Perusse, G. Theriault and C. Leblanc (1986). "Aerobic performance in brothers, dizygotic and monozygotic twins." Med Sci Sports Exerc **18**(6): 639-646.
Bouchard, C., M. A. Sarzynski, T. K. Rice, W. E. Kraus, T. S. Church, Y. J. Sung, D. C. Rao and T. Rankinen (2011). "Genomic predictors of the maximal $O_2$ uptake response to standardized exercise training programs." J Appl Physiol (1985) **110**(5): 1160-1170.
Bramble, D. M. and D. E. Lieberman (2004). "Endurance running and the evolution of Homo." Nature **432**(7015): 345-352.
Bratland-Sanda, S., F. G. Pedersen, M. N. Haave, J. Helgerud and Ø. Støren (2020). "Large Inter-Individual Differences in Responses to a Block of High Intensity Aerobic Interval Training: A Case Series in National-level Cyclists and Triathletes." Int J Exerc Sci **13**(2): 480-487.
Bray, M. S., J. E. Fulton, N. S. Kalupahana and J. T. Lightfoot (2011). Genetic epidemiology, physical activity, and inactivity. Genetic and Molecular Aspects of Sport Performance. Bouchard. C. and E. P. Hoffman, Wiley-Blackwell**:** 81-89.
Bretthauer, M., G. Gondal, I. Larsen, E. Carlsen, T. Eide, T. Grotmol, E. Skovlund, K. Tveit, M. Vatn and G. J. S. j. o. g. Hoff (2002). "Design, organization and management of a controlled population screening study for detection of colorectal neoplasia: attendance rates in the NORCCAP study (Norwegian Colorectal Cancer Prevention)." **37**(5): 568-573.
Buford, T. W., F. C. Hsu, T. E. Brinkley, C. S. Carter, T. S. Church, J. A. Dodson, B. H. Goodpaster, M. M. McDermott, B. J. Nicklas, V. Yank, J. A. Johnson and M. Pahor (2014). "Genetic influence on exercise-induced changes in physical function among mobility-limited older adults." Physiol Genomics **46**(5): 149-158.
Calvo, M., G. Rodas, M. Vallejo, A. Estruch, A. Arcas, C. Javierre, G. Viscor and J. L. Ventura (2002). "Heritability of explosive power and anaerobic capacity in humans." Eur J Appl Physiol **86**(3): 218-225.
Campos, G. E., T. J. Luecke, H. K. Wendeln, K. Toma, F. C. Hagerman, T. F. Murray, K. E. Ragg, N. A. Ratamess, W. J. Kraemer and R. S. Staron (2002). "Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones." Eur J Appl Physiol **88**(1-2): 50-60.
Carmelli, D., M. Kelly-Hayes, P. A. Wolf, G. E. Swan, L. M. Jack, T. Reed and J. M. Guralnik (2000). "The contribution of genetic influences to measures of lower-extremity function in older male twins." J Gerontol A Biol Sci Med Sci **55**(1): B49-53.
Cartee, G. D., R. T. Hepple, M. M. Bamman and J. R. Zierath (2016). "Exercise Promotes Healthy Aging of Skeletal Muscle." Cell Metab **23**(6): 1034-1047.
Chen, Y., D. Wang, P. Yan, S. Yan, Q. Chang and Z. Cheng (2019). "Meta-analyses of the association between the PPARGC1A Gly482Ser polymorphism and athletic performance." Biol Sport **36**(4): 301-309.
Clarkson, P. M., J. M. Devaney, H. Gordish-Dressman, P. D. Thompson, M. J. Hubal, M. Urso, T. B. Price, T. J. Angelopoulos, P. M. Gordon, N. M. Moyna, L. S. Pescatello, P. S. Visich, R. F. Zoeller, R. L. Seip and E. P. Hoffman (2005). "ACTN3 genotype is associated with increases in

muscle strength in response to resistance training in women." J Appl Physiol (1985) **99**(1): 154-163.
Cohen, J. (1988). Statistical power analysis for the behavioral sciences. Hillsdale, N. J, Laurence Erlbaum.
Collier, R. (2012). "Genetic tests for athletic ability: science or snake oil?" CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne **184**(1): E43-E44.
Correia, J. C., D. M. Ferreira and J. L. Ruas (2015). "Intercellular: local and systemic actions of skeletal muscle PGC-1s." Trends Endocrinol Metab **26**(6): 305-314.
Daniels, J. T. (1985). "A physiologist's view of running economy." Med Sci Sports Exerc **17**(3): 332-338.
Davis, J. A. (1985). "Anaerobic threshold: review of the concept and directions for future research." Med Sci Sports Exerc **17**(1): 6-21.
De Moor, M. H., T. D. Spector, L. F. Cherkas, M. Falchi, J. J. Hottenga, D. I. Boomsma and E. J. De Geus (2007). "Genome-wide linkage scan for athlete status in 700 British female DZ twin pairs." Twin Res Hum Genet **10**(6): 812-820.
Deckert, J., M. Catalano, Y. V. Syagailo, M. Bosi, O. Okladnova, D. Di Bella, M. M. Nothen, P. Maffei, P. Franke, J. Fritze, W. Maier, P. Propping, H. Beckmann, L. Bellodi and K. P. Lesch (1999). "Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder." Hum Mol Genet **8**(4): 621-624.
Defoor, J., L. Vanhees, K. Martens, G. Matthijs, A. Van Vlerken, D. Zielinska, D. Schepers, R. Vlietinck and R. Fagard (2006). "The CAREGENE study: ACE gene I/D polymorphism and effect of physical training on aerobic power in coronary artery disease." Heart **92**(4): 527-528.
Del Coso, J., D. Hiam, P. Houweling, L. M. Perez, N. Eynon and A. Lucia (2018). "More than a 'speed gene': ACTN3 R577X genotype, trainability, muscle damage, and the risk for injuries." Eur J Appl Physiol.
Delmonico, M. J., M. C. Kostek, N. A. Doldo, B. D. Hand, S. Walsh, J. M. Conway, C. R. Carignan, S. M. Roth and B. F. Hurley (2007). "Alpha-actinin-3 (ACTN3) R577X polymorphism influences knee extensor peak power response to strength training in older men and women." J Gerontol A Biol Sci Med Sci **62**(2): 206-212.
Deschamps, C. L., K. E. Connors, M. S. Klein, V. L. Johnsen, J. Shearer, H. J. Vogel, J. M. Devaney, H. Gordish-Dressman, G. M. Many, W. Barfield, E. P. Hoffman, W. E. Kraus and D. S. Hittel (2015). "The ACTN3 R577X Polymorphism Is Associated with Cardiometabolic Fitness in Healthy Young Adults." Plos One **10**(6).
Dey, D. K., I. Bosaeus, L. Lissner and B. Steen (2009). "Changes in body composition and its relation to muscle strength in 75-year-old men and women: a 5-year prospective follow-up study of the NORA cohort in Goteborg, Sweden." Nutrition **25**(6): 613-619.
di Prampero, P. E. (2003). "Factors limiting maximal performance in humans." Eur J Appl Physiol **90**(3-4): 420-429.
Dillon, L. M., A. P. Rebelo and C. T. Moraes (2012). "The role of PGC-1 coactivators in aging skeletal muscle and heart." IUBMB Life **64**(3): 231-241.
Distefano, G. and B. H. Goodpaster (2018). "Effects of Exercise and Aging on Skeletal Muscle." Cold Spring Harb Perspect Med **8**(3).
Döring, F. E., S. Onur, U. Geisen, M. R. Boulay, L. Pérusse, T. Rankinen, R. Rauramaa, B. Wolfahrt and C. Bouchard (2010). "ACTN3 R577X and other polymorphisms are not associated with elite endurance athlete status in the Genathlete study." J Sports Sci **28**(12): 1355-1359.
Ekelund, U., S. Brage, H. Besson, S. Sharp and N. J. Wareham (2008). "Time spent being sedentary and weight gain in healthy adults: reverse or bidirectional causality?" The American Journal of Clinical Nutrition **88**(3): 612-617.

Ekelund, U., E. Kolle, J. Steene-Johannessen, K. E. Dalene, A. K. O. Nilsen, S. A. Anderssen and B. H. Hansen (2017). "Objectively measured sedentary time and physical activity and associations with body weight gain: does body weight determine a decline in moderate and vigorous intensity physical activity?" International Journal of Obesity **41**(12): 1769-1774.
Erskine, R. M., G. Fletcher and J. P. Folland (2014). "The contribution of muscle hypertrophy to strength changes following resistance training." Eur J Appl Physiol **114**(6): 1239-1249.
Erskine, R. M., D. A. Jones, A. G. Williams, C. E. Stewart and H. Degens (2010). "Inter-individual variability in the adaptation of human muscle specific tension to progressive resistance training." Eur J Appl Physiol **110**(6): 1117-1125.
Erskine, R. M., A. G. Williams, D. A. Jones, C. E. Stewart and H. Degens (2014). "The individual and combined influence of ACE and ACTN3 genotypes on muscle phenotypes before and after strength training." Scand J Med Sci Sports **24**(4): 642-648.
Eynon, N., E. D. Hanson, A. Lucia, P. J. Houweling, F. Garton, K. N. North and D. J. Bishop (2013). "Genes for elite power and sprint performance: ACTN3 leads the way." Sports Med **43**(9): 803-817.
Eynon, N., Y. Meckel, M. Sagiv, C. Yamin, R. Amir, M. Sagiv, E. Goldhammer, J. A. Duarte and J. Oliveira (2010). "Do PPARGC1A and PPARalpha polymorphisms influence sprint or endurance phenotypes?" Scand J Med Sci Sports **20**(1): e145-150.
Eynon, N., J. R. Ruiz, P. Femia, V. P. Pushkarev, P. Cieszczyk, A. Maciejewska-Karlowska, M. Sawczuk, D. A. Dyatlov, E. V. Lekontsev, L. M. Kulikov, R. Birk, D. J. Bishop and A. Lucia (2012). "The ACTN3 R577X Polymorphism across Three Groups of Elite Male European Athletes." Plos One **7**(8).
Eynon, N., J. R. Ruiz, Y. Meckel, C. Santiago, C. Fiuza-Luces, F. Gómez-Gallego, J. Oliveira and A. Lucia (2011). "Is the -174 C/G polymorphism of the IL6 gene associated with elite power performance? A replication study with two different Caucasian cohorts." Exp Physiol **96**(2): 156-162.
Faude, O., W. Kindermann and T. Meyer (2009). "Lactate Threshold Concepts. How Valid are They?" Sports Medicine **39**(6): 469-490.
Fiatarone Singh, M. A., N. Gates, N. Saigal, G. C. Wilson, J. Meiklejohn, H. Brodaty, W. Wen, N. Singh, B. T. Baune, C. Suo, M. K. Baker, N. Foroughi, Y. Wang, P. S. Sachdev and M. Valenzuela (2014). "The Study of Mental and Resistance Training (SMART) study—resistance training and/or cognitive training in mild cognitive impairment: a randomized, double-blind, double-sham controlled trial." J Am Med Dir Assoc **15**(12): 873-880.
Folland, J., B. Leach, T. Little, K. Hawker, S. Myerson, H. Montgomery and D. Jones (2000). "Angiotensin-converting enzyme genotype affects the response of human skeletal muscle to functional overload." Exp Physiol **85**(5): 575-579.
Foy, C. A., L. J. McCormack, W. C. Knowler, J. H. Barrett, A. Catto and P. J. Grant (1996). "The angiotensin-I converting enzyme (ACE) gene I/D polymorphism and ACE levels in Pima Indians." J Med Genet **33**(4): 336-337.
Francis, P., M. Lyons, M. Piasecki, J. Mc Phee, K. Hind and P. Jakeman (2017). "Measurement of muscle health in aging." Biogerontology **18**(6): 901-911.
Friedlander, S. M., A. L. Herrmann, D. P. Lowry, E. R. Mepham, M. Lek, K. N. North and C. L. Organ (2013). "ACTN3 allele frequency in humans covaries with global latitudinal gradient." PLoS One **8**(1): e52282.
Fuentes, R. M., M. Perola, A. Nissinen and J. Tuomilehto (2002). "ACE gene and physical activity, blood pressure, and hypertension: a population study in Finland." J Appl Physiol (1985) **92**(6): 2508-2512.

Garatachea, N. and A. Lucia (2013). "Genes, physical fitness and ageing." Ageing Res Rev **12**(1): 90-102.
Garber, C. E., B. Blissmer, M. R. Deschenes, B. A. Franklin, M. J. Lamonte, I. M. Lee, D. C. Nieman, D. P. Swain and M. American College of Sports (2011). "American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise." Med Sci Sports Exerc **43**(7): 1334-1359.
Garton, F. C. and K. N. North (2016). "The Effect of Heterozygosity for the ACTN3 Null Allele on Human Muscle Performance." Med Sci Sports Exerc **48**(3): 509-520.
Gerovasili, V., I. T. Agaku, C. I. Vardavas and F. T. Filippidis (2015). "Levels of physical activity among adults 18-64 years old in 28 European countries." Prev Med **81**: 87-91.
Ghosh, S., J. C. Vivar, M. A. Sarzynski, Y. J. Sung, J. A. Timmons, C. Bouchard and T. Rankinen (2013). "Integrative pathway analysis of a genome-wide association study of (V)O(2max) response to exercise training." J Appl Physiol (1985) **115**(9): 1343-1359.
Gineviciene, V., A. Jakaitiene, M. O. Aksenov, A. V. Aksenova, A. M. Druzhevskaya, I. V. Astratenkova, E. S. Egorova, L. J. Gabdrakhmanova, L. Tubelis, V. Kucinskas and A. Utkus (2016). "Association analysis of ACE, ACTN3 and PPARGC1A gene polymorphisms in two cohorts of European strength and power athletes." Biol Sport **33**(3): 199-206.
Goh, K. P., K. Chew, A. Koh, M. Guan, Y. S. Wong and C. F. Sum (2009). "The relationship between ACE gene ID polymorphism and aerobic capacity in Asian rugby players." Singapore Med J **50**(10): 997-1003.
Goleva-Fjellet, S., A. M. Bjurholt, E. H. Kure, I. K. Larsen, O. Storen and M. Saebo (2020). "Distribution of allele frequencies for genes associated with physical activity and/or physical capacity in a homogenous Norwegian cohort- a cross-sectional study." BMC Genet **21**(1): 8.
Gomes, M., D. Figueiredo, L. Teixeira, V. Poveda, C. Paúl, A. Santos-Silva and E. Costa (2017). "Physical inactivity among older adults across Europe based on the SHARE database." Age Ageing **46**(1): 71-77.
Good, D. J., M. Li and K. Deater-Deckard (2015). "A Genetic Basis for Motivated Exercise." Exerc Sport Sci Rev **43**(4): 231-237.
Gordish-Dressman, H. and J. M. Devaney (2011). Statistical and methodological considerations in exercise genomics. Exercise genomics, Springer**:** 23-43.
Haff, G. (2019) "Will the genetic screening of athletes change sport as we know it?" The Conversation.
Hagerman, F. C., S. J. Walsh, R. S. Staron, R. S. Hikida, R. M. Gilders, T. F. Murray, K. Toma and K. E. Ragg (2000). "Effects of High-Intensity Resistance Training on Untrained Older Men. I. Strength, Cardiovascular, and Metabolic Responses." The Journals of Gerontology Series A: Biological Sciences and Medical Sciences **55**(7): B336-B346.
Hakkinen, K., A. Pakarinen, W. J. Kraemer, R. U. Newton and M. Alen (2000). "Basal concentrations and acute responses of serum hormones and strength development during heavy resistance training in middle-aged and elderly men and women." J Gerontol A Biol Sci Med Sci **55**(2): B95-105.
Hallal, P. C., L. B. Andersen, F. C. Bull, R. Guthold, W. Haskell and U. Ekelund (2012). "Global physical activity levels: surveillance progress, pitfalls, and prospects." The Lancet **380**(9838): 247-257.
Handelsman, D. J., A. L. Hirschberg and S. Bermon (2018). "Circulating Testosterone as the Hormonal Basis of Sex Differences in Athletic Performance." Endocr Rev **39**(5): 803-829.
Harvey, N. R., S. Voisin, P. J. Dunn, H. Sutherland, X. Yan, M. Jacques, I. D. Papadimitriou, L. J. Haseler, K. J. Ashton, L. M. Haupt, N. Eynon and L. R. Griffiths (2020). "Genetic variants

associated with exercise performance in both moderately trained and highly trained individuals." Molecular genetics and genomics : MGG: 10.1007/s00438-00019-01639-00438.
Harvey, N. R., S. Voisin, P. J. Dunn, H. Sutherland, X. Yan, M. Jacques, I. D. Papadimitriou, L. J. Haseler, K. J. Ashton, L. M. Haupt, N. Eynon and L. R. Griffiths (2020). "Genetic variants associated with exercise performance in both moderately trained and highly trained individuals." Molecular Genetics and Genomics **295**(2): 515-523.
Head, S. I., S. Chan, P. J. Houweling, K. G. Quinlan, R. Murphy, S. Wagner, O. Friedrich and K. N. North (2015). "Altered Ca2+ kinetics associated with α-actinin-3 deficiency may explain positive selection for ACTN3 null allele in human evolution." PLoS Genet **11**(2): e1004862.
Head, S. I., S. Chan, P. J. Houweling, K. G. R. Quinlan, R. Murphy, S. Wagner, O. Friedrich and K. N. North (2015). "Altered Ca2+ Kinetics Associated with alpha-Actinin-3 Deficiency May Explain Positive Selection for ACTN3 Null Allele in Human Evolution." Plos Genetics **11**(1).
Hegge, A. M., E. Bucher, G. Ettema, O. Faude, H. C. Holmberg and Ø. Sandbakk (2016). "Gender differences in power production, energetic capacity and efficiency of elite cross-country skiers during whole-body, upper-body, and arm poling." Eur J Appl Physiol **116**(2): 291-300.
Heggelund, J., M. S. Fimland, J. Helgerud and J. Hoff (2013). "Maximal strength training improves work economy, rate of force development and maximal strength more than conventional strength training." Eur J Appl Physiol **113**(6): 1565-1573.
Helgerud, J., K. Høydal, E. Wang, T. Karlsen, P. Berg, M. Bjerkaas, T. Simonsen, C. Helgesen, N. Hjorth, R. Bach and J. Hoff (2007). "Aerobic high-intensity intervals improve VO2max more than moderate training." Med Sci Sports Exerc **39**(4): 665-671.
Helgerud, J., F. Ingjer and S. B. Stromme (1990). "Sex differences in performance-matched marathon runners." Eur J Appl Physiol Occup Physiol **61**(5-6): 433-439.
Hills, A. P., S. J. Street and N. M. Byrne (2015). Chapter Three - Physical Activity and Health: "What is Old is New Again". Advances in Food and Nutrition Research. J. Henry, Academic Press. **75:** 77-95.
Hoff, J., J. Helgerud and U. Wisløff (1999). "Maximal strength training improves work economy in trained female cross-country skiers." Med Sci Sports Exerc **31**(6): 870-877.
Hoffmann, C. and C. Weigert (2017). "Skeletal Muscle as an Endocrine Organ: The Role of Myokines in Exercise Adaptations." Cold Spring Harbor perspectives in medicine **7**(11): a029793.
Hogarth, M. W., F. C. Garton, P. J. Houweling, T. Tukiainen, M. Lek, D. G. Macarthur, J. T. Seto, K. G. Quinlan, N. Yang, S. I. Head and K. N. North (2016). "Analysis of the ACTN3 heterozygous genotype suggests that α-actinin-3 controls sarcomeric composition and muscle function in a dose-dependent fashion." Hum Mol Genet **25**(5): 866-877.
Holmberg, H. C. (2015). "The elite cross-country skier provides unique insights into human exercise physiology." Scand J Med Sci Sports **25 Suppl 4**: 100-109.
Hong, F., S. Pan, Y. Guo, P. Xu and Y. Zhai (2019). "PPARs as Nuclear Receptors for Nutrient and Energy Metabolism." Molecules **24**(14).
Hopkins, W. G. (2016). "A New View of Statistics." Retrieved October 21st, 2020, from http://www.sportsci.org/resource/stats/index.html.
Houweling, P. J., I. D. Papadimitriou, J. T. Seto, L. M. Perez, J. D. Coso, K. N. North, A. Lucia and N. Eynon (2018). "Is evolutionary loss our gain? The role of ACTN3 p.Arg577Ter (R577X) genotype in athletic performance, ageing, and disease." Hum Mutat **39**(12): 1774-1787.
Hubal, M. J., H. Gordish-Dressman, P. D. Thompson, T. B. Price, E. P. Hoffman, T. J. Angelopoulos, P. M. Gordon, N. M. Moyna, L. S. Pescatello, P. S. Visich, R. F. Zoeller, R. L. Seip and P. M. Clarkson (2005). "Variability in muscle size and strength gain after unilateral resistance training." Med Sci Sports Exerc **37**(6): 964-972.

Hubal, M. J., M. L. Urso and P. M. Clarkson (2011). Genetic aspects of muscular strength and size. Exercise Genomics, Springer**:** 157-178.
Ingjer, F. (1991). "Maximal oxygen uptake as a predictor of performance ability in women and men elite cross-country skiers." Scandinavian Journal of Medicine & Science in Sports **1**(1): 25-30.
Ingjer, F. (1992). "Development of maximal oxygen uptake in young elite male cross-country skiers: a longitudinal study." J Sports Sci **10**(1): 49-63.
Jacques, M., S. Landen, S. Voisin and N. Eynon (2019). Summary Findings on Genetics and Sport Performance. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 347-356.
Jamshidi, Y., H. E. Montgomery, H. W. Hense, S. G. Myerson, I. P. Torra, B. Staels, M. J. World, A. Doering, J. Erdmann, C. Hengstenberg, S. E. Humphries, H. Schunkert and D. M. Flavell (2002). "Peroxisome proliferator--activated receptor alpha gene regulates left ventricular growth in response to exercise and hypertension." Circulation **105**(8): 950-955.
Jenkins, N. D. M., A. A. Miramonti, E. C. Hill, C. M. Smith, K. C. Cochrane-Snyman, T. J. Housh and J. T. Cramer (2017). "Greater Neural Adaptations following High- vs. Low-Load Resistance Training." Front Physiol **8**: 331.
Jones, A. and D. R. Woods (2003). "Skeletal muscle RAS and exercise performance." Int J Biochem Cell Biol **35**(6): 855-866.
Jones, N., J. Kiely, B. Suraci, D. J. Collins, D. de Lorenzo, C. Pickering and K. A. Grimaldi (2016). "A genetic-based algorithm for personalized resistance training." Biol Sport **33**(2): 117-126.
Joyner, M. J. (2019). "Genetic Approaches for Sports Performance: How Far Away Are We?" Sports Medicine **49**(S2): 199-204.
Kanegusuku, H., A. C. Queiroz, V. J. Silva, M. T. de Mello, C. Ugrinowitsch and C. L. Forjaz (2015). "High-Intensity Progressive Resistance Training Increases Strength With No Change in Cardiovascular Function and Autonomic Neural Regulation in Older Adults." J Aging Phys Act **23**(3): 339-345.
Kaplan, M. S., J. T. Newsom, B. H. McFarland and L. Lu (2001). "Demographic and psychosocial correlates of physical activity in late life." Am J Prev Med **21**(4): 306-312.
Kikuchi, N., S. Yoshida, S. K. Min, K. Lee, M. Sakamaki-Sunaga, T. Okamoto and K. Nakazato (2015). "The ACTN3 R577X genotype is associated with muscle function in a Japanese population." Applied Physiology Nutrition and Metabolism **40**(4).
Kohl, H. W., 3rd, C. L. Craig, E. V. Lambert, S. Inoue, J. R. Alkandari, G. Leetongin and S. Kahlmeier (2012). "The pandemic of physical inactivity: global action for public health." The Lancet **380**(9838): 294-305.
Kristiansen, B. and S. Guldteig Larsen (2016) "Gentest mente maratonvinner Sebastian (27) burde satse på sprint."
Lambert, C. P. and W. J. Evans (2002). "Effects of aging and resistance exercise on determinants of muscle strength." J Am Aging Assoc **25**(2): 73-78.
Lamichane, S., B. Dahal Lamichane and S. M. Kwon (2018). "Pivotal Roles of Peroxisome Proliferator-Activated Receptors (PPARs) and Their Signal Cascade for Cellular and Whole-Body Energy Homeostasis." Int J Mol Sci **19**(4).
Lasevicius, T., C. Ugrinowitsch, B. J. Schoenfeld, H. Roschel, L. D. Tavares, E. O. De Souza, G. Laurentino and V. Tricoli (2018). "Effects of different intensities of resistance training with equated volume load on muscle strength and hypertrophy." Eur J Sport Sci **18**(6): 772-780.
Laustsen, E. (2016) "Hos hele 18 prosent av befolkningen i Norge fungerer ikke dette genet som det skal." Dagens Næringliv.

Leblanc, A., B. A. Taylor, P. D. Thompson, J. A. Capizzi, P. M. Clarkson, C. Michael White and L. S. Pescatello (2015). "Relationships between physical activity and muscular strength among healthy adults across the lifespan." SpringerPlus **4**(1).
Lee, F. X., P. J. Houweling, K. N. North and K. G. Quinlan (2016). "How does α-actinin-3 deficiency alter muscle function? Mechanistic insights into ACTN3, the 'gene for speed'." Biochim Biophys Acta **1863**(4): 686-693.
Lee, F. X. Z., P. J. Houweling, K. N. North and K. G. R. Quinlan (2016). "How does α-actinin-3 deficiency alter muscle function? Mechanistic insights into ACTN3, the 'gene for speed'." Biochimica et Biophysica Acta (BBA) - Molecular Cell Research **1863**(4): 686-693.
Lee, I. M., E. J. Shiroma, F. Lobelo, P. Puska, S. N. Blair and P. T. Katzmarzyk (2012). "Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy." The Lancet **380**(9838): 219-229.
Lee, J. H. and H.-S. Jun (2019). "Role of Myokines in Regulating Skeletal Muscle Mass and Function." Frontiers in Physiology **10**(42).
Lemmer, J. T., D. E. Hurlbut, G. F. Martel, B. L. Tracy, F. M. Ivey, E. J. Metter, J. L. Fozard, J. L. Fleg and B. F. Hurley (2000). "Age and gender responses to strength training and detraining." Med Sci Sports Exerc **32**(8): 1505-1512.
Lemon, J. (2018) "China Will Begin Using Genetic Testing to Select Olympic Athletes." Newsweek.
Lewis, D. A., E. Kamon and J. L. Hodgson (1986). "Physiological differences between genders. Implications for sports conditioning." Sports Med **3**(5): 357-369.
LHNCBC (2020) "Help Me Understand Genetics: Genomic Research."
Li, R., J. Xia, X. I. Zhang, W. G. Gathirua-Mwangi, J. Guo, Y. Li, S. McKenzie and Y. Song (2018). "Associations of Muscle Mass and Strength with All-Cause Mortality among US Older Adults." Med Sci Sports Exerc **50**(3): 458-467.
Lightfoot, J. T. (2011). Can you be born a couch potato? The genomic regulation of physical activity. Exercise Genomics, Springer**:** 45-72.
Lightfoot, J. T., M. J. Hubal and S. M. Roth (2019). Introduction. Routledge Handbook of Sport and Exercise Systems Genetics. Abingdon, Oxon, Routledge**:** 1-4.
Lightfoot, J. T., A. C. Letsinger and J. Z. Granados (2019). The Evolution of Genetic Mechanisms Controlling Physical Activity. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 80-93.
Lindle, R. S., E. J. Metter, N. A. Lynch, J. L. Fleg, J. L. Fozard, J. Tobin, T. A. Roy and B. F. Hurley (1997). "Age and gender comparisons of muscle strength in 654 women and men aged 20–93 yr." Journal of Applied Physiology **83**(5): 1581-1587.
Lira, V. A., C. R. Benton, Z. Yan and A. Bonen (2010). "PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity." Am J Physiol Endocrinol Metab **299**(2): E145-161.
Lopez-Leon, S., C. Tuvblad and D. A. Forero (2016). "Sports genetics: the PPARA gene and athletes' high ability in endurance sports. A systematic review and meta-analysis." Biol Sport **33**(1): 3-6.
Losnegard, T., H. Myklebust, M. Spencer and J. Hallen (2013). "Seasonal variations in VO2max, O2-cost, O2-deficit, and performance in elite cross-country skiers." J Strength Cond Res **27**(7): 1780-1790.
Losnegard, T., D. Schäfer and J. Hallén (2014). "Exercise economy in skiing and running." Frontiers in Physiology **5**(5).

Lübs, L., J. Peplies, C. Drell and K. Bammann (2018). "Cross-sectional and longitudinal factors influencing physical activity of 65 to 75-year-olds: a pan European cohort study based on the survey of health, ageing and retirement in Europe (SHARE)." BMC Geriatrics **18**(1).
Ma, F., Y. Yang, X. Li, F. Zhou, C. Gao, M. Li and L. Gao (2013). "The association of sport performance with ACE and ACTN3 genetic polymorphisms: a systematic review and meta-analysis." PLoS One **8**(1): e54685.
MacArthur, D. G. and K. N. North (2004). "A gene for speed? The evolution and function of alpha-actinin-3." Bioessays **26**(7): 786-795.
Maciejewska-Karłowska, A. (2013). "Polymorphic variants of the PPAR (Peroxisome Proliferator-Activated Receptor) genes: relevancefor athletic performance." Trends in Sports Sciences **1 (20)**: 5-15.
Maciejewska-Karlowska, A., M. Sawczuk, P. Cieszczyk, A. Zarebska and S. Sawczyn (2013). "Association between the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma gene and strength athlete status." PloS one **8**(6): e67172-e67172.
Maestu, J., E. Latt, T. Raask, K. Sak, K. Laas, J. Jurimae and T. Jurimae (2013). "Ace I/D polymorphism is associated with habitual physical activity in pubertal boys." J Physiol Sci **63**(6): 427-434.
Mangine, G. T., J. R. Hoffman, A. M. Gonzalez, J. R. Townsend, A. J. Wells, A. R. Jajtner, K. S. Beyer, C. H. Boone, A. A. Miramonti, R. Wang, M. B. Lamonica, D. H. Fukuda, N. A. Ratamess and J. R. Stout (2015). "The effect of training volume and intensity on improvements in muscular strength and size in resistance-trained men." Physiological Reports **3**(8): e12472.
Marchini, J., L. R. Cardon, M. S. Phillips and P. Donnelly (2004). "The effects of human population structure on large genetic association studies." Nat Genet **36**(5): 512-517.
Martinez-Redondo, V., P. R. Jannig, J. C. Correia, D. M. Ferreira, I. Cervenka, J. M. Lindvall, I. Sinha, M. Izadi, A. T. Pettersson-Klein, L. Z. Agudelo, A. Gimenez-Cassina, P. C. Brum, K. Dahlman-Wright and J. L. Ruas (2016). "Peroxisome Proliferator-activated Receptor gamma Coactivator-1 alpha Isoforms Selectively Regulate Multiple Splicing Events on Target Genes." J Biol Chem **291**(29): 15169-15184.
Martinez-Redondo, V., A. T. Pettersson and J. L. Ruas (2015). "The hitchhiker's guide to PGC-1alpha isoform structure and biological functions." Diabetologia **58**(9): 1969-1977.
Mathew, J., K. Basheeruddin and S. Prabhakar (2001). "Differences in frequency of the deletion polymorphism of the angiotensin-converting enzyme gene in different ethnic groups." Angiology **52**(6): 375-379.
Maughan, R. J. (2005). "The limits of human athletic performance." Ann Transplant **10**(4): 52-54.
McArdle, W. D., V. L. Katch and F. I. Katch (2015). Exercise physiology : nutrition, energy, and human performance. Philadelphia,Baltimore, Lippincott Williams & Wilkins Wolters Kluwer Health**:** 461-497.
McNicol, A. J., B. J. O'Brien, C. D. Paton and W. L. Knez (2009). "The effects of increased absolute training intensity on adaptations to endurance exercise training." J Sci Med Sport **12**(4): 485-489.
Mendonca, G. V., P. Pezarat-Correia, J. R. Vaz, L. Silva and K. S. Heffernan (2017). "Impact of Aging on Endurance and Neuromuscular Physical Performance: The Role of Vascular Senescence." Sports Med **47**(4): 583-598.
Metcalf, B. S., J. Hosking, A. N. Jeffery, L. D. Voss, W. Henley and T. J. Wilkin (2011). "Fatness leads to inactivity, but inactivity does not lead to fatness: a longitudinal study in children (EarlyBird 45)." **96**(10): 942-947.

Mikami, E., N. Fuku, H. Murakami, H. Tsuchie, H. Takahashi, N. Ohiwa, H. Tanaka, Y. P. Pitsiladis, M. Higuchi, M. Miyachi, T. Kawahara and M. Tanaka (2014). "ACTN3 R577X genotype is associated with sprinting in elite Japanese athletes." Int J Sports Med **35**(2): 172-177.
Mikkelsen, K., L. Stojanovska, M. Polenakovic, M. Bosevski and V. Apostolopoulos (2017). "Exercise and mental health." Maturitas **106**: 48-56.
Miller, A. E. J., J. D. MacDougall, M. A. Tarnopolsky and D. G. Sale (1993). "Gender differences in strength and muscle fiber characteristics." European Journal of Applied Physiology and Occupational Physiology **66**(3): 254-262.
Miller, S. A., D. D. Dykes and H. F. Polesky (1988). "A simple salting out procedure for extracting DNA from human nucleated cells." Nucleic Acids Res **16**(3): 1215.
Mills, M., N. Yang, R. Weinberger, D. L. Vander Woude, A. H. Beggs, S. Easteal and K. North (2001). "Differential expression of the actin-binding proteins, alpha-actinin-2 and -3, in different species: implications for the evolution of functional redundancy." Human molecular genetics **10**(13): 1335-1346.
Miyamoto-Mikami, E., H. Zempo, N. Fuku, N. Kikuchi, M. Miyachi and H. Murakami (2018). "Heritability estimates of endurance-related phenotypes: A systematic review and meta-analysis." Scand J Med Sci Sports **28**(3): 834-845.
Montrezol, F. T., R. Marinho, G. Mota, V. D'almeida, E. M. de Oliveira, R. J. Gomes and A. Medeiros (2019). "ACE Gene Plays a Key Role in Reducing Blood Pressure in The Hyperintensive Elderly After Resistance Training." J Strength Cond Res **33**(4): 1119-1129.
Moore-Harrison, T. and J. T. Lightfoot (2010). "Driven to be inactive? The genetics of physical activity." Prog Mol Biol Transl Sci **94**: 271-290.
Moran, C. N., N. Yang, M. E. S. Bailey, A. Tsiokanos, A. Jamurtas, D. G. MacArthur, K. North, Y. P. Pitsiladis and R. H. Wilson (2007). "Association analysis of the ACTN3 R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks." European Journal of Human Genetics **15**(1): 88-93.
Morishima, M., N. Harada, S. Hara, A. Sano, H. Seno, A. Takahashi, Y. Morita and Y. Nakaya (2006). "Monoamine oxidase A activity and norepinephrine level in hippocampus determine hyperwheel running in SPORTS rats." Neuropsychopharmacology **31**(12): 2627-2638.
Mosti, M. P., T. Carlsen, E. Aas, J. Hoff, A. K. Stunes and U. Syversen (2014). "Maximal Strength Training Improves Bone Mineral Density and Neuromuscular Performance in Young Adult Women." **28**(10): 2935-2945.
Muñoz-Cánoves, P., C. Scheele, B. K. Pedersen and A. L. Serrano (2013). "Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword?" Febs j **280**(17): 4131-4148.
Myers, J., P. McAuley, C. J. Lavie, J. P. Despres, R. Arena and P. Kokkinos (2015). "Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: their independent and interwoven importance to health status." Prog Cardiovasc Dis **57**(4): 306-314.
Myerson, S., H. Hemingway, R. Budget, J. Martin, S. Humphries and H. Montgomery (1999). "Human angiotensin I-converting enzyme gene and endurance performance." J Appl Physiol (1985) **87**(4): 1313-1316.
Myerson, S. G., H. E. Montgomery, M. Whittingham, M. Jubb, M. J. World, S. E. Humphries and D. J. Pennell (2001). "Left ventricular hypertrophy with exercise and ACE gene insertion/deletion polymorphism: a randomized controlled trial with losartan." Circulation **103**(2): 226-230.
Mägi, A., E. Unt, E. Prans, L. Raus, J. Eha, A. Veraksitš, K. Kingo and S. Kõks (2016). "The Association Analysis between ACE and ACTN3 Genes Polymorphisms and Endurance Capacity in Young Cross-Country Skiers: Longitudinal Study." J Sports Sci Med **15**(2): 287-294.

