Search for evidence to improve symptom management and prevent complications in atrial fibrillation

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DOI:
10.21996/70de-es65

Publication date:
2023

Document version:
Forlagets udgivne version

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Preamble

This PhD looks at different aspects of symptom management and prevention of cardiovascular complications in patients with atrial fibrillation, with special attention given to women. The treatment of atrial fibrillation follows an A, B, C treatment algorithm which this thesis is built around. The 'A' represents avoid stroke/anticoagulation. In the PhD, we explore the difference in risk of stroke in men and women with atrial fibrillation. The 'B' represents better symptoms and is focused on optimization of rate and rhythm control in atrial fibrillation. In the PhD, this is explored in DanAF, a multicenter randomized trial comparing lenient rate control with strict rate control on quality of life and through a systematic review comparing different rate controlling drugs for atrial fibrillation. The 'C' represents optimization of comorbidity and risk factors, and the PhD here looks at the physical activity paradox where higher occupational physical activity leads to higher risk of cardiovascular disease whereas higher leisure time physical activity leads to lower risk of cardiovascular disease.

When conducting a randomized trial, the list of people who deserve appreciation is very long. I would like to thank the entire steering committee in DanAF. Your passion for driving investigator-initiated trials makes important, underfunded trials like DanAF possible.

Annika Christiansen, for helping me handle logistics and recognizing how tough it actually is to conduct clinical trials.

Ole Dyg Pedersen who put in many hours in recruiting participants, discussing logistics with me and listening when my frustrations were high.

Christian Gluud for being inspirational in always demanding the highest quality research and standard for published papers down to the last reference.

Anne Merethe Soja for her clinical expertise and encouraging words and for her willingness to become responsible for recruitment at Holbaek Hospital.

Isak Cold for making my job that much easier and challenging my assumptions about different aspects of medicine.

Ole Steen Mortensen who was both moral support and supervised the paper on physical activity paradox.

Anne Møller, who worked with me on my paper on physical activity, spending multiple hours with me at CSS working on the data.

Axel Brandes for making sure all aspects of the randomized trial lived up to the highest standards as well as all references to current guidelines were correct.

Ilan Raymond, who helped me plan how to recruit from Holbaek hospital and has helped facilitate recruitment from Hvidovre Hospital.

Michael Hecht Olsen. The list of help is very long, but the highlights include always being only a phone call away no matter the hour, being the clinical expert that always had my back and supervising my paper using data form the LIFE study.

Janus Christian Jakobsen for his belief in me, for his expertise in both conducting clinical trials and systematic reviews without which, this trial and my PhD could not have been planned or initiated.

Emil Eik Nielsen for his expertise on echocardiography, always willing to help, letting me have somewhere to vent my frustrations when facing the inevitable bureaucracy. He also opened doors for me that would otherwise be closed as his genuine interest in striking conversion with other people made it possible for me to connect to others for whom I would otherwise not have.

Mie Buron Feinberg, my wife, for giving me the most joy in my life of anything, preparing meals when I was too tired to think about food, and for enlightening me on many points of views of nurses.

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Study 2 – Systematic review of rate controlling drugs

Feinberg JB, Cold IM, Nielsen EE, et al. Rate controlling drugs for atrial fibrillation in the outpatient setting. A systematic review with meta-analysis and Trial Sequential Analysis. Will be submitted to JAMA Cardiology.

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1. JB Feinberg. Data protection in connection with the start op of an investigator initiated clinical trials – experience and hopes for the future. 2021. Danish Congress of Public Health Medicine.

Summary

Introduction

Atrial fibrillation is the most common arrhythmia of the heart in the world. Complications include stroke, heart failure, and death. Symptoms include shortness of breath, heart palpations, fatigue, and chest pain. Atrial fibrillation may be paroxysmal or non-paroxysmal. Depending on whether sinus rhythm is pursued or not, non-paroxysmal atrial fibrillation may be divided into persistent or permanent atrial fibrillation.

In the latest European guidelines for atrial fibrillation, treatment follows an A, B, C approach. The 'A' represents Anticoagulation/Avoid stroke, the 'B' represents Better symptom control, and the 'C' represents Comorbidity and includes optimizing of Cardiovascular risk factors and lifestyle.

Anticoagulation can be achieved using a new oral anticoagulant or a vitamin K antagonist. The risk of stroke and the indication for starting anticoagulation in atrial fibrillation is in clinical practice usually assessed using the CHA_2DS_2VASc score. Women who develop atrial fibrillation appear to have a higher risk of stroke compared with men. However, sex, is seen as an effect modifier, and not an independent risk factor. The mechanism behind this differential risk is unknown – theoretically, it could be related to residual confounding or biological sex.

Rate control is the main treatment for controlling symptoms in patients with permanent atrial fibrillation. Drugs include beta-blockers, calcium channel blockers, and digoxin. The optimal heart rate is currently not known for atrial fibrillation, neither is the best drug to achieve this heart rate control.

To prevent development of atrial fibrillation and prevent cardiovascular complications in patients with atrial fibrillation, physical activity is recommended. However, it seems the benefits of physical activity depend on if it is occupational physical activity (OPA) or leisure time physical activity (LTPA). This is called the physical activity paradox.

Objective

The objective of this PhD thesis was to search for evidence to improve symptom management and prevent complications in atrial fibrillation by looking at different aspects of the A, B, C guideline recommended approach where there are unanswered questions related to management of atrial fibrillation.

Methods

First and foremost, we designed, planned, initiated, and are currently conducting DanAF, a randomized clinical trial. 350 patients with persistent or permanent atrial fibrillation prior to inclusion are being randomized to either a lenient heart rate target (80-110 beats per minute (bpm)) or a strict heart rate

target (<80 bpm) based on five minutes resting electrocardiogram (ECG). The primary outcome is quality of life using the Short Form – 36 (SF-36) questionnaire, the physical health component score. Secondary outcomes include SF-36, the mental health component score, Atrial Fibrillation Effect on QualiTy of life (AFEQT) questionnaire, days alive outside hospital, and serious adverse events. The primary assessment time point is one year after randomization.

Secondly, in a systemic review with meta-analysis and Trial Sequential Analysis (TSA), we investigated the evidence for the drugs used for controlling the heart rate in atrial fibrillation. The focus was on the European guideline's first and second line drugs (calcium channel blockers, beta-blockers, and digoxin).

Thirdly, we investigated the phenomenon of women having a higher risk of stroke than men with atrial fibrillation using the patient cohort from the Losartan Intervention for Endpoint Reduction (LIFE) study. This study was a retrospective cohort study of a randomized clinical trial.

Fourthly, we wanted to evaluate one of the hypothesis for the physical activity paradox. In epidemiological studies, OPA does not seem to confer the same health benefits as LTPA. One of the hypotheses to explain this paradox is a differential response with regard to systemic inflammation which has been linked to atrial fibrillation development, progression, and complications. Using the Copenhagen Aging and Midlife Biobank (CAMB) cohort, we investigated whether the level of high sensitivity C reactive protein (hsCRP) was dependent on the context (which may in turn represent different types or intensity of physical activity, insufficient rest periods) of physical activity.

Results

Study 1 – Protocol for a randomized clinical trial, DanAF

At the time of writing, 75 out of 350 participants have been randomized, 20 participants have reached one-year follow-up. Three sites have recruited participants, with at least one more expected to start recruitment in May 2023.

Study 2 – Systematic review of rate controlling drugs

We included 51 trials. There was very limited data on all-cause mortality and serious adverse events for all comparisons. Likewise, there was very limited data for quality of life, non-serious adverse events and symptom scores for all comparisons.

Beta-blockers and calcium channel blockers appeared superior to digoxin in reducing maximal exertional heart rate but there was no difference in exercise capacity. There seemed to be no overall difference between beta-blockers and calcium channel blockers for resting heart rate or maximal exertional heart rate

control, but subgroup analysis suggest some beta-blockers may reduce maximal exertional heart rate more than calcium channel blockers and some less.

Beta-blockers may reduce exercise capacity compared with calcium channel blockers.

Study 3 – Retrospective cohort study on sex, atrial fibrillation and risk of stroke

Both the prevalence of a history with atrial fibrillation upon inclusion and the incidence of new-onset atrial fibrillation during the study was higher among men than women. The difference decreased with older age.

In patients with new-onset atrial fibrillation, the overall point estimate for the risk of stroke associated with female sex was higher but not statistically significant (Hazard ratios (HR) 1.52, CI 95% 0.95 – 2.43). In contrast, the point estimate for the risk of stroke associated with female sex in patients with a history of atrial fibrillation was insignificantly lower (HR 0.88, CI 95% 0.5 – 1.6).

In new-onset atrial fibrillation, the risk of stroke increased with older age in females whereas it fell in males. In patients with a history of atrial fibrillation, the risk of stroke increased with age for both females and males.

Study 4 – Cross-sectional study on the physical activity paradox

A total of 5304 participants were included in the analysis. Compared to low OPA, high OPA was associated with increased levels of hsCRP (6% increase, CI 95% 0% - 12%). In contrast, compared to high LTPA, low LTPA was also associated with a higher hsCRP (12% increase, CI 95% 6% - 18%).

Conclusion and perspectives

Study 1, the DanAF trial is ongoing comparing lenient rate control to strict rate control on quality of life measured using SF-36 physical component score. So far, 75 patients from three sites have been recruited.

In study 2, we found that there is very limited data on the best rate controlling drug to prevent all-cause mortality, serious adverse events, or improve quality of life. Beta-blockers and calcium channel blockers appeared superior to digoxin in reducing maximal exertional heart rate. It is uncertain if this translates to higher or lower exercise capacity. There seems to be no overall difference between beta-blockers and calcium channel blockers for heart rate control, but subgroup analysis suggest some beta-blockers may reduce maximal exertional heart rate more than calcium channel blockers and some beta-blockers less. Beta-blockers may reduce exercise capacity compared with calcium channel blockers.

In study 3 with participants with hypertension and left ventricular hypertrophy on ECG, only participants with new-onset atrial fibrillation had higher risk of stroke in women than in men, primarily in older women. The same relationship was not seen in patients with a history of atrial fibrillation.

Study 4 showed that hsCRP seems to depend on the context of the physical activity, and hence, a difference in systemic inflammation could be the mechanism behind the physical activity paradox.

Dansk resume

Introduktion

Atrieflimren er den mest almindelige rytmeforstyrrelse i verden. Hjertesvigt, stroke og død er komplikationer til atrieflimren. Symptomerne på atrieflimren er bl.a. åndenød, hjertebanken, træthed og brystsmerter. Atrieflimren deles op i paroxystisk og ikke-paroxystisk. Alt efter om man forsøger at opnå normal hjerterytme (sinusrytme), kan ikke-paroxystisk atrieflimren deles op i persisterende eller permanent atrieflimren.
I de seneste anbefalinger fra det europæiske selskab for kardiologi følger behandlingen en A, B, C metode. A'et står for undgå Apopleksi/Antikoagulation, B'et står for Bedre symptombehandling og C'et står for Komorbiditet (engelsk: comorbidity) og Kardiovaskulære (engelsk: cardiovascular) risikofaktorer.

Antikoagulation kan opnås med enten non-vitamin K antagonister eller med vitamin K antagonister. Typisk baseres opstart af antikoagulationsbehandling på risikoen for blodpropper vurderet ud fra et risikovurderingsredskab så som CHA_2DS_2VASc. Kvinder, som udvikler atrieflimren, ser ud til at have højere risiko for stroke end mænd. Dog ses køn aktuelt som en effektmodifikator. Mekanismen bag denne forskel er ukendt og kan skyldes både tilbageværende confounding eller en reel biologisk kønsforskel.

Frekvenskontrol er den primære strategi til at kontrollere symptomer hos patienter med permanent atrieflimren. Til at sænke frekvensen bruges beta-receptor-blokker, calcium-kanal antagonister, eller digoxin. Det er aktuelt uklart, hvad frekvensen bør være ved atrieflimren, og hvilke af ovenstående præparater man bør bruge til at opnå dette.

For at forebygge udviklingen af atrieflimren og forebygge kardiovaskulære komplikationer hos patienter med atrieflimren, anbefales fysisk aktivitet. Det ser dog ud til at de gavnlige effekter af fysisk aktivitet afhænger af om det er fysisk aktivitet i forbindelse med arbejde eller fysisk aktivitet i forbindelse med fritid. Dette kaldes det fysiske aktivitetsparadoks.

Formål

Formålet med denne afhandling er at søge efter evidens for at forbedre symptomerne og forebygge komplikationer hos mennesker med atrieflimren ved at kigge på forskellige aspekter af den nuværende A, B, C tilgang i de europæiske guidelines, hvor der er ubesvarede spørgsmål.

Metode

Der er fire delstudier.

1) Vi designede, planlagde, startede og er i gang med at gennemføre forsøget DanAF. DanAF er et klinisk, randomiseret forsøg, hvor 350 mennesker med persisterende eller permanent atrieflimren inden inklusion (når de inkluderes har de pr. definition permanent atrieflimren) randomiseres til løs (80-110 hjerteslag per minut) eller stram frekvenskontrol (<80 hjerteslag per minut), bedømt på elektrokardiogram (EKG) efter fem minutters hvile. Det primære endepunkt er fysisk livskvalitet målt ved spørgeskemaet Short Form 36 (SF-36). De sekundære endepunkter er mental livskvalitet målt ved spørgeskemaet SF-36, livskvalitet ved et sygdomsspecifikt spørgeskema "Atrial Fibrillation Effect on QualiTy of life (AFEQT)", hospitalsfri dage og alvorlige skadelige hændelser. Endepunkterne vurderes primært 1 år efter randomisering.
2) I en systematisk litteratur-gennemgang med meta-analyse og Trial Sequential Analysis (TSA) undersøgte vi effekten af de forskellige præparater, man bruger til at regulere hjertefrekvensen hos patienter med atrieflimren. Fokus var på de præparater man anbefaler som første- og andenvalgs præparater (beta-receptor-blokkere, calcium-kanal antagonister og digoxin).
3) Vi undersøgte baggrunden for den forhøjede risiko for stroke hos kvinder i forhold til mænd hos mennesker med atrieflimren. Vi brugte data fra Losartan Intervention for Endpoint Reduction (LIFE) studiet. Vores studie var et retrospektivt kohortestudie.
4) Vi undersøgte det fysiske aktivitetsparadoks. I epidemiologiske studier ser det ud til, at fysisk aktivitet i fritiden reducerer hjertekar død. Det virker dog til, at fysisk aktivitet i forbindelse med arbejde enten skader eller ikke giver den samme reduktion i hjertedød. Dette kaldes det fysiske aktivitetsparadoks. Én af hypoteserne bag paradokset er at paradokset er drevet af en forskel i systemisk inflammation alt efter om den fysiske aktivitet er arbejdsrelateret eller foregår i fritiden formodentligt fordi typen af fysisk aktivitet er forskellig. Systemisk inflammation er også vist at have en sammenhæng med atrieflimren udvikling, progression og komplikationer. Ved hjælp af data fra Copenhagen Aging and Midlife Biobank (CAMB) kohorten undersøgte vi associationen mellem konteksten af fysisk aktivitet (arbejde eller fritid) og høj sensitiv C reaktiv protein (hsCRP).

Resultater

Studie 1 – Protokol for det randomiserede forsøg, DanAF

Da denne afhandling blev skrevet, var der rekrutteret 75 ud af de ønskede 350 deltagere. 20 deltagere har fået foretaget 1 års besøget. Deltagerne var inkluderet på tre afdelinger. Yderligere en afdeling forventes at starte inklusion i maj 2023.

Studie 2 – Systematisk litteraturgennemgang af frekvenskontrollerende lægemidler

Vi inkluderede 51 forsøg. Det var meget sparsomt med data på død af alle årsager og alvorlige skadelige hændelser for alle sammenligninger. Ligeledes var det meget sparsomt med data på livskvalitet, ikke-alvorlige skadelige hændelser og symptom scores for alle sammenligninger.

Beta-receptor-blokkere og calcium-kanal antagonister virkede til at være bedre end digoxin til at sænke hjertefrekvensen ved maksimalt fysisk anstrengelse uden at der var en forskel i fysisk kapacitet. Der virkede ikke til at være en forskel mellem beta-receptor-blokkere og calcium-kanal antagonister på hvilefrekvensen eller hjertefrekvensen ved maksimal fysisk anstrengelse, men subgruppeanalyser tydede på, at nogle beta-receptor-blokkere reducerede hjertefrekvensen ved maksimal fysisk anstrengelse mere end calcium-kanal antagonister, og nogle reducerede mindre. Beta-receptor-blokkere reducerer muligvis fysisk kapacitet sammenlignet med calcium-kanal antagonister.

Studie 3 – Retrospektivt kohorte studie om køn, atrieflimren og risikoen for stroke

Både prævalensen af deltagere kendt med atrieflimren og incidensen af nyopstået atrieflimren var højere blandt mænd end kvinder. Forskellen faldt med alderen. Punktestimatet for risikoen for stroke var højere for kvinder med nyopstået atrieflimren end for mænd, men resultatet var ikke statistisk signifikant (HR 1.52, CI 95% 0.95 – 2.43). Modsat var punktestimatet for risikoen for stroke insignifikant lavere for kvinder hos deltagere med prævalent atrieflimren (HR 0.88, CI 95% 0.5 – 1.6). Hos deltagere med nyopstået atrieflimren steg risikoen for stroke med stigende alder hos kvinder, mens den faldt hos mænd. Hos deltagere med prævalent atrieflimren steg risikoen for stroke hos både mænd og kvinder.

Studie 4 – Tværsnitsstudie omhandlende det fysiske aktivitetsparadoks.

I alt blev 5304 deltagere inkluderet i analyserne. Høj fysisk aktivitet på arbejde var sammenlignet med lav fysisk aktivitet på arbejde associeret med højere hsCRP (6% forøgelse, CI 95% 0% - 12%). Modsat var lav fysisk aktivitet i fritiden associeret med højere hsCRP sammenlignet med høj fysisk aktivitet i fritiden (12% forøgelse, CI 95% 6% - 18%).

Konklusioner og perspektiver

Studie 1, DanAF forsøget, er i gang og sammenligner løs (80-110 hjerteslag per minut) med stram frekvenskontrol (<80 hjerteslag per minut) på livskvalitet målt ved SF-36 fysisk komponent score. 75 patienter er inkluderet fra 3 sites indtil videre.

I studie 2 fandt vi begrænset med data på valg af frekvenskontrollerende lægemiddel til at forebygge død, alvorlige skadelige hændelser eller forbedre livskvalitet. Vi fandt, at beta-receptor-blokkere og calcium-kanal antagonister er bedre end digoxin til at reducere hjertefrekvensen ved maksimalt fysisk arbejde. Det

er usikkert om dette giver bedre eller dårligere fysisk kapacitet. Der lader ikke til at være overordnet forskel mellem beta-receptor-blokkere og calcium-kanal antagonister i forhold til at kontrollere hjertefrekvensen, men subgruppe analyser indikerer, at nogle beta-receptor-blokkere måske er bedre end calcium-kanal antagonister og nogle dårligere.

I studie 3 hvor vi inkluderede deltagere med forhøjet blodtryk og forstørret venstre hjertekammer på EKG, fandt vi at kun hos deltagere med nyopstået atrieflimren havde kvinder højere risiko end mænd for stroke. Denne højere risiko var specielt tydelig hos ældre kvinder. Den samme sammenhæng så vi ikke hos patienter med prævalent atrieflimren.

I studie 4 fandt vi at hsCRP så ud til at være afhængig af konteksten for fysisk aktivitet og dermed, kan inflammation være den bagvedliggende mekanisme bag fysisk aktivitetsparadokset.

Abbreviations

AF: Atrial fibrillation

AFBHN: Variable name for the 3-level categorical variable with the categories being no atrial fibrillation, a history of atrial fibrillation and new-onset atrial fibrillation.

AFEQT: Atrial Fibrillation Effect on QualiTy of life

BMI: Body Mass Index

CAMB: Copenhagen Aging and Midlife Biobank

CI: confidence interval

COPD: Chronic Obstructive Pulmonary Disease

DanAF: Danish Atrial Fibrillation

ECG: electrocardiogram

ESC: European Society of Cardiology

HR: Hazard ratio.

