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The Effect of High-intensity Interval Training on Maximal Aerobic Capacity, DNA Methylation and Gene Expression in Healthy Elderly Men

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Abstract

Physical activity is generally beneficial to maintain good health and prevent diseases. Development of some diseases may include epigenetic changes. Epigenetic changes involve alterations to the genome without changing the DNA sequence itself. Different factors have been found to cause epigenetic changes in our DNA, such as age, gender, lifestyle and environment. DNA methylation is an epigenetic change described as the addition of a chemical methyl group (CH_3) to the DNA. Ordinarily, DNA methylation is naturally occurring and probably harmless, but sometimes these changes can alter gene expression and trigger diseases. A recent study discovered that exercise could provide an acute reduction in DNA methylation of specific genes, but that the methylation reoccurred with time.

In this project we wanted to investigate whether regular high-intensity interval training can change the DNA methylation in a more long-lasting state, and whether these changes affect the gene expression. Our null hypothesis was that no such exercise-induced changes occur. Healthy men (70-75 year old) were randomized into two groups: a control group (n=13) and a high-intensity interval training (HIIT) group (n=13). Testing was performed in both groups at baseline and after eight weeks. The HIIT group performed high-intensity interval training on treadmill three days a week for eight weeks, whereas the control group was instructed to live normally. To test our hypothesis DNA methylation and gene expression were analyzed in muscle tissue from participants. Additionally, specific health factors and possible complications of high-intensity interval training among elderly were investigated.

Significant differences was detected between the two groups, where the HIIT group increased their maximal aerobic capacity, reduced their body mass index and waist circumference, while the control group decreased their HDL cholesterol. Inverse relationships were detected between the groups regarding their change in DNA methylation of four specific CpG sites, but the relationships were not proven significant. No significant differences between the groups were observed in changes of gene expression of the CpG sites associated genes *MYH1, VDAC2, BNIP3,* and *COX16.* However, the results give reasons to believe that an improvement in VO_{2max} can affect the naturally occurring changes of DNA methylation in our genome. Still, due to study weaknesses, larger and more extensive studies are needed to determine whether high-intensity interval training actually changes DNA methylation, and to find out which consequences these changes have.

Sammendrag

Fysisk aktivitet er generelt gunstig for å opprettholde en god helse og forebygge sykdommer. I de siste årene har vi blitt klar over nye prosesser som kan påvirke arvematerialet vårt og som ser ut til å være nært knyttet til sykdomsutvikling. Disse prosessene kalles epigenetiske forandringer, og er endringer som skjer i genmaterialet vårt uten å endre selve DNA-sekvensen. Slike endringer er som oftest ufarlige, men i visse tilfeller kan de sannsynligvis bidra i utviklingen av sykdommer. Flere faktorer, som alder, kjønn, livsstil og miljø, har vist seg å kunne påvirke epigenetiske endringer. DNA-metylering er epigenetiske endringer hvor kjemiske metylgrupper (CH_3) fester seg til DNA-sekvensen. Metyleringen er dynamisk og har vist seg å kunne påvirke genekspresjon. Det er viktig å finne ut både hvordan DNA-metylering forekommer og hvilke konsekvenser dette har. Nylig viste en studie at én treningsøkt ga spontan reduksjon i DNA-metylering av enkelte gener, men at metyleringen økte igjen etter en viss tid.

Gjennom dette prosjektet ville vi å undersøke om regelmessig trening kan endre DNA-metyleringen av genomet vårt i en langvarig forstand, og om disse endringene kan påvirke genekspresjonen. Nullhypotesen vår var at ingen slike endringer oppstår som følge av trening. Friske menn i alderen 70-75 år ble randomisert til en kontrollgruppe (n=13) og en høyintensitets intervalltrenings (HIIT) gruppe (n=13). Tester ble utført i begge grupper ved oppstart og etter åtte uker. HIIT gruppen gjennomførte åtte uker med høyintensitets intervalltrening på tredemølle tre ganger i uken, mens kontrollgruppen ble oppfordret til å leve som normalt. For å teste hypotesen vår ble DNA-metylering og genekspresjon analysert i muskelvev fra deltakerne. I tillegg ble bestemte helsefaktorer målt og eventuelle komplikasjoner som følge av høyintensitets intervalltrening av de eldre undersøkt.

Treningsgruppen viste en signifikant økning i maksimal aerob kapasitet, og en signifikant reduksjon i BMI og midjemål, mens kontrollgruppen viste en signifikant reduksjon i HDL-kolesterol. Motsatte endringer ble påvist i gruppene når det gjaldt endringer i DNA-metylering av utvalgte CpG-seter. Det vil si at i de CpG-setene hvor DNA-metyleringen ble redusert i HIIT gruppen viste en økning kontrollgruppen, og motsatt. Resultatene gir grunn til å tro at en forbedring i VO_{2max} kan påvirke de naturlige endringene som skjer i DNA-metyleringen i arvematerialet vårt, men endringene ble ikke vist signifikante. Ingen signifikante forskjeller vistes mellom gruppene i endring av genekspresjon av *MYH1*, *VDAC2*, *BNIP3* og *COX16,* som var de de utvalgte CpG-setenes assosierte gener. På grunn av svakheter i studien er det behov for mer omfattende studier for å avgjøre om høyintensitets intervalltrening kan endre DNA-metyleringen i arvematerialet vårt, og for å finne ut hvilke effekter disse endringene har.

1 Introduction

1.1 An aging population

In Norway the life expectancy has increased about 30 years for the last 200 years[1]. The incidences of several diseases increase with age[2,3]. Some age-associated diseases are infection, cardiovascular disease (CVD), musculoskeletal weakness, cancer, and dementia[2,3]. As people continuously get older and diseases occur more frequently, health care centers are filled up and society's economy is challenged[2,4,5].

1.2 Physical activity and health

As people get older they are more physically inactive[3,6]. Several studies show that regular physical activity affects the health in a positive way and prevents diseases[3,5,7-14]. It is also known that exercise increases aerobic capacity[5]. In an article by Baldwin and Haddad the researchers address the importance of understanding the mechanisms behind exercise benefits. Furthermore, they mention that exercise and disease prevention probably will be the most important factors for the health industry in the next decades and beyond[13].

1.3 Aerobic capacity

A normal procedure to determine a person's aerobic capacity is by measuring the maximal oxygen uptake (VO_{2max}, $mL \cdot kg^{-1} \cdot min^{-1}$) by treadmill running or ergometer cycling to exhaustion[5]. VO_{2max} is often defined as *"the oxygen intake during an exercise intensity at which actual oxygen intake reaches a maximum beyond which no increase in effort can raise it"*[15]. High aerobic capacity and good physical health are associated with 46% lower risk of dying of any cause[6]. A low VO_{2max} is associated with higher risk of metabolic and CVD, as well as premature mortality[16]. People who perform 30 minutes of moderate daily activity, have been proven with significant higher VO_{2max} than those who are sedentary[6]. The most important factors that affect the VO_{2max} include gender, age, and body composition, whereas to improve the VO_{2max} important factors are the person's fitness at start and the exercise mode (intensity, duration, frequency)[17,18]. A study that included 6 weeks of high-intensity endurance training of sedentary young subjects presented an average increase in VO_{2max} of 14%, however about 20% of the subjects were found with less than 5% improvement in VO_{2max}[16]. Studies suggest that genes can be a contributor to an individual's ability to improve aerobic capacity following regular exercise[16-23].

1.4 Aging, trainability and their effect on aerobic capacity

Men normally have a higher VO_{2max} than women, and studies show that VO_{2max} typically decreases with age[5,6]. A recently published Norwegian study report a reduction in VO_{2max} of 11% for men >70 years old compared to 60-69 years old men[24]. Several other research groups have reported a similar decline in aerobic capacity by age of 13-25% decrease every decade[25-28]. The same Norwegian study also report that men above 70 years old have an average VO_{2max} of 35.3 $mL \cdot kg^{-1} \cdot min^{-1}$ [24]. Diseases are major causes of inactivity among elderly and reduces their aerobic capacity[5]. Additionally, among the elderly generation many have minimal knowledge about exercise or lack personal exercise experience, and therefore do not exercise at moderate- to high-intensities[5]. The ability to maintain a high aerobic capacity is a major determinant of functional independence among the elderly[6]. If a person's aerobic capacity gets really low, the performance of regularly daily activities can become a struggle[6].

To improve aerobic capacity, exercise is the solution[16]. Numerous studies have proven beneficial effects of exercise in elderly people[5,8-10,29-32]. The risk of exercise is demonstrated to be very low if the right precautions regarding warm-up and how the exercise is performed are taken into account[33]. A meta study by Kelley *et al.* found that aerobic exercise resulted in a significant decrease in body mass index (BMI, kg/m^2) and percentage body fat, while VO_{2max} increased by 13% in older adults[34]. Studies that have documented a 10-25% increase in peak VO_2, following endurance training in 60-80 year old adults[5,35-37].

The benefits of exercise occur through increased quantity and quality of physical activity, where factors as intensity, duration and frequency are important[11,38]. Intensity is often measured as percentage of maximum heart rate (HR_{max})[39]. A paper by Helgerud *et al.* concludes that: *"High-aerobic intensity endurance interval training is significantly more effective than performing the same total work at either lactate threshold or at 70% HR_{max}, in improving* VO_{2max}*."*[39]. This study also suggests that 4x4-minutes high-intensity interval training is one of the best exercise models for increasing ones VO_{2max}[39]. Furthermore, several other studies have proven that high-intensity exercise is superior to moderate- or low-intensity exercise in improving VO_{2max}[9,32,38]. Often exercise studies with elderly include moderate intensity, however high-intensity interval training studies with 65-70 years old men have been performed[9,30,32]. Still, more studies on elderly are needed to elucidate how high-intensity interval training affects aerobic capacity and health of the elderly *above* 70 years of age.

1.5 Effects of exercise on the lipid profile of cardiovascular health

A lipid profile can indicate health status and the risk of CVD[40]. Several risk markers can be analyzed in a single blood sample. Some risk markers of CVD are total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, glucose and high-sensitivity C-reactive protein (hs-CRP)[40]. The risk markers have a reference value and if they are detected outside their range the risk of a cardiac event increase (reference values are included in Table 3.2)[40,41]. Studies have shown that aerobic exercise can reduce TC, TG and LDL cholesterol, while it increases HDL cholesterol[34,42].

1.6 Epigenetics and gene expression

Through the last decades researchers have discovered molecular processes that can affect the human genome and participate in the development of several diseases[43-49]. These processes are called epigenetic changes. Epigenetic changes involve alterations of the genome, without changing the DNA sequence itself, and consists of chemical compounds that modify or mark the genome[50]. DNA methylation has been identified as an important epigenetic mechanism in the dynamic regulation of a genes expression, which is a complex but fine-tuned process where the information in the genome is used to synthesize a functional gene product[44,51-55].

DNA methylation

DNA methylation is an epigenetic modification, described as the addition of the chemical methyl group (CH_3) to the carbon number 5 of the nucleotide base cytosine in the DNA[43,44,48,50] (Figure 1.1). All cells in the human body contain essentially the same DNA, whereas the DNA methylation pattern may highly differ. DNA methylation is a dynamic but highly controlled process that forms different cell- and tissue types during development[50]. The methylation pattern can be passed on from cell to daughter cell, and they may be inherited from one generation to the next[50]. Furthermore, the process can also change during adult lifetime[44,50]. Hence, the patterns of DNA methylation are much like fingerprints, specific to each cell, tissue type, and individual[44].

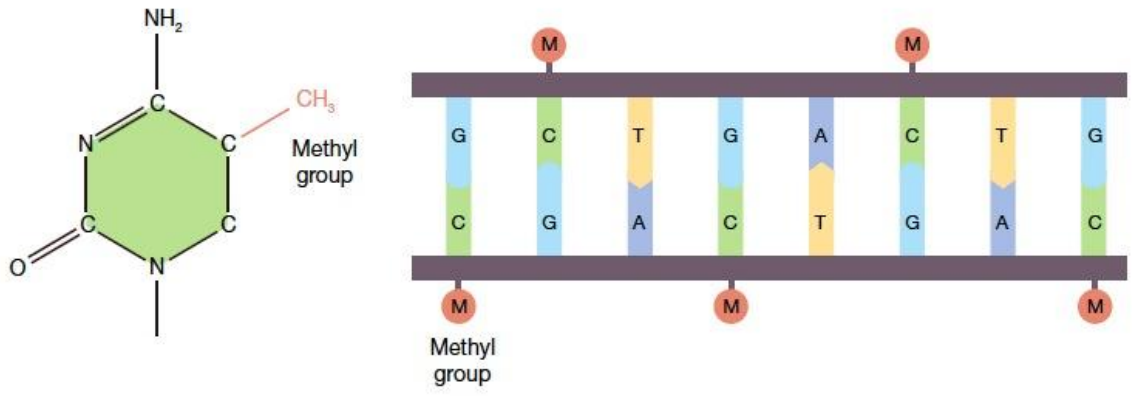

Figure 1.1: Left: Addition of a methyl group (CH_3) to carbon number 5 on the cytosine base creates 5-methylcytosine. Right: A small part of a DNA-strand. Cytosine bases in the DNA become methylated, often in CpG islands of the promoter regions. The figure is a reprint from the webpages of The Life Technologies[56]. O: oxygen; C: carbon; N: nitrogen; NH_2: amine group, CH_3: methyl group; M: methyl group; G: guanine, C: cytosine; T: thymine, A: adenine.

In humans, DNA methylation often occurs in CpG islands that are clusters of CpG dinucleotides (when a C and a G is connected with a phosphodiester bond) in GC-rich regions, and average about 1kb in length[44,53]. The human genome consists of approximately 30 000 000 CpG dinucleotides (or CpG sites) and about 6% of all CpG sites are located in the CpG islands[57]. There are estimated around 37 000 CpG islands within the human genome[53]. Approximately 65% of the CpG islands of the human genome are gene-associated, and around 11 000 of them overlap transcription start sites (TSS)[44,53]. The methyl groups affect the interactions between DNA and the cells transcription machinery and have important regulatory functions[44,50].

Numerous approaches have been developed to detect methylation patterns, like the *Illumina* bisulfite based array system[58]. Here, bisulfite converted DNA samples are added to an *Illumina microarray*, and methylation levels of 450 000 CpG sites can be detected within the same sample[58,59]. Sample throughput is however to some extent limited by the costs of the analysis[58]. One *Illumina microarray* often include 12 or 24 samples[59].

Gene expression

Gene expression is the process where the information from a gene is used in the synthesis of a functional gene product[51]. Gene expression is often divided into steps like transcription, RNA processing and translation[51]. Normally, transcription begins at the TSS in the promoter region of the encoding DNA sequence, and RNA is transcribed[51]. The RNA is then processed into a messenger RNA (mRNA), and follows translation into a functional product, which is usually one,

or several proteins[51]. Only a fraction of the genes in a cell is expressed at any one time and different cells express different combinations of genes[51]. The timing, location, and amount of gene expression profoundly affect the action and function of the gene in the organism[51]. Hence, gene expression is the most fundamental level at which genotype gives rise to the phenotype[51].

Numerous approaches have been developed to analyze gene expression. Gene expression is often investigated by quantitative (q) real-time reverse transcriptase (RT) polymerase chain reaction (PCR), which detect the transcription of specific genes at a certain time point with measurements of the amount of RNA in the sample[60].

1.7 Effects of DNA methylation on gene expression

It is generally accepted that a high levels of methylation in promoter CpG islands results in gene silencing and that CpG islands are normally unmethylated in actively transcribed genes (Figure 1.2)[57].

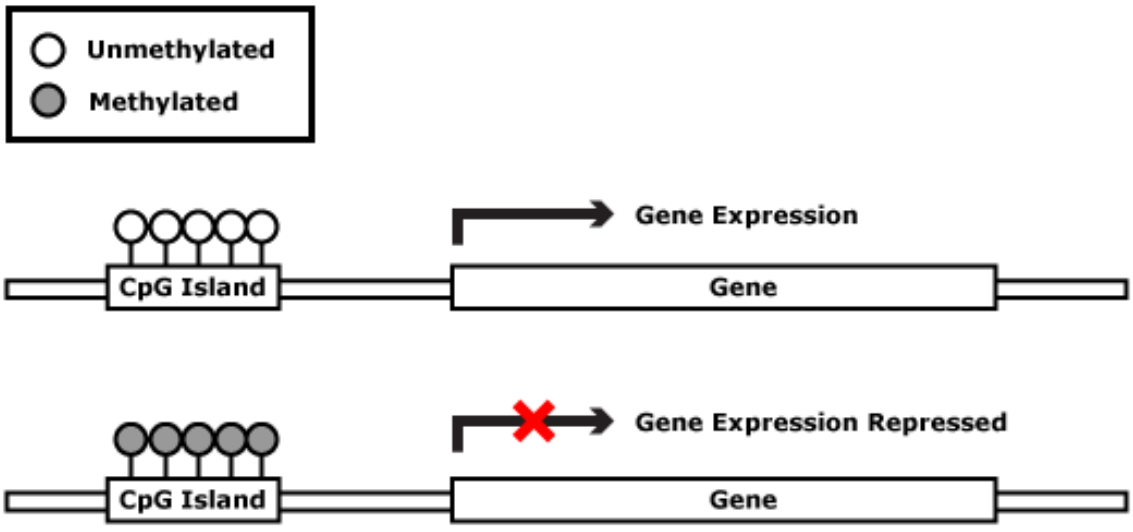

Figure 1.2 Unmethylated CpG islands are associated with an active gene, whereas a methylated CpG island is associated with repressed gene expression. The figure is a reprint from the webpage of The University of California[61].

Hypermethylation typically decreases transcription, whereas hypomethylation increases the transcription of downstream genes[44,52-54]. Hence, DNA methylation is an effective means by which gene expression is silenced, which may occur by different approaches[44,50,54,62]. DNA methylation of gene promoters may interfere with the binding of certain transcription factors

(TF's), it can attract methylated DNA-binding proteins that in turn recruit other modifying enzymes and hinder transcription, or it may lead to chromatin configuration that is unfavorable for gene expression[52,54,62].

Most changes of DNA methylation are probably harmless, but some changes have been proven to trigger or increase the severity of diseases[43,44]. This makes DNA methylation a vital part of efforts to better understand the human body and improve human health.

1.8 Causes of change in DNA methylation

Several factors have been proven to have an effect on DNA methylation, such as age, gender, lifestyle and environment[44,55,63]. DNA methylation is known to be higher in men, and in older people[55]. The cause of the variations is not fully understood[44]. Lifestyle and environmental factors can expose a person to chemical tags that change the epigenome[44,50,63]. Environmental factors such as diet, caffeine, and alcohol consumption have been shown to alter DNA methylation[44,63]. Additionally, exercise is believed to have an effect on DNA methylation as well[63]. A study by Barres *et al.* recently addressed this theory and proved that acute exercise caused transient changes in DNA methylation in adult skeletal muscle[63]. The workout demethylated the promoter regions of metabolic genes, whereas genes unrelated to the metabolism remained unmethylated[63]. The same study also found that the amount of demethylation of the promoters increased with the intensity of the exercise[63]. Furthermore, aberrant DNA methylation has been associated with various diseases, such as inflammation diseases, cancer or CVD[43-45,48,49,54].