NCBI. (2020a). "Reference SNP (rs) Report: rs1474347." Retrieved September 29, 2020, from https://www.ncbi.nlm.nih.gov/snp/rs1474347
NCBI. (2020b). "Reference SNP (rs) Report: rs1801282." Retrieved 14. September, 2020, from https://www.ncbi.nlm.nih.gov/snp/rs1801282
NCBI. (2020c). "Reference SNP (rs) Report: rs1815739." Retrieved September 30, 2020, from https://www.ncbi.nlm.nih.gov/snp/rs1815739
NCBI. (2020d). "Reference SNP (rs) Report: rs4253778." Retrieved September 29, 2020, from https://www.ncbi.nlm.nih.gov/snp/rs4253778
NCBI. (2020e). "Reference SNP (rs) Report: rs6552828." Retrieved September 29, 2020, from https://www.ncbi.nlm.nih.gov/snp/rs6552828
NCBI. (2020f). "Reference SNP (rs) Report: rs8192678." Retrieved September 16, 2020, from https://www.ncbi.nlm.nih.gov/snp/rs8192678
Norman, B., M. Esbjörnsson, H. Rundqvist, T. Osterlund, F. von Walden and P. A. Tesch (2009). "Strength, power, fiber types, and mRNA expression in trained men and women with different ACTN3 R577X genotypes." J Appl Physiol (1985) **106**(3): 959-965.
North, K. N. and A. H. Beggs (1996). "Deficiency of a skeletal muscle isoform of alpha-actinin (alpha-actinin-3) in merosin-positive congenital muscular dystrophy." Neuromuscul Disord **6**(4): 229-235.
North, K. N., N. Yang, D. Wattanasirichaigoon, M. Mills, S. Easteal and A. H. Beggs (1999). "A common nonsense mutation results in alpha-actinin-3 deficiency in the general population." Nat Genet **21**(4): 353-354.
Orysiak, J., P. Zmijewski, A. Klusiewicz, P. Kaliszewski, J. Malczewska-Lenczowska, J. Gajewski and A. Pokrywka (2013). "The association between ace gene variation and aerobic capacity in winter endurance disciplines." Biol Sport **30**(4): 249-253.
Papadimitriou, I. D., S. J. Lockey, S. Voisin, A. J. Herbert, F. Garton, P. J. Houweling, P. Cieszczyk, A. Maciejewska-Skrendo, M. Sawczuk, M. Massidda, C. M. Calò, I. V. Astratenkova, A. Kouvatsi, A. M. Druzhevskaya, M. Jacques, Ahmetov, II, G. K. Stebbings, S. Heffernan, S. H. Day, R. Erskine, C. Pedlar, C. Kipps, K. N. North, A. G. Williams and N. Eynon (2018). "No association between ACTN3 R577X and ACE I/D polymorphisms and endurance running times in 698 Caucasian athletes." BMC Genomics **19**(1): 13.
Papadimitriou, I. D., A. Lucia, Y. P. Pitsiladis, V. P. Pushkarev, D. A. Dyatlov, E. F. Orekhov, G. G. Artioli, J. P. Guilherme, A. H. Lancha, Jr., V. Gineviciene, P. Cieszczyk, A. Maciejewska-Karlowska, M. Sawczuk, C. A. Muniesa, A. Kouvatsi, M. Massidda, C. M. Calo, F. Garton, P. J. Houweling, G. Wang, K. Austin, A. M. Druzhevskaya, I. V. Astratenkova, Ahmetov, II, D. J. Bishop, K. N. North and N. Eynon (2016). "ACTN3 R577X and ACE I/D gene variants influence performance in elite sprinters: a multi-cohort study." BMC Genomics **17**: 285.
Pasqua, L. A., S. Bueno, G. G. Artioli, A. H. Lancha, Jr., M. Matsuda, M. V. Marquezini, A. E. Lima-Silva, P. H. Saldiva and R. Bertuzzi (2016). "Influence of ACTN3 R577X polymorphism on ventilatory thresholds related to endurance performance." J Sports Sci **34**(2): 163-170.
Pate, R. R. and A. Kriska (1984). "Physiological basis of the sex difference in cardiorespiratory endurance." Sports Med **1**(2): 87-98.
Pedersen, B. K. and B. Saltin (2015). "Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases." Scand J Med Sci Sports **25 Suppl 3**: 1-72.
Pedersen, K. (2014). Genforsker advarer mot å kjøpe gentest. Bergens Tidende. Bergen**:** 8-9.
Pereira, A., A. M. Costa, M. Izquierdo, A. J. Silva, E. Bastos and M. C. Marques (2013). "ACE I/D and ACTN3 R/X polymorphisms as potential factors in modulating exercise-related phenotypes in older women in response to a muscle power training stimuli." **35**(5): 1949-1959.

Pereira, A., A. M. Costa, M. Izquierdo, A. J. Silva, E. Bastos and M. C. Marques (2013). "ACE I/D and ACTN3 R/X polymorphisms as potential factors in modulating exercise-related phenotypes in older women in response to a muscle power training stimuli." Age (Dordr) **35**(5): 1949-1959.
Pereira, A., A. M. Costa, J. C. Leitao, A. M. Monteiro, M. Izquierdo, A. J. Silva, E. Bastos and M. C. Marques (2013). "The influence of ACE ID and ACTN3 R577X polymorphisms on lower-extremity function in older women in response to high-speed power training." BMC Geriatr **13**: 131.
Pérusse, L., J. Gagnon, M. A. Province, D. C. Rao, J. H. Wilmore, A. S. Leon, C. Bouchard and J. S. Skinner (2001). "Familial aggregation of submaximal aerobic performance in the HERITAGE Family study." Med Sci Sports Exerc **33**(4): 597-604.
Pescatello, L. S., L. M. Corso, L. P. Santos, J. Livingston and B. A. Taylor (2019). Angiotensin-Converting Enzyme and the Genomics of Endurance Performance. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 216-250.
Pescatello, L. S., L. M. L. Corso, L. P. Santos, J. Livingston and B. A. Taylor (2019). Angiotensin-Converting Enzyme and the Genomics of Endurance Performance. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 216-249.
Pescatello, L. S., J. M. Devaney, M. J. Hubal, P. D. Thompson and E. P. Hoffman (2013). "Highlights from the functional single nucleotide polymorphisms associated with human muscle size and strength or FAMuSS study." Biomed Res Int **2013**: 643575.
Pescatello, L. S., M. A. Kostek, H. Gordish-Dressman, P. D. Thompson, R. L. Seip, T. B. Price, T. J. Angelopoulos, P. M. Clarkson, P. M. Gordon, N. M. Moyna, P. S. Visich, R. F. Zoeller, J. M. Devaney and E. P. Hoffman (2006). "ACE ID genotype and the muscle strength and size response to unilateral resistance training." Med Sci Sports Exerc **38**(6): 1074-1081.
Petr, M., A. Maciejewska-Skrendo, A. Zajac, J. Chycki and P. Stastny (2019). "Association of Elite Sports Status with Gene Variants of Peroxisome Proliferator Activated Receptors and Their Transcriptional Coactivator." Int J Mol Sci **21**(1).
Petr, M., P. Stastny, O. Pecha, M. Šteffl, O. Šeda and E. Kohlíková (2014). "PPARA intron polymorphism associated with power performance in 30-s anaerobic Wingate Test." PLoS One **9**(9): e107171.
Petr, M., P. Stastny, A. Zajac, J. J. Tufano and A. Maciejewska-Skrendo (2018). "The Role of Peroxisome Proliferator-Activated Receptors and Their Transcriptional Coactivators Gene Variations in Human Trainability: A Systematic Review." International journal of molecular sciences **19**(5): 1472.
Petrella, J. K., J. S. Kim, S. C. Tuggle, S. R. Hall and M. M. Bamman (2005). "Age differences in knee extension power, contractile velocity, and fatigability." J Appl Physiol (1985) **98**(1): 211-220.
Phua, W. W. T., M. X. Y. Wong, Z. Liao and N. S. Tan (2018). "An aPPARent Functional Consequence in Skeletal Muscle Physiology via Peroxisome Proliferator-Activated Receptors." Int J Mol Sci **19**(5).
Pickering, C. and J. Kiely (2017). "ACTN3: More than Just a Gene for Speed." Front Physiol **8**: 1080.
Pickering, C. and J. Kiely (2018). "ACTN3, Morbidity, and Healthy Aging." Front Genet **9**: 15.
Pickering, C., J. Kiely, J. Grgic, A. Lucia and J. Del Coso (2019). "Can Genetic Testing Identify Talent for Sport?" Genes (Basel) **10**(12).
Pickering, C., B. Suraci, E. A. Semenova, E. A. Boulygina, E. S. Kostryukova, N. A. Kulemin, O. V. Borisov, S. A. Khabibova, A. K. Larin, A. V. Pavlenko, E. V. Lyubaeva, D. V. Popov, E. A. Lysenko, T. F. Vepkhvadze, E. M. Lednev, A. Leońska-Duniec, B. Pająk, J. Chycki, W. Moska, E. Lulińska-Kuklik, M. Dornowski, A. Maszczyk, B. Bradley, A. Kana-Ah, P. Cięszczyk, E. V. Generozov and

Ahmetov, II (2019). "A Genome-Wide Association Study of Sprint Performance in Elite Youth Football Players." J Strength Cond Res **33**(9): 2344-2351.
Pimenta, E. M., D. B. Coelho, C. E. Veneroso, E. J. Barros Coelho, I. R. Cruz, R. F. Morandi, A. P. G. De, M. R. Carvalho, E. S. Garcia and J. A. De Paz Fernandez (2013). "Effect of ACTN3 gene on strength and endurance in soccer players." J Strength Cond Res **27**(12): 3286-3292.
Pollock, M. L. (1977). "Submaximal and maximal working capacity of elite distance runners. Part I: Cardiorespiratory aspects." Ann N Y Acad Sci **301**: 310-322.
Prince, S. A., K. B. Adamo, M. E. Hamel, J. Hardt, S. Connor Gorber and M. Tremblay (2008). "A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review." Int J Behav Nutr Phys Act **5**: 56.
Prud'homme, D., C. Bouchard, C. Leblanc, F. Landry and E. Fontaine (1984). "Sensitivity of maximal aerobic power to training is genotype-dependent." Med Sci Sports Exerc **16**(5): 489-493.
Psilander, N., E. Eftestøl, K. T. Cumming, I. Juvkam, M. M. Ekblom, K. Sunding, M. Wernbom, H. C. Holmberg, B. Ekblom, J. C. Bruusgaard, T. Raastad and K. Gundersen (2019). "Effects of training, detraining, and retraining on strength, hypertrophy, and myonuclear number in human skeletal muscle." J Appl Physiol (1985) **126**(6): 1636-1645.
Puthucheary, Z., J. R. Skipworth, J. Rawal, M. Loosemore, K. Van Someren and H. E. Montgomery (2011). "The ACE gene and human performance: 12 years on." Sports Med **41**(6): 433-448.
Quindry, J. C. and M. D. Roberts (2019). Endurance Phenotype Primer. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 148-163.
Raichlen, D. A., J. T. Webber and H. Pontzer (2019). The evolution of the human endurance phenotype. Routledge Handbook of Sport and Exercise Systems Genetics, Taylor and Francis**:** 135-147.
Rankinen, T., N. Fuku, B. Wolfarth, G. Wang, M. A. Sarzynski, D. G. Alexeev, Ahmetov, II, M. R. Boulay, P. Cieszczyk, N. Eynon, M. L. Filipenko, F. C. Garton, E. V. Generozov, V. M. Govorun, P. J. Houweling, T. Kawahara, E. S. Kostryukova, N. A. Kulemin, A. K. Larin, A. Maciejewska-Karłowska, M. Miyachi, C. A. Muniesa, H. Murakami, E. A. Ospanova, S. Padmanabhan, A. V. Pavlenko, O. N. Pyankova, C. Santiago, M. Sawczuk, R. A. Scott, V. V. Uyba, T. Yvert, L. Perusse, S. Ghosh, R. Rauramaa, K. N. North, A. Lucia, Y. Pitsiladis and C. Bouchard (2016). "No Evidence of a Common DNA Variant Profile Specific to World Class Endurance Athletes." PLoS One **11**(1): e0147330.
Rankinen, T., L. Perusse, J. Gagnon, Y. C. Chagnon, A. S. Leon, J. S. Skinner, J. H. Wilmore, D. C. Rao and C. Bouchard (2000). "Angiotensin-converting enzyme ID polymorphism and fitness phenotype in the HERITAGE Family Study." J Appl Physiol (1985) **88**(3): 1029-1035.
Ratamess, N. A., B. A. Alvar, T. E. Evetoch, T. J. Housh, W. Ben Kibler, W. J. Kraemer, N. T. J. M. Triplett, s. i. sports and exercise (2009). "Progression models in resistance training for healthy adults." **41**(3): 687-708.
Raymond, M. J., R. E. Bramley-Tzerefos, K. J. Jeffs, A. Winter and A. E. Holland (2013). "Systematic review of high-intensity progressive resistance strength training of the lower limb compared with other intensities of strength training in older adults." Arch Phys Med Rehabil **94**(8): 1458-1472.
Reid, K. F. and R. A. Fielding (2012). "Skeletal muscle power: a critical determinant of physical functioning in older adults." Exerc Sport Sci Rev **40**(1): 4-12.
Reynolds, J. M., T. J. Gordon and R. A. Robergs (2006). "Prediction of one repetition maximum strength from multiple repetition maximum testing and anthropometry." J Strength Cond Res **20**(3): 584-592.

Rhea, M. R. (2004). "Determining the magnitude of treatment effects in strength training research through the use of the effect size." J Strength Cond Res **18**(4): 918-920.
Rhodes, J. S. (2019). Neurogenetics of motivation for physical activity. Routledge Handbook of Sport and Exercise Systems Genetics, Taylor and Francis**:** 94-106.
Rhodes, J. S., S. C. Gammie and T. Garland, Jr. (2005). "Neurobiology of Mice Selected for High Voluntary Wheel-running Activity." Integr Comp Biol **45**(3): 438-455.
Rigat, B., C. Hubert, F. Alhenc-Gelas, F. Cambien, P. Corvol and F. Soubrier (1990). "An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels." J Clin Invest **86**(4): 1343-1346.
Rigat, B., C. Hubert, P. Corvol and F. Soubrier (1992). "PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1)." Nucleic Acids Res **20**(6): 1433.
Rojer, A. G. M., E. M. Reijnierse, M. C. Trappenburg, R. C. van Lummel, M. Niessen, K. S. van Schooten, M. Pijnappels, C. G. M. Meskers and A. B. Maier (2018). "Instrumented Assessment of Physical Activity Is Associated With Muscle Function but Not With Muscle Mass in a General Population." J Aging Health **30**(9): 1462-1481.
Romero-Blanco, C., M. J. Artiga-González, A. Gómez-Cabello, S. Vila-Maldonado, J. A. Casajús, I. Ara and S. Aznar (2020). "Strength and Endurance Training in Older Women in Relation to ACTN3 R577X and ACE I/D Polymorphisms." International journal of environmental research and public health **17**(4): 1236.
Roth, S. M. (2012). "Genetic aspects of skeletal muscle strength and mass with relevance to sarcopenia." Bonekey Rep **1**: 58.
Roth, S. M. and M. A. Thomis (2011). Fundamental concepts in exercise genomics. Exercise Genomics, Springer**:** 1-22.
Roth, S. M., S. Walsh, D. Liu, E. J. Metter, L. Ferrucci and B. F. Hurley (2008). "The ACTN3 R577X nonsense allele is under-represented in elite-level strength athletes." European Journal of Human Genetics **16**(3): 391-394.
Ruas, J. L., J. P. White, R. R. Rao, S. Kleiner, K. T. Brannan, B. C. Harrison, N. P. Greene, J. Wu, J. L. Estall, B. A. Irving, I. R. Lanza, K. A. Rasbach, M. Okutsu, K. S. Nair, Z. Yan, L. A. Leinwand and B. M. Spiegelman (2012). "A PGC-1α isoform induced by resistance training regulates skeletal muscle hypertrophy." Cell **151**(6): 1319-1331.
Ruiz, J. R., A. Buxens, M. Artieda, D. Arteta, C. Santiago, G. Rodríguez-Romo, J. I. Lao, F. Gómez-Gallego and A. Lucia (2010). "The -174 G/C polymorphism of the IL6 gene is associated with elite power performance." J Sci Med Sport **13**(5): 549-553.
Rusko, H. K. (1992). "Development of aerobic power in relation to age and training in cross-country skiers." Med Sci Sports Exerc **24**(9): 1040-1047.
Sabol, S. Z., S. Hu and D. Hamer (1998). "A functional polymorphism in the monoamine oxidase A gene promoter." Hum Genet **103**(3): 273-279.
Sandbakk, O., G. Ettema and H. C. Holmberg (2014). "Gender differences in endurance performance by elite cross-country skiers are influenced by the contribution from poling." Scand J Med Sci Sports **24**(1): 28-33.
Sandbakk, O., A. M. Hegge, T. Losnegard, O. Skattebo, E. Tonnessen and H. C. Holmberg (2016). "The Physiological Capacity of the World's Highest Ranked Female Cross-country Skiers." Med Sci Sports Exerc **48**(6): 1091-1100.
Sandbakk, O. and H. C. Holmberg (2014). "A reappraisal of success factors for Olympic cross-country skiing." Int J Sports Physiol Perform **9**(1): 117-121.

Sandbakk, O. and H. C. Holmberg (2017). "Physiological Capacity and Training Routines of Elite Cross-Country Skiers: Approaching the Upper Limits of Human Endurance." Int J Sports Physiol Perform **12**(8): 1003-1011.
Sandbakk, O., H. C. Holmberg, S. Leirdal and G. Ettema (2011). "The physiology of world-class sprint skiers." Scand J Med Sci Sports **21**(6): e9-16.
Sandbakk, O., G. S. Solli and H. C. Holmberg (2018). "Sex Differences in World-Record Performance: The Influence of Sport Discipline and Competition Duration." Int J Sports Physiol Perform **13**(1): 2-8.
Saunders, P. U., D. B. Pyne, R. D. Telford and J. A. Hawley (2004). "Factors affecting running economy in trained distance runners." Sports Med **34**(7): 465-485.
Scarr, G. (2019) "DNA fitness testing and how it can help you train better."
Schnyder, S. and C. Handschin (2015). "Skeletal muscle as an endocrine organ: PGC-1α, myokines and exercise." Bone **80**: 115-125.
Schoenfeld, B. J., J. Grgic, D. Ogborn and J. W. Krieger (2017). "Strength and Hypertrophy Adaptations Between Low- vs. High-Load Resistance Training." Journal of Strength and Conditioning Research **31**(12): 3508-3523.
Schutte, N. M., I. Nederend, J. J. Hudziak, M. Bartels and E. J. de Geus (2016). "Twin-sibling study and meta-analysis on the heritability of maximal oxygen consumption." Physiol Genomics **48**(3): 210-219.
Schutte, N. M., I. Nederend, J. J. Hudziak, E. J. de Geus and M. Bartels (2016). "Differences in Adolescent Physical Fitness: A Multivariate Approach and Meta-analysis." Behav Genet **46**(2): 217-227.
Scott, R. A., R. Irving, L. Irwin, E. Morrison, V. Charlton, K. Austin, D. Tladi, M. Deason, S. A. Headley, F. W. Kolkhorst, N. Yang, K. North and Y. P. Pitsiladis (2010). "ACTN3 and ACE Genotypes in Elite Jamaican and US Sprinters." Medicine and Science in Sports and Exercise **42**(1): 107-112.
Serrano, A. L., B. Baeza-Raja, E. Perdiguero, M. Jardí and P. Muñoz-Cánoves (2008). "Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy." Cell Metab **7**(1): 33-44.
Seto, J. T., F. C. Garton, K. N. North and P. J. Houweling (2019). Alpha-actinin-3's role in the genetic control of muscle strength and performance. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 323-343.
Seto, J. T., K. G. Quinlan, M. Lek, X. F. Zheng, F. Garton, D. G. MacArthur, M. W. Hogarth, P. J. Houweling, P. Gregorevic, N. Turner, G. J. Cooney, N. Yang and K. N. North (2013). "ACTN3 genotype influences muscle performance through the regulation of calcineurin signaling." J Clin Invest **123**(10): 4255-4263.
Shanmugam, V., K. W. Sell and B. K. Saha (1993). "Mistyping ACE heterozygotes." PCR Methods Appl **3**(2): 120-121.
Shih, J. C. and R. F. Thompson (1999). "Monoamine oxidase in neuropsychiatry and behavior." Am J Hum Genet **65**(3): 593-598.
Shiraishi, H., A. Suzuki, T. Fukasawa, T. Aoshima, Y. Ujiie, G. Ishii and K. Otani (2006). "Monoamine oxidase A gene promoter polymorphism affects novelty seeking and reward dependence in healthy study participants." Psychiatr Genet **16**(2): 55-58.
Silva, M. S., W. Bolani, C. R. Alves, D. G. Biagi, J. R. Lemos, Jr., J. L. da Silva, P. A. de Oliveira, G. B. Alves, E. M. de Oliveira, C. E. Negrao, J. E. Krieger, R. G. Dias and A. C. Pereira (2015). "Elimination of influences of the ACTN3 R577X variant on oxygen uptake by endurance training in healthy individuals." Int J Sports Physiol Perform **10**(5): 636-641.

Skinner, J. S., K. M. Wilmore, J. B. Krasnoff, A. Jaskólski, A. Jaskólska, J. Gagnon, M. A. Province, A. S. Leon, D. C. Rao, J. H. Wilmore and C. Bouchard (2000). "Adaptation to a standardized training program and changes in fitness in a large, heterogeneous population: the HERITAGE Family Study." Med Sci Sports Exerc **32**(1): 157-161.
Skjelbred, C. F., M. Sæbø, H. Wallin, B. A. Nexø, P. C. Hagen, I. M. Lothe, S. Aase, E. Johnson, I. L. Hansteen, U. Vogel and E. H. Kure (2006). "Polymorphisms of the XRCC1, XRCC3 and XPD genes and risk of colorectal adenoma and carcinoma, in a Norwegian cohort: a case control study." BMC Cancer **6**: 67.
Solli, G. S., J. Kocbach, T. M. Seeberg, J. Tjonnas, O. M. H. Rindal, P. Haugnes, P. O. Torvik and O. Sandbakk (2018). "Sex-based differences in speed, sub-technique selection, and kinematic patterns during low- and high-intensity training for classical cross-country skiing." PLoS One **13**(11): e0207195.
Solli, G. S., E. Tønnessen and Ø. Sandbakk (2017). "The Training Characteristics of the World's Most Successful Female Cross-Country Skier." Frontiers in Physiology **8**.
Stastny, P., M. Lehnert, M. De Ste Croix, M. Petr, Z. Svoboda, E. Maixnerova, R. Varekova, M. Botek, M. Petrek, L. Kocourkova and P. Cięszczyk (2019). "Effect of COL5A1, GDF5, and PPARA Genes on a Movement Screen and Neuromuscular Performance in Adolescent Team Sport Athletes." J Strength Cond Res **33**(8): 2057-2065.
Steinbacher, P., R. G. Feichtinger, L. Kedenko, I. Kedenko, S. Reinhardt, A. L. Schonauer, I. Leitner, A. M. Sanger, W. Stoiber, B. Kofler, H. Forster, B. Paulweber and S. Ring-Dimitriou (2015). "The single nucleotide polymorphism Gly482Ser in the PGC-1alpha gene impairs exercise-induced slow-twitch muscle fibre transformation in humans." PLoS One **10**(4): e0123881.
Steves, C. J., M. M. Mehta, S. H. Jackson and T. D. Spector (2016). "Kicking Back Cognitive Ageing: Leg Power Predicts Cognitive Ageing after Ten Years in Older Female Twins." Gerontology **62**(2): 138-149.
Strasser, B. and M. Burtscher (2018). "Survival of the fittest: VO(2)max, a key predictor of longevity?" Front Biosci (Landmark Ed) **23**: 1505-1516.
Stromme, S. B., F. Ingjer and H. D. Meen (1977). "Assessment of maximal aerobic power in specifically trained athletes." J Appl Physiol Respir Environ Exerc Physiol **42**(6): 833-837.
Stubbe, J. H., D. I. Boomsma, E. J. J. M. de Geus, S. i. Sports and Exercise (2005). "Sports participation during adolescence: a shift from environmental to genetic factors." **37**(4): 563-570.
Stubbe, J. H., D. I. Boomsma, J. M. Vink, B. K. Cornes, N. G. Martin, A. Skytthe, K. O. Kyvik, R. J. Rose, U. M. Kujala, J. Kaprio, J. R. Harris, N. L. Pedersen, J. Hunkin, T. D. Spector and E. J. de Geus (2006). "Genetic influences on exercise participation in 37,051 twin pairs from seven countries." PLoS One **1**(1): e22.
Stumvoll, M. and H. Häring (2002). "The peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism." Diabetes **51**(8): 2341-2347.
Støren, Ø., J. Helgerud, M. Saebo, E. M. Stoa, S. Bratland-Sanda, R. J. Unhjem, J. Hoff and E. Wang (2017). "The Effect of Age on the V O2max Response to High-Intensity Interval Training." Med Sci Sports Exerc **49**(1): 78-85.
Støren, Ø., J. Helgerud, E. M. Stoa and J. Hoff (2008). "Maximal strength training improves running economy in distance runners." Med Sci Sports Exerc **40**(6): 1087-1092.
Støren, Ø., B. R. Rønnestad, A. Sunde, J. Hansen, S. Ellefsen and J. Helgerud (2014). "A Time-Saving Method to Assess Power Output at Lactate Threshold in Well-Trained and Elite Cyclists." Journal of Strength and Conditioning Research **28**(3): 622-629.

Sunde, A., J.-M. Johansen, M. Gjøra, G. Paulsen, M. Bråten, J. Helgerud and Ø. Støren (2019). "Stronger Is Better: The Impact of Upper Body Strength in Double Poling Performance." Frontiers in Physiology **10**.
Sunde, A., O. Storen, M. Bjerkaas, M. H. Larsen, J. Hoff and J. Helgerud (2010). "Maximal strength training improves cycling economy in competitive cyclists." J Strength Cond Res **24**(8): 2157-2165.
Tamburus, N. Y., R. Verlengia, V. C. Kunz, M. C. Cesar and E. Silva (2018). "Apolipoprotein B and angiotensin-converting enzyme polymorphisms and aerobic interval training: randomized controlled trial in coronary artery disease patients." Braz J Med Biol Res **51**(8): e6944.
Tanaka, H. and D. R. Seals (2008). "Endurance exercise performance in Masters athletes: age-associated changes and underlying physiological mechanisms." J Physiol **586**(1): 55-63.
Tharabenjasin, P., N. Pabalan and H. Jarjanazi (2019). "Association of PPARGC1A Gly428Ser (rs8192678) polymorphism with potential for athletic ability and sports performance: A meta-analysis." PLoS One **14**(1): e0200967.
Tharabenjasin, P., N. Pabalan and H. Jarjanazi (2019). "Association of the ACTN3 R577X (rs1815739) polymorphism with elite power sports: A meta-analysis." PLoS One **14**(5): e0217390.
Thomis, M. (2019). Heritability of Muscle Size and Strength Traits. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 253-263.
Thomis, M. A., G. P. Beunen, H. H. Maes, C. J. Blimkie, M. Van Leemputte, A. L. Claessens, G. Marchal, E. Willems and R. F. Vlietinck (1998). "Strength training: importance of genetic factors." Med Sci Sports Exerc **30**(5): 724-731.
Tjernshaugen, A. (2014) "For første gang tilbys gentester over disk i Norge.".
Tomás, M. T., A. Galán-Mercant, E. A. Carnero and B. Fernandes (2018). "Functional Capacity and Levels of Physical Activity in Aging: A 3-Year Follow-up." Frontiers in medicine **4**: 244-244.
Tonnessen, E., O. Sylta, T. A. Haugen, E. Hem, I. S. Svendsen and S. Seiler (2014). "The road to gold: training and peaking characteristics in the year prior to a gold medal endurance performance." PLoS One **9**(7): e101796.
Tracy, B. L., F. M. Ivey, D. Hurlbut, G. F. Martel, J. T. Lemmer, E. L. Siegel, E. J. Metter, J. L. Fozard, J. L. Fleg and B. F. Hurley (1999). "Muscle quality. II. Effects of strength training in 65- to 75-yr-old men and women." Journal of Applied Physiology **86**(1): 195-201.
Tural, E., N. Kara, S. A. Agaoglu, M. Elbistan, M. Y. Tasmektepligil and O. Imamoglu (2014). "PPAR-α and PPARGC1A gene variants have strong effects on aerobic performance of Turkish elite endurance athletes." Mol Biol Rep **41**(9): 5799-5804.
Unhjem, R., R. Lundestad, M. S. Fimland, M. P. Mosti and E. Wang (2015). "Strength training-induced responses in older adults: attenuation of descending neural drive with age." Age (Dordr) **37**(3): 9784.
Unhjem, R., M. Nygard, L. T. van den Hoven, S. K. Sidhu, J. Hoff and E. Wang (2016). "Lifelong strength training mitigates the age-related decline in efferent drive." J Appl Physiol (1985) **121**(2): 415-423.
Valdivieso, P., D. Vaughan, E. Laczko, M. Brogioli, S. Waldron, J. Rittweger and M. Fluck (2017). "The Metabolic Response of Skeletal Muscle to Endurance Exercise Is Modified by the ACE-I/D Gene Polymorphism and Training State." Front Physiol **8**: 993.
Van Der Mee, D. J., I. O. Fedko, J. J. Hottenga, E. A. Ehli, V. D. Z. MD, L. Ligthart, V. A. N. B. TCEM, G. E. Davies, M. Bartels, J. G. Landers and D. E. G. EJC (2018). "Dopaminergic Genetic Variants and Voluntary Externally Paced Exercise Behavior." Med Sci Sports Exerc **50**(4): 700-708.

Van Der Zee, M. D. and E. De Geus (2019). Is physical activity regulated by genetics? Evidence from studies in humans. Routledge handbook of sport and exercise systems genetics, Taylor and Francis AS**:** 67-79.
Varley-Campbell, J., C. Cooper, D. Wilkerson, S. Wardle, J. Greeves and T. Lorenc (2018). "Sex-Specific Changes in Physical Performance Following Military Training: A Systematic Review." Sports Med **48**(11): 2623-2640.
Vaughan, D., F. A. Huber-Abel, F. Graber, H. Hoppeler and M. Flück (2013). "The angiotensin converting enzyme insertion/deletion polymorphism alters the response of muscle energy supply lines to exercise." European Journal of Applied Physiology **113**(7): 1719-1729.
Venezia, A. C. and S. M. Roth (2019). The Scientific and Ethical Challenges of Using Genetic Information to Predict Sport Performance. Routledge Handbook of Sport and Exercise Systems Genetics, Routledge**:** 442-452.
Viken, H., N. P. Aspvik, J. E. Ingebrigtsen, N. Zisko, U. Wisløff and D. Stensvold (2016). "Correlates of Objectively Measured Physical Activity Among Norwegian Older Adults: The Generation 100 Study." Journal of Aging and Physical Activity **24**(3): 369-375.
Vlahovich, N., D. C. Hughes, L. R. Griffiths, G. Wang, Y. P. Pitsiladis, F. Pigozzi, N. Bachl and N. Eynon (2017). "Genetic testing for exercise prescription and injury prevention: AIS-Athlome consortium-FIMS joint statement." BMC genomics **18**(Suppl 8): 818-818.
Wang, E., S. K. Nyberg, J. Hoff, J. Zhao, G. Leivseth, T. Torhaug, O. S. Husby, J. Helgerud and R. S. Richardson (2017). "Impact of maximal strength training on work efficiency and muscle fiber type in the elderly: Implications for physical function and fall prevention." Exp Gerontol **91**: 64-71.
Wang, G., S. Padmanabhan, B. Wolfarth, N. Fuku, A. Lucia, Ahmetov, II, P. Cieszczyk, M. Collins, N. Eynon, V. Klissouras, A. Williams and Y. Pitsiladis (2013). "Genomics of elite sporting performance: what little we know and necessary advances." Adv Genet **84**: 123-149.
Wetterstrand, K. (2020) "DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP)."
Weyerstraß, J., K. Stewart, A. Wesselius and M. Zeegers (2018). "Nine genetic polymorphisms associated with power athlete status - A Meta-Analysis." J Sci Med Sport **21**(2): 213-220.
WHO (2010). Global recommendations on physical activity for health. Geneva, World Health Organization.
WHO (2019). Global action plan on physical activity 2018-2030: more active people for a healthier world, World Health Organization.
Williams, A. G., S. H. Day, J. P. Folland, P. Gohlke, S. Dhamrait and H. E. Montgomery (2005). "Circulating angiotensin converting enzyme activity is correlated with muscle strength." Med Sci Sports Exerc **37**(6): 944-948.
Williams, A. G. and J. P. Folland (2008). "Similarity of polygenic profiles limits the potential for elite human physical performance." J Physiol **586**(1): 113-121.
Williams, A. G., H. Wackerhage and S. H. Day (2016). Genetic testing for sports performance, responses to training and injury risk: practical and ethical considerations. Genetics and Sports, Karger Publishers. **61:** 105-119.
Wilson, G. C., Y. Mavros, L. Tajouri and M. F. Singh (2019). "The Role of Genetic Profile in Functional Performance Adaptations to Exercise Training or Physical Activity: A Systematic Review of the Literature." J Aging Phys Act **27**(4): 594-616.
Wilson, M., R. O'Hanlon, S. Basavarajaiah, K. George, D. Green, P. Ainslie, S. Sharma, S. Prasad, C. Murrell, D. Thijssen, A. Nevill and G. Whyte (2010). "Cardiovascular function and the veteran athlete." Eur J Appl Physiol **110**(3): 459-478.

Winnicki, M., V. Accurso, M. Hoffmann, R. Pawlowski, F. Dorigatti, M. Santonastaso, D. Longo, B. Krupa-Wojciechowska, X. Jeunemaitre, A. C. Pessina, V. K. Somers and P. Palatini (2004). "Physical activity and angiotensin-converting enzyme gene polymorphism in mild hypertensives." Am J Med Genet A **125a**(1): 38-44.
Woods, D. R., M. World, M. P. Rayson, A. G. Williams, M. Jubb, Y. Jamshidi, M. Hayward, D. A. Mary, S. E. Humphries and H. E. Montgomery (2002). "Endurance enhancement related to the human angiotensin I-converting enzyme I-D polymorphism is not due to differences in the cardiorespiratory response to training." Eur J Appl Physiol **86**(3): 240-244.
Wu, R., E. Delahunt, M. Ditroilo, M. Lowery and G. De Vito (2016). "Effects of age and sex on neuromuscular-mechanical determinants of muscle strength." AGE **38**(3).
Yamin, C., O. Amir, M. Sagiv, E. Attias, Y. Meckel, N. Eynon, M. Sagiv and R. E. Amir (2007). "ACE ID genotype affects blood creatine kinase response to eccentric exercise." J Appl Physiol (1985) **103**(6): 2057-2061.
Yang, N., F. Garton and K. North (2009). "alpha-actinin-3 and performance." Med Sport Sci **54**: 88-101.
Yang, N., D. G. MacArthur, J. P. Gulbin, A. G. Hahn, A. H. Beggs, S. Easteal and K. North (2003). "ACTN3 Genotype Is Associated with Human Elite Athletic Performance." The American Journal of Human Genetics **73**(3): 627-631.
Yang, N., D. G. Macarthur, B. Wolde, V. O. Onywera, M. K. Boit, S. Y. M. A. Lau, R. H. Wilson, R. A. Scott, Y. P. Pitsiladis and K. North (2007). "The ACTN3 R577X polymorphism in east and west African athletes." Medicine and Science in Sports and Exercise **39**(11): 1985-1988.
Yoshihara, A., T. Tobina, T. Yamaga, M. Ayabe, Y. Yoshitake, Y. Kimura, M. Shimada, M. Nishimuta, N. Nakagawa, M. Ohashi, N. Hanada, H. Tanaka, A. Kiyonaga and H. Miyazaki (2009). "Physical function is weakly associated with angiotensin-converting enzyme gene I/D polymorphism in elderly Japanese subjects." Gerontology **55**(4): 387-392.
Yvert, T., Z. H. He, C. Santiago, Y. Hu, Y. C. Li, F. Gomez-Gallego, C. Fiuza-Luces, Z. Verde, C. A. Muniesa, J. Olivan, A. Santalla, J. R. Ruiz and A. Lucia (2012). "Acyl coenzyme A synthetase long-chain 1 (ACSL1) gene polymorphism (rs6552828) and elite endurance athletic status: a replication study." PLoS One **7**(7): e41268.
Zempo, H., E. Miyamoto-Mikami, N. Kikuchi, N. Fuku, M. Miyachi and H. Murakami (2017). "Heritability estimates of muscle strength-related phenotypes: A systematic review and meta-analysis." Scand J Med Sci Sports **27**(12): 1537-1546.
Zettel-Watson, L., M. Suen, L. Wehbe, D. N. Rutledge and B. J. Cherry (2017). "Aging well: Processing speed inhibition and working memory related to balance and aerobic endurance." Geriatr Gerontol Int **17**(1): 108-115.
Zhai, G., C. Ding, J. Stankovich, F. Cicuttini and G. Jones (2005). "The genetic contribution to longitudinal changes in knee structure and muscle strength: A sibpair study." Arthritis & Rheumatism **52**(9): 2830-2834.
Zhang, B., H. Tanaka, N. Shono, S. Miura, A. Kiyonaga, M. Shindo and K. Saku (2003). "The I allele of the angiotensin-converting enzyme gene is associated with an increased percentage of slow-twitch type I fibers in human skeletal muscle." Clin Genet **63**(2): 139-144.

## Paper 1

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# Distribution of allele frequencies for genes associated with physical activity and/or physical capacity in a homogenous Norwegian cohort- a cross-sectional study

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## Abstract

**Background:** There are large individual differences in physical activity (PA) behavior as well as trainability of physical capacity. Heritability studies have shown that genes may have as much impact on exercise participation behavior as environmental factors. Genes that favor both trainability and participation may increase the levels of PA. The present study aimed to assess the allele frequencies in genes associated with PA and/or physical capacity, and to see if there is any association between these polymorphisms and self-reported PA levels in a cohort of middle-aged Norwegians of Scandinavian descent ($n = 831$; mean age mean age (± SD) 55.5 ± 3.8 years).

**Results:** The genotype distributions of the *ACTN3* R577X, *ACE* I/D and *MAOA* uVNTR polymorphisms were similar to other populations of European descent. When comparing the genotype distribution between the low/medium level PA group (LMPA) and high level PA groups (HPA), a significant difference in *ACTN3* 577X allele distribution was found. The X allele frequency was 10% lower in the HPA level group ($P = 0.006$). There were no differences in the genotype distribution of the *ACE* I/D or *MAOA* uVNTR polymorphism. Education and previous participation in sports or outdoor activities was positively associated with the self-reported PA levels ($P \leq 0.001$).

**Conclusions:** To the best of our knowledge, this is the first study to report association between *ACTN3* R577X genotype and PA level in middle-aged Scandinavians. Nevertheless, the contribution of a single polymorphism to a complex trait, like PA level, is likely small. Socioeconomic variables, as education and previous participation in sports or outdoor activities, are positively associated with the self-reported PA levels.

**Keywords:** Genes, Polymorphism, *ACTN3*, Physical activity

## Background

Physical activity (PA) is a complex behavior [1], influenced by both genetic and environmental variables [2–4]. The health effects of PA are well described [5–7], as are the negative consequences of inactivity [8–10]. Insufficient PA levels have been linked to an increased risk of many chronic diseases [11]. Physical inactivity is a modifiable risk factor meaning that increased PA may have a positive effect on several diseases, e.g. diabetes and hypertension [5, 12]. Recommendation for maintaining good health is aerobic PA for a minimum of 150 min per week at moderate intensity or a minimum of 75 min per week at high intensity [10]. Despite strong evidence for genetic influence on PA [1], it is complex and not yet fully understood.