HsCRP: high sensitivity C reactive protein

GRADE: Grading of Recommendations, Assessment, Development and Evaluation

LIFE: Losartan Intervention for Endpoint Reduction

LTPA: leisure time physical activity

LVEF: Left ventricular ejection fraction

MI: Myocardial Infarction

OPA: occupational physical activity

RACE II: Rate Control Efficacy in Permanent Atrial Fibrillation: a Comparison between Lenient versus Strict Rate Control II

SF-36: Short Form – 36

TIA: Transient Ischemic Attack

TSA: Trial Sequential Analysis

Introduction

Epidemiology

Atrial fibrillation is the most common arrhythmia of the heart.[1] Currently, the prevalence in around 2-4% but with large differences across different regions.[1,2] In general women have lower incidence than men, but have a higher risk of stroke.[1] The number of people with atrial fibrillation is projected to rise to around the double in Europe and the United States primarily due to an aging population and the fact that the risk of atrial fibrillation increases with age.[1,2]

Classification

Atrial fibrillation may be classified into the following categories according to the European Society of Cardiology (ESC) 2020 guidelines[1]:

1. New-onset atrial fibrillation.[1] This is the first time the patient is diagnosed with atrial fibrillation.[1]
2. Paroxysmal atrial fibrillation.[1] If atrial fibrillation is terminated within 7 days either spontaneously or due to an intervention e.g. direct current conversion.[1]
3. Persistent atrial fibrillation. Atrial fibrillation which persists beyond 7 days. Rhythm control is by definition still pursued.[1]
4. Long-standing persistent atrial fibrillation: Atrial fibrillation which persist beyond 1 year. Rhythm control is by definition still pursued.[1]
5. Permanent atrial fibrillation.[1] By definition, rhythm control is no longer pursued and symptoms are instead controlled with rate control.[1] If for any reason rhythm control is again pursued, the atrial fibrillation will be reclassified to long standing persistent atrial fibrillation.[1] This could e.g. be the case if the patient develops severe heart failure.[1]

Clinical presentations

Symptoms of atrial fibrillation include heart palpations, shortness of breath, dizziness, fatigue, chest pain, and edema of the lower extremities.[1] Patients may also present as initially asymptomatic.[1] Atrial fibrillation is associated with decreased quality of life.[1]

Risk factors for developing atrial fibrillation

The most important co-morbidities that may contribute to the development of atrial fibrillation are hypertension, heart failure, valvular heart disease, diabetes, and ischemic heart disease.[1,3] Hence treatment of these conditions may help prevent atrial fibrillation. Lifestyle related risk factors that may be altered with lifestyle changes includes smoking, alcohol consumption, level of physical activity, and obesity.[1] Another very important risk factor is age.[1,4]

Pathophysiology

Atrial fibrillation is thought to be the end result of various risk factors leading to electrical and structural remodeling in the atria.[1,5] Changes include atrial fibrosis, altered channel functions, conduction disturbances and alterations, altered contractility, changes in the autonomic nervous system, and atrial dilation.[1,5,6] These changes as well as the development of atrial fibrillation itself may aggravate the changes, further promoting atrial fibrillation.[1] For atrial fibrillation to develop, one usually requires a trigger and a mechanism to sustain AF which are described below.[7]

The electropathophysiology of atrial fibrillation

Atrial fibrillation is the result of ectopic firing of significant frequency or/and the result of reentry.[8] Ectopic firing is thought to be the result of difference in calcium handling (including leakage from the sarcoplasmic reticulum) and changes to other currents affecting the differences phases of the action potential.[7,9] Atrial fibrillation in itself can promote electrical remodeling.[5,10] The primary foci of the initial ectopic firing are usually the pulmonary veins due to the cardiac myocytes in this area having different properties such as higher resting potential, shorter duration action potentials (as a result of size differences in potassium channels) and difference in intracellular calcium handling.[10] As a result, early afterdepolarization (triggering of an action potential before resting potential has been achieved) and delayed afterdepolarization (early depolarization after resting potential has been achieved and a result of diastolic calcium leakage) is triggered resulting in abnormal focal firing.[5,10,11]

Structural remodeling includes enlargement of the atria, fibrosis and myocyte hypertrophy.[7] Fibrosis may promote atrial fibrillation by blocking the normal propagation of the action potential across the atria.[7] This means that localized reentry circuits may develop.[7] Fibrosis may be both extracellular fibrosis or fibrosis as the result of cellular death.[7] Structural remodeling also includes atrial dilation, which both promote atrial fibrillation reentry by either increasing the time of an electrical conduction circuit or enabling multiple parallel conduction circuits.[5] There are several explanations for the structural and remodeling thought to ultimately result in atrial fibrillation. Inflammation, hemodynamic changes, genetic disposition, among other things may play a role.[10,12,13] The fact that both the risk of ectopic firing and reentry at the pulmonary veins is large has led this area to be a primary target of catheter ablation (rhythm control described further below).[7]

Quality of life for patients with atrial fibrillation

Many patients (50-60%) with atrial fibrillation have reduced quality of life.[1,14,15] Quality of life is especially lower in women with atrial fibrillation.[1,16,17] There may be many reasons for the reduced quality of life including symptoms themselves, co-morbidity, demographic factors, and the psychological response to the

disease.[1,16,17] Specific patient characteristics associated with lower quality of life in patients with atrial fibrillation are younger age, higher heart rate, and obstructive sleep apnea.[18] New-onset atrial fibrillation is also associated with worse quality of life compared to the other types of atrial fibrillation.[18] Atrial fibrillation is also associated with depression and anxiety, which may also lead to lower quality of life.[1]

There is no consensus on how to measure quality of life in atrial fibrillation.[19] Quality of life may be assessed both with a generic questionnaire such as SF-36 or a disease-specific questionnaire such as Atrial Fibrillation Effect on QualiTy of life (AFEQT).[19] There are advantages and disadvantages to using the different types of questionnaire.[19] Generic questionnaires are typically more validated and used and have a more holistic interpretation, whereas disease-specific may be better at capturing specific responses e.g. to treatment.[19]

The link between the pathophysiology related to atrial fibrillation and symptoms is generally not very precisely modelled.[1,20] There is a poor correlation between ECG changes and subjective well-being.[1] Different mechanisms are thought to be behind different symptoms.[4] Palpations are thought to be directly related to the heart rate, although the direct association is inconsistent and other factors such as psychosocial factors appear to play a role.[20] The reduced exercise capacity is thought to be related to reduced cardiac output described below.[20]

Electro-cardiomechanical effects of atrial fibrillation

The normal cycle of the left ventricular has a systolic phase and a diastolic phase.[21] The diastolic phase can be divided into four distinct phases: 1) relaxation of left ventricle without a change in the volume of the left ventricle (isovolumetric relaxation), 2) early diastolic filling of the ventricular from the passive flow of blood after the mitral valve opens due to pressure differences between the left atria and ventricle, 3) diastasis, where the pressures in the left atria and ventricular are similar and blood flow ceases, 4) atrial systole where atrial contraction results in increased pressure in the atrium and resulting flow from the atrium to the ventricle.[21] Atrial fibrillation results in the loss of the 'atrial kick' which is the coordinated contraction of the atria in the last part of the ventricular diastolic phase.[22,23] This may particular important in specific conditions such as mitral stenosis, and heart failure with preserved ejection fraction due to the passive flow of blood being reduced.[23,24] Other changes associated with atrial fibrillation are increased mean diastolic pressure in the atria, shorter intervals for passive filling of the ventricles, and an irregular ventricular beat.[22] Ultimately, these changes may lead to reduced cardiac output and induce remodeling, including remodeling of the ventricles.[22]

Atrial fibrillation and heart failure

One of the most commonly associated diseases with atrial fibrillation is heart failure. Development of atrial fibrillation in patients with heart failure is associated with a worse prognosis, and vice-versa.[25] The incidence of heart failure with preserved ejection fraction among patients with atrial fibrillation is between 8% - 24%.[26] In heart failure with preserved ejection fraction, the prevalence of atrial fibrillation is between 15% - 41%.[26] Heart failure may increase the risk of atrial fibrillation by increasing the pressure in the atrium leading to fibrosis and enlargement, structural changes that may make it possible for local reentry pathways to develop.[7,27] The same changes may also induce electrical remodeling including shortening of the refractory period and promoting ectopic firing.[28] Heart failure may also promote atrial fibrillation by inducing neurohormonal change e.g. through the renin-angiotension-aldosterone system that further promotes atrial fibrillation.[28]

Atrial fibrillation may also promote heart failure (and directly reduce cardiac output as described above). Atrial fibrillation induces left ventricular fibrosis, which can lead to heart failure with preserved ejection fraction.[26] A specific case of atrial fibrillation promoting heart failure is tachycardia-induced cardiomyopathy.[28] The tachycardia may induce heart failure through different mechanisms including myocardial energy depletion, matrix remodeling, ischemia and change to calcium handling.[28] In the case of tachycardia-induced cardiomyopathy, rapid improvement is seen upon heart rate or rhythm control in the first weeks and further gradual improvement is seen in the following month.[28]

Inflammation and atrial fibrillation including inflammatory markers

Inflammation is thought to be one of the key mechanism behind the development of atrial fibrillation.[1] As described further above, fibrosis may play a role in establishing the basis for atrial fibrillation. In turn, fibrosis may be the result of inflammation or be stretch induced.[1] There are several possible sources of inflammation, which are associated with the previously described risk factors (see 'Risk factors for developing atrial fibrillation' above). The source of inflammation may be systemic or local with significant overlap possible.[29] One source is from systemic diseases such as hypertension or obesity.[29] Obesity acts as a systemic source of inflammation by stimulating the production of pro-inflammatory markers.[29,30] Increased release of pro-inflammatory markers in the pericardial fat has also been associated with atrial fibrillation.[29,31] Hypertension may act in several ways. Hypertension may influence the susceptibility to atrial fibrillation through the Renin-Angiotension-Aldosterone system, which has been shown to be able to both increase pro-inflammatory cytokines but also by activating immunecells.[29,32] It may also induce direct inflammation into the atria through atrial strech.[29] Once atrial fibrillation has been established, atrial fibrillation may in itself further promote inflammation, creating a vicious circle.[29]

The classical inflammatory markers are tumor necrosis factor alpha (TNF-α) and CRP.[33,34] The role of IL-6 is controversial, with some arguing that it may be an upstream target promoting inflammation whilst others arguing it is anti-inflammatory and the epidemiological associated with disease is only an association not a causality.[33,34] It may also be that the function of Il-6 depends on the specific circumstances IL-6.[30,31] TNF-α may promote atrial fibrillation by stimulating a signaling pathway through TGF-β.[35] Genetic differences in expression of TNF or TGF-β1 in cardiac tissue have also been linked to increased susceptibility to atrial fibrillation as well as higher levels are seen in patients with atrial fibrillation.[29]

Especially CRP has been linked to atrial fibrillation in epidemiological studies. Increased levels of CRP are associated with incidence of atrial fibrillation.[29,36] Increased levels of CRP have also been found in patients who are currently in atrial fibrillation, compared with patients who are currently no in sinus rhythm.[29] Higher CRP levels are also seen in patients with recurrent atrial fibrillation after a procedure to restore sinus rhythm and higher CRP levels are associated with development of atrial fibrillation after cardiac surgery.[29]

Studies in animal models also point to a link between inflammation and atrial fibrillation, where several studies have found lower atrial fibrillation duration with the use of prednisolone in induced atrial fibrillation.[29]

Taken together, several pieces of evidence points to a role of inflammation in atrial fibrillation.

Possible link between exercise, inflammation, and atrial fibrillation including inflammatory markers

As described above, systemic, low grade inflammation and the development and progression of atrial fibrillation appear linked. Exercise may be one possible way to counteract this low grade inflammation.[30] After strenuous exercise there is an acute phase response including an increase in CRP and IL-6.[30] However, following this initial acute phase response which leads to higher levels of systemic inflammatory markers, this phase is followed by an anti-inflammatory response which lowers the amount of systemic inflammation.[30] IL-6 has suggested to play a major part in inducing the anti-inflammatory response following exercise even though it has classically been thought of as a pro-inflammatory marker.[37] The absence of TNF alpha and IL-β may play a role in the dual action of IL-6 as well as the source of IL-6 may play a role.[37]

Exercise may, however, influence many different aspects of possible pathways to atrial fibrillation including modification of risk factors, positive cardiac remodeling, and influence on the autonomic nervous system.[38] Exercise for one may reduce inflammation from fatty tissue especially visceral fat as high levels of

abdominal fat has been link to various chronic diseases in epidemiological studies.[37] Physical inactivity has been associated with an increase in abdominal fat without increasing the total fat mass.[39]

Epidemiological literature has focused on exercise and prevention on atrial fibrillation. The relationship has been described both as U-shaped, J-shaped, and no relationship at all.[40-42] Likewise, the type of exercise and the intensity have also been studied.[41] Possible sex differences have also been suggested.[43,44]

The context (and underlying nature) of physical activity may also influence atrial fibrillation development. A cohort study using data from the Copenhagen City Heart Study found that high and very high OPA increased the risk of developing atrial fibrillation, whereas LTPA did not.[42] Although plausible mechanisms for reduction of inflammation directly and indirectly from exercise exist, clinical effects have not yet been documented in randomized trials.[45] In observational studies, physical activity in patients with atrial fibrillation has shown to reduce atrial fibrillation recurrence and all-cause mortality.[38]

Complications of atrial fibrillation

There are several complications associated with atrial fibrillation.[1,46] Three of the most feared complications are death, heart failure, and stroke.[1] The risk of mortality is increased around 1.5 – 3.5 mortality in patients with atrial fibrillation compared to people without atrial fibrillation.[1] The risk of stroke is higher in women than in men.[1] However, female sex is not considered an independent risk factor, as much as an effect modifier, and hence, female sex alone is not enough to warrant anticoagulation theapy.[1] This risk seems to vary between regions and between studies.[47] Unlike the risk of stroke associated with atrial fibrillation, most studies have not found any sex difference for the risk of heart failure.[47] Other co-morbidities associated with atrial fibrillation are depression, sleep apnea, and dementia (related to stroke).[1,47]

Possible mechanisms behind sex differences between men and women with atrial fibrillation

Several phenomena have been observed that may explain some of the sex differences seen in atrial fibrillation.[47,48] It may be that structural differences in the atria between women and men contribute to the difference in risk of stroke.[48] It may also be related to increased levels of endothelial dysfunction in women with atrial fibrillation.[49] In the LIFE study, despite having left ventricular hypertrophy, the ejection fraction was still higher in women.[50]

Sex hormones may also play a role.[47] Several studies have found an association between episodes of supraventricular tachycardia and phases of the menstrual cycle where an inverse relationship between estrogen and duration of supraventricular episode.[47] However, in a sub publication of the randomized trial 'The Women's Health Initiative ' assessing the risk of cardiovascular complications when using hormone

replace therapy, the risk of atrial fibrillation was higher in the group receiving hormone replacement therapy.[51]

Genetics have also been proposed to play a role.[47] The risk of developing atrial fibrillation associated with having a parent with atrial fibrillation is increased with 2%.[47] However, currently, the research into specific genes have not lead to the identification of genes that may explain sex differences.[47]

It may be that some of these proposed sex differences affecting the risk of developing atrial fibrillation may also be the reason for the increased risk of stroke seen in women compared with men.[47]

Another possibility is that a difference in risk factors may explain the higher risk of stroke seen in women compared with men.[47] Ultimately, the increased risk of stroke may be the result of confounding.[47] Women develop atrial fibrillation later than men and may therefore have accumulated more risk factors.[47,52]

Guideline recommended treatment of atrial fibrillation

'A' – Anticoagulation/avoid stroke

The cornerstone of avoiding stroke in diagnosed atrial fibrillation is proper anticoagulation.[1] The first step is to assess the risk of stroke.[1] This is often done by using a tool for assessing risk, such as the CHA_2DS_2VASc.[1] Patients who have low risk based on a tool such as CHA_2DS_2VASc do not need anticoagulation therapy.[1] In patients who are not low risk, anticoagulation therapy should be considered (1 point on the CHA_2DS_2VASc for men and 2 points for women) or recommended (≥ 2 for men or ≥3 for women).[1] Sex is considered an effect modifier, not a risk factor in itself and hence, (as described above) sex alone is not enough to initiate anticoagulation therapy without other risk factors.[1]

Assessment of the risk of stroke must be accompanied by an assessment of risk factors for bleeding, which can be done using a risk tool such as the HAS-BLED.[1] Modifiable risk factors should be attempted addressed and resolved but should not in itself lead to avoiding anticoagulation therapy.[1]

Anticoagulation therapy for atrial fibrillation usually consists of a non-vitamin K antagonist administered orally.[1] The drugs approved are apixaban, dabigatran, edoxaban, and rivaroxaban.[1] Previously, anticoagulation was achieved with a vitamin K antagonist, which required periodic measurement of International normalized ratio (INR).[1] Specific conditions warrant vitamin K antagonist such as patients with a mechanical heart valve.[1]

'B' – Better Symptom control

Rhythm vs rate control of atrial fibrillation

According to the ESC 2020 guidelines, the primary indication for rhythm control is to improve atrial fibrillation symptoms and quality of life.[1] Since atrial fibrillation symptoms often are unspecific, and

patients possibly unconsciously may be unaware of reduced quality of life, the guideline recommends an attempt at cardioversion in case of no symptoms or if it is unclear if symptoms are related to atrial fibrillation.[1] Factors that favor rhythm control in general are patient preference, young age, tachycardia-induced heart failure, a high burden of symptoms, or failure of rate control to control symptoms.[1] However, the results of the EAST trial have not yet been incorporated into the guidelines and is expected to widen the indication for rhythm control.[53] From a physiological standpoint, as described further above, there are several possible detrimental effects including decreased cardiac output from loss of 'atrial kick', irregularity of the rhythm, possible adverse remodeling of the myocardium possibly leading to heart failure, and increased pressure in the atrium strengthening the argument for rhythm control.[7,27] However, a holistic view of the patient, including possible beneficial effects of remodeling in the medium term and harms of medication to achieve rhythm control must also be considered.

Currently, either as first line therapy, as a complement to rhythm control or if rhythm control fails, rate control can be initiated.[1]

Rhythm control

Long-term rhythm control comes in different forms: medical rhythm control and invasive rhythm control (catheter ablation and surgical ablation).[1] Electrical cardioversion may supplement both types of rhythm control in case of relapse providing immediate cardioversion.[1] The choice between medical and invasive rhythm control depends on the type of atrial fibrillation and the risk of atrial fibrillation relapse.[1] In case of paroxysmal or persistent atrial fibrillation and heart failure with reduced ejection fraction, catheter ablation is recommended over medical therapy.[1] In case of persistent atrial fibrillation with major risk factors for relapse, only in rare cases should ablation be recommended over medical therapy, whereas persistent atrial fibrillation without major risk factors for relapse ablation may be considered.[1] In case of paroxysmal atrial fibrillation, there is given a IIa recommendation for catheter ablation which means ablation should be considered. The choice should always be discussed with the patient considering patient preferences and risks associated with the procedure.[1]

Invasive rhythm control

Catheter ablation is the usual first line ablation technique.[1] Pulmonary vein isolation is core to catheter ablation.[1] In many cases, however, this is insufficient and multiple other targets could be considered.[1] Catheter ablation can be achieved by radiofrequency catheter ablation or cryoballoon catheter ablation.[1] Recurrence after catheter ablation may be followed up by surgical ablation.[1]

Medical rhythm control

Medical rhythm control may be part of a rhythm control strategy including catheter ablation or it may be isolated medical rhythm control.[1] The recommended drug is based on the presence of structural heart disease. If the patient has heart failure with reduced ejection fraction, amiodarone is recommended.[1] If the patient has coronary artery disease, heart failure with preserved ejection fraction, or clinically significant valvular disease then either amiodarone or dronedarone may be used.[1] If there are no signs of structural heart disease, dronedarone, flecainide or propafenone are recommended.[1]

Rate control target

The current optimal resting heart rate target in atrial fibrillation is unknown.[1] Previously it was recommended to target a resting heart rate below 80 bpm and a heart rate below 110 bpm during exercise.[54-56]. However, the Rate Control Efficacy in Permanent Atrial Fibrillation: a Comparison between Lenient versus Strict Rate Control II (RACE II) trial showed that there was non-inferiority on a composite outcome of either death from cardiovascular causes, hospitalization for heart failure, and stroke, systemic embolism, bleeding, and life-threatening arrhythmic events between lenient (<110 bpm) and strict rate control (<80 bpm).[54] There were several limitations to the RACE II trial. Specifically, for quality of life, only 71% of the data were available at maximum follow-up.[57] Hence, the RACE II trial has not led firm conclusions regarding the optimal heart rate target in atrial fibrillation.[1]

Rate control and the 'clinical approach'

In clinical practice, many aspects may be considered when choosing rate control therapy in patients with atrial fibrillation and cardiac comorbidity such as heart failure including type of heart failure (left versus right), type of left sided heart failure (heart failure with reduced ejection fraction versus heart failure without reduced ejection fraction), severity of heart failure, and valvular pathology. A short description of the theoretical impact of the different scenarios is given below.

Left ventricular failure

Patients with atrial fibrillation and reduced ejection fraction are recommended the highest tolerated dose of beta-blocker (up to 200 mg for metoprolol, 10 mg for bisoprolol/day).[58] This is despite that Kotecha et al. showed in an individual patient data meta-analysis including 18,254 patients that the prognostic benefit of beta-blockers seems to be absent in participants with atrial fibrillation and heart failure with reduced ejection fraction.[59] The rationale for still recommending beta-blockers was that the individual patient data meta-analysis was a post-hoc, retrospective analysis and beta-blockers have not shown to cause harm.[58] However, if lenient rate control target is superior in terms of quality of life, this recommendation may in fact reduce quality of life without any prognostic benefit.

Right ventricular failure

In right ventricular failure, beta blocker therapy is not recommended.[58] This is because the right ventricle has reduced contractile reserve, and hence, if output from the right ventricle is to be preserved, this can only be achieved by increasing the heart rate.[60,61] Instead, reducing afterload through fluid restriction and diuretics is the main focus.[60,61]

Valvular heart disease

When considering hemodynamic theory, different types of valvular disease may require a higher or lower heart rate, which makes randomization not possible. The stenosis or insufficiency must be of such a degree that it has hemodynamic consequences, so the stenosis/insufficiency should least be at least moderate grade to have consequences for participation in DanAF.

Rate controlling drugs

Treatment to control the heart rate is most commonly achieved using a beta-blocker, a calcium channel blocker, digoxin, or amiodarone.[1]

They exert much of their effect by modulating the normal phases of an action potential in the heart.[62] A brief and simplified description of the normal cycle of the action potential is as follows: Phase 0 is the rapid depolarization phase, driven by inward flow of sodium ions.[63] This is followed by rapid repolarization phase, phase 1 where potassium briefly flows out of the cardiac cell.[63] Phase 2 is the plateau phase, where potassium and calcium channels offset each other.[63] Phase 3 is another repolarization phase driven by multiple potassium channels.[63] Phase 4 is the resting phase.[63]
The most commonly used way to classify the different type of actions drugs used for both rate and rhythm control can have on the heart is the Vaughan Williams classification.[64,65] The Vaughan Williams classification groups drug actions into four different groups I through IV.[64,65] Different additions to the Vaughan Williams classification have been made.[62]

Type 1 drugs affect the 0 phase of the action potential in the heart through acting on the sodium channels.[64,65] This property is typical of rhythm controlling drugs such as quinidine and flecainide.[62]

Type 2 antiarrhythmic actions are defined by modulating the autonomic nerves system.[62] Beta-blockers, which are commonly prescribed for atrial fibrillation, work by blocking b_1-receptors in the SA node and AV node.[62] Downstream they are believed to act through a g-protein coupled receptor to influence calcium channel currents.[62] Ultimately, beta-blockers slow the heart rate.[62]

Type 3 antiarrhythmic actions are defined by modulating potassium channels.[62] Examples of this are amiodarone, dronedarone, sotalol, and ibutilide.[62]

Type 4 antiarrhythmic actions are defined by modulating calcium channels.[62] Verapamil and diltiazem function in this way.[62]

Type 0 drugs include ivabradine that modulate the pacemaker current (sinus node).[62]

It should be noted, however, that the idea of a clear-cut distinction of the mechanism of action may not always hold true. E.g. researchers suggest that ivabradine may function in other ways than only modulating the pacemaker current and a randomized trial has been identified to explore its use in atrial fibrillation.[66]

Beta blockers

For atrial fibrillation selective $beta_1$ blockers are the most commonly prescribed.[1,54,67] These are typically metoprolol or bisoprolol.[1,68] Other commonly used beta-blockers are the non-selective beta blockers such as carvedilol.[1] Clinically, you may first try one beta-blocker, and switch to a different one if the patients report side effects to the first one.[69] The most commonly reported side effects are headache, fatigue, and edema of the lower extremities.[69]

Calcium channel blockers

The other first line therapy according to ESC is a calcium channel blocker.[1] If the patients has heart failure with reduced ejection fraction calcium channel blockers should not be used.[1] There are two different calcium channel blockers used, verapamil and diltiazem.[1,70] The most commonly reported side effects include constipation, headache, peripheral edema, and hypotension.[71]

Digoxin

Digoxin is used as second line therapy for long-term management of atrial fibrillation and may also be used safely as acute rate control as it may be administered to patients with heart failure.[1] Digoxin is one of the oldest drugs used for the treatment of atrial fibrillation.[72] The use of digoxin has declined in recent years probably because observational studies showed digoxin was harmful as well as other drugs had shown great benefits such as beta-blockers for heart failure with reduced ejection fraction.[1,54,67] In clinical practice, there may also be hesitation to use digoxin in patients with left ventricular hypertrophy because of considerations towards digoxin stimulating further myocyte hypertrophy although I found no study documenting this.

