Master's thesis

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

Daniel Estil Brissach

The association between cardiorespiratory fitness and incidence of Alzheimer's disease in older adults

A prospective cohort study

Master's thesis in Physical Activity and Health
Supervisor: Ulrik Wisløff
Co-supervisor: Atefe R. Tari
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Infographic

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GENERASJON 100

Background

Alzheimer's disease is the most common form of dementia. Age is the greatest risk factor.

By year 2050...

- The number of older adults aged >60 doubles
- Prevalence of Alzheimer's disease triples

Thus, Alzheimer's disease is a global health challenge

Aim

To assess the association between objectively measured cardiorespiratory fitness (CRF) and incidence of Alzheimer's disease in older adults participating in the large exercise trial, **The Generation 100 Study**

Hypothesis

Those who...

- Have a high CRF a baseline
- Maintained or increased their CRF over one-year

...Have a reduced risk of Alzheimer's disease

Study design and study population

Prospective cohort study

941 participants

Mean age: 72.2 ±2.2

Sex: 52.3% women

Participants were apparently healthy and had complete data at on baseline and one-year follow-up testing

Comparison

CRF at baseline and after one-year, and one-year changes in CRF

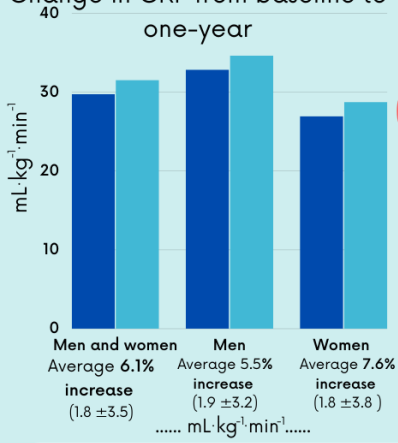
Outcome

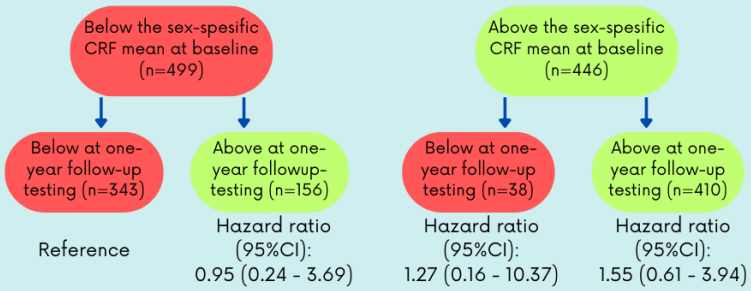
Alzheimer's disease

Overall, 24 participants developed Alzheimer's disease during the mean follow-up time of 6.3 years (50% women).

Results

Change in CRF from baseline to one-year





Association between CRF levels at baseline, and after one year:
Baseline: Odds ratio 0.99 (95%CI: 0.93 - 1.07)
One-year: Odds ratio: 1.02 (95%CI: 0.95 - 1.09)

Conclusion

We found no association between CRF and incidence of Alzheimer's disease, either at CRF at baseline and after one year, or between one-year changes in CRF

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Selected abbreviations

AD	Alzheimer's Disease
BMI	Body mass index
CPET	Cardiopulmonary exercise test
CRF	Cardiorespiratory fitness
CVD	Cardiovascular disease
HUNT	The Trøndelag Health Study
VO_2	Oxygen uptake
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Peak oxygen uptake

Definitions

Dementia encompasses various neurological diseases with a set of related symptoms causing impaired cognition and functional abilities that interfere with daily life. Dementia is more accurately characterized as a syndrome rather than one particular disease (3).

Alzheimer's disease (AD) is the most common form of dementia, and account for 50-70% of all dementia cases. AD is progressive neurogenerative disease, characterized by memory loss and cognitive impairment. Pathologically, AD is characterized by accumulation and deposition of amyloid-β into extracellular plaques and hyperphosphorylated tau proteins into intracellular neurofibrillary tangles in the brain (5, 6).

Cardiovascular disease (CVD) encompasses a range of cardiovascular disorders involving hart and blood vessels, such as cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis and pulmonary embolism, and coronary heart disease. Coronary heart disease includes stable angina, unstable angina, sudden cardiac death, and myocardial infarction (50).

Cardiorespiratory fitness (CRF) CRF reflects the ability of the circulatory and respiratory system to supply oxygen to the muscle mitochondria during endurance exercise (18). The gold standard to quantify CRF is to measure maximal oxygen uptake (VO_{2max}) using cardiopulmonary exercise testing (CPET).

Cardiopulmonary exercise testing (CPET) is a clinical procedure that measures an individual's cardiovascular and respiratory system responses during exercise (20). It involves monitoring various parameters such oxygen consumption, carbon dioxide production, and ventilation to assess the efficiency and capacity of the cardiopulmonary system. The test is usually performed on treadmill or ergometer cycle.

Maximal oxygen uptake (VO_{2max}) refers to the highest rate at which an individual can transport and utilize oxygen during serve dynamic muscle work with large muscle mass (18). Maximal oxygen uptake is commonly expressed as liters per minute or related to body mass or in milliliters of oxygen per kilogram of body mass per minute ($mL \cdot kg^{-1} \cdot min^{-1)}$. To reach the true maximal oxygen uptake the oxygen uptake must level off despite increase in work load during a cardiopulmonary exercise test.

Peak oxygen uptake (VO_{2peak}) is a term often used instead of maximal oxygen uptake because maximal effort does not necessarily always give a leveling-off in oxygen uptake during a cardiopulmonary exercise test.

Physical activity is a term that encompasses any activity involving movement performed by skeletal muscles and resulting in an increase in energy expenditure above resting values (13).

The Generation 100 Study is a large randomized exercise trial that evaluates the effect of regular exercise on morbidity and mortality in older adults aged between 70-77 years at inclusion in the study. The aim of the study was to assess if exercise gives older adults a longer and healthier life, and also to compare the effect of moderate and high-intensity training (29,30).

Abstract

Purpose: To assess the association between cardiorespiratory fitness (CRF) and incidence of Alzheimer's disease (AD) in older adults aged 70-77 years at baseline.

Methods: We included 941 older adults (72.2±2.0 years at baseline) that took part in the large exercise trail, The Generation 100 Study and had complete data on CRF and important confounding factors (socioeconomic-, and smoking status) at baseline and after one-year. We assessed CRF using gold standard ergospirometry. AD incidence was identified by review of the participants hospital records. Binary logistic regression model was used to assess the risk (Odds ratios) of AD from baseline and one-year CRF levels. Participants was classified into CRF categories according to the sex-specific mean based on baseline CRF value: below- or above mean. To assess the association between one-year changes in CRF and AD incidence, we assessed the changes in CRF categories from baseline to one-year follow-up testing: Below mean at both baseline and one-year testing, below mean at baseline and above mean at one-year testing, above mean at baseline and below mean at one-year testing, and above mean at both baseline and one-year testing. Cox proportional hazard analysis was used to estimate hazard ratios for AD incidence related to changes in CRF.

Results: Baseline CRF levels for all participants were 29.7±6.4 $mL \cdot kg^{-1} \cdot min^{-1}$ (26.9±4.9 $mL \cdot kg^{-1} \cdot min^{-1}$ for women, and 32.8±6.4 $mL \cdot kg^{-1} \cdot min^{-1}$ for men). After one-year CRF on average increased by 6.1% (1.8±3.5 $mL \cdot kg^{-1} \cdot min^{-1}$, $p<0.001$), with similar increase in both genders. During a mean follow-up of 6.3 years, there were 24 incidents of AD. CRF for those who stayed healthy throughout the follow-up period were 29.7±6.4 $mL \cdot kg^{-1} \cdot min^{-1}$ at baseline, and 31.5±6.8 $mL \cdot kg^{-1} \cdot min^{-1}$ after one-year. Those who later developed AD had a mean CRF of 29.9±6.6 $mL \cdot kg^{-1} \cdot min^{-1}$ at baseline, and 32.5±8.4 $mL \cdot kg^{-1} \cdot min^{-1}$ after one-year. Baseline CRF values were not associated with incidence of AD (Odds ratio: 0.99, 95% Confidence interval: 0.92–1.07). After one year there were still no association (Odds ratio: 1.02, 95% Confidence interval: 0.95–1.09). One-year changes in CRF were not associated with incidence of AD. Compared with participants below the mean at both baseline and one-year testing, a maintained high CRF (Hazard ratio: 1.55, 95% Confidence interval: 0.61–3.94) or increased CRF (Hazard ratio: 0.95, 95% Confidence interval: 0.24–3.67) was not found to be associated with AD incidence.