1.9 The future of epigenetic and exercise in relation to health

In an article by You *et al.* the author address that in the future, new knowledge about the epigenome may benefit human health and provide new opportunities for improvement of therapies, and adds that the epigenome needs more research[49]. The mentioned study by Barres *et al.* addressed a potential relationship between exercise and epigenetics, and proved an acute decrease in DNA methylation in response to a single-bout of endurance training[63]. More research is needed to determine whether regular exercise can change the DNA methylation in a more long-lasting state, and to determine the causes and consequences of these exercise-induced changes.

1.10 The research hypothesis

In the present project we wanted to investigate whether regularly high-intensity interval training could provide long-term changes in DNA methylation of the human genome, and whether these changes could affect gene expression. Our null hypothesis was that no such changes in DNA methylation or gene expression could be detected.

1.11 Research objectives

- The ***primary research objective*** was to uncover non-acute changes in DNA methylation in promoter regions of specific genes in muscle tissue from high-intensity interval training subjects.
- The ***secondary research objective*** was to expose an inverse relationship between the change in DNA methylation of a specific gene and the expression of the gene.

Furthermore, ***additional research objectives*** were to explore the effects of high-intensity interval training on specific health factors and address possible complications of exercising considering the age of the subjects.

2 Experimental procedures and methods

To test the research hypothesis we measured the changes in DNA methylation and gene expression in skeletal muscle tissue before and after an exercise intervention, in old men. Furthermore, we measured the changes in VO_{2max}, BMI, waist circumference, fat percentage, and risk markers of CVD in blood from the same subjects. We analyzed the changes *within* each group (cases and controls, from baseline to follow-up), and also compared the difference in changes *between* the groups.

2.1 Subjects

This project was performed in collaboration with a study called Generation100 (Gen100), at NTNU, Faculty of Medicine, Department of Circulation and Medical Imaging. Gen100 addresses whether exercise has an effect on morbidity and mortality among elderly in Norway. Men and women in Trondheim, born in 1936-1942, were asked to participate in the Gen100-project. Participants were randomized into one of three groups; control group, moderate-intensity exercise training group, or high-intensity interval training (HIIT) group.

Twenty-six men (70-75 years old) were recruited from Gen100 to the present project, where participants were already randomized into groups. Thirteen men were recruited for each of the following groups: (1) HIIT group or (2) control group. The number of participants was based on estimations from previous studies at NTNU and similar studies from literature, with reservations of subjects withdrawing. A purpose of the project was to recruit participants at same age and gender, and train them with the same intensity, duration and frequency to avoid differences of these important factors affecting the results.

2.1.1 Inclusion and exclusion criteria

All participants had to approve their participation and sign a written, informed consent before their involvement in the study (Appendix A). Participants were free to withdraw from the study at any time and without further questioning. Further inclusion and exclusion criteria are presented in Table 2.1.

Table 2.1: Inclusion and exclusion criteria for participants in the project.

Inclusion criteria	▪ Male ▪ 70-75 years old ▪ Randomized to HIIT or control group in Gen100
Exclusion criteria	▪ Incompatible with exercise ▪ Known cardiac disease ▪ Active cancer ▪ Significant pulmonary disease ▪ Uncontrolled hypertension ▪ Use of blood thinning medicine (except Albyl-E) ▪ Drug abuse ▪ Alcohol abuse ▪ Finishing <80% of the exercise sessions (this only complies to the HIIT group)

HIIT: high-intensity interval training; Gen100: The Generation100-study

Ten men finished the project period, whereas three men dropped out in each group. Two men withdraw from the HIIT group and did not complete the exercise period. The first withdraw from personal reasons, and the second was unable to perform exercise because of severe back-pain (not caused by this project). Another man was later excluded from the HIIT group because he was diagnosed with significant pulmonary disease. Three men from the control group were excluded; two because they were unable to finish the follow-up tests because of personal reasons and one for changing his dietary pattern and exercise habits significantly during the project (lost 13 kg). For the distinctive laboratory analysis specific numbers of participants were included, and some subjects had to be excluded during data analyses, more details in Table 2.2.

Table 2.2: Number of included and excluded subject within all analyses, as well as the final valid number of subjects in the analyses, separated by groups: control and high-intensity interval training (HIIT) group.

	Included subjects		Excluded subjects in data analysis		Valid number of subjects in analysis	
Analyses	**HIIT**	**Control**	**HIIT**	**Control**	**HIIT**	**Control**
BMI, waist circumference, body fat, VO_{2max}, and blood markers	13	13	3	3	10	10
DNA methylation analyses	6∫	6∫	0	3‡	6	3
Gene expression of *MYH1*	10	9◊	2†	1†	8	8
Gene expression of *VDAC2*	10	9◊	1†	0	9	9
Gene expression of *BNIP3*	10	9◊	1†	1†	9	8
Gene expression of *COX16*	10	9◊	0	0	10	9

∫: Restricted numbers of samples included because of the high costs of performing this analysis; ‡: Excluded because they became "outliers" during the analysis on the *Illumina microarray* and were found fallacious; ◊: There was not enough muscle tissue from the last control to isolate RNA from; †: Excluded because of inadequate parallels; BMI: body mass index; VO_{2max}: maximal aerobic capacity; *MYH1:* Myosin Heavy Chain 1; *VDAC2:* voltage-dependent anion channel 2*; BNIP3:* BCL2/adenovirus E1B 19kDa interacting protein 3*; COX16:* Cytochrome C Oxidase Assembly Homolog.

2.2 Sampling procedure

Baseline measurement of height, weight, BMI, body fat, waist, VO_{2max}, and HR_{max} were performed in collaboration with Gen100 before the participants were randomized, and involved in the present project. A second baseline day was arranged to collect blood and skeletal muscle samples particularly for the present project. After baseline tests were performed the HIIT group started a eight weeks exercise period, whereas the control group was instructed to live normally. Then, individual follow-up tests were performed continuously. The follow-up test of VO_{2max} and HR_{max} was performed as the individuals last exercise session in the HIIT group, and after 8 weeks for the control group. The follow-up measurements of weight, BMI, body fat, waist, as well as collection of blood samples and muscle biopsies were scheduled two days later, to avoid acute effects of the treadmill test in the measurements. Therefor the participants were also specifically requested to avoid exercise during the 48-72 hours between these two tests. All measurements of weight, BMI, body fat, waist circumference, as well as blood samples and muscle biopsies were collected on a fasting stomach, whereas tests on treadmill to obtain measurements of VO_{2max} and HR_{max} were performed non-fasting. The project procedure is illustrated in Figure 2.1.

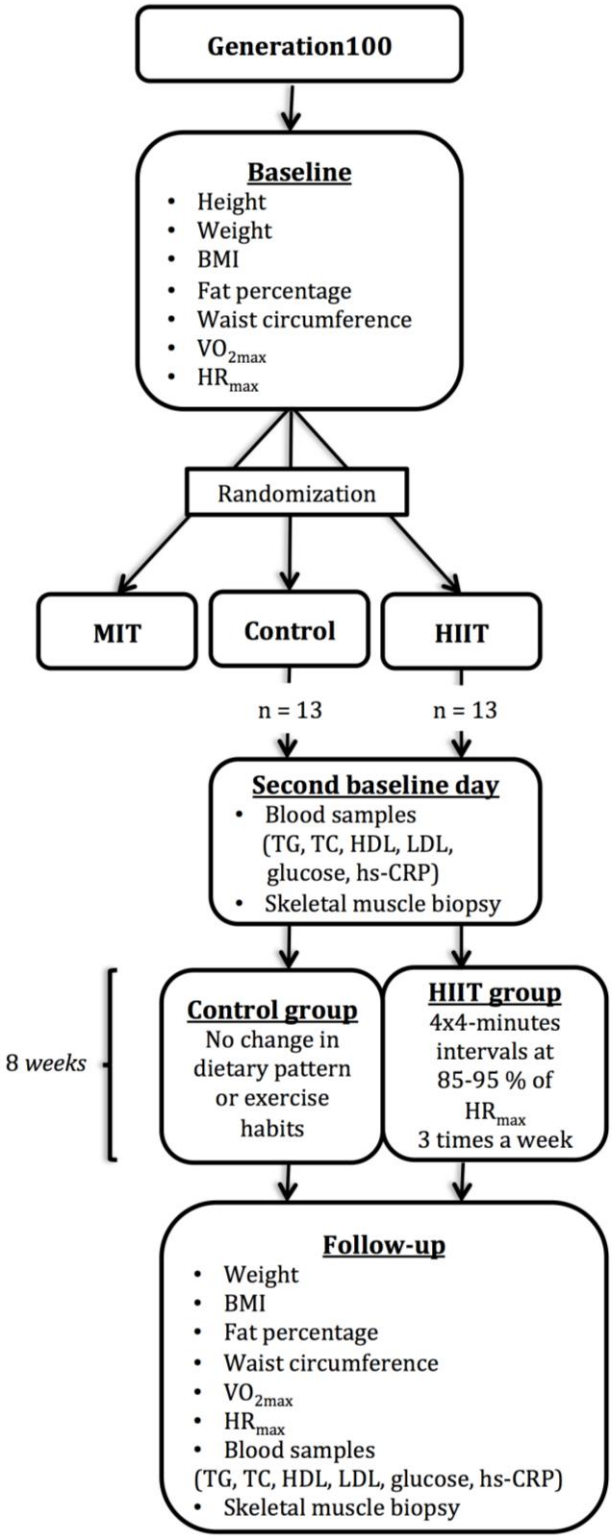

Figure 2.1: Project procedure. The inclusion and baseline tests were performed in collaboration with Gen100. Then, the participants were randomized, and included to the present study. The intervention period lasted for 8 weeks. BMI: body mass index; VO_{2max}: Maximal aerobic capacity; HR_{max}: Maximal heart rate; MIT: Moderate-intensity training group; HIIT: High-intensity interval training group; n: Number of subjects; TG: Triglycerides; TC: Total cholesterol; HDL: High-density lipoprotein cholesterol; LDL: Low-density lipoprotein cholesterol; hs-CRP: High-sensitivity C-reactive protein.

2.2.1 Height, weight, body mass index, body fat and waist circumference

The standard procedures at *The Clinical Research Facility* at the St. Olavs Hospital were used to measure the height, weight, BMI, body fat percentage and waist circumference of the participants. The height was measured in meters (m) with one decimal and adjusted to the nearest 0.5 centimeter (cm). Weight, BMI and body fat percentage was measured using an instrument called *Inbody 720* (BIOSPACE, Body Analysis AS, The southern region, Korea). *Inbody 720* also provided other measurements, as left-leg muscle mass that was used for decisions regarding latter DNA methylation analysis. Participants wore light clothes (top and trousers) and no shoes. The weight was measured in kilograms (kg) with one decimal. Body fat percentage was measured to the nearest 0.1%. BMI was automatically calculated as weight divided by square of height (kg/m^2). The waist circumference was measured in cm with a regular measuring band, one cm over the navel and adjusted to the nearest 0.5 cm. It was ensured that all measurements during follow-up tests were taken at the same time of day as the baseline tests.

2.2.2 Blood analyses of risk markers of cardiovascular disease

Health personnel collected blood samples following standard procedures at St. Olavs Hospital. A 3 mL Li-Heparin-tube was collected, sent and directly analyzed at *The Medical Biochemistry Laboratory* at St. Olavs Hospital. Parameters that were analyzed were TC, HDL cholesterol, LDL cholesterol, TG, glucose, and hs-CRP.

2.2.3 Maximal aerobic capacity and maximal heart rate

Measurements of VO_{2max} and HR_{max} were completed during walking or running on the treadmill. The test was performed using *MetaMaxII* (CORTEX Biophysik, Leipzig, Germany) and its software *MetaSoft3*. To perform the maximal aerobic test on the treadmill the participants wore a mask, covering both their nose and mouth. The test began with 10 minutes warm-up, where the incline and speed was individually adjusted, based on the participants' fitness level. After warm-up the next level was either to increase the incline of the treadmill by 2% or increase the speed by 1 km/h. The first increase was after 2-3 minutes for the values to stabilize. The increase continued when the level of oxygen uptake stabilized, approximately every one or two minutes. This protocol was carried out until the participant was no longer able to increase workload and had to stop because of exhaustion, as well as the criteria for maximal oxygen uptake had been reached. The best way to ensure that the participants had reached their true VO_{2max} was to see that the oxygen curve had a straightening out or drop in spite of increased

workload on the treadmill. In combination, a respiratory exchange ratio (RER, the ratio of carbon dioxide produced, to oxygen consumed) higher than 1.05, was used as criteria for exhaustion and that true VO_{2max} had been reached. However, it is important to mention that reaching this value were not a criterion to stop the test. RER was only a factor to control how close to fatigue the participants were during the tests, so the test continued till the participant was exhausted and had to stop. Most of the participants reached RER-values of 1.15-1.20. During the working period measurements of oxygen uptake and heart rate were continuously monitored, using *MetaMaxII* and *Polar Heart Rate Monitors*, respectively. The three highest achieved oxygen uptakes during the test were used as average, and termed as VO_{2max}. The highest heart rate during the test was ascribed 5 additional beats and assigned as HR_{max} (this is the procedure in Gen100 and has previously been done in another study[64]). The HR_{max} from the baseline test was used to calculate the individual heart rate during the exercise sessions. HR_{max} and RER-values did not change significantly from baseline to follow-up, which are good indications that the tests were performed in a right manner.

2.2.4 Muscle biopsies from *vastus lateralis*

Muscle biopsies were collected from all participants in both groups before and after the exercise period. The biopsy was obtained from the skeletal muscle *vastus lateralis* at the same site of the participants left thigh. The procedure was performed by a medical doctor using a sterile 5-mm-diameter biopsy needle under local anesthesia (2% Lidocaine)[65]. First, a 5–10 mm incision was made and the biopsy needle was introduced into the muscle tissue without using suction. One additional person assisted the medical doctor during the surgical intervention. Three small muscle biopsies were collected from each participant, directly frozen in liquid nitrogen and stored at −80 °C in the biobank of Gen100 for later analyses. After the muscle tissue was collected, the patient got 2-3 stiches and compression for 10 minutes before finishing. The participants were followed up after the surgical procedure and the stiches were removed after 7-10 days.

2.3 The exercise period

The HIIT group performed individually supervised high-intensity interval training on treadmills, three days a week for eight weeks at the exercise-facility at NTNU/St. Olavs Hospital. The control group had no follow-up on exercise during the 8 weeks.

2.3.1 High-intensity interval training group

The HIIT group was instructed to complete exercise sessions of 4x4-minutes intervals at 85-95% of HR_{max}, which was selected as the exercise intervention based on previous studies presenting great improvement of VO_{2max} with this type of exercise[5,10,32,34,39]. This type of exercise include 10 minutes warm-up, followed by four intervals of four minutes high-intensity (85-95% of HR_{max}), with three minutes active breaks (70% of HR_{max}) between each interval, and ending with seven minutes cool-down. An exercise session last for 42 minutes, where the participants should breathe heavily and feel exhausted four times. Exercise intensity was supervised through the use of *Polar Heart Rate Monitors*, and speed and incline were adjusted continuously to ensure that each session was performed at the assigned intensity. Participants had a personal instructor following them at every exercise session. If participants could for some reason not show up for their exercise sessions they were rescheduled. All participants were able to finish all sessions. Most of the participants of the HIIT group were unfamiliar with treadmills and high-intensity interval training. However, they quickly got familiar with this type of exercise and managed to control the treadmills themselves.

2.3.2 Control group

The control group followed the baseline and follow-up tests, and was asked to live normally and not change their dietary pattern or exercise habits during their participation in this project. Based on the well-known beneficial effects of exercise on general health, it was regarded unethical to demand the control group to desist from any types of physical activity during the project period. They got no further follow-up on exercise.

2.4 Wet laboratory analyses

The collected muscle tissue was used for wet laboratory analyses of DNA methylation and gene expression. DNA was isolated from muscle tissue, bisulfite converted, and DNA methylation was analyzed by microarray analysis. Furthermore, RNA was isolated from muscle tissue, and gene expression was measured by quantitative real-time reverse transcriptase polymerase chain reaction (real-time qRT-PCR). The wet laboratory analyses process is illustrated in Figure 2.2.

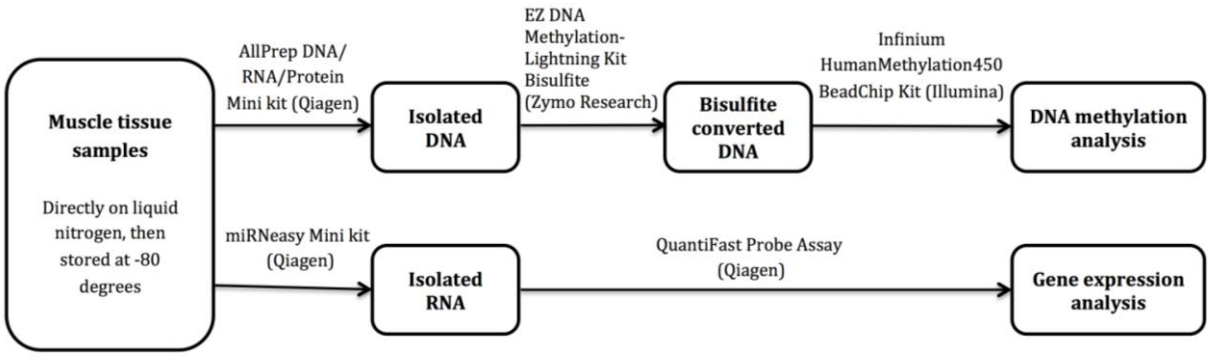

Figure 2.2: Flow chart of the wet laboratory process, including the names of the kits used to analyze the muscle tissue samples.

2.4.1 DNA methylation analysis

Because of high costs of the DNA methylation analysis the number of samples had to be restricted, where change in VO_{2max} and left-leg muscle mass were used as criteria for inclusion. From the control group, the subjects with the most stable VO_{2max} and left-leg muscle mass were chosen to be included. From the HIIT group the subjects with the greatest improvements in VO_{2max}, to be sure that they have had optimal benefits from the exercise intervention, and minor changes in left-leg muscle mass were included. It was assumed that these subjects would show the largest changes in methylation status after the exercise intervention. A stable left-leg muscle mass was a criteria to avoid changes of muscle mass to disturb the potential findings of change in DNA methylation.