There are large inter-individual differences in PA levels [13] and trainability [14, 15]. Genes influence response to exercise as well as intrinsic behavior like motivation for activity [16, 17]. Thus, genes that favor both trainability and participation may increase the levels of PA [4, 14]. With increased age, heredity may have an even larger impact on exercise participation

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behavior [18]. Twin studies have shown that up to 62% of PA levels may be explained by genetic factors [4]. However, PA level is affected by the interaction of many genes, most of them with only a small effect each [13].

The two genes most studied in relation to trainability of cardiovascular traits [7, 19], physical function [20] and muscle strength [21] are α-actinin-3 (*ACTN3)* and angiotensin-converting enzyme (*ACE). ACTN3* is a member of the alpha-actin binding protein family. It is predominately expressed in fast twitch muscle fibers [22]. The wild type RR genotype has been reported to be more common among athletes in sprint/power sporting disciplines [23, 24]. The bases for many previous studies have been ethnically highly heterogenous cohorts [25]. Around 18% of the European population are homozygous for the minor allele R577X polymorphism, a premature stop codon [23], with large differences in the minor allele frequency among populations. The frequency of the X allele covaries with the latitude gradient [26]. Absence of the α-actinin-3 protein has been associated with a range of alterations in muscle function [27], including more efficient muscle metabolism [28], increased post-exercise muscle damage and risk of injuries [25]. The R577X polymorphism is one of only two known loss-of-function polymorphisms in humans known to have a selective advantage [27], it is more frequently observed in athletes participating in endurance disciplines [23, 24].

*ACE* codes for the angiotensin-converting enzyme and plays a role in blood pressure regulation [29]. It also influences skeletal muscle metabolism [30] and thus aerobic capacity [31, 32]. Aerobic capacity has been shown to be an important determinant for PA levels [33]. The *ACE* insertion/deletion (I/D) polymorphism is a length polymorphism, where a 287-bp *Alu* repeat is either present or absent [34]. The D allele is mostly associated with sprinting performance ability [35], while the I allele is associated with endurance performance ability [35, 36]. Studies conducted on non-athletes have revealed that the *ACE* I/D polymorphism may also influence responses to strength training [37]. Even though it has been suggested to influence the PA level [38, 39], the results are inconclusive [40].

In addition to genes influencing physiological exercise responses, genes altering PA motivation may also influence the PA level [16]. The dopaminergic system has been a subject to a number of studies on voluntary PA due to its role in reward systems and motor movement, [16, 41–43] and it has been shown to influence the inherent motivation to run in female mice [42].

Monoamine oxidase A (*MAOA*) is one of the genes in the dopaminergic pathways [44] that have been found to influence sedentary behavior [16]. It codes for an enzyme involved in oxidation of neurotransmitters, especially, serotonin, norepinephrine and dopamine [45], and may thus play a role in behavior [42, 44]. The *MAOA* gene is located on the X chromosome and a variable number of tandem repeat sequence upstream from *MAOA* (*MAOA* uVNTR) has been shown to influence the transcription levels of the enzyme [44]. Six alleles have previously been reported [46] with the major *MAOA* allele 3 having lower transcriptional activity (TA) than the other common alleles 3.5 and 4 (high TA alleles) [44, 47] It has therefore been hypothesized that individuals with *MAOA* high TA alleles degrade monoamine neurotransmitters more rapidly ultimately leading to lower PA levels [16]. Also the ACE gene may play a role in the dopaminergic pathways [48, 49], as the renin-angiotensin system, which the enzyme is a part of, and the dopaminergic system interacts [50]. Thus, *ACE* might be involved in the neurobiological regulation of exercise motivation [13, 16]. However, evidence for such relationships is still weak.

Identifying the role of genes and investigating their effect on PA behavior may contribute to further understanding of the large individual differences in PA behavior. Since many studies have been performed on either athletes, well-trained participants or patient groups, they generally have a low number of participants, and seldom represent the general population. In addition, there are large variations in allele frequencies between populations. In order to advance the knowledge, studies on larger and more homogenous cohorts are needed.

The functional *ACTN3* R577X polymorphism has been associated with a range of different exercise and performance related phenotypes, but few studies, if any, have investigated its relation to PA levels. On the other hand, both *ACE* I/D and *MAOA* uVNTR polymorphisms have been studied in relation to PA phenotypes, however, the results have been inconsistent. Therefore, the aim of the present study was to assess *ACTN3*, *ACE* and *MAOA* allele frequencies in a cohort of middle-aged Norwegians of mainly Scandinavian descent, and to investigate any associations between these genes and self-reported PA levels. In addition, the authors wanted to look for associations between socioeconomic variables, such as education and previous participation in sports or outdoor activities, and self-reported PA levels.

## Results

### Demographics

Blood samples and questionnaire data for 416 males and 415 females were available in this study. The mean age of the subjects was 55.5 ± 3.8 years. Participants were slightly overweight, as the mean BMI was 26.1 ± 3.8. However, the BMI in the HPA group was significantly lower than in the LMPA level group ($P = 0.001$; Table 1). The proportion of the cohort with higher education was 24.7%.

**Table 1** Anthropometric data according to self-reported PA level

| | Low/medium PA level | | | High PA level | | |
|---|---|---|---|---|---|---|
| Variable | All ($n = 215$) | Females ($n = 82$) | Males ($n = 133$) | All ($n = 616$) | Females ($n = 334$) | Males ($n = 282$) |
| Age (y) | 55.8 ± 3.8 | 56.0 ± 4.3 | 55.6 ± 3.5 | 55.5 ± 3.7 | 55.3 ± 3.7 | 55.6 ± 3.8 |
| Weight (kg) | 83.3 ± 15.6 | 76.6 ± 15.8 | 87.4 ± 14.0 | 76.9 ± 12.9 | 71.0 ± 11.4 | 83.8 ± 11.1 |
| Height (cm) | 174.7 ± 8.1 | 167.7 ± 5.0 | 179.1 ± 6.4 | 172.5 ± 8.6 | 166.7 ± 5.7 | 179.4 ± 6.0 |
| BMI (kg/m$^2$) | 27.3 ± 4.5 | 27.3 ± 5.5 | 27.3 ± 3.7 | 25.7 ± 3.5* | 25.5 ± 3.8* | 26.0 ± 3.0* |

Data are presented as mean ± SD. n- number of subjects; PA- physical activity; BMI- Body Mass Index. *$P \leq 0.009$ different from low/medium PA level

### Physical activity data

Of the 831 participants, 25.9 and 74.1% reported LMPA and HPA, respectively. Females reported a significantly higher PA level compared to males ($P < 0.01$). Regular participation in sports or outdoor activities at a younger age was reported to be 51.7%, and participation was higher among males (59.4%) than females (44.0%; $P < 0.01$). Prior participation in sports and outdoor activities was positively associated with reported PA level later in life ($P < 0.01$). Similarly, higher education was positively associated with higher PA levels ($P < 0.01$).

### Genotype and allele frequency distribution for ACE, ACTN3 and MAOA

Out of the 831 participants, 822 were successfully genotyped for the *ACTN3*, 721 for the *MAOA* and 616 for the *ACE* gene. For the *ACE* gene, 24.5% ($n = 151$) were homozygous for the D allele, 52.6% ($n = 324$) were heterozygous, and 22.9% ($n = 141$) were homozygous for the I allele (Table 2). Allele frequencies for the *ACE* gene were 50.8 and 49.2% for the D and I allele, respectively. Genotype distribution for the *ACTN3* gene was 30.8% ($n = 253$), 50.5% ($n = 415$), 18.7% ($n = 154$) for the RR, RX and XX genotype, respectively (Table 2). The frequency for the R allele was 56.0, and 44.0% for the X allele. The observed genotype frequencies were in HWE for all genotypes. Genotype frequencies did not differ significantly between male and female subjects for either *ACTN3* R577X or *ACE* I/D polymorphisms. For the *MAOA* uVNTR polymorphism, five alleles were found: 2, 3, 3.5, 4 and 5 repeat alleles. Frequencies of the rare alleles were as follows: allele 2 was found in one heterozygous female; allele 5 in one hemizygous male and four heterozygous females; allele 3.5 in two hemizygous males and seven heterozygous females (1%). Common allele frequencies were 38.6 and 61.4% for the alleles 3 and 4, respectively. The observed heterozygosity for the common alleles was 42.5%.

The *ACTN3* X allele frequency was 10.0% lower in individuals with HPA than those with LMPA level

**Table 2** Genotype distribution for the *ACTN3*, *ACE* and *MAOA* gene according to PA level

| | Low/medium PA level | | | High PA level | | |
|---|---|---|---|---|---|---|
| Genotype | All ($n = 215$) | Females ($n = 82$) | Males ($n = 133$) | All ($n = 616$) | Females ($n = 334$) | Males ($n = 282$) |
| *ACTN3* | | | | | | |
| Total (n) | 214 | 82 | 132 | 608 | 330 | 278 |
| RR | 23.4%* | 28.0% | 20.5% | 33.4%* | 34.2% | 32.4% |
| RX | 58.9%* | 57.3% | 59.8% | 47.5%* | 47.0% | 48.2% |
| XX | 17.8% | 14.6% | 19.7% | 19.1% | 18.8% | 19.4% |
| *ACE* | | | | | | |
| Total (n) | 161 | 65 | 96 | 455 | 250 | 205 |
| DD | 25.5% | 29.2% | 22.9% | 24.2% | 26.4% | 21.5% |
| ID | 52.8% | 46.2% | 57.3% | 52.5% | 52.8% | 52.2% |
| II | 21.7% | 24.6% | 19.8% | 23.3% | 20.8% | 26.3% |
| *MAOA* | | | | | | |
| Total (n) | 187 | 70 | 117 | 534 | 290 | 244 |
| Low TA[1] | 29.4% | 14.3% | 38.5% | 27.7% | 15.2% | 42.6% |
| High TA[2] | 55.1% | 44.3% | 61.5% | 49.1% | 42.1% | 57.4% |
| Heterozygotes[3] | 15.5% | 41.4% | – | 23.2% | 42.8% | – |

Data are presented as frequency and percentages. n- number of subjects, TA- transcriptional activity genotype. [1]3-repeat allele male carriers and female homozygotes; [2]3.5- or 4-repeat allele male carriers and female homozygotes or heterozygotes; [3]females only; *$P < 0.01$ difference in *ACTN3* genotype distribution between low/medium and high PA level

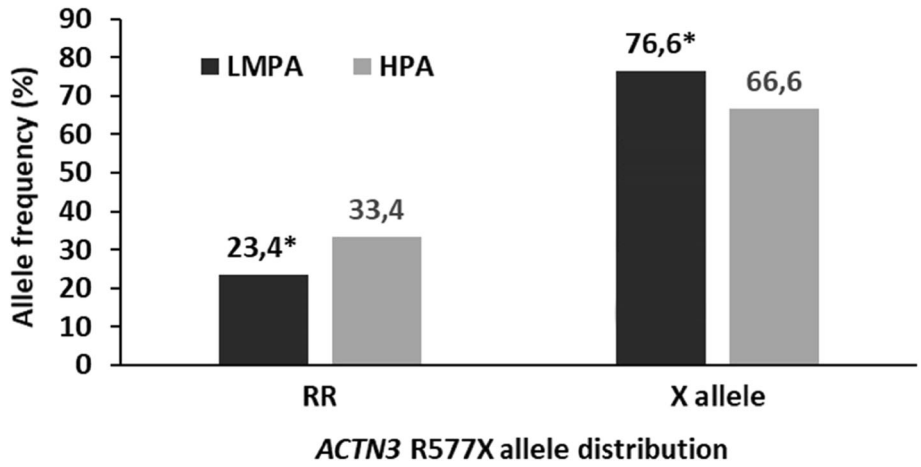


**Fig. 1** *ACTN3* R577X allele distribution differences between the low/medium and high physical activity groups. *significantly different from the high PA level group ($P = 0.006$). LMPA-low/medium physical activity group; HPA- high physical activity group

($P = 0.006$, Fig. 1). When stratified by sex, significant difference in the X allele was only seen in males ($P = 0.013$).

No associations were found between the ACE I/D (Fig. 2) or MAOA uVNTR (Fig. 3) polymorphisms and the PA level (Table 2).

### Logistic regression models

Gender was found to significantly influence the likelihood of belonging to one of the two PA level groups, i.e. either to LMPA or to HPA level group ($P < 0.001$; Table 3). Males were less likely (OR: 0.47) to belong into the HPA level group compared to females. The BMI was more likely to be lower among subjects in the HPA level group compared to the LMPA level group counterparts ($p < 0.01$; OR = 0.92). Education level showed a statistically significant association ($P < 0.01$) with the PA level. Participants having completed higher education were 2.2 times more likely to belong to the HPA group than the participants with secondary (or lower) education level. Also, subjects who participated in sports/outdoor activities earlier in life were 1.8 times more likely to belong to the HPA level group compared to those that had not ($P < 0.01$).

In the second logistic regression model, the genotype data were added to the socioeconomic factors tested previously. Those socioeconomic variables that contributed significantly to the PA level, remained significantly associated in the second model (Table 4). In addition, the *ACTN3* R577X polymorphism was significantly associated with the PA levels ($P < 0.01$). Subjects with the RX genotype were more likely to belong to the LMPA level group ($P = 0.001$; OR = 0.43) compared to the RR

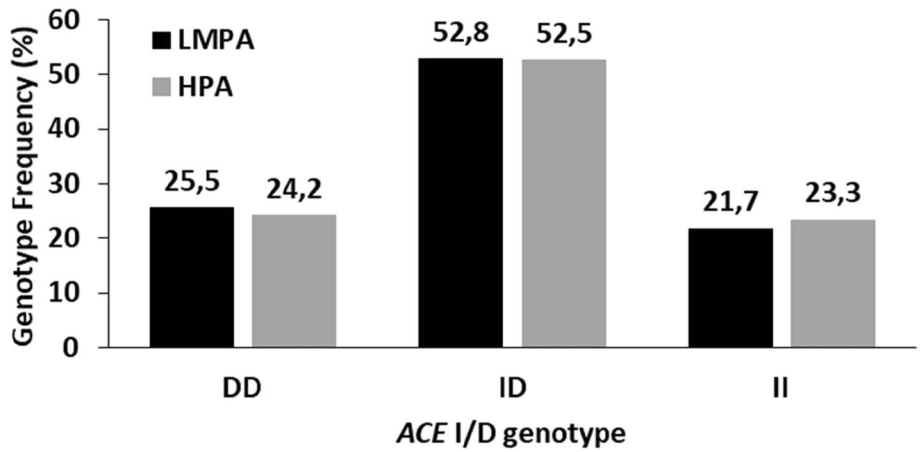


**Fig. 2** *ACE* I/D genotype distribution in the low/medium and high physical activity groups. LMPA-low/medium physical activity group; HPA- high physical activity group

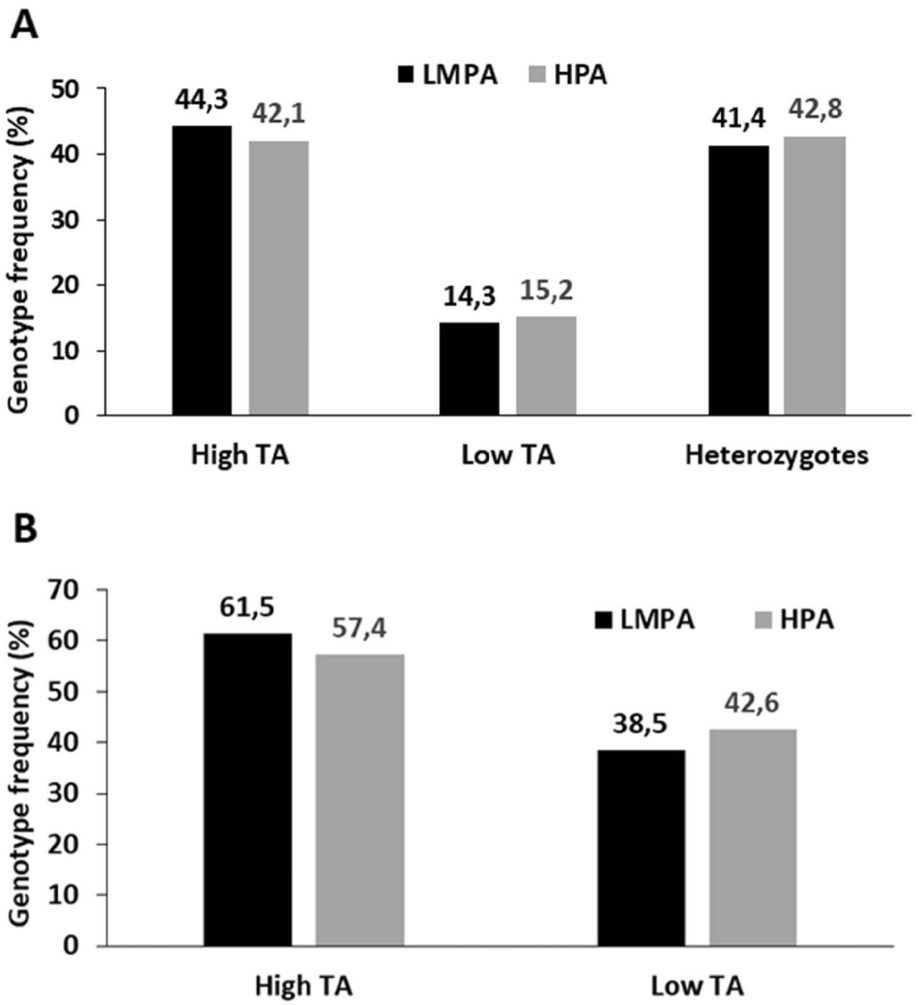


**Fig. 3** *MAOA* uVNTR genotype distribution in the low/medium and high physical activity groups among female (**a**) and male (**b**) participants. **a** TA- transcriptional activity genotype. Low TA represents 3-repeat allele homozygotes; High TA represents 3.5- or 4-repeat allele homozygotes and heterozygotes carrying one of each high TA alleles; heterozygotes- carriers of one low TA and one high TA alleles. **b** TA- transcriptional activity genotype. Low TA represents 3-repeat allele hemizygotes; High TA represents 3.5- or 4-repeat allele hemizygotes

**Table 3** Variables entered on step 1: gender, age, BMI, education (2 categories) and participation in sports/outdoor activities earlier in life (2 categories)

| Variables included in the equation | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Gender (Male/Female[r.c.]) | −0.753 | 0.205 | 13.513 | 1 | **0.000** | 0.471 | 0.315 | 0.704 |
| Age | −0.035 | 0.027 | 1.731 | 1 | 0.188 | 0.965 | 0.915 | 1.018 |
| BMI | −0.067 | 0.026 | 6.791 | 1 | **0.009** | 0.935 | 0.889 | 0.983 |
| Education (Higher/Secondary[$r.c.]) | 0.805 | 0.263 | 9.358 | 1 | **0.002** | 2.236 | 1.335 | 3.745 |
| Sports earlier in life (Yes/No[r.c.]) | 0.599 | 0.203 | 8.702 | 1 | **0.003** | 1.821 | 1.223 | 2.711 |
| Constant | 4.080 | 1.723 | 5.606 | 1 | 0.018 | 59.173 | | |

Significant values ($p < 0.05$) are indicated in bold; [r.c.]- reference category; [$] - Secondary education or lower

**Table 4** Variables entered on step 2: gender, age, BMI, education (2 categories), participation in sports/outdoor activities earlier in life (2 categories), *ACE* I/D polymorphism (3 categories), *ACTN3* R577X (3 categories) and *MAOA* uVNTR (3 categories)

| Variables included in the equation | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Gender (Male/Female[r.c.]) | −0.769 | 0.253 | 9.218 | 1 | **0.002** | 0.464 | 0.315 | 0.704 |
| Age | −0.029 | 0.027 | 1.134 | 1 | 0.287 | 0.971 | 0.915 | 1.018 |
| BMI | −0.071 | 0.026 | 7.252 | 1 | **0.007** | 0.932 | 0.889 | 0.983 |
| Education (Higher/Secondary[$r.c.]) | 0.843 | 0.267 | 9.944 | 1 | **0.002** | 2.324 | 1.335 | 3.745 |
| Sports earlier in life (Yes/No[r.c.]) | 0.643 | 0.207 | 9.635 | 1 | **0.002** | 1.902 | 1.223 | 2.711 |
| *ACE* (all genotypes) | | | 0.611 | 2 | 0.737 | | | |
| *ACE* (ID/DD[r.c.]) | 0.183 | 0.248 | 0.545 | 1 | 0.461 | 1.201 | 0.738 | 1.953 |
| *ACE* (II/DD[r.c.]) | 0.188 | 0.297 | 0.402 | 1 | 0.526 | 1.207 | 0.675 | 2.16 |
| *ACTN3* (all genotypes) | | | 11.473 | 2 | **0.003** | | | |
| *ACTN3* (XX/RR[r.c.]) | −0.491 | 0.322 | 2.325 | 1 | 0.127 | 0.612 | 0.326 | 1.15 |
| *ACTN3* (RX/RR[r.c.]) | −0.843 | 0.251 | 11.268 | 1 | **0.001** | 0.430 | 0.263 | 0.704 |
| *MAOA* (all genotypes) | | | 0.777 | 2 | 0.678 | | | |
| *MAOA* (Low TA/High TA[r.c.]) | 0.211 | 0.242 | 0.758 | 1 | 0.384 | 1.235 | 0.768 | 1.987 |
| *MAOA* (Heterozygotes/High TA[r.c.]) | 0.089 | 0.317 | 0.079 | 1 | 0.778 | 1.093 | 0.588 | 2.035 |
| Constant | 4.816 | 1.719 | 7.852 | 1 | 0.005 | 123.475 | | |

Significant values ($p < 0.05$) are indicated in bold; [r.c.]- reference category; [$] - Secondary education or lower

genotype subjects. Neither *ACE* I/D nor *MAOA* uVNTR polymorphism showed a significant association with the PA levels.

## Discussion

Allele frequencies for the *ACTN3* R577X and the *ACE* I/D polymorphisms has been reported to be highly variable between different ethnic groups, and the X allele has previously been reported to be much more common in Japanese (55%) [51] than it is in Kenyans (9%) [52]. Prevalence of the *ACTN3* X allele in the present study was similar to populations of European descent, with around 45% of individuals being carriers of the minor allele [23].

The frequency distributions for the *ACE* alleles in the present study are also in line with other populations of European descent [30] i.e. 25–50% - 25% for the II, ID and DD alleles respectively. For the ACE I/D polymorphism, distribution of the D allele ranges from 10% for the D allele in Samoans [53], to around 60% in African Americans [54]. Genotype frequencies in other studies on Norwegian subjects were comparable with the frequencies in the present study [55, 56]. However, in one of the studies, the DD genotype was reported to be more prevalent than in other European populations. This is likely due to the preferential amplification of the D allele in heterozygotes, leading to mistyping of some heterozygotes as homozygotes for D allele [57]. That particular study did not used the insertion-specific primers to avoid mistyping of the ID genotype. The large variations in allele frequencies among different ethnicities is important to take into account when doing candidate gene studies [58]. Thus, analyzing homogenous cohorts [54], or accounting for the stratification [58] may improve study power. For the *MAOA* uVNTR polymorphism the allele frequencies were similar to those previously observed in Europeans [44, 47].

The present study found differences in the *ACTN3* R577X allele distribution between the LMPA and the HPA level group. The logistic regression model indicated that the RX allele carriers were more likely to belong to the LMPA level group compared to the RR counterparts. Furthermore, individuals reporting HPA demonstrated higher frequency of the R allele compared to those reporting LMPA. Interestingly, when analyzed by gender, only males demonstrated significant differences in allele distributions between the two PA level groups. Although the authors have not found other studies reporting a relationship between the *ACTN3* gene and PA levels in the general public, it has been suggested to be a potential candidate gene for PA behavior in mice models [13]. The *ACTN3* R577X polymorphism has been linked to trainability of various cardiovascular traits [7, 19] which could, in turn, influence PA behavior [4]. Furthermore, the polymorphism has been associated with traits like sarcopenia [59], muscle function [51] and strength [60]. Previous research indicates that these may be important correlates of PA phenotypes [61–64]. Animal studies have shown changes in signaling and metabolism, among other things [27], which might help

to explain the differences in phenotypes between the different genotypes.

Only few studies have been performed on *ACE* I/D polymorphism and PA levels in adults. Similarly to Fuentes et al. [40], the present study could not find any relationship between the *ACE* I/D polymorphism and PA levels, although some previous studies have found an association [38, 39].

The *MAOA* uVNTR polymorphism has a potential to be a candidate gene for influencing PA due to the phenotypic differences in transcriptional activity. Higher TA is expected to lead to higher monoamine oxidase activity and thus lower levels of monoamine neurotransmitters [44]. This, in turn, may lead to different PA level phenotypes [16]. However, the present study could not confirm the findings of Good et al. [16] who observed higher levels of PA in girls homozygous for the low TA allele compared to the high TA allele counterparts. It is still unclear whether the high and low TA alleles influence the monoamine oxidase A enzyme activity in the brain, as Fowler et.al [65]. was not able to measure significant enzyme activity differences in brains of healthy male participants.

Results from the present study showed a strong association between the present PA level and PA at younger ages ($P < 0.01$). Education also correlated with PA levels in the present study ($P < 0.01$). Both education level [66, 67], and PA activity level at younger ages [66, 68], have in previous studies been shown to correlate positively with present PA levels.

A large proportion of the present cohort reported high PA level (74%). This could be, in part, due to the use of questionnaires as a method for determining the PA levels in the present study. Questionnaire-based methods have been shown to over or underestimate PA behavior compared to the objectively measured PA. The subjective nature of questionnaires may explain the large variation in PA levels observed between different studies [69]. Nevertheless, due to cost efficiency, questionnaires are often used in large epidemiologic studies, including genetic studies [1]. Another questionnaire-based study on a large European cohort of older subjects reported relatively low proportion of participants with no vigorous/moderate physical activity. The overall prevalence of inactivity in the cohort was reported to be 12.5%, with the Scandinavian countries demonstrating some of the lowest rates, i.e. 4.9 and 7.5% in Sweden and Denmark, respectively [64].

The present cohort was randomly drawn from NORCCAP, a homogenous Scandinavian population study with a high attendance rate [70]. The data from the questionnaires allowed the authors to map the ethnicity of the participants. Out of the 831 participants, only two did not have grandparents of Scandinavian descent or lacked the information about ethnicity. The remaining 829 (99, 8%) had grandparents of Scandinavian descent. According to Marchini, Cardon [71], a well-known problem with genetic association studies is the undetected population structure such as heterogeneous ethnicity. In the present study, the material may be regarded as ethnically homogenous based on the results from the questionnaires. This can be regarded as one of the strengths of the present study, as a homogenous cohort reduces the chances of both false positive results and failures to detect genuine associations [71]. A further stratification based on ethnicity was therefore not necessary or possible.

Although study population was overweight, the differences in the BMI between the LMPA and HPA groups may also indicate that the self-reported PA levels are reliable [72]. Increase in adiposity (BMI) has been reported to be the cause of decrease in PA levels, as opposed to being the consequence of inactivity [43, 73, 74]. The design of the present study would have been strengthened by including direct measurements of the PA levels to validate the questionnaire data [69].

## Conclusions

The present study demonstrates a novel finding that the X allele of the *ACTN3* gene is underrepresented among participants reporting high PA levels. Genotype data from the present study can be used as a control population in future intervention studies on subjects of European descent. Consistently with previous reports, PA levels in adulthood are associated with factors like education and participation in exercise or outdoor activities earlier in life.

## Methods

### Participants

Blood samples and self-reported PA data were available for 831 individuals from the cohort "Kolorektal cancer, Arv og Miljø (KAM)", a molecular epidemiological study partly based on the screening group of the Norwegian Colorectal Cancer Prevention Study (The NORCCAP study) in the county of Telemark, Norway [70, 75]. The study design, inclusion/exclusion criteria, participation rates and other relevant information about the NORCCAP study is described in Bretthauer, Gondal [70]. The study was approved by the Regional Medical Ethics Committee of South-Eastern Norway and the Data Inspectorate (REK 3087, S-98052 and S-98190), and is registered in Clinical Trials [76] with the identifier NCT00119912. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical

standards. Informed written consent was obtained from all individual participants included in the study. Only control subjects (polyp free or polyps with mild grade dysplasia) were included in the present study. Socioeconomic data, including education data, were available for all the subjects. Characteristics of the participants are presented in Table 1.

### Assessment of PA level

PA data was obtained by questionnaire used in the KAM study [75]. The following questions had graded responses on weekly frequency of the activity: "In the last five years, have you walked or bicycled to or from work?", "Do you hike (cross country)?", "How often do you exercise for at least 20 minutes?". Questions "If you exercise, do you perspire?" and "Were you regularly participating in sports or outdoor activities at a younger age?" were dichotomous.

Since the demand for energy differs between various types of activities [77, 78], the different activities were scaled as for example: Hiking = 1 (used as reference, and representing moderate to vigorous intensity), Walking/bicycling = 0.5 (representing low to moderate intensity), Exercise = 1.5 (representing vigorous intensity). By summing the frequencies per week of the scaled activities, each person achieved an activity score. The American College of Sports Medicine (ACSM) has recommended moderate-intensity cardiorespiratory exercise training for at least 30 min, at least five days per week, or at least 20 min of vigorous-intensity cardiorespiratory exercise training for at least three days per week, or a combination of the two training modalities [79]. The activity score of 3 in the present study thus represents the minimum for accomplishing the ACSM physical activity recommendations. For example, a person performing exercise training of 45 min two times per week, or hiking three times per week, or performing walking or cycling six times per week, will reach the activity score 3. Therefore, participants who achieved a score under 3 were defined as inactive or untrained (possessing LMPA) and those who achieved a score ≥ 3 was defined as active/trained (possessing HPA). These PA level groups were assessed for any associations with the ACE I/D, ACTN3 R577X and MAOA uVNTR genotypes, as well as for relationships with gender, education level and previous participation in sports or outdoor activities.

### DNA collection and genotyping

The genomic DNA was extracted from venous EDTA blood stored at -20 °C by using a salting out procedure [80] with minor modifications [75].

The *ACE* I/D polymorphism was genotyped using the Eppendorf Mastercycler Gradient (Eppendorf AG, Germany). Each reaction mixture of 25.5 μl contained 2% DMSO, 1 x PCR buffer, 0.2 mM dNTP, 2 mM MgCl, 0.2 pmol/μl of each primer, 0.5 U/μl Taq polymerase, and 1 μl of DNA (~ 100 ng). Forward and reverse primer were 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and 5′-GATGTGGCCAT-CACATTCGTCAGAT-3′, respectively [34]. Initial denaturation at 95 °C for 3 min was followed by 30 cycles of denaturation (95 °C; 15 s), hybridization (53 °C; 45 s) and extension (72 °C; 30 s). After final elongation (72 °C; 5 min) the PCR products were stored at 4 °C. These were separated by 6% polyacrylamide gel electrophoresis (PAGE) for 30 min at 150 V, and resulted in three possible outcomes (DD, ID and II).

The ACE I allele is often weakly amplified in heterozygotes. Samples with the DD genotype were therefore reanalyzed by using a different PCR reaction in order to avoid mistyping of heterozygotes as DD. Insertion specific forward primer 5′-TTTGAGACGGAGTCTCGCTC-3′ and standard reverse primer [57] were used. Each reaction of 25.0 μl contained 12.5 μl AmpliTaq Gold® PCR Master Mix (Thermo Fisher Scientific, Inc.; MA, USA), 5% DMSO, 0.2 pmol/μl of each primer, and ~ 100 ng template DNA. PCR reaction conditions were as follows: initial denaturation at 95 °C for 3 min followed by 30 cycles of denaturation (92 °C), hybridization (61 °C) and extension (72 °C) for 1 min each. After final elongation (72 °C; 7 min) the PCR products were visualized by 6% PAGE. Insertion specific PCR reaction yielded a 408 bp long DNA fragment in carriers of the I-allele and no PCR product in DD subjects. 215 (25.8%) samples yielded no genotype results ever after repeated PCR runs.

Genotyping of the *ACTN3* R577X polymorphism was carried out with TaqMan® SNP Genotyping Assay, assay ID C____590093_1 (Applied Biosystems®, CA, USA) on the StepOnePlus™ Real-Time PCR System (Applied Biosystems®, CA, USA). Genotype calling was performed by StepOne Software v2.0. Each 15 μl reaction genotyping mixture contained 8.44 μl Genotyping Master Mix, 0.42 μl Assay mix (40x), 6.33 μl distilled $H_20$ and ~ 150 ng of DNA template. Reaction conditions were as follows: 30 s at 60 °C was followed by initial denaturation stage for 10 min at 95 °C; denaturation at 95 °C for 15 s followed by annealing at 60 °C for 1 min in cycling stage with 40 cycles altogether; finally post read temperature was kept at 60 °C for 30 s. Nine samples (1.1%) yielded no genotype results.

The *MAOA* promoter polymorphism was amplified by PCR followed by capillary electrophoresis on an Applied Biosystems 3130xl genetic analyzer using GeneMapper® (Applied Biosystems®, CA, USA) Software 5. Each 15 μl reaction contained 5% DMSO, 1x PCR buffer, 0.2 mM dNTP, 2.5 mM MgCl, 0.4 mM of each primer, 1 U/μl Taq polymerase. The PCR conditions were as follows: initial denaturation at 95 °C (2 min) followed by 35 cycles

of denaturation at 95 °C (1 min), annealing at 55.5 °C (1 min) and elongation at 72 °C (2 min), and a final elongation at 72 °C (5 min). Primer sequences have been described earlier [44], and were as follows: a FAM labeled forward primer 5′-ACAGCCTGACCGTGGAGAAG-3′ and a reverse primer 5′-GAACGGACGCTCCA TTCGGA-3′. 110 (13.2%) samples yielded no results even after repeated PCR run.

In order to check for reproducibility for *ACE* I/D and *MAOA* uVNTR polymorphisms, approximately 10% of the samples were re-analyzed. In addition, those samples that yielded inconclusive results were re-run. They were excluded from further data analysis if the samples did not yield any genotype or if they remained inconclusive. For *ACTN3* R577X, all samples were run as duplicates.

## Statistical analysis

The material was tested for normality and corrected for multiple testing (Bonferroni method), where appropriate. Association between the BMI and PA level groups was analyzed by using two-tailed independent sample t-test. Pearson's Chi-square test ($\chi^2$) was applied to test for the Hardy-Weinberg equilibrium (HWE) for the *ACTN3* R577X, *ACE* I/D genotype, and the differences in categorical variables, including genotype and allelic frequencies between the PA level groups. *MAOA* genotypes were analyzed by dividing genotypes into groups, based on the TA of the alleles [44, 47]. Males carrying the 3-repeat allele and homozygous females for the 3-repeat allele were grouped into low TA, while males carrying either 3.5- or 4-repeat alleles were grouped into high TA group. Similarly, females homozygous for either 3.5 or 4-repeat-alleles and females heterozygous for 3.5 or 4-repeat-alleles were grouped into high TA group. Heterozygous females carrying one 3-repeat and either 3.5- or 4-repeat allele were placed into the heterozygous group. Individuals carrying the rare alleles were excluded from the analysis.

To test the contribution of socioeconomic factors (Gender, Age, BMI, Education, Participation in sports/outdoor activities earlier in life) and genetic variables (*ACTN3* R577X, *ACE* I/D and *MAOA* uVNTR genotypes) to the PA level, binomial logistic regression was used. For this purpose, two models were analyzed: 1. socioeconomic factors only; 2. socioeconomic and genotype data together. Odds ratio (OR) were calculated for the significant associations in the logistic regression. Significance was set at 0.05 for all tests. Results are presented as mean ± SD. All statistical analysis was performed in IBM SPSS Statistics, version 25 (Chicago, IL, USA).

### Abbreviations

*ACE*: Angiotensin-converting enzyme; *ACTN3*: α-actinin-3; BMI: Body mass index; HPA: High physical activity; HWE: Hardy-Weinberg equilibrium; KAM: «Kolorektal cancer, Arv og Miljø» study; LMPA: Low/medium physical activity; *MAOA*: Monoamine oxidase A; PA: Physical activity; PAGE: Polyacrylamide gel electrophoresis; PCR: Polymerase chain reaction; TA: Transcriptional activity

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### Authors' contributions

SGF carried out the genetic studies, interpreted the study results and drafted the manuscript, AMB converted the questionnaire responses to PA level scores, interpreted the study results and also drafted the manuscript, EHK, IKL, MS and ØS participated in designing the study, interpreting the results, writing and supervising the writing of the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

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### Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to Norwegian legislation but are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

The Regional Medical Ethics Committee of South-Eastern Norway and the Data Inspectorate (REK 3087, S-98052 and S-98190) approved the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. Informed written consent was obtained from all individual participants included in the study.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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### References

1. Bray MS, Fulton JE, Kalupahana NS, Lightfoot JT. Genetic Epidemiology, Physical Activity, and Inactivity. Genet Mol Aspects Sport Performance. 2011:81–9.
2. De Moor MH, Liu YJ, Boomsma DI, Li J, Hamilton JJ, Hottenga JJ, et al. Genome-wide association study of exercise behavior in Dutch and American adults. Med Sci Sports Exerc. 2009;41(10):1887–95.
3. Lightfoot JT. Current understanding of the genetic basis for physical activity. J Nutr. 2011;141(3):526–30.
4. Stubbe JH, Boomsma DI, Vink JM, Cornes BK, Martin NG, Skytthe A, et al. Genetic Influences on Exercise Participation in 37.051 Twin Pairs from Seven Countries. PloS one. 2006;1(1):e22.
5. Bird SR, Hawley JA. Exercise and type 2 diabetes: new prescription for an old problem. Maturitas. 2012;72(4):311–6.
6. Ehsani AA, Martin WH 3rd, Heath GW, Coyle EF. Cardiac effects of prolonged and intense exercise training in patients with coronary artery disease. Am J Cardiol. 1982;50(2):246–54.