Conclusion: The present study found no association between either CRF levels at baseline and after one year, or between one-year changes in CRF and incidence of AD.

Abstrakt

Formål: Å undersøke sammenhengen mellom kardiorespiratorisk fitness (CRF) og forekomst av Alzheimers sykdom (AD) blant eldre i alderen 70-77 år ved baseline.

Metode: Vi inkluderte 941 eldre (72.2±2.0 år ved baseline) som deltok i den store treningsstudien, Generasjon 100 Studien hvor vi hadde fullstendige data på CRF og viktige konfunderende faktorer (sosioøkonomisk status og røykevaner) ved baseline og etter ett år. Vi målte CRF ved bruk av gullstandardmetoden ergospirometri. AD forekomst ble identifisert ved gjennomgang av deltakernes sykehusjournaler. Binær logistisk regresjonsmodell ble brukt til å estimere risiko (Odds ratio) for AD basert på baseline og ett-års CRF nivåer. Deltakerne ble klassifisert i CRF kategorier basert på kjønnsspesifikt gjennomsnittlig CRF ved baseline: under- eller over gjennomsnittet. For å undersøke assosiasjonen mellom ett-års endringer i CRF og forekomst av AD, undersøkte vi endringer i CRF kategori fra baseline til ett-års oppfølgingstesting: under gjennomsnittet ved både baseline og ett-års testing, under gjennomsnittet ved baseline og over gjennomsnittet ved ett-års testing, over gjennomsnittet ved baseline og under gjennomsnittet ved ett-års testing, og over gjennomsnittet ved både baseline og ett-års-testing. Cox proporsjonal hazard modell ble brukt for å estimere hazard ratios for forekomst av AD relatert til endringer i CRF.

Resultat: CRF ved baseline for alle deltakerne var 29.7±6.4 $mL \cdot kg^{-1} \cdot min^{-1}$ (26.9±4.9 $mL \cdot kg^{-1} \cdot min^{-1}$ for kvinner, og 32.8±6.4 $mL \cdot kg^{-1} \cdot min^{-1}$ for menn). Etter ett år økte gjennomsnittlig CRF med 6.1% (1.8±3.5 $mL \cdot kg^{-1} \cdot min^{-1}$, $p<0.001$), med lik økning mellom kjønnene. I løpet av en gjennomsnittlig oppfølgingsperiode på 6.3 år, var det 24 tilfeller av AD. CRF for de som ikke utviklet AD gjennom oppfølgingsperioden var 29.7±6.4 $mL \cdot kg^{-1} \cdot min^{-1}$ ved baseline, og 31.5±6.8 $mL \cdot kg^{-1} \cdot min^{-1}$ etter ett år. De som senere utviklet AD hadde en gjennomsnittlig CRF på 29.9±6.6 $mL \cdot kg^{-1} \cdot min^{-1}$ ved baseline, og 32.5±8.4 $mL \cdot kg^{-1} \cdot min^{-1}$ etter ett år. CRF ved baseline var ikke assosiert med forekomst av AD (Odd ratio: 0.99, 95% konfidensintervall: 0.92–1.07). Etter ett år var det fortsatt ingen assosiasjon (Odds ratio: 1.02, 95% konfidensintervall: 0.95–1.09). Ett-års endringer i CRF ble ikke funnet å ha sammenheng med forekomst av AD. Sammenlignet med deltakere under gjennomsnittet ved både baseline og ett-års testing, var en opprettholdt høy CRF (hazard ratio: 1.55, 95% konfidensintervall: 0.61–3.94), eller økt CRF (HR: 0.95, 95% konfidensintervall: 0.24–3.67) funnet å være assosiert med forekomst av AD.

Konklusjon: Denne studien fant ingen assosiasjon mellom hverken CRF ved baseline eller etter ett år, eller mellom ett-ås endringer i CRF og forekomst av AD.

Introduction

The world population is rapidly aging, and according to World Health Organization, the number of people aged 60 years and older is expected to double, from current 1 billion to reaching 2 billion by 2050 (1). This demographic shift is largely due to increased life expectancy, and with an older population comes age-related diseases, such dementia (1,2). The number of people developing dementia is expected to triple from the current 50 million cases worldwide, to more than 150 million by the year 2050 (2). With age being the greatest risk factor for developing dementia, the increasing dementia burden is a worldwide challenge and the global cost is expected to increase from today's 1 trillion US dollars pr. year, to more than 9 trillion US dollars by year 2050 (2). Alzheimer's disease (AD) is the most common form of dementia, and accounts for approximately 50-70% of all cases (3). Despite many efforts, there is no cure for the disease and drug trials has proven to the be difficult with a failure rate of >99 percent (4).

Thus, AD is considered one of the greatest scientific and health challenges of our time, and emphasize the importance of optimizing treatment, diagnosis, and prevention of the disease (4).

Alzheimer's disease

AD is a progressive neurodegenerative disease, characterized by memory loss and cognitive impairment (5). Pathologically, AD is characterized by the accumulation and deposition of amyloid-β into extracellular plaques and hyperphosphorylated tau proteins into intracellular neurofibrillary tangles in the brain (6). A growing body of evidence suggest that risk factors for AD are coincident with risk factors known to increase the risk of cardiovascular diseases (CVD), such as low education level, smoking, depression, diabetes, hypertension, obesity and physical inactivity (7). It is estimated that around 40-50% of all dementia cases worldwide are caused by modifiable risk factors, and 12.7% of worldwide AD cases is estimated to be caused by physical inactivity alone (8,9).

Physical activity and Alzheimer's disease

Meta-analysis has shown that performing regular physical activity is associated with 30-45% reduced risk of developing AD compared to being inactive or performing low levels of physical activity (10-12). In a prospective cohort study by Tari and colleagues, they used Personal Activity Intelligence (PAI) to assess the association between physical activity levels and risk of developing dementia and dementia mortality. PAI is a robust metric for physical activity tracking and translates heart rate during daily activity into a weekly score (14). A weekly PAI >100 has in other studies been found to be associated with a reduced risk of premature morbidity, and CVD (15,16). Here (14) they demonstrated that individuals that maintained a high PAI score (>100) and increased the PAI score over time (from <100 to >100) had a reduced risk of incident dementia and dementia related mortality (14). Another important finding was the observation of about 2 years delayed onset of dementia in individuals that maintained a high PAI score compared to inactive individuals, with a low PAI score. Although there is considerable evidence that being physically active at both midlife (<65 year), and older age (>65 year) reduces the risk of dementia and AD, there is evidence

that this may be explained by the effect of physical activity in improving the cardiorespiratory fitness (CRF) (17).

Cardiorespiratory fitness

CRF reflects the ability of the circulatory and respiratory system to supply oxygen to the muscle mitochondria during endurance exercise (18). The gold standard to quantify CRF is to measure maximal oxygen uptake (VO_{2max}) using cardiopulmonary exercise testing (CPET) (20). VO_{2max} refers to the highest rate at which an individual can transport and utilize oxygen during serve dynamic work with large muscle mass (18). VO_{2max} mirrors the cardiac, pulmonary, vasculature and skeletal muscle system, and thus considered to be a reflection of overall health status (19, 20). VO_{2max} has been proposed to be a vital clinical sign that should be evaluated in all people regularly (19).

Multiple studies have reported an inverse relationship between CRF and all-cause mortality (17). The American heart association has also stated that "CRF is as strong predictor of mortality as the established risk factors cigarette smoking, hypertension, high cholesterol, and type 2 diabetes - combined" (19). In line with this, several studies suggest that CRF should be given more emphasis in the healthcare profession (19-21). CRF declines with age, but it can be improved by exercise training and can therefore be used as an independent, and objective measure to predict health risk (22).

Cardiorespiratory fitness and Alzheimer's disease

A high mid-, and later life CRF is well documented to be associated with a reduced risk of developing dementia. DeFina and colleagues showed that, in a generally healthy cohort of 15 485 men and 4100 women (mean age of 49.8 years), the risk of developing dementia was 36% lower in those within the highest quintile of CRF compared to the lowest (23). Similar findings have also been shown in studies including women only (mean age of 50 years) (25). Population based cohort studies have showed that improving CRF over time reduced the risk of dementia and dementia-related mortality (24, 28). These studies, including participants with a mean age of 50-60 years at inclusion, showed that maintaining a high CRF reduces the risk of AD, and also that improving CRF can reduce the risk compared to a maintained low CRF (24, 28). Of interest, longitudinal studies have reported that exercise and an improved CRF can help preserve brain tissue and enhance the volume of the hippocampus by ensuring proper regulation of cerebral blood flow and hence delivery of essential oxygen, nutrients, and other neuroprotective factors to the brain (26, 27, 22)

Most studies on exercise and physical activity on AD are observational studies, and conclusion on causation must therefore be drawn with caution. No randomized controlled trials on exercise and AD have shown clear beneficial effects on the occurrence of the disease.