DNA isolation

DNA, total RNA and proteins were isolated from muscle tissue samples (~20 mg) following the standards of the protocol *AllPrep DNA/RNA/Protein Mini Kit* (Qiagen, Hilden, Germany)[66]. Briefly, the muscle tissue was first lysed and homogenized in a buffer, containing guanidine-isothiocyanate[66]. This buffer is highly denaturing and immediately inactivates DNases and RNases[66]. The lysate was then passed through an AllPrep DNA spin column[66]. In combination with high-salt buffer, the column selectively binds the genomic DNA from the tissue sample[66]. After washing, ready-to-use DNA was eluted from the column[66]. Furthermore, this kit also isolates total RNA and proteins from the same muscle tissue sample. The isolated RNA had low quantity, and thus another kit was used to isolate RNA to use in this project (see section 2.4.2). Isolated proteins were not used in this project. Therefore, both isolated RNA and proteins from this kit were directly stored at -80 degrees for prospect work. A *NanoDrop 2000c/2000 UV-Vis Spectrophotometer* (Thermo Fisher Scientific, Wilmington, USA) was used to measure the

260/280 Ratio and 260/230 Ratio, which determine the quantity and the purity of the isolated DNA[67]. All samples were found at acceptable levels, around 1.8 for the 260/280 Ratio and 2.0-2.2 for the 260/230 Ratio[67].

Bisulfite conversion of DNA

The isolated DNA was bisulfite converted following the standards of the protocol *EZ DNA Methylation-Lightning™ Kit Bisulfite* (Zymo Research, Irvine, USA)[68]. The kit converts unmethylated cytosine to uracil while methylated cytosine remain unchanged[68]. Quantity and purity of the bisulfite converted DNA was tested on the *NanoDrop*, whereas *Bioanalyzer 2100* (Agilent, California, USA) was used to control the degradation, to assure the quality of the samples. The quantity, purity and quality of the bisulfite converted DNA was found acceptable in all samples.

Microarray analysis

Bisulfite converted DNA was further administered by *The Genomics Core Facility* at NTNU, and DNA methylation analysis was performed following standards of the protocol *Illumina Infinium HumanMethylation450 BeadChip Kit* (Illumina, San Diego, USA)[59].

The *Illumina HumanMethylation450 BeadChip* is a microarray used for detection of DNA methylation level within more than 450 000 CpG sites of the human genome, both within and outside of genes and CpG island regions[59]. In general, microarray is a method where synthesized probes (a fragment of DNA or RNA that is able to hybridize to a specific target) are attached to a solid surface and used to detect nucleotide sequences of interest from a sample that are complimentary to the sequence in the probe[69]. A microarray can contain tens of thousands of probes, located within different "spots" on the array[69]. The target bind to the specific probe in a spot and the amount of target in a sample can be detected[69].

The *HumanMethylation450 chip* used in this project have micro wells where the individual samples are applied, and each well contain DNA methylation specific probes linked to specific bead types[70,71]. Each bead-bonded probe corresponds to the methylated or unmethylated state of each specific CpG site[70,71]. Basically, depending on the methylation status of the DNA sample, the sample will either bind to the methylation specific or the unmethylated specific probe[70]. The *Illumina microarray* is a trusted DNA analysis platform and contains 600 internal quality

controls[59,72]. The results from the *Illumina microarray* represent the DNA methylation level of the DNA samples and are presented as Beta values (the ratio of signal from the methylated probe relative to the sum of both methylated and unmethylated probes)[55]. The Beta value ranges from 0 (unmethylated) to 1 (completely methylated)[55].

To run statistical analyses of the results from the *Illumina microarray* the Beta values were converted to M-values as derived in the article of Du *et al.* from 2010[73]. The conversion was done because of the heteroscedasticity of the Beta values, and to make them more suitable for statistical analyses[73,74]. Furthermore, the data was analyzed and a "Ranged list" was created, containing the CpG sites that had changed their DNA methylation status the most among the individuals. The subjects were compared to each other in terms of their change in VO_{2max}. This means the individual samples were not compared considering which group they belonged to, but the change in DNA methylation was rather analyzed as a correlation to the individuals change in VO_{2max}. This was considered the best way to compare the samples, because this method would also take the gradational amount of change of VO_{2max} into account.

Because of the high number of analyzed CpG sites and the low number of samples, *Bonferroni corrections* were performed (This method divides the critical p-value by the number of comparisons being made, to adjust the statistical analyses for multiple testing)[75]. This gives a significance level of these tests a p-value$<1.1 \times 10^{-7}$ (0.05/450 000). The "Ranged list" contains an insecurity of faulty detected CpG sites. Also, the list can be prepared in a many different ways considering its purpose. Thus, a literature search was performed to pick the CpG sites from the "Ranged list" considered of best biological interest, as well as most believable to be changed following an exercise intervention. The chosen CpG site were all part of CpG islands of promoter regions, which was also the case in the study of Barres *et al.*, the central article of the present research hypothesis.

The selected four CpG sites from the "Ranged list" became (Illumina ID): cg16842280, cg26801646, cg08079580, and cg23562388, presented with details in Appendix B. These four CpG sites are respectively associated with the promoter region of the genes *MYH1*, *VDAC2*, *BNIP3*, and *COX16*. *MYH1* (Myosin Heavy Chain 1) is highly expressed in the skeletal muscle, whereas *VDAC2* (voltage-dependent anion channel 2), *BNIP3* (BCL2/adenovirus E1B 19kDa interacting protein 3), and *COX16* (Cytochrome C Oxidase Assembly Homolog) are all genes that encode proteins of the mitochondrial membrane. Both skeletal muscle and mitochondria are highly

active during exercise and therefore these CpG sites were considered of our biological interest, because of the projects exercise intervention.

The change in DNA methylation of the four CpG sites from baseline to follow-up is presented in this report as delta values (follow-up Beta value/baseline Beta value). A delta ratio >1 indicates an increase in DNA methylation from baseline to follow-up, whereas a ratio <1 indicates a decrease in DNA methylation.

2.4.2 Gene expression analyses

RNA was isolated from muscle tissue samples and analyzed by real-time qRT-PCR to detect gene expression of the four chosen genes *MYH1*, *VDAC2*, *BNIP3,* and *COX16*.

RNA isolation

RNA was isolated from muscle tissue samples (~30 mg) following the standards of the protocol *miRNeasy Mini kit* (Qiagen, Hilden, Germany)[76]. Briefly, the muscle tissue was lysed and homogenized in the *QIAzol Lysis Reagent*, using beads and the TissueRuptor *Mixer Mill Type MM 301* (Retsch GmbH & Co., Haan, Germany). Then, spin columns was used to separate sections, then bind, wash, and elute high-quality RNA from the samples[76]. The *QIAzol Lysis Reagent* in this kit is a solution of phenol and guanidine thiocyanate, which inhibits RNases from degrading the unstable RNA, and also removes most of the cellular DNA and proteins from the lysate[76]. The *NanoDrop* was used to measure the 260/280 Ratio and the 260/280 Ratio to determine the concentration and purity of the isolated RNA[67]. All samples were found at acceptable levels, around 2.0 for the 260/280 Ratio and 2.0-2.2 for the 260/230 Ratio[67]. After RNA concentrations were measured every sample was diluted to a concentration of 90 ng/µL by adding appropriate amounts of RNase free water. The RNA concentrations and dilution details are added in Appendix C.

Quantitative real-time reverse transcriptase polymerase chain reaction

The isolated RNA with a concentration of 90 ng/µL was analyzed by real-time qRT-PCR, detecting the expression of the four genes *MYH1*, *VDAC2*, *BNIP3*, and *COX16*. Using *QuantiFast Probe Assay* (Qiagen, Hilden, Germany) in combination with *QuantiFast Probe RT-PCR Plus Kit* (Qiagen, Hilden, Germany) the PCR process was performed following the standard protocol for

one-step duplex detection[77,78]. The assay contains premixed and specific primer pairs, and is designed to detect hydrolysis probes in a single step RT-PCR[77]. Briefly, the principle of these hydrolysis probes and the PCR process is explained in Figure 2.3[77].

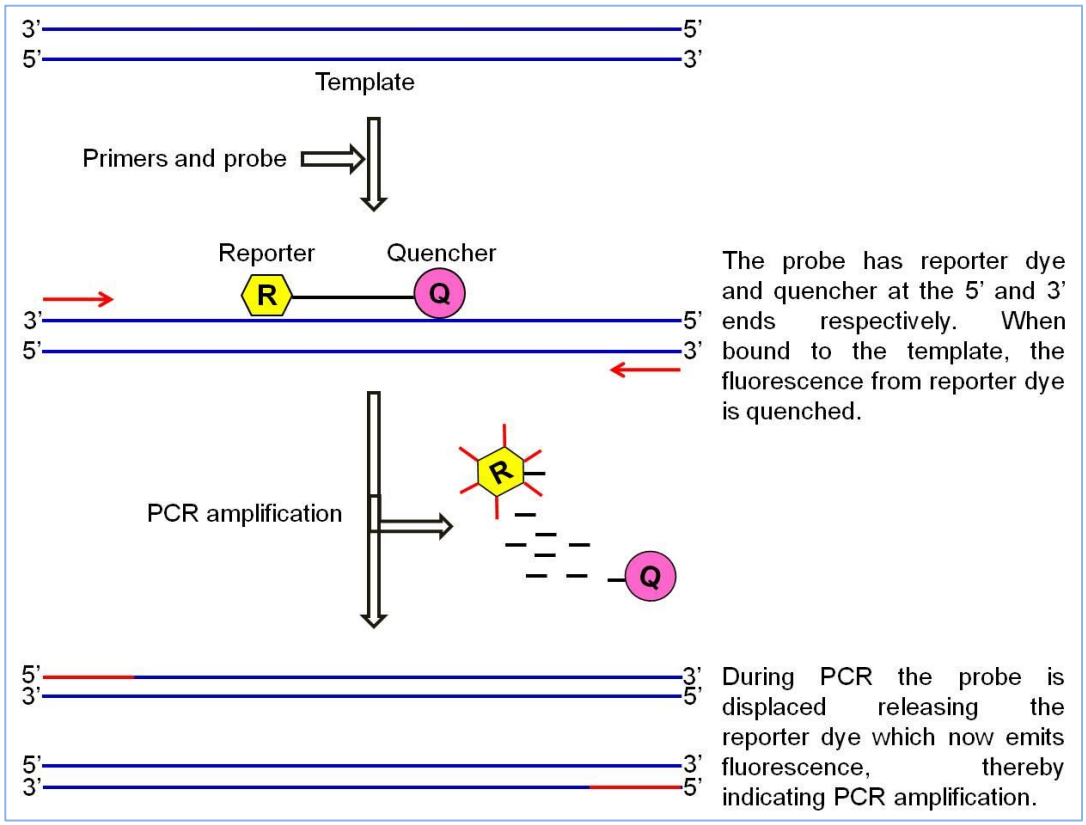

Figure 2.3: The principle of the real-time qRT-PCR and the detecting of fluorescence from the probes during PCR amplification. Top: During the PCR annealing step, both the probe and the primers anneal to the RNA target sequence (template). The proximity of the fluorophore (R) with the quencher (Q) prevents the probe from fluorescing. Bottom: During the PCR extension step, DNA polymerase extends the primer. When DNA polymerase reaches the probe, the fluorophore is cleaved from the probe and emit a fluorescent detectable signal. This results in a detectable fluorescence that is proportional to the amount of accumulated PCR product. The figure is a reprint from the article of Ali and Ali[79].

Four assays were performed in total, one for each of the four chosen genes *MYH1*, *VDAC2*, *BNIP3,* and *COX16*. 96-wells plates were used, and all samples and experimental controls were run in parallels. The set-up for the real-time qRT-PCR analyses can be found in Appendix D. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as the reference gene. Carboxyfluorescein (FAM) was used as reporter dyes for the target gene and hexachloro-6-carboxy-fluoresceine (HEX) was used as reporter dyes for the reference gene. The choice of *GAPDH* as the reference gene was supported by a study that concluded that *GAPDH* and β2M were the most stably expressed reference genes in skeletal muscle following endurance exercise[80]. In the present project *GAPDH* were stable and showed no significant variation in the

gene expression analyses. Also, a no-template control (NTC) and a no-reverse transcriptase (NRT) control were included into the assay, where no amplification should be detected, to be able to prove that the signals obtained from the target samples were valid and specific. To run the real-time qRT-PCR process the *BioRad CFX96 real-time PCR thermal cycler* (BioRad Laboratories Inc., California, USA) was used. The *Bio-Rad CFX Manager Software 2.0* (2009) delivered the results that were subsequently handled manually.

The cycle threshold (C_t) were detected for every well, which is the cycle number where the fluorescent signal of the reaction crosses the set threshold (manually adjusted)[60]. The C_t value was used to calculate the initial amount of the specific RNA in each sample[60]. The C_t value is inversely related to the starting amount of your target[60]. If the C_t value increase with one cycle the sample will contain double of the starting amount (assuming 100% efficiency)[60]. Figure 2.4 shows how the results from the real-time qRT-PCR are illustrated, which include threshold line, and detected fluorescence's (both for the reporter gene and the target gene) in every individual samples and the controls.

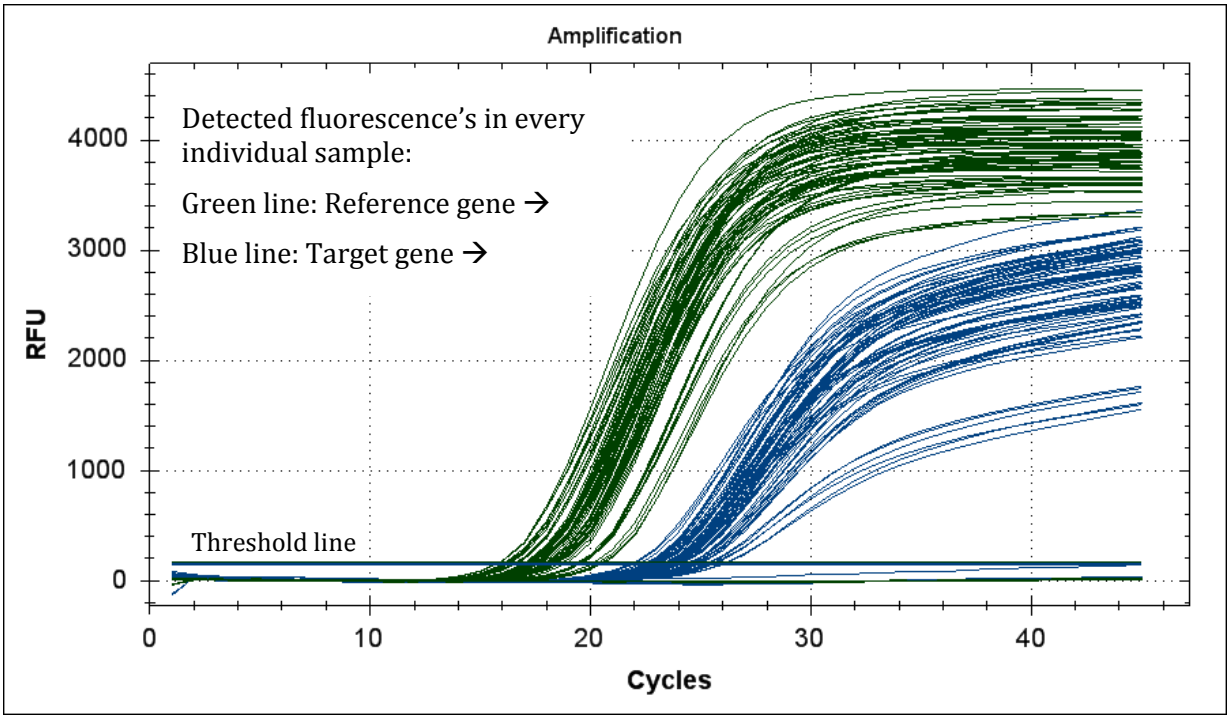

Figure 2.4: Graphical results from duplex run of real-time RT-qPCR. Threshold is here shown as a straight, horizontal line at approximately 200 relative fluorescence units (RFU), right above the detected background and controls. Green lines represent the reference gene (HEX as reporter dye) and blue lines represent the target gene (FAM as reporter dye), and are both detected in every individual sample.

The C_t values were analyzed using comparative quantification where the $\Delta\Delta C_t$-algorithms were used[60]. The method compares the results from the experimental samples (here: the HIIT group

samples) with both a calibrator (here: the control group samples) and a normalizer (here: the reference gene *GAPDH*)[60]. Mean values of the parallels were used. Using this method, the C_t value for the gene of interest (GOI) in both the experimental samples (s) and calibrator samples (c) were adjusted in relation to a normalizer (norm) gene's C_t from the same two samples[60]. The $\Delta\Delta C_t$ values were incorporated to determine the fold change in gene expression, following these equations (1-4)[60]:

$$\text{Fold change} = 2^{-\Delta\Delta Ct} \quad (1)$$

$$\Delta C_{t\ sample} - \Delta C_{t\ calibrator} = \Delta\Delta C_t \quad (2)$$

$$C_{t\ GOI}\ s - C_{t\ norm}\ s = \Delta C_{t\ sample} \quad (3)$$

$$C_{t\ GOI}\ c - C_{t\ norm}\ c = \Delta C_{t\ calibrator} \quad (4)$$

A fold change <1 denote a decrease in gene expression from baseline to follow-up, whereas a fold change >1 represent an increase.

2.5 Ethics

All subjects recruited for the study participated voluntarily. Participants got written information about the project and had to sign an informed consent before their involvement in the study, which also included that their biological material could be used in this project. The study personnel had to ensure that every subject understood what participation in this study involved, and also informed about possible side effects that could occur. Participants were free to withdraw from the study without reason, and were free to choose whether collected data could be used in further research or not. If risk markers of CVD were detected outside reference intervals in blood samples, the results were reported back to the subject with a requirement to contact his family doctor. All data was treated confidentially to protect the participants' privacy. All employees in the project had professional secrecy. The samples and data that were registered about the participants were only used in accordance with the purpose of the study. Gen100 and the present project were both undertaken in accordance with the Helsinki declaration, and approved by the Regional Ethical Committee (REC, Gen100 ref. nr: 2012/381, Present project ref. nr: 2012/823b). The REC approval for the present project is added in Appendix E.

2.6 Biobank

The biobank for Gen100 was used for storage of muscle tissue samples. This biobank was located at *Heart and Lung Center*, St. Olavs Hospital. Professor Ulrik Wisløff had the responsibility for the biobank. All the data and samples were processed without name, ID number or other directly recognizable type of information. A serial number linked each participant to their data and samples through a list of names. All personal information and data collected were stored in a locked place, and only the responsible researchers had access to the list that linked the serial number to personal information.