Digoxin increases the contractility of the myocardium through its inhibition of the ATPase pump which ultimately leads to increased intracellular calcium, and decreases the heart rate through activation of the parasympathetic nervous system.[73]

One of the main reasons for digoxin to be a second line therapy is that it is generally accepted that digoxin provides inadequate rate control during activity. This evidence in general comes from small trials (<30 participants), and clinical experience.[74]

Amiodarone

Amiodarone is considered third line therapy for outpatient rate control.[1] It can also be used for acute rate control if the patient has severe left ventricular systolic function.[1] Before starting treatment with amiodarone an ECG must be taken checking for QT prolongation.[1] Outpatient treatment with amiodarone requires periodic checks of the lungs, thyroid and liver. Amiodarone may cause deposits in the eyes and optic neuropathy.[75]

'C' – Optimization of cardiovascular risk factors and comorbidities

The 'C' represents identification and optimization of treatment of comorbidities such as hypertension, obesity, diabetes, heart failure, and sleep apnea.[1] As described further above, risk factors may act through increased systemic and local inflammation to promote atrial fibrillation. Hypertension is an important risk factor both for the development of atrial fibrillation and associated complications.[1] It is associated with an 1.7 times increased risk of developing atrial fibrillation.[1] Most patients with atrial fibrillation should target an office blood pressure of <130/80 mmgHG.[1] Depending how the blood pressure is measured and the age, the target blood pressure varies.[76] Sleep apnea is also highly present among individuals with atrial fibrillation and is an important risk factor for cardiovascular complications.[1] It has also been associated with less success of rhythm control.[1] It may be treated with continuous positive airway pressure and successful treatment has been linked to improvement of rhythm control.[1] The 'C' also represents optimization of lifestyle including alcohol and physical activity.[1]

Physical activity is recommended across guidelines to improve both current physical function in patients with atrial fibrillation as well as prevent future cardiovascular complications.[1] It is also important in the prevention of atrial fibrillation. The current ESC guideline on atrial fibrillation recommends moderate exercise/physical activity.[1] It does not appear to distinguish between occupational physical activity (OPA) and leisure time physical activity (LTPA).[1] This is important, as the risk of cardiovascular complications seems to depend on whether physical activity occurs during occupation or leisure time.[77] This is called the physical activity paradox.[77] Six mechanisms have been proposed to explain the underlying cause of the physical activity paradox.[77] Differences in intensity, type or duration as well as difference in rest time may be possible reasons.[77] Another proposed mechanism is a difference in effect on systemic inflammation although there is some overlap.[77] Understanding what drives the paradox may improve recommendations regarding physical activity and ultimately preventing atrial fibrillation and reducing cardiovascular

complications in women and men with atrial fibrillation.[77] In clinical practice, patients who have high OPA often use this as an argument for not doing LTPA. Understanding the mechanism behind the physical activity paradox is important as most people will not change their habits without rationale and hence, being able to present a scientifically based model of the physical health paradox is key.

Objectives

The objective of this PhD thesis was to search for evidence to improve symptom management and prevent complications in atrial fibrillation by looking at different aspects of the A, B, C guideline recommended approach where there are unanswered questions related to treatment of atrial fibrillation.

Study 1 – Protocol for a randomized clinical trial, DanAF

The aim of this study was to assess whether lenient rate control was superior to strict rate control on quality of life in patients with persistent or permanent atrial fibrillation upon inclusion as this is currently not known. Clarifying if one of the rate control strategies is superior will improve the 'B, better symptoms' aspect of atrial fibrillation management. The trial does not attempt to clarify for whom rhythm and rate control is appropriate, as this decision is made before inclusion in the trial. One of the main subgroup analyses will be to compare the effect in women and in men.

Study 2 – Systematic review of rate controlling drugs

The aim was to compare the different available rate controlling drugs for atrial fibrillation and if feasible and possible, rank them according to their effect on all-cause mortality, serious adverse events, and quality of life. Our hypothesis was that not all drugs are equally efficient, and that clinicians should choose one drug over another. The results could potentially improve the 'B, better symptoms' aspect of atrial fibrillation management.[1] One of the main tests for subgroup differences planned was to compare women with men.

Study 3 – Retrospective cohort study on sex, atrial fibrillation and risk of stroke

The aim was to explore the increased risk of stroke seen in women with atrial fibrillation in a population with left ventricular hypertrophy and hypertension. Further, we wished to explore whether the apparent difference in risk was more likely a result of confounding or a 'true' sex related difference by dividing the participants into those with new-onset atrial fibrillation (less risk of residual confounding) and those with a history of atrial fibrillation (higher risk of residual confounding). The results would help guide future research within the 'A, avoid stroke' part of atrial fibrillation management, particularly for women.

Study 4 – Cross-sectional study on the physical activity paradox

The aim was to explore systemic inflammation as one of the possible mechanisms behind the physical activity paradox.[77] We hypothesized that hsCRP would be negatively associated with higher LTPA but positively associated with higher OPA.[77] The results would perhaps in the future improve our management of the 'C, optimization of risk factors' by ultimately improving our recommendations regarding physical activity.[1,77]

Method

Study 1 – Protocol for a randomized clinical trial, DanAF

Choice of study design

When attempting to answer the question of how to treat patients, the best way to answer the question is in most cases to conduct a randomized clinical trial.[78,79] In contrast to observational studies, randomized trials conducted with low risk of bias will suffer much less from confounding than observational studies.[79] In some cases, if the risk is either very high of an adverse event, or the intervention results in quick (minutes to seconds) of undisputable relief, or an intervention effect is extreme (risk ratio above 10) a randomized trial may not be necessary.[78] None of necessary factors are present for atrial fibrillation and rate control to alleviate symptoms and improve prognosis, at least in the outpatient setting. Symptoms from atrial fibrillation are not very specific for atrial fibrillation.[1] Palpitations, shortness of breath, dizziness, and fatigue are all symptoms that can be affected by many other conditions, psychosocial factors, time of the year, and ultimately the patient experienced burden of atrial fibrillation corresponds poorly to the ECG-assessed burden.[1] Hence, it will not be possible based on patient reported symptoms of the individual patient and clinical expertise and experience, to assess whether a treatment has had an effect or not.[79]

The design of the trial has been published and is the basis for the following description of the methods used in the DanAF trial which in turn was inspired by the RACE II trial.[54,80] The rationale behind conducting an additional trial to RACE II was that the RACE II trial was designed to be a non-inferiority trial on a composite cardiovascular outcome. However, an important part of rate control is symptom management. Given the physiological expectations that strict rate control would give fewer symptoms, the fact that RACE II had 29% missing data on quality of life, the results of RACE II have not led to consensus in the atrial fibrillation guidelines, to account for the risk of a type II error, and the importance of this question for long term management of permanent atrial fibrillation, we found it scientifically valid to conduct DanAF to test for superiority on quality of life.

Importantly, the DanAF trial does not seek to answer the question if rate or rhythm control is the treatment strategy of choice. Prior to inclusion in DanAF, the decision to pursue rate control has been made by the treating physician unrelated to inclusion in the trial.

Design

Randomized, parallel, two-arm trial. At the design stage, we also considered designing the trial as a factorial design, as the optimal choice of rate controlling drug is also unclear and we wanted to compare beta-blockers versus calcium antagonist/digoxin (depending on heart failure status). However, as the intervention is already a complex intervention, this was abandoned. Participants are randomized 1:1 to

include the lowest amount of participants to be able to assess the difference specified in the sample size calculation. We did not foresee participants not willing to join the trial based on the risk of being randomized to a certain group, which is sometimes the idea behind a 2:1 randomization in placebo-controlled trials. Participants were randomized to either lenient or strict rate control.

Randomization

Randomization is stratified for site, type of atrial fibrillation, and whether LVEF isreduced or not (<40%). Stratification is used to ensure a balancing of important variables across the two groups. Although perhaps not necessary with a sufficiently large sample size, an uneven distribution of key stratification variables by chance might impede on the overall validity of the trial results. In practice, stratification means separate randomization lists are generated based on all the combinations of stratifying variables. Specifically, for this trial we initially wished to stratify for age, as this is an important factor for quality of life and hard outcomes.[18] However, after peer review we instead use type of atrial fibrillation prior to inclusion in DanAF. This accounts both for the nature of atrial fibrillation, underlying treatment strategy as well as somewhat for age, as participants with permanent atrial fibrillation tend to be older than participants with persistent atrial fibrillation. Site is used to make sure randomization is balanced across sites as site specific differences may play an important role. Choosing site to be a stratification factor allowed for both balanced randomization across sites as well as ensuring any treatment differences are balanced. Lastly, LVEF is an important prognostic factor in terms of quality of life as well as hard outcomes.[81] Further, LVEF status is an important determinant of the drugs used to control the heart rate.[1]

The randomization list was produced using an online web program, Sealed Envelope (https://www.sealedenvelope.com/). The list is a randomly generated sequence specifying if a participant should be allocated to lenient or strict rate control. The sequence used block randomization, with varying block sizes of 6, 8, and 10. A block means that within the next 6, 8 or 10 participants, half will be allocated to lenient rate control and half will be allocated to strict rate control. Block randomization ensures an even distribution of treatment allocation, and the varying block size of a sufficient size ensures that the randomization sequence remain hidden.

A statistician from OPEN uploaded the sequence to RedCap. Randomization is done automatically after entering the stratification variables in RedCap. The randomization list is kept secret from the randomizing investigators as is required to achieve low risk of bias. If the list was not kept secret investigators could in theory allocate participants based on participant factors by being able to foresee what the next participant will be randomized into and this may ultimately bias the outcome results of the trial.

Blinding

All the possible parties that could be blinded were attempted to be blinded in order to reduce introducing systematic bias in to the trial results. Participants were not informed of their heart rates during the trial nor were they informed of the heart rate target. This was to avoid any perception of direct heart rate influence on symptoms.

Treatment providers in charge of adjusting the dose of rate controlling drugs were aware of the treatment allocation. Outcome assessors were not aware of the heart rate target. In theory, awareness of allocation may influence the outcome assessment if e.g. an outcome assessor has a strong belief that strict (or lenient) is better, this may consciously or unconsciously affect the assessment. Further, the assessment of clinical outcomes and serious adverse events will at the end of the trial be performed by a blinded adjudication committee, to further eliminate any bias from allocation.

When the trial is complete, a blinded statistician will perform the analysis. Based on the blinded analysis, two manuscripts will be written, one assuming that group 'A' is lenient rate control, and one assuming group 'B' is lenient rate control. Together with the published statistical analysis plan, this should avoid any bias as described for Cochrane risk of bias domain 5 (see description of the different types of bias in the section on study 2).

Participants

Adults (>18 years old) with either persistent or permanent atrial fibrillation prior to inclusion where rate control is the primary strategy going forward for better symptoms management, who can provide informed consent are eligible for inclusion. A description of the informed consent is described in the ethics section further below. By definition, when the participants are included, they have permanent atrial fibrillation.[1]

Exclusion criteria are no informed consent, heart rate at rest below 80 bpm, if they have not been treated with appropriate anticoagulation therapy for less than 3 weeks, or not being able to be randomized into either group based on an individual assessment.

We chose to exclude participants with a heart rate below 80 as they would either a) not be able to achieve lenient rate control if on no rate controlling medication without being given rate increasing medication, which is currently not part of the atrial fibrillation guidelines for long-term management, or b) will have to be decreased perhaps significantly in their rate controlling medication, which by design, would change the nature of the study.

Participants who were not treated with appropriate anticoagulation were also excluded. The important wording here is appropriate, as not all participants in theory would receive anticoagulants. A further

advantage of the exclusion criteria when we expected more participants with new-onset/persistent atrial fibrillation, was that it increased the likelihood that participants truly did have a non-paroxysmal form of atrial fibrillation.

The last inclusion criteria changed a lot during the protocol phase and in the initial start of the trial. The idea was to exclude participants who from a physiological and clinical perspective were not fit for randomization. In the trial author group's experience, this is often determined based on the results of an echocardiography, the symptom severity, age, and medical history. This also implies that there will be differences among sites, which is also what would be expected in the real world. It will therefore be important to account for the type of participants who actually end up in the study to make it clear who the trial results apply to.

Intervention

Lenient rate control

Participants randomized to lenient rate control will have a heart rate target of 80 – 110 bpm. If the heart rate is below 90 bpm at rest, the treatment provider is encouraged to reduce heart rate controlling medication. This provision was in to facilitate a difference in heart rate between lenient and strict rate control. If symptoms or other reasons required further heart rate reductions as deemed necessary by the treatment provider, this was permitted.

Strict rate control

Participants randomized to strict rate control will have a heart rate target of <80 bpm. In general, treatment providers are encouraged to target a heart rate of 70 bpm or below. No lower boundary was in place as both from the internal discussion in the trial author group and our knowledge of the clinical heterogeneity did not allow us to put a lower boundary, although 60 bpm was discussed. In the end, we followed the RACE II trial which did not put a lower boundary on strict rate control. It was also discussed if there should be a provision to Holter monitor all participants in the strict group, as well as perform exercise test as was done in the RACE II trial. However, it was deemed that this was not in line with current practice.

Rate controlling drugs

Treatment providers are recommended to follow guidelines for choice of rate controlling drug. In general, in participants with reduced ejection fraction, beta-blockers are recommended as first line therapy for heart rate control.[1] In patients without reduced ejection fraction, participants will in general receive beta-blockers or calcium antagonist as first line therapy.[1] Both patients with and without reduced ejection fraction could receive digoxin and amiodarone as second or third line therapy.[1] A more specific treatment algorithm was discussed, however, to make sure the trial mimicked real world practice as much as possible, we chose the rate controlling drug to be at the discretion of the treatment provider. The drawback is more

clinical heterogeneity and less ability to specifically point out the reasoning for the difference, should a difference arise.

Outcome

The primary outcome is SF-36 physical component score. Quality of life was chosen as the primary outcome as it is an important patient centered outcome. In the protocol phase, we discussed a bicycle test as the primary outcome. Quality of life was chosen over the bicycle test as a difference on the bicycle test would be of questionable patient relevance, whereas quality of life is more directly patient relevant and finding a difference on quality of life should impact clinical decision making directly. SF-36 was chosen for its previous widespread use and the fact that it is a generic vis-à-vis a disease specific questionnaire, which if a change is found, is more patient relevant.

Secondary outcomes include SF-36 mental health component score, AFEQT score, serious adverse events, and days alive outside of hospital.

SF-36 mental health component score was chosen to complement the SF-36 physical component score as it measures the mental aspect of quality of life. As we expect the heart rate to most directly influence the physical quality of life based on physiological considerations, this outcome was only a secondary outcome.

AFEQT was chosen to supplement the generic quality of life questionnaire SF-36 with a disease specific questionnaire. Disease specific questionnaires tend to be more responsive to change. Originally, we planned to use the University of Toronto Atrial Fibrillation Severity Scale (AFSS), but since this questionnaire has not been validated in Danish, and the Danish ethics committee requires such a questionnaire to be available in Danish, we chose instead to use the AFEQT questionnaire, which has also been extensively used in the literature and is validated in Danish.

Serious adverse events was chosen for it obvious patient relevance and the fact it captures holistically any difference in complications. This is supplemented with a description and table of the type of serious adverse events encountered in the trial.

Days alive outside hospital may be important measure both from a health care system point of you and a patient point of view, although whether a shorter or longer duration hospitalization is considered best for the individual patient may vary. The idea to use days alive outside hospital instead of hospital duration is that the outcome captures if any reduction in length of stay comes at the cost of mortality.

Exploratory outcomes include heart failure leading to hospitalization, hospital admissions, walking distance achieved during 6 minutes, a composite outcome of cardiac arrest, mortality, myocardial infarction, and ischemic or hemorrhagic stroke. The individual outcomes of the composite outcomes are assessed as well.

The cost associated with the two interventions is also planned to be assessed. These outcomes were important, but given the power associated with these outcomes, or their indirect patient relevance (6 minute walking distance), they were only exploratory.

A biobank has also been created as well as several echocardiographic outcomes assessed. These two outcomes, especially the echocardiography was important in terms of possible explaining any difference in quality of life and tying the results with physiological parameters to gain greater insight in the mechanisms behind symptoms in atrial fibrillation and atrial fibrillation as a disease.

The last two exploratory outcomes are the number of participants who are started on rhythm control and the number of participants who have either a pacemaker or defibrillator inserted. These outcomes may catch failure in the adequacy of the target rate control.

Statistics

A statistical analysis plan has been accepted for publication in the journal Trials (not part of the Thesis, but supervised partly by me).[82] The statistical analysis plan was published before recruitment was finished and before aggregate participants data was looked at.[82] A brief account of the most important aspects are covered below.

General principles

Data will be analyzed according to the intention-to-treat principle for the primary analysis. This means that all participants are analyzed according to the randomization, regardless of the heart rate they end up in or the intervention they received. This is to ensure not to introduce bias especially from differences in underlying prognostic variables.[82]

As a supplement to assess the impact of lack of achievement of the randomization target, if more than 5% do not achieve their assigned heart rate, we will conduct an analysis were participants are analyzed according to the actual heart rate they achieved.[82]

To account for impact of missing data (attrition), if the missing data is missing at random, and there is less than 5% of participants with missing data, we will only use the data from the participants with follow-up data. If there is more than 5% of participants with missing data, we will use multiple imputations.[82] If the data is not missing at random, we will investigate the pattern of missing data. If necessary, we will undertake best-worst, worst-best sensitivity analysis.[82]

If a participant is dead, we will not include such a participant in the analysis for quality of life or impute data for this participant. Instead, we will conduct a sensitivity analysis were participants who died have the imputed value of 0 for quality of life.[82]

Sample size and power calculations

The sample size is calculated based on the primary outcome SF-36 physical component score. Previous trials with participants with atrial fibrillation suggested that the standard deviation to be expected in this population is 10.[57,83,84] The difference is set to 3 points which is considered the minimally relevant difference based on a small difference using a distribution method but perhaps it should have been 0.5 SD which is a moderate change. This will be discussed when the trial is completed. Alpha was set to 0.05 based on conventional standard of a 5% family wise type I error, and beta to 0.80 which should perhaps have been 0.90 which many trials today use to avoid type II error. Based on these parameters, the estimated sample size is 350 participants. We did not choose to account for attrition (i.e. missing data/drop outs/death). The argument was that if we keep missing data to a minimum, we will not need to account for the missing data and instead unnecessarily inflate the recruitment number. Even if the missing data was above 5%, if it was missing at random we could use multiple imputation to account for the missing data.[80,85]

Data analysis

Data for continuous outcomes will be presented as means and standard deviations. Continuous data will be compared using linear regression with mixed effects. The type of analysis is based on the type of dependent variable. Linear regression assumes a linear dependency between the dependent and independent variables.[86] They will be adjusted for the stratification variables (site, type of atrial fibrillation, LVEF) and the baseline value of the outcome. Count data will be analyzed using van Elteren's test adjusted for the site of randomization. Binary data will be analyzed using logistic regression.

Subgroup analyses

To explore if the results differ according to important participants characteristics, the following subgroups are preplanned:

- Women versus men (based on biological sex and not gender identification)
- Duration of atrial fibrillation
- Heart failure status
- Age ≥ 75 versus <75

Ethics

As part of designing the trial, ethical aspects had to be considered. The Helsinki declaration is at the center of such considerations.[87] As a general rule, the possible benefits should outweigh the risks and burdens.[87] When conducting a randomized trial, the foundation of patient participation is the informed consent. In DanAF, only adults were included, whereas specific procedures and considerations arise in the case of children. As part of ensuring possible participants understand what they are accepting by enrolling in the

trial, both written and oral information must be given. The written information must be in Danish and is reviewed by the regional ethics committee. Consideration towards how participants are included (no coercion, not participating must not infringe on treatment rights) is very important.

The potential harms of the trial must be considered and are described in the written information for DanAF. An important potential harm is if participants are randomized to a group which turns out to result in a worse outcome. Other potential harms are the time and effort involved with study procedures including extra blood samples taken as part of the trial.

To ensure participants rights, the decision to include was not made by me, but rather by the treating physician or by another physician not directly involved in the trial.

Other considerations are participant rights in term of data protection rights, which is also in this trial based on informed consent. Before the trial stated, a process with internal registration of what data is stored, and a written description to the participants of what data is handled, participant rights and who to contact in case of doubts about rights or other inquires.

Study 2 – Systematic review of rate controlling drugs

Choice of study design

Before a randomized trial is to be undertaken it is important that there is a systematic review or at least a search of previous publications to ensure that a research question is unanswered. In the case of the optimal rate controlling drug, to our knowledge and what is cited in the European guidelines, no systematic review has compared the different rate controlling drugs, but instead recommendations are based on individual smaller trials. Hence the choice of a systematic review to answer this research question.

The following is a summary of a planned submission.[88] It reflects the methods described on the Prospero registration (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022310938).[89] We followed the PRISMA guidelines.[90]

Participants

Adults (18+) with non-paroxysmal atrial fibrillation. This could be permanent or persistent atrial fibrillation. If a trial had less than 40% of participants with paroxysmal atrial fibrillation or atrial flutter we still included the trial. This was done in order not to lose valuable information but at the same time ensure that there was not too much noise from participants we did not wish to include. Ideally, if such trials were found, we would request individual data from the trial authors.

Intervention

We included any intervention we considered rate control. We did not specify specific drugs as to ensure no valuable data were overlooked. An alternative could have been to specifically search for the drugs used in current practice for rate control in atrial fibrillation. However, since no previous review had been done before, we found it most scientifically correct to include all rate controlling drugs.

Comparison

We included any intervention, we considered rate control, no intervention or placebo. Again, we could have chosen to be more specific, but as this is the first review of its kind, we thought it was important to give the full picture.

Outcomes

Primary outcomes were all-cause mortality and serious adverse events according to ICH-GCP.[91] These outcomes were chosen, as they are the most patient relevant outcomes and any difference in these will in many cases be the most important.

Secondary outcomes were quality of life (health related). The questionnaire could be either disease specific or generic; symptom score such as the New York heart Association (NYHA) or European Heart Rhythm Association (EHRA); and non-serious adverse events.

The first three outcomes were chosen to capture quality of life and symptoms reported both by the patient and assessed by the physician. In contrast to DanAF, they were secondary outcomes here. If one drug held benefit in terms of all-cause mortality or serious adverse events, we would expect this to be the primary basis for clinical decision making including patient preference. Secondarily, quality of life would come into play. However, one could also argue that quality of life should have been a primary outcome, as some patient may choose to base their choice of treatment primarily on anticipated effect on quality of life. We chose ultimately to let the outcome be a secondary outcome and hence, conclusions will have to be downplayed accordingly, as we do not adjust for the family wise type I error accordingly.

Tertiary outcomes were resting heart rate, exertional heart rate, exercise capacity, ejection fraction, successful achievement of resting heart rate.

These outcomes are part of clinical decision-making, but clinical decision-making would not be expected to be guided by these if evidence were available for primary and secondary outcomes. However, as for now, the parameters are used in clinical practice (as surrogate for achieving patient well-being and optimal cardiac output). They also serve a different function: the relationship between these outcomes and the more patient relevant outcomes will be important in understanding the coupling between physiology and patient centered outcomes.

All outcomes were assessed at maximum follow-up.