7. Myerson SG, Montgomery HE, Whittingham M, Jubb M, World MJ, Humphries SE, et al. Left ventricular hypertrophy with exercise and ACE gene insertion/deletion polymorphism: a randomized controlled trial with losartan. Circulation. 2001;103(2):226–30.
8. Levine JA. Lethal sitting: homo sedentarius seeks answers. Physiology (Bethesda, Md). 2014;29(5):300–1.
9. McGuire DK, Levine BD, Williamson JW, Snell PG, Blomqvist CG, Saltin B, et al. A 30-year follow-up of the Dallas Bedrest and training study: I. effect of age on the cardiovascular response to exercise. Circulation. 2001;104(12): 1350–7.
10. World Health Organization. Global recommendations on physical activity for health. Geneva: World Health Organization; 2010.
11. Hallal PC, Andersen LB, Bull FC, Guthold R, Haskell W, Ekelund U, et al. Global physical activity levels: surveillance progress, pitfalls, and prospects. Lancet. 2012;380(9838):247–57.
12. Hagberg JM, Park JJ, Brown MD. The role of exercise training in the treatment of hypertension - an update. Sports Med. 2000;30(3):193–206.
13. Lightfoot JT. Can you be born a couch potato? The genomic regulation of physical activity. Exercise Genomics: Springer; 2011. p. 45–72.
14. Nicklas BJ. Heterogeneity of physical function responses to exercise in older adults: possible contribution of variation in the Angiotensin-1 converting enzyme (ACE) gene? Perspect Psychol Sci. 2010;5(5):575–84.
15. Sarzynski MA, Rankinen T, Bouchard C. Twin and Family Studies of Training Responses. 2011. In: genetic and molecular aspects of sport performance [internet]. Wiley-Blackwell.
16. Good DJ, Li M, Deater-Deckard K. A genetic basis for motivated exercise. Exerc Sport Sci Rev. 2015;43(4):231–7.
17. Simonen RL, Rankinen T, Perusse L, Leon AS, Skinner JS, Wilmore JH, et al. A dopamine D2 receptor gene polymorphism and physical activity in two family studies. Physiol Behav. 2003;78(4–5):751–7.
18. Stubbe JH, Boomsma DI, De Geus EJ. Sports participation during adolescence: a shift from environmental to genetic factors. Med Sci Sports Exerc. 2005;37(4):563–70.
19. Deschamps CL, Connors KE, Klein MS, Johnsen VL, Shearer J, Vogel HJ, et al. The ACTN3 R577X Polymorphism Is Associated with Cardiometabolic Fitness in Healthy Young Adults. PloS one. 2015;10(6):e0130644.
20. Pereira A, Costa AM, Leitao JC, Monteiro AM, Izquierdo M, Silva AJ, et al. The influence of ACE ID and ACTN3 R577X polymorphisms on lower-extremity function in older women in response to high-speed power training. BMC Geriatr. 2013;13:131.
21. Pereira A, Costa AM, Izquierdo M, Silva AJ, Bastos E, Marques MC. ACE I/D and ACTN3 R/X polymorphisms as potential factors in modulating exercise-related phenotypes in older women in response to a muscle power training stimuli. Age. 2013;35(5):1949–59.
22. Mills M, Yang N, Weinberger R, Vander Woude DL, Beggs AH, Easteal S, et al. Differential expression of the actin-binding proteins, alpha-actinin-2 and -3, in different species: implications for the evolution of functional redundancy. Hum Mol Genet. 2001;10(13):1335–46.
23. Yang N, Garton F, North K. alpha-actinin-3 and performance. Med Sport Sci. 2009;54:88–101.
24. Ahmetov II, Egorova ES, Gabdrakhmanova LJ, Fedotovskaya ON. Genes and athletic performance: an update. Med Sport Sci. 2016;61:41–54.
25. Pickering C, Kiely J. ACTN3: more than just a gene for speed. Front Physiol. 2017;8:1080.
26. Friedlander SM, Herrmann AL, Lowry DP, Mepham ER, Lek M, North KN, et al. ACTN3 allele frequency in humans covaries with global latitudinal gradient. PLoS One. 2013;8(1):e52282.
27. Lee FXZ, Houweling PJ, North KN, Quinlan KGR. How does α-actinin-3 deficiency alter muscle function? Mechanistic insights into ACTN3, the 'gene for speed'. Biochim Biophys Acta. 2016;1863(4):686–93.
28. Head SI, Chan S, Houweling PJ, Quinlan KGR, Murphy R, Wagner S, et al. Altered Ca2+ Kinetics Associated with alpha-Actinin-3 Deficiency May Explain Positive Selection for ACTN3 Null Allele in Human Evolution. PLoS genetics. 2015;11(1).
29. Coates D. The angiotensin converting enzyme (ACE). Int J Biochem Cell Biol. 2003;35(6):769–73.
30. Jones A, Woods DR. Skeletal muscle RAS and exercise performance. Int J Biochem Cell Biol. 2003;35(6):855–66.
31. Goh KP, Chew K, Koh A, Guan M, Wong YS, Sum CF. The relationship between ACE gene ID polymorphism and aerobic capacity in Asian rugby players. Singap Med J. 2009;50(10):997–1003.
32. Tamburus NY, Verlengia R, Kunz VC, Cesar MC, Silva E. Apolipoprotein B and angiotensin-converting enzyme polymorphisms and aerobic interval training: randomized controlled trial in coronary artery disease patients. Braz J Med Biol Res. 2018;51(8):e6944.
33. Novak CM, Escande C, Gerber SM, Chini EN, Zhang M, Britton SL, et al. Endurance capacity, not body size, determines physical activity levels: role of skeletal muscle PEPCK. PLoS One. 2009;4(6):e5869.
34. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res. 1992;20(6):1433.
35. Myerson SG, Hemingway H, Budget R, Martin J, Humphries S, Montgomery H. Human angiotensin I-converting enzyme gene and endurance performance. J Appl Physiol. 1999;87(4):1313–6.
36. Hruskovicova H, Dzurenkova D, Selingerova M, Bohus B, Timkanicova B, Kovacs L. The angiotensin converting enzyme I/D polymorphism in long distance runners. J Sports Med Physical Fitness. 2006;46(3):509–13.
37. Folland J, Leach B, Little T, Hawker K, Myerson S, Montgomery H, et al. Angiotensin-converting enzyme genotype affects the response of human skeletal muscle to functional overload. Exp Physiol. 2000;85(5):575–9.
38. Maestu J, Latt E, Raask T, Sak K, Laas K, Jurimae J, et al. Ace I/D polymorphism is associated with habitual physical activity in pubertal boys. J Physiol Sci. 2013;63(6):427–34.
39. Winnicki M, Accurso V, Hoffmann M, Pawlowski R, Dorigatti F, Santonastaso M, et al. Physical activity and angiotensin-converting enzyme gene polymorphism in mild hypertensives. Am J Med Genet A. 2004;125A(1):38–44.
40. Fuentes RM, Perola M, Nissinen A, Tuomilehto J. ACE gene and physical activity, blood pressure, and hypertension: a population study in Finland2002 2002-06-01 00:00:00. 2508–12 p.
41. Knab AM, Bowen RS, Hamilton AT, Gulledge AA, Lightfoot JT. Altered dopaminergic profiles: implications for the regulation of voluntary physical activity. Behav Brain Res. 2009;204(1):147–52.
42. Park YM, Kanaley JA, Padilla J, Zidon T, Welly RJ, Will MJ, et al. Effects of intrinsic aerobic capacity and ovariectomy on voluntary wheel running and nucleus accumbens dopamine receptor gene expression. Physiol Behav. 2016;164(Pt A):383–9.
43. Friend DM, Devarakonda K, O'Neal TJ, Skirzewski M, Papazoglou I, Kaplan AR, et al. Basal ganglia dysfunction contributes to physical inactivity in obesity. Cell Metab. 2017;25(2):312–21.
44. Sabol SZ, Hu S, Hamer D. A functional polymorphism in the monoamine oxidase a gene promoter. Hum Genet. 1998;103(3):273–9.
45. Shih JC, Thompson RF. Monoamine oxidase in neuropsychiatry and behavior. Am J Hum Genet. 1999;65(3):593–8.
46. Huang YY, Cate SP, Battistuzzi C, Oquendo MA, Brent D, Mann JJ. An association between a functional polymorphism in the monoamine oxidase a gene promoter, impulsive traits and early abuse experiences. Neuropsychopharmacology. 2004;29(8):1498–505.
47. Deckert J, Catalano M, Syagailo YV, Bosi M, Okladnova O, Di Bella D, et al. Excess of high activity monoamine oxidase a gene promoter alleles in female patients with panic disorder. Hum Mol Genet. 1999;8(4):621–4.
48. Jenkins TA, Mendelsohn FAO, Chai SY. Angiotensin-converting enzyme modulates dopamine turnover in the striatum. J Neurochem. 1997;68(3):1304–11.
49. Nyberg F, Terenius L. Degradation of Neuropeptides In: Henriksen JH, editor. Degradation of Bioactive Substances: Physiology and Pathophysiology Boca Raton, Florida: CRC Press, Inc; 1991. p. 189–200.
50. Labandeira-Garcia JL, Rodriguez-Pallares J, Rodriguez-Perez AI, Garrido-Gil P, Villar-Cheda B, Valenzuela R, et al. Brain angiotensin and dopaminergic degeneration: relevance to Parkinson's disease. Am J Neurodegenerative Dis. 2012;1(3):226–44.
51. Kikuchi N, Yoshida S, Min SK, Lee K, Sakamaki-Sunaga M, Okamoto T, et al. The ACTN3 R577X genotype is associated with muscle function in a Japanese population. Appl Physiol Nutr Metabol. 2015;40(4):316–22.
52. Yang N, MacArthur DG, Wolde B, Onywera VO, Boit MK, Lau SY, et al. The ACTN3 R577X polymorphism in east and west African athletes. Med Sci Sports Exerc. 2007;39(11):1985–8.
53. Barley J, Blackwood A, Carter ND, Crews DE, Cruickshank JK, Jeffery S, et al. Angiotensin converting enzyme insertion/deletion polymorphism: association with ethnic origin. J Hypertens. 1994;12(8):955–7.
54. Mathew J, Basheeruddin K, Prabhakar S. Differences in frequency of the deletion polymorphism of the angiotensin-converting enzyme gene in different ethnic groups. Angiology. 2001;52(6):375–9.

55. Bohn M, Berge KE, Bakken A, Eriksen J, Berg K. Insertion/deletion (I/D) polymorphism at the locus for angiotensin I-converting enzyme and myocardial infarction. Clin Genet. 1993;44(6):292–7.
56. Tronvik E, Stovner LJ, Bovim G, White LR, Gladwin AJ, Owen K, et al. Angiotensin-converting enzyme gene insertion/deletion polymorphism in migraine patients. BMC Neurol. 2008;8:4.
57. Shanmugam V, Sell KW, Saha BK. Mistyping ACE heterozygotes. PCR Methods Applications. 1993;3(2):120–1.
58. Gordish-Dressman H, Devaney JM. Statistical and methodological considerations in exercise genomics. Exercise Genomics: Springer; 2011. p. 23–43.
59. Cho J, Lee I, Kang H. ACTN3 gene and Susceptibility to sarcopenia and osteoporotic status in older Korean adults. Biomed Res Int. 2017;2017: 4239648.
60. Erskine RM, Williams AG, Jones DA, Stewart CE, Degens H. The individual and combined influence of ACE and ACTN3 genotypes on muscle phenotypes before and after strength training. Scand J Med Sci Sports. 2014;24(4):642–8.
61. Viken H, Aspvik NP, Ingebrigtsen JE, Zisko N, Wisloff U, Stensvold D. Correlates of objectively measured physical activity among Norwegian older adults: the generation 100 study. J Aging Phys Act. 2016;24(2): 369–75.
62. Leblanc A, Pescatello LS, Taylor BA, Capizzi JA, Clarkson PM, Michael White C, et al. Relationships between physical activity and muscular strength among healthy adults across the lifespan. SpringerPlus. 2015;4:557.
63. Rojer AGM, Reijnierse EM, Trappenburg MC, van Lummel RC, Niessen M, van Schooten KS, et al. Instrumented assessment of physical activity is associated with muscle function but not with muscle mass in a general population. J Aging Health. 2018;30(9):1462–81.
64. Gomes M, Figueiredo D, Teixeira L, Poveda V, Paúl C, Santos-Silva A, et al. Physical inactivity among older adults across Europe based on the SHARE database. Age Ageing. 2017;46(1):71–7.
65. Fowler JS, Alia-Klein N, Kriplani A, Logan J, Williams B, Zhu W, et al. Evidence that brain MAO a activity does not correspond to MAO a genotype in healthy male subjects. Biol Psychiatry. 2007;62(4):355–8.
66. Bauman AE, Reis RS, Sallis JF, Wells JC, Loos RJ, Martin BW. Correlates of physical activity: why are some people physically active and others not? Lancet. 2012;380(9838):258–71.
67. Lubs L, Peplies J, Drell C, Bammann K. Cross-sectional and longitudinal factors influencing physical activity of 65 to 75-year-olds: a pan European cohort study based on the survey of health, ageing and retirement in Europe (SHARE). BMC Geriatr. 2018;18(1):94.
68. Wichstrom L, von Soest T, Kvalem IL. Predictors of growth and decline in leisure time physical activity from adolescence to adulthood. Health Psychol. 2013;32(7):775–84.
69. Prince SA, Adamo KB, Hamel ME, Hardt J, Connor Gorber S, Tremblay M. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. Int J Behav Nutr Phys Activity. 2008;5: 56.
70. Bretthauer M, Gondal G, Larsen K, Carlsen E, Eide TJ, Grotmol T, et al. Design, organization and management of a controlled population screening study for detection of colorectal neoplasia: attendance rates in the NORCCAP study (Norwegian colorectal Cancer prevention). Scand J Gastroenterol. 2002;37(5):568–73.
71. Marchini J, Cardon LR, Phillips MS, Donnelly P. The effects of human population structure on large genetic association studies. Nat Genet. 2004;36(5):512–7.
72. Piirtola M, Kaprio J, Waller K, Heikkila K, Koskenvuo M, Svedberg P, et al. Leisure-time physical inactivity and association with body mass index: a Finnish twin study with a 35-year follow-up. Int J Epidemiol. 2016.
73. Ekelund U, Brage S, Besson H, Sharp S, Wareham NJ. Time spent being sedentary and weight gain in healthy adults: reverse or bidirectional causality? Am J Clin Nutr. 2008;88(3):612–7.
74. Metcalf BS, Hosking J, Jeffery AN, Voss LD, Henley W, Wilkin TJ. Fatness leads to inactivity, but inactivity does not lead to fatness: a longitudinal study in children (EarlyBird 45). Arch Dis Child. 2011;96(10):942–7.
75. Skjelbred CF, Saebo M, Wallin H, Nexo BA, Hagen PC, Lothe IM, et al. Polymorphisms of the XRCC1, XRCC3 and XPD genes and risk of colorectal adenoma and carcinoma, in a Norwegian cohort: a case control study. BMC Cancer. 2006;6:67.
76. Clinical Trials. NORCCAP: Norwegian Colorectal Cancer Prevention Trial: U.S. National Library of Medicine; [Available from: https://clinicaltrials.gov/show/NCT00119912.
77. Ainsworth BE, Haskell WL, Leon AS, Jacobs DR Jr, Montoye HJ, Sallis JF, et al. Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sports Exerc. 1993;25(1):71–80.
78. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR Jr, Tudor-Locke C, et al. 2011 compendium of physical activities: a second update of codes and MET values. Med Sci Sports Exerc. 2011;43(8):1575–81.
79. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc. 2011;43(7):1334–59.
80. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3): 1215.


## Publisher's Note

## Paper 2

Hans Torvild Kittilsen*, Sannija Goleva-Fjellet*, Baard I. Freberg, Iver Nicolaisen, Eva M Støa, Solfrid Bratland-Sanda, Jan Helgerud, Eivind Wang, Mona Sæbø, Øyvind Støren. Early responses to maximal strength training were not influenced by age, gender or initial training status

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# Early responses to maximal strength training were not influenced by age, gender or initial training status

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## ABSTRACT

**Purpose:** The present study aimed to investigate the potential impact of age, gender, initial training status on maximal strength training (MST) adaptations. **Methods:** 49 subjects (22 men, 27 women) aged 20-76 years, divided into five age groups, completed an eight weeks MST intervention. Each MST session consisted of 4 sets with 4 repetitions at ~85-90% of one-repetition maximum (1RM) intensity in leg-press, three times per week. 1RM was tested pre- and post the intervention and blood samples were drawn to genotype candidate polymorphisms *ACE* I/D, *ACTN3* R577X and *PPARGC1A* rs8192678. **Results:** All age groups increased leg-press 1RM ($p<0.01$), with a mean improvement of 24.2±14.0%. There were no differences in improvements between the five age groups or between male and female participants, and there were no non-responders. Initial training status did not correlate with 1RM improvements. *PPARGC1A* rs8192678 T allele carriers had a 15% higher age- and gender corrected baseline 1RM than the CC genotype ($p<0.05$). C allele carriers improved 1RM(%) by 34.2% more than homozygotes for the T allele ($p<0.05$). **Conclusion:** Leg-press maximal strength improved regardless of age, gender or initial training status, and with only a minor impact from key genes. This suggests good health and performance benefits from MST for all healthy individuals.

**Keywords:** AGING, 1RM, GENES, POLYMORPHISMS, MST

## INTRODUCTION

Maximal muscle strength is important for everyday functionality in all age groups [33]. From the age of approximately 40, maximal muscle strength decreases steadily [6, 56-58], and the decrease seems to accelerate from the age of 50 to 70 [1]. As a consequence of this, elevated risk of physical frailty, reduction in general motor function, decline in functional movement, poor balance, falls, risk of fracture, and decline in quality of life has been reported [1-4]. Strength training has been recommended to delay or reverse the structural and functional changes that occur with ageing in the neuro-muscular system [1, 5, 6]. Maximal strength training (MST) above 85% of one-repetition maximum (1RM) has been suggested to be more effective than low-intensity training regimens to improve muscle strength in both young and old [7]. In previous strength training interventions, the effect of age on strength training adaptions has been studied in young versus old [1, 8, 9] or middle-aged versus old [5, 10], but not in a large cohort ranging from young via middle-aged to old, with the same initial training status, typical for what is observed in the population. A study showed that age affects changes in 1RM with young subjects having a greater increase in 1RM compared with older subjects [11]. Some MST studies have included both males and females; however, these studies have not reported any difference between genders [11-13]. There may also be large inter-individual variability in different muscle strength-related phenotypes, as a response to the same strength training [14]. Heritability estimates for general muscle strength have been reported to range from 30% to 60% [15]. However, heritability impact on responses

to strength training seems to depend highly on the measured phenotypes [16-19]. The genetic component in muscle strength-related phenotypes seems thus to be strong, but not fully understood [20]. Some of the most extensively studied polymorphisms in association with various aspects of exercise genetics are *ACE* I/D, *ACTN3* R577X and *PPARGC1A* rs8192678 [21].

*ACE* gene codes for the angiotensin-converting enzyme involved in the regulation of blood pressure [22] and exhibiting a local effect on skeletal muscle function [23]. The insertion/deletion (I/D) polymorphism within the *ACE* gene has been widely investigated in relation to various skeletal muscle phenotypes [24-26]. *ACTN3,* coding for α-actinin-3 protein, has been described as "gene for speed" [27, 28]. SNP (R577X), leading to a premature stop codon, have been associated with various muscle phenotypes in athletes [29, 30] as well as in the general population [24, 31-33]). Around 18 % of the global population are homozygous for the 577X allele, thus lacking the α-actinin-3 [34]. This affects several aspects of muscle metabolism, leading to lower muscle strength and mass, among others [35]. *PPARGC1A* gene codes for the peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), enriched in metabolically active tissues [36]. PGC-1α have a range of functions, including being a master regulator of mitochondrial biogenesis [37]. Several PGC-1α isoforms exist, exhibiting actions through different pathways [38]. Although most of the findings relate to adaptations to aerobic exercise [39, 40] and athletic ability [41], PGC-1α may also mediate the adaptations to resistance training [42, 43]. A common coding SNP within the *PPARGC1A* gene is the rs8192678 polymorphism [44], more known as Gly482Ser, a missense mutation where serine (Ser) substitutes glycine (Gly; NCBI [45]). As most previous studies have been cross-sectional [43, 46-50], it is uncertain how key candidate genes may influence MST responses in a cohort with a training status typically for their age.

Previously, MST has been shown to effectively improve maximal muscle strength in different cohorts ranging from patients to athletes, and from young to old of both genders [8, 12, 13, 51, 52]. However, it remains unclear to what extent the strength training response may be influenced by age, gender and key genes following a short-term intervention. This may be important for the health and functional benefits an individual can expect. Thus, the purpose of the present study was to investigate the effect of age, gender, initial training status, and candidate gene status on the adaptability to leg-press MST. Specifically, we hypothesized that 1) MST would lead to an increase in maximal strength in all age cohorts; 2) The increase in maximal strength would not be different between males and females; 3) MST would improve maximal strength more in young (20-29 and 30-39) than in middle-aged (40-49 and 50-59) or old (60+); 4) the improvements in maximal strength after MST would not be affected by the selected key genes.

## METHODS

*Subjects.*

A total 76 healthy subjects (33 men; 43 women) with age ranging from 20 to 76 years, were included in the present study. Subjects' characteristics are presented in table 2, 3 and 4. Subjects were divided into five age groups with 10 years age-span in each, except the oldest group ranging from 60-76 years. Each age group was matched for baseline 1RM in leg-press, corrected for age, gender (Table 1) and body mass. The correction was based on previously reported values in males and females with different age [2, 5, 6, 8, 55, 58, 70, 71], and mean age and gender differences were calculated based the results and the number of participants in these previous studies.

Table 1 Age and gender correction table for maximal strength in leg-press

| Age group | | 20-29 | 30-39 | 40-49 | 50-59 | 60-70+ |
|---|---|---|---|---|---|---|
| Gender | Male | 1 | 1 | 1 | 1 | 1 |
| | Female | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| Age | | 1 | 0.98 | 0.92 | 0.88 | 0.82 |

*Values are based on means of the results i.e. gender differences, adjusted for the number of participants, from the following studies: Dey, Bosaeus [70], Hakkinen, Pakarinen [5], Lindle, Metter [56], Petrella, Kim [6], Reid, Naumova [71], Reynolds, Gordon [59], Unhjem, Nygard [1], Wang, Nyberg [8].*

Inclusion criteria were general good health status with no contra-indications for MST and testing, assessed by the study`s physician, and compliance of at least 80% of all training sessions. Exclusion criteria included any injury or illness that could prevent subjects from performing in MST or testing in leg-press or compliance of less than 80 % of all training sessions. Informed consent was obtained from all subjects, and the study was approved by the institutional review board of Telemark University College (now the University of South-Eastern Norway) and the Norwegian Centre for Research Data (NSD, reg 45185/3/AH). The study was also registered in Clinical trials (NCT02589990).

*Study timeline*

The subjects performed pre-testing 2-4 days before the 8-wk MST intervention, and post-tests 2-5 days after the last training session. Subjects were instructed not to exercise the last 24 hours before the test days, not to eat within 2-4 hours before the tests, and only to drink water for the last 2 hours before the testing procedures.

*Testing*

Pre- and post-tests were identical and performed at the same time of day ± 1 hour. A general warm-up for 10 min performed as cycling, walking or running was performed at a moderate intensity. After the general warm-up, a specific warm-up was performed in the leg-press machine (OPS161 interchangeable leg-press, Vertex USA). This included sets of 10, 5 and 3 repetitions at approximately 50 %, 60 %, and 70 % of 1RM respectively. The estimates of 1RM before the first 1 RM test were based on age, gender, body weight and training history. There were three minutes of rest between each set. Following this, 1RM was assessed by first one repetition at approximately 80 % 1RM, and then one and one repetition at weight loads increased by 5-15 kg from the previous lift, separated by three minutes rest until reaching 1RM. Each lift was performed with a controlled slow eccentric phase, a complete stop of movement for approximately 1 s in the lowest position (90 degrees between femur and tibia), followed by a maximal mobilization of force in the concentric phase, as described in Støren et al [12, 13]. Lifting time and distance were measured using the Muscle lab system (Ergotest Innovation A.S., Porsgrunn, Norway) to control the work distance.

*Maximal strength training (MST)*

The MST intervention lasted for eight weeks and included three MST-sessions per week with at least one day of rest between each session. Participants were instructed to maintain their habitual training as normal, and both the MST intervention and habitual training was logged. Each session consisted of a general warm-up for 10 min at moderate intensity and then three 10-repetition warm-up sets in leg-press with increasing load (30-70% 1RM). After the warm-up, participants performed four sets of 4RM in the leg-press, with 90 degrees between femur and tibia, divided by 3 min of rest between sets. Every time a subject managed to do five repetitions during a set, 2.5-5 kg were added for the next set. Guidance and instruction were given to all subjects during the training period.

*DNA sampling and genotyping.*

Venous blood was collected in EDTA tubes from all participants prior to the admission to the exercise intervention. The samples were stored at -20$^{o}$C until the genomic DNA was extracted from 100 µl of blood using the DNeasy Blood & Tissue Kit (Qiagen, MD, USA) according to the manufacturer's instructions.

The rs4343 polymorphism in the *ACE* gene, which might be the best proxy to *ACE* I/D polymorphism [53], was analyzed to determine the I/D genotype. Genotyping for all polymorphisms was performed using TaqMan® SNP Genotyping Assay. Assay ID were as follows: C__11942562_20 for *ACE* rs4343; C____590093_1 for the *ACTN3* R577X and C___1643192_20 for the *PPARGC1A* rs8192678 (Thermo

Fisher Scientific, MA, USA). qPCR was carried out on the StepOnePlus™ Real-Time PCR System (Applied Biosystems®, CA, USA), and genotype calling was performed by StepOne Software v2.0. The final reaction volume was 15 µl and contained 8.44 µl Genotyping Master Mix, 0.42 µl Assay mix (40x), 6.33 µl distilled $H_20$ and ~100 ng of DNA template. Following cycling conditions were used: 30 s at 60°C followed by initial denaturation step for 10 min at 95°C; 40 cycles of denaturation at 95°C for 15 s followed by annealing at 60°C for 1 min in cycling stage, and a final post-read step for 30 s at 60°C.

*Statistical analysis*

Data were tested for normality by use of QQ-plot and the Kolmogorov-Smirnov test and found to be normally distributed for the main variable 1RM, corrected for age and gender (1RMcorr). A general linear model with Tukey post-hoc analyses for age groups was used to assess 1RM and Δ1RM results. Independent t-tests were used to compare males and females overall since the sample size was too low to assess potential gender differences in each age group. Associations between the genotypes and continuous variables, and the alleles and continuous variables were analysed by one-way ANOVA and two-tailed independent sample t-tests, respectively. Correlation analyses were performed by use of the Pearson correlation test. Pearson's Chi-square test ($\chi^2$) was applied to test for the Hardy-Weinberg equilibrium (HWE) for all polymorphisms and the differences in categorical variables. The significance level was set to $p<0.05$ in two-tailed tests. All statistical analysis were performed by the use of IBM SPSS Statistics, version 25 (Chicago, IL, USA).

In the present study, the sample size for genetic association studies is relatively small. Therefore, to determine the magnitude of differences, also Cohen's d effect size was calculated for baseline strength (1RMcorr) and Δ1RM (%) across phenotypes. Effect sizes were interpreted as: d= <0.35 (trivial), d= 0.35-0.80 (small); d= 0.80-1.50 (moderate); d= >1.50 (large effect size) [54].

## RESULTS

Forty-nine subjects (22 males and 27 females) aged 20 to 76 years (45.3±16.0) completed the eight-week three times per week MST intervention. There was no difference in baseline characteristics between completers and non-completers.

Baseline 1RM in absolute values (kg) decreased with increasing age ($p<0.01$) from group 2 (33.9 ± 2.8 years). Baseline 1RM corrected for age, gender and body mass scaled to the power of 0.67 ($kg \cdot kg^{-0.67}$) was not significantly different between any of the age groups (Table 2).

**Table 2 Age, body weight (BW) at baseline, and maximal strength (1RM) in leg-press at pre- and post-tests, and percentage improvements (Δ1RM) in maximal strength**

| Group | 1 (n = 10) | 2 (n=9) | 3 (n=12) | 4 (n=8) | 5 (n=10) | Total (n= 49) |
|---|---|---|---|---|---|---|
| Age (yrs) | 25.6±2.8 | 33.9±2.8 | 44.2±3.2 | 53.5±3.0 | 70.3±4.3 | 45.3 ± 16.0 |
| BW (kg) | 74.4±8.9 | 83.5±11.8 | 74.4±12.5 | 80.8±15.1 | 70.3±4.2 | 76.3±12.2 |
| Pre-1RM (kg) | 224.5±53.3# | 362.2±135.3 | 255.8±86.9 | 240.6±77.6 | 191.0±50.8# | 253.3±99.6 |
| 1RMcorr (kg) | 18.1±4.7 | 20.8±4.9 | 20.5±4.3 | 18.3±3.4 | 19.5±5.5 | 19.5±4.6 |
| Post-1RM (kg) | 267.0±59.1* | 443.9±136.7* | 333.13±115.2* | 290.63±99.6* | 231.5±53.2* | 313.3±118.4* |
| Δ1RM (%) | 19.5±7.4 | 25.5±15.0 | 30.9±19.2 | 20.2±9.3 | 22.86±13.3 | 24.2±14.0 |

*Data are presented as mean ± SD, standard deviation. BW, body weight. Yrs, years. Kg, kilograms. 1RM, one-repetition maximum. corr., baseline 1RM corrected for age, gender and body weight raised to the power of 0.67*
**p<0.01different from pre-test*

After the intervention, there was a mean improvement in 1RM leg-press of 24.2±14.0 % ($p<0.01$), with no participants having less than 7% improvement. In relative terms (%), there were no significant differences in Δ1RM between any of the age groups (Table 2, Figure 1). No changes in body mass in any of the groups were found following the intervention.

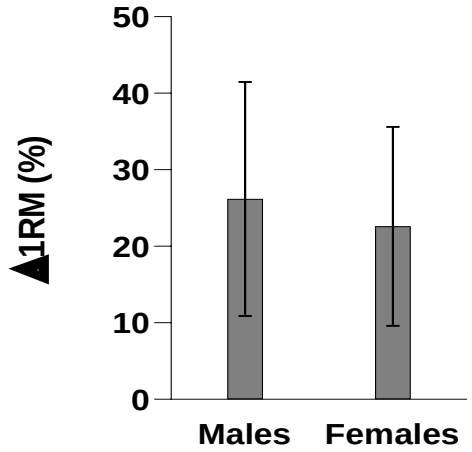


*Figure 1 Mean improvements (%) in one-repetition maximum (Δ1RM) ± standard deviation (SD) following an 8-week maximal strength training program by gender.*

At baseline, males were heavier and had higher 1RM in absolute values (kg) than females (p<0.01). Independent of age groups, males improved 1RM by 26.2 % ±15.3 %, whereas the females improved 1RM by 22.6 % ± 13.0 % (Table 3, Figure 2), which was not significantly different (p=0.56). There was no significant correlation between baseline 1RM and relative improvement in Δ1RM (%) (r=0.25, p=0.08).

Table 3. Age, body weight (BW) at baseline, the percentage change in BW, and leg-press maximal strength (1RM) and percentage improvements (Δ1RM) in maximal strength by gender

(N=49, 22 males and 27 females)

| | Age (yrs) | BW(kg)pre | ΔBW(%) | 1RM(kg)pre | Δ1RM(%) |
|---|---|---|---|---|---|
| Males | 43.3±13.8 | 83.1±11.8 | 1.1±3.0 | 315.2±112.6 | 26.2±15.3 |
| Females | 47.0±17.7 | 70.7±9.5* | -0.3±2.3* | 202.8±46.4* | 22.6±13.0 |

*Results are mean ± SD, standard deviation, and percent change from pre to post-intervention. Yrs, years; BW, body weight; 1RM, one-repetition maximum in leg-press; Kg, kilograms; % percent.*

**P<0.01 different from males.*

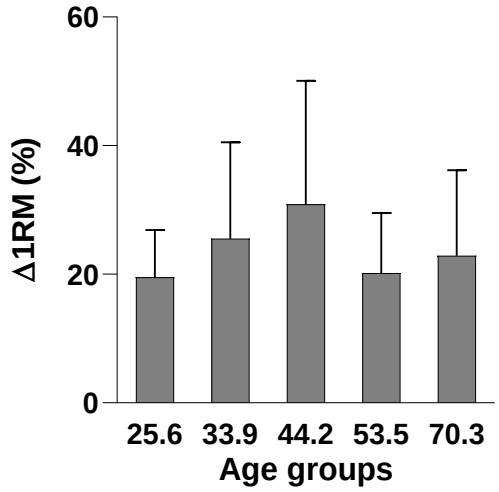


*Figure 2 Age group improvements (%) in one-repetition maximum (Δ1RM) ± standard deviation (SD).*

All three gene polymorphisms were successfully genotyped (*ACE* I/D, *ACTN3* R577X, *PPARGC1A* Gly482Ser). Genotype distributions for all polymorphisms are displayed in Table 4. Minor allele frequencies for these polymorphisms were 52% for the *ACE* I allele, 48% for the *ACTN3* X allele and 40% for the *PPARGC1A* T (Ser) allele. The genotype frequencies were consistent with Hardy-Weinberg Equilibrium (P > 0.05).

**Table 4. Genotype distributions for *ACE* I/D, *ACTN3* R577X and *PPARGC1A* rs8192678 polymorphisms (N=49)**

| *ACE* | | *ACTN3* | | *PPARGC1A* | |
|---|---|---|---|---|---|
| DD | 25 % | RR | 27 % | CC | 41 % |
| ID | 45 % | RX | 51 % | CT | 39 % |
| II | 30 % | XX | 22 % | TT | 20 % |
| | | | | | |
| D al. | 48 % | R al. | 52 % | C al. | 60 % |
| I al. | 52 % | X al. | 48 % | T al. | 40 % |

*Results are displayed as percentages (%); Al.-allele*.

*PPARGC1A* Gly482Ser T allele carriers demonstrated 15.0 % higher baseline 1RMcorr compared to the CC genotype. Also, the participants with CT genotype were 17.9% stronger at baseline (1RMcorr) compared to the wild type CC counterparts ($p<0.05$) (Table 5). C-allele carriers, on the contrary, showed 34.2 % higher improvements in Δ1RM (%), compared to the homozygotes for the minor allele i.e. the TT genotype ($p<0.05$) (Table 5). 1RMpre and 1RMpost values for the *PPARGC1A* rs8192678, *ACTN3* R577X and *ACE* I/D polymorphisms are displayed as supplementary data (Figure S1).

**Table 5. Associations between the *PPARGC1A* rs8192678 polymorphism and leg-press maximal strength and percentage improvements in maximal strength.**

| | 1RMcorr (kg) | Δ1RM (%) |
|---|---|---|
| *PPARGC1A* | | |
| CC | **17.8 ± 4.4*/#** | 29.3 ± 17.5 |
| CT | **21.3 ± 4.5#** | 22.0 ± 11.7 |
| TT | 20.0 ± 4.1 | **18.2 ± 4.6¤** |
| C allele | 19.5 ± 4.7 | **25.7 ± 15.2¤** |
| T allele | **20.7 ± 4.4*** | 20.7 ± 9.9 |

*Results are mean ± SD, standard deviation, and change (Δ) in percent.; Corr, corrected for age, gender and body weight raised to the power of 0.67; 1RM, one-repetition maximum in leg-press; p-values are corrected for multiple testing where appropriate (Tukey): *p=0.027, #p=0.042, ¤p=0.011*

No significant associations were found between the *ACTN3* R577X and *ACE* I/D, and baseline 1RMcorr or Δ1RM (%). However, participants with *ACTN3* RR genotype demonstrated a non-significant 46.5 % larger increase in Δ1RM on average, compared to their XX counterparts. This corresponds to a moderate effect size measured in Cohen's d. A summary table of all genotype/allele combinations for the *ACTN3* R577X, *PPARGC1A* Gly482Ser and *ACE* I/D polymorphisms, and 1RMcorr and Δ1RM (%) can be found in the supplementary data (Table S1).

## DISCUSSION

The main findings of the present study were that MST-induced increases in leg-press 1RM were similar regardless of age, gender, initial training status or most of the selected candidate genes. The first hypothesis that all age cohort would improve in maximal strength was confirmed. The second hypothesis that an increase in maximal strength would not be different between males and females were also confirmed. The third hypothesis that young would improve more than old was rejected, and

the fourth hypothesis that improvement in maximal strength would not be affected by the selected single genes was confirmed.

The results from the present study show the same results in maximal strength adaptations as previously found in $VO_{2max}$ adaptations in Støren et al. (2016). Although middle-aged and old had lower baseline values in these two studies, the relative improvements were just as good in untrained and moderately trained at older ages. To our knowledge, this is the first study to report similar training responses in all age groups from young adults in their twenties and thirties via middle-aged in their forties and fifties and up to older adults in their sixties and seventies.

That MST was an effective method to improve maximal strength was in the present study shown by no non-responders to the MST Program, with the smallest improvement being 7.4 %. Furthermore, the rather homogenous improvements in maximal strength, with a coefficient of variance of 8.7 %, were found to be more or less independent of the same inter-individual variability in polymorphisms for the selected genes as in the general population in this geographic area [55].

*The impact of age, gender and selected candidate genes on baseline 1RM*

As expected, 1RM (kg) decreased with advancing age at baseline (table 2). The 1.3% decrease per year in the current study from young (33.9 years) to old (70.3 years) is in line with previous studies [6, 55-57], but the decrease in the present study is evenly distributed among age groups. The results show a 1.2 % decrease from 53.5 years to 70.3 years of age, while some studies also show an accelerated drop in muscle strength from 50-70 years [6, 56-58].In the present study, males were 56% stronger than females, expressed in absolute values (kg). This corresponds well with the findings of Reynolds, Gordon [59] and Petrella, Kim [6], showing approximately 50-65% higher 1RM in lower extremities in males than in females.