2.7 Statistics

Statistical analyses were performed using the software program *SPSS, version 20.0* (IBM SPSS, New York, USA). Means and standard deviations (SD) for all variables were computed using descriptive statistics. Delta values (follow-up/baseline) were calculated for each variable, and represent a fold change. Generally, a fold change>1 shows an increase of the variable from baseline to follow-up, whereas fold changes<1 represents a decrease. All variables were tested with Shapiro-Wilk test, but because of the low number of participants all variables were accepted as nonparametric. Wilcoxon signed rank test was used to detect exact significant changes within the groups, from baseline to follow-up. Delta values were analyzed by Mann-Whitney U test, to find exact significant difference in changes between the two groups. Correlations of delta values were analyzed by Crosstab correlation statistics using Pearson test, where DNA methylation of the four CpG sites and the respective genes expression data were correlated. Exact 2-tailed significant p-values were used for all the tests. Significance levels for all tests were set to $p<0.05$ and trends were set to $p<0.10$. Figures were made using Chart builders in SPSS or excel. All the statistical tests were performed with advices and support from the *Unit for Applied Clinical Research* at The Faculty of Medicine.

The *Genomics Core Facility*, NTNU, provided the microarray service, and bioinformatics scientist Einar Ryeng performed the statistical analyses of the results from the *Illumina microarray*. Considering the projects selections and requests he performed statistical analyses using Principal Component Analysis (PCA) and Linear Models for Microarray Data (Limma). To adjust for the high number of detected CpG sites *Bonferroni corrections* were used[75]. Thus, the significance level for these analyses were set to $p<1.1x10^{-7}$.

3 Results

3.1 Body mass index, body fat and waist circumference

Descriptive data of BMI, body fat and waist circumferences for the study population is summarized in Table 3.1. The results present a significant decrease in BMI ($p<0.05$) and waist circumference ($p<0.01$) in the HIIT group, with an average decrease of 1.7% and 5.5%, respectively. There was also detected a trend of 0.6% decrease in body fat within the HIIT group ($p<0.1$). A significant decrease in waist circumference was detected in the control group with an average decrease of 2.5% ($p<0.05$), however, the HIIT group experienced a significantly larger decline in waist circumference compared to the control group. No significant changes were detected for BMI or body fat percentage in the control group. The individual changes of weight, BMI, body fat percentage and waist circumference are added in Appendix F.

Table 3.1: Mean values of body mass index, body fat and waist circumferences at baseline and follow-up.

	HIIT group (n=10)		**Control group (n=10)**	
	Baseline (±SD)	**Follow-up (±SD)**	**Baseline (±SD)**	**Follow-up (±SD)**
BMI, kg/m²	28.9 ±3.8	28.4 ±3.7 **§	26.5 ±2.2	26.5 ±2.3
Body fat, %	29.8 ±6.9	29.2 ±6.9	25.8 ±4.0	26.0 ±3.7
Waist, cm	103.7 ±10.6	97.9 ±8.2 **§	100.3 ±7.4	97.8 ±9.2 *

*: Significantly different from baseline, $p<0.05$; **: Significantly different from baseline, $p<0.01$; §: Significantly different from control group in change from baseline to follow-up, $p<0.05$. Values are mean±SD. SD: Standard deviation; HIIT group: High-intensity interval training group; n: number of participants; BMI: Body mass index.

3.2 Circulating blood markers of cardiovascular health

Descriptive data of the blood marker analyses is presented in Table 3.2. The control group was found with a significant 8% decrease in HDL cholesterol ($p<0.01$), and the change was significantly different from the HIIT group ($p<0.05$). The blood markers TC, LDL, TG, glucose and hs-CRP did not change significantly. All mean values of the groups were detected inside the reference intervals[41]. Individual data on the blood markers are presented in Appendix G.

Table 3.2: Blood markers of cardiovascular health at baseline and follow-up. Reference values from St. Olavs Hospital where the normality of the population spans are presented.

	HIIT group (n=10)		**Control group (n=10)**		***Reference values from St. Olavs Hospital[41]***
	Baseline (±SD)	**Follow-up (±SD)**	**Baseline (±SD)**	**Follow-up (±SD)**	
Total cholesterol (mmol/L)	5.50 ±0.77	5.46 ±0.81	5.73 ±1.18	5.93 ±1.43	*3.90-7.80*
LDL cholesterol (mmol/L)	3.40 ±0.63	3.46 ±0.79	3.53 ±1.26	3.90 ±1.36	*2.00-5.30*
HDL cholesterol (mmol/L)	1.58 ±0.52	1.55 ±0.51 **§**	1.71 ±0.51	1.57 ±0.47 **	*0.80-2.10*
Triglycerides (mmol/L)	1.15 ±0.40	1.02 ±0.32	1.09 ±0.26	1.02 ±0.16	*0.45-2.60*
Glucose (mmol/L)	5.89 ±0.47	5.82 ±0.86	5.90 ±1.19	6.06 ±1.52	*4.20-6.30*
hs-CRP (mg/L)	4.70 ±10.27	1.67 ±1.00	2.47 ±2.44	2.47 ±1.98	*0.00-5.00*

**: Significantly different from baseline, p<0.01; §: Significantly different from control group in change from baseline to follow-up, p<0.05. Values are mean±SD. SD: Standard deviation; HIIT group: High-intensity interval training group; n: number of participants; LDL cholesterol: Low-density lipoprotein cholesterol; HDL cholesterol: High-density lipoprotein cholesterol; hs-CRP: High-sensitivity C-reactive protein.

3.3 Maximal aerobic capacity

The HIIT group had a significant increase in VO_{2max} (p<0.01), and the change was also significant compared to the control group (p<0.01) (Table 3.3).

Table 3.3: Maximal aerobic capacity, heart rate, and respiratory exchange ratio at baseline and follow-up.

	HIIT group (n=10)		**Control group (n=10)**	
	Baseline (±SD)	**Follow-up (±SD)**	**Baseline (±SD)**	**Follow-up (±SD)**
VO_{2max}, $mL \cdot kg^{-1} \cdot min^{-1}$	29.4 ±4.4	33.7 ±5.1**§§	31.6 ±5.1	32.6 ±5.5
HR_{max}, heartbeats $\cdot min^{-1}$	167.2±12.9	167.7±13.5	153.9±19.8	156.9±14.1
RER	1.16±0.07	1.13±0.05	1.13±0.03	1.11±0.05

**: Significantly different from baseline, p<0.01; §§: Significantly different from control group in change from baseline to follow-up, p<0.01. Values are mean±SD. SD: Standard deviation; HIIT group: High-intensity interval training group; n: number of participants; VO_{2max}: Maximal aerobic capacity; HR_{max}: maximum heart rate; RER: Respiratory exchange ratio.

The participants in the HIIT group had an average improvement of 14.6% in VO_{2max} after the exercise intervention. The fold change in VO_{2max} (follow-up/baseline) within the two groups is shown in Figure 3.1. A fold change>1 represents an improvement in VO_{2max}, whereas fold change<1 represents a decrease from baseline to follow-up.

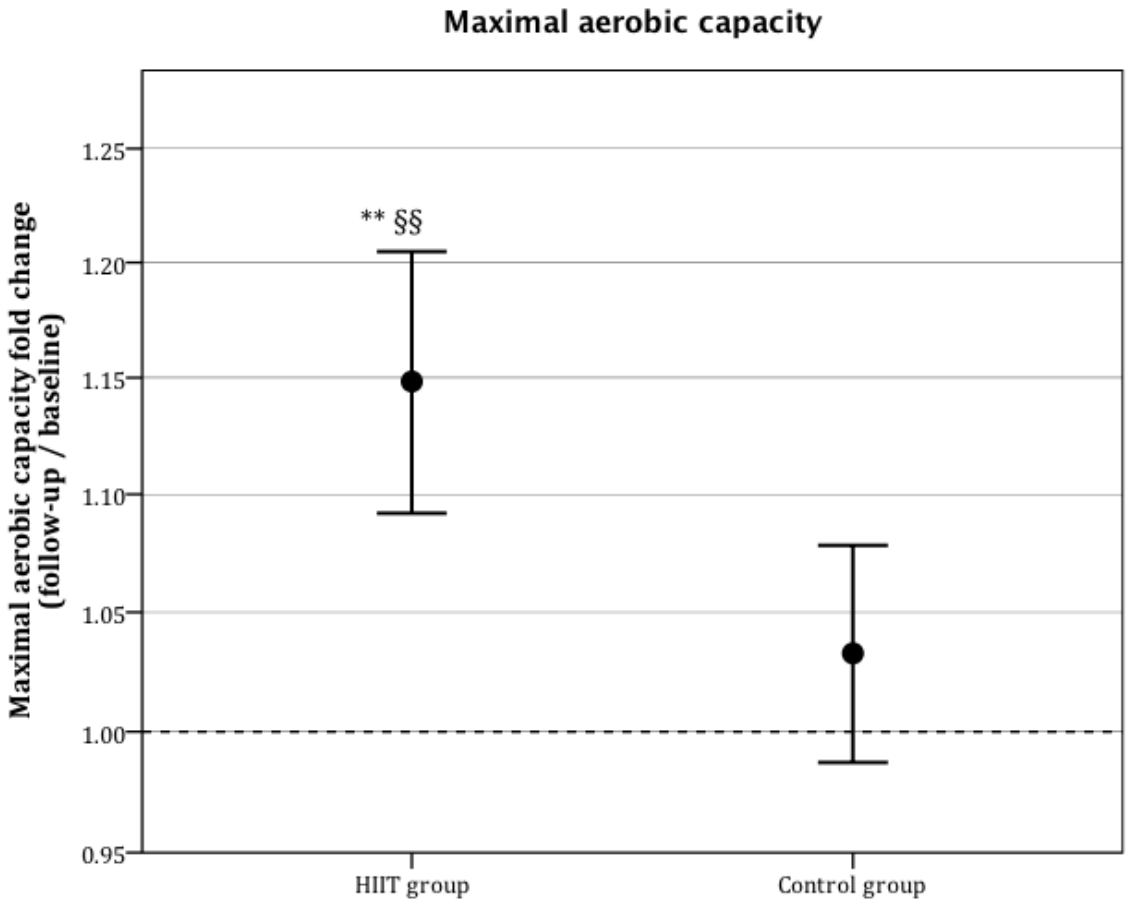

Figure 3.1: Fold change in maximal aerobic capacity in high-intensity interval training (HIIT) group and control group. Mean value ± SD. SD: Standard deviation **: Significantly different from baseline, p<0.01; §§: Significantly different from control group in change from baseline to follow-up, p<0.01.

The individual improvements of VO_{2max} in the HIIT group ranged from a 3% to a 28% increase. The controls individually ranged from a 7% decrease to a 12% increase, but the group had no significant change. Figure 3.2 shows mean values±SD of VO_{2max} within groups illustrated in bars, at baseline and follow-up. Additionally, it includes lines representing each individual, illustrating the differences in initial VO_{2max} and the change in VO_{2max}. Individual data of VO_{2max} are presented in Appendix H.

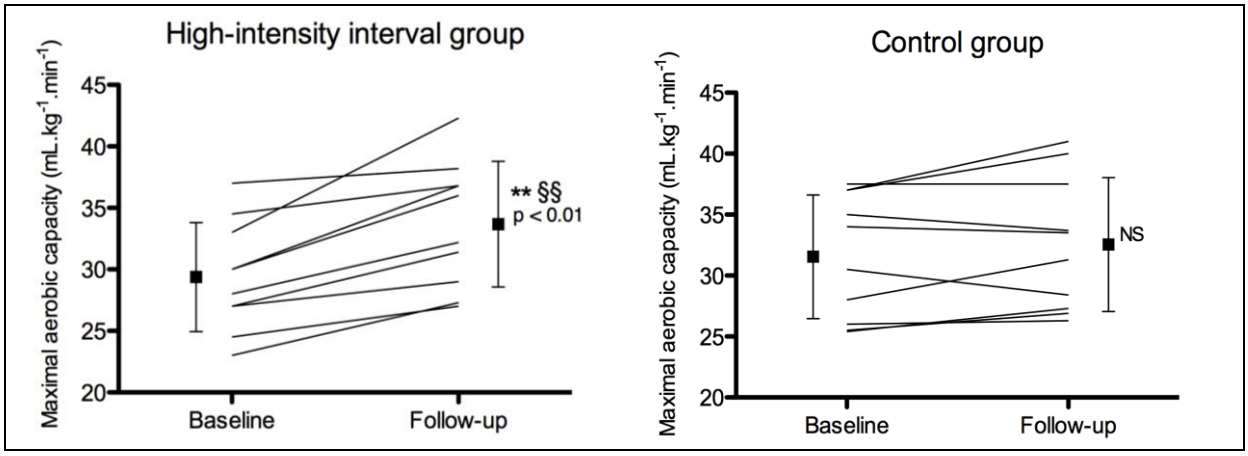

Figure 3.2: Bars represent changes in VO_{2max} (mean ± SD) at baseline and follow-up. Lines represent individuals and their change from baseline to follow-up. Left: High-intensity interval training group with a significant change of VO_{2max} within the group (**) as well as compared to the change of the control group (§§), with p<0.01. Right: Control group show no significant (NS) change in VO_{2max}. SD: Standard deviation.

3.4 DNA methylation

The DNA methylation delta ratios from the *Illumina microarray* for the four chosen CpG sites are presented in Figure 3.3. The detected *global* DNA methylation from the *Illumina microarray* was about 51% for every sample, only separated by the seventh decimal. However, differences were detected between the groups in terms of changes in DNA methylation of the four chosen CpG sites in the promoter region of *MYH1, VDAC2, BNIP3,* and *COX16,* where all four CpG sites changed in opposite directions in the two groups, but no significance could be detected. In more details, the DNA methylation of the CpG site in the promoter region of *MYH1*, *VDAC2*, and *COX16* decreased in the HIIT group (13.5%, 14.3% and 15%, respectively) whereas it increased in the control group (23.4%, 13.6% and 12.5%, respectively). The DNA methylation of the promoter region of *BNIP3* increased with 25.7% in the HIIT group whereas it decreased 10% in the control group. Individual data on DNA methylation are presented in Appendix I. In the individual results from DNA methylation two subjects especially stood out from the others, one subject from each group, discussed in section 4.3.

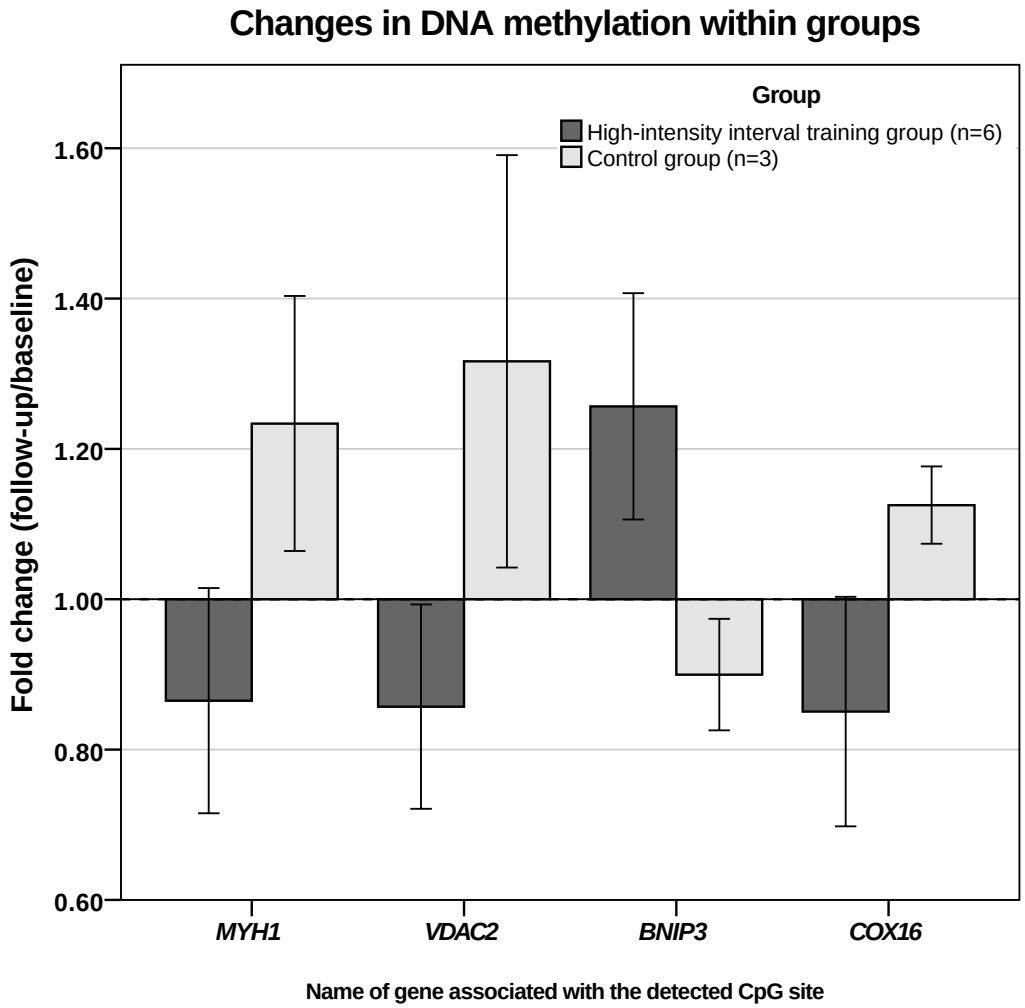

Figure 3.3: Fold change of DNA methylation in the four CpG sites of the promoter regions of the genes *MYH1*, *VDAC2*, *BNIP3,* and *COX16*. Figure shows mean Beta values ± SD within both groups. SD: Standard deviation; n: Number of subjects.

3.5 Gene expression

Fold change of gene expression ($2^{-\Delta\Delta Ct}$) is shown for *MYH1*, *VDAC2*, *BNIP3,* and *COX16* in Figure 3.4. A significant change in gene expression was detected for *MYH1* within the control group ($p<0.05$), where the expression increased by 3.25-fold during the study period. The expression of the same gene increased1.47-fold after the exercise intervention in the HIIT group, but the change was not significant. No significant changes of gene expression were detected for *VDAC2, BNIP3* or *COX16* within neither of the two groups. Furthermore, no significant changes were detected in gene expression of *MYH1, VDAC2, BNIP3* or *COX16 between* the groups either. Individual data on gene expression is shown in Appendix J. One subject from the control group clearly differed from the other subjects in change of *MYH1* gene expression.