Search, extraction of data and risk of bias

We searched common registries such as Pubmed, Excerpta Medica database, Latin American and Caribbean Health Sciences Literature, Cochrane Central Register of Controlled Trials, Medical Literature Analysis and Retrieval System Online, Conference Proceedings Citation Index—Science. We did not search Chinese data bases. The reason based on many peer review and editors comments is that results from Chinese trials and Chinese clinical practice may be too different from western trials and hence, pooling them may introduce too much heterogeneity. The drawback is that relevant data may not have been included in this paper.

We also searched trial registries such as clinicaltrials.gov, www.ema.europa.eu, www.who.int/ictrp, www.fda.gov. This is both to find ongoing trials but also trials may have unpublished data here.

There we no restrictions on language. Again, this is to ensure all available data is used. Considerations must then be taken towards whether this introduces clinical heterogeneity if data from many different countries is used. This also means that outside expertise may be needed if there is a trial in a language we do not possess in the author group. The databases were searched from inception until the date of the search.

After the search was completed, three authors looked through the references. All references were reviewed by two authors. If there was disagreement the authors would discuss the difference in assessment. The third author was consulted if necessary. This process is to ensure no paper is overlooked and the correct papers are included.

A data extraction sheet was prepared and tested on three different trials with different trial design (parallel or cross-over). The data extraction sheet was made in excel for its ease of use. All authors were encouraged to provide feedback.

Data extraction and risk of bias was done by five authors. As lead author, I looked at all extracted data and all trials to ensure that there was knowledge dissemination among authors in terms of how to handle different subjects.

Risk of bias was assessed using Risk of Bias 2 created by Cochrane.[89,92] The risk of bias is described at the outcome level vis-à-vis the trial level, since two outcomes from the same trial may have different risk of bias.[89,92]

The Cochrane risk of bias tool 2 is a tool to assess the risk of systematic error. The tool consists of five domains. Each domain consists of signaling questions that through an algorithm results in a risk of bias from that domain.[92] The algorithm, however, may be overridden by the review authors. The resulting assessment of the five domains gives rise to a classification of the overall risk of bias.[92]

Bias from the randomization process

The first domain assesses risk of bias from the randomization process. The domain contains three signaling questions from which bias from this domain may originate from.[92] The first question concerns bias from generation of the random sequence. Low risk of bias process to generate the randomization sequence are e.g. random number table and a computer generated sequence. Classical examples of a high risk of bias process is using date of birth (even/odd) or date of inclusion.

The second questions concerns the risk of bias from the concealment of the random sequence.[92] A sequence may be random, but if the investigators are aware of the order, there is a high risk of bias. Examples of inappropriate concealment may include using envelopes that are transparent, too small blocks in block randomization, or the randomization list freely available.

The third question is not a bias in itself, but may serve as a signal of bias. Imbalances in the baseline table may signal potential problems with the randomization or potentially fraud.[92] However, imbalances by chance must be carefully considered when answering this question.

Bias from deviations from the planned interventions

This domain intends to capture bias due to participants not receiving the intervention as planned.[92] The first two signaling questions assess whether involved parties (participants, personnel) in delivering/receiving the interventions were blinded.

If they were blinded, the second part of the domain assesses whether the analysis are appropriate i.e. did the trial use intention to treat or per protocol analysis (or something in between).[92] If not all parties were blinded, one is supposed to assess if the deviations were a result of fact that participants received the intervention in the trial and not in real life. If not, then this does not give rise to bias. However, if deviations are a result of participants being in a trial, then this is considered a risk of bias since the results might then be different, if they happened outside the trial. The assessment also involves assessing whether these deviations were balanced (less bias) or imbalanced (more bias).[92] As the case for when parties were blinded, the last part of the domain focuses on assessing the type of analysis (intention to treat versus per protocol).

Bias from incomplete outcome data

The third domain assesses bias from missing data.[92] The first signaling questions assess whether there is full data for all participants. Missing data above 5% may be considered a general rule of thumb for preceding to the next set of signaling questions.[92] Signaling question 3.2 assesses whether there is evidence that the outcome was not biased as a result of the missing data.[92] Evidence may come in the form of sensitivity analysis e.g. best-worst, worst-best showing the missing data could not change the overall result even in extreme scenarios. Evidence may also come from robust analysis methods to correct for bias. E.g. last observation carried forward will be considered inadequate. 3.3 assesses whether the missing data could be a result of the value of the missing event e.g. if an intervention increases adverse events leading to participants to drop out, and this then leads to missing assessments, this would mean the missingness depends on its true value.[92] 3.4 assesses whether the missingness is likely to be a result of the true value of an outcome.[92]

Bias from the assessment of the outcome

This bias domain attempts to assess bias from the assessment of the individual outcome.[92] The first signaling question assesses whether the instrument/device or assessment method in general is appropriate.[92] An unvalidated questionnaire or using a machine insufficient to assess clinically relevant changes are examples.[92] Signaling question 4.2 assesses whether the assessment process could have differed for the intervention groups. For example, if the intervention is longer for one of the intervention groups and the assessment time point for AE was 'end of treatment' this will lead to bias since one of the intervention groups would have a longer period of time for the event to happen. Instead a fix time point

should be used. 4.3 assesses whether outcome assessor were blinded.[92] 4.4 and 4.5 assess the impact of any lack of blinding.[92] In general, more objective outcome such as death are considered less likely to be influenced by lack of blinding.[92]

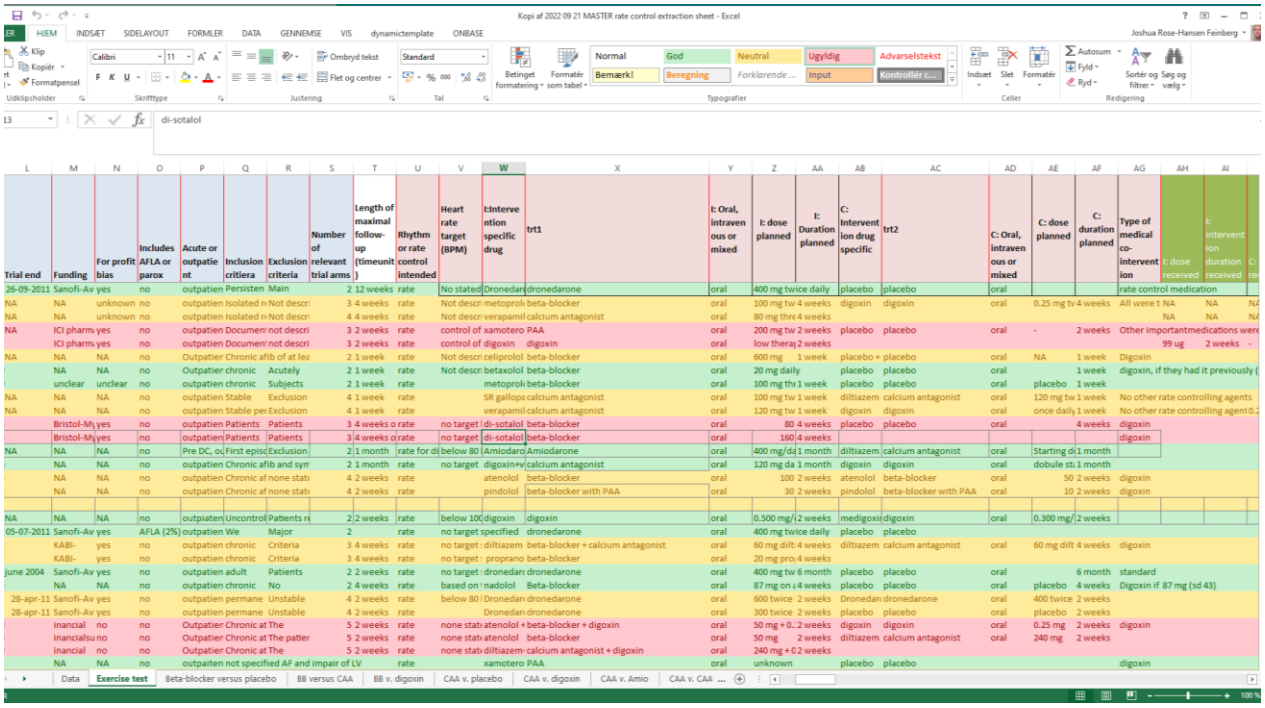
Bias from selective outcome reporting

This domain assesses whether bias was introduced from selectively reporting outcomes, time points, scales, analysis methods or similar.[92] 5.1 assesses whether a statistical analysis plan (or similar) was available before unblinded data was available. 5.2 assesses whether the choice of outcome, time point or similar were based on the result. 5.3 is the same but for the analysis method. 5.2 and 5.3 should be assessed against the statistical analysis plan. If no plan is available, the answer is 'no information'.

Overall risk of bias

The grading of the different domains leads to an overall risk of bias. If all domains are low risk of bias, the overall risk of bias is low.[92] If one domain is judged as 'some concern' or 'high risk' the overall risk of bias has the same risk. If multiple domains have some concern, this will also lead to 'high risk'.[92]

A screen shot of part of the excel sheet is provided below.



Besides our outcomes, we extracted information to describe the type of studies included, type of participants included, length of follow-up and how outcomes were measured.

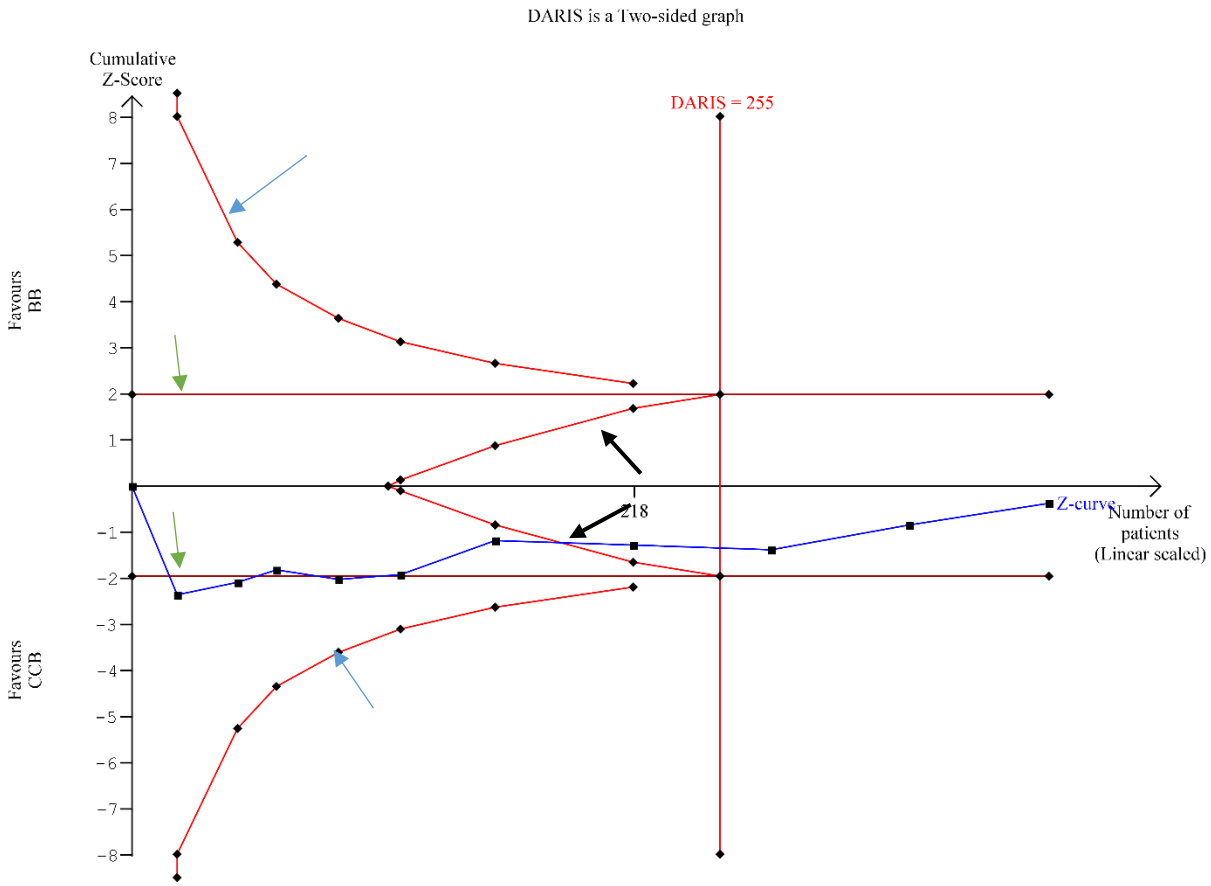
Statistical analysis

Meta-analysis in itself is a way of pooling results from several studies. The reason for conducting a meta-analysis is to achieve increased power to detect a difference especially in the case of several small trials. A meta-analysis may also investigate subgroup differences based on clinical setting, patient population, etc. A meta-analysis can either be performed using published trial level data, or be done as an individual patient data meta-analysis allowing more powerful analysis.[93] Before undertaking the meta-analysis it must be considered whether a meta-analysis is appropriate.[94] For every comparison, it was first assessed if meta-analysis made sense from a clinical and outcome heterogeneity point of view. Heterogeneity was assessed by visual inspection of the forest plot and I^2 statistics (see further below). If meta-analysis was considered feasible, we conducted both random-effects meta-analysis using the methods described by Der Simonian and Laird and fixed effect meta-analysis.[95] Meta-analysis uses a weighted average and the way the weighted average is calculated is different as well as the assumption behind the way it is calculated. The weight is usually based on the standard error of the study, meaning the weighted average takes into account the number of participants in the study and the precision. The idea behind random-effects meta-analysis is that the effect of an intervention is not fixed and may depend on the population, setting, etc.[96] The included studies are a sample of a true population. The fixed-effect meta-analysis assumes there is one 'true' effect, all the different studies are trying to assess.[96] There are several different types of random-effects meta-analysis, which differ based on how they calculate the between study variability.[96] We performed both random effects and fixed effect analyses to ensure results were robust to the type of analysis used to ensure we did not make a type I or type 2 error.[95] If there were differences, these differences would be highlighted.

Trial sequential analysis

To reduce the risk of random error, the meta-analysis were supplemented with TSA.[97,98] TSA ensures that premature conclusions are not made by calculating the information size based on either realistic intervention effects or minimal important difference.[97,98] Premature conclusions can be both premature false acceptance of the null hypothesis (type II) which instead can be due to lack of data or premature conclusion of falsely rejecting the null hypothesis (type I error). TSA may also allow authors to conclude with it is futile to conduct additional trials. [97,98] In our review we chose to base our TSA calculation on minimal important differences.[97,98] Additionally, to calculate TSA boundaries, an alpha (risk of type I error), beta (risk of type II error), and any heterogeneity adjustments must be specified. Similar choices for meta-analysis method must also be made as described above. If the required information size has not been reached, the threshold for significance was adjusted accordingly via monitoring boundries.[97,98] The adjustments both reduces the risk associated with repetitive testing and risk of chance producing false

results. After the parameters have been chosen, both a visual representation as well as TSA adjusted confidence intervals are calculated. Below is a copy of figure 2b. The cured bright red lines with the blue arrows pointing to them are the upper and lower monitoring boundary respectively. They are the visual representation of the threshold for significance that has been adjusted due to the diversity adjusted required information size (DARIS) having not been met.[99] The dark red line with the green arrows pointing to them are the conventional 0.05 boundary for statistical significance. The blue line is the visual representation of the cumulative z-score. Therefore, if the blue line crosses the dark red line, this indicate that the meta-analysis results is statistically significant using the conventional $P < 0.05$ as the threshold. This happens in the figure below. If one were to look at the meta-analysis result at this time, one would erroneously conclude that one of the two intervention compared was superior to the other (type I error). This is exactly what TSA is meant to prevent.[99] At a later point, the cumulative z-score (blue line) crosses the inner wedges for futility (black arrows). This signifies that it is now futile to conduct additional trials. If one had conducted a meta-analysis at this point and the accompanying TSA, one would have been able to conclude that no additional trials were needed to assess the specified change in the TSA (e.g. a 3 point change on SF-36). Hence, additional resources could be spent better elsewhere. A possibility would remain to find a difference on a smaller change, but if the change is then clinically relevant should come into consideration. If the cumulative Z score reaches the DARIS without crossing any of the monitoring boundaries, the conventional significance level can now be used and results concluded accordingly.



Trial sequential analysis – parameters for required information size

For all-cause mortality and serious adverse events: TSA boundaries were calculated using a risk ratio reduction of 25%. The alpha was adjusted for multiplicity to 0.033 as described by Jakobsen et al.[95] This is done to account for the increased risk of type I errors associated with testing for multiple outcomes. The method described by Jakobsen et al is a compromise between the Bonferroni adjustment and no adjust for multiplicity. The basis for the adjustment is a conventional alpha level of 5%. The beta was set at 0.10. For the measure of statistical heterogeneity, we used the diversity found in the meta-analysis with the chosen model with the most conservative estimate.[95]

For other dichotomous outcomes, the beta and the measure of statistical heterogeneity was the same. We calculated the required information size using an alpha of 0.05, as all the other outcomes were considered hypothesis generating only.

For continuous outcomes: a mean difference of SD/2 calculated from the included trials was considered the minimal important difference.[100] As all continuous outcomes were hypothesis generating, we used an alpha of 0.05, a beta of 0.10 and used the diversity from the meta-analysis.[95]

The SD/2 (0.5 SD) was chosen based on distribution method, which states a 0.5 SD is a moderate change.[101] We could have used an anchor-based difference, however, we anticipated that many different scales would be used, and hence, it made more sense to use a distribution method.

Presentation of data

Dichotomous outcomes were analyzed as risk ratios. Risk ratios are the ratio between the risk associated with treatment one versus the risk associated with treatment two. It is more easily interpretable than odds ratio although odds ratio may hold other benefits.[102] Data was presented with CIs (95%) and p value. Continuous outcomes were primarily analyzed as mean difference with CIs (95%). If deemed feasible, we also used standardized mean difference. The standardized mean difference standardizes the mean difference to the standard deviation, allowing comparison of measurement of the same concept but using different scales/measurements.[103] Care should be taken in ensuring that it is actually the same concept being measured, and not different concepts, as results will then be erroneous.

Statistical heterogeneity

Statistical heterogeneity was assessed by visual inspection of the forest plots and as a supplement, we looked at I^2.

A rough guide for interpretation of I^2 is the following suggested by the Cochrane handbook[103]:
0% to 40%: might not be important;

30% to 60%: may represent moderate heterogeneity;

50% to 90%: may represent substantial heterogeneity;

75% to 100%: considerable heterogeneity

Network meta-analysis

We had planned to conduct a network meta-analysis. We planned to include all rate controlling interventions, placebo, or no interventions arms. This was not possible.

Subgroup analyses

We conducted subgroup analyses to explore if there was a difference in effects based on the specific rate controlling drug and if there was a difference between the time periods.

The following test for subgroup differences that were planned were not performed either due to insufficient data or all trials were in the same group: test for subgroup differences by heart failure status (reduced ejection fraction versus preserved), high risk of bias versus low risk of bias, different doses of rate controlling drugs, different heart rate targets, and different routes of administration.

We added post-hoc a test for subgroup difference where we compared trials with a cross-over design with trials with a parallel design.

Summary of findings table

We had planned to create a summary of findings table of our primary and secondary outcomes. However, there was very limited data available for our secondary outcomes and hence, we instead included some of our exploratory outcomes (resting heart rate, exertional heart rate and exercise capacity). To assess the certainty of evidence, we used the five domains of Grading of Recommendations, Assessment, Development and Evaluations (GRADE) approach for systematic reviews.[104] The GRADE approach is a way of assessing the certainty of evidence i.e. how reliable is the effect estimate. There are five domains (study limitations, inconsistency, indirectness, imprecision, and publication bias). Study limitation (risk of bias) was assessed using the Cochrane risk of bias 2 tool. Indirectness was done by assessing the setting, interventions, patient populations and outcome in contrast to clinical practice and determine how direct the evidence is applicable to clinical practice.[104,105] Inconsistency, a measure of how consistent the results are e.g. do results differ across subgroups, was assessed both by subgroup analysis accounting for important intervention and patient characteristics, as well as visual inspection of the forest plot and I^2 test. Publication bias that is whether we found evidence that possibly results showing specific results were not published, was assessed using funnel plots. Publication bias arises from the fact that positive trial results are easier to publish and perhaps also more frequently submitted. Imprecision, a measure of how precise a result is and whether important benefits/harms have been ruled in/out was assessed using TSA.

Software

Data extraction was performed in excel. Meta-analysis and any analysis of single trial results were done using STATA 17, performed by me.[106] TSA were done using the TSA software, performed by me. Network meta-analysis was planned to be done in STATA 17.

Ethics

When using trial level data, ethical considerations both in terms of participants' rights and data protection is not especially relevant as this should have been considered at the trial level. However, if individual patient data is obtained, considerations towards participants right and data protection must be considered: Have the participants consent to their data being shared with a third party, what data is necessary to conduct the study, where the minimal level possible to achieve the scientific results sought should be shared. In the systematic review, no individual patient data was obtained.

Study 3 – Retrospective cohort study on sex, atrial fibrillation and risk of stroke

Choice of study design

For the question of sex differences in risk of stroke among patients with atrial fibrillation, we chose a cost efficient study design as we had a well-suited cohort (patients with hypertension and hypertrophy of the heart, two risk factors for developing atrial fibrillation) to answer the question. The LIFE study also had yearly ECG measurements to detect new-onset atrial fibrillation, consistently measured blood pressure data and smoking status which is not always available in registers. While the choice of design does not confer the same confidence in any estimates found compared to a new randomized clinical trial or a new prospective cohort study, it is a more cost-efficient way in the preliminary stages of developing an understanding of a research question.

Population

This study used data from the Losartan Intervention for Endpoint Reduction (LIFE) trial.[107,108] The LIFE trial was a multicenter randomized trial including 9193 participants from June 1995 to May 1997.[107,108]

To be included, participants had to have both left ventricular hypertrophy and hypertension.[107,108] The diagnosis of left ventricular hypertrophy was made by electrocardiogram and diagnosis of hypertension was defined as a sitting systolic blood pressure of 160-200 mmHg or a diastolic blood pressure of 95-115 mmHg.[107,108]

Important exclusion criteria included heart failure with reduced ejection fraction and recent (<6 month) myocardial infarction or stroke.[107,108] Follow-up was at least 4 years.[107,108]

Statistical analysis

Description of included population

To describe the included population, we presented clinical and treatment characteristics and compared the groups. For dichotomous variables, we used chi-square test. This is a test used for dichotomous/categorical variables, comparing the distribution of one variable between different samples.[109] The expected numbers in case of no difference in distribution is compared to the actual numbers yielding the χ^2 and from a table (chi square distribution table) depending on the degrees of freedom, a corresponding p-value may be obtained.[109] For continuous variables, we used either unpaired Student t-test or ANOVA with post-hoc estimation made with Tukey's test.[110] Student t-test was used for to compare means between two groups which directly gives a p-value between the two groups.[111] One-way ANOVA was used when more than two groups were compared for one independent variable (e.g. no atrial fibrillation vs new-onset atrial fibrillation vs history of atrial fibrillation).[111] To avoid problems with multiplicity, the post-hoc Tukey's test

was used. There are many other post estimation tests to use, but Tukey's is a commonly used, and considered robust.[110]

Risk factors for either having a history of atrial fibrillation or developing new-onset atrial fibrillation

We used logistic regression to test for associations between possible risk factors and either having a history of atrial fibrillation or developing new-onset atrial fibrillation during the trial. Logistic regression assumes the dependent variable is binary. It also assumes observations are independent. Since different individuals were used, the assumption was not violated. Multicollinearity i.e. strong correlation between independent variables should also be avoided.[112]

Associations between variables and clinical outcomes

To test associations between variables of interest and clinical outcomes of interest we used Cox regression. In the Cox regression we included age, sex, systolic blood pressure, body mass index, smoking status, diabetes, previous transient ischemic attack or stroke, previous myocardial infarction, and previous heart failure. These were variables that are important possibly confounding factors when assessing atrial fibrillation and the clinical outcomes. To account for the randomization, we also included treatment allocation which is standard when conducting retrospective cohort studies from randomized trials. Results were reported as Hazard Ratios. We reported the p-values from the Wald test of possible interactions and subsequently performed stratified analysis according to sex and age.