Aims and hypothesis

The aim of this thesis was to assess the association between CRF and incidence of AD in older adults aged between 70-77 years at baseline. We hypothesized that **1)** a high CRF at baseline were associated with a reduced risk of developing AD, and **2)** those who maintained

a high, or increased their CRF over one year had a reduced risk of developing AD in the follow-up period of up to 7 years.

Material and methods

Study design

A prospective cohort study with data from the randomized controlled exercise trail, the Generation 100 Study (REK: 2012/381, ClinicalTrials.gov: NCT01666340) (29, 30). The Generation 100 Study was a phase IIb clinical trial with the primary aim to investigate the effects of exercise on all-cause mortality in older adults (29, 30). Inclusion and baseline testing started in august 2012 and the initial intervention period ran until 2018, which included follow-up testing of the participants after, one-, three- and five years. In September 2022, ten-year follow-up testing of the participants started.

Participants

In 2012 all men and women with a permanent address in Trondheim born between 1936 and 1942 were invited to participate in the study (n=6966). Exclusion criteria were diseases or disabilities that could prevent exercise or completion of the study, other diseases or diagnosed dementia (30). Detailed inclusion and exclusion criteria are described elsewhere (30). In total 1567 (777 men and 790 women) were included at baseline. After baseline testing, the participants were randomized 1:1 into an exercise training group or to a control group. The exercise group was further randomized 1:1 into moderate intensity or high intensity training. The control group was instructed to follow the Norwegian recommendations for physical activity of 30 minutes of moderate-level physical activity every day. The moderate intensity training group was instructed to complete 50 minutes of continuous moderate-intensity exercise corresponding to 70% of peak heart rate, twice a week. The high intensity training group was instructed to complete two weekly ~40 minutes long workouts consisting of high intensity interval training. They were supervised to perform a 4x4 high intensity interval session, consisting of 10 minutes warm-up, followed by 4-minute working periods (intervals) with an intensity of 85-95% of peak heart rate, interspersed by 3 min active break, exercising at ~60-70% of peak heart rate. Which exercise group they were randomized to is not taken into consideration in this study, as the number who developed AD during the follow-up period in the respective groups was too few. (9 participants from the control group, 6 from the moderate intensity group, and 9 from the high intensity exercise group developed AD in the follow-up period).

For the present study, we excluded participants who had a history of CVD (n=166). CVD was defined as self-reported history of myocardial infarction, heart failure, angina pectoris or stroke earlier in life. We also excluded those who did not have available CRF data (n=30) and had missing data on smoking and educational status (n=115), leaving 1256 participants in the study cohort at baseline testing. Between baseline testing and the one-year follow up testing, 6 reported a new incident of CVD and was therefore excluded. Furthermore, 261 was excluded because of missing CRF data on one-year testing, and 44 for missing data on

smoking status at one-year follow-up. Six participants were excluded for onset of symptoms before 2014.

A total of 941 participants, presumably healthy men and women at both baseline and one-year follow-up testing was found eligible for statistical analysis of the association between CRF and AD (Figure 1).

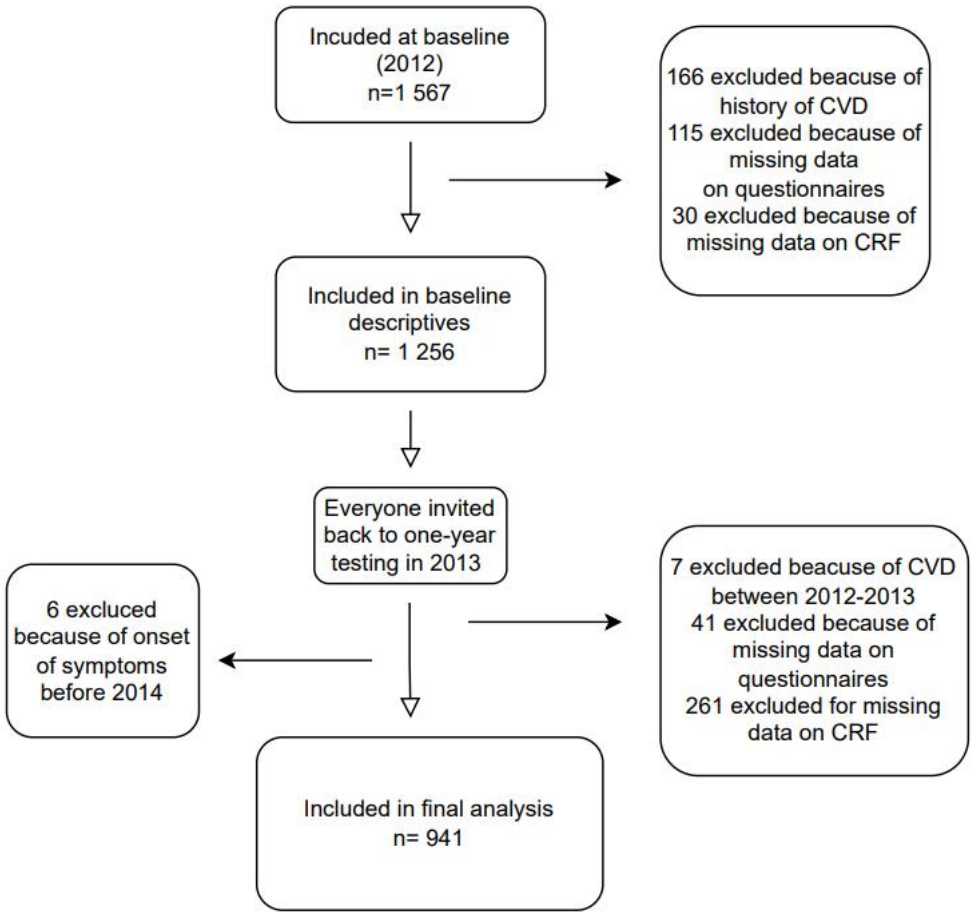

Figure 1: Flowchart of included and excluded participants
In total, 1567 was included in the baseline testing. 941 was found eligible to have complete data on both baseline and one-year follow-up testing.

Alzheimer's disease incidence (outcome and follow-up)

To identify participants that developed AD, the participants' hospital records were reviewed following a protocol developed by neurologists at St. Olavs University Hospital, Trondheim, Norway. The following diagnosis codes (International Classification of Disease, 10th edition) were searched and looked for: F00-F03, G20, G30-G31, F10.7, R41.8, G12.2 in contact overview, discharge summary and journal notes. Words in free text were also searched, these include words/conditions associated with AD (cognitive, memory, dementia, demented, alzheimer, Alzheimer's, normal pressure hydrocephalus, "mess up", forgetful, disoriented, oriented, delirious, confusion, cholinesterase) and different dementia

medications (Donepezil, Aricept, Galantamine, Reminyl, Rivastigmine, Exelon, Memantine, Ebixa, Nemdatine). For participants with mentioned diagnosis codes or search terms, the medical record was reviewed by a neurologist, and if cognitive impairment, a neurodegenerative condition or dementia was suspected, relevant information from the medical history was recorded in an Excel document. It was then determined whether the patient met the criteria for AD using the 2011 Albert and McKhann criteria (31, 32). Those who met these criteria and had onset of symptoms 01.01.2014 or later were included. Participants with onset of cognitive failure before 2014 were excluded.

This review was carried out according to an approved protocol and the work was validated by a neurologist at the Department of Neurology at St. Olavs University Hospital, Trondheim, Norway. End of follow-up was set at the end of December 2021 as this was the last day with updated patient records of AD incidence and health status.

Date of participation in 1-year testing was defined as baseline for follow up of AD incidence, and participants were followed until onset of symptoms of AD, death, or end of follow-up in December 2021 (31.12.2021). Onset of symptoms was calculated as the first day mentioned by either patient or relatives. If there was discrepancy between the date from patient and relatives, the date from the relatives was used.

Test protocol

A full description of the test protocol assessments has been described previously (30). In brief, participants filled out a questionnaire regarding health status, social demographic environment and lifestyle. Information on education level, smoking status and self-reported incidents of myocardial infarctions, angina pectoris, stroke or heart failure was obtained from the questionnaires.

Resting health assessment

Clinical measurements, including blood pressure measurements, anthropometrics (weight, height) and blood chemistry were performed by trained technicians, using standardized laboratory procedures. Devices and laboratories used is under the quality control at St. Olavs University Hospital, Trondheim, Norway where quality controls are frequency performed.