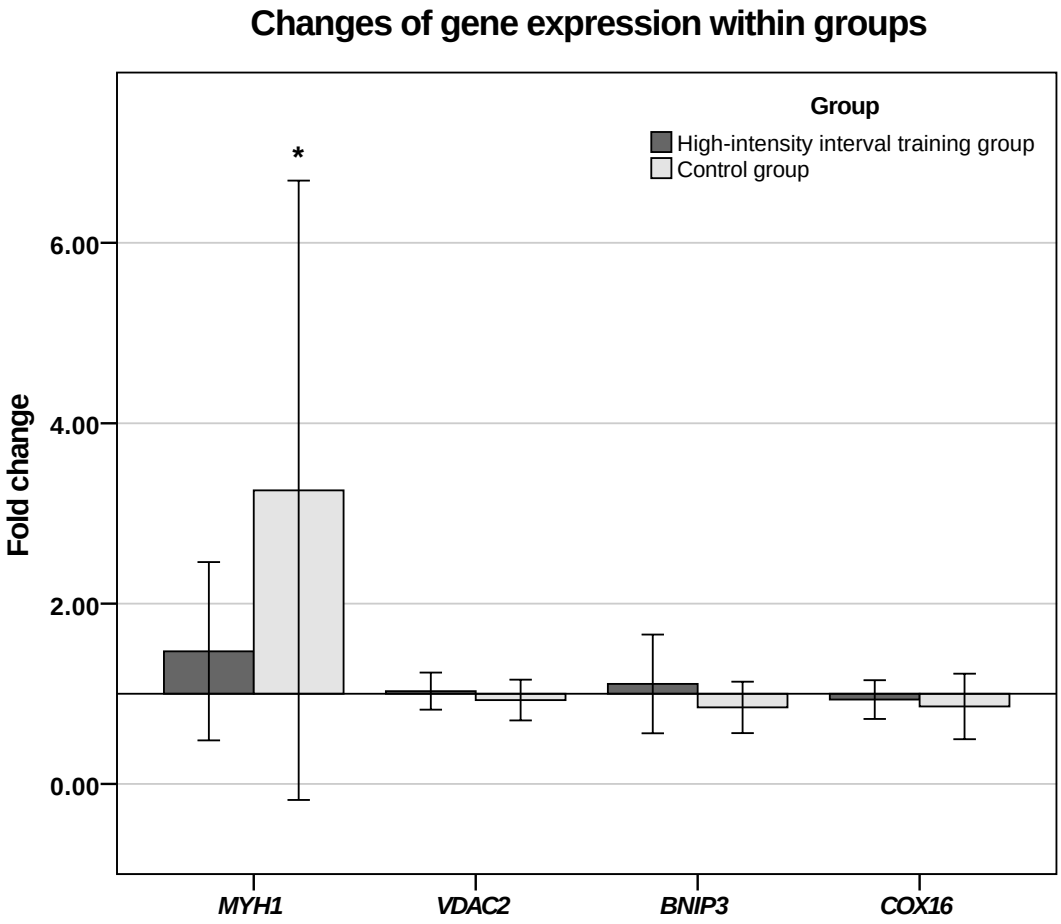

Figure 3.4: Fold change ($2^{-\Delta\Delta Ct}$) of gene expression for the four genes *MYH1*, *VDAC2*, *BNIP3,* and *COX16*, within each group. *: Significantly different from baseline, $p<0.05$. Figure shows mean values ± SD. SD: Standard deviation; C_t: cycle threshold.

3.6 Correlation analyses of changes in DNA methylation and changes in gene expression

No significant correlations were detected in either group when correlating changes in DNA methylation of the four specific CpG sites and the changes in expression of the four respective genes; *MYH1, VDAC2, BNIP3,* and *COX16*.

4 Discussion

The main findings of this study were that eight weeks of high-intensity interval training in elderly men resulted in a significant increase in VO_{2max} and a significant decrease of BMI and waist circumference. These changes are likely to have improved the participant's general and cardiovascular health, even though we were not able to show any changes in blood parameter's reflecting cardiovascular health[5,34,40]. Furthermore, no adverse side effects were detected by the exercise intervention, supporting earlier evidence that high-intensity interval training is a safe prevention strategy even in men aged 70-75 years old[33].

Regarding DNA methylation, the *Illumina microarray* analysis indicated a possible correlation between changes in VO_{2max} and changes in DNA methylation of the promoter regions of the genes *MYH1, VDAC2, BNIP3,* and *COX16*. Despite changes in DNA methylation of a CpG site in the promoter of these genes, no significant correlations were found between the changes in DNA methylation and the changes of gene expression. Additionally, no significant difference could be detected between the groups in changes of gene expression. This may indicate that the change in DNA methylation of these particular CpG sites did not have an effect on gene expression of these particular genes.

Following, the results give reasons to believe that an improved VO_{2max} can affect the naturally occurring changes of DNA methylation. However, larger and more extensive studies are needed to determine whether high-intensity interval training actually changes DNA methylation, and whether these changes have functional effects on transcription.

4.1 High-intensity interval training in elderly

The project demonstrates that exercise at intensities above 85% of HR_{max} was not associated with any specific complications or injuries among 70-75 year old men. Explaining the principles of 4x4-minutes intervals for the participants seemed to be a motivating factor for the subjects to perform the exercise sessions. Also, the efficiency of the exercise sessions (only 42 minutes to finish), along with the variation of regulating speed and incline during the sessions appeared as encouraging for the subjects. Furthermore, five months after the exercise period ended, HIIT-participants could tell that they still perform the same 4x4-minutes regime 2-3 times a week on their own, because they liked the efficacy of the session and the effects this type of exercise give them. Many elderly share the opinion that exercise at high intensities are only for young people[5].

However, this project address that with the right motivating factors, also the elderly are able to perform high-intensity interval training and can benefit from it.

4.2 Positive health effects of high-intensity interval training

This project showed that eight weeks of high-intensity interval training resulted in a likely improvement of the participant's general and cardiovascular health, which was anticipated considering earlier studies[34,81,82].

Effects on body mass index, waist circumference and body fat percentage

At baseline both groups were classified as overweight, regarding their BMI, body fat percentage and waist circumference, which are all well-known factors to increase with age[81-84]. Furthermore, high BMI or large waist circumference are known as important risk factors of several diseases, like heart disease, type-2 diabetes, and high blood pressure[81,82,84]. The present study found an exercise-induced decrease in these risk factors, which may reduce the participant's risk of CVD[84]. Additionally, it may improve the respiratory function, sleep, functional capacity, the daily activity ability, depressive symptoms, sense of well-being and quality of life[84]. However, it is still not clear which cut-off values of BMI, waist circumference and body fat percentage that predicts the best health risks in obese elderly[84]. No significant change was detected in body fat percentage within the groups. This is supported by a study by Nybo *et al.* where also no significant change in fat percentage was detected after 12 weeks of high-intensity interval training[82]. In contrast, the same study also included subjects performing prolonged moderate endurance training, which induced a significant reduction in fat percentage[82]. This indicates that the duration of the exercise, rather than high-intensity, is a more important factor for improving fat percentage.

Effects on blood markers of cardiovascular health

According to the detected values of the blood markers no participants were considered at high risk of CVD. However, the control group showed a significant decrease in HDL cholesterol, which is considered non-beneficial for their health[41]. Furthermore, only small changes were detected in the blood markers within both groups, where the SD's express the natural variations between the individuals. Such individual variations were clearly shown in the hs-CRP at baseline in the HIIT group, where the SD was detected at 10.27. This SD reflects that one subject clearly differed from the others; the reason is probably that this subject had an uncomplicated viral respiratory

tract infection, or "the flue", which often occur among Norwegians at these times of year. This is supported by the fact that the same individual was detected at normal levels of hs-CRP in the follow-up test, and therefore was not considered at higher risk of CVD. A raised hs-CRP could have affected the VO_{2max}-test, but the RER-values and HR_{max} measured during the tests indicate no support to these possibilities. The raised hs-CRP is not considered to be of high influence on any of the other factors or tests.

The reasons why few changes occurred in the blood markers may be that the duration of the exercise period or that each exercise session was too short. This is supported by a study that indicates that exercise volume rather than intensity is more important for the improvement of the lipid profile[82]. Other previous studies have detected decreased levels of TC, TG, LDL cholesterol, and increased levels of HDL cholesterol, but here the exercise intervention have been more than 8 weeks[5,34,42]. Summarized, it is more likely that blood markers had changed if the intervention period had continued for several months, or with increased duration of each exercise session[5,34,42,82].

Effects on maximal aerobic capacity

The individuals in both groups had a similar initial $VO_{2max,}$ in the range of 25-35 mL · kg^{-1} · min^{-1}. The HIIT group significantly ($p<0.01$) increased their VO_{2max} during the exercise period, which was as anticipated considering previous related studies[18,32,34]. The benefits of a higher aerobic capacity are many, but the managing of daily activities may be the best-perceived effect for these elderly men. All the subjects in the HIIT group increased their VO_{2max}, individually increasing by 3-28%. These results are comparable to the earlier mentioned studies, which detected a increase in VO_{2max} by 10-25% following high-intensity training[5,35-37]. In a study where eleven men (73.5+/-4.2 years) performed 14 weeks of high-intensity interval training on ergometer cycle, the subjects improved their VO_{2max} by 18.6% (26.8±4.4 to 31.8±5.2 mL · kg^{-1} · min^{-1}, $p<0.01$)[85]. These results are very similar to our eight weeks study, where the HIIT group improved their VO_{2max} with 14.6% (29.4±4.4 to 33.7±5.1 mL · kg^{-1} · min^{-1}, $p<0.01$). Our results clearly prove that high-intensity interval training is efficient to increase VO_{2max} in 70-75 year old men. From being almost 14% lower than the general average for men >70 years old, at 35.3 mL · kg^{-1} · min^{-1}, the HIIT group were able to nearly reach the average[24]. The individual differences of improvement in VO_{2max} seen in participants of the HIIT group might be explained by differences in initial VO_{2max}-levels, body composition, or genes[5,13,16,18,32,34]. A main criterion to improve the VO_{2max} is exercise intensity[5,17]. All participants of the HIIT group were instructed to keep an

intensity of 85-95% of HR_{max} during every exercise session, and were personally followed. However, if a participant constantly exercised in the upper level (95%) versus the lower level (85%) of the HR_{max}, this may give individual difference of improvement in VO_{2max}.

The control group did not change their VO_{2max} significantly, but shows great individual variations. The subject with the minimum change had a decrease of 7%, whereas the best individual improvement was an increase of 12%. The undetermined differences in activity level among the controls is most probably the reason for the individual variation of change in VO_{2max}, see more in section 4.4. Furthermore, the effects of joining an exercise study, even though you are randomized to the control group, may in some situations encourage the participants to perform more physical activity as a consequence of the information provided in the participant information.

4.3 Epigenetic and genetic effects of high-intensity interval training

Effects on DNA methylation levels

The aim of this project was to explore if high-intensity interval training could induce changes in DNA methylation in skeletal muscle. Whether an up-regulation or down-regulation of DNA methylation of genes is positive or not is still unclear. The theory was that sedentary people and active people would have opposite effects on the DNA methylation of specific genes, whereas some genes would also remain unchanged. It is known that DNA methylation increases with age[44,55]. If exercise were able to modulate DNA methylation, the theory suggests that it would also be capable of suppressing the natural changes of DNA methylation caused by age. These theories are supported in the article by Nakajima *et al.,* which found that DNA methylation in a CpG island of the gene *ASC* was higher in active elderly people than in sedentary elderly[86]. This study also showed that *ASC* was expressed differently according to the subjects' age and physical activity[86]. Furthermore, the DNA methylation level of another gene that the same study analyzed, *P15*, was not affected by either age or exercise[86]. The same theories were also supported in the present project, since we observed that HIIT altered DNA methylation in *MYH1*, *VDAC2*, *BNIP3,* and *COX16* in the opposite direction of the control group. However, no significance was detected, and the lack of validation of the chosen CpG sites makes the inverse relationship insecure. Our suggestion is that the observed changes in DNA methylation should have been validated by DNA methylation specific PCR, and in a larger population to be confirmed.

It is interesting that the most extreme changes in DNA methylation are detected in individuals of opposite groups; subject number 4135 in the control group and subject number 3505 in the HIIT group (see Appendix I). The control subject showed the maximum individual increase in DNA methylation of the CpG sites at *MYH1* and *VDAC2*, and the second highest increase of the CpG site at *COX16*, as well as the utmost decrease in DNA methylation of the CpG site at *BNIP3*. Furthermore, the greatest opposites were found within the HIIT-subject. The interesting part of this is that this control subject also had the utmost decrease in VO_{2max}, whereas this HIIT-subject had the maximum increase in VO_{2max}. These opposite findings of changes in DNA methylation, both between these individuals and the two groups in general, support that an improvement in VO_{2max} takes part in suppression of the DNA methylation natural changes.

Effects on gene expression

A research objective was to explore if a probable change in DNA methylation of a gene could inversely affect the genes expression. The article of Nakajima *et al.* supports this theory where they proved that a high amount of DNA methylation (of seven CpG sites in a CpG island near exon 1 of the gene *ASC*) was associated with a lower gene expression in elderly people[86]. Our theory was that these potential changes in DNA methylation and gene expression would be associated with exercise in a positive manner. However, it is worth mentioning that whether this means more or less gene expression depends on which gene is considered. These theories are supported in the article by Timmons *et al*., which proved that 6-20 weeks of endurance training resulted in several variations in how genes were expressed and had a significant effect on how fit people became[16]. This is also supported by the Nakajima study, which found that moderate exercise of older subjects might restore the DNA methylation levels in *ASC* to comparably younger levels[86]. Accordingly, exercise have former been proven to affect DNA methylation and gene expression in a positive manner. However, in our study no significant difference in change of gene expression was detected between the groups, which support our null hypothesis that no exercise-induced changes in gene expression of *MYH1, VDAC2, BNIP3,* and COX16 occur.

Along with no changes in gene expression, also no significant correlations could be detected for the changes in DNA methylation and the changes in gene expression for the genes *MYH1*, *VDAC2, BNIP3,* and *COX16,* in either group. In the HIIT group, a detected significant correlation would support our research hypothesis and the second research objective. Also, such a detection would have been a tool in the confirmation or debunk of the findings of change in DNA methylation from the *Illumina microarray*.

4.4 Limitations of the study

The main concerns of the study are the small study population, the multiple testing problems in the statistical analyses of the results from the *Illumina microarray*, and the lack of a validating step of the DNA methylation analysis. Further limitations of the study may be the duration of the exercise period, the lack of reports on dietary pattern, and varied exercise habits within the control group.

The first limitation of the project is the small number of participants. As mentioned, the numbers of participants were considered sufficient at start but with several exclusions, both for the DNA methylation analysis and generally during data analyses, the number of participants became scarce. The *Illumina microarray* analysis should optimally have included all the participants; however this was limited by economy. In general, with a low number of participants the project got limitations regarding statistically significant findings. However, some new theories could appear based on the project's findings, which again may give life to new projects. A larger number of subjects in the project would have enhanced the statistical power. The data found significant should therefore be reproduced in a larger population to potentially have an impact on the guidelines of physical activity in this group of elderly people.

A second limitation is the multiple testing problems in the statistical analyses of the *Illumina microarray*, and the lack of a validating step of these results. Dealing with a small number of participants, and the detection of 450 000 CpG sites in each sample, made the multiple testing problems prominent. Therefore, it always exist a possibility of false positives within the created "Ranged list". This makes the results from the *Illumina microarray* not strong enough to give reliable results about DNA methylation levels alone. Hence, a validating step of the detected changes in DNA methylation levels should have been included, and may have been performed by DNA methylation specific PCR on the chosen specific CpG sites. DNA methylation specific PCR analyses were meant to be performed in this present project, but due to the lack of commercial available kits for this purpose, and limited amount of time to develop a custom-made procedure, this were not done. With no validation, the choice of CpG sites from the "Ranged list" was only based on a literature search. Optimally, as many as possible of the CpG sites from the "Ranged list" should have been investigated by DNA methylation specific PCR. Then, the associated genes of the validated CpG sites could have been further examined by gene expression specific PCR.

Further limitations may be the duration of the exercise period. Exercise is known to have positive health effects when performed regularly, thus a longer exercise period may have increased the possibilities of exploring positive health effects. Also, the lack of information on participants' dietary habits, smoking habits, alcohol consumption, as well as more detailed information about medical use may also be a limitation; because coffee, alcohol and smoking are possible affecters of DNA methylation[44,45,63]. Finally, the activity levels in the control group may be a limitation for the study. The project includes controls intended to behave as normally, and not change their dietary pattern or exercise habits. Hence, there may be great variability in exercise habits among the controls. The theory was that the intervened change in exercise would be the possibility of finding differences between the groups. However, to be able to explore possible changes in DNA methylation and gene expression it may have been better to include a sedentary control group, and therefore rather analyze the extremes. Then, the results (if significant) could explain the outcomes of an active lifestyle versus an inactive one.

4.5 Future directions

First, as briefly done in the present project but with alternative directions; the results from the *Illumina Infinium HumanMethylation450 BeadChip* can be analyzed following multiple procedures, where alternative perspectives and inputs can be used to produce multiple "Ranged lists" from the same *Illumina microarray* results. First, one alternative way of searching for candidate CpG sites is to look for *multiple CpG sites that are related to the same gene* in the top of the "Ranged list". This may lead to a great chance of finding a correlation between change in DNA methylation and regulation of gene expression. A second alternative is to make several "Ranged list" by different inputs and perspectives, and then chose the *CpG sites that are present in several lists* for further analyses. A third alternative is to *make a new general group* (consisting of the originally control group at baseline and follow-up, and the originally HIIT group at baseline), and make a "Ranged list" by comparing this new group to the originally HIIT group at follow-up. Then, the assumptions would be that the control subjects and the HIIT-subjects were similar at start, and that no changes occurred in the control group during the exercise period. This third alternative may be a good alternative to reveal only the CpG sites that actually changed because of the exercise. Generally for all three alternatives, whichever CpG sites are chosen they should all be validated by DNA methylation specific PCR in an expanded number of subjects. Thereafter, gene expression specific PCR should be performed on the respective genes to explore whether the change in DNA methylation affected the expression of the genes.

Then, considering other future directions of epigenetics in general, possibilities seem enormous. DNA methylation of CpG sites and genes should be thoroughly investigated. In the future the status of DNA methylation may increase the molecular understanding of different diseases. Maybe disease-associated genes can be detected and exercise can be introduced as a preventive or treating intervention for these diseases. Or maybe disease-associated pathways that are triggered by epigenetic changes can be detected, and these pathways can be introduced as drug targets for the pharmacological industry. Surely, these are just ideas and lots remain to derive these theories.

5 Conclusion

Eight weeks of high-intensity interval training of 70-75 year old men was well tolerated, and gave significant improvements in maximal aerobic capacity. The HIIT group significantly reduced their waist circumference and body mass index, whereas the control group significantly decreased their HDL cholesterol. An inverse relationship was detected between the groups regarding their change in DNA methylation of four specific CpG sites, but the relationships were not proven significant. Furthermore, no significant differences were detected between the groups' changes in gene expression of *MYH1, VDAC2, BNIP3,* and *COX16*, which are the genes associated with the four specific CpG sites. However, the results give reasons to believe that an improvement in VO_{2max} can affect the naturally occurring changes of DNA methylation in our genome. Still, larger and more extensive studies are needed to determine whether high-intensity interval training actually changes DNA methylation, and to find out which consequences these changes have.

References

1. Levealder - faktaark med statistikk om forventa levealder i Noreg. 2012. (Accessed November 2nd, 2012, at http://www.fhi.no/artikler/?id=70805.)