We performed two sensitivity analyses for the analysis of stroke, our primary outcome of interest: 1) Fine-Gray regression for the overall analysis and 2) a Cox regression where new-onset atrial fibrillation was considered a time-varying constant.

The Fine-gray regression was done to account for competing risk. Competing risk arises when a competing event (usually death) makes another event (e.g. stroke) not possible. Not accounting for the competing event may bias the result, at least in terms of incidence and risk, but not so much rate/hazard.[113] The competing risk situation is a difficult one, and hence, we present also the result of the fine-gray regression, to illustrate any discrepancies.[113] Since this was an etiological research question, we put emphasis on the cox regression.[114]

There are underlying assumptions for cox regression. One assumption is the proportional hazard assumption which assumes the hazard is equal over time.[115] Schoenfield residuals were used to test the assumption of proportional hazards.

Ethics

The study involved data collected on a cohort of patients, where the dataset contained no obvious identifiers. The dataset is now completely anonymized. The participants who were part of the LIFE cohort had all given written consent to participate in the study after having received oral and written information.[108] The study was approved by local ethics committees before the trial started.[108] Further the study was overseen by an independent safety monitoring committee.[108]

Study 4 – Cross-sectional study of the physical activity paradox

Choice of study design

There were several factors that played into the study design and choice of cohort. There is a close relationship between inflammation and atrial fibrillation as previously described. We thought specifically looking at hsCRP as part of the systemic inflammatory response was of interest. CRP in particular has been linked to atrial fibrillation as a downstream marker of the systemic inflammatory response. Physical activity is part of the 'C' part of the recommendation for management of atrial fibrillation and the rationale behind is partly to lower systemic inflammation as described in the background section. In clinical practice, patients will often argue that the need for physical activity in their leisure time is not necessary if they have a physically demanding job.

Physical activity's role in preventing atrial fibrillation has also been widely studied. However, most, but not all previous studies have either not distinguished between LTPA and OPA or focused exclusively on LTPA.[40,41] The CAMB cohort distinguished between the context of physical activity and also contained a detailed description of job history which combined with a job exposure matrices meant we could improve on the precision of the exposure compared to a previous study on the matter.[42,116] Further, the CAMB cohort had blood samples for hsCRP analysis taken which meant we could specifically explore one of the six hypothesis explaining the physical activity paradox.[77] For these reasons the cohort was chosen.

Ideally, it would have been optimal with a population of patients with atrial fibrillation or at least a subset of patients diagnosed with atrial fibrillation during study. However, although this evidence will be indirect, it may serve as a platform for atrial fibrillation specific studies in future.

Cohort

The following is based on the methods described in my previously published article.

This study was a cross sectional study using data from the Copenhagen Aging and Midlife Biobank (CAMB) cohort.[117] The CAMB cohort consisted of participants from three previously established cohorts.[117] For this study, we only used participants from two of the subcohorts: "The Metropolit Cohort" and the "Danish Longitudinal Study on Work, Unemployment and Health" cohort.[117,118] In total, 12656 participants were

invited, 7243 participants answered the questionnaire (57.2%) and 5304 participants also had blood samples taken.

Exposure

The two exposure variables were leisure time physical activity (LTPA) and occupational physical activity (OPA). LTPA was based on a question in the CAMB questionnaire with four possible answers (translated by me from the Danish questionnaire)[117]:

1) "Hard exercise and competitive sports regularly and multiple times a week".[117]
2) "Armature sports, strenuous housework or gardening at least four times a week".[117]
3) "Stroll, ride a bicycle, or other light exercise at least four times a week (count also Sunday strolls, light housework or gardening and riding your bicycle/walking to and from work)".[117]
4) "Read, watch TV, or other sedentary activities".[117]

Heavy lifting was used as a surrogate for OPA. OPA was estimated based on a detailed self-reported job history. The job title was then coded according to the Danish Version of the international Standard Classification of Occupations registry.[117] This was then combined with a job exposure matrix, where experts based on their experience and consensus agreement had agreed upon the level of OPA associated with different job titles.[116,117] This was done to more accurately provide a measure of OPA, as basing OPA on only self-reported assessment of OPA exposure is inaccurate.[119] OPA was measured as ton-years (lifting a 1000 kg/day for a year).

Outcome

High sensitivity C reactive protein (hsCRP) measured in mg/L was used as a downstream marker of systemic inflammation. Consideration towards using other biomarkers including more upstream biomarkers were considered, but ultimately this biomarker was chosen due to its downstream presence, and widespread use. At this stage of the hypothesis testing of inflammation being a player in the physical activity paradox we did not want to complicate the design with multiple additional biomarkers.

Covariates

Covariates identified based on prior knowledge of systemic inflammation were Body Mass Index (kg/m^2), age, sex, smoking measured as pack years (one pack year = smoking 20 cigarettes a day for a year), alcohol measured in units (1 unit = 8g of pure alcohol), number of chronic diseases (0, 1, or more than 2). We also considered including social class, but given the expected correlation with many of the other covariates, we chose not to.

Statistical analysis

We performed linear regression since our dependent variable was continuous. Linear regression attempts to model the relationship between the continuous dependent variable. We transformed both LTPA and OPA to binary outcomes, which were then combined to a four level categorical outcome. This was done to better illustrate any interaction between the outcomes. However, during the final stages of peer review, we removed the four level categorical outcome as the peer reviewers found it unnecessary as we found no interaction.

When using linear regression, several assumption are made. One assumption is homoscedasticity meaning that the variance is constant across values, implying that there is a linear relationship and linear regression is the right model.[120] We did not detect heteroscedasticity in our plot of fitted values vs residuals.

Linear regression also requires no correlation between the error of covariates and hence, independent observations are required.[120]

Multicollinarity should also be avoided.[120] This is also why we avoided social class as a co-variate but instead chose to analyze it as a stratifying factor.

Another assumption is normality of the residuals.[120] This may be and was assessed by a QQ plot. HsCRP was log transformed as residuals were not normally distributed. Therefore, when going back to non-log scale, they should be interpreted as a factor increase (percentage change). The data was analyzed using statistical analysis software (SAS). The analysis were performed by me, supervised by Anne Møller and Volkert Siersma.

Post-hoc analysis requested pr. Peer review

At the request of the peer reviewers, we performed two post-hoc analysis. We performed stratified analysis according to social class. And we analyzed the exposure outcomes as continuous outcomes instead of binary outcomes.

Ethics

The study involved data collected on a cohort of patients, where the dataset contained no obvious identifiers, but is not completely anonymized, only pseudo anonymized. Prior to working with the data, our author group had to submit a written proposal to justify access to the data. The statistical analysis were conducted at a specific location with access to ensure data protection rights of the participants. The participants who were part of the CAMB cohort had all given written consent to participate in the study after having received oral and written information.

Results

Study 1 – Protocol for a randomized clinical trial, DanAF

As of now, 75 participants have been recruited, 20 participants have had their 1-year visit. The participants have been recruited from Zealand University Hospital – Roskilde and Holbaek University Hospital. As recruitment is still under way, the data is locked. The first patient was recruited on 24.03.21.

Steering group meetings

During the trial, monthly steering group meetings were held. The steering committee consisted of the key members from the participating sites as well as experts on trial conduction and methodology. Before each steering meeting, an agenda was sent out by the coordinating investigator (me). The coordinating investigator led the meetings including time management.

Initiation of sites

Before a site could begin recruitment, at least one start-up visit by the coordinating investigator was done. This included going through key study documents in the site file such as the study schedule, standard operating procedures for the investigations, procedure of informed consent. Sparring was provided about how other sites have accomplished producing the setup and how an appropriate model for the corresponding site could be reached. Any questions or comments regarding how to comply with the protocol was also directed to me.

Recruitment from Holbaek hospital

Screening for participants was performed among patients from the cardiology ward as well as the outpatient clinic. At least two times a week, I screened the list of hospitalized patients for possible candidates as well as asked the present attending physicians if they had any potential candidates. Pocket size screening cards were made with inclusion and exclusion criteria as well as contact information for me.

To facilitate the recruitment from Holbaek Hospital, I held two meetings for the attending physicians where the premise of the trial and the inclusion and exclusion criteria were presented. I also presented the study for the nurses at the ward. I also presented for the entire medical department twice.

After a patient was included, I treated the patients according to the randomization and initially did most of the baseline outcome assessment. Later, we had a medical student do the outcome assessments, who I trained in the assessment procedures. Besides attempting to reach the heart rate target, I also treated any cardiovascular comorbidity.

Inclusion of additional sites

To facilitate the possibility of additional sites, I presented at a national meeting of the Danish Cardiology Society. Bispebjerg-Frederiksberg was added as a site. I was further in preliminary talks with two additional sites.

Data management

I created our electronic case report form using RedCap. There was made several updates to it along the way. I was also in charge of adding new persons who should have access to entering data and helping trial personal with any trouble shooting. I also created a data management plan.

Study 2 – Systematic review of different rate controlling drugs

Results of the search

The search was first performed on 28/01/22 and then again on 01/09/22. We began screening the records in Endnote on the 15th of January.

We included 51 trials.[69,121-184] A characteristics of included studies table and a Prisma flow chart is included in the appendix in the article planned to be submitted to JAMA Cardiology. 11 trials were conducted in the UK, 8 trials in the United States, 5 trials in Italy, 5 in Japan, 4 in Sweden. All other countries had 2 or less trials.

Most trials described their participants as "chronic atrial fibrillation". The shortest duration of atrial fibrillation described was 3 weeks and the longest 8 years. Only 34.5% were female.

There were multiple drugs used for rate control. The focus here was on beta-blockers, calcium channel blockers, and digoxin. They were chosen because beta-blockers and calcium channel blockers are 1st line therapy. Digoxin is second line, but this notion has recently been challenged by the RATE-AF trial.[69]

There was very limited data on our primary and secondary outcomes, and hence, the results are not reported in the main Thesis. The Rate-AF trial is part of the discussion as it is the largest trial to date assessing digoxin versus bisoprolol and reporting on hard outcomes.[69]

Beta-blockers versus placebo or in addition to another rate controlling agent

Exploratory outcomes

Resting heart rate

Meta-analysis of 11 trials showed that beta-blockers on average reduced the resting heart rate with 11.27 bpm (CI 95% -14.97 – -7.57, P < 0.0001). We found no suggestion of heterogeneity through visual inspection of the forest plots, I^2 statistics, nor test for subgroup differences. TSA showed that we had enough information to assess our predefined threshold for minimal important difference (11 bpm = SD/2). The evidence was of low certainty and high risk of bias.

Maximal exertional heart rate

Meta-analysis of 11 trials showed that beta-blockers on average reduced the maximal exertional heart rate by 34 bpm (CI 95 -41.57 to -26.42, P < 0.0001). There was moderate heterogeneity assessed by visual inspection of the forest plot and I^2 statistics (62.9%). Test for subgroup differences according to type of beta-blocker was statistically significant. TSA confirmed the result. The evidence was of low grade and high risk of bias.

Assessing each beta-blocker separately, the heterogeneity was almost resolved. The effect size of the different beta-blockers are presented in figure 2a below. None of the drugs included are used in normal clinical practice for rate control in atrial fibrillation.

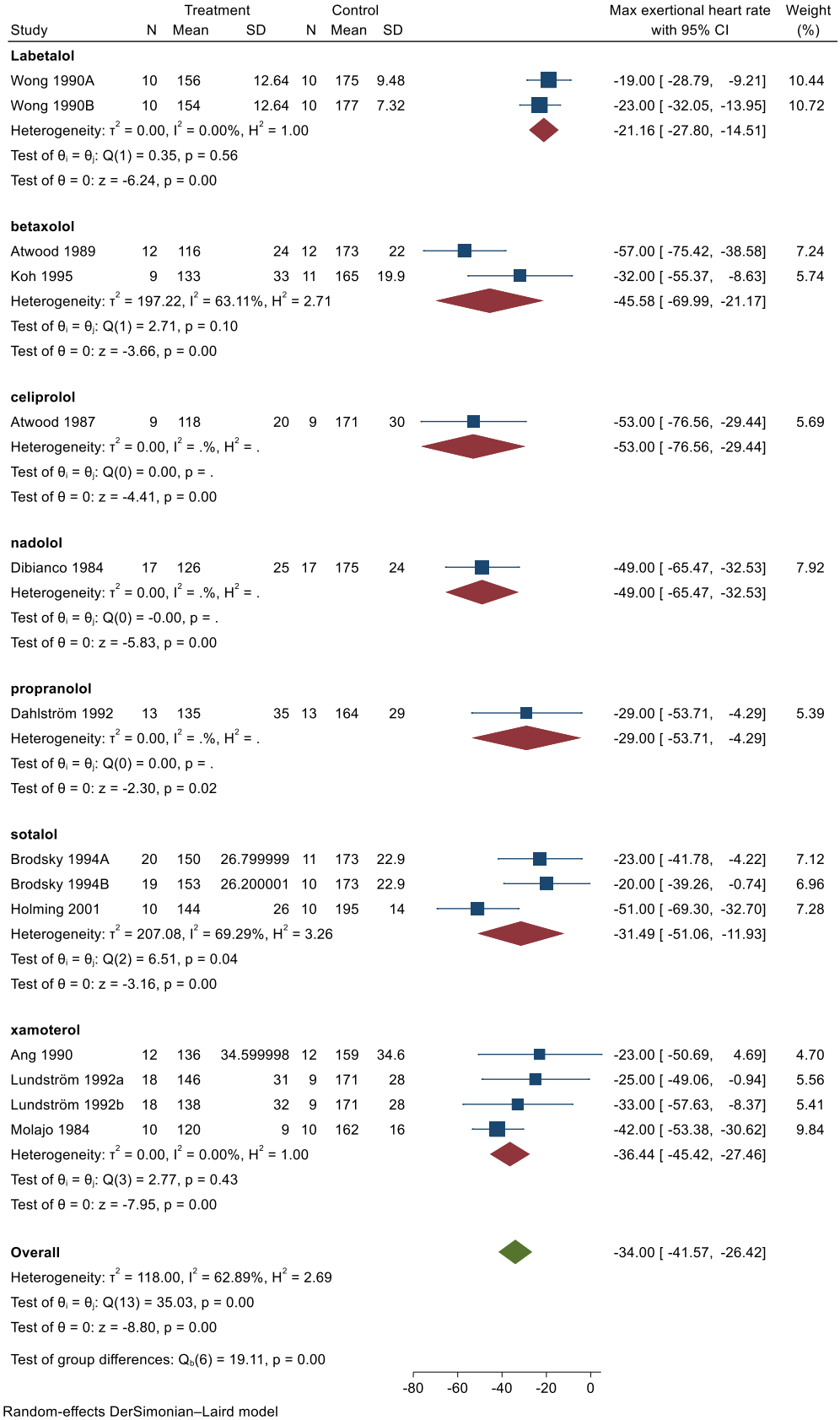


Figure 2a - forest plot by specific beta-blocker used

Exercise capacity

Meta-analysis of 10 trials using standardized mean difference was not statistically different (SMD 0.05 CI 95% -0.26 to 0.36, P value = 0.77). The trials used different ways of assessing exercise capacity and different measuring units.

Beta-blockers versus calcium channel blockers

Exploratory outcomes

Resting heart rate

Meta-analysis of 5 trials showed no statistical difference between beta-blockers and calcium channel blockers on average resting heart rate, with the point estimate being a 2.16 bpm increase (CI 95% -1.25 – 5.56, P = 0.22). We found low heterogeneity through visual inspection of the forest plots, I^2 statistics (22.72%). Tests for subgroup differences were not statistically significant. TSA showed that additional trials are futile to show a difference of 7.5 bpm or more (figure 2b). The evidence was of low certainty and high risk of bias.

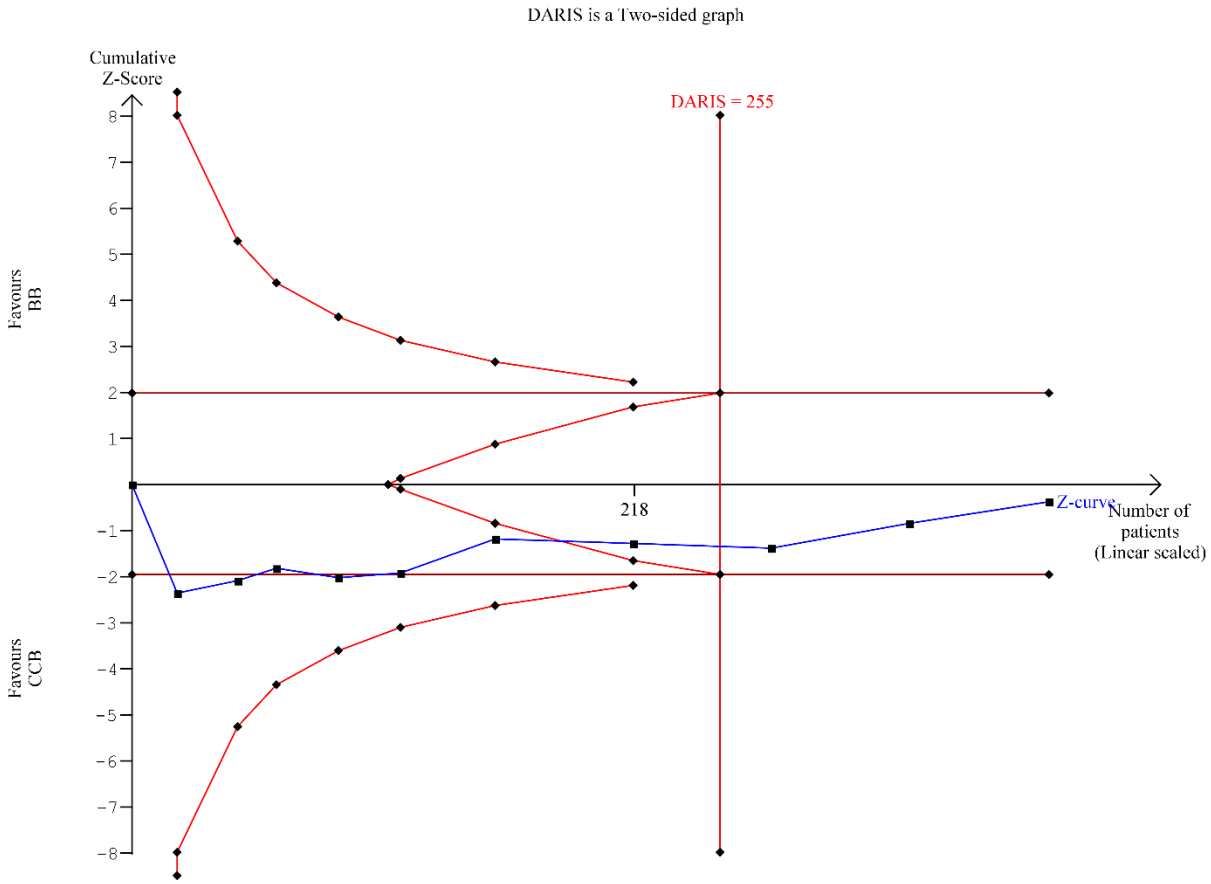


Figure 2b – TSA

Maximal exertional heart rate

Meta-analysis of 6 trials showed no statistical difference between beta-blockers and calcium channel blockers on maximal exertional heart rate, with the point estimate being a 0.52 bpm decrease (CI 95% -6.87 – 5.82, P = 0.87). We found moderate heterogeneity through visual inspection of the forest plots, I^2 statistics (33.07%). Tests for subgroup differences according to the beta-blocker used was statistically significant. The different effects according to the beta-blocker used are presented in figure 2c below. For a better visual presentation, a forest plot including only drugs used in clinical practice is presented (figure 2c*). Metoprolol appeared to be less efficient than calcium channel blocker, whereas carvedilol or a combination of bisoprolol, atenolol or metoprolol may reduce maximal heart rate more than calcium channel blockers. The evidence was of low certainty and high risk of bias.