Homozygotes for the *PPARGC1A* Gly482Ser C allele had lower 1RMcorr at baseline compared to both CT genotype counterparts and T-allele carriers. This may indicate that the Ser-encoding allele might be favourable for baseline muscle strength not only in athletes but also in the general population. Gly482Ser polymorphism has been associated with differences in *PPARGC1A* mRNA expression, with lower expression among carriers of Ser-allele [60]. Gene expression responses may be important for muscle adaptations in response to different modes of exercise [61].

*Improvements in 1RM*

The average relative improvements in 1RM by ~24 %, was not different between the age groups after 8 weeks of MST. The size of the average improvement is in line with comparable studies on MST, showing improvements in the range of 23-33% [12, 13, 52].

To our knowledge, this is the first study to report similar training responses in all age groups from young adults in their twenties and thirties via middle-aged in their forties and fifties and up to older adults in their sixties and seventies. Actually, the oldest group improved 1RM to the same extent as the mean of the other four age groups. The present results are in line with some studies comparing young and old, like Hakkinen, Pakarinen [5], but differ from Petrella, Kim [6] and Lemmer, Hurlbut [12] showing better adaptations in young than old.

That males and females improved relative 1RM to the same extent was as expected, and in line with previous studies. No gender differences in $\Delta$1RM % were found in Hakkinen, Pakarinen [5], Storen, Helgerud [12], Sunde, Storen [13], Kanegusuku, Queiroz [4], Berg, Kwon [62] or Winther, Foss [63].

When corrected for age, gender and body mass (1RMcorr), baseline 1RM indicates the participant`s initial training status. In light of this, it was somewhat surprising that initial training status did not significantly affect 1RM improvements. In a previous study on $VO_{2max}$ adaptations to endurance training in different age groups [64], initial training status was found to significantly affect training adaptations. This should also be expected in MST interventions, as untrained and trained in previous studies have shown rapid improvements in neural adaptations during the first 2-4 weeks of this type of training [1, 5, 8, 9, 12].

Bodyweight did not change in the present study, and this may support the assumption that it is predominately the neural adaptions and changes in recruitment patterns, which have led to increased 1RM. However, any change in body composition cannot be excluded in the present study and this is in line several other studies [1, 11, 13, 52].

T allele carriers of the *PPARGC1A* Gly482Ser polymorphism had higher baseline 1RMcorr compared to the CC genotype. The T allele, more widely known as the Ser allele, has been associated with strength/power athlete status [43], indicating an advantageous effect on muscle strength not only in athletes but also among the general public. On the other hand, C allele carriers, possessing lower 1RMcorr at baseline, demonstrated larger improvements in 1RM compared to the TT genotype in the present study. These differences could theoretically be attributable to a larger potential for improvements, due to lower muscle strength at baseline in C allele carriers. However, baseline 1RM and improvements in 1RM did not correlate in the present study. Resistance training has been shown to induce expression of an isoform of the protein coded by the *PPARGC1A* gene (i.e. PGC-1α4) that regulates muscle hypertrophy [42]. The polymorphism is known to influence mRNA expression [60]. However, to the best of authors' knowledge, it is not known whether the Gly482Ser polymorphism may influence the expression of the hypertrophy-specific isoform.

No significant associations between *ACTN3* R577X and *ACE* I/D polymorphisms and baseline or Δ1RM were found in the present study. Of these, especially the *ACTN3* R577X polymorphism has been shown to have a range of effects on various muscle phenotypes, such as improvements in strength or muscle function [31, 32, 65]. Previous studies indicate that the R allele may be advantageous for an increased maximal dynamic strength [66-68]. That this association was not significant in the present study could in part be a result of the relatively low sample size. A low number of participants in genetic association studies investigating complex traits tend to be vulnerable to type II error [69]. Therefore, the effect size of these relationships was also reported in the present study (tables S1, S2). Cohen's d for differences in Δ1RM between the RR and XX genotypes indicates a moderate negative effect for the latter group. The indications of greater response to resistance training in R allele carriers are thus in line with the overall impression from studies on resistance training [35]. Genotype frequencies of the *ACTN3* and the *ACE* polymorphisms were in line with those reported previously in a Scandinavian population from the same geographical region [55], indicating that the participants in the present study were genetically representative for the population in this region.

*Practical implications*

The present results demonstrated that MST is effective in improving maximal strength in most healthy people capable of performing MST. There were no differences in drop out between the age groups, and the dropout rate may be considered to be in line with previous MST studies such as [8]. Improved muscle strength has been shown to better general motor function, maintain or increase functional movement, balance, independence and quality of life [1-4], especially among old. We, therefore, recommend MST 2-3 times per week in leg-press, squats or deadlift at all ages to delay the age-related decline in muscle strength and health. However, cautions should be taken as some may experience muscle or joint pain from this kind of exercise.

*Conclusion*

Improvements in 1RM leg-press were found in all age groups from 20 to 76 years and were not affected by age, gender or initial training status, and with minimal impact from selected genes. These findings imply that most healthy people have great potential for maximal strength improvements and that MST may be used as a strategy for healthy ageing.

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## REFERENCES


1. Unhjem R, Nygard M, van den Hoven LT, Sidhu SK, Hoff J and Wang E. Lifelong strength training mitigates the age-related decline in efferent drive. Journal of applied physiology (Bethesda, Md : 1985). 2016; 121(2):415-423.
2. Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, Roberts SB, Kehayias JJ, Lipsitz LA and Evans WJ. Exercise training and nutritional supplementation for physical frailty in very elderly people. The New England journal of medicine. 1994; 330(25):1769-1775.
3. Kirkendall DT and Garrett WE, Jr. The effects of aging and training on skeletal muscle. The American journal of sports medicine. 1998; 26(4):598-602.
4. Kanegusuku H, Queiroz AC, Silva VJ, de Mello MT, Ugrinowitsch C and Forjaz CL. High-Intensity Progressive Resistance Training Increases Strength With No Change in Cardiovascular Function and Autonomic Neural Regulation in Older Adults. Journal of aging and physical activity. 2015; 23(3):339-345.
5. Hakkinen K, Pakarinen A, Kraemer WJ, Newton RU and Alen M. Basal concentrations and acute responses of serum hormones and strength development during heavy resistance training in middle-aged and elderly men and women. The journals of gerontology Series A, Biological sciences and medical sciences. 2000; 55(2):B95-105.
6. Petrella JK, Kim JS, Tuggle SC, Hall SR and Bamman MM. Age differences in knee extension power, contractile velocity, and fatigability. Journal of applied physiology (Bethesda, Md : 1985). 2005; 98(1):211-220.
7. Heggelund J, Fimland MS, Helgerud J and Hoff J. Maximal strength training improves work economy, rate of force development and maximal strength more than conventional strength training. Eur J Appl Physiol. 2013; 113(6):1565-1573.
8. Wang E, Nyberg SK, Hoff J, Zhao J, Leivseth G, Torhaug T, Husby OS, Helgerud J and Richardson RS. Impact of maximal strength training on work efficiency and muscle fiber type in the elderly: Implications for physical function and fall prevention. Experimental gerontology. 2017; 91:64-71.
9. Unhjem R, Lundestad R, Fimland MS, Mosti MP and Wang E. Strength training-induced responses in older adults: attenuation of descending neural drive with age. Age. 2015; 37(3):9784.
10. Sillanpaa E, Laaksonen DE, Hakkinen A, Karavirta L, Jensen B, Kraemer WJ, Nyman K and Hakkinen K. Body composition, fitness, and metabolic health during strength and endurance training and their combination in middle-aged and older women. Eur J Appl Physiol. 2009; 106(2):285-296.
11. Lemmer JT, Hurlbut DE, Martel GF, Tracy BL, Ivey FM, Metter EJ, Fozard JL, Fleg JL and Hurley BF. Age and gender responses to strength training and detraining. Med Sci Sports Exerc. 2000; 32(8):1505-1512.
12. Storen O, Helgerud J, Stoa EM and Hoff J. Maximal strength training improves running economy in distance runners. Med Sci Sports Exerc. 2008; 40(6):1087-1092.
13. Sunde A, Storen O, Bjerkaas M, Larsen MH, Hoff J and Helgerud J. Maximal strength training improves cycling economy in competitive cyclists. Journal of strength and conditioning research / National Strength & Conditioning Association. 2010; 24(8):2157-2165.
14. Hubal MJ, Urso ML and Clarkson PM. (2011). Genetic Aspects of Muscular Strength and Size. In: Pescatello LS and Roth SM, eds. Exercise Genomics. (Totowa, NJ: Humana Press), pp. 157-178.
15. Perusse L, Lortie G, Leblanc C, Tremblay A, Theriault G and Bouchard C. Genetic and environmental sources of variation in physical fitness. Ann Hum Biol. 1987; 14(5):425-434.
16. Arden NK and Spector TD. Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. J Bone Miner Res. 1997; 12(12):2076-2081.
17. Calvo M, Rodas G, Vallejo M, Estruch A, Arcas A, Javierre C, Viscor G and Ventura JL. Heritability of explosive power and anaerobic capacity in humans. Eur J Appl Physiol. 2002; 86(3):218-225.
18. Thomis MA, Beunen GP, Maes HH, Blimkie CJ, Van Leemputte M, Claessens AL, Marchal G, Willems E and Vlietinck RF. Strength training: importance of genetic factors. Med Sci Sports Exerc. 1998; 30(5):724-731.

19. Thomis MAI, Beunen GP, Leemputte MV, Maes HH, Blimkie CJ, Claessens AL, Marchal G, Willems E and Vlietinck RF. Inheritance of static and dynamic arm strength and some of its determinants. Acta physiologica Scandinavica. 1998; 163(1):59-71.
20. Roth SM. Genetic aspects of skeletal muscle strength and mass with relevance to sarcopenia. Bonekey Rep. 2012; 1:58.
21. Ahmetov, II and Fedotovskaya ON. Current Progress in Sports Genomics. Adv Clin Chem. 2015; 70:247-314.
22. Coates D. The angiotensin converting enzyme (ACE). Int J Biochem Cell B. 2003; 35(6):769-773.
23. Jones A and Woods DR. Skeletal muscle RAS and exercise performance. Int J Biochem Cell Biol. 2003; 35(6):855-866.
24. Pereira A, Costa AM, Leitao JC, Monteiro AM, Izquierdo M, Silva AJ, Bastos E and Marques MC. The influence of ACE ID and ACTN3 R577X polymorphisms on lower-extremity function in older women in response to high-speed power training. BMC Geriatr. 2013; 13:131.
25. Erskine RM, Williams AG, Jones DA, Stewart CE and Degens H. The individual and combined influence of ACE and ACTN3 genotypes on muscle phenotypes before and after strength training. Scandinavian journal of medicine & science in sports. 2014; 24(4):642-648.
26. Wagle JP, Carroll KM, Cunanan AJ, Wetmore A, Taber CB, DeWeese BH, Sato K, Stuart CA and Stone MH. Preliminary Investigation Into the Effect of ACTN3 and ACE Polymorphisms on Muscle and Performance Characteristics. Journal of strength and conditioning research. 2018.
27. MacArthur DG and North KN. A gene for speed? The evolution and function of α-actinin-3. BioEssays. 2004; 26(7):786-795.
28. North KN, Yang N, Wattanasirichaigoon D, Mills M, Easteal S and Beggs AH. A common nonsense mutation results in alpha-actinin-3 deficiency in the general population. Nature genetics. 1999; 21(4):353-354.
29. Yang N, Garton F and North K. alpha-actinin-3 and performance. Med Sport Sci. 2009; 54:88-101.
30. Eynon N, Hanson ED, Lucia A, Houweling PJ, Garton F, North KN and Bishop DJ. Genes for elite power and sprint performance: ACTN3 leads the way. Sports medicine. 2013; 43(9):803-817.
31. Pickering C and Kiely J. ACTN3, Morbidity, and Healthy Aging. Frontiers in genetics. 2018; 9:15.
32. Del Coso J, Hiam D, Houweling P, Perez LM, Eynon N and Lucia A. More than a 'speed gene': ACTN3 R577X genotype, trainability, muscle damage, and the risk for injuries. Eur J Appl Physiol. 2018.
33. Houweling PJ, Papadimitriou ID, Seto JT, Perez LM, Coso JD, North KN, Lucia A and Eynon N. Is evolutionary loss our gain? The role of ACTN3 p.Arg577Ter (R577X) genotype in athletic performance, ageing, and disease. Hum Mutat. 2018; 39(12):1774-1787.
34. Mills M, Yang N, Weinberger R, Vander Woude DL, Beggs AH, Easteal S and North K. Differential expression of the actin-binding proteins, alpha-actinin-2 and -3, in different species: implications for the evolution of functional redundancy. Human molecular genetics. 2001; 10(13):1335-1346.
35. Seto JT, Garton FC, North KN and Houweling PJ. (2019). Alpha-Actinin-3's Role in the Genetic Control of Muscle Strength and Performance: Routledge).
36. Liang H and Ward WF. PGC-1alpha: a key regulator of energy metabolism. Adv Physiol Educ. 2006; 30(4):145-151.
37. Di Meo S, Iossa S and Venditti P. Skeletal muscle insulin resistance: role of mitochondria and other ROS sources. The Journal of endocrinology. 2017; 233(1):R15-r42.
38. Martinez-Redondo V, Jannig PR, Correia JC, Ferreira DM, Cervenka I, Lindvall JM, Sinha I, Izadi M, Pettersson-Klein AT, Agudelo LZ, Gimenez-Cassina A, Brum PC, Dahlman-Wright K, et al. Peroxisome Proliferator-activated Receptor gamma Coactivator-1 alpha Isoforms Selectively Regulate Multiple Splicing Events on Target Genes. The Journal of biological chemistry. 2016; 291(29):15169-15184.
39. Steinbacher P, Feichtinger RG, Kedenko L, Kedenko I, Reinhardt S, Schonauer AL, Leitner I, Sanger AM, Stoiber W, Kofler B, Forster H, Paulweber B and Ring-Dimitriou S. The single nucleotide

polymorphism Gly482Ser in the PGC-1alpha gene impairs exercise-induced slow-twitch muscle fibre transformation in humans. PloS one. 2015; 10(4):e0123881.
40. Lira VA, Benton CR, Yan Z and Bonen A. PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. American journal of physiology Endocrinology and metabolism. 2010; 299(2):E145-161.
41. Tharabenjasin P, Pabalan N and Jarjanazi H. Association of PPARGC1A Gly428Ser (rs8192678) polymorphism with potential for athletic ability and sports performance: A meta-analysis. PloS one. 2019; 14(1):e0200967.
42. Ruas JL, White JP, Rao RR, Kleiner S, Brannan KT, Harrison BC, Greene NP, Wu J, Estall JL, Irving BA, Lanza IR, Rasbach KA, Okutsu M, et al. A PGC-1alpha isoform induced by resistance training regulates skeletal muscle hypertrophy. Cell. 2012; 151(6):1319-1331.
43. Gineviciene V, Jakaitiene A, Aksenov MO, Aksenova AV, Druzhevskaya AM, Astratenkova IV, Egorova ES, Gabdrakhmanova LJ, Tubelis L, Kucinskas V and Utkus A. Association analysis of ACE, ACTN3 and PPARGC1A gene polymorphisms in two cohorts of European strength and power athletes. Biol Sport. 2016; 33(3):199-206.
44. Nitz I, Ewert A, Klapper M and Döring F. Analysis of PGC-1α variants Gly482Ser and Thr612Met concerning their PPARγ2-coactivation function. Biochemical and Biophysical Research Communications. 2007; 353(2):481-486.
45. NCBI. (2018). Reference SNP (rs) Report: rs8192678. (MD, USA: National Center for Biotechnology Information).
46. Kim H, Song KH and Kim CH. The ACTN3 R577X variant in sprint and strength performance. Journal of exercise nutrition & biochemistry. 2014; 18(4):347-353.
47. Fluck M, Kramer M, Fitze DP, Kasper S, Franchi MV and Valdivieso P. Cellular Aspects of Muscle Specialization Demonstrate Genotype - Phenotype Interaction Effects in Athletes. Frontiers in physiology. 2019; 10:526.
48. Ginszt M, Michalak-Wojnowska M, Gawda P, Wojcierowska-Litwin M, Korszen-Pilecka I, Kusztelak M, Muda R, Filip AA and Majcher P. ACTN3 Genotype in Professional Sport Climbers. Journal of strength and conditioning research / National Strength & Conditioning Association. 2018; 32(5):1311-1315.
49. Ben-Zaken S, Eliakim A, Nemet D and Meckel Y. Genetic Variability Among Power Athletes: The Stronger vs. the Faster. Journal of strength and conditioning research / National Strength & Conditioning Association. 2019; 33(6):1505-1511.
50. Ben-Zaken S, Meckel Y, Nemet D and Eliakim A. Genetic score of power-speed and endurance track and field athletes. Scandinavian journal of medicine & science in sports. 2015; 25(2):166-174.
51. Helgerud J, Rodas G, Kemi OJ and Hoff J. Strength and endurance in elite football players. International journal of sports medicine. 2011; 32(9):677-682.
52. Barrett-O'Keefe Z, Helgerud J, Wagner PD and Richardson RS. Maximal strength training and increased work efficiency: contribution from the trained muscle bed. Journal of applied physiology (Bethesda, Md : 1985). 2012; 113(12):1846-1851.
53. Abdollahi MR, Huang S, Rodriguez S, Guthrie PA, Smith GD, Ebrahim S, Lawlor DA, Day IN and Gaunt TR. Homogeneous assay of rs4343, an ACE I/D proxy, and an analysis in the British Women's Heart and Health Study (BWHHS). Dis Markers. 2008; 24(1):11-17.
54. Rhea MR. Determining the magnitude of treatment effects in strength training research through the use of the effect size. Journal of strength and conditioning research / National Strength & Conditioning Association. 2004; 18(4):918-920.
55. Goleva-Fjellet S, Bjurholt AM, Kure EH, Larsen IK, Støren Ø and Sæbø M. Distribution of allele frequencies for genes associated with physical activity and/or physical capacity in a homogenous Norwegian cohort- a cross-sectional study. BMC Genetics. 2020; 21(1):8.
56. Lindle RS, Metter EJ, Lynch NA, Fleg JL, Fozard JL, Tobin J, Roy TA and Hurley BF. Age and gender comparisons of muscle strength in 654 women and men aged 20-93 yr. Journal of applied physiology (Bethesda, Md : 1985). 1997; 83(5):1581-1587.

57. Lambert CP and Evans WJ. Effects of aging and resistance exercise on determinants of muscle strength. Journal of the American Aging Association. 2002; 25(2):73-78.
58. Distefano G and Goodpaster BH. Effects of Exercise and Aging on Skeletal Muscle. Cold Spring Harbor perspectives in medicine. 2018; 8(3).
59. Reynolds JM, Gordon TJ and Robergs RA. Prediction of one repetition maximum strength from multiple repetition maximum testing and anthropometry. Journal of strength and conditioning research / National Strength & Conditioning Association. 2006; 20(3):584-592.
60. Vandenbeek R, Khan NP and Estall JL. Linking Metabolic Disease With the PGC-1alpha Gly482Ser Polymorphism. Endocrinology. 2018; 159(2):853-865.
61. Silvennoinen M, Ahtiainen JP, Hulmi JJ, Pekkala S, Taipale RS, Nindl BC, Laine T, Hakkinen K, Selanne H, Kyrolainen H and Kainulainen H. PGC-1 isoforms and their target genes are expressed differently in human skeletal muscle following resistance and endurance exercise. Physiological Reports. 2015; 3(10).
62. Berg OK, Kwon OS, Hureau TJ, Clifton HL, Thurston T, Le Fur Y, Jeong EK, Amann M, Richardson RS, Trinity JD, Wang E and Layec G. Maximal strength training increases muscle force generating capacity and the anaerobic ATP synthesis flux without altering the cost of contraction in elderly. Experimental gerontology. 2018; 111:154-161.
63. Winther SB, Foss OA, Husby OS, Wik TS, Klaksvik J and Husby VS. A randomized controlled trial on maximal strength training in 60 patients undergoing total hip arthroplasty. Acta Orthopaedica. 2018; 89(3):295-301.
64. Storen O, Helgerud J, Saebo M, Stoa EM, Bratland-Sanda S, Unhjem RJ, Hoff J and Wang E. The Effect of Age on the V O2max Response to High-Intensity Interval Training. Med Sci Sports Exerc. 2017; 49(1):78-85.
65. Pickering C and Kiely J. ACTN3: More than Just a Gene for Speed. Frontiers in physiology. 2017; 8:1080.
66. Pereira A, Costa AM, Izquierdo M, Silva AJ, Bastos E and Marques MC. ACE I/D and ACTN3 R/X polymorphisms as potential factors in modulating exercise-related phenotypes in older women in response to a muscle power training stimuli. Age. 2013; 35(5):1949-1959.
67. Weyerstrass J, Stewart K, Wesselius A and Zeegers M. Nine genetic polymorphisms associated with power athlete status - A Meta-Analysis. Journal of science and medicine in sport / Sports Medicine Australia. 2018; 21(2):213-220.
68. Delmonico MJ, Kostek MC, Doldo NA, Hand BD, Walsh S, Conway JM, Carignan CR, Roth SM and Hurley BF. Alpha-actinin-3 (ACTN3) R577X polymorphism influences knee extensor peak power response to strength training in older men and women. The journals of gerontology Series A, Biological sciences and medical sciences. 2007; 62(2):206-212.
69. Hong EP and Park JW. Sample size and statistical power calculation in genetic association studies. Genomics Inform. 2012; 10(2):117-122.
70. Dey DK, Bosaeus I, Lissner L and Steen B. Changes in body composition and its relation to muscle strength in 75-year-old men and women: a 5-year prospective follow-up study of the NORA cohort in Göteborg, Sweden. Nutrition (Burbank, Los Angeles County, Calif). 2009; 25(6):613-619.
71. Reid KF, Naumova EN, Carabello RJ, Phillips EM and Fielding RA. Lower extremity muscle mass predicts functional performance in mobility-limited elders. The journal of nutrition, health & aging. 2008; 12(7):493-498.

SUPPLEMENTARY DATA

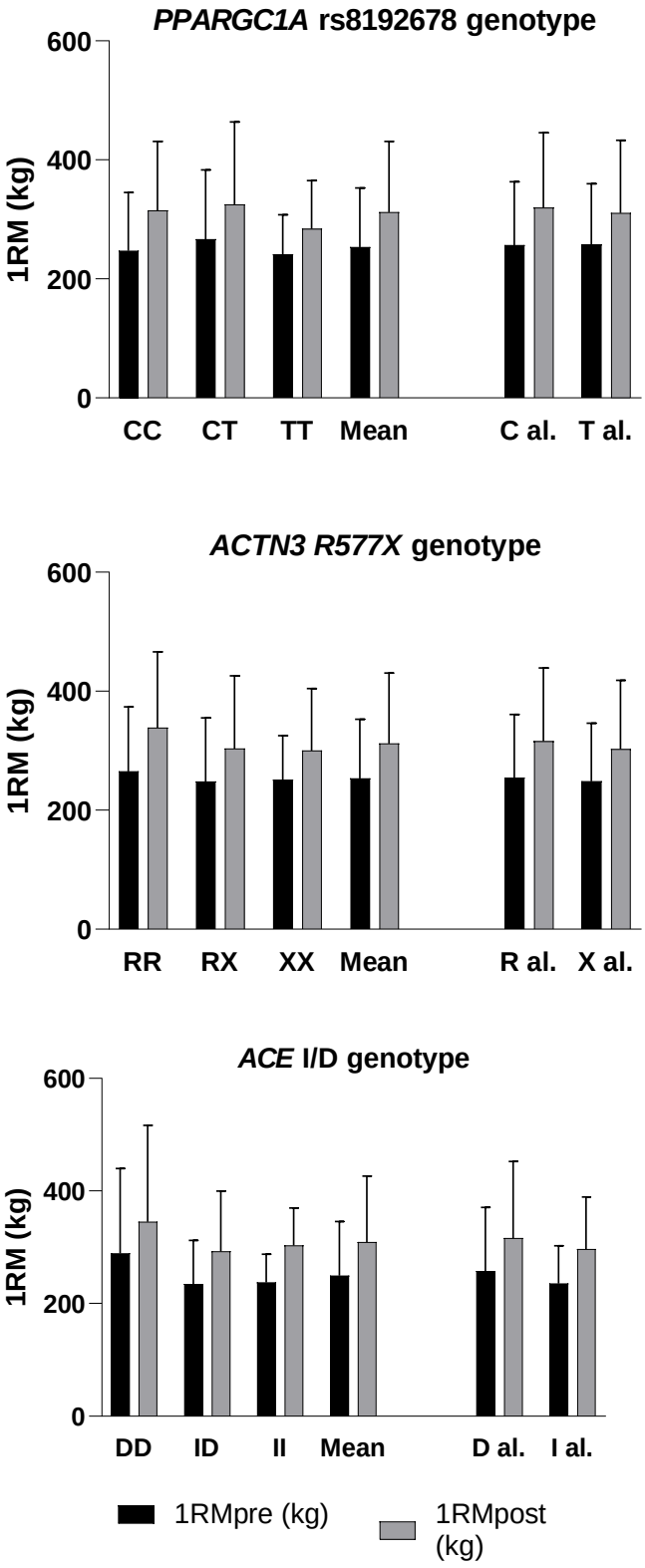


*Figure S1 One-repetition maximum at baseline (1RMpre) and after (1RMpost) an 8-week maximal strength training program expressed in kilograms (kg) by genotype and alleles (al.) for PPARGC1A rs8192678, ACTN3 R577X and ACE I/D polymorphisms.*

Table S1 Pairwise genotype and allele comparisons for the *ACTN3* R577X, *PPARGC1A* rs8192678 and *ACE* I/D polymorphisms, 1RMcorr (baseline) and Δ1RM(%).

| | | 1RMcorr (baseline) | | | | | | Δ1RM % | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pair comparisons | N | Means | SD | Diff. | % diff. | Cohen's d | P-value | Means | SD | Diff. | % diff. | Cohen's d | P-value |
| *ACTN3* | | | | | | | | | | | | | |
| RR/RX | 13/25 | 18.01/20.23 | 3.71/4.90 | 2.22 | 11.61 | 0.50 | 0.336 | 30.00/23.59 | 18.17/13.36 | 6.41 | 23.92 | 0.43 | 0.368 |
| RR/XX | 13/11 | 18.01/19.52 | 3.71/4.60 | 1.50 | 8.04 | 0.37 | 0.700 | 30.00/18.68 | 18.17/6.66 | 11.33 | 46.54 | **0.82** | 0.121 |
| RX/XX | 25/11 | 20.23/19.52 | 4.90/4.60 | 0.71 | 3.58 | 0.15 | 0.902 | 23.59/18.68 | 13.36/6.66 | 4.92 | 23.27 | 0.42 | 0.587 |
| R al./XX | 38/11 | 19.47/19.52 | 4.60/4.60 | 0.05 | 0.25 | 0.01 | 0.976 | 25.79/18.68 | 15.24/6.66 | 7.11 | 31.99 | 0.52 | 0.141 |
| RR/X al. | 13/36 | 18.01/20.01 | 3.71/4.76 | 2.00 | 10.53 | 0.45 | 0.177 | 30.00/22.09 | 18.17/11.85 | 7.91 | 30.38 | 0.58 | 0.082 |
| *PPARGC1A* | | | | | | | | | | | | | |
| CC/CT | 20/19 | 17.77/21.26 | 4.37/4.49 | 3.49 | 17.89 | **0.80** | 0.042* | 29.26/22.00 | 17.54/11.73 | 7.25 | 28.30 | 0.49 | 0.230 |
| CC/TT | 20/10 | 17.77/19.53 | 4.37/4.11 | 1.76 | 9.46 | 0.42 | 0.554 | 29.26/18.21 | 17.54/4.62 | 11.05 | 46.55 | 0.77 | 0.102 |
| CT/TT | 19/10 | 21.26/19.53 | 4.49/4.11 | 1.73 | 8.47 | 0.40 | 0.573 | 22.00/18.21 | 11.73/4.62 | 3.80 | 18.87 | 0.39 | 0.756 |
| C al./TT | 39/10 | 19.47/19.53 | 4.71/4.11 | 0.06 | 0.33 | 0.01 | 0.969 | 25.72/18.21 | 15.25/4.62 | 7.51 | 34.21 | 0.55 | 0.011* |
| CC/T al. | 20/29 | 17.77/20.66 | 4.37/4.37 | 2.90 | 15.07 | 0.67 | 0.027* | 29.26/20.70 | 17.54/9.93 | 8.56 | 34.28 | 0.67 | 0.058 |
| *ACE* | | | | | | | | | | | | | |
| DD/ID | 12/21 | 19.47/19.98 | 5.20/3.64 | 0.51 | 2.60 | 0.12 | 0.948 | 21.54/23.74 | 11.15/11.50 | 2.20 | 9.74 | 0.20 | 0.904 |
| DD/II | 12/14 | 19.47/18.91 | 5.20/5.20 | 0.56 | 2.91 | 0.11 | 0.948 | 21.54/28.49 | 11.15/19.32 | 6.96 | 27.81 | 0.44 | 0.433 |
| ID/II | 21/14 | 19.98/18.91 | 3.64/5.20 | 1.07 | 5.50 | 0.25 | 0.776 | 23.74/28.49 | 11.50/19.32 | 4.75 | 18.19 | 0.32 | 0.599 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D al./II | 31/16 | 19.83/18.97 | 4.23/5.02 | 0.86 | 4.41 | 0.19 | 0.54 | 23.22/27.26 | 11.55/18.33 | 4.04 | 15.99 | 0.29 | 0.36 |
| DD/I al. | 12/35 | 19.47/19.56 | 5.20/4.29 | 0.09 | 0.43 | 0.02 | 0.956 | 21.54/25.64 | 11.15/15.04 | 4.11 | 17.40 | 0.29 | 0.392 |

*Corr- corrected for age, gender and body weight raised to the power of 0.67; N- number of subjects; SD- standard deviation; Diff.- difference; al.- allele; P-values are corrected for multiple testing where appropriate (Tukey); Cohen's d effect size: d= <0.35 (trivial), d= 0.35-0.80 (small); d= 0.80-1.50 (moderate; as defined by Rhea [54] specifically for strength training); * P<0.05*

## Paper 3

Jan-Michael Johansen, Sannija Goleva-Fjellet, Arnstein Sunde, Lars Erik Gjerløw, Lars Arne Skeimo, Baard I. Freberg, Mona Sæbø, Jan Helgerud, Øyvind Støren. No change – no gain; the effect of age, sex, selected genes and training on physiological and performance adaptations in cross-country skiing. Front. Physiol., 26 October 2020; https://www.frontiersin.org/articles/10.3389/fphys.2020.581339/full

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# No Change – No Gain; The Effect of Age, Sex, Selected Genes and Training on Physiological and Performance Adaptations in Cross-Country Skiing

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The aim was to investigate the effect of training, sex, age and selected genes on physiological and performance variables and adaptations before, and during 6 months of training in well-trained cross-country skiers. National-level cross-country skiers were recruited for a 6 months observational study (pre – post 1 – post 2 test). All participants were tested in an outside double poling time trial ($TT_{DP}$), maximal oxygen uptake in running (RUN-$VO_{2max}$), peak oxygen uptake in double poling (DP-$VO_{2peak}$), lactate threshold (LT) and oxygen cost of double poling ($C_{DP}$), jump height and maximal strength (1RM) in half squat and pull-down. Blood samples were drawn to genetically screen the participants for the *ACTN3* R577X, *ACE* I/D, *PPARGC1A* rs8192678, *PPARG* rs1801282, *PPARA* rs4253778, *ACSL1* rs6552828, and *IL6* rs1474347 polymorphisms. The skiers were instructed to train according to their own training programs and report all training in training diaries based on heart rate measures from May to October. 29 skiers completed all testing and registered their training sufficiently throughout the study period. At pre-test, significant sex and age differences were observed in $TT_{DP}$ ($p < 0.01$), DP-$VO_{2peak}$ ($p < 0.01$), $C_{DP}$ ($p < 0.05$), MAS ($p < 0.01$), $LT_v$ ($p < 0.01$), 1RM half squat ($p < 0.01$), and 1RM pull-down ($p < 0.01$). For sex, there was also a significant difference in RUN-$VO_{2max}$ ($p < 0.01$). No major differences were detected in physiological or performance variables based on genotypes. Total training volume ranged from 357.5 to 1056.8 min per week between participants, with a training intensity distribution of 90–5–5% in low-, moderate- and high-intensity training, respectively. Total training volume and ski-specific training increased significantly ($p < 0.05$) throughout the study period for the whole group, while the training intensity distribution was maintained. No physiological or performance variables improved during the 6 months of training for the whole group. No differences were observed in training progression or training adaptation between sexes

or age-groups. In conclusion, sex and age affected physiological and performance variables, with only a minor impact from selected genes, at baseline. However, minor to no effect of sex, age, selected genes or the participants training were shown on training adaptations. Increased total training volume did not affect physiological and performance variables.

**Keywords: endurance training, skiing performance, training adaptations, double poling, maximal oxygen uptake, lactate threshold, work economy, genomics**

## INTRODUCTION

Cross-country skiing is regarded as one of the most demanding aerobic endurance sports, where male and female athletes have displayed some of the highest maximal oxygen uptakes ($VO_{2max}$) ever recorded (Sandbakk and Holmberg, 2017). $VO_{2max}$, often measured in running (RUN-$VO_{2max}$), is suggested as a main predictor for cross-country skiing and overall endurance performance (Pate and Kriska, 1984; Ingjer, 1991; di Prampero, 2003; Støren et al., 2013; Sandbakk and Holmberg, 2017; Sunde et al., 2019; Johansen et al., 2020). However, in cases where RUN-$VO_{2max}$ is relatively homogenous or held constant, differences in work economy (C) (Conley and Krahenbuhl, 1980; di Prampero, 2003) and/or maximal strength (Hoff et al., 2002; Støren et al., 2008; Sunde et al., 2010, 2019) are regarded as major contributors for differentiating performance in endurance athletes.

Although the main determining factors for cross-country skiing performance are relatively clear, the best way to develop these physiological factors over longer periods in every individual skier is still under investigation (Stöggl and Sperlich, 2015). Traditionally, endurance training makes up almost 90% of the total training for competitive cross-country skiers, while the rest is strength training and speed training (Losnegaard et al., 2013; Stöggl and Sperlich, 2015; Sandbakk et al., 2016). The endurance training during season preparation for both junior and senior cross-country skiers is characterized with high volumes of low-intensity training (LIT) and low to moderate volumes of moderate- (MIT) and high-intensity training (HIT). This has been regarded as an "optimal" intensity distribution for developing higher performance capacity in cross-country skiers (Ingjer, 1992; Seiler and Kjerland, 2006; Sandbakk et al., 2016; Solli et al., 2017). Stöggl and Sperlich (2014) suggests that a polarized training intensity distribution, with high LIT volumes (∼80%) and relatively high HIT volumes (∼20%) with low volumes of MIT, would be more beneficial for further improvements of well-trained endurance athletes, compared to training models with higher volumes of MIT. Additionally, higher volumes of HIT are considered as a more efficient way to elevate $VO_{2max}$ compared to LIT, both in well-trained to elite cross-country skiers and recreational skiers (Nilsson et al., 2004; Helgerud et al., 2007; Støren et al., 2012; Rønnestad et al., 2014, 2016; Stöggl and Sperlich, 2014; Johansen et al., 2020).

Ingjer (1992) observed that young cross-country skiers started to level-off in $VO_{2max}$ at age 19–20 following a training regime similar to that described above, at least in values relative to body mass. Following the same training pattern year after year has not proven to be an effective strategy to increase $VO_{2max}$ further in well-trained and elite adult cross-country skiers (Gaskill et al., 1999; Solli et al., 2017). In Gaskill et al. (1999) and Støren et al. (2012), major changes in the relative intensity distribution of the endurance training led to significant improvements in $VO_{2max}$ and performance in well-trained endurance athletes. However, a recent study showed substantial differences in training response to the same HIT protocol among well-trained cyclists (Bratland-Sanda et al., 2020). This points to the need for better individualization of training programs.

Earlier studies have mainly explored training characteristics in cross-country skiers retrospectively, with no opportunity to investigate the direct physiological effect of the athlete's training. However, the study of Losnegaard et al. (2013) performed several tests through the preparation phase and the competitive season in elite male cross-country skiers competing at an international and national level. The study revealed improvements in skiing economy (V2 skating), $O_2$-deficit and skating performance on a time trial on a roller-skiing treadmill. No improvements were observed in $VO_{2max}$. These were the results of a traditional high volume LIT and low to moderate volume of MIT and HIT regime. However, mainly retrospective studies have been performed on sub-elite and junior cross-country skiers over longer time periods (>10 weeks). No studies have investigated training characteristics and the subsequent physiological effects in both sub-elite senior and junior cross-country skiers competing at a national and regional level over longer periods.

Sex differences in performance determining factors in cross-country skiing is generally reported to be between 10 and 30%, where greater sex differences are shown when the upper-body is used more extensively (Sandbakk et al., 2014; Hegge et al., 2016; Sunde et al., 2019). Sex differences have been examined in recent years among cross-country skiers, however, sex comparisons in training responses to a similar training regimen is not well examined in well-trained cross-country skiers. Previous investigations have revealed no difference in training responses between males and females following the same training program in both sedentary and well-trained individuals (Astorino et al., 2011; Støren et al., 2017; Varley-Campbell et al., 2018), suggesting that this may also be the case for well-trained cross-country skiers. Although both junior and senior skiers have been investigated separately (Ingjer, 1992; Sandbakk et al., 2010, 2016; Losnegaard et al., 2013), direct comparisons of training responses in these age-groups have not been executed previously in cross-country skiers. Investigations of both sex and age-related

differences in training responses may be crucial to understand differences in training adaptations, and further improve the quality of the individualization of training programs.