Blood pressure was measured after 5 minutes resting in a chair with a Philips IntelliVue MP50 (Philips medizin systeme, Boeblingen, Germany). Blood pressure was measured twice in both arms, with one minute break between tests, starting with the left arm. A third measurement was taken if the blood pressure differed >10 mmHg for systolic-, and >6mmHg for diastolic blood pressure. The mean of the two last measurements in the dominant arm was used to determine hypertension status in this study. Hypertension was defined as systolic blood pressure ≥140 mm Hg, and/or diastolic blood pressure ≥90 mm Hg (13), or self-reported use of antihypertensive mediations, or any combination of these.

Height was measured by participants standing barefoot with their feet placed against the wall, shoulder-width apart (Seca 222, Hamburg, Germany). Measured height was nearest millimeter.

Weight was measured on bioelectrical impedance (Inbody 720, BIOSPACE, Seoul, Korea). From here only weight was used to calculate the BMI ($kg \cdot m^2$).

Blood sampling was taken from arm vein (≥ 8 h postprandial) in laboratory at St.Olavs University Hospital, Trondheim, Norway. Measurements used is in this study include total cholesterol (high cholesterol defined as >7.8 $mmol \cdot L^{-1}$) (13), and HDL-cholesterol.

Assessment of cardiorespiratory fitness

CPET testing was performed at the Core Facility NeXt Move at NTNU (Norwegian University of Science and Technology, Trondheim, Norway) on a treadmill (Woodway USA Inc., Waukesha, WI, USA), or a bicycle (Monark Ergomedic 839 E, Sweden). The CPET performed with two different systems: Oxycon Pro (Erich Jaeger, Hoechberg, Germany), and Cortex MetaMax II (Leipzig, Germany) (30). Participants were tested on the same system on both baseline and one-year testing. Before the start of testing, both systems were calibrated against a motorized standardized mechanical lung (Motorized Syringe with Metabolic Calibration Kit; VacuMed, AkuMed AS, Oslo). Gas calibration was performed before the start of each test day, and after every fifth test (or if ambient air measurements was rejected), including both ambient air-, and a reference gas mixture of known content (15.00% O_2 and 5.00% CO_2) measurements. Volume, using a 3 L volume syringe (Hans Rudolph Inc., Kansas City, MO, USA) and inspiratory flowmeter were calibrated before each test.

The CPET protocol consisted of a 10-minute warmup and familiarization on the treadmill. Twenty-eight of the participants performed the CPET on a bicycle due to reduced functionality or leg pain. Warmup was conducted at an individually submaximal level based on 1) self-reported physical activity level, 2) heart rate, and 3) the participant's own perceived intensity. Heart rate was measured by Polar RS100 (Polar Electro Oy, Kempele, Finland) attached to the participant before the start of testing. After warm up, an individualized protocol was performed to measure VO_{2max}. Incline or speed was increased by respectively 2% or 1 $km \cdot h^{-1}$ approximately every minute until exhaustion or until criteria for VO_{2max} were met. Criteria for reaching VO_{2max} was defined as leveling of VO_2 (defined as not increasing by more than 2 $mL \cdot kg^{-1} \cdot min^{-1}$) despite increased workload, and a respiratory exchange ratio ≥ 1.05. The average of the three highest measurements was determined as VO_{2max}. Forty-one percent of the participants did not meet criteria for VO_{2max}, and their highest value at voluntary exhaustion was determined as peak oxygen uptake (VO_{2peak}). VO_{2peak} is further used as a collective term for both VO_{2max} and VO_{2peak}.

Statistical analysis

Descriptive data are shown as mean ± standard deviation for continuous variables, and number (%) for categorical variables. Visual inspection of QQ-plots and histograms determined normality of residuals. Baseline characteristics were compared using χ^2 test for categorical variables and Student's t-test for continuous variables. Difference in mean between the fitness groups were compared using One-Way ANOVA and Post – Hoc analysis for continuous variables and χ^2 for categorical variables.

All analyses were performed with relevant adjustments for covariates chosen due to their potential association to both CRF and AD. Due to limited statistical power, we were only able

to adjust for two variables. Based upon previous research (34), we considered socioeconomic (educational level) and smoking status to be among the two most important ones. Of those, smoking status were found to have the biggest impact on the results, but with no notable difference between the two variables. Therefore, all analyses are presented adjusted for gender and smoking status.

Binary logistic regression was used to estimate the odds ratio (OR) and 95% confidence intervals (CI) form CRF at baseline and at one-year. To assess the association between change in CRF following one year of exercise and incidence of AD, Cox proportional hazard regression analysis was used. For AD incidence, time to event was calculated from date of one-year testing, to date of onset of symptoms, otherwise considered as censored. Effect estimates is presented as hazard ratios (HRs) with 95% CIs. All statistical tests were 2. sided, and significance level was set at 5%.

Participants were classified into CRF categories depending on sex-specific VO_{2peak} mean at baseline. Those who were below the sex-specific mean at baseline was determined at "below mean", and those above was determined as "above mean". To assess the association between change in CRF following one year of exercise and AD incidence, we used 4 categories of change depending on mean VO_{2peak} at baseline: Below mean at both baseline and one-year, below mean at baseline – above mean at one year, above mean at baseline – below mean at one-year, and above mean at both baseline and one-year.

IBM SPSS, version 26 (Statistical Package for Social Science, Chicago, IL) was used for descriptive and statistical analyses. Canva 1.42.0 (App, Perth, Australia) was used for infographic visualization. Flowchart (Figure 1) was made in Diagrams (JGraph, Queensbridge, Northampton, England).

Ethical considerations

The Generation 100 study was approved by the Regional ethical Committee (REK, 2012/381), and fulfills the principles of the Declaration of Helsinki. Participation was voluntary, and all participants gave written consent before participation. The study is registered in the clinical trials registry (ClinicalTrials.gov, Identifier: NCT01666340).

Results

Overall, 29 participants developed AD during the follow-up of 6.3±1.1 years (range: 1-7 years). Of these, 3 were excluded for self-reported history of CVD, and 2 for missing data (smoking status). Thus, 24 participants (12 men and 12 women) who later developed AD were available in the final analysis.

Descriptive and physiological characteristics according to CRF level at baseline are shown in Table 1. The mean VO_{2peak} at baseline were 29.7±6.4 $mL \cdot kg^{-1} \cdot min^{-1}$ for men and women combined. The mean VO_{2peak} for women and men separately were 26.9±4.9 $mL \cdot kg^{-1} \cdot min^{-1}$, and 32.8±6.4 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively.

VO_{2peak} for participants below, and above the mean (men and women combined) is presented in Table 1. Participants above the mean were significantly younger than those below, had a lower BMI, lower prevalence of hypertension and high cholesterol, and a less proportion were current or former smokers (Table 1).

Association between CRF and risk of developing AD

Binary logistic regression models, adjusted for gender and smoking status, showed no significant association between VO_{2peak} as a continuous variable and incidence of AD, either at baseline, or after one year. At baseline the observed OR was 0.99 (95% CI: 0.93–1.07). After one year the association was still not significant, OR: 1.02 (95%CI: 0.95–1.09). Among participants that stayed healthy or later developed AD there was a big range in CRF levels- both at baseline and after one year.

VO_{2peak} in participants that did not develop AD ranged from 12.7 $mL \cdot kg^{-1} \cdot min^{-1}$, to 52.8 $mL \cdot kg^{-1} \cdot min^{-1}$ at baseline, and from 14.2 $mL \cdot kg^{-1} \cdot min^{-1}$, to 57.5 $mL \cdot kg^{-1} \cdot min^{-1}$ after one year, men and women combined. VO_{2peak} in among those who later developed AD ranged from 15.6 $mL \cdot kg^{-1} \cdot min^{-1}$ to 41.5 $mL \cdot kg^{-1} \cdot min^{-1}$ at baseline, and from 14.1 $mL \cdot kg^{-1} \cdot min^{-1}$ to 48.2 $mL \cdot kg^{-1} \cdot min^{-1}$ after one year, men and women combined. (Figure 2, Appendix 1).

Table 1: Descriptive and physiological characteristics according to CRF at baseline.