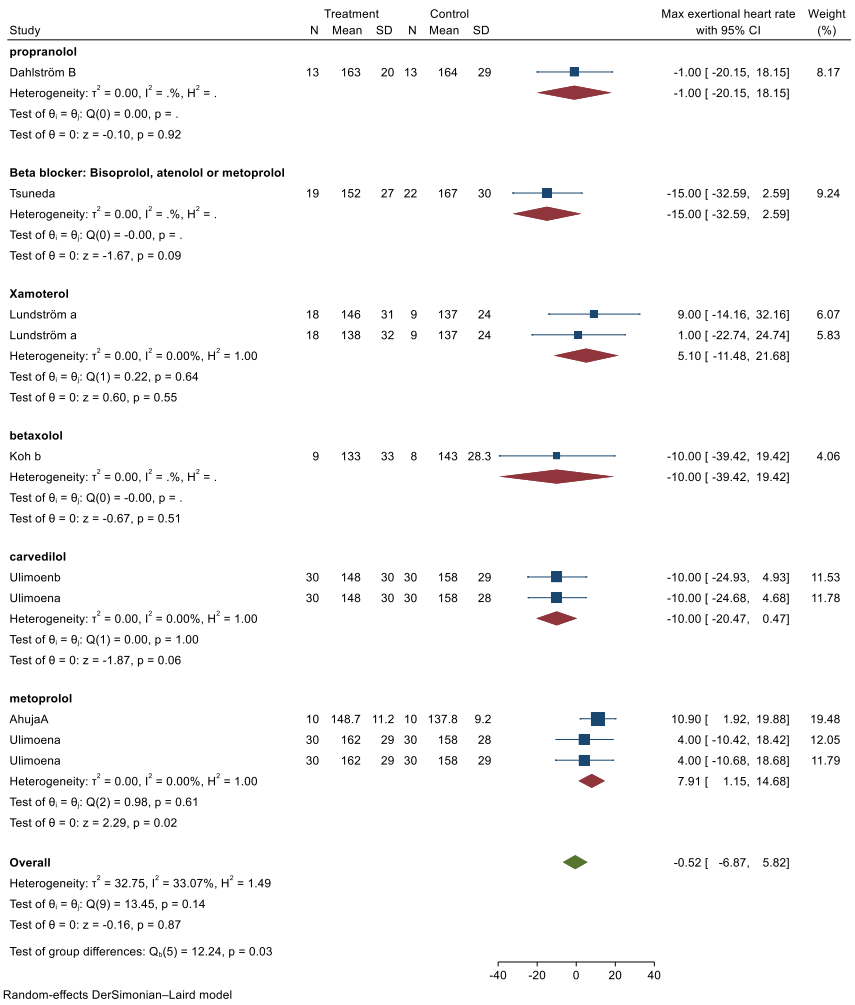


Figure 2c - forest plot by specific beta-blocker used

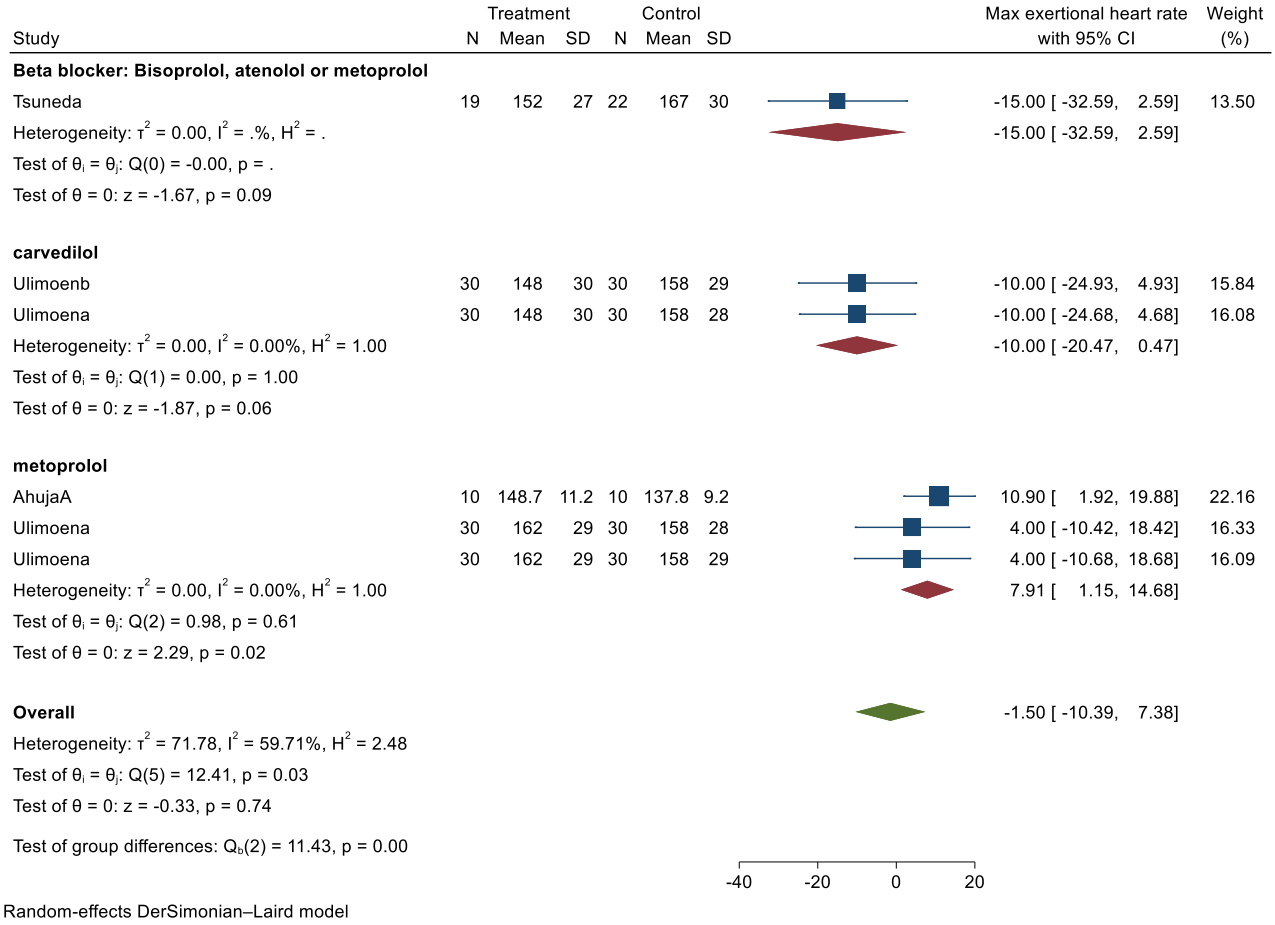


Figure 2c - forest plot by specific beta-blocker used in clinical practice*

Exercise capacity

Meta-analysis of 7 trials using standardized mean difference was statistically different SMD -0.26 CI 95% -0.45 to -0.08, P value = 0.01). The trials used different ways of assessing exercise capacity and different measuring units. The results according to the different measuring units and according to each beta-blocker are presented in figure 2d and 2e measured as mean differences.

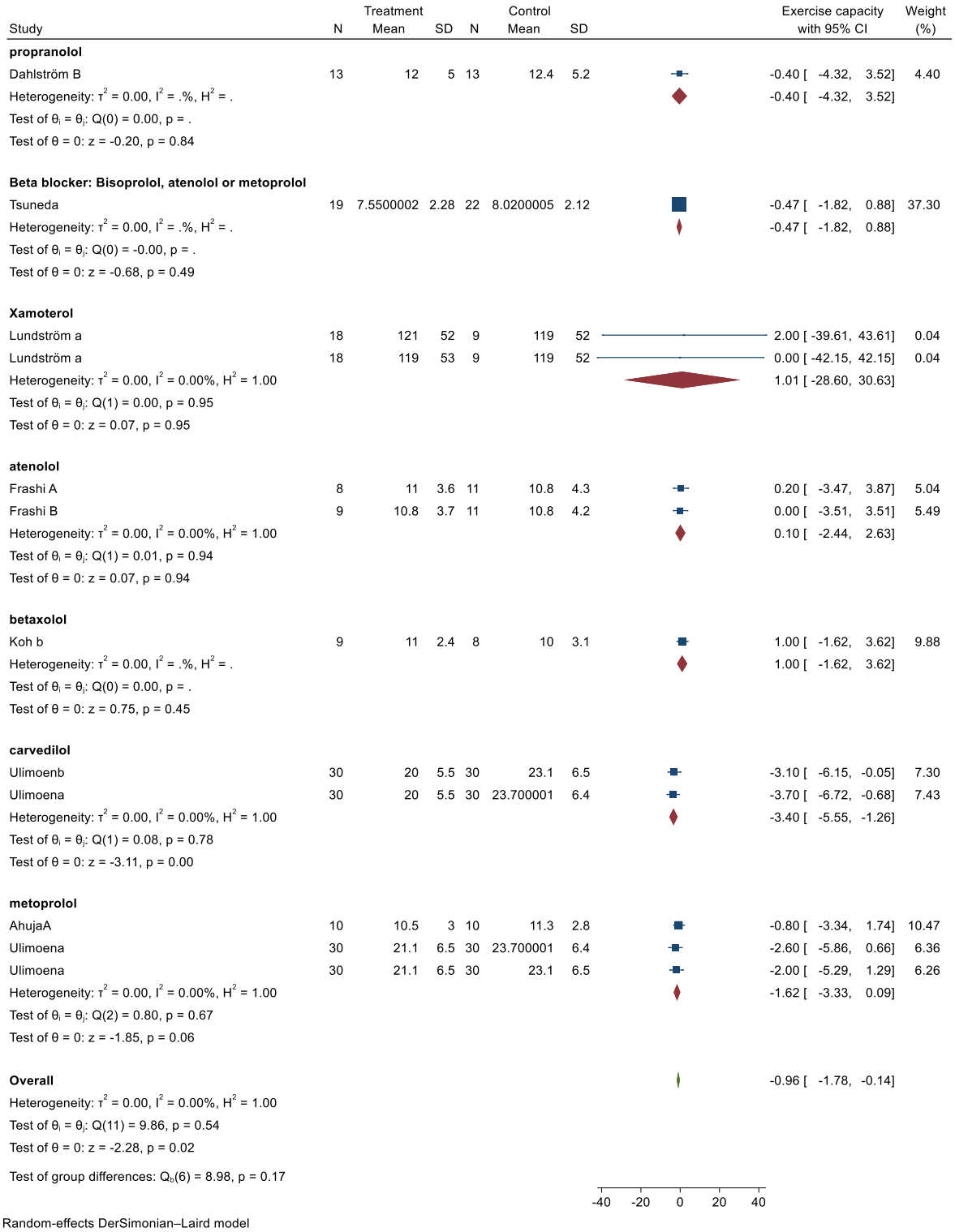


Figure 2d - forest plot by specific beta-blocker used

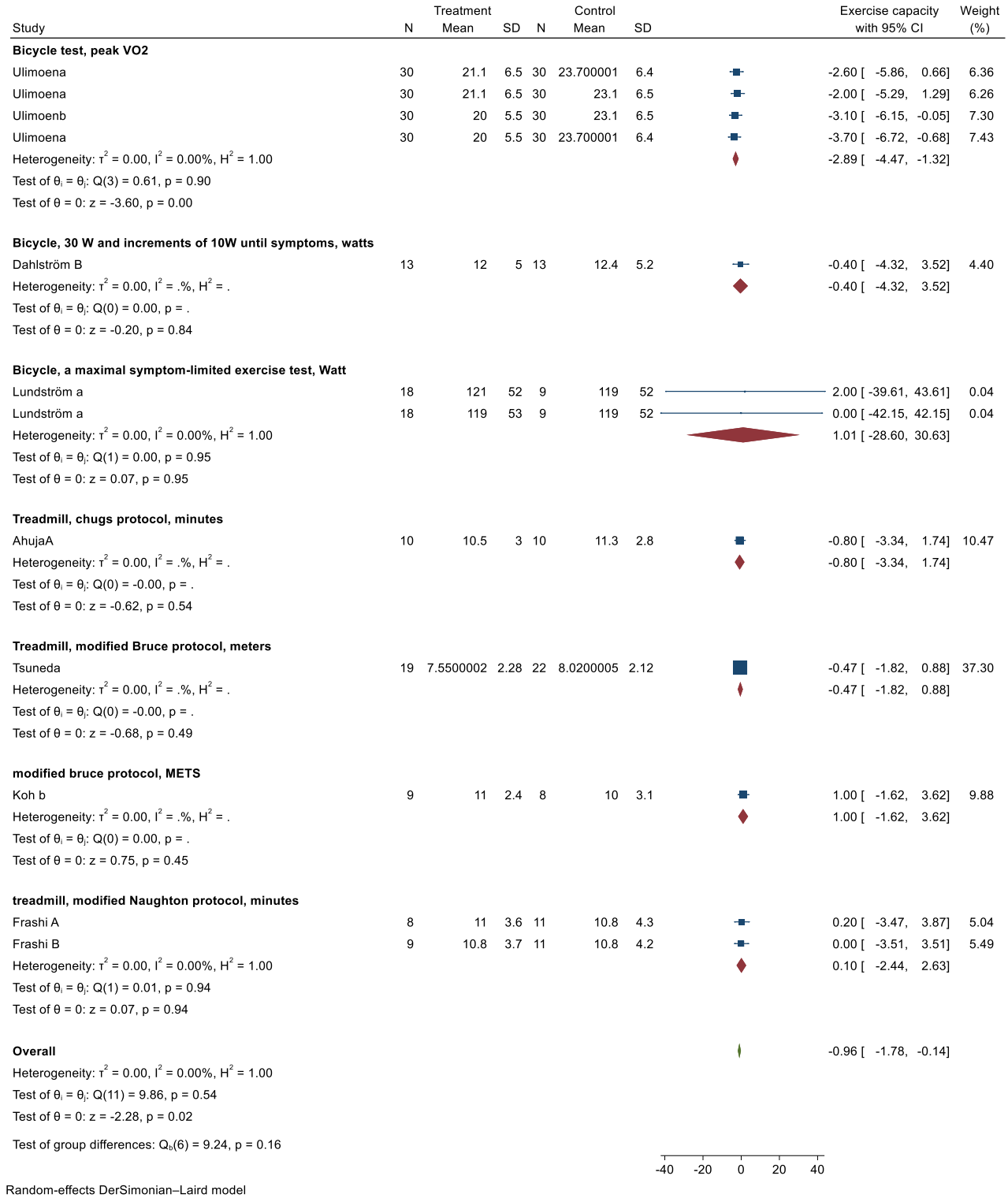


Figure 2e - forest plot by exercise capacity test used

Beta-blockers versus digoxin

Exploratory outcomes

Resting heart rate

Meta-analysis of 5 trials showed no statistical difference between beta-blockers and digoxin on average resting heart rate, with the point estimate being a 1.49 bpm decrease (CI 95% -6.05 – 3.07, P = 0.52). We found moderate heterogeneity through visual inspection of the forest plots, I^2 statistics (34.24%). Tests for subgroup differences were not statistically significant. Despite this, we presented each beta-blocker separately to in the submitted paper in response to the moderate statistical heterogeneity (figure 2f). TSA showed that additional trials are futile to show a difference of 6.5 bpm or more. The evidence was of very low certainty and high risk of bias.

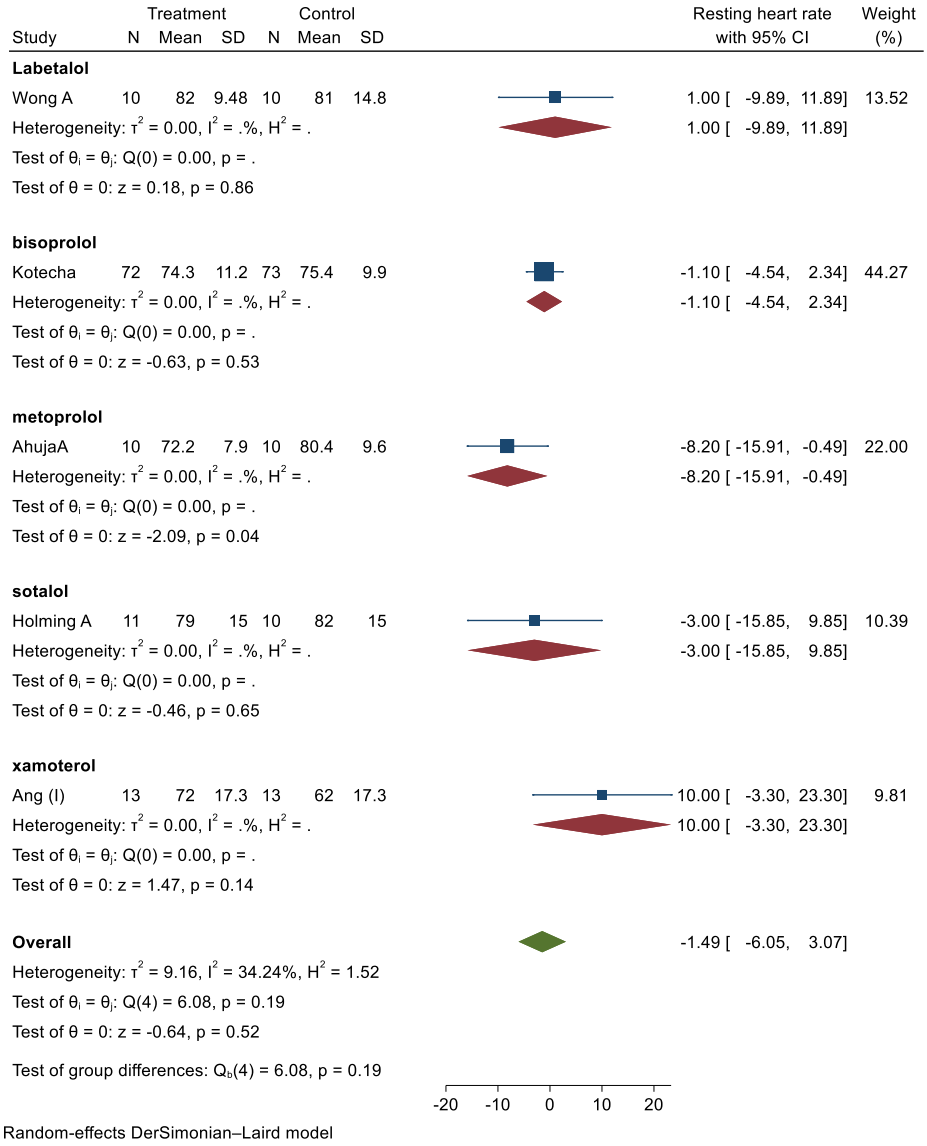


Figure 2f - forest plot by specific beta-blocker used

Maximal exertional heart rate

Meta-analysis of 4 trials showed that beta-blockers on average reduced the maximal exertional heart rate with 33.5 bpm (CI 95% -51.23 – -15.78, P < 0.001). We found substantial heterogeneity through visual inspection of the forest plots, I^2 statistics (91.58%). Tests for subgroup differences according to the beta-blocker used was statistically significant. The effect according to the specific beta-blocker is presented in figure 2g. The evidence was of low certainty and high risk of bias.

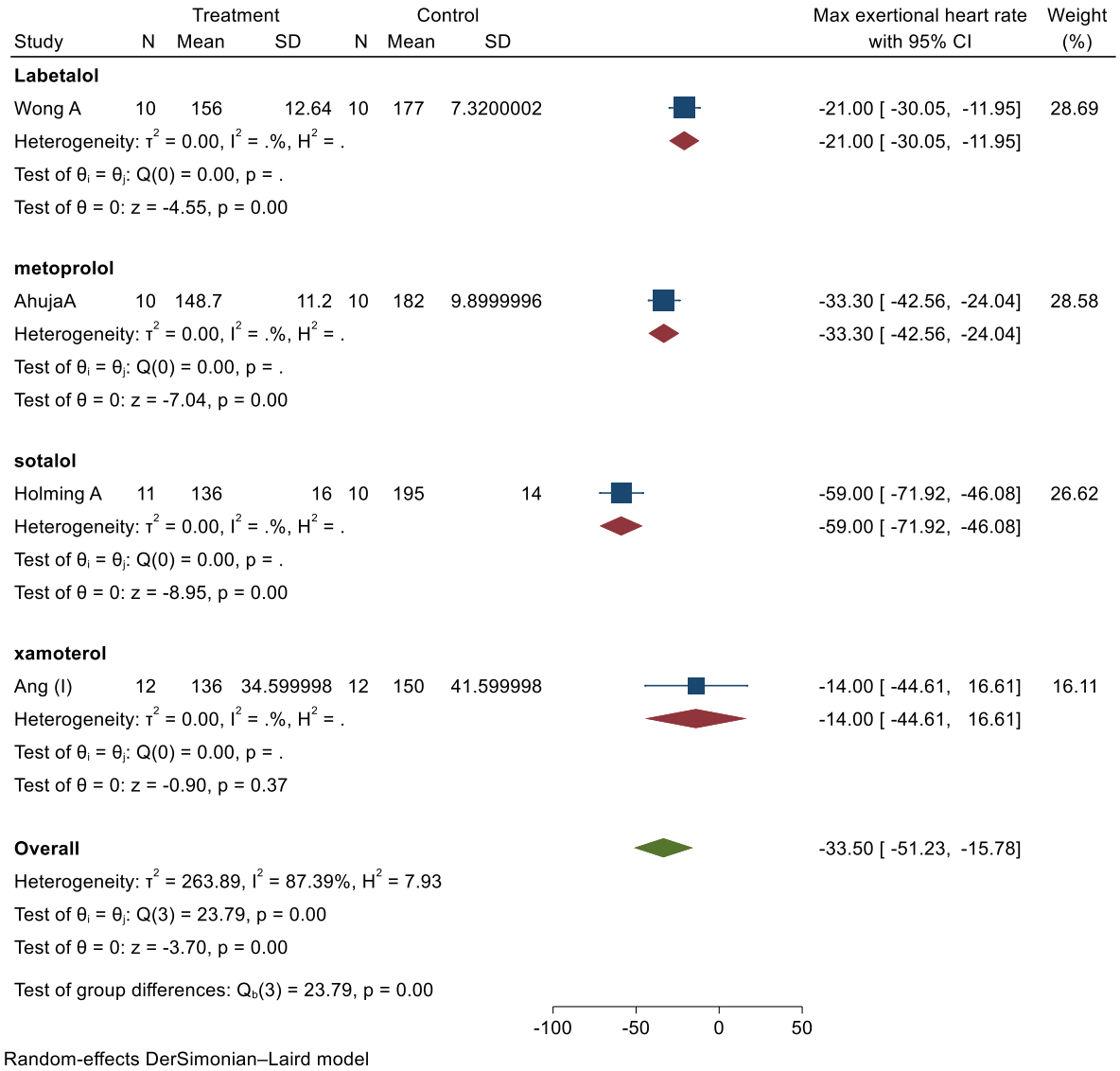


Figure 2g - forest plot by specific beta-blocker used

Exercise capacity

Meta-analysis of 6 trials using standardized mean difference was not statistically different (SMD 0.37 CI 95% -0.01 to 0.74, P value = 0.05). The trials used different ways of assessing exercise capacity and different measuring units.

Calcium channel blockers versus placebo or in addition to another rate controlling agent

Exploratory outcomes

Resting heart rate

Meta-analysis of 6 trials showed that calcium channel blockers on average reduced the resting heart rate with 17.37 bpm (CI 95% -22.22 – -12.53, P < 0.0001). We found no heterogeneity through visual inspection of the forest plots, I^2 statistics (0.00%). Tests for subgroup differences were not significant.

TSA showed that we had information to assess our predefined threshold for minimal important difference (10 bpm = SD/2).

The evidence was of low certainty and high risk of bias.

Maximal exertional heart rate

Meta-analysis of 6 trials showed that calcium channel blockers on average reduced the maximal exertional heart rate with 29.83 bpm (CI 95% -36.49 – -23.18, P < 0.001). We found no heterogeneity through visual inspection of the forest plots, I^2 statistics (0.00%). Tests for subgroup differences were not significant. TSA showed that we had information to assess our predefined threshold for minimal important difference (12 bpm = SD/2). The evidence was of low certainty and high risk of bias.

Exercise capacity

Meta-analysis of 6 trials using standardized mean difference was not statistically different (SMD 0.34 CI 95% -0.06 to 0.73, P value = 0.09). The trials used different ways of assessing exercise capacity and different measuring units.

Calcium channel blockers versus digoxin

Exploratory outcomes

Resting heart rate

Meta-analysis of 5 trials showed that calcium channel blockers on average reduced the resting heart rate with 6.46 bpm (CI 95% -12.16 – -0.77, P = 0.03). We found moderate heterogeneity through visual inspection of the forest plots, I^2 statistics (55%) which could not be resolved. Tests for subgroup differences were not statistically significant.

TSA showed that we had information to assess our predefined threshold for minimal important difference (6 bpm = SD/2). The evidence was of very low certainty and high risk of bias.

Maximal exertional heart rate

Meta-analysis of 4 trials showed that calcium channel blockers on average reduced the maximal exertional heart rate with 21.74 bpm (CI 95% -36.61 – -6.87, P = 0.0042).