The genetic component of sports performance and trainability has received increasing attention the last two decades. Sports performance is considered a complex trait, influenced by many genes. A number of single nucleotide polymorphisms (SNPs) have been associated with various aspects of athletic ability and sports performance. Two polymorphisms that have been intensely investigated are the *ACTN3* R577X and *ACE* I/D (Jacques et al., 2019). The *ACTN3* gene codes for α-actinin-3, a protein expressed in fast-twitch muscle fibers. The common R577X polymorphism leads to the deficiency of the protein in individuals with the XX genotype (North et al., 1999), which is the case for around 19% of Caucasians (Roth et al., 2008; Goleva-Fjellet et al., 2020). Lack of the α-actinin-3 has been associated with increased muscle endurance, and decreased maximal power generation (MacArthur et al., 2008). The *ACE* gene encodes the angiotensin I-converting enzyme, having a role in the regulation of blood pressure, fluid-electrolyte balance and affecting the muscle function (Puthucheary et al., 2011; Pescatello et al., 2019). *ACE* seems to play a role in exercise induced adaptations and the I allele has been regarded as the endurance allele (Ma et al., 2013; Pescatello et al., 2019). Few studies have investigated these polymorphisms in relation to cross-country skiing performance. Magi et al. (2016) found higher frequencies of the *ACTN3* RR and *ACE* ID genotype in male skiers compared to controls. In addition, male skiers with XX genotype tended to exhibit greater increase in $VO_{2peak}$ over a 5-year period. The same finding applied to female skiers with the ID genotype. Orysiak et al. (2013), on the other hand, did not find any associations between the *ACE* I/D and $VO_{2max}$ in well trained winter sports athletes. No previous studies have compared the genotype distribution for selected genes between regional to national cross-country skiers and the normal population within the same region. Goleva-Fjellet et al. (2020) genotyped *ACE* and *ACTN3* in a cohort representing the region of South East Norway, making it possible to compare this with an athletic cohort.

The *PPARGC1A* rs8192678 SNP has also gained attention in exercise genetics. The protein encoded by the gene, PGC1α (peroxisome proliferator-activated receptor gamma co-activator-1-alpha), induce the mitochondrial biogenesis and modulate the composition and functions of the mitochondria (Austin and St-Pierre, 2012). Recent reviews have concluded that the rs8192678 polymorphism is associated with aerobic trainability and sports performance (Petr et al., 2018, 2020; Tharabenjasin et al., 2019). Peroxisome proliferator-activated receptor genes, e.g., *PPARG* (rs1801282) and *PPARA (*rs4253778), have also been investigated in relation to trainability and athletic ability (Petr et al., 2018, 2020). According to Bouchard et al. (2011) the rs6552828 SNP of the acyl-CoA synthase long-chain member 1 gene (*ACSL1*) could explain around 6% of the training response of $VO_{2max}$ to standardized exercise training programs. A recent study by Harvey et al. (2020) reported that the rs1474347 polymorphisms in the interleukin-6 (*IL6*) gene was associated with training induced improvements in $VO_{2max}$ in both moderately and well trained participants.

To the best of our knowledge, no study have investigated effects of sex, age, training and selected genes on physiological and performance adaptations in the same study. Therefore, the primary aim of this study was to investigate training adaptations in physiological and performance variables in well-trained cross-country skiers after 6 months of training during season preparation (i.e., May to October). Secondly, we wanted to investigate possible differences between gender and age groups in baseline values and training adaptations during the study period. Thirdly, we wanted to investigate the effects of specific candidate genes on physiological and performance variables at baseline. We hypothesized that age and sex would influence on baseline values, but not training adaptations, and that differences in training would impact training adaptations. Further, we hypothesized that the distribution of the selected genetic variants would represent the distribution of the general population for this region and not impact physiological or performance values at baseline.

# MATERIALS AND METHODS

## Experimental Approach

The main purpose of this study was to evaluate changes in physiological and performance variables after 6 months of training (May to October) in well-trained cross-country skiers. We also wanted to compare baseline values and training induced changes in males and females, and young and older skiers, as well as in skiers with different genotypes. Therefore, the participants were instructed to train according to their own training programs worked out by themselves or their coaches prior to the research project, and report their daily training for the whole 6 months period. They were tested for a number of physiological, strength and performance variables over 2 days at three occasions; before (PRE), mid-way (POST1) and after (POST2) the study period. The test battery consisted of measurements of RUN-$VO_{2max}$, $VO_{2peak}$ in double poling (DP-$VO_{2peak}$), time to exhaustion (TTE), oxygen cost of double poling ($C_{DP}$), lactate threshold in double poling (LT), jump height, 1RM and maximal power tests in half squat and pull-down and performance in a 5.64 km double poling time trial ($TT_{DP}$). At baseline, blood samples were drawn to assess gene status in selected genes.

## Subjects

A total of 46 well-trained cross-country skiers (30 males and 16 females), differing in age (16–48 years) and performance-level, were recruited for the whole study. The study's medical doctor approved all participants for participation. However, 17 skiers were excluded because they were not able to fulfill the requirements of three testing sessions during the study period due to sickness or injuries or did not report their training habits sufficiently. Thus, 29 skiers were included in the statistical analyzes. To investigate age-related effects the included skiers were divided in two age groups (16–18 and ≥19 years). These groups were defined as either in, or above puberty, and also corresponding to in, or above high-school age. The ≥19 group included skiers from 19 to 48 years. All subjects were recruited by invitation to high-schools for skiers in Southeastern Norway

or regional cross country ski teams. The included skiers differed substantially in performance level, from medium-junior level to top national level. The best male and female skiers had finished top 10 in numerous VISMA ski classics races (i.e., Vasaloppet and Marcialonga) and/or top 30 in the Norwegian national championship, and the slowest skiers finished in the lower part of national junior competitions. Subjects' characteristics are summarized in **Table 1**.

The study was conducted in accordance with the Declaration of Helsinki, and evaluated and approved by the regional ethics committee of Southeast Norway (REK 2017/2522) and the institutional research board at the University of South-Eastern Norway (former University College of South-Eastern Norway). After having received information about the study, all participants gave their written informed consent before participation. Parental written consent was collected for skiers below 18 years.

## Test Procedures

In order to evaluate changes in physiological and performance variables related to the skiers training, all participants were tested at three separate occasions. PRE were performed in April/May, POST1 were performed in July/August, and POST2 were conducted in October/November. All testing procedures were the same at all testing sessions.

All tests were performed on two consecutive days. The participants were instructed to do only light training the last 24 h before testing, and no food or nutritious drinks were allowed 1 h before the first test. In between tests, the participants were allowed to eat a light meal of energy-rich food and drinks. The last meal before testing and food intake in-between tests were registered, and all participants were asked to consume the same food in the subsequent testing sessions (POST1 and POST2). All preparation procedures were the same at all three testing sessions. The tests were also conducted at approximately the same time of day (±2 h) at PRE, POST1 and POST2 to avoid circadian differences.

The first day of testing consisted of three maximal jump height tests, an incremental running test for determining RUN-$VO_{2max}$, and a $TT_{DP}$. Before the jump tests, the participants performed a self-conducted warm-up procedure of at least 10 min. This warm-up was registered and repeated at POST1 and POST2. Then they performed three separate jump tests in the following order: squat jump (SJ), counter-movement jump (CMJ) and counter-movement jump with arm swing (CMJas). For the SJ tests, the knee-angle were 90° and this was controlled by the same test leader at all tests. No counter-movements were allowed in this particular test, whereas no counter-movement restrictions were given for the CMJ and CMJas tests. All participants were given at least three consecutive attempts in each jump-test, and the best attempt was registered as the result. At least 3 min of rest were given between the separate jump tests to ensure sufficient restitution. All jump-tests were performed by use of a force platform (Ergotest Innovation, Porsgrunn, Norway) for jump height measurements. The force platform was calibrated in accordance with the manufacturers' manual before each test. Jump height was calculated by the following equation,

$$h = \frac{v_v^2}{2 \times g} \quad (1)$$

where $h$ is jump height, $v$ is the velocity at take-off, which again is based on calculation of force multiplied with time divided by mass, and $g$ is gravitation (Ergotest Innovation, Porsgrunn, Norway).

After at least 20 min of rest, the participants started a 10 min self-conducted warm-up procedure before an incremental $VO_{2max}$ test in running. This warm-up was registered and repeated at POST1 and POST2. The RUN-$VO_{2max}$ test was conducted by the same procedures as presented in Sunde et al. (2019). Briefly, the participants started at an intensity of 6% inclination and 7–8 km · $h^{-1}$ and 9–10 km · $h^{-1}$ for female and male, respectively. The test started with 1% increase in inclination every 30 s until 8% was reached, whereas only speed was increased by 0.5 km · $h^{-1}$ every 30 s after that. All participants were instructed to run to voluntary fatigue, and the three highest subsequent $VO_2$ measurements were used to calculate $VO_{2max}$. Heart rate (HR) ≥ 98% of $HR_{max}$, respiratory exchange ratio (RER) ≥1.05, blood lactate concentration ($[La^-]_b$) ≥ 8.0 mmol · $L^{-1}$, rate of perceived exertion (Borg scale 6–20) ≥17, and flattening of the $VO_2$ curve was used to evaluate if $VO_{2max}$ was reached. The metabolic test system, MetaLyzer II Cortex

**TABLE 1 |** Subjects characteristics.

| Variable | Total ($n$ = 29) | Males ($n$ = 17) | Females ($n$ = 12) | 16–18 years ($n$ = 16) | ≥19 years ($n$ = 13) |
|---|---|---|---|---|---|
| Age (yr) | 22.1 ± 8.4 | 24.1 ± 10.2 | 19.3 ± 4.1 | 17.3 ± 0.8 | 28.0 ± 9.8 |
| Weight (kg) | 69.4 ± 9.3 | 73.2 ± 8.6 | 64.0 ± 7.8** | 64.4 ± 6.7 | 75.5 ± 8.5§§ |
| Height (cm) | 176.2 ± 8.9 | 181.1 ± 7.1 | 169.3 ± 6.3** | 173.8 ± 7.7 | 179.2 ± 9.7 |
| **RUN-$VO_{2max}$** | | | | | |
| mL · $kg^{-1}$ · $min^{-1}$ | 62.9 ± 8.0 | 67.4 ± 6.7 | 56.5 ± 4.5** | 61.1 ± 8.0 | 65.2 ± 7.7 |
| L · $min^{-1}$ | 4.38 ± 0.88 | 4.92 ± 0.68 | 3.60 ± 0.37** | 3.94 ± 0.70 | 4.92 ± 0.79§§ |
| **Training** | | | | | |
| min · $week^{-1}$ | 241.0 ± 162.6 | 604.2 ± 153.1 | 462.1 ± 142.9* | 529.4 ± 180.6 | 557.9 ± 138.7 |

*Values are mean and SD. Yr, years. Kg, kilograms. Cm, centimeters. RUN-$VO_{2max}$, maximal oxygen uptake in running. mL · $kg^{-1}$ · $min^{-1}$, milliliters per kilogram bodyweight per minute. L · $min^{-1}$, liters per minute. $min^{-1}$ week, average weekly training the last 3 months in minutes. *$p < 0.05$ significantly different from male value. **$p < 0.01$ significantly different from male value. §§ $p < 0.01$ significantly different from 16 to 18 years value.*

(Biophysic GmbH, Leipzig, Germany) was used for all $VO_2$ measurements, with measurements every 10 s. Before testing the $O_2$-analyzer were calibrated with ambient air and certified calibration gases (16% $O_2$/4% $CO_2$), while the flow sensors were calibrated with a 3-L calibration syringe (Biophysic GmbH, Leipzig, Germany) before each test. The treadmill used was a Woodway PPS 55 sport (Waukesha, WI, United States), calibrated for speed and incline. HR were registered by the participants own heart rate monitors or by Polar s610 HR monitors (Kempele, Finland).

After at least 1 h of rest, a 5.64 km $TT_{DP}$ test was performed in a paved roller ski course track of 940 m. The TT procedures have been previously presented in Sunde et al. (2019). Only the DP technique was allowed throughout the test. The TT was organized with individual starts, and 30 s starting intervals. Drafting was not allowed. The subjects used their own roller-skis for classic skiing and poles and were instructed to use wheel type 2 for the time trial test. All subjects used the same pair of roller skis at PRE, POST1, and POST2. Differences in temperature and humidity may influence the rolling resistance of the roller skis, and thus the results of this test. Therefore, we used the same procedures for calculating a correction factor described previously in Sunde et al. (2019).

The second day of testing consisted first of sub-maximal $VO_2$ and $[La^-]_b$ measurements in DP, in order to determine $C_{DP}$ and LT. This was, after 5 min of active recovery, followed by a ramp protocol to exhaustion to determine DP-$VO_{2peak}$. After 1 h of rest, the second day of testing ended with two maximal strength tests in half-squat and pull-down.

The DP tests were performed on a motorized treadmill specialized for cross-country skiing (Rodby RL 2700E, Rodby Innovation, Vänge, Sweden). Every participant performed one 30-min workout for familiarization to the DP treadmill before testing, as previously used in Sunde et al. (2019). All participants used the same pair of roller skis at all DP tests during the study period (Swenor Fiberglass, Sarpsborg, Norway) with the same binding system (NNN, Rottefella, Klokkarstua, Norway). The subjects were allowed to use their own poles and additional skiing equipment, which was the same in all three test sessions. During treadmill testing, the participants were attached to a safety harness, connected to the roof, to avoid falling. Three to six 4-min work periods, with registration of $VO_2$ and HR measurements the last minute, were conducted for calculating $C_{DP}$ at LT intensity and LT. Work periods were only separated by 1-min for measurements of $[La^-]_b$. Whole blood lactate values were measured by a Lactate Scout+ (SensLab GmbH, Leipzig, ray Inc., Kyoto, Japan). The subjects started the first work period at a work intensity assumed to be 50–70% of their DP-$VO_{2peak}$. This corresponded to 10–11.5 $km \cdot h^{-1}$ and 4% inclination for males and 6–8 $km \cdot h^{-1}$ and 4% inclination for females. In the following work periods, the speed increased by 1–3 $km \cdot h^{-1}$, and the test terminated after $[La^-]_b$ levels exceeding the subjects' LT. Warm up lactate value (i.e., the lowest measured lactate value) + 2.3 $mmol \cdot L^{-1}$ were used to define LT. This is in accordance with the protocol from Helgerud et al. (1990) and described and discussed in detail in Støren et al. (2014) and Sunde et al. (2019).

After 5-min of active rest, the subjects performed the RAMP protocol to exhaustion for determining DP-$VO_{2peak}$. The starting intensity was set to 6% inclination and 7 $km \cdot h^{-1}$ for both genders. The inclination was constant through the whole test, while speed increased by 1 $km \cdot h^{-1}$ every 60 s. All participants received motivational feedback throughout the test. The test terminated when the skiers slowly moved backward, despite intense motivational feedback, and reached a pre-defined mark 1 m behind the subjects starting position on the treadmill. TTE was registered and the DP-$VO_{2peak}$ was defined as the mean of the two highest subsequent $VO_2$-measurements. Maximal aerobic speed (MAS) in double poling were calculated in the same way as presented in Sunde et al. (2019) and Johansen et al. (2020), i.e., DP-$VO_{2peak}/C_{DP}$.

A 60-min rest period were given prior to the tests of 1RM and maximal power output in half-squat (Smith-machine, PreCore, Woodinville, WA, United States) and pull-down (Gym 2000, Vikersund, Norway). Pilot testing in Støren et al. (2008) showed no deterioration in 1RM half-squat 30 min after maximal aerobic tests, thus we considered 60-min to be more than sufficient to give valid maximal strength results. The strength tests protocol is identical to the protocol used in Sunde et al. (2019). Both strength tests started with 10 reps at approximately 50% of 1RM. After this, the following sets were performed at approximately 60% (5 reps), 70% (3 reps), and 80% (2 reps), only separated by 3 min rest periods. All repetitions were performed with a slow eccentric phase with a complete stop of movement in the lowest position (half-squat) or the highest position (pull-down) of approximately 1 s. This was followed by a maximal mobilization in the concentric phase. The MuscleLab system (Ergotest Innovation, Porsgrunn, Norway) calculated power output by measurements of lifting time and distance of work. After the sub-maximal series, the participants performed at least 1 rep at their estimated 1RM. From there on: 1 rep, and load increments of 2.5–10 kg from the subsequent lift, were conducted until 1RM was reached.

## Training Registration

The participants were instructed to train according to their own training plans worked out by themselves or by their coaches throughout the study period, without any influence or interventional instructions from the research personnel. All participants recorded training data in digital training diaries, i.e., in an online diary from the Norwegian Olympic Federation, or in Polar Flow. The athletes had all used digital training diaries for at least 1 year prior to the study. Every training session and competition was recorded and controlled by the same research personnel throughout the study period, and 3-months prior to PRE. The two training periods between PRE to POST1 and POST1 to POST2 were defined as 1st training period ($P_1$) and 2nd training period ($P_2$). In order to investigate potential changes in training inside $P_1$ and $P_2$, the periods have been further divided into a total of four periods where appropriate ($P_{1A}$, $P_{1B}$, $P_{2A}$, and $P_{2B}$).

All training data were systemized based on training modality and training intensity. Training modality was either endurance,

strength, speed/jump or other, and activity was running, roller-skiing, cross-country skiing or cycling. Roller-skiing and cross-country skiing on snow were defined as ski-specific training, while running and cycling was defined as unspecific training. Endurance training intensity were monitored as HR "time in zone," and categorized into three intensity zones: (1) low-intensity training (LIT; ≤81% of HRmax), (2) moderate-intensity training (MIT; 82–87% of HRmax), and (3) high-intensity training (HIT; ≥88% of HRmax). All endurance training and competitions were performed with the skiers' personal heart rate monitors. This is in accordance with the procedures used in Støren et al. (2008) and Sunde et al. (2010).

Strength training consisted mainly of maximal strength training and/or general strength training. Maximal strength training was targeting large muscle groups, i.e., 1–6 repetitions in, i.e., half squat, pull-down or deadlift. General strength training was performed with 10–30 repetitions and with a main purpose of increase stability and general strength in the upper-body and trunk. The duration of strength training sessions where quantified as the time between the first set of the first exercise and last set of the last exercise, including rest periods between sets and exercises. Additional warm-up and cool-down were registered as LIT, while stretching where included in "other training." Jump training (i.e., 1–6 box-jumps or jump exercises in stairs) was quantified in the same manner as strength training. Speed training during LIT- or MIT-sessions was mainly performed during ski-specific training. The number of sprints were multiplied by 1.5 min since the period after each sprint was performed at a very low intensity. The monitoring of strength-, speed-, and jump training is in accordance with the quantification procedures used in Sandbakk et al. (2016).

## DNA Sampling and Genotyping

Venous blood was drawn when the participants first attended to the laboratory before the physiological testing procedures at the first testing session (April/May). The EDTA tubes were stored at −20°C. Before the DNA extraction, the samples were thawed at room temperature. DNeasy Blood & Tissue Kit (Qiagen, MD, United States) was used to extract the DNA from 100 μl of blood following the manufacturer's instructions.

*ACE* I/D polymorphism, rs4343 polymorphism in the *ACE* gene was genotyped as it might be the best proxy to I/D polymorphism (Abdollahi et al., 2008), than analyzed to determine the I/D genotype. Genotyping for all polymorphisms was performed using TaqMan® SNP Genotyping Assay. Assay IDs were as follows: C__11942562_20 for *ACE* rs4343; C____590093_1 for *ACTN3* R577X; C__30469648_10 for *ACSL1* rs6552828; C___1643192_20 for the *PPARGC1A* rs8192678; C___1839698_20 for *IL6* rs1474347; C___1129864_10 for *PPARG* rs1801282 and C___2985251_20 for *PPARA* rs4253778 polymorphism (Thermo Fisher Scientific, MA, United States). StepOnePlus™ Real-Time PCR System (Applied Biosystems®, CA, United States) was used to carry out the qPCR. Genotype calling was performed by StepOne Software v2.0. 15 μl of final reaction volume contained 8.44 μl Genotyping Master Mix, 0.42 μl Assay mix (40×), 6.33 μl double distilled $H_2O$ and ∼100 ng of DNA template. Cycling conditions were as follows: 30 s at 60°C was followed by initial denaturation step for 10 min at 95°C; then, 40 cycles of denaturation at 95°C for 15 s were followed by annealing at 60°C for 1 min in cycling stage, finishing with the final post-read step for 30 s at 60°C.

## Statistical Analyzes

Normality tests and Q-Q plots were used to evaluate normal distribution for main variables ($TT_{DP}$, RUN-$VO_{2max}$ and MAS). In all cases, a normal distribution was observed, thus parametric statistics were used. Values were expressed as mean ± SD, and inter-individual variability in training and physiological variables were expressed as coefficient of variance (CV). To evaluate potential changes in physiological response and training characteristics for the total group, within sexes and within age groups, a Univariate General Linear Model (GLM) test with Tukey *Post Hoc*-tests was used. To examine potential differences between sexes and age groups in physiological response and training characteristics during the study period, GLM Univariate with pairwise comparisons and independent sample *t*-tests were conducted. For correlations between baseline values, and between differences between different test points (delta correlations), correlation coefficients *r* was used from Pearson's bivariate tests. Correlation coefficients were evaluated in accordance with Hopkins (2000), which are presented in detail previously (Sunde et al., 2019). Since the participants represented both female and male skiers, also partial correlations were conducted corrected for sex and age.

One-way ANOVA with Tukey *Post Hoc*-tests was used to assess the associations between the genotypes and physiological and performance variables at baseline. To assess the effects of the alleles on these variables, a two-tailed independent sample *t*-test was applied. In order to test for the Hardy-Weinberg equilibrium (HWE) for all polymorphisms and to compare the genotype frequencies to those of other studies, Pearson's Chi-square test ($\chi^2$) was used. When analyzing effects of different genotypes on physiological parameters, all female values from the physiological tests were multiplied according to the average gender difference between males and females in the present study. This was conducted to avoid bias effects of different gender representation for the different candidate genes and genotypes. In order to promote comparability between candidate gene studies, effect size (Cohen's *d*) was calculated using Microsoft® Excel® (Redmond, WA, United States) for the gender corrected variables across the genotypes (**Supplementary Table 6**). The effect size was interpreted as follows: below 0.50 – small effect, 0.5 and above – moderate effect, 0.8 and above – large effect (Cohen, 1988). As the participants were following individual training programs, genetic analyzes of trainability were not performed. For all statistical analyzes performed, the statistical package for social science version 26 (SPSS, IBM, Chicago, IL, United States) was used. A $p$ value $< 0.05$ was accepted as statistically significant in all tests (two-tailed).

Power calculations prior to the study revealed that with a between-group difference in the selected physiological variables of 5%, and with a common standard deviation of the same size, a sample size of 12 to 16 subjects were needed in each age- and gender group in order accomplish a significant level of 0.05 and

a power of 80%. Regarding the genetic variables, the material is under-powered in order to accomplish full genetic analyses. Multivariate ANOVA analyzes between the different genotypes and the different physiological variables were thus not performed. However, the material was still interesting in order to see if there were substantial differences in physiological variables related to single genes. Also, the material was sufficient to investigate if the cohort of skiers differentiated from a general population from the same geographical area in genotype and allele frequencies.

# RESULTS

## Training Characteristics

The skiers training was registered for 23.4 ± 2.2 weeks from PRE to POST2. From PRE to POST1 the skiers trained for 12.7 ± 1.7 weeks, and for 10.7 ± 1.4 weeks from POST1 to POST2. In total, 8460 training sessions were registered, with 5957 inside the 6-months study period. The remaining sessions registered were conducted in the 3 months before PRE. This corresponded to an average of 205 ± 48 sessions per skier during the study period, and 292 ± 72 sessions per skier when the training period before PRE were included.

Training characteristics for the whole group in $P_1$ and $P_2$ are presented in **Table 2**, while the sub-periods ($P_{1A}$, $P_{1B}$, $P_{2A}$, and $P_{2B}$) are presented in **Supplementary Table 3**. The mean total training volume in $P_1$ was 701.5 ± 169.8 min · week$^{-1}$ and increased significantly to 753.2 ± 137.6 min · week$^{-1}$ in $P_2$ ($p < 0.05$). Total endurance training accounted for 86.9 ± 6.6 and 84.4 ± 7.1% of total training volume in $P_1$ and $P_2$, respectively. The relative intensity distribution in the endurance training was 90.0 ± 4.3, 4.8 ± 2.2, and 5.2 ± 3.0% in LIT, MIT, and HIT, respectively, in $P_1$. In $P_2$, LIT, MIT, and HIT represented 89.6 ± 3.2, 4.8 ± 2.2, and 5.7 ± 2.4%, respectively. The relative intensity distribution did not change significantly throughout the 6-months training period. Ski-specific training accounted for 49.7 ± 13.6 and 55.7 ± 10.5% of total endurance training in $P_1$ and $P_2$, respectively. Total ski-specific training and ski-specific LIT increased significantly from $P_1$ to $P_2$ ($p < 0.01$), while ski-specific MIT and HIT remained unchanged. In total, 65.2 ± 18.0 and 62.4 ± 17.7% of ski-specific training was performed as classic skiing, while the remaining 34.8 ± 17.3 and 37.6 ± 17.7% was performed as freestyle-skiing in $P_1$ and $P_2$, respectively. Most of the remaining volume of total endurance training were performed either as running (40.1 ± 9.8% in $P_1$, 38.6 ± 9.0% in $P_2$) or as cycling (9.9 ± 14.7% in $P_1$, 5.6 ± 7.0% in $P_2$).

Strength training was performed regularly with 1–3 sessions per week throughout the study period. In $P_1$, strength training accounted for 8.8 ± 4.0% of the total training volume while in $P_2$, 10.3 ± 3.8% of total training volume was strength training. The amount of strength training increased significantly from $P_1$ to $P_2$ ($p < 0.01$). Speed/jump and other training stayed unchanged throughout the whole training period while accounting for 1.2 ± 1.3 and 3.1 ± 4.8% in $P_1$ and 1.3 ± 1.4 and 4.0 ± 4.1% in $P_2$, respectively.

**TABLE 2 |** Training characteristics during the 6 months study period ($n$ = 29).

| Variable | $P_1$ (May to July) | $P_2$ (August to October) |
|---|---|---|
| Duration (weeks) | 12.7 ± 1.7 | 10.7 ± 1.4 |
| **Training (min · week$^{-1}$)** | | |
| Total training volume | 701.5 ± 169.8 | 753.2 ± 137.6* |
| **Endurance training** | | |
| LIT | 548.7 ± 148.2 | 569.1 ± 116.9 |
| MIT | 29.4 ± 11.4 | 30.4 ± 14.7 |
| HIT | 31.8 ± 15.7 | 36.0 ± 17.2 |
| Total | 609.8 ± 154.1 | 635.5 ± 126.3 |
| **Training mode** | | |
| Ski specific | 303.1 ± 120.1 | 353.8 ± 105.4** |
| $LIT_{ski}$ | 270.2 ± 108.0 | 313.6 ± 91.0** |
| $MIT_{ski}$ | 15.7 ± 8.4 | 19.4 ± 12.4 |
| $HIT_{ski}$ | 14.0 ± 11.7 | 17.5 ± 10.6 |
| Running | 244.5 ± 77.6 | 245.4 ± 71.8 |
| Cycling | 60.5 ± 95.5 | 35.3 ± 46.2 |
| Strength training | 61.7 ± 30.5 | 77.8 ± 31.4** |
| Speed/jump training | 8.2 ± 8.4 | 9.6 ± 10.4 |
| Other | 21.7 ± 41.8 | 30.5 ± 33.9 |

*Values are mean and SD with coefficient of variance in percentage. min · week$^{-1}$, minutes per week. $P_1$, first training period from May to July. $P_2$, second training period from August to October. LIT, low-intensity training. MIT, moderate-intensity training, HIT, high-intensity training. *$p < 0.05$ significantly different from $P_1$ value. **$p < 0.01$ significantly different from $P_1$ value.*

## Physiological Adaptations

Results in physiological and performance variables at the three testing sessions (PRE, POST1, and POST2) are presented in **Table 3**. No significant changes were observed in physiological and performance variables in the whole group from PRE to POST1, from POST1 to POST2, except for $RER_{RUN}$ ($p < 0.05$), or PRE to POST2.

Correlations between physiological and performance variables at baseline and between delta values in physiological, performance and training variables is presented in **Tables 4–6**. Strong correlations were observed between $TT_{DP}$ and DP-$VO_{2peak}$ ($r = -0.79$, $p < 0.01$), MAS ($r = -0.79$, $p < 0.01$), $LT_v$ ($r = -0.82$, $p < 0.01$), RUN-$VO_{2max}$ ($r = -0.68$, $p < 0.01$), and 1RM pull-down ($r = -0.64$, $p < 0.01$) at baseline for the whole group. Corrected for gender, strong significant correlations were still apparent between $TT_{DP}$ and DP-$VO_{2peak}$ ($r = -0.63$, $p < 0.01$), MAS ($r = -0.58$, $p < 0.01$), and $LT_v$ ($r = -0.64$, $p < 0.01$) at baseline. Corrected for age-groups, the similar strong correlations as seen for the whole group were almost at same level between $TT_{DP}$ and RUN-$VO_{2max}$ ($r = -0.68$, $p < 0.01$), $LT_v$ ($r = -0.77$, $p < 0.01$), DP-$VO_{2peak}$ ($r = -0.76$, $p < 0.01$), MAS ($r = -0.75$, $p < 0.01$), and 1RM pull-down ($r = -0.52$, $p < 0.01$). A strong correlation was also apparent between MAS and $LT_v$, both independent ($r = 0.93$, $p < 0.01$) and dependent ($r = 0.85$, $p < 0.01$ and $r = 0.89$, $p < 0.01$) of gender and age, respectively.

No delta correlations were observed between $\Delta TT_{DP}$ and any delta values of the physiological or training variables (**Tables 5**, **6**). ΔMAS revealed strong significant correlations to $\Delta LT_v$ ($r = 0.57$, $p < 0.01$) and $\Delta C_{DP}$ ($r = -0.85$, $p < 0.01$). Δski specific

**TABLE 3 |** Physiological and performance characteristics during the study period ($n$ = 29).

| Variable | PRE | | POST1 | | POST2[a] | |
|---|---|---|---|---|---|---|
| BW (kg) | 69.4 ± 9.3 | (13.4) | 69.0 ± 8.6 | (12.5) | 69.6 ± 8.3 | (11.9) |
| **$TT_{DP}$** | | | | | | |
| seconds | 875.1 ± 92.8 | (10.6) | 866.9 ± 91.4 | (10.5) | 845.9 ± 88.2 | (10.4) |
| **RUN-$VO_{2max}$** | | | | | | |
| $mL \cdot kg^{-1} \cdot min^{-1}$ | 62.9 ± 8.0 | (12.7) | 64.7 ± 7.7 | (11.9) | 64.1 ± 8.8 | (13.7) |
| $L \cdot min^{-1}$ | 4.38 ± 0.87 | (19.9) | 4.48 ± 0.85 | (19.0) | 4.47 ± 0.86 | (19.2) |
| $mL \cdot kg^{-0.67} \cdot min^{-1}$ | 254.6 ± 36.1 | (14.2) | 261.4 ± 35.4 | (13.5) | 259.7 ± 38.8 | (14.9) |
| HR | 196.6 ± 10.6 | (5.3) | 195.5 ± 10.6 | (5.4) | 193.6 ± 10.9 | (5.6) |
| RER | 1.12 ± 0.03 | (2.7) | 1.11 ± 0.05 | (4.5) | 1.14 ± 0.04* | (3.5) |
| $[La^-_b]$ | 10.1 ± 2.3 | (22.8) | 11.3 ± 2.6 | (23.0) | 10.0 ± 2.1 | (21.0) |
| RPE | 17.2 ± 1.7 | (9.9) | 17.9 ± 1.2 | (6.7) | 17.6 ± 1.4 | (7.9) |
| **DP-$VO_{2peak}$** | | | | | | |
| $mL \cdot kg^{-1} \cdot min^{-1}$ | 54.3 ± 7.3 | (13.4) | 54.6 ± 7.2 | (13.2) | 55.5 ± 7.3 | (13.2) |
| $L \cdot min^{-1}$ | 3.79 ± 0.79 | (20.8) | 3.80 ± 0.73 | (19.2) | 3.89 ± 0.74 | (19.0) |
| $mL \cdot kg^{-0.67} \cdot min^{-1}$ | 220.0 ± 33.0 | (15.0) | 221.0 ± 31.7 | (14.3) | 225.2 ± 32.3 | (14.3) |
| %RUN-$VO_{2max}$ | 86.5 ± 7.3 | (8.4) | 84.4 ± 5.8 | (6.9) | 86.9 ± 5.7 | (6.6) |
| HR | 190.8 ± 9.8 | (5.1) | 190.9 ± 9.9 | (5.2) | 190.8 ± 9.9 | (5.2) |
| RER | 1.10 ± 0.06 | (5.4) | 1.11 ± 0.05 | (4.5) | 1.13 ± 0.05 | (4.4) |
| $[La^-_b]$ | 9.2 ± 2.0 | (21.8) | 9.0 ± 1.9 | (21.1) | 9.0 ± 1.7 | (18.9) |
| RPE | 17.5 ± 1.2 | (6.9) | 17.6 ± 1.1 | (6.3) | 17.5 ± 1.4 | (8.0) |
| TTE (s) | 494.3 ± 125.4 | (25.4) | 524.0 ± 127.9 | (24.4) | 542.9 ± 124.0 | (22.8) |
| **$C_{DP}$ at LT** | | | | | | |
| $mL \cdot kg^{-1} \cdot m^{-1}$ | 0.198 ± 0.021 | (10.6) | 0.193 ± 0.019 | (9.8) | 0.193 ± 0.020 | (10.4) |
| $mL \cdot kg^{-0.67} \cdot m^{-1}$ | 0.800 ± 0.078 | (9.8) | 0.779 ± 0.070 | (9.0) | 0.780 ± 0.066 | (8.5) |
| **MAS** | | | | | | |
| $m \cdot min^{-1}$ | 278.1 ± 52.6 | (18.9) | 285.4 ± 45.1 | (15.8) | 290.3 ± 44.2 | (15.2) |
| $km \cdot h^{-1}$ | 16.7 ± 3.2 | (19.2) | 17.1 ± 2.7 | (15.8) | 17.4 ± 2.7 | (15.5) |
| **LT** | | | | | | |
| %DP-$VO_{2peak}$ | 82.3 ± 6.5 | (7.9) | 82.4 ± 6.3 | (7.6) | 81.6 ± 5.6 | (6.9) |
| HR | 175.4 ± 11.5 | (6.6) | 173.2 ± 11.9 | (6.9) | 172.3 ± 11.9 | (6.9) |
| $VO_2$ | 44.6 ± 6.6 | (14.8) | 44.9 ± 6.4 | (14.3) | 45.3 ± 6.8 | (3.9) |
| $[La^-_b]$ | 4.6 ± 0.6 | (13.0) | 4.7 ± 0.7 | (14.9) | 4.5 ± 0.6 | (13.3) |
| Speed ($km \cdot h^{-1}$) | 13.7 ± 2.5 | (18.7) | 14.1 ± 2.2 | (15.6) | 14.2 ± 2.1 | (14.8) |
| **Strength** | | | | | | |
| 1RM half squat (kg) | 120.8 ± 21.9 | (18.1) | 129.7 ± 24.2 | (18.7) | 131.1 ± 23.3 | (17.8) |
| 1RM pull-down (kg) | 87.4 ± 16.5 | (18.9) | 87.9 ± 15.6 | (17.7) | 89.8 ± 16.2 | (18.0) |
| **Maximal power** | | | | | | |
| Half squat (w) | 808.6 ± 207.6 | (25.7) | 816.6 ± 177.4 | (21.7) | 831.8 ± 180.7 | (21.7) |
| Pull-down (w) | 473.9 ± 152.8 | (32.2) | 469.7 ± 119.1 | (25.4) | 490.3 ± 124.0 | (25.3) |
| SJ (cm) | 28.0 ± 5.1 | (18.2) | 26.8 ± 4.7 | (17.5) | 27.2 ± 4.8 | (17.6) |
| CMJ (cm) | 31.5 ± 5.5 | (17.5) | 31.6 ± 4.2 | (13.3) | 30.7 ± 5.0 | (16.3) |
| CMJas (cm) | 35.9 ± 5.5 | (15.3) | 35.0 ± 4.9 | (14.0) | 33.6 ± 5.2 | (15.5) |

*Values are mean and SD with coefficient of variance in percentage in parenthesis. [a]no effect size for delta physiological variables from PRE to POST2 over 0.5. BW, body-weight. Kg, kilograms. $TT_{DP}$, double poling time trial. RUN-$VO_{2max}$, maximal oxygen uptake in running. $mL \cdot kg^{-1} \cdot min^{-1}$, milliliters per kilogram bodyweight per minute. $L \cdot min^{-1}$, liters per minute. $mL \cdot kg^{-0.67} \cdot min^{-1}$, milliliters per kilogram raised to the power of −0.67 per minute. HR, heart rate. RER, respiratory exchange ratio. $[La^-_b]$, blood lactate concentration. RPE, rate of perceived exertion. %RUN-$VO_{2max}$, fractional utilization of RUN-$VO_{2max}$ at DP-$VO_{2peak}$. TTE, time to exhaustion. $C_{DP}$, oxygen cost of double poling at lactate threshold. $mL \cdot kg^{-1} \cdot m^{-1}$, milliliters per kilogram per meter. $mL \cdot kg^{-0.67} \cdot m^{-1}$, milliliters per kilogram raised to the power of −0.67 per meter. MAS, maximal aerobic speed. LT, lactate threshold. $VO_2$, oxygen uptake. Km, kilometers. H, hours. 1RM, one repetition maximum. W, watt. SJ, squat jump. CMJ, counter movement jump. CMJas, counter movement jump with armswing. Cm, centimeters. *$p < 0.05$ significantly different from POST1 value.*

training and $\Delta LIT_{ski}$ showed low significant correlations to $\Delta LT_v$ ($r = 0.48$, $p < 0.01$ and $r = 0.45$, $p < 0.05$, respectively), while $\Delta LIT_{ski}$ showed a low significant correlation to $\Delta C_{DP}$ ($r = -0.41$, $p < 0.05$).