	Below mean at baseline	Above mean at baseline	Total	p-value
Male	241 (48.7%)	206 (46.2%)	447 (47.5%)	..
Female	254 (51.3%)	240 (53.8%)	494 (52.5%)	<0.001
Age (years)	72.5±3.1	71.9±2.1	72.2±2.2	<0.001
BMI ($kg \cdot m^2$)	27.2±3.2	24.2±2.7	25.7±3.4	<0.001
Total cholesterol ($mmol \cdot L^{-1}$)	5.71±1.09	5.92±1.05	5.87±1.11	<0.001
High cholesterol				
Yes	19 (3.8%)	9 (2%)	28 (3.1%)	..
No	476 (96.2%)	437 (98.0%)	913 (96.9%)	<0.001
HDL cholesterol ($mmol \cdot L^{-1}$)	1.68±0.53	1.89±0.56	1.78±0.54	0.003
Systolic blood pressure (mmHg)	135±16	133±17	134±16	<0.001
Diastolic blood pressure (mmHg)	75±10	75.3±10	75±9	0.234
Hypertension				
Yes	284 (57.4%)	187 (42.2%)	471 (50.1%)	..
No	211 (42.6%)	259 (57.8%)	470 (49.9%)	<0.001
Education				
<10 years	65 (13.2%)	56 (12.6%)	121 (12.9%)	..
10-12 years	167 (33.7%)	144 (32.3%)	311 (33.1%)	..
>12 years	263 (53.1%)	245 (54.9%)	508 (54.0%)	<0.001
Smoking status				
Never	225 (45.5%)	265 (59.3%)	490 (52.0%)	..
Former	239 (48.3%)	166 (37.4%)	405 (42.9%)	..
Current	31 (6.3%)	15 (3.4%)	46 (4.9%)	<0.001
VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)	25.5±3.9	34.3±5.5	29.7±6.4	<0.001
VO_{2peak} ($L \cdot min^{-1}$)	2.02±0.48	2.42±0.63	2.21±0.54	<0.001

Data are reported as mean ± standard deviation *for continuous variables, and number (%) for categorical variables. Difference between the groups was examined by independent sample t-test for continuous variables, and χ^2-test for categorical variables. BMI was calculated as weight divided by height in m^2. High cholesterol was defined as total cholesterol concentrations ≥7.8 $mmol \cdot L^{-1}$. Hypertension was defined as systolic blood pressure ≥140 mmHg, systolic blood pressure ≥90 mmHg or taking antihypertensive medication, or any combinations of these. Abbreviations: HDL cholesterol=high-density lipoprotein cholesterol, BMI=body mass index, VO_{2peak}=peak oxygen uptake.*

One-year change in cardiorespiratory fitness and risk of Alzheimer's disease

Table 2 present the descriptive and physiological characteristics of the participants according to one-year changes in CRF. The mean VO_{2peak} increased by 6.1% (1.8±3.5 $mL \cdot kg^{-1} \cdot min^{-1}$, p<0.001) from baseline to one-year follow-up in the whole study population. The mean VO_{2peak} after one year were 34.6±6.9 $mL \cdot kg^{-1} \cdot min^{-1}$ for men (5.5% increase, p<0.001), and 28.7±5.5 $mL \cdot kg^{-1} \cdot min^{-1}$ for women (6.7% increase, p<0.001).

Table 2: Descriptive and physiological characteristics according to one-year changes in CRF.

	Below mean at baseline		**Above mean at baseline**		**p-value**
	Below mean at one-year	Above mean at one-year	Below mean at one-year	Above mean at one-year	
Male	176 (51.6%)	65 (42.2%)	18 (47.4%)	188 (46.1%)	..
Female	165 (48.4%)	89 (57.8%)	20 (52.6%)	220 (53.9%)	<0.001
Age (years)	73.6±2.1	73.3±2.0	73.0±2.0	72.9±1.8[#]	<0.001
BMI ($kg \cdot m^2$)	27.3±3.6	25.5±3.2	25.0±3.3	24.0±2.6[#]	<0.001
Total cholesterol ($mmol \cdot L^{-1}$)	5.50±1.14	5.58±1.08	5.73±1.03	5.76±1.14[#]	<0.001
High cholesterol					
Yes	5 (1.5%)	3 (1.9%)	-	13 (3.2%)	..
No	336 (98.5%)	151 (98.1%)	38 (100%)	395 (96.8%)	<0.001
HDL cholesterol ($mmol \cdot L^{-1}$)	1.68±0.49	1.78±0.53	1.78±0.47	1.91±0.53[#]	0.003
Systolic blood pressure (mmHg)	134±15	132±17	131±15	129±17[#]	<0.001
Diastolic blood pressure (mmHg)	74±10	73±9	74±9	73±9	0.234
Hypertension					
Yes	196 (57.5%)	73 (47.4%)	15 (60.5%)	151 (37.1%)[#]	..
No	145 (42.5%)	81 (52.6)	23 (39.5%)	257 (62.9%)	<0.001
Education					
<10 years	48 (14.1%)	17 (11.0%)	7 (19.5%)	49 (12.0%)	..
10-12 years	118 (34.6%)	49 (31.8%)	12 (30.5%)	133 (32.6%)	..
>12 years	175 (51.3%)	88 (57.1%)	19 (50.0%)	226 (55.4%)	<0.001
Smoking status					
Never	150 (44.3%)	85 (55.2%)	17 (44.7%)	257 (63.0%)[#]	..
Former	167 (49.7%)	65 (42.2%)	16 (42.1%)	141 (34.6%)	..
Current	24 (7.0%)	4 (2.6%)	5 (13.2%)	10 (2.5%)	<0.001
$\mathbf{VO_{2peak}}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	25.7±3.9	32.1±3.9	28.2±3.4	36.5±5.8[#]	<0.001
$\mathbf{VO_{2peak}}$ ($L \cdot min^{-1}$)	2.05±0.52	2.38±0.62	2.05±0.49	2.54±0.58[#]	<0.001

Data are presented as mean ± standard deviation for continuous variables, and n (%) for categorical variables. Difference between groups was tested with one-way ANOVA, and post hoc analysis for continuous variables, and χ^2-test for categorical variables. [#]Determines significant difference between below the mean at both baseline and one-year follow-up testing, and above the mean at both baseline and one-year follow-up testing. Body mass index was calculated as weight divided by height in m^2. High cholesterol was defined as total cholesterol concentrations ≥7.8 $mmol \cdot L^{-1}$*. Hypertension was defined as systolic blood pressure ≥140 mmHg, systolic blood pressure ≥90 mmHg or taking antihypertensive medication – or any combinations of these. Abbreviations: HDL cholesterol= high-density lipoprotein cholesterol, BMI=body mass index,* $VO2_{peak}$*=peak oxygen uptake.*

We observed that 341 participants (36%) were below their sex specific mean at both baseline and one-year follow-up testing, 154 participants (16%) increased their CRF from below the mean at baseline, to above after one year, 38 participants (4%) decreased their

CRF, from above the mean at baseline to below after one year, 408 participants (43%) were above the mean at both baseline and one-year follow-up testing. Table 3 presents the HRs according to one-year changes in CRF, and incidence of AD. Below the mean at both baseline and one-year follow-up testing was used as reference in the analysis. Of those who later developed AD, 7 maintained a low CRF over one year (3 men and 4 women), 3 increased their CRF (2 men and 1 women), and 13 maintained a high CRF (7 men and 6 women). Only 1 who later developed AD decreased in CRF from baseline to one-year follow-up.

Table 3*:* HRs of Alzheimer's disease incidence by one-year changes in CRF.

	Above mean at one-year	**Below mean at one-year**
Above mean at baseline		
Events	13	1
HR (95% CI)	1.55 (0.61 – 3.94)	1.27 (0.16 – 10.37)
Below mean at baseline		
Events	3	7
HR (95%CI)	0.95 (0.24 – 3.69)	Reference

Hazard ratios (HRs) is adjusted for gender and smoking status. 95% CI: 95% confidence interval.

VO_{2peak} for participants in the different CRF categories is shown in Table 2. Compared to participants below the CRF mean at both baseline and one-year follow-up testing, there were no association between incidence of AD and a maintained high CRF over one year, or increased CRF (Table 3). Participants that did not develop the disease during the follow-up period had an average VO_{2peak} of 29.7±6.4 $mL \cdot kg^{-1} \cdot min^{-1}$ at baseline, and 31.5±6.8 $mL \cdot kg^{-1} \cdot min^{-1}$ after one-year (6.1% increase, $p<0.001$). The mean VO_{2peak} for those who later developed AD were 29.9±6.6 $mL \cdot kg^{-1} \cdot min^{-1}$ at baseline, and 32.5±8.4 $mL \cdot kg^{-1} \cdot min^{-1}$ after one-year (8.7% increase, $p<0.001$).

We compared excluded participants to included. Excluded participants were older, had a higher BMI, were more likely to be men (331 men were excluded, compared to 294 women) and were less educated compared to included. Participants with available data on baseline testing but did not meet for one-year follow-up testing were older, had a lower VO_{2peak} and higher BMI at baseline compared to included. Excluded participants due to history of CVD was of similar age to included, but had a lower VO_{2peak} at baseline, and had a lower increase VO_{2peak} from baseline to one-year, higher BMI and a higher percentage were classified as having hypertension at baseline. There were more men than women excluded for CVD (71.9% men).