We found substantial heterogeneity through visual inspection of the forest plots, I^2 statistics (76.95%). When we removed one trial, the heterogeneity was removed. Calcium channel blockers then on average reduced the maximal exertional heart rate with 16.03 bpm (CI 95% -25.80 – -6.26, P = 0.0013). Tests for subgroup differences were not statistically significant.

TSA showed that we did not have enough information to assess our predefined threshold for minimal important difference (11 bpm = SD/2). The evidence was of low certainty and high risk of bias.

Exercise capacity

Meta-analysis of 4 trials using standardized mean difference was not statistically different (SMD 0.52 CI 95% -0.35 to 1.39, P value = 0.24). The trials used different ways of assessing exercise capacity and different measuring units.

Study 3 – Retrospective cohort study on sex, atrial fibrillation and risk of stroke

Table 3a describes the included population:

Table 3a

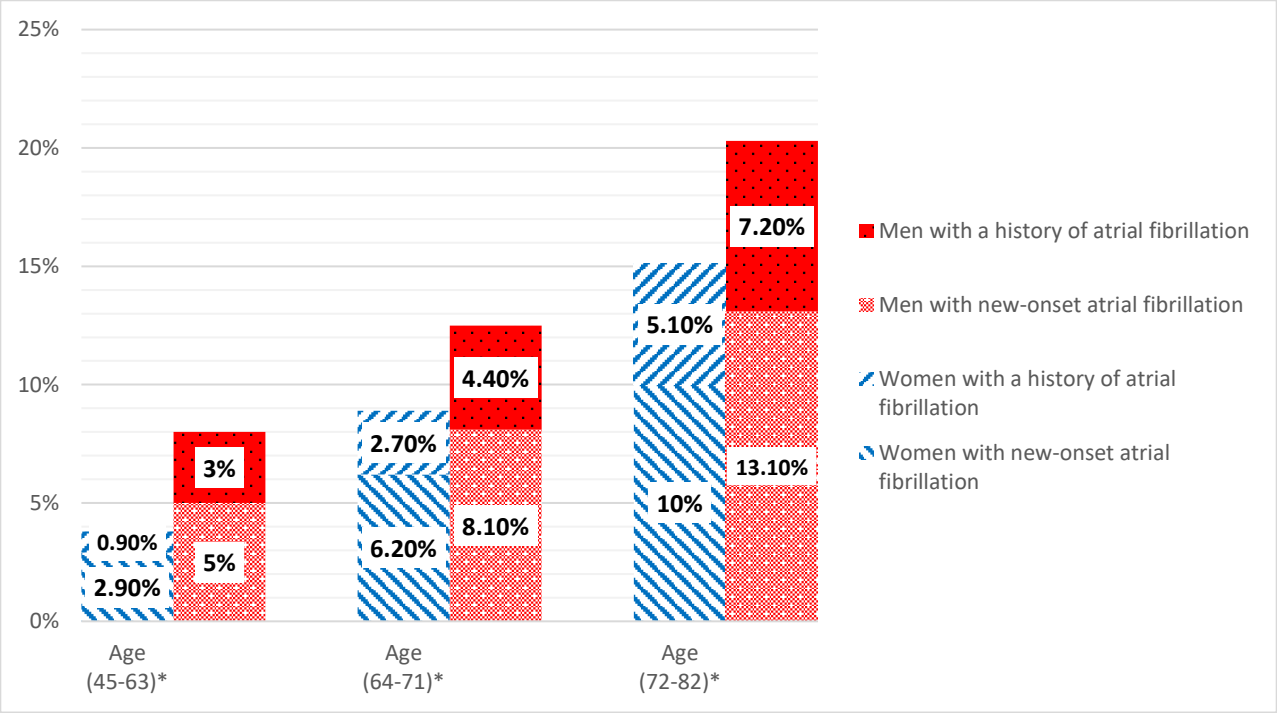
Baseline characteristics	Non-AF (n= 8.851)	New-onset AF (n = 669)	History of AF (n = 342)	Females new-onset AF (n = 323)	Males new-onset AF (n = 346)	Females with a history of AF (n = 147)	Males with a history of AF (n = 195)
Age (years)	66.6 ± 7.0	69.8 ± 6.6	70.3 ± 6.5*	71.0 ± 6.2	68.7 ± 6.7*	72.0 ± 5.8	69.0 ± 6.7*
White	7544 (92.4%)	636 (95.1%)	323 (94.7%)NS	309 (95.7%)	327 (94.1%)NS	140 (95.2%)	183 (93.8%)NS
Losartan	4136 (50.3%)	312 (46.6%)	157 (46.0%)*	152 (47.1%)	160 (46.2%)NS	64 (43.5%)	93 (47.7%)NS
Systolic blood pressure (mmHg)	174 ± 14	177±14	176 ± 14*	178.5	175.5*	176 ± 14	175 ± 14 NS
Diastolic blood pressure (mmHg)	98 ± 10	97 ± 9	96 ± 10*	96.4	97.3 NS	94 ± 10	98 ± 10*
Total cholesterol	6.1 ± 1.1	5.9 ± 1.1	5.7 ± 1.1*	6.2±1.1	5.6±1.1*	5.9 ± 1.1	5.5 ± 1.1NS
Hdl	1.5 ± 0.4	1.5 ± 0.4	1.5 ± 0.4NS	1.6 ± 0.4	1.4 ± 0.4*	1.5 ± 1.4	1.3 ± 3.8*
Creatinine	86.6 ± 19.9	87.7 ± 21.6	94.6 ± 21.9*	81.4 ± 24.1	93.4 ± 17.0*	85.8 ± 18.8	101.1 ± 21.9*
Glucose	6.0 ± 2.2	6.1 ± 2.1	6.4 ± 2.4¤	6.0 ± 2.3	6.1 ± 1.9 NS	6.5 ± 2.8	6.4 ± 2.3NS
BMI (kg/m²)	28.0 ± 4.8	28.1 (5.0%)	27.4 ± 4.6NS	28.4 ± 5.5	27.8 ± 4.4NS	27.4 ± 5.5	27.4 ± 3.7NS
Current smoker	1349 (16.5%)	101 (15.1%)	49 (14.3%)NS	42 (13.0%)	59 (17.1%)NS	15 (10.2%)	34 (17.4%)NS
Ischaemic heart disease	1215 (14.9%)	146 (21.82%)	108 (31.6%)*	55 (17.0%)	91 (26.3%)*	42 (28.6%)	66 (33.8%)NS
Previous MI	472 (5.8%)	57 (8.5%)	40 (11.7%)*	18 (5.6%)	39 (11.2%)*	8 (5.4%)	32 (16.4%)*
Heart failure	108 (1.3%)	21 (3.14%)	37 (3.1%)*	10 (3.1%)	11 (3.2%)NS	21 (14.3%)	16 (8.2%)NS
Stroke/TIA	615 (7.5%)	64 (9.6%)	49 (14.3) *	29 (9.0%)	35 (10.1%)NS	24 (16.3%)	25 (12.8%)NS
COPD	328 (4.0%)	32 (4.78%)	25 (7.3%) ¤	14 (4.3%)	18 (5.2%)NS	6 (4.1%)	19 (9.7%)*
Diabetes	1016 (12.6%)	95 (14.2%)	84 (24.6%) ¤	45 (13.9%)	50 (14.5%)NS	41 (27.9%)	43 (22.1%)NS

In the latter four columns, males versus females for new-onset atrial fibrillation and patients with a history of atrial fibrillation, respectively. * P < 0.05 for a difference between both patients with a history of atrial fibrillation and with new-onset atrial fibrillation compared with patients with no atrial fibrillation. ¤ P < 0.05 for a difference between patients with a history of atrial fibrillation compared with patients with no atrial fibrillation. Latter four columns use t-test/chi squared test.

AF = Atrial Fibrillation. BMI = Body Mass Index, COPD = Chronic Obstructive Pulmonary Disease, MI = Myocardial Infarction, TIA = Transient Ischemic Attack

Participants with a history of atrial fibrillation were older and had more comorbidity. Men with atrial fibrillation had more ischemic heart disease than women, whereas women were older. More men than women had a history of atrial fibrillation as well as developed new-onset atrial fibrillation during the study. The difference between sexes decreased with age which is presented in figure 3a below.

Figure 3a – distribution of atrial fibrillation across ages



Digoxin was the only drug of the ones compared that there was a difference between men and women. The data is presented below in table 3b.

Table 3b. Concomitant therapies

Concomitant therapy	Men with atrial a history fibrillation n = 195		Women with a history atrial fibrillation n = 147		Men with new-onset atrial fibrillation n = 346		Women with new-onset atrial atrial fibrillation n = 323	
	Previous treatment	At study end	Previous treatment[a]	At study end[b]	Previous treatment	At study end	Previous treatment	At study end[b]
K-vitamin antagonist	39 (20.0%)	85 (43.6%)	30 (20.4%)[NS]	64 (43.5%)[NS]	14 (4.1%)	149 (43.1%)	7 (2.2%)[NS]	134 (41.5%)[NS]
Aspirin, clopiodgrel, dipyridamole, ticlide	4 (2.1%)	13 (6.7%)	1 (0.68%)[NS]	10 (6.8%)[NS]	1 (0.3%)	22 (6.4%)	1 (0.3%)[NS]	21 (6.5%)[NS]
Beta-blocker	63 (32.3%)	52 (26.7%)	48 (32.6%)[NS]	50 (34.0%)[NS]	112 (32.4%)	150 (43.4%)	101 (31.3%)[NS]	146 (45.2%)[NS]
Digoxin	98 (50.3%)	104 (53.3%)	88 (59.9%)[NS]	92 (62.6%)[NS]	15 (4.3%)	138 (39.9%)	24(7.4%)[NS]	158 (48.9%)[¤]
Verapamil	14 (7.2%)	9 (4.6%)	9 (6.1%)[NS]	17 (11.6%)[¤]	7 (2.0%)	28 (8.1%)	9 (2.8%)[NS]	28 (8.7%)[NS]
Diltiazem	6 (3.1%)	18 (9.2%)	9 (6.1%)[NS]	11 (7.5%) [NS]	25 (7.2%)	32 (9.3%)	13 (4.0%)[NS]	33 (10.2%)[NS]
Class IA antiarrhythmic drug	9 (4.6%)	10 (5.1%)	9 (6.1%)[NS]	12 (8.2%)[NS]	2 (0.6%)	7 (2.0%)	0 (0%)[NS]	5 (1.6%)[NS]
Class IC antiarrhythmic drug	7 (3.6%)	6 (3.1%)	5 (3.4%)[NS]	6 (4.1%)[NS]	1 (0.3%)	16 (4.6%)	1 (0.3%)[NS]	15 (4.6%)[NS]
Class III antiarrhythmic drug	2 (1.0%)	12 (6.2%)	2 (1.4%)[NS]	8 (5.4%)[NS]	1 (0.3%)	25 (7.2%)	0 (0%)[NS]	16 (5.0%) [NS]

[a] Statistical significance compared with previous treatment in men

[b] Statistical significance compared with treatment at study end in men

[¤] P <0.05

Results of cox regression

Both participants with a history of atrial fibrillation and participants with new-onset atrial fibrillation had higher risk of our outcomes compared with participants without atrial fibrillation. Data are presented in table 3c.

Table 3c. Hazard ratios comparing a history of atrial fibrillation/new-onset atrial fibrillation with no atrial fibrillation.

End point	No AF (n = 8182)		History of AF (n = 342)	New-onset AF (n = 669)			Unadjusted History of AF	Adjusted history of AF	unadjusted new-onset AF	Adjusted new-onset AF	P value new-onset AF versus a history of AF
	Rate (per 1000 years)	n (%)	Rate (per 1000 years)	n (%)	Rate (per 1000 years)	n (%)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	
Stroke	9.7	391 (4.8%)	34.5	54 (15.8%)	29.6	94 (14.1%)	3.54 (2.67 – 4.71)	2.64 (1.95 – 3.58)	3.05 (2.43 – 3.82)	2.31 (1.81 – 2.95)	P = 0.456
All-cause mortality	16.0	639 (7.8%)	50.2	79 (23.1%)	29.6	96 (14.3%)	3.19 (2.52 – 4.03)	1.96 (1.52 – 2.52)	1.86 (1.50 – 2.30)	1.33 (1.05 – 1.67)	P = 0.016
Composite of death, MI, and stroke	20.8	833 (10.2%)	64.5	101 (29.5%)	50.4	160 (23.9%)	3.13 (2.55 – 3.85)	2.16 (1.73 – 2.70)	2.45 (2.07 – 2.90)	1.86 (1.55 – 2.23)	P = 0.270

Hazard ratios for stroke, all-cause mortality and the composite outcome comparing 1) a history of atrial fibrillation with no atrial fibrillation, and 2) new-onset atrial fibrillation with no atrial fibrillation. The multivariate analysis was adjusted for treatment allocation, age, sex, systolic blood pressure, cholesterol, body mass index, smoking, diabetes, history of transient ischemic attack/stroke, previous MI, and history of heart failure. There was no interaction term.

AF = atrial fibrillation. MI = Myocardial infarction.

Participants with a history of atrial fibrillation

The risk of all-cause mortality (HR 1.96, CI 95% 1.52– 2.52), stroke (HR 2.64, CI 95% 1.95–3.58) and the composite cardiovascular outcome of cardiovascular death, non-fatal stroke, and myocardial infarction (HR 2.16, CI 95% 1.73 – 2.70) were all higher for participants with a history of atrial fibrillation. In the adjusted models, there was no interaction between sex, age, and a history of atrial fibrillation. The results of the test for interaction are presented in the supplementary appendix of article 3.

Participants with new-onset atrial fibrillation

The risk of all-cause mortality (HR 1.33, CI 95% 1.05 – 1.67), stroke (2.31, CI 95% 1.81 – 2.95) and the composite cardiovascular outcome (HR 1.86, CI 95% 1.55 – 2.23) were all significantly higher for participants with a history of atrial fibrillation. In the adjusted models, there was significant interaction between sex and new-onset atrial fibrillation on the risk of mortality, stroke and the composite outcome and of age and new-onset atrial fibrillation on the risk of mortality, stroke and the composite outcome (tables included in supplemental appendix of article 3).

Stratified cox regression of participants with new-onset atrial fibrillation

In stratified analysis only including participants with new-onset atrial fibrillation, the point estimate for the risk of stroke was higher for women than men but the result was not statistically significant (HR 1.52, CI 95% 0.95 – 2.43).

When further stratifying for sex, the risk of stroke was higher for women above 64 years of age than below. The same tendency was not seen for men, where the risk of stroke was lower with increasing age. These two factors together contributed to the increased risk of being a women compared to being a man with increasing age. Table 3d and table 3e below summarizes the results.

Table 3d. Age stratified adjusted HRs comparing incidence rates of stroke in females and in males with new-onset atrial fibrillation or history of atrial fibrillation

Age tertiles	**Adjusted risk of stroke associated with a new-onset atrial fibrillation in males**	**Adjusted risk of stroke associated with a new-onset atrial fibrillation in females**	**Adjusted risk of stroke for females versus males with new-onset atrial fibrillation**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Age 55*– 63	2.63 (1.19 – 5.86)	0.87 (0.12 – 6.18)	0.16 (0.01 – 2.01)
Age 64 – 71	1.94 (1.12 – 3.35)	5.28 (3.01 – 9.08)	1.54 (0.73 – 3.22)
Age 72 – 82	1.10 (0.58 – 2.10)	2.39 (1.55 – 3.69)	1.86 (0.84 – 4.10)
Age tertiles	**Adjusted risk of stroke associated with a history of atrial fibrillation in males**	**Adjusted risk of stroke associated with a history of atrial fibrillation in females**	**Adjusted risk of stroke for females versus males with a history of atrial fibrillation**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Age 55* – 63	1.71 (0.53 – 5.89)	Too few events	-
Age 64 – 71	1.89 (0.96 – 3.73)	1.18 (0.35 – 4.00)	-
Age 72 – 82	2.65 (1.43 – 4.92)	4.14 (2.53 – 6.79)	-
Hazard ratios for stroke stratified according to age. Multivariate analysis was adjusted for treatment allocation, systolic blood pressure, cholesterol, body mass index, smoking, diabetes, history of transient ischemic attack/stroke, previous myocardial infarction, and history of heart failure. *There were under 100 participants between 45-55 who were protocol violators and were included.			

Table 3e. Age stratified HRs comparing incidence rates of composite cardiovascular outcome in women and in men with new-onset AF or a history of atrial fibrillation

Age tertiles	Adjusted risk of composite cardiovascular outcome associated with new-onset AF in men	Adjusted risk of composite cardiovascular outcome associated with new-onset AF in women	Adjusted risk of composite cardiovascular outcome for women versus men with new-onset AF
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Age 45-63	1.79 (1.01 – 3.17)	1.38 (0.35 – 19.36)	0.40 (0.11 – 1.52)
Age 64-71	1.76 (1.19 – 2.61)	3.92 (2.49 – 6.17)	0.91 (0.51 – 1.62)
Age 72-82	1.29 (0.86 – 1.93)	1.74 (1.23 – 2.45)	1.11 (0.64 – 1.93)
Age tertiles	**Adjusted risk of composite cardiovascular outcome associated with a history of AF in men**	**Adjusted risk of composite cardiovascular outcome associated with a history of AF in women**	**Adjusted risk of composite cardiovascular outcome for women versus men with a history of AF**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Age 45-63	1.19 (0.52 – 2.74)	2.59 (0.35 – 19.35)	-
Age 64-71	1.80 (1.12 – 2.90)	1.65 (0.70 – 3.88)	-
Age 72-82	2.52 (1.64 – 3.84)	3.05 (2.05 – 4.55)	-
Hazard ratios for composite cardiovascular outcome stratified according to age. Multivariate analysis was adjusted for treatment allocation, systolic blood pressure, cholesterol, body mass index, smoking, diabetes, history of transient ischemic attack/stroke, previous myocardial infarction, and history of heart failure. AF = Atrial fibrillation.			

Stratified cox regression of participants with a history of atrial fibrillation

In participants with a history of atrial fibrillation, the overall risk in stroke was similar among males and females (HR 0.88, CI 95% 0.5 – 1.6). The risk of stroke increased with age in both males and females. Similar results were seen for the composite cardiovascular outcome. The results are presented in the table 3d and table 3e above.

Study 4 – Cross-sectional study of the physical activity paradox

A summary of the main characteristics of the population is given in table 4a below.

Table 4a: Characteristics of the study population, exposures and outcome.

Variable	N	
Age (years), *mean (SD)*	5304	54.42 (4.2)
Sex, n (%)	5304	
Men		3644 (68.7)
Women		1660 (31.3)
Duration of working life (years), *mean (SD)*	5185	29.2(8.3)
hsCRP (mg/L), *mean (SD)*	5304	1.75 (1.8)
Ton years[1], *mean (SD)*	5185	9.46 (19.16)
Smoking, pack-years, *mean (SD)*	5015	15.65 (22.4)
Alcohol consumption, units/week, *mean (SD)*	5191	11.95 (12.37)
Body mass index (kg/m^2), mean (SD)	5175	25.9 (4.01)
Chronic diseases[2] , *n (%)*	5247	
No disease		1792 (34.2)
1 disease		1792 (34.2)
2 or more diseases		1663 (31.7)
Leisure-time physical activity[3],*n (%)*	5221	
Medium/hard		1718 (32.9)
Light		3020 (57.8)
Sedentary		483 (9.3)
Ton-years, *n (%)*	5185	
0 ton-years		2542 (49.0)
>0-10 ton-years		1282 (24.7)
>10 ton-years		1361 (26.3)

[1] Amount of lifting during working life. One ton-year is lifting 1000 kg/day for a year

[2] Asthma, diabetes, hypertension, angina, stroke, bronchitis, chronic obstructive pulmonary disease, rheumatoid arthritis, osteoarthritis, cancer, anxiety, depression, psychiatric diseases, and back disease

[3] Medium/ hard: > 4 hours a week, light: <4 hours a week, sedentary: reading/watching television in leisure-time.

Before adjusting the result for possible confounding, both low LTPA (27% increase, CI 95% 21% - 35%) and high OPA (23% increase, CI 95% 16% - 31%) were associated with higher hsCRP. Table 4b below is a copy of the table in the draft submitted to BMJ Sportsmedicine with some modifications:

After adjustment, high OPA was still associated with a slightly higher hsCRP (6% increase, CI 95% 0% - 12%) compared to low OPA. Low LTPA was also associated with a higher hsCRP (12% increase, CI 95% 6% - 18%).

Table 4b	**Results of analyses**				
	HsCRP (mg/l)	unadjusted		Adjusted for potential confounders	
	Median (IQR)	Percentage increase in hsCRP (95%CI)*	p-value	Percentage increase in hsCRP (95%CI)	p-value
Model for occupational physical activity					
Low *occupational physical activity*	1.0 (0.5-2.1)	Ref		Ref	
High *occupational physical activity*	1.3 (0.7-2.6)	1.23 (1.16 - 1.31)	<0.0001	1.06 (1.00 – 1.12)	0.0477
Model for leisure time physical activity					
Low *leisure time physical activity*	1.2 (0.6-2.4)	1.27 (1.21 – 1.35)	<0.0001	1.12 (1.06 – 1.18)	<0.0001
High *leisure time physical activity*	0.9 (0.5–1.8)	Ref		Ref	

If OPA and LTPA were instead treated as a continuous variable, only LTPA was associated with a change in hsCRP.

Discussion - Search for evidence to improve symptom management and prevent complications in atrial fibrillation

Searching for evidence to avoid stroke and improving the 'A' part of the atrial fibrillation guidelines

This Thesis has approached searching for evidence for improving symptom management and preventing complications using different study types. The basis for the chosen studies has been the European guidelines and gaps in the evidence identified.

One of the key complications to avoid in patients with atrial fibrillation in stroke. This has traditionally been pursued through proper anticoagulation. From a physiological standpoint, the successful achievement of rhythm control should also reduce the risk of stroke. To date, however, the indication for rhythm control has not been a reduction of stroke or improvement of clinical outcome, but only an improvement in symptoms.[1,185] From an epidemiological standpoint, sex has been identified as an effect modifier for the risk of stroke associated with atrial fibrillation.[1] We explored this possible phenomenon in study 3.

Including participants with left ventricular hypertrophy and hypertension, we found that the prevalence and incidence of atrial fibrillation increased more with age in females compared with males. There was no interaction between having a history of atrial fibrillation, age and sex. The event rates for stroke in patients with a history of atrial fibrillation and patients with new-onset atrial fibrillation were similar, depending on choice of analysis method. The risk associated with new-onset atrial fibrillation depended on sex and age with results indicating females below the age of 64 having a lower risk of stroke than males, but females above 64 years of age had a higher risk of stroke. The event rate for men with new-onset atrial fibrillation seemed to fall with age, whereas the risk for women seemed to peak in women aged 64-71, and was lower in women aged 72-82.

Before discussing how this study fits into the current evidence for management of atrial fibrillation, a short description of the limitations and strength of study 3 is warranted.