2. Eldres helse - 65 år og over. 2010. (Accessed November 2nd, 2012, at http://www.fhi.no/artikler/?id=85146)

3. Woods JA, Wilund KR, Martin SA, Kistler BM. Exercise, inflammation and aging. Aging and disease 2012;3:130-40.

4. Andelen personer over 65 år i befolkningen. Folkehelseinstituttet, 2008. (Accessed June 22, 2013, at http://www.fhi.no/artikler/?id=70828.)

5. Fleg JL. Aerobic exercise in the elderly: a key to successful aging. Discovery medicine 2012;13:223-8.

6. Anderssen SAH, B.S.; Kolle, E.; Lohne-Seiler, H.; Edvardsen, E.; Holme, I. Fysisk form blant voksne og eldre i Norge - Resultater fra en kartlegging i 2009/-2010. http://www.helsedirektoratet.no; 2010 October 2010.

7. Khan KM, Thompson AM, Blair SN, et al. Sport and exercise as contributors to the health of nations. Lancet 2012;380:59-64.

8. Fielding RA, Rejeski WJ, Blair S, et al. The Lifestyle Interventions and Independence for Elders Study: design and methods. The journals of gerontology Series A, Biological sciences and medical sciences 2011;66:1226-37.

9. Nemoto K, Gen-no H, Masuki S, Okazaki K, Nose H. Effects of high-intensity interval walking training on physical fitness and blood pressure in middle-aged and older people. Mayo Clinic proceedings Mayo Clinic 2007;82:803-11.

10. Langlois F, Vu TT, Chasse K, Dupuis G, Kergoat MJ, Bherer L. Benefits of Physical Exercise Training on Cognition and Quality of Life in Frail Older Adults. The journals of gerontology Series B, Psychological sciences and social sciences 2012.

11. Mendes R, Sousa N, Barata JL. [Physical activity and public health: recommendations for exercise prescription]. Acta medica portuguesa 2011;24:1025-30.

12. Paffenbarger RS, Jr., Hyde RT, Wing AL, Lee IM, Jung DL, Kampert JB. The association of changes in physical-activity level and other lifestyle characteristics with mortality among men. The New England journal of medicine 1993;328:538-45.

13. Baldwin KM, Haddad F. Research in the exercise sciences: where we are and where do we go from here--Part II. Exercise and sport sciences reviews 2010;38:42-50.

14. Rehn TA, Winett RA, Wisloff U, Rognmo O. Increasing physical activity of high intensity to reduce the prevalence of chronic diseases and improve public health. The open cardiovascular medicine journal 2013;7:1-8.

15. THE PEAK VERSUS MAXIMUM OXYGEN UPTAKE ISSUE. (Accessed at http://www.cpxinternational.com/attachments/028_BJW - Vo2 Peak vs Max final 2.pdf.)

16. Timmons JA, Knudsen S, Rankinen T, et al. Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. J Appl Physiol 2010;108:1487-96.

17. Katch VL, McArdle, W.D. AND Katch, F.I. Essentials of exercise physiology. 4th ed: Lippincott Williams & Wilkins; 2011.

18. Bouchard C, Sarzynski MA, Rice TK, et al. Genomic predictors of the maximal O(2) uptake response to standardized exercise training programs. J Appl Physiol 2011;110:1160-70.

19. Rice TK, Sarzynski MA, Sung YJ, et al. Fine mapping of a QTL on chromosome 13 for submaximal exercise capacity training response: the HERITAGE Family Study. European journal of applied physiology 2012;112:2969-78.

20. Bouchard C. Genomic predictors of trainability. Experimental physiology 2012;97:347-52.

21. Keller P, Vollaard NB, Gustafsson T, et al. A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. J Appl Physiol 2011;110:46-59.

22. Bouchard C, Rankinen T, Chagnon YC, et al. Genomic scan for maximal oxygen uptake and its response to training in the HERITAGE Family Study. J Appl Physiol 2000;88:551-9.

23. Bouchard C, An P, Rice T, et al. Familial aggregation of VO(2max) response to exercise training: results from the HERITAGE Family Study. J Appl Physiol 1999;87:1003-8.

24. Loe H, Rognmo O, Saltin B, Wisloff U. Aerobic capacity reference data in 3816 healthy men and women 20-90 years. PloS one 2013;8:e64319.

25. Sanada K, Kuchiki T, Miyachi M, McGrath K, Higuchi M, Ebashi H. Effects of age on ventilatory threshold and peak oxygen uptake normalised for regional skeletal muscle mass in Japanese men and women aged 20-80 years. European journal of applied physiology 2007;99:475-83.

26. Fleg JL, Morrell CH, Bos AG, et al. Accelerated longitudinal decline of aerobic capacity in healthy older adults. Circulation 2005;112:674-82.

27. Hollenberg M, Ngo LH, Turner D, Tager IB. Treadmill exercise testing in an epidemiologic study of elderly subjects. The journals of gerontology Series A, Biological sciences and medical sciences 1998;53:B259-67.

28. Weiss EP, Spina RJ, Holloszy JO, Ehsani AA. Gender differences in the decline in aerobic capacity and its physiological determinants during the later decades of life. J Appl Physiol 2006;101:938-44.

29. Yang PY, Ho KH, Chen HC, Chien MY. Exercise training improves sleep quality in middle-aged and older adults with sleep problems: a systematic review. Journal of physiotherapy 2012;58:157-63.

30. Hagerman FC, Walsh SJ, Staron RS, et al. 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 2000;55:B336-46.

31. Carroll JF, Pollock ML, Graves JE, Leggett SH, Spitler DL, Lowenthal DT. Incidence of injury during moderate- and high-intensity walking training in the elderly. Journal of gerontology 1992;47:M61-6.

32. Makrides L, Heigenhauser GJ, Jones NL. High-intensity endurance training in 20- to 30- and 60- to 70-yr-old healthy men. J Appl Physiol 1990;69:1792-8.

33. Rognmo O, Moholdt T, Bakken H, et al. Cardiovascular risk of high- versus moderate-intensity aerobic exercise in coronary heart disease patients. Circulation 2012;126:1436-40.

34. Kelley GA, Kelley KS, Tran ZV. Exercise, lipids, and lipoproteins in older adults: a meta-analysis. Preventive cardiology 2005;8:206-14.

35. Seals DR, Hagberg JM, Hurley BF, Ehsani AA, Holloszy JO. Endurance training in older men and women. I. Cardiovascular responses to exercise. J Appl Physiol 1984;57:1024-9.

36. Vaitkevicius PV, Ebersold C, Shah MS, et al. Effects of aerobic exercise training in community-based subjects aged 80 and older: a pilot study. Journal of the American Geriatrics Society 2002;50:2009-13.

37. Hagberg JM, Graves JE, Limacher M, et al. Cardiovascular responses of 70- to 79-yr-old men and women to exercise training. J Appl Physiol 1989;66:2589-94.

38. Wenger HA, Bell GJ. The interactions of intensity, frequency and duration of exercise training in altering cardiorespiratory fitness. Sports Med 1986;3:346-56.

39. Helgerud J, Hoydal K, Wang E, et al. Aerobic high-intensity intervals improve VO2max more than moderate training. Medicine and science in sports and exercise 2007;39:665-71.

40. Cardiac Risk Assessment. 2012. (Accessed 6th December 2012, 2012, at http://labtestsonline.org/understanding/analytes/cardiac-risk/.)

41. Analyser og undersøkelser. 2013. (Accessed May 27, 2013, at http://www.helse-midt.no/ftp/stolav/labhandboker/Medisinsk_biokjemi/ambbok.html.)

42. Kelley GA, Kelley KS. Aerobic exercise and lipids and lipoproteins in men: a meta-analysis of randomized controlled trials. The journal of men's health & gender : the official journal of the International Society for Men's Health & Gender 2006;3:61-70.

43. Stenvinkel P, Karimi M, Johansson S, et al. Impact of inflammation on epigenetic DNA methylation - a novel risk factor for cardiovascular disease? J Intern Med 2007;261:488-99.

44. Liu L, Wylie RC, Andrews LG, Tollefsbol TO. Aging, cancer and nutrition: the DNA methylation connection. Mechanisms of ageing and development 2003;124:989-98.

45. Barres R, Zierath JR. DNA methylation in metabolic disorders. The American journal of clinical nutrition 2011;93:897S-900.

46. Kim M, Long TI, Arakawa K, Wang R, Yu MC, Laird PW. DNA methylation as a biomarker for cardiovascular disease risk. PloS one 2010;5:e9692.

47. Ogoshi K, Hashimoto S, Nakatani Y, et al. Genome-wide profiling of DNA methylation in human cancer cells. Genomics 2011;98:280-7.

48. Richardson B. DNA methylation and autoimmune disease. Clin Immunol 2003;109:72-9.

49. You JS, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? Cancer cell 2012;22:9-20.

50. Auclair G, Weber M. Mechanisms of DNA methylation and demethylation in mammals. Biochimie 2012;94:2202-11.

51. Alberts B JA, Lewis J, et al. . Studying Gene Expression and Function. In: Molecular biology of the cell. 4th ed. New York: Garland Science; 2002.

52. Newell-Price J, Clark AJ, King P. DNA methylation and silencing of gene expression. Trends in endocrinology and metabolism: TEM 2000;11:142-8.

53. Han L, Zhao Z. CpG islands or CpG clusters: how to identify functional GC-rich regions in a genome? BMC bioinformatics 2009;10:65.

54. Francisco A. CpG Islands and DNA Methylation. ENCYCLOPEDIA OF LIFE SCIENCES 2007.

55. Boks MP, Derks EM, Weisenberger DJ, et al. The relationship of DNA methylation with age, gender and genotype in twins and healthy controls. PloS one 2009;4:e6767.

56. Methylation Analysis by Sequencing. 2013. (Accessed May 9, 2013, at http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Sequencing/Epigenetic-Sequencing/Methylation-Analysis.html.)

57. Weng YI, Huang TH, Yan PS. Methylated DNA immunoprecipitation and microarray-based analysis: detection of DNA methylation in breast cancer cell lines. Methods Mol Biol 2009;590:165-76.

58. Beck S, Rakyan VK. The methylome: approaches for global DNA methylation profiling. Trends in genetics : TIG 2008;24:231-7.

59. Illumina. Infinium® HumanMethylation450 BeadChip- The ideal solution for affordable, large sample–size genome-wide DNA methylation studies. In.

60. Technologies L. Real-time PCR handbook. In; 2012:3-7, 43-4.

61. Epigenetics. 2007. (Accessed May 9, 2013, at http://missinglink.ucsf.edu/lm/genes_and_genomes/methylation.html.)

62. Ling C, Groop L. Epigenetics: a molecular link between environmental factors and type 2 diabetes. Diabetes 2009;58:2718-25.

63. Barres R, Yan J, Egan B, et al. Acute exercise remodels promoter methylation in human skeletal muscle. Cell metabolism 2012;15:405-11.

64. Schjerve IE, Tyldum GA, Tjonna AE, et al. Both aerobic endurance and strength training programmes improve cardiovascular health in obese adults. Clin Sci (Lond) 2008;115:283-93.

65. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. Scandinavian journal of clinical and laboratory investigation 1975;35:609-16.

66. Qiagen. AllPrep® DNA/RNA/Protein Mini Handbook. In; 2011.

67. Assessment of Nucleic Acid Purity. (Accessed 12 February, 2013, at http://www.nanodrop.com/Library/T042-NanoDrop-Spectrophotometers-Nucleic-Acid-Purity-Ratios.pdf.)

68. Research Z. EZ DNA Methylation-LightningTM Kit. In: Research Z, ed.

69. Miller MB, Tang YW. Basic concepts of microarrays and potential applications in clinical microbiology. Clinical microbiology reviews 2009;22:611-33.

70. Illumina. Infinium HD Methylation Assay Guide. In; 2011.

71. Laird PW. Principles and challenges of genomewide DNA methylation analysis. Nature reviews Genetics 2010;11:191-203.

72. Illumina. HumanMethylation450 BeadChip Achieves Breadth of Coverage Using Two Infinium® Chemistries. In.

73. Du P, Zhang X, Huang CC, et al. Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. BMC bioinformatics 2010;11:587.

74. Plume JM, Beach SR, Brody GH, Philibert RA. A cross-platform genome-wide comparison of the relationship of promoter DNA methylation to gene expression. Frontiers in genetics 2012;3:12.

75. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310:170.

76. Qiagen. miRNeasy Mini Handbook. In; 2012.

77. Qiagen. QuantiFast Probe Assay Handbook. In; 2011.

78. Qiagen. QuantiFast Probe RT-PCR Plus Kit Quick-startProtocol. In; 2012:4.

79. Differential Gene Expression and Its Possible Therapeutic Implications, Gene Therapy - Developments and Future Perspectives. InTech, 2011. (Accessed June 20, 2013, at http://www.intechopen.com/books/gene-therapy-developments-and-future-perspectives/differential-gene-expression-and-its-possible-therapeutic-implications.)

80. Mahoney DJ, Carey K, Fu MH, et al. Real-time RT-PCR analysis of housekeeping genes in human skeletal muscle following acute exercise. Physiological genomics 2004;18:226-31.

81. Riedl I, Yoshioka M, Nishida Y, et al. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Experimental gerontology 2010;45:896-903.

82. Nybo L, Sundstrup E, Jakobsen MD, et al. High-intensity training versus traditional exercise interventions for promoting health. Medicine and science in sports and exercise 2010;42:1951-8.

83. WHO. The challenge of obesity in the WHO European region and the strategies for response. In. Copenhagen: WHO; 2007.

84. Mathus-Vliegen EM. Prevalence, pathophysiology, health consequences and treatment options of obesity in the elderly: a guideline. Obesity facts 2012;5:460-83.

85. Pichot V, Roche F, Denis C, et al. Interval training in elderly men increases both heart rate variability and baroreflex activity. Clinical autonomic research : official journal of the Clinical Autonomic Research Society 2005;15:107-15.

86. Nakajima K, Takeoka M, Mori M, et al. Exercise effects on methylation of ASC gene. International journal of sports medicine 2010;31:671-5.

Appendixes

Appendix A: Informed consent

All the participants had to approve their participation and sign this written, informed consent before their involvement in the study. The information is written in Norwegian. For information only, this informed consent also contains some material not included in this report, because it is part of another project.

Forespørsel om deltakelse i forskningsprosjektet

"Epigenetiske endringer ved moderat og høy intensiv trening"

Studien er godkjent av Regional Komité for Medisinsk og Helsefaglig Forskningsetikk, Midt-Norge.

Bakgrunn og hensikt

Dette er et spørsmål til deg om å delta i et underprosjekt til «Generasjon 100». Vi ønsker å undersøke om åtte uker med trening kan påvirke genuttrykk i blod og muskelvev, og videre om dette eventuelt skyldes epigenetiske endringer målt som DNA-metylering. Vi ønsker å undersøke om det er ulik effekt om du trener med moderat versus høy intensitet. Du inviteres herved til å delta i denne treningsstudien som gjennomføres ved St. Olavs Hospital og NTNU.

Hva innebærer studien?

Dersom du takker ja til å delta i studien, vil du bli innkalt til pre-testing, deretter gjennomgå en åtte ukers treningsperiode ved St. Olavs Hospital, for så å gjøre post-tester. De som deltar i studien vil, etter pre-testing (se info lenger ned), bli tilfeldig plassert i en av de tre følgende gruppene: kontroll, trening med moderat intensitet, eller trening med høy intensitet. Treningsgruppene skal trene tre ganger i uken i åtte uker, på St. Olavs Hospital, hvor en treningsveileder vil være tilstede under alle treningene. Kontrollgruppen skal bare fortsette med sine daglige aktiviteter, uten å drastisk endre kosthold eller mosjonsvaner. Pre- og post-testene vil i hovedsak inneholde blodprøvetaking og muskelbiopsi. Se utdypende forklaring om hva studien innebærer lenger ned.

Mulige fordeler og ulemper

Det er svært gode holdepunkter for at regelmessig trening gir bedre helse enn om man ikke trener. Ved å delta i prosjektet vil du bidra til viktig forskning, samt få kunnskap om trening, fysisk aktivitet og helse. Deltakerne vil få utført en rekke kliniske undersøkelser og oppfølging som de ellers ikke ville fått (gjelder også kontrollgruppen). Alle metodene som skal benyttes i prosjektet er godt utprøvd tidligere, både på friske mennesker og i ulike risikogrupper. De anses derfor ikke for å være risikable eller å ha negative bivirkninger.

Ulemper kan likevel være forbigående stølhet i muskler og ledd etter trening, særlig i starten hvis du ikke er vant til å trene. Et par dager etter muskelbiopsi, vil man og kunne oppleve forbigående muskelømhet (som å ha fått en «lårhøne»). Risikoen for en eventuell infeksjon ved inngrepet ansees som liten og forebygges ved bruk av sterile teknikker, samt at prøven tas av lege. Inngrepet føres inn i den enkeltes pasientjournal. Skulle det oppstå en infeksjon vil dette bli undersøkt og behandlet av lege. Medisinsk ansvarlig for studien er MD Erlend Hassel, ISB, NTNU.

Hva skjer med prøvene og informasjonen om deg?

Hvis du sier ja til å delta i studien, har du alltid rett til å få innsyn i hvilke opplysninger som er registrert om deg. Opplysninger og prøver merkes med et løpenummer og behandles uten navn, fødselsnummer eller andre opplysninger som gjør at den enkelte pasient kan gjenkjennes. En kode knytter deg til dine opplysninger og prøver til en navneliste. Navnelisten vil bli slettet i 2020, etter dette kan ingen opplysninger som er lagret, knyttes til deg. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Det vil ikke være mulig å identifisere deg i resultatene av studien ved en eventuell publikasjon. Alle medarbeidere i studien har taushetsplikt.

Frivillig deltakelse

Det er frivillig å delta i studien. Du kan når som helst trekke ditt samtykke til å delta i studien uten å oppgi noen grunn. Dette vil ikke få konsekvenser for din videre behandling, eller deltakelse i hovedprosjektet «Generasjon 100». Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte:

Kine Andenæs på telefon 48008411 (epost: kinean@stud.ntnu.no)

Ali Shahab på telefon 47661223 (epost: alims@stud.ntnu.no)

Anja Bye på telefon 92644422 (epost: anja.bye@ntnu.no)

Dorthe Stensvold på telefon 92092856 (epost: dorthe.stensvold@ntnu.no)

Ytterligere informasjon om studien finnes i kapittel *A* – *utdypende forklaring av hva studien innebærer.*

Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel B – *Personvern, biobank, økonomi og forsikring.*

Samtykkeerklæring følger etter kapittel B.