In sensitivity analysis done to check for reverse causality, we excluded the first year of follow-up, leaving 21 AD cases for analysis. These exclusions did not affect the results. Also, we identified 120 cases of other neurogenerative disease and cognitive issues within the study cohort (other forms of dementia, Parkinson disease, brain tumors, major brain

diseases, multiple sclerosis or mild cognitive impairment). Sensitivity analysis showed that they did not affect the results. (data not shown).

Discussion

Contrary to our hypothesis, the main findings from this study were that we observed no association between CRF and incidence of AD in older adults aged 70-77 years at baseline. Our results do not reinforce the well-established positive association between CRF and the risk of developing AD in mid- and later life observed in both men and women in other studies (23,24,25,28,33). To the best of our knowledge, no other studies have assessed the association between objectively measured CRF and incidence of AD in older adults >70 years.

CRF at baseline and after one year and risk of developing AD

Unlike findings from other studies (23,25,33), we did not observe any association between CRF levels either at baseline or after one year and later development of AD. A study by Müller and colleagues objectively assessed CRF in over 6100 participants aged 59.2 at baseline (33). After a follow-up time of 10.3 years, they showed that a low CRF (defined as a CRF estimate of <6 metabolic equivalent of task; METs) were associated with a 4 times greater risk of AD compared to a high CRF (>12 METs), (1 MET equals 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$) (33). Higher levels of CRF have also been found to be associated with reduced cognitive decline in healthy older adults, and in older adults at risk of AD due to genetic predisposition (26). Hence, given the link between CRF, cognitive decline and AD development, our findings were unexpected based on most relevant literature. Although the reason for the different results compared to most relevant literature is not known, it seems reasonable to speculate that it may be due to our participants higher age and our smaller sample size. As all participants were healthy from baseline to one-year follow-up, longer follow-up time may be needed to detect clear associations.

It may be that older adults that sign up to participate in an exercise intervention study over 5 years, already is familiar with exercise, and thereby represent a healthier part of the general population of older adults. Included participants in the Generation 100 main study were found to be more active, and showed a better overall heath compared to non-included participants. In fact, the mortality rate in the Generation 100 main study was found to be 4.6%, which is less than half of the expected mortality rate in the general population of older adults in Norway of the same age (36). Thus, selection bias might clearly have influenced our results, and support the suggestion that longer follow-up is needed to give a more conclusive answer to our hypothesis. It seems fair to argue that included participants already are more protected due to their better overall health compared to non-included. Based on these assumptions it seems natural to speculate that individuals in this population develop AD at a later age compared to the general population.

Interestingly, findings from this study also shows that those who participated in the baseline testing but did not meet for the one-year follow-up testing had a lower VO_{2peak} at baseline

when compared to those that took part at both timepoints. When looking for AD cases within the Generation 100 population, it was only looked at participants with available CRF data at both baseline-, and one-year follow-up testing. Given the knowledge of the importance of a high CRF and reduced risk of developing AD, and the possibility that non-included participants in the Generation 100 Study had an overall poorer health it is possible that those who did not participate in the study had a lower CRF and thereby an increased risk of AD. Also, there may be incidents of AD among the participants that did not have complete CRF data on both baseline and one-year follow-up testing. This remains, however, speculative at present.

One-year changes in CRF and risk of developing AD

The association between change in CRF and risk of AD have previously been studied in population-based studies, with estimated changes in CRF based on self-reported physical activity levels (24, 28). Contrary to these studies, we found no association between one-year changes in CRF and incidence of AD.

In a prospective cohort study by Tari and colleagues, they used data from the first and second wave of the Trøndelag health study (HUNT), and estimated CRF with a validated non-exercise model (24). They further assessed temporal changes in CRF and risk of dementia incidence and dementia-related mortality in 14 550 men and 19 925 women (24). During a median follow-up time of 7.6 years, dementia incidence in this study was found to be reduced by 40-50% in those who maintained their CRF above the least fit quantile within participants of the same age and gender group, or increased their CRF from the least fit quantile at first wave, to above in the second wave. Those who maintained or improved their CRF during the ten years between the two HUNT waves were also found to have a delayed onset of dementia of about 2 years. This study suggests that the association between CRF and AD (or dementia of any cause) is modifiable, also after mid-life. In our study the mean follow-up time was 6.3 years, which is similar to the study by Tari and colleagues. Contrary to the study by Tari and colleagues, all the participants were >70 years in our study at baseline, compared to about 60 years in Tari and colleagues. We also assessed changes in CRF over only one year of exercise, whereas Tari and colleagues assessed changes over 10 years (between HUNT1 and HUNT2). Importantly, Tari and colleagues had substantially more participants than our study. Finally, we assessed the association between CRF and AD specifically, and not all cause dementia.

The onset of symptoms was close to the one-year testing, with 3 of the participants having onset in the first year of follow-up. In sensitivity analysis we excluded the first year of follow-up and this was found to not affect the results. However, the short time period between one-year testing and the onset of symptoms along with the notably higher age of the participants in our study, may partly explain why our study did not find any associations between changes in CRF and AD risk. Considering that the pathological changes in AD may occur even decades before clinical symptoms (3), the disease may already have progressed into a stage where it is “too late” to experience the same positive modifying association

between CRF and AD (and other form of dementia) as seen in in other studies with younger participants (24, 28).

Previous studies have suggested early-stage AD individuals to have a similar CRF as non-demented counterparts (35). A study by Burns and colleagues tested the feasibility of maximal exercise testing in an early-stage AD population with a mean age of 73.6±6.5 (35). They compared 31 early-stage AD individuals to 31 non-demented individuals of the same age and same distribution of gender, and found that physically healthy individuals in early-stage of AD had similar CRF levels as non-demented individuals (mean VO_{2peak} of 21.2 $mL \cdot kg^{-1} \cdot min^{-1}$ in nondemented vs. 19.8 $mL \cdot kg^{-1} \cdot min^{-1}$ in early-stage AD). Their finding of no significant difference between early-stage AD and non-demented of the same age may be relevant to our findings. Given the aforementioned pathological changes that occur decades before clinical symptoms of AD, and the short period of time between one-year testing and onset of symptoms it is reasonable to assume that the progression of the disease already had started in our participants. Although the study by Burns and colleagues did not show any significant associations between early-stage AD and non-demented participants, the cross-sectional design of the study limits the interpretation of the results. Also, they had relatively few participants, with a notably lower VO_{2peak} compared to our participants (around 20 $mL \cdot kg^{-1} \cdot min^{-1}$, compared to around 30 $mL \cdot kg^{-1} \cdot min^{-1}$ in our study). Some studies have also shown that individuals in early-stage AD is able to improve their CRF following exercise (36, 37). Our results at least indicate that in this population CRF was not the best predictor for AD incidence. Based on our findings, it would be interesting to see if the results were different if we started the intervention earlier in life.

In our data, we observed an average increase in CRF by 1.8 $mL \cdot kg^{-1} \cdot min^{-1}$ from baseline to one-year, with similar increase in both genders. This finding is similar to other studies of one-year of exercise in older adults >70 years (38). The VO_{2peak} in this population is line with a previous study on Norwegian adults of the same age (39). Data from the HUNT study reported an average VO_{2max}, measured with CPET on treadmill, of 32.4 $mL \cdot kg^{-1} \cdot min^{-1}$ in men and women >70 years old (39). This number include the average of 76 men (35.4 $mL \cdot kg^{-1} \cdot min^{-1}$), and 53 females (28.3 $mL \cdot kg^{-1} \cdot min^{-1}$). After one year, the mean VO_{2peak} in this Generation 100 population was similar to the findings in the HUNT study (31.5 $mL \cdot kg^{-1} \cdot min^{-1}$ in our study vs. 32.4 $mL \cdot kg^{-1} \cdot min^{-1}$ in HUNT). The HUNT study is conducted in habitants in the same demographic area in Norway as the participants in the Generation 100 Study, and the HUNT population is shown to be representative of the Norwegian population in general (40). The HUNT population has also been used in other countries as a validation cohort to replicate their findings (41).