## Sex Differences

Male skiers trained significantly higher volumes than females 3 months before pre-tests ($p < 0.05$). Additionally, no statistical difference was observed in LIT, MIT, HIT, total endurance

**TABLE 4 |** Baseline correlations between performance and physiological variables ($n$ = 29).

| Variable | $TT_{DP}$ | $VO_{2max}$ ($mL^{-1}$ $kg^{-0.67} \cdot min^{-1}$) | MAS |
|---|---|---|---|
| Age | −0.09 (0.23) | 0.15 (0.25) | 0.07 ( − 0.28) |
| BW | −0.41* ( − 0.01) | 0.43* ( − 0.02) | 0.40* ( − 0.03) |
| **$TT_{DP}$** | | | |
| seconds | – | −0.73** ( − 0.39) | −0.79** ( − 0.58**) |
| **RUN-$VO_{2max}$** | | | |
| $mL \cdot kg^{-1} \cdot min^{-1}$ | −0.68** ( − 0.33) | 0.96** (0.92**) | 0.69** (0.34) |
| $L \cdot min^{-1}$ | −0.71** ( − 0.35) | 0.92** (0.77**) | 0.72** (0.27) |
| $mL \cdot kg^{-0.67} \cdot min^{-1}$ | −0.73** ( − 0.39) | – | 0.75** (0.37) |
| HR | −0.07 ( − 0.07) | −0.02 ( − 0.003) | −0.03 ( − 0.01) |
| RER | −0.14 ( − 0.001) | −0.01 ( − 0.34) | 0.08 ( − 0.15) |
| $[La^{-}_{b}]$ | 0.26 (0.24) | 0.08 (0.27) | −0.22 ( − 0.23) |
| RPE | −0.36 ( − 0.45*) | 0.06 (0.03) | 0.16 (0.21) |
| **DP-$VO_{2peak}$** | | | |
| $mL \cdot kg^{-1} \cdot min^{-1}$ | −0.79** ( − 0.63**) | 0.75** (0.44*) | 0.81** (0.65**) |
| $L \cdot min^{-1}$ | −0.78** ( − 0.57**) | 0.77** (0.34) | 0.79** (0.48*) |
| $mL \cdot kg^{-0.67} \cdot min^{-1}$ | −0.83** ( − 0.69**) | 0.80** (0.46*) | 0.85** (0.68**) |
| %RUN-$VO_{2max}$ | −0.24 ( − 0.32) | −0.20 ( − 0.46*) | 0.24 (0.32) |
| HR | −0.06 (0.08) | −0.001 ( − 0.12) | 0.09 (0.03) |
| RER | 0.18 (0.49*) | 0.11 ( − 0.09) | −0.03 ( − 0.29) |
| $[La^{-}_{b}]$ | 0.08 (0.21) | 0.09 ( − 0.10) | 0.11 ( − 0.04) |
| RPE | −0.01 ( − 0.16) | −0.03 (0.14) | −0.04 (0.13) |
| TTE (s) | −0.84** ( − 0.72**) | 0.78** (0.43*) | 0.93** (0.85**) |
| **$C_{DP}$ at LT** | | | |
| $mL \cdot kg^{-1} \cdot m^{-1}$ | 0.41* (0.17) | −0.40* ( − 0.07) | −0.69** ( − 0.63**) |
| $mL \cdot kg^{-0.67} \cdot m^{-1}$ | 0.27 (0.17) | −0.23 ( − 0.08) | −0.56** ( − 0.62**) |
| **MAS** | | | |
| $m \cdot min^{-1}$ | −0.79** ( − 0.58**) | 0.75** (0.37) | – |
| $km \cdot h^{-1}$ | −0.79** ( − 0.58**) | 0.75** (0.37) | – |
| **LT** | | | |
| %DP-$VO_{2peak}$ | −0.05 ( − 0.13) | −0.03 (0.17) | −0.24 ( − 0.19) |
| HR | 0.08 (0.01) | −0.25 ( − 0.19) | −0.22 ( − 0.11) |
| $VO_2$ | −0.72** ( − 0.56**) | 0.66** (0.46*) | 0.59** (0.37) |
| $[La^{-}_{b}]$ | 0.25 (0.51**) | −0.06 ( − 0.26) | −0.23 ( − 0.51**) |
| Speed ($km \cdot h^{-1}$) | −0.82** ( − 0.64**) | 0.77** (0.48*) | 0.91** (0.84**) |
| **Strength** | | | |
| 1RM half squat | −0.49** ( − 0.15) | 0.59** (0.33) | 0.55** (0.29) |
| 1RM pull-down | −0.64** ( − 0.27) | 0.61** (0.07) | 0.60** (0.13) |
| Power half squat | −0.48** ( − 0.12) | 0.54** (0.10) | 0.49** (0.05) |
| Power pull-down | −0.53** ( − 0.27) | 0.50** ( − 0.14) | 0.54** (0.07) |

*Values are correlation coefficients r and partial correlation coefficients corrected for sex in parenthesis. BW, body-weight. Kg, kilograms. $TT_{DP}$, double poling time trial. RUN-$VO_{2max}$, maximal oxygen uptake in running. $mL \cdot kg \cdot min^{-1}$, milliliters per kilogram bodyweight per minute. $L \cdot min^{-1}$, liters per minute. $mL \cdot kg^{-0.67} \cdot min^{-1}$, milliliters per kilogram raised to the power of −0.67 per minute. HR, heart rate. RER, respiratory exchange ratio. $[La^{-}_{b}]$, blood lactate concentration. RPE, rate of perceived exertion. %RUN-$VO_{2max}$, fractional utilization of RUN-$VO_{2max}$ at DP-$VO_{2peak}$. TTE, time to exhaustion. $C_{DP}$, oxygen cost of double poling at lactate threshold. $mL \cdot kg^{-1} \cdot m^{-1}$, milliliters per kilogram per meter. $mL \cdot kg^{-0.67} \cdot m^{-1}$, milliliters per kilogram raised to the power of −0.67 per meter. MAS, maximal aerobic speed. LT, lactate threshold. $VO_2$, oxygen uptake. Km, kilometers. H, hours. 1RM, one repetition maximum. *$p < 0.05$ significant correlation. **$p < 0.01$ significant correlation.*

training, ski-specific training, running, strength training or other training between males and females in either $P_1$ or $P_2$. Males trained significantly more cycling in $P_2$ ($p < 0.05$), and females trained significantly higher volumes of speed/jump training, both in $P_1$ ($p < 0.01$) and $P_2$ ($p < 0.05$). No statistical difference was observed in training progression throughout the 6-months period between males and females, except for other training ($p < 0.05$). Differences in training characteristics between males and females are shown in **Figures 1A,B** and **Supplementary Table 4**.

Significant sex differences in physiological and performance variables were observed at PRE-tests. Results from physiological and performance tests are presented in **Supplementary Table 1**. Males had on average 14.7% ($p < 0.01$, effect size = 2.28) better $TT_{DP}$ performance, 19.3% ($p < 0.01$, effect size = 1.91) higher RUN-$VO_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$), 19.3% ($p < 0.01$,

**TABLE 5 |** Delta correlations between physiological and performance variables and training ($n$ = 29).

| Variables | RUN-$VO_{2max}$ | DP-$VO_{2peak}$ | $C_{DP}$ | MAS | $LT_v$ | $TT_{DP}$ |
|---|---|---|---|---|---|---|
| Total training | 0.11 | −0.01 | −0.25 | 0.19 | 0.35 | −0.13 |
| Ski specific training | 0.08 | −0.03 | −0.34 | 0.28 | 0.48** | −0.06 |
| $LIT_{ski}$ | 0.07 | −0.11 | −0.41* | 0.29 | 0.45* | −0.05 |
| Strength training | 0.15 | −0.08 | −0.30 | 0.16 | 0.36 | −0.32 |

*Values are correlation coefficient r for delta physiological values from POST1 to POST2, and delta training values that showed significant differences from 1st training period to 2nd training period. RUN-$VO_{2max}$, maximal oxygen uptake in running in milliliters per kilogram body weight raised to the power of −0.67 per minute. DP-$VO_{2peak}$, peak oxygen uptake in double poling in milliliters per kilogram body weight raised to the power of −0.67 per minute. $C_{DP}$, oxygen cost of double poling in milliliters per kilogram bodyweight per meter. MAS, maximal aerobic speed. $LT_v$, velocity at lactate threshold. $TT_{DP}$, double poling time trial. $LIT_{ski}$, ski-specific low intensity training. *$p < 0.05$ significant correlation. **$p < 0.01$ significant correlation.*

effect size = 1.72) higher DP-$VO_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$), 9.1% ($p < 0.01$, effect size = 0.97) better $C_{DP}$ ($mL \cdot kg^{-1} \cdot m$), 30.2% ($p < 0.01$, effect size = 2.04) higher $LT_v$ and a 32.3% ($p < 0.01$, effect size = 2.16) higher MAS than females at baseline. In addition, males were 21.3% ($p < 0.01$, effect size = 1.22) and 30.5% ($p < 0.01$, effect size = 1.86) stronger than females in half squat and pull-down, respectively, and displayed 34.7% ($p < 0.01$, effect size = 1.37) and 45.9% ($p < 0.01$, effect size = 1.37) higher power values in half squat and pull-down, respectively. No significant gender differences were apparent in HR, RER, $[La^-]_b$ or RPE in running or double-poling, %RUN-$VO_{2max}$ or LT%, at baseline (all effect sizes <0.7).

No sex differences were observed in physiological or performance adaptations from PRE to POST2, except for $[La^-]_b$ in RUN-$VO_{2max}$ ($p < 0.05$, effect size = 0.93). From PRE to POST1, only $RER_{RUN}$ ($p < 0.05$, effect size = 0.88), $C_{DP}$ ($p < 0.05$, effect size = 0.77), and LT% ($p < 0.05$, effect size = 0.91) changed significantly different between males and females. However, no gender differences were observed in physiological or performance adaptations from POST1 to POST2. Training adaptations for males and females in key physiological variables are presented in **Figure 2**.

## Age-Group Differences

No age differences were observed in total training volume 3-months before PRE. Total MIT volume was significantly higher in the ≥19 years group ($p < 0.05$) in both $P_1$ and $P_2$, while other training volume was significantly lower in the same group compared to the 16–18 years group. Speed/jump and strength training was significantly lower in the ≥19 years group in $P_2$ ($p < 0.05$ and $p < 0.01$, respectively), while no difference was apparent in $P_1$. No training differences between age groups were displayed in total training volume, LIT, HIT, ski-specific training, running, or cycling during the whole training period. No age group differences were observed in delta training values throughout the whole period. Training characteristics for the two age groups are presented in **Figures 3A,B** and **Supplementary Table 5**.

Results from physiological and performance tests among age-groups are presented in **Supplementary Table 2**. At PRE

**TABLE 6 |** Delta correlations between performance and physiological variables ($n$ = 29).

| Variable | Δ$TT_{DP}$ Δ PRE–POST2 | ΔRUN-$VO_{2max}$ Δ PRE–POST2 | ΔMAS Δ PRE–POST2 |
|---|---|---|---|
| ΔBW | 0.34 | −0.24 | −0.31 |
| **Δ$TT_{DP}$** | | | |
| seconds | – | −0.05 | −0.09 |
| **ΔRUN-$VO_{2max}$** | | | |
| $mL \cdot kg^{-1} \cdot min^{-1}$ | −0.11 | 0.99** | −0.21 |
| $L \cdot min^{-1}$ | 0.08 | 0.93** | −0.20 |
| $mL \cdot kg^{-0.67} \cdot min^{-1}$ | −0.05 | – | −0.21 |
| HR | −0.11 | 0.19 | −0.39* |
| **ΔDP-$VO_{2peak}$** | | | |
| $mL \cdot kg^{-1} \cdot min^{-1}$ | −0.19 | 0.02 | −0.08 |
| $L \cdot min^{-1}$ | 0.03 | −0.11 | −0.11 |
| $mL \cdot kg^{-0.67} \cdot min^{-1}$ | −0.12 | −0.03 | −0.10 |
| %RUN-$VO_{2max}$ | −0.08 | −0.72** | 0.16 |
| HF | 0.10 | −0.25 | −0.15 |
| TTE (s) | −0.25 | 0.30 | −0.02 |
| **Δ$C_{DP}$ at LT** | | | |
| $mL \cdot kg^{-1} \cdot m^{-1}$ | −0.02 | 0.19 | −0.85** |
| $mL \cdot kg^{-0.67} \cdot m^{-1}$ | 0.01 | 0.16 | −0.79** |
| **ΔMAS** | | | |
| $m \cdot min^{-1}$ | −0.09 | −0.21 | – |
| $km \cdot h^{-1}$ | −0.09 | −0.21 | – |
| **ΔLT** | | | |
| %DP-$VO_{2peak}$ | −0.09 | 0.24 | −0.53** |
| HF | 0.12 | −0.09 | −0.46* |
| $VO_2$ | −0.16 | 0.23 | −0.49** |
| $[La^-_b]$ | 0.004 | −0.10 | −0.17 |
| Speed ($km \cdot h^{-1}$) | −0.16 | 0.05 | 0.57** |
| **ΔStrength** | | | |
| Half squat | −0.20 | −0.23 | −0.01 |
| Pull-down | −0.28 | 0.10 | −0.20 |
| **ΔPower** | | | |
| Half squat | 0.30 | −0.15 | −0.17 |
| Pull-down | 0.05 | 0.13 | −0.28 |
| SJ | −0.24 | 0.42* | 0.22 |
| CMJ | −0.12 | 0.13 | −0.12 |
| CMJas | −0.11 | −0.12 | 0.20 |

*Values are correlation coefficients r. ΔBW, change in body-weight. Kg, kilograms. Δ$TT_{DP}$, change in double poling time trial. ΔRUN-$VO_{2max}$, change in maximal oxygen uptake in running. $mL \cdot kg^{-1} \cdot min^{-1}$, milliliters per kilogram bodyweight per minute. $L \cdot min^{-1}$, liters per minute. $mL \cdot kg^{-0.67} \cdot min^{-1}$, milliliters per kilogram raised to the power of −0.67 per minute. HR, heart rate. RER, respiratory exchange ratio. $[La^-_b]$, blood lactate concentration. RPE, rate of perceived exertion. ΔDP-$VO_{2peak}$, change in peak oxygen uptake in double poling. %RUN-$VO_{2max}$, fractional utilization of RUN-$VO_{2max}$ at DP-$VO_{2peak}$. TTE, time to exhaustion. Δ$C_{DP}$, change in oxygen cost of double poling at lactate threshold. ΔMAS, change in maximal aerobic speed. ΔLT, change in lactate threshold. $VO_2$, oxygen uptake. Km, kilometers. H, hours. 1RM, one repetition maximum. SJ, squat jump. CMJ, counter-movement jump. CMJas, counter-movement jump with armswing. *$p < 0.05$ significant delta correlation. **$p < 0.01$ significant delta correlation.*

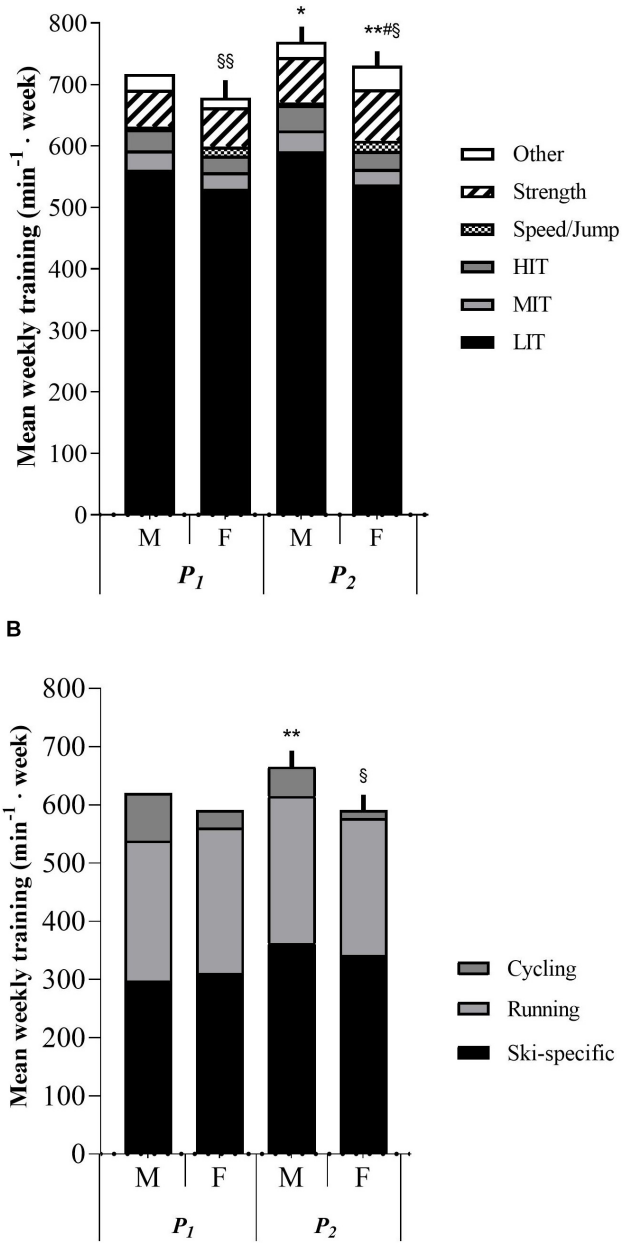


**FIGURE 1 | (A)** Sex differences between males and females in training characteristics in minutes per week. M, males. F, females. $P_1$, first training period from May to July. $P_2$, second training period from August to October. LIT, low-intensity endurance training. MIT, moderate-intensity endurance training. HIT, high-intensity training. min$^{-1}$ week, minutes per week. $^{*}p < 0.05$ significant difference from $P_1$ in strength and other training. $^{**}p < 0.01$ significant difference from $P_1$ in strength training. $^{\#}p < 0.05$ significantly different in delta values in other training from male value. $^{\S}$ $p < 0.05$ significantly different from male value in speed/jump training volume. $^{\S\S}$ $p < 0.01$ significantly different from male value in speed/jump training volume. **(B)** Sex differences between males and females in endurance training mode in minutes per week. $^{**}p < 0.01$ significantly different from $P_1$ value in ski-specific training. $^{\S}$ $p < 0.05$ significantly different from male value in cycling.

the ≥19 years group had a 9.6% ($p < 0.01$) better $TT_{DP}$ performance, 12.0% ($p < 0.05$) higher DP-$VO_{2peak}$ (mL · kg$^{-1}$ · min$^{-1}$), 7.3% ($p < 0.05$) better $C_{DP}$, 23.4% ($p < 0.01$) higher $LT_v$ and 21.6% ($p < 0.01$) higher MAS than the younger skiers. In addition, the oldest skiers were significantly stronger in half squat (15.9%, $p < 0.05$) and pull-down (26.5%, $p < 0.01$), and had higher maximal power values both in half squat (35.1%, $p < 0.01$) and pull-down (41.5%, $p < 0.01$). No differences were observed between age groups in RUN-$VO_{2max}$ (mL· kg$^{-1}$ · min$^{-1}$), RER, $[La^-]_b$, %RUN-$VO_{2max}$ or LT%.

No differences in delta values was observed between age groups from PRE to POST2, except for BW ($p < 0.05$, effect size = 0.94), $[La^-]_b$ in running ($p < 0.05$, effect size = 0.92), $LT_v$ ($p < 0.05$, effect size = 0.87) and power in half squat ($p < 0.05$, effect size = 0.88). Additionally, differences in delta values were observed in RUN-$VO_{2max}$ (mL · kg$^{-1}$ · min$^{-1}$, $p < 0.05$, effect size = 0.87) and power in half squat ($p < 0.01$, effect size = 1.22) from PRE to POST1. No differences were observed in physiological and performance adaptations from POST1 to POST2. Training adaptations in key variables for the two age-groups are presented in **Figure 2**.

## Impact of the Selected Genes

All polymorphisms were successfully genotyped, and were at Hardy-Weinberg equilibrium ($p > 0.05$). Genotype frequencies are displayed in **Table 7**. Minor allele frequencies (MAF) for the genotyped polymorphisms were as follows: 53% for *ACTN3*, 41% for *ACE* and *IL6*, 40% for *ACSL1,* 38% for *PPARGC1A,* 19% for *PPARA* and 9% for *PPARG* polymorphism.

Key physiological and performance results among genotypes in *ACTN3* and *ACE* at baseline is presented in **Table 8**. All genotype and allele data is presented in **Supplementary Table 6**. There were no differences in physiological and performance results between the three *ACTN3* genotypes when analyzing the 29 included skiers. The same picture was shown when analyzing all successfully genotyped participants ($n = 40$), except for a significantly higher DP-$VO_{2peak}$ in the RX genotype compared to the RR genotype (**Supplementary Table 8**). When testing X allele carriers compared to the RR genotype, DP-$VO_{2peak}$ (mL·kg$^{-1}$·min$^{-1}$), both independent of- and corrected for gender, was, respectively, 12.4 and 8.8% higher ($p < 0.05$, effect sizes >0.80).

For the *ACE* I/D genotypes, individuals with the DD genotype displayed an 18.4% better $C_{DP}$ (mL · kg$^{-0.67}$ · m$^{-1}$) compared to those with the II genotype ($p < 0.01$, effect size = 1.42). However, corrected for gender this difference was no longer significant. Individuals with DD genotype also displayed significantly higher values in 1RM pull-down from the II counterparts, both dependent (34%, $p = 0.05$, effect size = 1.67) and independent (23.4%, $p < 0.05$, effect size = 2.66) of gender (**Table 8**). Although the same picture was apparent, all significant associations disappeared when analyzing all 40 successfully genotyped participants (**Supplementary Table 8**). When comparing II genotypes to D carriers we detected a 9.2% higher RUN-$VO_{2max}$ (mL · kg$^{-1}$ · min$^{-1}$), when corrected for

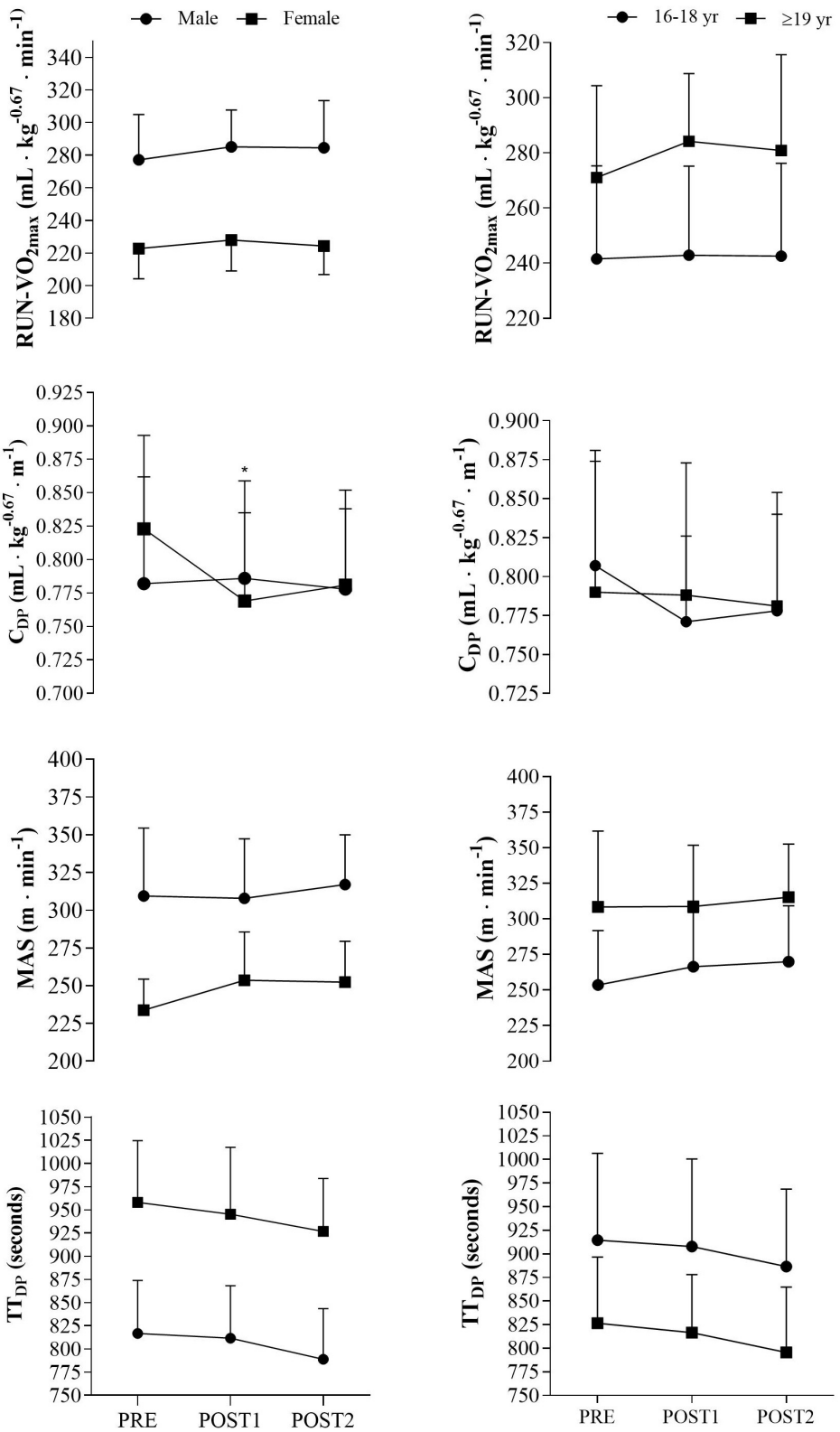


**FIGURE 2 |** Sex and age differences in key physiological and performance variables. RUN-$VO_{2max}$, maximal oxygen uptake in running. mL · $kg^{-0.67}$ · $min^{-1}$, milliliters per kilogram raised to the power of –0.67 per minute. $C_{DP}$, oxygen cost of double poling at lactate threshold. mL · $kg^{-0.67}$ · $m^{-1}$, milliliters per kilogram raised to the power of –0.67 per meter. MAS, maximal aerobic speed. m · $min^{-1}$, meter per minute. $TT_{DP}$, time trial performance in double poling. *$p < 0.05$ significant different from male delta value.

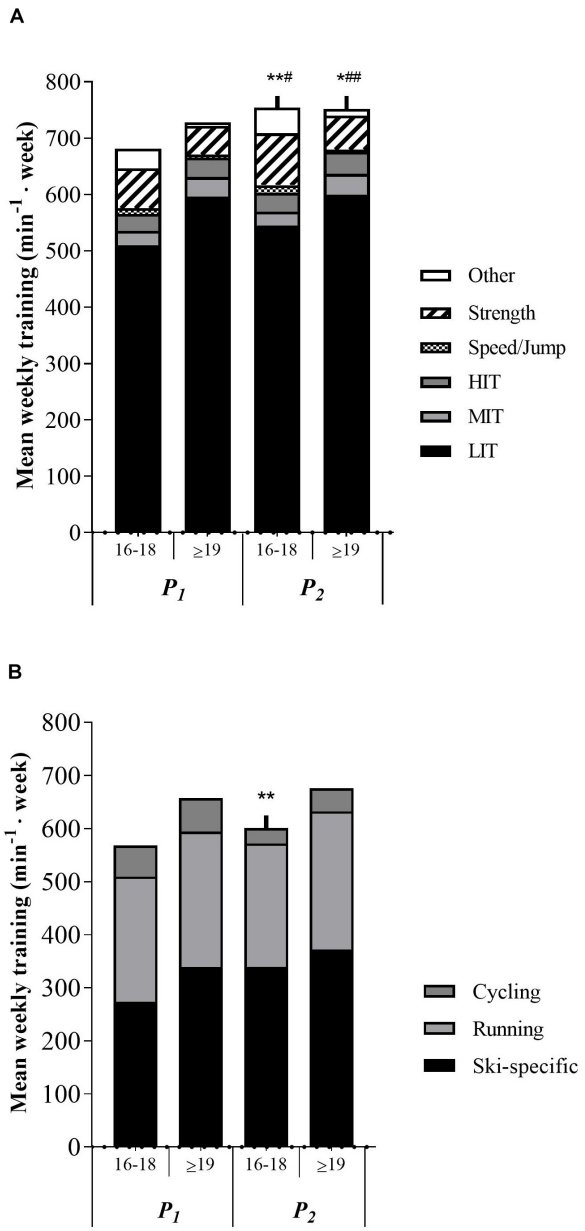


**FIGURE 3 | (A)** Age differences in training characteristics in minutes per week. $P_1$, first training period from May to July. $P_2$, second training period from August to October. LIT, low-intensity endurance training. MIT, moderate-intensity endurance training. HIT, high-intensity training. $min^{-1}$ week, minutes per week. $^{*}p < 0.05$ significantly different from $P_1$ in strength training. $^{**}p < 0.01$ significantly different from $P_1$ in total training volume and strength training. $^{\#}p < 0.05$ significantly different from ≥19 years value in speed/jump training. $^{\#\#}p < 0.01$ significantly different from 16 to 18 years value in strength and other training. **(B)** Age differences in endurance training mode in minutes per week. $^{**}p < 0.01$ significantly different from $P_1$ in ski-specific training.

gender ($p < 0.05$, effect size = 1.14). Overall, D allele carriers were 27.2% stronger than individuals with the II genotype ($p < 0.05$), and the association remained significant when corrected for gender (17.4%, $p < 0.01$, effect size = 1.27).

**TABLE 7 |** Genotype distributions for selected genes ($n$ = 29).

| *ACTN3* | | *ACE* | | *ACSL1* | | *IL6* | |
|---|---|---|---|---|---|---|---|
| RR | 7 (24.1) | DD | 9 (31.0) | GG | 11 (37.9) | CC | 7 (24.1) |
| RX | 13 (44.8) | ID | 16 (55.2) | GA | 13 (44.8) | AC | 20 (69.0) |
| XX | 9 (31.0) | II | 4 (13.8) | AA | 5 (17.2) | AA | 2 (6.9) |
| ***PPARGC1A*** | | ***PPARG*** | | ***PPARA*** | | | |
| CC | 8 (27.6) | GG | 24 (82.8) | GG | 20 (69.0) | | |
| CT | 20 (69.0) | CG | 5 (17.2) | CG | 7 (24.1) | | |
| TT | 1 (3.4) | CC | 0 (0.0) | CC | 2 (6.9) | | |

*Values are presented as n with percentage genotype distribution in parenthesis. Genotypes are ACTN3 R577X, ACE I/D, ACSL1 rs6552828, IL6 rs1474347, PPARGC1A rs819267, PPARG rs1801282, and PPARA rs4253778 polymorphisms.*

There were no significant associations between genotypes of the *ACSL1* rs6552828 polymorphism and physiological variables. However, when corrected for gender, an 8.4% difference was observed in RUN-$VO_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$ and $mL \cdot kg^{-0.67} \cdot min^{-0.67}$; $p < 0.05$) between A allele carriers compared to the GG genotype. As to the *PPARGC1A* rs8192678, the highest RUN-$VO_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$, $L \cdot min^{-1}$ and $mL \cdot kg^{-0.67} \cdot min^{-1}$) was exhibited by the only individual with TT. Significant differences were observed in RUN-$VO_{2max}$ ($p < 0.05$), DP-$VO_{2peak}$ ($p < 0.05$) and 1RM pull-down ($p < 0.05$) between genotypes and allele carriers in *PPARA* rs4253778, *IL6* rs1474347 and *PPARG* rs1801282 independent of gender. However, when corrected for gender, these differences disappeared.

# DISCUSSION

## Main Findings

This is the first study to investigate effects of age, sex, selected genes and training on physiological and performance characteristics and adaptations in well-trained cross-country skiers. Throughout the 6 months period, the skiers displayed no differences in relative distribution of training intensity, although the total training volume and relative amount of ski-specific training increased. This led to no significant differences in physiological and performance variables. Neither was there observed any differences between groups in training adaptations throughout the 6 months training period. At baseline, the male skiers trained more than the female skiers did, but with approximately the same distribution regarding training modalities and intensity zones. We did not detect any major differences in physiological or performance variables based on genotypes.

## Training Characteristics

The participants in the present study were all well-trained and competitive skiers, although not international elite athletes. All skiers competed at national events, and some skiers competed at Scandinavian or international long-distance events (i.e., Vasaloppet and Marcialonga). Thus, all athletes planned

**TABLE 8 |** Physiological baseline results divided by *ACTN3* and *ACE* genotypes.

| | *ACTN3* | | |
|---|---|---|---|
| **Genotype (N)** | **RR (7)** | **RX (13)** | **XX (9)** |
| **Independent of gender** | | | |
| RUN-$VO_{2max}$ | 237.9 ± 43.9 | 259.2 ± 36.2 | 261.1 ± 28.7 |
| DP-$VO_{2peak}$ | 201.5 ± 32.2 | 226.1 ± 36.9 | 225.6 ± 24.6 |
| $C_{DP}$ | 0.796 ± 0.047 | 0.779 ± 0.082 | 0.829 ± 0.066 |
| 1RM half squat | 113.6 ± 19.5 | 120.2 ± 21.6 | 127.2 ± 24.5 |
| 1RM pull-down | 89.3 ± 19.7 | 84.2 ± 16.7 | 90.6 ± 14.7 |
| **Corrected for gender** | | | |
| RUN-$VO_{2max}$ | 267.1 ± 22.9 | 285.7 ± 25.2 | 273.6 ± 22.4 |
| DP-$VO_{2peak}$ | 227.4 ± 21.5 | 249.3 ± 28.7 | 236.0 ± 10.9 |
| $C_{DP}$ | 0.773 ± 0.042 | 0.760 ± 0.074 | 0.820 ± 0.064 |
| 1RM half squat | 126.1 ± 14.7 | 131.7 ± 20.2 | 132.8 ± 24.7 |
| 1RM pull-down | 102.6 ± 13.2 | 94.8 ± 16.5 | 95.1 ± 7.8 |
| | *ACE* | | |
| **Genotype (N)** | **DD (9)** | **ID (16)** | **II (4)** |
| **Independent of gender** | | | |
| RUN-$VO_{2max}$ | 254.8 ± 48.4 | 256.3 ± 29.5 | 247.6 ± 38.6 |
| DP-$VO_{2peak}$ | 220.9 ± 44.0 | 222.6 ± 28.9 | 207.3 ± 24.3 |
| $C_{DP}$ | 0.755 ± 0.070 | 0.812 ± 0.067 | 0.844 ± 0.051 |
| 1RM half squat | 121.4 ± 27.8 | 122.7 ± 16.1 | 112.5 ± 30.7 |
| 1RM pull-down | 92.2 ± 16.4* | 89.4 ± 15.2 | 68.8 ± 11.1 |
| **Corrected for gender** | | | |
| RUN-$VO_{2max}$ | 278.1 ± 29.0 | 273.9 ± 23.7 | 290.1 ± 13.1 |
| DP-$VO_{2peak}$ | 240.8 ± 24.1 | 238.4 ± 27.0 | 244.2 ± 11.0 |
| $C_{DP}$ | 0.738 ± 0.066 | 0.799 ± 0.065 | 0.811 ± 0.035 |
| 1RM half squat | 131.3 ± 23.2 | 130.8 ± 17.9 | 128.5 ± 25.9 |
| 1RM pull-down | 102.7 ± 9.8* | 96.8 ± 14.8 | 83.2 ± 3.3 |

*Values are mean ± SD. RUN-$VO_{2max}$, maximal oxygen uptake in running expressed in milliliters per kilogram bodyweight raised to the power of −0.67 per minute. DP-$VO_{2peak}$, peak oxygen uptake in double poling expressed in milliliters per kilogram bodyweight raised to the power of −0.67 per minute. $C_{DP}$, oxygen cost of double poling expressed in milliliters per kilogram bodyweight raised to the power of −0.67 per meter. 1RM, one repetition maximum expressed in kilograms. *$p < 0.05$ significantly different from ACE II genotype.*

and performed their training with a goal of developing better performance capacity. Compared to training data from elite cross-country skiers (Losnegaard et al., 2013; Tønnessen et al., 2014; Sandbakk et al., 2016; Solli et al., 2017, 2018), most of the participants displayed lower training volumes. This may partly be explained by the fact that the skiers in the present study had to perform their daily training beside other work duties, studies or family obligations. The national level skiers in the study of Sandbakk et al. (2016) are thus more comparable to these participants. Secondly, approximately 50% of the skiers in the present study were still 16 to 18 years, and their total training volume was not statistically different from the older (≥19 years) skiers. This may be because the younger skiers were attending different skiing high schools, were training was an incorporated part of the school schedule. In previous studies, total training volume of younger skiers were lower than in older skiers (Sandbakk et al., 2010, 2013, 2016; Losnegaard et al., 2013; Solli et al., 2017).

The relative distribution of training intensity in the present study was in line with general recommendations for cross-country ski training (Sandbakk and Holmberg, 2017). Cross-country skiers are generally recommended to train with high amounts of LIT and low to moderate amounts of MIT and HIT during the preparation period (May to October). In the study of Sandbakk et al. (2016) national-class cross-country skiers performed on average 90% of their total endurance training at <81% of $HR_{max}$, 4% at 82–87% $HR_{max}$ and 6% at 88% of $HR_{max}$ or higher from May to October. The skiers in the present study displayed, respectively 90, 5, and 5% at the same intensity zones from May to July, showing almost exactly the same intensity distribution from August to October (88, 5, and 6%, respectively). However, different training quantification methods were used in the present study compared to Losnegaard et al. (2013) and Sandbakk et al. (2016). In the present study, training was registered as "time in zone," meaning the exact time in each intensity zone regardless of how the training may have been planned. In Losnegaard et al. (2013) and Sandbakk et al. (2016), the session-goal approach was used, meaning that average HR during either continuous workouts or interval bouts is used to determine the intensity distribution during sessions. This makes the training data difficult to compare with the results in the present study, at least in the higher intensity zones. Sylta et al. (2014) has suggested a conversion factor of 1:3 for comparison of "time-in-zone" training data to session-goal training data for HIT training. By use of this factor on training data from Losnegaard et al. (2013) and Sandbakk et al. (2016), the HIT training should be only a third of what was reported. The study of Tønnessen et al. (2014) shows more comparable "time in zone" data for MIT and HIT in Olympic-medal winning skiers as the skiers in the present study. However, their study showed higher volumes of both HIT and total training volume compared to the present study.

In the present study, the skiers displayed almost no progression in training volume or relative distribution of training intensity during the 6 months period. These findings are in contrast to the study of Sandbakk et al. (2016), where the national level skiers showed a linear increase in training volume from May to September, generally due to higher volumes of LIT. However, the elite international skiers in the studies of Sandbakk et al. (2016) and Losnegaard et al. (2013) showed progression more similar to the skiers in the present study from May to October, but with an increase in HIT and a decrease in LIT during the competitive season, i.e., November to April. The latter may also be the case for the skiers in the present study, but training during the competitive season was not investigated here.