Kapittel A- utdypende forklaring av hva studien innebærer

Bakgrunn for studien

Aldring er preget av funksjonelle og fysiologiske endringer og omfatter nedgang i maksimalt oksygenopptak, redusert muskelmasse og nedsatt funksjonsevne. I tillegg øker risikoen for sykdomsutvikling og forekomsten av både type-2 diabetes, kreft, KOLS, samt psykiske plager som depresjon og demens med alderen. I dette studiet ønsker vi å se på DNA-metylering i forhold til trening. DNA er menneskets arvemateriale. DNA-metylering er en kjemisk modifisering av DNAet som forekommer naturlig i alle levende organismer. Metylering av DNA påvirker mange funksjoner i kroppen vår, blant annet hvordan genene reguleres. Aldring er en av flere faktorer som påvirker DNA-metyleringen, og eldre mennesker har vist seg å ha høyere nivåer av DNA-metylering enn yngre. Økt fysisk aktivitet har en positiv effekt på mange av de plager og sykdommer som ofte opptrer hos eldre, men det er fortsatt ikke klart om fysisk aktivitet kan påvirke DNA-metylering. Hovedmålet med dette prosjektet er å undersøke om åtte uker med utholdenhetstrening kan endre den globale metylering av DNA hos eldre, og eventuelt om trening av ulik intensitet påvirker DNA-metyleringen forskjellig. I tillegg til å måle global metylering i blod og muskelvev, vil det og kunne bli målt DNA-metylering av noen spesifikke gener involvert i metabolismen.

Hvem kan delta i studien?

Vi ønsker å rekruttere 45 friske menn mellom 70-75 år, med BMI på 20-30,og som har bostedsadresse i Trondheim kommune. Tidligere studier har vist at DNA-metyleringen hos menn og kvinner er forskjellig, derfor ønsker vi bare å inkludere ett kjønn. I hovedprosjektet «Generasjon 100» vil alle mellom 70-75 år i Trondheim kommune bli invitert til å delta. Det er et tilfeldig utvalg av de som deltar i «Generasjon 100" og som innfrir våre inklusjonskriterier, som vil blir forespurt om å delta i dette underprosjektet. Eksklusjonskriterier for studien er: kjent hjertesykdom, aktiv kreftsykdom, betydelig lungesykdom, ukontrollert hypertensjon, funksjonshemninger som gjør at man ikke kan trene, motvilje til å delta i studiet eller å underskrive erklæringsskjemaet, narkotika og/eller alkoholmisbruk.

Hvordan foregår studien:

Deltakerne vil etter inklusjon bli testet og randomisert til enten en treningsgruppe eller en kontrollgruppe. Treningsgruppene vil gjennomføre åtte uker med moderat eller høy intensiv trening, tre ganger i uken. Treningen vil foregå ved gange/løp på tredemølle, og det vil være en treningsveileder tilstede ved hver treningsøkt. Avhengig av hvilken treningsgruppe man tilhører er det lagt opp til bestemte treningsprogram. Kontrollgruppa får ikke tilbud om veiledet trening og blir bedt om å fortsette sine daglige aktiviteter som før. Etter treningsperioden blir det ny testing, for alle gruppene, av de samme parameterne som før treningsperioden (disse er nevnt nedenfor). Kontrollgruppa vil få tilbud om en time treningsveiledning etter studieslutt.

Følgende tester blir gjort før og etter åtte uker med trening:

Maksimal oksygenopptak

Alle målingene av oksygenopptak foretas under gange på tredemølle. Tredemølleprotokollen består av kontinuerlig gange til utmattelse. Hastigheten vil være 4-5 km/t og stigningen på mølla starter på 0 % og økes med 2 % for hvert andre minutt. Protokollen gjennomføres til deltakeren har nådd sitt maksimale oksygenopptak eller til utmattelse.

Muskelbiopsi

Det vil bli tatt ut en liten muskelprøve fra lårmuskulaturen før og etter åtte uker med trening. Det vil bli gitt lokal bedøvelse før selve inngrepet. Veletablerte prosedyrer fra vår forskningsgruppe blir brukt, metoden ansees som forsvarlig og sikker, og blir utført av lege.

Andre tester

Det vil bli tatt en fastende blodprøve fra venen i armen. I tillegg vil vi måle vekt, blodtrykk, hvilepuls og kroppssammensetning. Deltakerne skal også fylle ut et spørreskjema som omhandler livskvalitet, aktivitetsnivå og funksjonsnivå (samme spørreskjema som brukt i studien «generasjon 100» som er godkjent av REK: 2012/381).

Fordeler ved å delta i studien

Alle deltagerne får en grundig helseundersøkelse samt opplæring i hvordan man kan trene. I tillegg vil man få veiledet trening tre ganger i uken i 8 uker.

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern

Opplysninger som registreres om deg er resultatet av alle prøver, spørreskjema og oppfølging i forbindelse med treningen. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det er kun 4 autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Navnelisten vil slettes i 2020, etter dette kan ingen opplysninger som er lagret knyttes til deg. Prosjektlederne Anja Bye (Post-doc) og Dorthe Stensvold (Post-doc), ved NTNU, er databehandlingsansvarlig.

Biobank

Blodprøvene som blir tatt og analyseresultatene vil bli lagret i en allerede opprette biobank (G-100). Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultatene inngår i biobanken. Ulrik Wisløff er ansvarshavende for forskningsbiobanken. Dataene i biobanken er avidentifisert, og listen som kobler sammen løpenummer og personidentitet vil bli slettet i 2020. I likhet med materiale fra «Generasjon 100» ønsker vi å oppbevare biologisk materiale og avidentifiserte opplysninger til 2025. Vi vil søke REK før nedleggelse av biobank.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver

Du kan når som helst velge å trekke deg fra studien uten å oppgi grunner for dette. Hvis du sier ja til å delta i studien, har du rett til innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Oppfølging

Etter de planlagte åtte ukene med trening for denne studien skal deltakerne fortsette sin deltakelse i «Generasjon 100». For å se på langtidseffektene av trening ønsker «Generasjon 100» å ta nye biopsier etter ett år og etter tre år, altså ved undersøkelse to og undersøkelse tre i «Generasjon 100». Vi minner om at du som deltaker da må gi et nytt samtykke for at en ny biopsi skal kunne tas.

Finansiering

Studien og biobanken er finansiert gjennom forskningsmidler fra St. Olav Hospital, NTNU og Helse Midt-Norge.

Forsikring

Pasientene som deltar i studien, er alle dekket mot eventuelle komplikasjoner i Norsk pasientskadeerstatning, slik som ved vanlig medisinsk behandling.

Informasjon om utfallet av studien

Alle som har deltatt i studien har rett til å få informasjon av resultatet. Det vil sannsynligvis være klart mot slutten av 2014. Resultatene vil bli offentliggjort i medisinske tidsskrifter og vil trolig også bli omtalt i pressen, men dette vil ta litt tid. Ta gjerne kontakt for informasjon.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Appendix B: Details of the selected CpG sites

Table B: Details of the selected four CpG sites from the "Ranged list".

CpG site (Illumina ID)	**Cg16842280**	**Cg26801646**	**Cg08079580**	**Cg23562388**
Associated gene name	MYH1	VDAC2	BNIP3	COX16
NCBI Reference Sequence	NM_005963.3	NM_003375.3	NM_004052.2	NM_016468.6
HGVS name	NC_000017.10:g.10422189	NC_000010.10:g.76970496	NC_000010.10:g.133795885	NC_000014.8:g.70826455
Associated TSS *	TSS1500	TSS200	TSS1500	TSS200
Associated to CpG island	Yes	Yes	Yes	Yes
Position on "Ranged list"	15	6	218	279
Mean DNA methylation ratio in control group (follow-up/baseline)	1.233745244	1.316470055	0.899568165	1.125208116
Mean DNA methylation ratio in HIIT group (follow-up/baseline)	0.8649630	0.8570032	1.2565574	0.8504299
Why our interest: Because its translated protein is part of…	Skeleton muscle contraction	Mitochondrial membrane	Mitochondrial membrane	Mitochondrial membrane

*: The number represents the CpG sites position in number of nucleotides from the transcription starting site (TSS); NCBI: The National Center for Biotechnology Information; HGVS: Human genome variation society; *MYH1:* Myosin Heavy Chain 1; *VDAC2:* voltage-dependent anion channel 2*; BNIP3:* BCL2/adenovirus E1B 19kDa interacting protein 3*; COX16:* Cytochrome C Oxidase Assembly Homolog; HIIT: High-intensity interval training.

Appendix C: Measured RNA concentrations and dilution details

Table C.1: Measured RNA concentration and dilution details for the control group.

Control group Subject number	Measured RNA concentration at NanoDrop (ng/µL)	Amount of RNA (µL)	Amount of RNase free water (µL)	Total volume (µL)	Final concentration of RNA (ng)
Baseline					
182	123	9.00	16.45	18.45	90
2141	156	7.00	16.04	17.54	90
2574	84	13.00	15.90	18.90	90
2875	127	9.00	17.10	19.10	90
3879	172	7.00	17.79	19.29	90
4057	64	15.00	13.30	16.80	90
4086	234	5.00	16.51	17.51	90
4135	82	13.00	15.47	18.47	90
4595	105	11.00	17.19	19.69	90
Follow-up					
182	338	5.00	24.35	25.35	90
2141	324	5.00	23.29	24.29	90
2574	74	13.00	13.56	16.56	90
2875	59	17.00	13.55	17.55	90
3481	211	7.00	22.27	23.77	90
3879	179	7.00	18.65	20.15	90
4057	93	11.00	14.96	17.46	90
4086	212	7.00	22.29	23.79	90
4135	238	5.00	16.83	17.83	90
4595	126	9.00	16.95	18.95	90

Table C.2: Measured RNA concentration and dilution details for the high-intensity interval training (HIIT) group.

HIIT Group Subject number	Measured RNA concentration at NanoDrop (ng/µL)	Amount of RNA (µL)	Amount of RNase free water (µL)	Total volume (µL)	Final concentration of RNA (ng)
Baseline					
969	130	9.00	17.49	19.49	90
1085	139	9.00	18.84	20.84	90
2049	248	5.00	17.60	18.60	90
2170	175	7.00	18.18	19.68	90
2445	76	15.00	16.48	19.98	90
3505	124	9.00	16.53	18.53	90
3615	145	9.00	19.71	21.71	90
3651	159	7.00	16.43	17.93	90
3853	59	17.00	13.79	17.79	90
4776	73	15.00	15.61	19.11	90
Follow-up					
969	223	7.00	23.55	25.05	90
1085	120	9.00	15.94	17.94	90
2049	167	7.00	17.31	18.81	90
2170	97	11.00	15.67	18.17	90
2445	106	11.00	17.41	19.91	90
3505	168	7.00	17.43	18.93	90
3615	47	21.00	12.74	17.74	90
3651	100	11.00	16.29	18.79	90
3853	148	9.00	20.22	22.22	90
4776	57	19.00	14.57	19.07	90

Appendix D: Set-up for real-time qRT-PCR

Step 1: DNA polymerase activating step

Step 2: Denaturation

Step 3: Annealing

Step 4: Extension

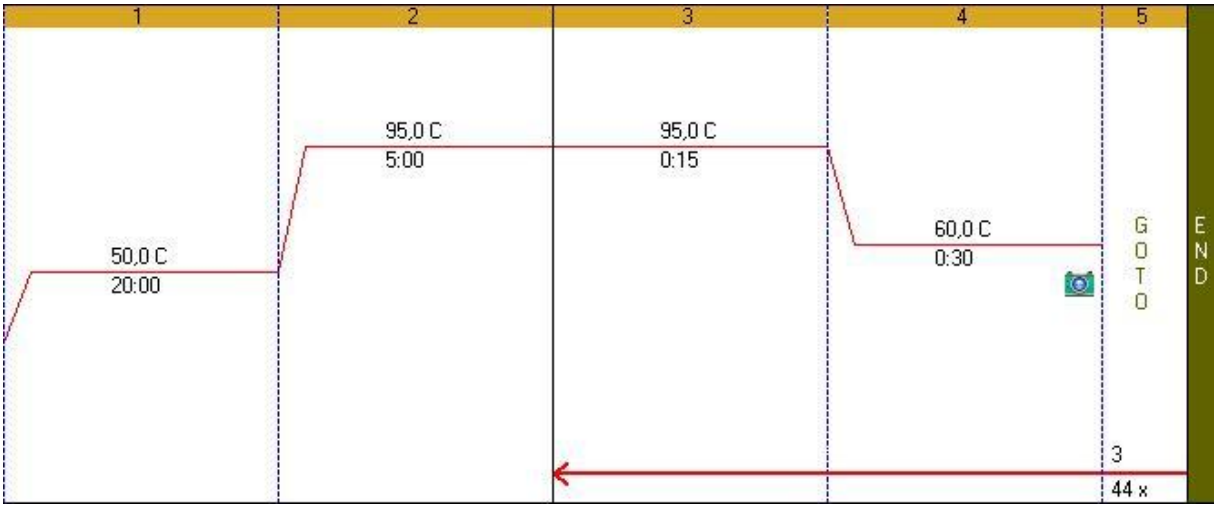

Figure D.1: Temperature and duration (minutes) of the four PCR-steps, where step 3 and 4 are repeated 44 times.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	NTC	NRT	C4 Pre	C4 Post	C8 Pre	C8 Post	H3 Pre	H3 Post	H7 Pre	H7 Post	EMPTY	EMPTY	**A**
B	NTC	NRT	C4 Pre	C4 Post	C8 Pre	C8 Post	H3 Pre	H3 Post	H7 Pre	H7 Post	EMPTY	EMPTY	**B**
C	C1 Pre	C1 Post	C5 Pre	C5 Post	C9 Pre	C9 Post	H4 Pre	H4 Post	H8 Pre	H8 Post	EMPTY	EMPTY	**C**
D	C1 Pre	C1 Post	C5 Pre	C5 Post	C9 Pre	C9 Post	H4 Pre	H4 Post	H8 Pre	H8 Post	EMPTY	EMPTY	**D**
E	C2 Pre	C2 Post	C6 Pre	C6 Post	H1 Pre	H1 Post	H5 Pre	H5 Post	H9 Pre	H9 Post	EMPTY	EMPTY	**E**
F	C2 Pre	C2 Post	C6 Pre	C6 Post	H1 Pre	H1 Post	H5 Pre	H5 Post	H9 Pre	H9 Post	EMPTY	EMPTY	**F**
G	C3 Pre	C3 Post	C7 Pre	C7 Post	H2 Pre	H2 Post	H6 Pre	H6 Post	H10 Pre	H10 Post	EMPTY	EMPTY	**G**
H	C3 Pre	C3 Post	C7 Pre	C7 Post	H2 Pre	H2 Post	H6 Pre	H6 Post	H10 Pre	H10 Post	EMPTY	EMPTY	**H**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	

Figure D.2: 96-well plate with information of the contents in each well (numbered 1-12 and A-H). NTC: No template control; NRT: No-reverse transcriptase control; C1-C9: Control subject 1-9; H1-H9: Exercise subject 1-9; Pre: Baseline sample; Post: Follow-up sample.

Appendix E: REC approval

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Hege Holde Andersson	22845514	01.06.2012	2012/823 REK sør-øst B
			Deres dato:	Deres referanse:
			24.04.2012	

Vår referanse må oppgis ved alle henvendelser

Dorthe Stensvold
NTNU, Institutt for sirkulasjon og bildediagnostikk

2012/823b Epigenetikk og trening

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk, REK sør-øst B, i møtet 09.05.2012.

Prosjektleder: Dorthe-Stensvold
Forskningsansvarlig: NTNU

Prosjektomtale
I dette mastergradsprosjektet, som er et underprosjekt til "Generasjon 100", skal det undersøkes om fysisk aktivitet kan påvirke DNA metylering. Hovedmålet med prosjektet er å undersøke om 10 uker med utholdenhetstrening kan endre den globale metyleringen av DNA hos eldre, og eventuelt om trening av ulik intensitet påvirker DNA metyleringen forskjellig. I tillegg til å måle den globale metylering i blod og muskelvev, vil det også bli målt DNA metylering av noen spesifikke gener involvert i metabolismen.

Det planlegges å inkludere 60 friske menn mellom 70 - 75 år bosatt i Trondheim kommune. Disse skal gjennomføre noen tester, og deretter bli randomisert i 3 grupper (en kontrollgruppe, en som skal trene med høy intensitet og en som skal trene med moderat intensitet). Treningen skal gjennomføres ved St. Olavs hospital. Aktuelle tester som skal gjennomføres er bl.a. måling av oksygenopptak, blodprøver, spørreskjema og muskelbiopsier. Prosjektet er samtykkebasert, og blodprøver og analyseresultater skal inngå i en allerede opprettet biobank ("Gen 100").

Komiteens vurdering
Komiteen har ingen innvendinger til at prosjektet skal gjennomføres. Den antar at metylering undersøker regulering av genuttrykk, uttrykt folkelig som om et gen er skrudd på eller av, i forhold til om trening kan forandre global metylering av DNA, og om treningsintensitet kan vise forskjeller. Hvorvidt gener skrur seg av eller på reiser i denne sammenhengen ikke spesielle problemstillinger som må tas hensyn til.

Forskningsbiobank
Blodprøver og muskelbiopsier vil inngå i en allerede godkjent biobank: Gen 100, med ansvarshavende Ulrik Wisløff.

Vedtak
Komiteen godkjenner prosjektet med hjemmel i helseforskningsloven § 9.

Godkjenningen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden.

Besøksadresse: Gullhaug torg 4A, Nydalen, 0484 Oslo
Telefon: 22845511
E-post: post@helseforskning.etikkom.no
Web: http://helseforskning.etikkom.no/

Godkjenningen gjelder til 15.06.2013.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder "*Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren*"

Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK.

Prosjektet skal sende sluttmelding på eget skjema, se helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jfr. helseforskningsloven § 10, 3 ledd og forvaltningsloven § 28. En eventuell klage sendes til REK sør-øst B. Klagefristen er tre uker fra mottak av dette brevet, jfr. forvaltningsloven § 29.

Komiteens avgjørelse var enstemmig.

Vi ber om at alle henvendelser sendes inn via vår saksportal: http://helseforskning.etikkom.no eller på e-post til post@helseforskning.etikkom.no. Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Stein Opjordsmoen Ilner
Professor dr. med
Komitéleder

Hege Holde Andersson
Komitésekretær

Kopi til: Instituttleder Øyvind Ellingsen, NTNU, Institutt for sirkulasjon og bildediagnostikk
Biobankregisteret

Appendix F: Individual changes in weight, body mass index, waist circumference and body fat percentage

Table F.1: Individual data on weight and body mass index for both groups at baseline, follow-up, and delta values (follow-up/baseline).