Due to limited AD cases in total, and limited number of AD cases within each CRF category, we did not present sex-specific results. There is a notable sex-difference in AD prevalence, where women represent a clear majority of AD cases worldwide (42). However, there were equal numbers of men and women (12 men and 12 women) in this study. As of now, it seems too soon for this kind of study in this population. With an expected increase in the prevalence of AD, we will have more statistical power to detect significant associations in the future. In the years to come, we also have the possibility to assess changes in CRF over a

longer period of time (from three-, five- or ten-year follow-up testing), and with longer follow-up time of AD incidence (or AD mortality). Little is known on the effect of intensity of exercise and risk of AD (or dementia of any kind). With the well demonstrated benefit of a high at CRF mid-, and later life and risk of AD, it would be interesting to use findings from the Generation 100 Study to assess differences in risk of AD within the different exercise groups. If the reported association of CRF and AD remains the same in this population also in the years to come, it will have a huge clinical impact.

On one hand, our findings do not change the belief that a high CRF is of importance for risk of AD in later life, whereas on the other hand it might be that when above a certain age, CRF is not the best indication of AD. This remains speculative for now, but our findings are a step in the direction of understanding the association of CRF and AD in a population of older adults.

The big individual difference in CRF levels between those who later developed AD further emphasizes the difficulties of finding any association between CRF and AD in this population at this time point and can along with the small sample size explain the wide confidence intervals. It seems reasonable to speculate a larger sample size and longer follow-up time may be needed to detect significant associations. Visual inspection of scatter plots seemed to show a protective effect around 10-15% above sex-specific mean for both men and women. Due to the limited statistical power, we were unable to investigate this further. No men >41.5 $mL \cdot kg^{-1} \cdot min^{-1}$, or women >38 $mL \cdot kg^{-1} \cdot min^{-1}$ at baseline later developed AD. The chosen CRF categories were used because of similar use in other studies (24, 43), and to ensure sufficient number of participants in each CRF category.

Potential underlying mechanisms between CRF and development of AD

Available literature suggests that the effect of exercise and a high CRF may be linked to the changes in hippocampal perfusion and structure (44-46). The reduced brain atrophy seen in those with a high CRF may partly explain the reduced risk of AD (or dementia of any kind) compared to individuals with a lower CRF (46). Also, systemic neuroprotective factors can be induced by exercise, and these circulating biomolecules might cross the blood-brain barrier and be important to protect against AD (47).

Relevant to the findings in this study, a study by Pani and colleagues, also using data from the Generation 100 Study, did not find any changes in CRF to be associated with brain volume changes (27). Higher CRF at baseline was however found to be associated with greater cortical volume at one-, three-, and five-year follow-up. Within the Generation 100 Study population, 105 of the participants accepted to be included in an additional sub-study, assessing the structural brain health. Forty-eight from the control group, twenty-five from the moderate intensity training group, and thirty-three from the high intensity training group were included. Interestingly, the high intensity interval group showed greater hippocampal atrophy compared to the control group in this study. All groups were well within normal annual reductions in hippocampal volume, but this underlines the evidence that exercise and CRF can modulate the hippocampus, and that the underlying mechanisms

of exercise and CRF needs to be further assessed to fully understand effect of exercise and CRF on AD prevention and treatment in older adults.

Another potential mechanism that could have affected the results is genetics. Genes have been found to determine around fifty percent of our CRF, and they are also found to have an impact on the response of exercise in terms of improving our CRF levels (48). Importantly, heredity and genes (especially APOE genotype) have a huge impact in the risk of developing AD as well (49). These assumptions suggest that genetics and genetic predisposition have a role in the response or in the improvement of CRF levels in the participant of this study. We have the possibility to study this further in biological material from the Generation 100 Study in the future.

Strength and weaknesses

We utilized data from the world's largest randomized exercise trial in older adults, The Generation 100 Study, which involved objective measurements of CRF at both baseline and after one year of exercise. In contrast, many other studies examining the relationship between CRF and AD (or dementia of any form) either rely on a single baseline measurement of CRF or estimate of CRF based on self-reported physical activity. Additionally, our study specifically assessed the association between CRF and AD and did not combine that with other types of dementias. All participants in our study were healthy individuals aged 70-77 years at baseline and engaged in an exercise intervention.

These strengths highlight the robustness and novelty of the study. However, it is important to acknowledge that this study also has several limitations that should be considered. Firstly, the sample size for AD incidence was small, leading to limited statistical power in analyses. Secondly, the assessment of changes in CRF was conducted over a relatively short period of time (baseline to one year). Thirdly, our study includes a relatively homogenous group of participants. Fourthly, it is possible that not all AD cases within the Generation 100 population were identified. Fifthly, the observational design of our study limits the ability to establish causality.

Conclusion

We found no association between CRF and incidence of AD in this population. Baseline and one-year CRF levels were not associated with development of AD. One-year changes in CRF were also found to not have any association with later development of AD. In the future, studies should aim to incorporate the underlying mechanisms in combination with factors evaluated in this thesis to fully understand the association between CRF and development of AD in older adults. Larger sample size, and longer follow-up time is needed to further assess the association between CRF and incidence of AD in the Generation 100 population.

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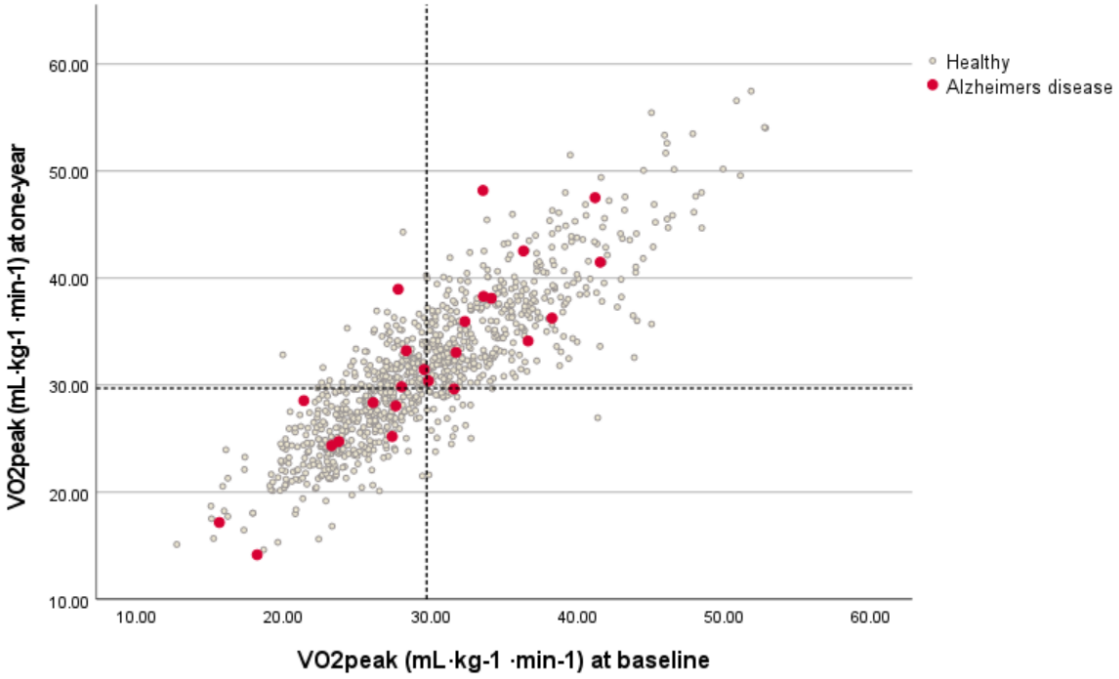
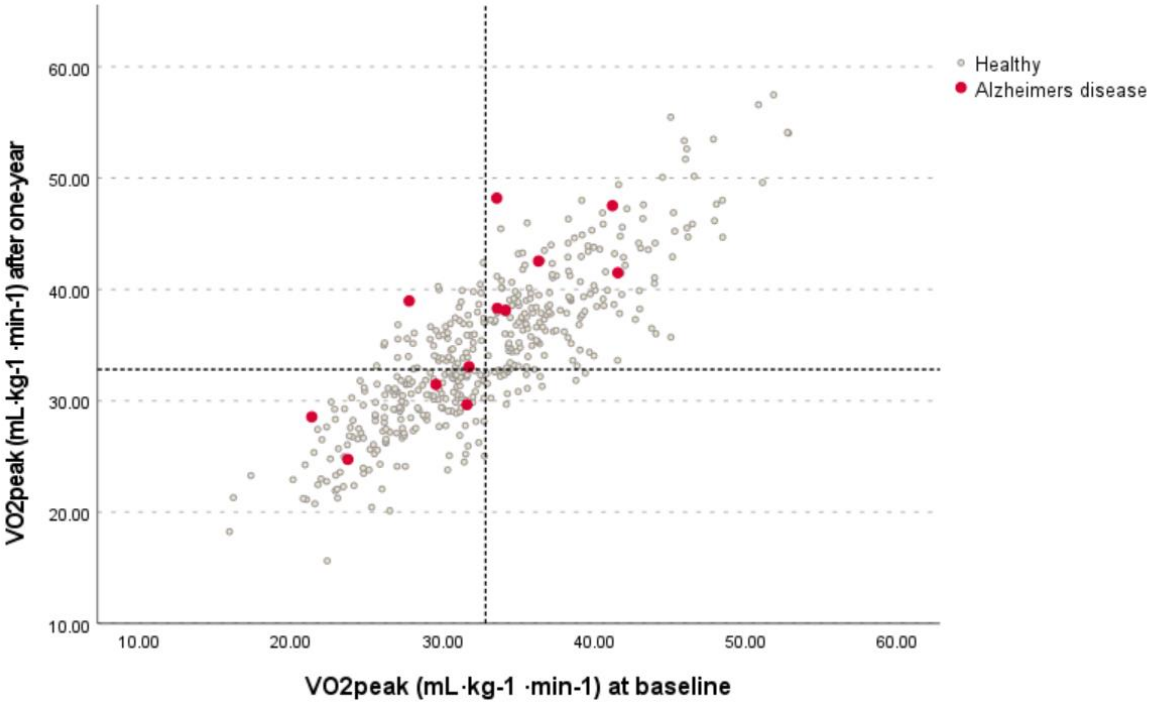
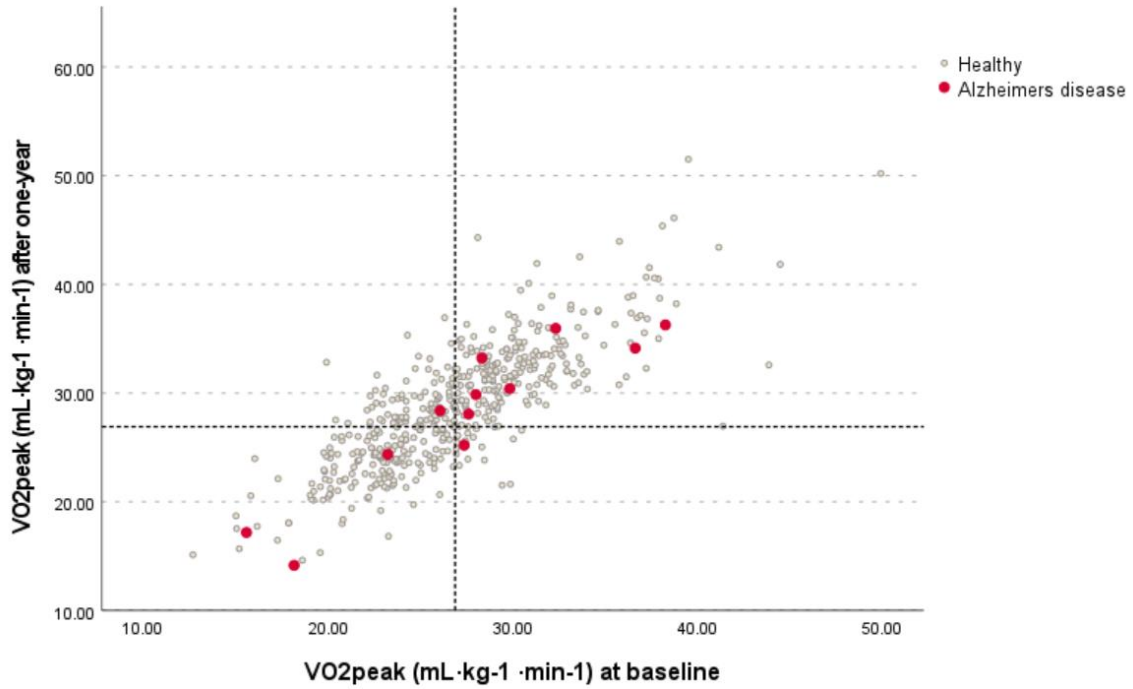
Appendix