The skiers in the present study performed on average 50% of their total endurance training on ski-specific training, mainly roller skiing. The other half was devoted for the most part to running. These volumes and relative distribution of ski-specific vs. unspecific training are in close agreement with previous studies on cross-country skiers, both non-elite and elite (Losnegaard et al., 2013; Tønnessen et al., 2014; Sandbakk et al.,

2016; Solli et al., 2017). Ski-specific training may target the ski-specific aerobic capacity, work economy and technical factors better than general endurance training (Johansen et al., 2020). The skiers added approximately 60 min per week of ski-specific training from $P_1$ to $P_2$, while running was held relatively constant throughout the whole training period. This may be a result of a desire to elevate ski-specific capacities (i.e., work economy or technical factors) closer to the competitive season, in line with training characteristics from Losnegaard et al. (2013) and Sandbakk et al. (2016).

The relative distribution of strength and speed/jump training was comparable to the training volumes observed previously (Losnegaard et al., 2013; Tønnessen et al., 2014). This is in line with the increased focus on the upper- and lower body strength and speed in modern cross-country skiing (Sandbakk and Holmberg, 2017; Sunde et al., 2019).

## Training Adaptations

The 6 months of training from May to October led to no significant improvements in physiological and performance variables for the skiers in the present study. Since the training volume and intensity distribution was almost constant throughout the study period, this was no surprise. It is still noteworthy that junior- and sub-elite cross-country skiers that train a total of approximately 300 h from May to October show no improvements in physiological factors and only minor improvements in performance. On the other hand, the training performed was sufficient to maintain physiological and performance variables throughout the study period.

Strong correlations ($p < 0.01$) were found at baseline between TT performance and MAS ($r = -0.79$), DP-$VO_{2peak}$ ($r = -0.83$) and 1RM pulldown ($r = -0.72$). When corrected for gender all these correlations were still significant. This is in line with other studies examining performance determining factors in endurance sports, i.e., running, cycling and cross-country skiing (Pate and Kriska, 1984; Ingjer, 1991; di Prampero, 2003; Støren et al., 2013; Sunde et al., 2019; Johansen et al., 2020). Several studies have also observed better performance after improved MAS (Støren et al., 2008, 2012; Sunde et al., 2010; Johansen et al., 2020) or improved maximal strength (Hoff et al., 2002; Støren et al., 2008; Sunde et al., 2010). However, no significant relationships between changes in $TT_{DP}$ performance and changes in physiological variables were found in the present study.

The 3.3% non-significant improvement in TT performance in the present study is approximately half of that reported in Losnegaard et al. (2013). That study observed a 6% improvement from June to October in a 1000-meter TT in V2 skating in elite cross-country skiers, despite no improvements in $VO_{peak}$ in V2 skating. However, C in V2 skating was significantly improved suggesting that the improvement in performance was due to an improvement in MAS in that study. Losnegaard et al. (2013) also explained the better performance by increased anaerobic capacity, measured as $\Sigma O_2$-deficit. Anaerobic capacity was not measured directly in the present study. However, compared to the 1000 m TT used in Losnegaard et al. (2013) the anaerobic capacity should be of lesser importance in the 5.6 km TT used in the present study.

Overall aerobic capacity (RUN-$VO_{2max}$) and specific (DP-$VO_{2peak}$) aerobic capacity was not improved significantly from May to October in the present study. These findings are in line with studies investigating training patterns and development in $VO_{2max}$ in well-trained or elite cross-country skiers maintaining similar training routines (volume and intensity distribution) over longer periods (Rusko, 1987; Ingjer, 1992; Jones, 1998; Gaskill et al., 1999; Losnegaard et al., 2013; Solli et al., 2017). Further improvements of extremely high $VO_{2max}$ in elite endurance athletes have shown to be challenging. Compared to their elite counterparts, the skiers in the present study had approximately 20% lower aerobic capacities (Tønnessen et al., 2015; Sandbakk and Holmberg, 2017). This suggests that the potential for further improvements should be higher for the skiers in the present study, at least for the younger skiers. There is much evidence supporting that HIT may effectively improve $VO_{2max}$, both in recreational and elite endurance athletes (Nilsson et al., 2004; Støren et al., 2012; Sandbakk et al., 2013; Rønnestad et al., 2014, 2016; Johansen et al., 2020). However, these interventional studies include longer or shorter periods of higher amounts of HIT, and lower total training volume. Stöggl and Sperlich (2014) reported superior adaptations in well-trained endurance athletes after 9 weeks of polarized training (56% LIT, 3% MIT, and 26% HIT) and HIT (43% LIT, 0% MIT, and 57% HIT) in $VO_{2max}$, compared to training models with no training at HIT intensities and higher training volumes. This is well in line with the studies of Støren et al. (2012) and Gaskill et al. (1999), where endurance athletes experienced great improvements with a training program with higher amounts of HIT, with the same, or reduced total training volumes. Thus, we may speculate that more HIT training during pre-season may be crucial for further development of aerobic capacity in junior- and sub-elite cross-country skiers.

No statistically significant improvements in $C_{DP}$ were observed. However, like most of the other physiological variables, a slightly better average $C_{DP}$ was seen, although not significant. Losnegaard et al. (2013) reported improved C from June to October in elite cross-country skiers and this could be due to the increased ski-specific training. A significant correlation was also observed between change in total ski-specific training and $\Delta C_{DP}$ in the present study, suggesting that adaptation is specifically to the movement patterns used in training (Scrimgeour et al., 1986; McMillan et al., 2005; Johansen et al., 2020). Previous studies have reported improved C after MST in both running (Støren et al., 2008), cycling (Sunde et al., 2010) and cross-country skiing (Hoff et al., 2002; Østerås et al., 2002). However, this relationship was not observed for the whole group in the present study, since MST and thus $C_{DP}$ did not change during the 6 month period.

Several previous studies have reported no training adaptations in LT in % of $VO_{2max}$ after shorter or longer periods of endurance or strength training (Helgerud et al., 2001, 2007; Støren et al., 2008, 2012; Sunde et al., 2010; Rønnestad et al., 2014). This is in line with results in the present study, since the skiers had almost exactly the same LT% at all test points. The present study also showed a strong correlation at baseline between MAS and $LT_v$ ($r = 0.93$, $p < 0.01$) indicating a close relationship, which have

previously been reported (Støren et al., 2014; Sunde et al., 2019). Consequently, to elevate $LT_v$ skiers should aim to improve MAS ($VO_{2max}$ and C).

## Sex Differences

Males had higher training volumes than females preceding the baseline tests in the present study. However, from May to October no significant sex differences were observed in total training volume, relative intensity distribution, endurance training, ski-specific training, strength training or other training. These training characteristics are in line with the findings in elite cross-country skiers from Solli et al. (2018), where males tended to train more in total than females throughout a whole year (∼ 90 h), although not significant. In Solli et al. (2018), strength and speed training was similar for males and females, as observed in the present study regarding strength training. However, in the present study the amount of speed and jump training was four times higher in females than males.

Males displayed significantly higher values than females in RUN-$VO_{2max}$ (19%), DP-$VO_{2peak}$ (19%), and MAS (32%), had better $C_{DP}$ (9%) and $TT_{DP}$ (15%) at baseline in the present study. These sex differences are in line with previous results (Sandbakk et al., 2014; Andersson et al., 2019; Sunde et al., 2019). Since MAS is the product of DP-$VO_{2peak}$ and $C_{DP}$ it was no surprise that the sum of sex differences in these two variables equalled almost exactly the difference seen in MAS. The gender difference in $TT_{DP}$ seemed to correspond to the 32% difference in MAS. This is further supported by the correlation between MAS and $TT_{DP}$ ($r = -0.58$, $p < 0.01$) at baseline corrected for gender.

Interestingly, the sex differences in DP-$VO_{2peak}$ was the same as in RUN-$VO_{2max}$ in the present study. Sandbakk et al. (2014) and Hegge et al. (2016), found the sex differences to be larger with increased contribution of upper-body musculature, i.e., larger in DP-$VO_{2peak}$ than in RUN-$VO_{2max}$. Regarding 1RM strength variables, the gender differences were larger in 1RM pull-down (30%) compared to 1RM half-squat (21%) in the present study. These sex differences in 1RM strength are in line with previous results (Sandbakk et al., 2014; Sunde et al., 2019).

The sex differences at baseline in the present study were maintained in $TT_{DP}$, RUN-$VO_{2max}$, and DP-$VO_{2peak}$ from May to October due to no significant differences in training progression between males and females in this period. This may suggest that males and females do not differ in physiological and performance adaptations to a similar training pattern, which is in line with previous studies (Astorino et al., 2011; Støren et al., 2017; Varley-Campbell et al., 2018). However, sex difference in $C_{DP}$ declined significantly from PRE to POST1, due to a significantly improved $C_{DP}$ in females while the males maintained their pre-values. This may be explained by the lower training volumes in females 3-months prior to pre-test, resulting in a greater sex difference in $C_{DP}$ at PRE. From May to July, males and females trained more similar, at least in terms of ski-specific training, and this may have reduced the initial gap. Another explanation for the improved $C_{DP}$ observed in females, may be the relationship observed earlier between improved maximal strength and improved C in running, cycling and cross-country skiing (Hoff et al., 2002; Støren et al., 2008; Sunde et al., 2010). In the present study, a significant correlation was observed between Δ1RM pull-down and $\Delta C_{DP}$ ($r = -0.60$, $p < 0.05$) in the female skiers, which supports this explanation. However, further improvements in $C_{DP}$ was not observed in females or males from August to October.

## Age-Related Differences

To the best of our knowledge, no studies have investigated age-related differences in training characteristics and training adaptations between younger (junior athletes) and older skiers (senior athletes). In the present study, the age groups did not differ significantly in total training volume 3-months prior to pre-test. No differences were apparent in total training volume or in LIT, HIT, ski-specific training, running, cycling, strength or speed/jump training during the preparation period from May to October. The only difference between 16 and 18 years old compared to ≥19 years old was the amount of MIT, where the average difference were ∼ 20 min per week. Compared to other studies examining either junior- or adult skiers, the 16–18 years old skiers in the present study show similar training volumes as seen in previous studies on junior athletes, however, with a slightly lower amount of MIT and HIT (Sandbakk et al., 2011, 2013). However, the older skiers had lower training volumes compared to age-matched adult elite cross-country skiers (Losnegaard et al., 2013; Tønnessen et al., 2014; Sandbakk et al., 2016; Solli et al., 2017).

From May to October, the young and adult skiers did not differ significantly in training progression. The oldest skiers displayed almost no progression in all training variables, throughout the study period. This is in accordance to earlier observations of training progression in adult elite cross-country skiers (Losnegaard et al., 2013; Sandbakk et al., 2016).

At baseline, the adult skiers were 15% heavier than the younger skiers. A significant age-related difference was also apparent in $TT_{DP}$ (10.6%), which was followed by a 17.8% difference in MAS. Corrected for age, MAS showed a strong correlation to $TT_{DP}$ at baseline ($r = -0.77$, $p < 0.01$). The difference in MAS was almost exactly the same as in $LT_v$, supporting that DP-$VO_{2peak}$ and $C_{DP}$ are the main predictors for $LT_v$. The age difference in MAS is a consequence of the 10.7% difference in DP-$VO_{2peak}$ and the 7.9% difference in $C_{DP}$. The age difference in RUN-$VO_{2max}$ and DP-$VO_{2peak}$ may be attributed to incomplete development of the cardiac system and muscle mass in the younger skiers still in puberty (Rusko, 1992). Additionally, the lower number of years of training in the young skiers may be an explanation for the observed difference. The difference in $C_{DP}$ may also be a result of less training years and experience in the younger skiers. In addition, the adult skiers had 21% higher 1RM pull-down than the younger skiers. Stronger skiers have shown to have higher peak forces in DP, lower DP frequency and shorter contact time (Sunde et al., 2019). However, all these age differences should be handled with great caution as they are most probably due to the sex differences in the two age groups. When analyzing age differences in males and females separately in the two groups, young and adult females differed in the same physiological and performance variables observed for the whole group, except for $C_{DP}$ and strength variables. For the males, almost every

significant age-related difference disappeared, except for 1RM pull-down and DP-$VO_{2peak}$ in absolute values.

## Effect of Selected Genes

We did not detect any major effects for the selected genes on physical and performance variables at baseline. Based on the low number of participants and the expected influence by single genes, this was not unexpected. However, we did find some minor effects.

In the present study the common *ACTN3* R577X, X allele carriers demonstrated higher DP-$VO_{2peak}$ than participants with the RR genotype at PRE. This is in accordance to Pimenta et al. (2013) that observed that soccer players with the XX genotype had the highest $VO_{2max}$. According to Yang et al. (2003) the X allele is overrepresented among endurance athletes, especially females. The importance of the advantageous allele is also likely dependent on the performance level (Eynon et al., 2012). However, others have not been able to confirm this (Papadimitriou et al., 2018). The X allele frequency in the present study was slightly higher (44 vs. 53%) among athletes than the general population from the same geographical area (Goleva-Fjellet et al., 2020). For the *ACE* gene, skiers with the II genotype exhibited higher RUN-$VO_{2max}$ compared with carriers of the D allele. However, participants with DD genotype demonstrated ∼15% better $C_{DP}$ and had ∼28% higher 1RM pull-down compared to the II genotype. The observed superior $C_{DP}$ among skiers with the DD genotype could be explained by gender differences in 1RM (Sunde et al., 2019). The I allele frequency among the cross-country skiers included in the present study was 14.6% higher than in a Norwegian cohort from the same geographic region (Goleva-Fjellet et al., 2020).

For the *ACSL1* rs6552828, the A allele carriers had 8.4% higher RUN-$VO_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$ and $mL \cdot kg^{-0.67} \cdot min^{-1}$; $p < 0.05$) compared to the GG genotype. A relatively large effect of 6% on the training response of $VO_{2max}$ have been reported previously with the carriers and the common G allele exhibiting larger increase than the homozygotes of the less common A allele (Bouchard et al., 2011). The differences between the findings in the present study and the study of Bouchard et al. (2011) might be due to the different study population profiles, i.e., highly trained athletes vs. sedentary adults, respectively. The latter group have a larger potential of increasing their $VO_{2max}$ as a result of standardized exercise-training programs compared to athletes. The present study did not measure a significant increase in the $VO_{2max}$ throughout the testing period.

All associations between *PPARGC1A* rs8192678 and physiological and performance variables dissapeared when correcting for gender. The C allele (Gly) have been suggested to be an elite status endurance allele favorable to athletic ability (Tharabenjasin et al., 2019; Petr et al., 2020). Homozygotes of the C allele are generally more responding aerobic training compared to the T allele (Ser) (Petr et al., 2018). In the present study, only a single male participant possesed the least favorable genotype for endurance performance, i.e., TT. Despite possessing an unfavorable genotype to endurance performance, he demonstrated the highest RUN-$VO_{2max}$ of all participants. This points at carefullness when interpreting physiological performance based on single genes.

No significant associations were found for either of the following polymorphisms in the present study when corrected for gender: *PPARA* rs4253778, *IL6* rs1474347, and *PPARG* rs1801282. For muscle function and jumping capacity, this is well in line with previous findings in other sports, at least for the *PPARA* rs4253778 polymorphism (Stastny et al., 2019). However, Stastny et al. (2019) found significant associations to other muscle parameters, such as reactive muscle index.

Despite previous findings on the effects of the *ACE* I/D and the *ACTN3* R577X polymorphisms on athletic ability and trainability, the impact of these are not strong enough predictors to determine the athletic ability (Venezia and Roth, 2019). Results from the present study confirms that genotype frequencies for the two most investigated and replicated polymorphisms (i.e., *ACE* I/D and *ACTN3* R577X) among the cross-country skiers were similar to those from a large general Scandinavian cohort (Goleva-Fjellet et al., 2020). Furthermore, there was the case of the one skier that possessed the least favorable endurance genotype for the *PPARGC1A* SNP, but still demonstrated the highest $VO_{2max}$. These findings may indicate that possessing the optimal alleles of the different polymorphisms may be beneficial for endurance performance, but it is not critical for the athletic ability (Venezia and Roth, 2019; Petr et al., 2020). This may be especially true for athletes competing at a national level compared to world-class elite athletes (Eynon et al., 2012; Papadimitriou et al., 2016). However, the results from the present study should be treated with some caution due to the limited sample size. Some genotypes within the selected genes were either not present or only apparent in 1–2 participants, and may therefore influence our results. The material is thus prone to false negative results (type 2 errors), and we can only state that there were minor associations between some genotypes and physiological variables in our cohort of 29 skiers. This should be taken into account when interpreting the genetic results from the present study. Also, these athletes were already well trained and could be argued to not represent a good sample population to detect associations between genotype variants and physiological or performance characteristics.

## Practical Implications

In the present study, maintaining the same training intensity distribution, and only increase total training volume was not sufficient to further improve aerobic capacity and cross-country skiing performance significantly throughout 6 months of training. This suggests that training programs with the same training intensity distribution, only differing in training volume, may not ensure optimal development of each individual skier independent of age and sex (Gaskill et al., 1999). For the individual well trained athlete, substantial changes in training volume and training intensity distribution could be necessary to facilitate further improvements, as observed in earlier studies (Gaskill et al., 1999; Støren et al., 2012; Bratland-Sanda et al., 2020). This is important knowledge for trainers of talented cross-country skiers that have faced stagnation.

An interesting finding in the present study is that our cohort of skiers did not differentiate genetically in two of the most

investigated polymorphisms in association to athletic ability compared to a general Scandinavian cohort from the same geographical area. This may suggest that one might be able to reach a high national level in cross-country skiing without having the optimal genotypes in selected genes, with sufficient and individualized training.

## CONCLUSION

Sex and age did influence physiological and performance variables at baseline, but did not influence training adaptations. Since the skiers in the present study did not display major changes in training, it was no surprise that no adaptations occurred in physiological or performance variables either. The genotype variants of selected genes were not critical determinants for physiological and performance variables in national and sub-elite cross-country skiers in the present study.

## DATA AVAILABILITY STATEMENT

Restrictions apply to the datasets: the datasets presented in this article are not readily available due to the Norwegian legislation regarding the publication of genetic data. Requests to access the datasets should be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Ethics Commitee of South-Eastern Norway, Telemark, Norway. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## AUTHOR CONTRIBUTIONS

J-MJ, SG-F, ØS, AS, MS, and JH all participated significantly in the planning and design of the study, as well as the data analyzing and the writing of the article. J-MJ, AS, ØS, SG-F, LG, LS, BF, and MS participated in the data collection. LG, LS, and BF also participated in the writing of the article. All authors read and approved the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.581339/full#supplementary-material

## REFERENCES

Abdollahi, M. R., Huang, S., Rodriguez, S., Guthrie, P. A., Smith, G. D., Ebrahim, S., et al. (2008). Homogenous assay of rs4343, an ACE I/D proxy, and an analysis in the British Women's Heart and Health study (BWHHS). *Dis. Markers* 24, 11–17. doi: 10.1155/2008/813679

Andersson, E. P., Govus, A., Shannon, O. M., and McGawley, K. (2019). Sex differences in performance and pacing strategies during sprint skiing. *Front. Physiol.* 10:295.

Astorino, T. A., Allen, R. P., Roberson, D. W., Jurancich, M., Lewis, R., McCarthy, K., et al. (2011). Adaptations to high-intensity training are independent of gender. *Eur. J. Appl. Physiol.* 111, 1279–1286. doi: 10.1007/s00421-010-1741-y

Austin, S., and St-Pierre, J. (2012). PGC1α and mitochondrial metabolism – emerging concepts and relevance in ageing and neurodegenerative disorders. *J. Cell Sci.* 125, 4963–4971. doi: 10.1242/jcs.113662

Bouchard, C., Sarzynski, M. A., Rice, T. K., Kraus, W. E., Church, T. S., Sung, Y. J., et al. (2011). Genomic predictors of the maximal $O_2$ uptake response to standardized exercise training programs. *J. Appl. Physiol.* 110, 1160–1170. doi: 10.1152/japplphysiol.00973.2010

Bratland-Sanda, S., Pedersen, F. G., Haave, M. N., Helgerud, J., and Støren, Ø (2020). Large inter-individual differences in responses to a block of high intensity aerobic interval training: A case series in national-level cyclists and triathletes. *Int. J. Exerc. Sci.* 13, 480–487.

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*, 2 Edn. Hillsdale, NJ: Laurence Erlbaum Associates.

Conley, D. L., and Krahenbuhl, G. S. (1980). Running economy and distance running performance of highly trained athletes. *Med. Sci. Sports Exerc.* 12, 357–360. doi: 10.1249/00005768-198012050-00010

di Prampero, P. E. (2003). Factors limiting maximal performance in humans. *Eur. J. Appl. Physiol.* 90, 420–429. doi: 10.1007/s00421-003-0926-z

Eynon, N., Ruiz, J. R., Femia, P., Pushkarev, V. P., Cieszczyk, P., Maciejewska-Karlowska, A., et al. (2012). The ACTN3 R577X polymorphism across three groups of elite male European athletes. *PLoS One* 7:e43132. doi: 10.1371/journal.pone.0043132

Gaskill, S. E., Serfass, R. C., Bacharach, D. W., and Kelly, J. M. (1999). Responses to training in cross-country skiers. *Med. Sci. Sports Exerc.* 31, 1211–1217. doi: 10.1097/00005768-199908000-00020

Goleva-Fjellet, S., Bjurholt, A. M., Kure, E. H., Larsen, I. K., Støren, Ø, and Sæbø, M. (2020). Distribution of allele frequencies for genes associated with physical activity and/or physical capacity in a homogenous Norwegian cohort-a cross-sectional study. *BMC Genetics* 21:8.

Harvey, N. R., Voisin, S., Dunn, P. J., Sutherland, H., Yan, X., Jacques, M., et al. (2020). Genetic variants associated with exercise performance in both moderately trained and highly trained individuals. *Mol. Genet. Genomics.* 295, 515–523. doi: 10.1007/s00438-019-01639-8

Hegge, A. M., Bucher, E., Ettema, G., Faude, O., Holmberg, H. C., and Sandbakk, Ø (2016). Gender differences in power production, energetic capacity and efficiency of elite cross-country skiers during whole-body, upper-body, and arm poling. *Eur. J. Appl. Physiol.* 116, 291–300. doi: 10.1007/s00421-015-3281-y

Helgerud, J., Engen, L. C., Wisløff, U., and Hoff, J. (2001). Aerobic endurance training improves soccer performance. *Med. Sci. Sports Exerc.* 33, 1925–1931. doi: 10.1097/00005768-200111000-00019

Helgerud, J., Høydal, K. L., Wang, E., Karlsen, T., Berg, P. R., Bjerkaas, M., et al. (2007). Aerobic high-intensity intervals improve VO2max more than moderate training. *Med. Sci. Sports Exerc.* 39, 665–671. doi: 10.1249/mss.0b013e3180304570

Helgerud, J., Ingjer, F., and Strømme, S. B. (1990). Sex differences in performance-matched marathon runners. *Eur. J. Appl. Physiol.* 61, 433–439. doi: 10.1007/bf00236064

Hoff, J., Gran, A., and Helgerud, J. (2002). Maximal strength training improves aerobic endurance performance. *Scand. J. Med. Sci. Sports.* 12, 288–295. doi: 10.1034/j.1600-0838.2002.01140.x

Hopkins, W. G. (2000). *A Scale of Min: A New View of Statistics*. Available online at: http://www.sportsci.org/resource/stats/index.html (accessed May 28, 2020).

Ingjer, F. (1991). Maximal oxygen uptake as a predictor of performance ability in women and men elite cross-country skiers. *Scand. J. Med. Sci. Sports* 1, 25–30. doi: 10.1111/j.1600-0838.1991.tb00267.x

Ingjer, F. (1992). Development of maximal oxygen uptake in young elite male cross-country skiers: a longitudinal study. *J. Sports Sci.* 10, 49–63. doi: 10.1080/02640419208729906

Jacques, M., Landen, S., Voisin, S., and Eynon, N. (2019). "Summary findings on genetics and sport performance," in *Routledge Handbook of Sport and Exercise Systems Genetics*, eds T. Lightfoot, M. J. Hubal, and S. M. Roth (Abingdon: Routledge), 347–356. doi: 10.4324/9781315146287-30

Johansen, J.-M., Eriksen, S., Sunde, A., Slettemeås, ØB., Helgerud, J., and Støren, Ø (2020). Improving utilization of maximal oxygen uptake and work economy in recreational cross-country skiers with high-intensity double-poling intervals. *Int. J. Sports Physiol. Perform*. doi: 10.1123/ijspp.2019-0689 [Epub ahead of print].

Jones, A. M. (1998). A five year physiological case study of an Olympic runner. *Br. J. Sports Med.* 32, 39–43. doi: 10.1136/bjsm.32.1.39

Losnegaard, T., Myklebust, H., Spencer, M., and Hallen, J. (2013). Seasonal variations in $VO_{2max}$, $O_2$-cost, $O_2$-deficit, and performance in elite cross-country skiers. *J. Strength Cond. Res.* 27, 1780–1790. doi: 10.1519/jsc.0b013e31827368f6

Ma, F., Yang, Y., Li, X., Zhou, F., Gao, C., Li, M., et al. (2013). The association of sport performance with ACE and ACTN3 genetic polymorphisms: a systematic review and meta-analysis. *PLoS One* 8:e54685. doi: 10.1371/journal.pone.0054685

MacArthur, D. G., Seto, J. T., Chan, S., Quinlan, K. G., Raftery, J. M., Turner, N., et al. (2008). An Actn3 knockout mouse provides mechanistic insights into the association between alpha-actinin-3 deficiency and human athletic performance. *Hum. Mol. Genet.* 17, 1076–1086. doi: 10.1093/hmg/ddm380

Magi, A., Unt, E., Prans, E., Raus, L., Eha, J., Veraksits, A., et al. (2016). The association Analysis between ACE and ACTN3 genes polymorphisms and endurance capacity in young cross-country skiers: longitudinal study. *J. Sports Sci. Med.* 15, 287–294.

McMillan, K., Helgerud, J., MacDonald, R., and Hoff, J. (2005). Physiological adaptations to soccer specific endurance training in professional youth soccer players. *Br. J. Sports Med.* 39, 273–277. doi: 10.1136/bjsm.2004.012526

Nilsson, J. E., Holmberg, H. C., Tveit, P., and Hallén, J. (2004). Effects of 20-s and 180-s double poling interval training in cross-country skiers. *Eur. J. Appl. Physiol.* 92, 121–127. doi: 10.1007/s00421-004-1042-4

North, K. N., Yang, N., Wattanasirichaigoon, D., Mills, M., Easteal, S., and Beggs, A. H. (1999). A common nonsense mutation results in alpha-actinin-3 deficiency in the general population. *Nat. Genet.* 21, 353–354. doi: 10.1038/7675

Orysiak, J., Zmijewski, P., Klusiewicz, A., Kaliszewski, P., Malczewska-Lenczowska, J., Gajewski, J., et al. (2013). The association between ace gene variation and aerobic capacity in winter endurance disciplines. *Biol. Sport* 30, 249–253. doi: 10.5604/20831862.1077549

Østerås, H., Helgerud, J., and Hoff, J. (2002). Maximal strength-training effects on force-velocity and force-power relationships explain increases in aerobic performance in humans. *Eur. J. Appl. Physiol.* 88, 255–263. doi: 10.1007/s00421-002-0717-y

Papadimitriou, I. D., Lockey, S. J., Voisin, S., Herbert, A. J., Garton, F., Houweling, P. J., et al. (2018). No association between ACTN3 R577X and ACE I/D polymorphisms and endurance running times in 698 Caucasian athletes. *BMC Genomics*. 19:13.

Papadimitriou, I. D., Lucia, A., Pitsiladis, Y. P., Pushkarev, V. P., Dyatlov, D. A., Orekhov, E. F., et al. (2016). ACTN3 R577X and ACE I/D gene variants influence performance in elite sprinters: a multi-cohort study. *BMC Genomics* 17:285.

Pate, R. R., and Kriska, A. (1984). Physiological basis of the sex difference in cardiorespiratory endurance. *Sports Med.* 1, 87–98. doi: 10.2165/00007256-198401020-00001

Pescatello, L. S., Corso, L. M. L., Santos, L. P., Livingston, J., and Taylor, B. A. (2019). "Angiotensin-converting enzyme and the genomics of endurance performance," in *Routledge Handbook of Sport and Exercise Systems Genetics*, eds T. Lightfoot, M. J. Hubal, and S. M. Roth (Abingdon: Routledge), 216–249. doi: 10.4324/9781315146287-21

Petr, M., Maciejewska-Skrendo, A., Zajac, A., Chycki, J., and Stastny, P. (2020). Association of elite sports status with gene variants of peroxisome proliferator activated receptors and their transcriptional coactivator. *Int. J. Mol. Sci.* 21:162. doi: 10.3390/ijms21010162

Petr, M., Stastny, P., Zajac, A., Tufano, J. J., and Maciejewska-Skrendo, A. (2018). The role of peroxisome proliferator-activated receptors and their transcriptional coactivators gene variations in human trainability: a systematic review. *Int. J. Mol. Sci.* 19:1472. doi: 10.3390/ijms19051472

Pimenta, E. M., Coelho, D. B., Veneroso, C. E., Barros Coelho, E. J., Cruz, I. R., Morandi, R. F., et al. (2013). Effect of ACTN3 gene on strength and endurance in soccer players. *J. Strength Cond. Res.* 27, 3286–3292. doi: 10.1519/jsc.0b013e3182915e66

Puthucheary, Z., Skipworth, J. R., Rawal, J., Loosemore, M., Van Someren, K., and Montgomery, H. E. (2011). The ACE gene and human performance: 12 years on. *Sports Med.* 41, 433–448. doi: 10.2165/11588720-000000000-00000

Rønnestad, B. R., Ellefsen, S., Nygaard, H., Zacharoff, E. E., Vikmoen, O., Hansen, J., et al. (2014). Effects of 12 weeks of block periodization on performance and performance indices in well-trained cyclists. *Scand. J. Med. Sci. Sports.* 24, 327–335. doi: 10.1111/sms.12016

Rønnestad, B. R., Hansen, J., Thyli, V., Bakken, T. A., and Sandbakk, Ø (2016). 5-week block periodization increases aerobic power in elite cross-country skiers. *Scand. J. Med. Sci. Sports* 26, 140–146. doi: 10.1111/sms.12418

Roth, S. M., Walsh, S., Liu, D., Metter, E. J., Ferrucci, L., and Hurley, B. F. (2008). The ACTN3 R577X nonsense allele is under-represented in elite-level strength athletes. *Eur. J. Hum. Genet.* 16, 391–394. doi: 10.1038/sj.ejhg.5201964

Rusko, H. K. (1987). Development of aerobic power in relation to age and training in cross-country skiers. *Med. Sci. Sports Exerc.* 24, 1040–1047.

Rusko, H. K. (1992). Development of aerobic power in relation to age and training in cross-country skiers. *Med. Sci. Sports Exerc.* 24, 1040–1047.

Sandbakk, Ø., Ettema, G., and Holmberg, H.-C. (2014). Gender differences in endurance performance by elite cross-country skiers are influenced by the contribution from poling. *Scand. J. Med. Sci. Sports* 24, 28–33. doi: 10.1111/j.1600-0838.2012.01482.x

Sandbakk, Ø, Hegge, A. M., Losnegaard, T., Skattebo, Ø, Tønnessen, E., and Holmberg, H. C. (2016). The physiological capacity of the world's highest ranked female cross-country skiers. *Med. Sci. Sports Exerc.* 48, 1091–1100. doi: 10.1249/mss.0000000000000862

Sandbakk, Ø, and Holmberg, H. C. (2017). Physiological capacity and training routines of elite cross-country skiers: approaching the upper limits of human endurance. *Int. J. Sports Physiol. Perform.* 12, 1003–1011. doi: 10.1123/ijspp.2016-0749

Sandbakk, Ø, Holmberg, H. C., Leirdal, S., and Ettema, G. (2011). The physiology of world-class sprint skiers. *Scand. J. Med. Sci. Sports* 21, e9–e16.

Sandbakk, Ø, Sandbakk, S. B., Ettema, G., and Welde, B. (2013). Effects of intensity and duration in aerobic high-intensity interval training in highly training junior cross-country skiers. *J. Strength Cond. Res.* 27, 1974–1980. doi: 10.1519/jsc.0b013e3182752f08

Sandbakk, Ø, Welde, B., and Holmberg, H. C. (2010). Endurance training and sprint performance in elite junior cross-country skiers. *J. Strength Cond. Res.* 25, 1299–1305. doi: 10.1519/jsc.0b013e3181d82d11

Scrimgeour, A. G., Noakes, T. D., Adams, B., and Myburgh, K. (1986). The influence of weekly training distance on fractional utilization of maximum aerobic capacity in marathon and ultramarathon runners. *Eur. J. Appl. Physiol.* 55, 202–209. doi: 10.1007/bf00715006

Seiler, S., and Kjerland, G. Ø (2006). Quantifying trainng intensity distribution in elite endurance atheletes: is there evidence for an "optimal" distribution? *Scand. J. Med. Sci. Sports* 16, 49–56. doi: 10.1111/j.1600-0838.2004.00418.x

Solli, G. S., Kocbach, J., Seeberg, T. M., Tjønnås, J., Rindal, O. M. H., Haugnes, P., et al. (2018). Sex-based differences in speed, sub-technique selection, and

kinematic patterns during low- and high-intensity training for classical cross-country skiing. *PLoS ONE* 13:e0207195. doi: 10.1371/journal.pone.0207195

Solli, G. S., Tønnessen, E., and Sandbakk, Ø (2017). The training characteristics of the world's most successful female cross-country skier. *Front. Physiol.* 8:1069.

Stastny, P., Lehnert, M., De Ste Croix, M., Petr, M., Svoboda, Z., Maixnerova, E., et al. (2019). Effect of *COL5A1, GDF5,* and *PPARA* genes on a movement screen and neuromuscular performance in adolescent team sport athletes. *J. Strength Cond. Res.* 33, 2057–2065. doi: 10.1519/jsc.0000000000003142

Stöggl, T. L., and Sperlich, B. (2014). Polarized training has greater impact on key endurance variables than threshold, high intensity, or high volume training. *Front. Physiol.* 5:33.

Stöggl, T. L., and Sperlich, B. (2015). The training intensity distribution among well-trained and elite endurance athletes. *Front. Physiol.* 6:295.

Støren, Ø, Bratland-Sanda, S., Haave, M., and Helgerud, J. (2012). Improved VO2max and time trial performance with more high aerobic intensity interval training and reduced training volume: a case study on an elite national cyclist. *J. Strength Cond. Res.* 26, 2705–2711. doi: 10.1519/jsc.0b013e318241deec

Støren, Ø, Helgerud, J., Sæbø, M., Støa, E. M., Bratland-Sanda, S., Unhjem, R. J., et al. (2017). The effect of age on the $VO_{2max}$ response to high-intensity interval training. *Med. Sci. Sports Exerc.* 49, 78–85.

Støren, Ø, Helgerud, J., Støa, E. M., and Hoff, J. (2008). Maximal strength training improves running economy in distance runners. *Med. Sci. Sports Exerc.* 40, 1087–1092. doi: 10.1249/mss.0b013e318168da2f

Støren, Ø, Rønnestad, B. R., Sunde, A., Hansen, J., Ellefsen, S., and Helgerud, J. (2014). A time-saving method to assess power output at lactate threshold in well-trained and elite cyclists. *J. Strength Cond. Res.* 28, 622–629. doi: 10.1519/jsc.0b013e3182a73e70

Støren, Ø, Ulevåg, K., Larsen, M. H., Støa, E. M., and Helgerud, J. (2013). Physiological determinants of the cycling time trial. *J. Strength Cond. Res.* 27, 2366–2373. doi: 10.1519/jsc.0b013e31827f5427

Sunde, A., Johansen, J.-M., Gjøra, M., Paulsen, G., Bråten, M., Helgerud, J., et al. (2019). Stronger is better: The impact of upper-body strength in double poling performance. *Front. Physiol.* 10:1091.

Sunde, A., Støren, Ø, Bjerkaas, M., Larsen, M. H., Hoff, J., and Helgerud, J. (2010). Maximal strength training improves cycling economy in competitive cyclists. *J. Strength Cond. Res.* 24, 2157–2165. doi: 10.1519/jsc.0b013e3181aeb16a

Sylta, Ø, Tønnessen, E., and Seiler, S. (2014). From heart-rate data to training quantification: a comparison of 3 methods of training-intensity analysis. *Int. J. Sports Physiol. Perform.* 9, 100–107. doi: 10.1123/ijspp.2013-0298

Tharabenjasin, P., Pabalan, N., and Jarjanazi, H. (2019). Association of PPARGC1A Gly428Ser (rs8192678) polymorphism with potential for athletic ability and sports performance: a meta-analysis. *PLoS One* 14:e0200967.

Tønnessen, E., Haugen, T. A., Hem, E., Leirstein, S., and Seiler, S. (2015). Maximal aerobic capacity in the winter-olympics endurance disciplines: olympic-medal benchmarks for the time period 1990-2013. *Int. J. Sports Physiol. Perform.* 10, 835–839.

Tønnessen, E., Sylta, Ø, Haugen, T. A., Hem, E., Svendsen, I. S., and Seiler, S. (2014). The road to gold: training and peaking characteristics in the year prior to a gold medal endurance performance. *PLoS One* 9:e101796.

Varley-Campbell, J., Cooper, C., Wilkerson, D., Wardle, S., Greeves, J., and Lorenc, T. (2018). Sex-specific changes in physical performance following military training: a systematic review. *Sports Med.* 48, 2623–2640.

Venezia, A. C., and Roth, S. M. (2019). "The scientific and ethical challenges of using genetic information to predict sport performance," in *Routledge Handbook of Sport and Exercise Systems Genetics*, eds T. Lightfoot, M. J. Hubal, and S. M. Roth (Abingdon: Routledge), 442–452.

Yang, N., MacArthur, D. G., Gulbin, J. P., Hahn, A. G., Beggs, A. H., Easteal, S., et al. (2003). ACTN3 genotype is associated with human elite athletic performance. *Am. J. Hum. Genet.* 73, 627–631.

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