Subject number	Group	Weight (kg)			BMI (kg/m²)		
		Baseline	*Follow-up*	*Delta*	*Baseline*	*Follow-up*	*Delta*
182	Control	87.1	88.0	1.01	27.5	27.9	1.01
2141	Control	86.0	86.1	1.00	26.3	26.3	1.00
2574	Control	89.3	88.2	0.99	28.8	28.6	0.99
2875	Control	95.6	95.8	1.00	28.5	28.8	1.01
3481	Control	71.4	69.3	0.97	26.2	25.6	0.98
3879	Control	65.7	65.5	1.00	21.5	21.6	1.00
4057	Control	91.2	89.9	0.99	26.9	27.0	1.00
4086	Control	83.5	85.0	1.02	26.7	27.0	1.01
4135	Control	100.9	99.4	0.99	28.2	28.3	1.00
4595	Control	65.3	64.6	0.99	24.0	23.8	0.99
969	HIIT	82.9	80.1	0.97	29.4	28.7	0.98
1085	HIIT	103.2	100.1	0.97	34.5	33.6	0.97
2049	HIIT	79.9	78.0	0.98	24.7	24.2	0.98
2170	HIIT	92.0	90.9	0.99	34.2	34.4	1.01
2445	HIIT	86.1	84.2	0.98	27.2	26.7	0.98
3505	HIIT	78.0	75.1	0.96	26.4	25.6	0.97
3615	HIIT	81.0	80.6	1.00	28.0	28.1	1.00
3651	HIIT	114.9	108.5	0.94	32.9	31.2	0.95
3853	HIIT	72.5	72.3	1.00	23.7	23.5	0.99
4776	HIIT	95.5	94.2	0.99	28.2	28.0	0.99

BMI: Body mass index; HIIT: High-intensity interval training.

Table F.2: Individual data on waist circumference and body fat percentage for both groups, at baseline, follow-up, and delta values (follow-up/baseline).

Subject number	Group	Waist circumference (cm)			Body fat percentage (%)		
		Baseline	*Follow-up*	*Delta*	*Baseline*	*Follow-up*	*Delta*
182	Control	105.5	103.0	0.98	20.0	21.4	1.06
2141	Control	101.0	102.5	1.01	24.0	24.1	1.00
2574	Control	107.5	106.5	0.99	32.3	32.0	0.99
2875	Control	109.0	106.5	0.98	23.9	24.5	1.03
3481	Control	91.5	85.0	0.93	28.1	28.9	1.03
3879	Control	92.5	90.5	0.98	19.0	20.2	1.06
4057	Control	102.5	99.0	0.97	27.2	29.8	1.10
4086	Control	101.5	101.0	1.00	28.7	26.3	0.92
4135	Control	105.0	103.0	0.98	27.4	27.3	1.00
4595	Control	87.0	80.5	0.93	27.1	25.0	0.92
969	HIIT	108.0	97.5	0.90	32.8	32.0	0.98
1085	HIIT	116.5	111.0	0.95	42.8	41.8	0.98
2049	HIIT	92.0	90.0	0.98	23.0	21.3	0.93
2170	HIIT	112.0	106.5	0.95	36.9	37.1	1.01
2445	HIIT	100.5	98.0	0.98	25.5	24.8	0.97
3505	HIIT	90.0	87.0	0.97	24.8	23.3	0.94
3615	HIIT	103.0	97.0	0.94	31.6	32.2	1.02
3651	HIIT	119.0	105.0	0.88	33.5	32.1	0.96
3853	HIIT	90.0	87.0	0.97	20.6	21.3	1.03
4776	HIIT	106.0	100.0	0.94	26.4	26.4	1.00

HIIT: High-intensity interval training.

Appendix G: Individual changes in circulating blood markers of cardiovascular health

Table G.1: Individual data on total cholesterol and LDL cholesterol for both groups at baseline, follow-up, and delta values (follow-up/baseline).

Subject number	Group	Total cholesterol (mmol/L)			LDL cholesterol (mmol/L)		
		Baseline	*Follow-up*	*Delta*	*Baseline*	*Follow-up*	*Delta*
182	Control	6.80	7.60	1.12	5.19	5.92	1.14
2141	Control	5.30	5.90	1.11	2.43	3.36	1.38
2574	Control	4.60	6.10	1.33	2.29	3.84	1.68
2875	Control	6.10	6.10	1.00	4.19	4.32	1.03
3481	Control	6.80	7.60	1.12	3.85	4.66	1.21
3879	Control	4.40	3.70	0.84	2.30	1.94	0.84
4057	Control	6.50	6.60	1.02	4.43	4.73	1.07
4086	Control	5.90	5.10	0.86	3.40	2.93	0.86
4135	Control	7.20	7.00	0.97	5.33	5.35	1.00
4595	Control	3.70	3.60	0.97	1.87	1.91	1.02
969	HIIT	5.00	5.10	1.02	3.22	3.29	1.02
1085	HIIT	7.50	7.50	1.00	4.84	5.13	1.06
2049	HIIT	5.50	5.10	0.93	3.53	3.24	0.92
2170	HIIT	5.30	5.20	0.98	3.31	3.19	0.96
2445	HIIT	5.60	5.60	1.00	3.60	3.73	1.04
3505	HIIT	5.40	5.10	0.94	2.35	2.07	0.88
3615	HIIT	5.30	5.50	1.04	3.17	3.54	1.12
3651	HIIT	5.00	4.50	0.90	3.22	2.90	0.90
3853	HIIT	5.70	5.90	1.04	3.72	4.02	1.08
4776	HIIT	4.70	5.10	1.09	3.08	3.46	1.12

LDL: Low-density lipoprotein; HIIT: High-intensity interval training.

Table G.2: Individual data on HDL cholesterol and triglycerides for both groups at baseline, follow-up, and delta values (follow-up/baseline).

Subject number	Group	HDL cholesterol (mmol/L)			Triglycerides (mmol/L)		
		Baseline	***Follow-up***	***Delta***	***Baseline***	***Follow-up***	***Delta***
182	Control	1.16	1.14	0.98	0.99	1.21	1.22
2141	Control	2.49	2.18	0.88	0.85	0.81	0.95
2574	Control	1.83	1.73	0.95	1.07	1.18	1.10
2875	Control	1.28	1.27	0.99	1.39	1.14	0.82
3481	Control	2.57	2.54	0.99	0.85	0.88	1.04
3879	Control	1.37	1.31	0.96	1.62	1.00	0.62
4057	Control	1.51	1.30	0.86	1.24	1.26	1.02
4086	Control	2.14	1.79	0.84	0.80	0.84	1.05
4135	Control	1.36	1.22	0.90	1.14	0.95	0.83
4595	Control	1.41	1.26	0.89	0.94	0.95	1.01
969	HIIT	1.15	1.19	1.03	1.40	1.38	0.99
1085	HIIT	2.30	1.99	0.87	0.80	0.85	1.06
2049	HIIT	1.63	1.53	0.94	0.76	0.74	0.97
2170	HIIT	1.66	1.66	1.00	0.74	0.77	1.04
2445	HIIT	1.52	1.33	0.88	1.06	1.21	1.14
3505	HIIT	2.60	2.76	1.06	0.99	0.61	0.62
3615	HIIT	1.24	1.27	1.02	1.98	1.53	0.77
3651	HIIT	1.10	1.14	1.04	1.51	1.02	0.68
3853	HIIT	1.54	1.54	1.00	0.98	0.75	0.77
4776	HIIT	1.04	1.05	1.01	1.28	1.30	1.02

HDL: High-density lipoprotein; HIIT: High-intensity interval training.

Table G.3: Individual data on glucose and hs-CRP for both groups at baseline, follow-up, and delta values (follow-up/baseline). By a mistake hs-CRP was not detected in the blood sample of subject number 3615 at follow-up, and thus this cell and the respective delta cell are empty.

Subject number	Group	Glucose (mmol/L)			Hs-CRP (mg/L)		
		Baseline	***Follow-up***	***Delta***	***Baseline***	***Follow-up***	***Delta***
182	Control	5.30	5.10	0.96	1.71	1.76	1.03
2141	Control	5.70	5.30	0.93	2.09	1.39	0.67
2574	Control	7.40	6.80	0.92	2.54	2.31	0.91
2875	Control	6.00	6.10	1.02	1.25	2.13	1.70
3481	Control	5.20	5.00	0.96	9.19	7.74	0.84
3879	Control	5.50	5.40	0.98	2.24	1.69	0.75
4057	Control	8.60	10.10	1.17	2.11	3.00	1.42
4086	Control	5.40	5.80	1.07	1.09	1.73	1.59
4135	Control	5.00	5.60	1.12	1.98	2.52	1.27
4595	Control	4.90	5.40	1.10	0.48	0.40	0.83
969	HIIT	5.90	6.20	1.05	1.04	1.28	1.23
1085	HIIT	6.40	6.10	0.95	33.81	3.84	0.11
2049	HIIT	5.10	4.90	0.96	2.45	2.21	0.90
2170	HIIT	5.90	6.20	1.05	2.57	1.68	0.65
2445	HIIT	5.10	5.30	1.04	1.56	2.22	1.42
3505	HIIT	6.10	7.40	1.21	0.51	1.06	2.08
3615	HIIT	6.30	6.50	1.03	0.92	-	-
3651	HIIT	5.90	5.20	0.88	0.71	0.89	1.25
3853	HIIT	5.80	4.50	0.78	0.62	0.46	0.74
4776	HIIT	6.40	5.90	0.92	2.78	1.40	0.50

Hs-CRP: High-sensitivity C-reactive protein; HIIT: High-intensity interval training.

Appendix H: Individual changes in maximal aerobic capacity

Table H: Individual data on maximal aerobic capacity for both groups at baseline, follow-up, and delta values (follow-up/baseline).

Subject number	Group	VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)		
		Baseline	***Follow-up***	***Delta***
182	Control	35.0	33.7	0.96
2141	Control	28.0	31.3	1.12
2574	Control	25.4	27.3	1.07
2875	Control	37.0	40.0	1.08
3481	Control	37.0	41.0	1.11
3879	Control	34.0	33.5	0.99
4057	Control	25.5	26.9	1.05
4086	Control	37.5	37.5	1.00
4135	Control	30.5	28.4	0.93
4595	Control	26.0	26.3	1.01
969	HIIT	30.0	36.8	1.23
1085	HIIT	27.0	31.4	1.16
2049	HIIT	30.0	36.0	1.20
2170	HIIT	23.0	27.3	1.19
2445	HIIT	37.0	38.2	1.03
3505	HIIT	33.0	42.3	1.28
3615	HIIT	28.0	32.2	1.15
3651	HIIT	24.5	27.0	1.10
3853	HIIT	34.5	36.8	1.07
4776	HIIT	27.0	29.0	1.07

VO_{2max}: maximal aerobic capacity; HIIT: High-intensity interval training.

Appendix I: Individual changes in DNA methylation

Table I.1: Individual data on DNA methylation of CpG site in the promoter region of the associated genes *MYH1* and *VDAC2,* for both groups at baseline, follow-up, and delta values (follow-up/baseline). Empty cells means the subject was not included in the analysis.

Subject number	Group	DNA methylation *MYH1* (Beta values)			DNA methylation *VDAC2* (Beta values)		
		Baseline	***Follow-up***	***Delta***	***Baseline***	***Follow-up***	***Delta***
182	Control	0.301587	0.376211	1.25	0.097700	0.108197	1.11
2141	Control	-	-	-	-	-	-
2574	Control	-	-	-	-	-	-
2875	Control	-	-	-	-	-	-
3481	Control	-	-	-	-	-	-
3879	Control	-	-	-	-	-	-
4057	Control	-	-	-	-	-	-
4086	Control	-	-	-	-	-	-
4135	Control	0.319541	0.446094	1.40	0.060906	0.099101	1.63
4595	Control	0.306837	0.324557	1.06	0.070816	0.086031	1.21
969	HIIT	0.311132	0.246104	0.79	0.084283	0.066202	0.79
1085	HIIT	0.369342	0.321640	0.87	0.090983	0.077543	0.85
2049	HIIT	0.384279	0.285639	0.74	0.073180	0.073852	1.01
2170	HIIT	0.310213	0.350430	1.13	0.087684	0.073898	0.84
2445	HIIT	-	-	-	-	-	-
3505	HIIT	0.324660	0.236960	0.73	0.136645	0.088837	0.65
3615	HIIT	0.315300	0.291687	0.93	0.083402	0.083583	1.00
3651	HIIT	-	-	-	-	-	-
3853	HIIT	-	-	-	-	-	-
4776	HIIT	-	-	-	-	-	-

MYH1: Myosin Heavy Chain 1; *VDAC2:* voltage-dependent anion channel 2*;* HIIT: High-intensity interval training.

Table I.2: Individual data on DNA methylation of CpG site in the promoter region of the associated genes *BNIP3* and *COX16*, for both groups at baseline, follow-up and delta values (follow-up/baseline). Empty cells means the subject was not included in the analysis.

Subject number	Group	DNA methylation *BNIP3* (Beta values)			DNA methylation *COX16* (Beta values)		
		Baseline	***Follow-up***	***Delta***	***Baseline***	***Follow-up***	***Delta***
182	Control	0.035173	0.034621	0.98	0.078243	0.084121	1.08
2141	Control	-	-	-	-	-	-
2574	Control	-	-	-	-	-	-
2875	Control	-	-	-	-	-	-
3481	Control	-	-	-	-	-	-
3879	Control	-	-	-	-	-	-
4057	Control	-	-	-	-	-	-
4086	Control	-	-	-	-	-	-
4135	Control	0.041227	0.035797	0.87	0.057603	0.064672	1.12
4595	Control	0.047426	0.040128	0.85	0.065881	0.077593	1.18
969	HIIT	0.038877	0.046719	1.20	0.067692	0.050114	0.74
1085	HIIT	0.031149	0.040451	1.30	0.057637	0.053950	0.94
2049	HIIT	0.031863	0.041107	1.29	0.059835	0.052250	0.87
2170	HIIT	0.038658	0.043651	1.13	0.072642	0.059845	0.82
2445	HIIT	-	-	-	-	-	-
3505	HIIT	0.025801	0.039130	1.52	0.208823	0.134941	0.65
3615	HIIT	0.034279	0.037814	1.10	0.045588	0.049370	1.08
3651	HIIT	-	-	-	-	-	-
3853	HIIT	-	-	-	-	-	-
4776	HIIT	-	-	-	-	-	-

BNIP3: BCL2/adenovirus E1B 19kDa interacting protein 3*; COX16:* Cytochrome C Oxidase Assembly Homolog; HIIT: High-intensity interval training.

Appendix J: Individual changes of gene expression

Table J.1: Individual data on gene expression of *MYH1* and *VDAC2*, for both groups at baseline, follow-up, and delta values (follow-up/baseline). Empty cells means the subject was excluded from the data analyses.

Subject number	Group	Gene expression *MYH1*			Gene expression *VDAC2*		
		Baseline ***(ΔC_t)***	***Follow-up*** ***(ΔC_t)***	***Delta*** ***($2^{-\Delta\Delta Ct}$)***	***Baseline*** ***(ΔC_t)***	***Follow-up*** ***(ΔC_t)***	***Delta*** ***($2^{-\Delta\Delta Ct}$)***
182	Control	0.571141	-0.234525	1.75	5.018883	4.745515	1.21
2141	Control	2.373681	0.724295	3.14	4.399667	4.714605	0.80
2574	Control	-	-	-	5.074679	4.499753	1.49
2875	Control	3.448027	-0.068182	11.44	4.288813	4.930594	0.64
3481	Control	-	-	-	-	-	-
3879	Control	-0.707098	-1.263126	1.47	5.467263	5.295227	1.13
4057	Control	0.331089	-1.163683	2.82	5.201937	5.712226	0.70
4086	Control	1.775380	1.307970	1.38	4.922813	4.978713	0.96
4135	Control	-0.234469	0.219265	0.73	4.749615	4.490690	1.20
4595	Control	1.416239	-0.316416	3.32	4.440838	4.757796	0.80
969	HIIT	-0.690777	0.693191	0.38	4.943945	4.678077	1.20
1085	HIIT	0.075539	0.443740	0.77	4.946180	5.102590	0.90
2049	HIIT	1.624573	0.919883	1.63	4.410948	4.837720	0.74
2170	HIIT	-0.352780	-1.390774	2.05	4.704365	4.679816	1.02
2445	HIIT	1.313972	-0.479176	3.47	4.425322	4.769386	0.79
3505	HIIT	1.154427	0.585278	1.48	4.885610	4.484857	1.32
3615	HIIT	0.538679	0.037079	1.42	5.598107	5.414517	1.14
3651	HIIT	-	-	-	-	-	-
3853	HIIT	0.409417	1.237056	0.56	5.088331	4.910000	1.13
4776	HIIT	-	-	-	4.494548	4.360000	1.10

MYH1: Myosin Heavy Chain 1; *VDAC2:* voltage-dependent anion channel 2*;* HIIT: High-intensity interval training; C_t: cycle threshold.

Table J.2: Individual data on gene expression of *BNIP3* and *COX16,* for both groups at baseline, follow-up, and delta values (follow-up/baseline). Empty cells means the subject was excluded from the data analyses.

Subject number	Group	Gene expression *BNIP3*			Gene expression *COX16*		
		Baseline ***(ΔC_t)***	***Follow-up*** ***(ΔC_t)***	***Delta*** ***($2^{-\Delta\Delta Ct}$)***	***Baseline*** ***(ΔC_t)***	***Follow-up*** ***(ΔC_t)***	***Delta*** ***($2^{-\Delta\Delta Ct}$)***
182	Control	8.142539	7.813787	1.26	6.095964	6.184691	0.94
2141	Control	6.554335	7.467028	0.53	5.136140	6.040526	0.53
2574	Control	-	-	-	5.653051	5.283407	1.29
2875	Control	6.857820	7.304058	0.73	5.406255	6.030156	0.65
3481	Control	-	-	-	-	-	-
3879	Control	8.243578	8.164110	1.06	6.760049	6.035310	1.65
4057	Control	7.105399	8.084176	0.51	5.517188	6.359217	0.56
4086	Control	7.449079	7.336849	1.08	6.016058	6.167627	0.90
4135	Control	6.946155	6.946378	1.00	5.698213	5.736199	0.97
4595	Control	6.321473	7.011842	0.62	5.434199	6.009006	0.67
969	HIIT	7.058693	7.323145	0.83	5.555350	5.826860	0.83
1085	HIIT	6.781217	6.985312	0.87	5.814319	6.146984	0.79
2049	HIIT	6.391804	7.335266	0.52	5.376178	5.985891	0.66
2170	HIIT	6.487051	6.782428	0.81	5.623651	5.690485	0.95
2445	HIIT	6.789155	7.118723	0.80	5.352601	5.673736	0.80
3505	HIIT	7.466215	7.121903	1.27	5.804218	5.784298	1.01
3615	HIIT	8.806499	7.670701	2.20	6.695814	6.273776	1.34
3651	HIIT	7.156362	7.819605	0.63	5.903714	6.165746	0.83
3853	HIIT	7.877529	7.219458	1.58	6.070505	5.937033	1.10
4776	HIIT	-	-	-	5.310185	4.360000	1.93

BNIP3: BCL2/adenovirus E1B 19kDa interacting protein 3*; COX16:* Cytochrome C Oxidase Assembly Homolog; HIIT: High-intensity interval training; C_t: cycle threshold.