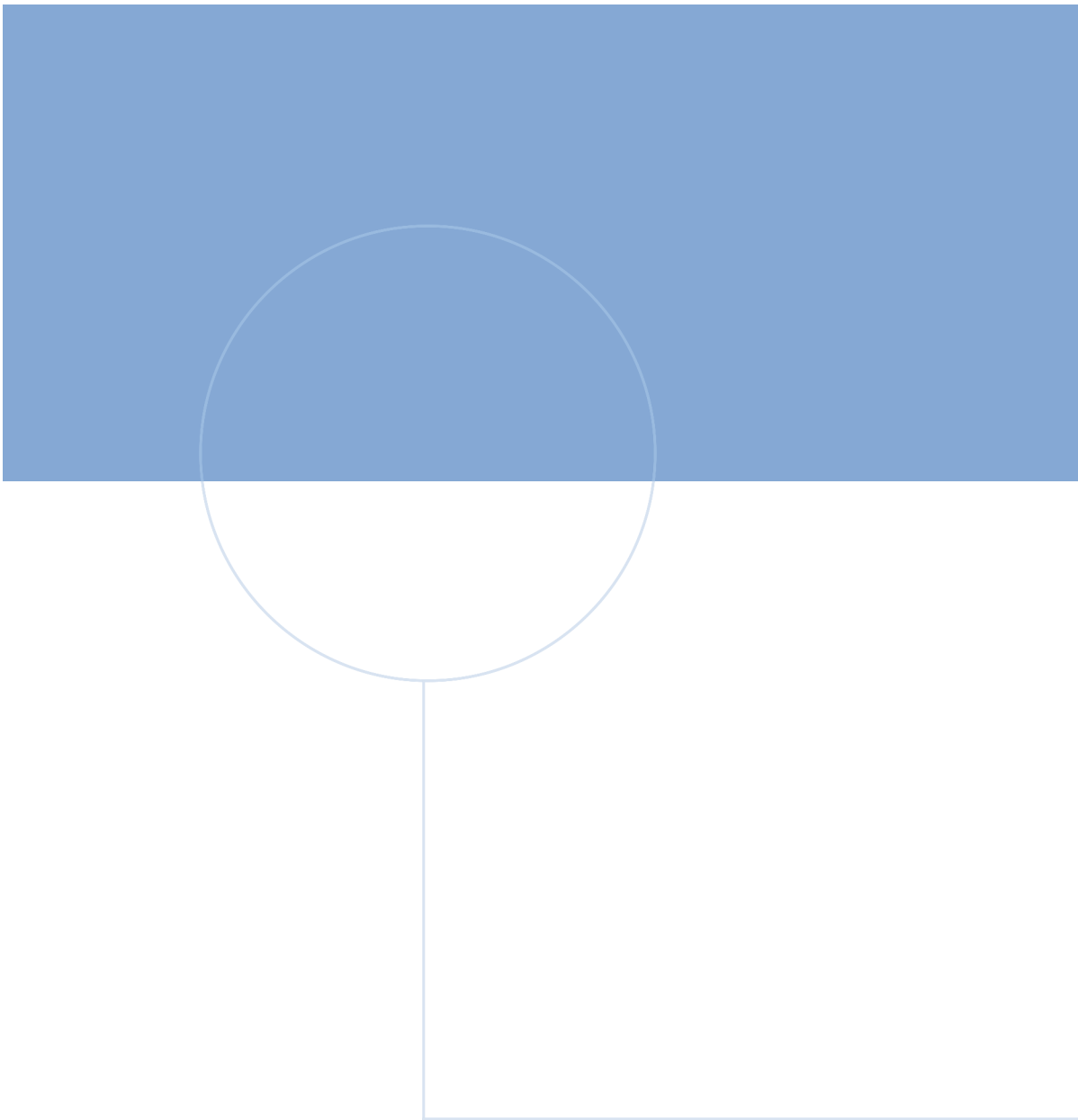
Appendix 1, Figure 2: CRF at baseline and after one-year (men and women combined)
Scatter plot of CRF measured as VO_{2peak} at baseline and after one-year, men and women combined. Dotted lines represent mean VO_{2peak} for the whole population at baseline (29.7 mL·kg^{-1} ·min^{-1}), used to determine and illustrate the CRF categories.



Appendix 2, Figure 3: CRF at baseline and after one-year (men)
Scatter plot of CRF measured as VO_{2peak} at baseline and after one-year for men. Dotted lines represent mean VO_{2peak} at baseline (32.8 mL·kg^{-1} ·min^{-1}), used to determine and illustrate the CRF categories.



Appendix 3, Figure 4: CRF at basline and after one-year (women)
Scatter plot of CRF measured as VO_{2peak} at baseline and after one-year for women. Dotted lines represent mean VO_{2peak} at baseline (26.9 mL·kg^{-1} ·min^{-1}), used to determine and illustrate the CRF categories.



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