Strength and limitations of study 3

A strength of the study is the type of participants and the size of the patient population. The participants had hypertrophy and hypertension, two conditions closely related to atrial fibrillation. The length of follow-up was long. As the patient population is rather homogenous, the findings are more likely to be true findings although the external validity to other types of patients with atrial fibrillation may be limited.

There are several limitations to the study. The study was not part of the original plan for study publications of the LIFE trial. The family wise type I error increase with increasing number of studies, and we did not

adjust our p-values. In general with regard to the use of p-values, there are two very different school of thought: One approach allows for formal scientific dismissal of a hypothesis.[186] The contrasting approach states that there is an uncertainty and hence, a rigid p-value threshold cannot be prespecified and solely relied on.[187] These two schools of thought regarding scientific interpretation are important to bear in mind. Whatever approach is taken, it is important that our results be viewed as hypothesis generating and should be confirmed in other studies before being accepted as a true causal relationship.

Another limitation is the lack of echocardiography confirming the diagnosis of left ventricular hypertrophy, which is common by today's standards. However, in approximately 13% of the LIFE patients an echocardiography was performed confirming that most of the LIFE patients had some indication of left ventricular hypertrophy and dysfunction.[188] Another limitation is that the LIFE study was performed 20-25 years ago in a time period where the main anticoagulation was a vitamin-K antagonist. However, this may not be a problem because we saw no indication that the sex difference is related to the anticoagulative treatment. Therefore, we hope our findings can be tested in newer cohorts.

It should also be noted that confidence intervals in general were wide probably because of the low number of events in each stratum. Hence, interpretation of the results and the fact that risk for stroke among men with new-onset atrial fibrillation seems to fall age should be done with caution.

There is a risk of misclassification in this study. There are two instances of possible misclassification. The diagnosis of atrial fibrillation may in many cases be missed, especially in case of paroxysmal atrial fibrillation and first be diagnosed after a clinical event. It may require several days of Holter monitoring and patient outpatient visits with ECG to confirm the diagnosis. It may also result in general underreporting of the prevalence and incidence of atrial fibrillation.

The other instance of possible misclassification was that in our analyses, we grouped participants as having new-onset atrial fibrillation from the start, even if they developed it several years after the study start. In our sensitivity analysis, we accounted for this by including new onset atrial fibrillation as a time varying covariate, which resulted in a slightly higher risk of stroke for participants with new-onset atrial fibrillation than those without atrial fibrillation (HR 3.06, CI 95% 2.27 – 4.14). Taken together with the other type of misclassification, it is hard to definitively state what this means for the study results.

Comparing Thesis study results to other studies

Study 3 makes it more likely that the difference in risk are the result of true sex difference vis-à-vis residual confounding. Currently, anticoagulation is the main way to avoid stroke. However, the recent EAST trial found that early rhythm-control reduced the primary composite outcome of death from cardiovascular

causes, stroke, hospitalization for worsening of heart failure or hospitalization for acute coronary syndrome.[53] Interestingly, in subgroup analyses the point estimate for women and older people was more favorable for early rhythm control than men and younger people respectively. There was no combined subgroup to see if older women in particular had increased risk. These results together with the results of study 3 may indicate that there is a specific risk associated with being an older female with atrial fibrillation, and that this risk may be reduced with rhythm control. Considering current clinical practice that tends to favor rhythm control in a younger population, this practice may need to be revisited.

The relationship between favorable outcome of early rhythm control may also be particular important, since a recent prospective cohort study using the PREFER registry including 6412 patients found that women receive less rhythm control despite being more symptomatic.[189]

Considering the possible mechanism behind the apparent difference in risk of stroke it may be important to look at the whether the finding only holds true for stroke. Study 3 suggests that this is a particular interaction between age, sex and atrial fibrillation for stroke but also a somewhat similar interaction for the composite cardiovascular outcome including cardiovascular death, stroke, and myocardial infarction. A meta-analysis of both randomized trials and cohort studies by Marzona et al found that in cohort studies there was also an increased risk CV death among women with atrial fibrillation whereas in randomized trials there was a decreased risk.[190] This risk, however, seemed to be tied to the amount of anticoagulation administered, and the difference disappeared with proper anticoagulation use.[190] Hence, the association with stroke seems the strongest.

As described in the background section, several physiological differences as well as residual confounding may explain the increased risk and the consideration of sex as an effect modifier for the risk of stroke in patients with atrial fibrillation. Differences in sex hormones, structural heart differences, inflammation and fibrosis may account for the differences in stroke risk in older women with atrial fibrillation.[48] In an attempt to target inflammation as a therapeutic target, Sardu et al performed a randomized trial of alpha lipoic acid on atrial fibrillation recurrence after ablation.[191] Despite reducing markers of systemic inflammation, this did not lead to a statistically significant reduction in atrial fibrillation recurrence.[191] A better understanding of the physiological basis of the sex differences seems warranted to better identify the best therapeutic approach.[48]

Searching for evidence for managing symptoms as part of the 'B' part of atrial fibrillation guidelines

Continuing to the B of the A, B, C guidelines, and searching for additional evidence there, both the randomized clinical trial, DanAF, and the systematic review may contribute to additional evidence on how to manage symptoms in atrial fibrillation. The trial is not complete and hence, at the moment the trial does not directly contribute with evidence for the optimal treatment of symptoms in atrial fibrillation. This is a major limitation of the Thesis ability to provide additional evidence for managing symptom in atrial fibrillation. A discussion of the barriers to the conduction of DanAF, randomized trials in general and implications for evidence-based medicine are discussed further below.

As stated in the background section, both a rhythm and a rate control strategy may be chosen. When choosing a rate control strategy, one must both choose a rate control target and the drugs to achieve the target. In the Thesis, the choice of drug for rate control was investigated in Study 2 which was a systematic review with meta-analyses. Several findings were of interest for choosing the rate-controlling drug. Although we included 51 trials, there was very limited data available for assessment of all-cause mortality and serious adverse events. There was also very limited data available for our secondary outcomes quality of life, non-serious adverse events, and symptom scores for all comparisons.

Only for our exploratory outcomes was there sufficient data available for meta-analysis. We found that beta-blockers, calcium channel blockers and digoxin all reduce resting heart rate while beta-blockers and calcium channel blockers reduce maximal exertional heart rate more than digoxin. We found no difference between beta-blockers and calcium channel blockers on both resting heart rate and maximal exertional heart rate in overall analysis. In subgroup analyses, we found that some beta-blockers (carvedilol, bisoprolol, atenolol) may maximal exertional heart rate more than calcium channel blockers whereas metoprolol may reduce maximal exertional heart rate less than calcium channel blockers.

It is uncertain if the reduced maximal exertional heart rate found with using beta-blockers or calcium channel blockers compared with either digoxin, placebo or in addition to another rate controlling drug leads to an improvement in exercise capacity. Beta-blockers may cause a reduction in exercise capacity compared with calcium channel blockers but the certainty of evidence is very low. Subgroup analyses suggest metoprolol and carvedilol reduce exercise capacity the most.

Before moving on to discuss what, if any, the Thesis adds to current guidelines and how our results relate to other studies, the strength and limitations of the systematic review will be discussed.

Our review has several strengths. It follows a predefined methodology described on Prospero including following the 8-step procedure as described by Jakobsen et al.[95] We assessed both the risk of systematic error (bias risk) and the risk of random error by using TSA.[98] It is to our knowledge the first systematic review with meta-analysis assessing all available evidence for the optimal rate-controlling drug.

Our review also has several limitations. There was very little information on our primary and secondary outcomes which were chosen to be patient relevant outcomes compared with our exploratory outcome, which have comparatively less obvious value to the patient (e.g. resting heart rate vs mortality). The p-value for exploratory outcomes was not adjusted as these outcomes were seen as hypothesis generating and not with the goal of basing clinical decisions on them.

The included trials used very different drugs of the same drug class, and different doses of the same drug as well as different doses of co-interventions (often digoxin). Hence, it was not possible to conduct a network meta-analysis and several meta-analyses had statistical heterogeneity. Further, the trials were often conducted using a specific dose. In contrast, usual clinical practice targets a specific heart rate and/or attempts to reduce perceived symptoms of the patient. The doses used in the included trials were often not comparable from a clinical point of view. i.e. one drug in one trial arm might be 50% of the maximal recommended dose of a drug whereas the other drug may be the highest recommended dose, making a comparison difficult. Many of the included drugs are not used for rate control in atrial fibrillation in current clinical practice. However, since this is a systemic review providing overview of the available evidence, these drugs were still included.

The trials also used different ways to assess exercise capacity and this should also be considered when assessing the results where standardized mean difference was used. Are the different tests actually measuring the same thing which is the premise for using standardized mean difference?

Another limitation is that the participants are younger compared to atrial fibrillation patient seen in clinical practice and hence, differences in hemodynamic properties may mean the results are not applicable to many of the patients seen in clinical practice. Only 34% of the included participants were women, and separate reporting of data was not reported, making it impossible for us to identify any sex differences.

Comparing Thesis study results to other studies

The outcomes from the systematic review with meta-analysis that were best for making clinical decisions had very limited data available. Despite the choice being of interest for treating patients with atrial fibrillation, it seems different barriers exist for conducting randomized trials to be able to assess these crucial endpoints.

Although not directly science, but still important if the scientific community wishes to conduct randomized trials to answer clinical questions, are the surrounding logistics, regulatory requirements and general barriers to conducting randomized clinical trials. In DanAF, many different barriers were encountered, which may help explain why so few trials of sufficient size are conducted. Using DanAF as an example, different barriers to conducting randomized clinical trials will be covered in the context of evidence based medicine (EBM).

Barriers to conducting randomized clinical trials

Several barriers exist to achieve EBM and conduction of randomized trials in particular.[192] For this Thesis, EBM refers to the concept of clinical decision-making besides being based on patient preferences and physician experience, should rely on scientific studies considering the evidence hierarchy as the basis for judging inferential powers.[193] The same hierarchy also is the basis for the evidence level attributed to the recommendation in guidelines.[1] It may be that this hierarchy can be supplemented or reconsidered in the future, but currently, this is the basis of the Thesis and a discussion of whether the classical evidence hierarchy should be modified is outside the scope of this Thesis.[194] DanAF encountered several barriers. The barriers described below in DanAF will be compared to other studies in the literature describing barriers to conducting clinical trials.

Barriers to recruitment for randomized clinical trials

An important aspect of randomized trials (and other study designs) is recruitment.[195] In a systematic review by Fletcher et al, including 8 quantitative studies and 11 qualitative studies aiming at increasing recruitment, several barriers were identified. Eight themes were extracted from the qualitative studies.[195] Communication (between clinician and both patients and the trial coordinator), perceived patient barriers, and possible impact of attempting recruitment on patient-clinician relationship were important barriers. Two additional important aspects with particular relevance for DanAF, were also identified by Fletcher et al: Possible effect of the intervention on the patient and effect of the trial on clinical practice.[195] The possible negative consequence for the individual patient had to be weighed against the possible positive effect for the future group of patients as a whole. The impact of these two aspects are covered below in DanAF.

Possible effect (both beneficial and harmful effect) of the intervention using DanAF as example

It became evident that inclusion of patients with atrial fibrillation who also had heart failure was harder than expected at some sites, but not all. There were two problems. Some outpatient physicians considered it unethical to include heart failure patients with reduced ejection fraction, as they considered beta-blockers first line therapy irrespective of atrial fibrillation. This is despite that the evidence for beta-blockers for patients with atrial fibrillation based on the results from the individual patient data meta-

analysis by Kotecha et al showing there seems to be no benefit from beta-blockers for patients with heart failure and atrial fibrillation.[148] Further, it was deemed by the attending physicians that most heart failure patients should either have a heart rate above 80 or below 80 (based on the physiological considerations concerning cardio output described in the introduction) and therefore were not eligible to be randomized in DanAF. Besides the physiological rationale, a recent observational study by Hess et al also supported a lower rate for heart failure patients.[196] However, since the study was observational in nature, the results may be the result of confounding and the authors recommended high quality trials of rate control and specific agents.[196] In contrast, a different registry study by Song et al in 11,104 patients in Korea, found the optimal heart rate to be 88 bpm in participants with heart failure with preserved ejection fraction on death or hospitalization and provided a physiological rationale for the lack of benefit of beta-blockers and lower heart rate.[197]

This recruitment problem highlights several important considerations when designing a randomized trial: Evidence can be interpreted differently, as well as clinicians may differ in their opinion on whether evidence from randomized trials or consideration based on physiological knowledge and clinical experience should be the dominating framework for making treatment decisions.[198] It is therefore important that the question being asked by a clinical trial takes current practice into consideration and it incorporates the physiological logic(s) behind how the different intervention might work. In hindsight, we should have made sure that there were no reservations among the cardiologists at any of the participating sites before initiating the trial, as this would have made it abundantly clear that our capacity for inclusion would not be satiated with patients from the cardiology department alone. It may be that our research question should have been framed differently to accommodate current practice, especially concerning participants with heart failure and the physiological theory behind a higher or lower rate.[79,198] One could also argue that we from the start should have focused more on recruitment from outside the hospital, where patients with atrial fibrillation in general have less comorbidity.

Disruption of clinical practice

During the trial, it became apparent that at some participating sites, patients with uncomplicated atrial fibrillation where a rate control strategy was chosen were not followed at the hospital, contrary to patients where rhythm control was chosen. Further, if a patient was admitted to the ward with atrial fibrillation with a high ventricular rate (>110 bpm), they would at some participating sites usually leave with a rate around 60 – 70 bpm and hence, not be eligible for DanAF, unless the patient was first reduced in rate control medication. It was not feasible to recruit them during their admission to the hospital ward, as it would disrupt the flow of the daily clinical work as has been a barrier previously identified.[199] Many considerations

had to be made to reduce the rate-controlling drug including a thorough discussion with the patient. This was a time-consuming affair and in many cases ultimately did not result in additional recruitment.

Regulatory barriers to conducting randomized clinical trials

Depending on the type of trial, trialists may require approval from the regional ethics committee, the Danish Medicines Agency, and the participating Regions as well as the central Danish Data Protection Agency. When conducting a multicenter randomized trial ruled by the law of the ethics committee, if any of the following changes are made to the protocol, the ethics committee must approve the change before they can be implemented: A new site, changes to recruitment material, measures to increase recruitment, new funding, and changes to inclusion or exclusion criteria. This list is not exhaustive, but these are very common measures that need to be adapted as a trial progresses. This administrative burden means conducting randomized trials, especially multicenter randomized trials require great resources as well as a longtime frame to accommodate the wait time from submitting an application to receiving approval.

Delay due to regulatory approvals

During our trial, as it is for many trials, it became necessary to make amendments to our original protocol. On 23.12.21 we submitted an amendment to the regional ethics committee (version 2.01). The main focus of the amendments was to increase recruitment. The three main changes/additions we wished to make were:

1) We needed the ability to contact potential candidates in a non-intrusive way. On site screening was only made the cardiology department. However, if we had the ability to send 'E-boks' (an electronic, secure mail delivery system, commonly used to communicate information from public institutions such as the hospital) we would be able to contact an even larger audience, since we could also contact patients in other hospital wards. This had the further advantage that these patients typically not had been seen by a cardiologist for some time and the patients could benefit from additional optimization of therapy besides rate control therapy.
2) We needed to be able to make advertisements in local newspapers, through patient advocacy groups and social media.
3) We needed to be able to recruitment from general practitioners. It became clear that many of the sites who were part of the trial did in fact not see patients who could be included (please see further above). Recruiting from general practice had the further advantage of increasing our external validity to patients who are not followed in the cardiology department.

In March 2022 we submitted another amendment, where we requested that Bispebjerg – Frederiksberg be added as a site.

Unfortunately, the regional ethics committee first approved the changes in October 2022. This large delay in regulatory approval, made it very hard to adapt to any oversights on our behalf as well as any developing events that required change.

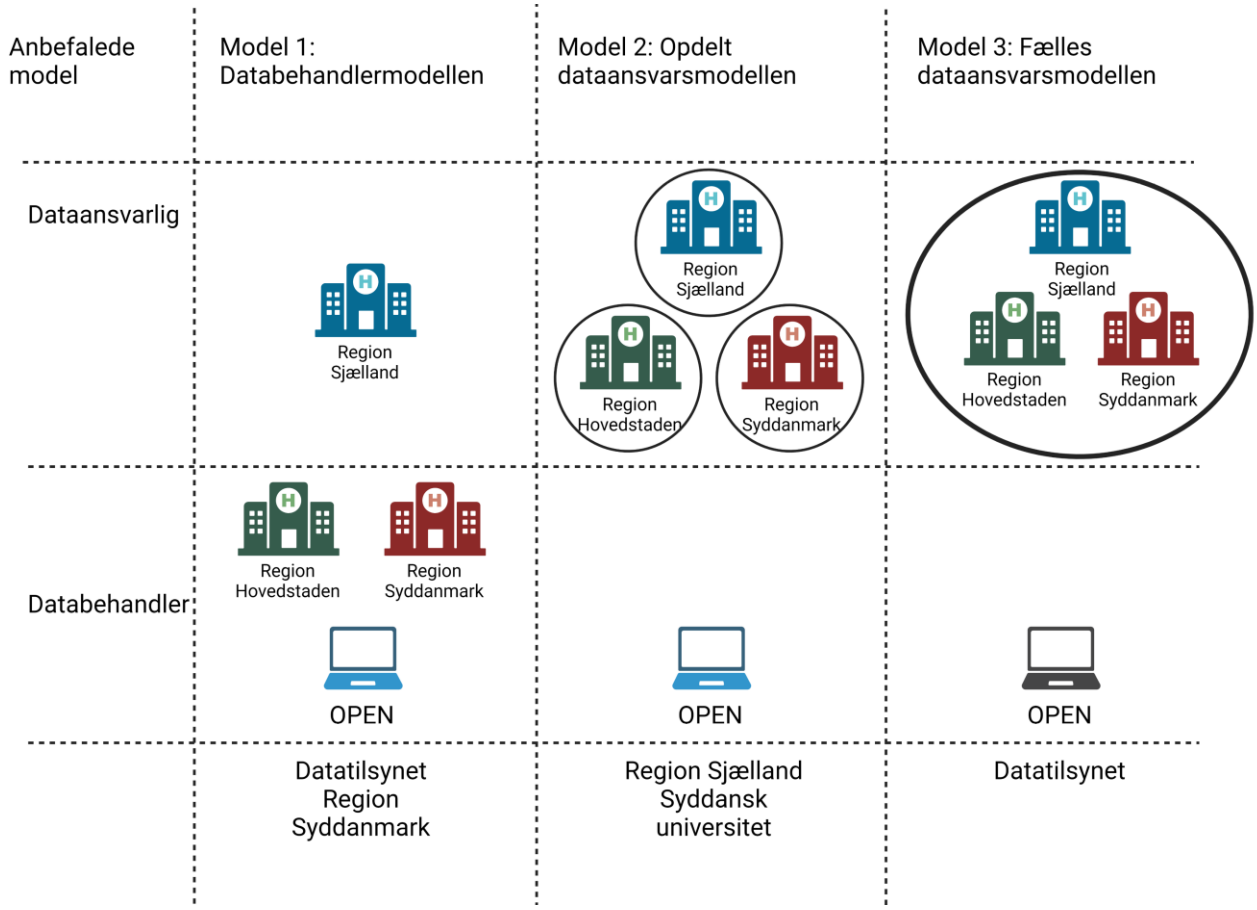
Abstract presented at the Danish congress for public health medicine 2021.

During 2021, I presented a poster at the Danish congress for public health medicine. The aim of the poster was to convey the difference in legal opinion across different Region in Denmark as well as the central Danish Data Protection Agency.

Initially, the trial was granted permission to handle data under the assumption of model 1 in December 2020. However, during the legal procedure to establish a contract between Region Zealand and Region of Southern Denmark, the judicial office at the University of Southern Denmark, who previously advised Region Zealand on legal matters, became aware of our data construction model. Despite me telling the lawyer that the model was approved by the lawyers at Region Zealand, she demanded we change model to model 2 and contacted Region Zealand to inform them that they had to change model. After much correspondence and change in the wording (but ultimately, patients felt no difference), we were now approved using model 2 in Region Zealand and Region of Southern Denmark.

Because we in the trial use a biobank, we then needed approval from the central Danish Data Protection Agency to transfer biological material from one region to another. They then commented on the new model we used (model 2) and said, we should reconsider either switching to model 1 or to model 3. Model 3 was never considered in option, as a consensus paper between all the different region and the umbrella organization for all the regions recommended strongly against model 3.

Ultimately, the Danish Data Protection Agency approved the transfer of biological material from the Region of Southern Denmark to Region Zealand.



Additional general considerations for the initiation of trials

Guidelines are usually the foundation for clinical practice, although ultimately the treatment is determined by the treating physician in the optimal world in consultation with the patient.

Several barriers to the conduct of DanAF were identified above. The systematic review highlighted the lack of trials providing data on important patient related outcomes. Several other consideration may come into play even before the trial is initiated:

1) Is there really need of a randomized clinical trial? E.g. don't we already know that beta-blockers and calcium channel blockers are more efficient than digoxin for reducing exertional heart rate? In the case of this particular question, it seems we already did. But the systematic review raises an important question regarding exercise capacity and the relationship with heart rate control. However, there are many treatment questions that we believe we know the answers to based on physiological reasoning and clinical experience, but once a randomized trial has been conducted, it

may turn out that the trial does not support the physiological reasoning or the picture was incomplete.[200,201]

2) The effort (time, money, logistics, regulatory requirements) to conduct properly large trials to find differences on mortality. In our systematic review, most trials focused on surrogate outcomes such as heart rate instead of patient relevant outcomes such as mortality or serious adverse events. Likewise, in DanAF, we did not attempt to find superiority in terms of mortality as the number of participants required in a single randomized trial is very large. The effort may be even larger when conducting a complex intervention as the one in DanAF, where there is a target, but many ways to achieve the target vis-à-vis a fixed dose trial. The design of DanAF is in contrast to most of the trials included in the systematic review, which compared fixed doses of a drug. Although DanAF is not the most complex intervention, the design allows for physician variation at the same site, and variation among sites. Administered drugs may also depend on the drugs the patient is already receiving and co-morbidity. The obvious advantage of this design is that it mimics real clinical practice. Real clinical practice is heterogeneous and any difference identified between groups allow for robust conclusions. On the other hand, the risk of non-compliance increases. In DanAF in particular a significant risk to the trial results is if the difference in heart rate on average is not sufficient large enough. If e.g. too many participants have a heart rate of 81 when they enter the study on no rate controlling medication, the actual difference in heart rate will be small. If there is a difference, it may also be more difficult to assess what component of an intervention that contributed to the effect seen.
3) Rhythm control intuitively must be better than rate control and hence, is more interesting. If one looks at the amount of trials assessing rhythm control, there seems to be an overweight of trials despite 40-50% of patients are treated with rate control.[202,203] This is also a view from a funding perspective, where rejection of our proposals have been argued from the perspective of "what is new?". This is unfortunate as only one non-superiority trial for a so prevalent disease with a key management question, seems insufficient.

Ultimately, one must also consider the trade off and synergy between research and treating patients: If one treats with a treatment with no effect, then it is a waste of resources. If one only does research, but the results are not implemented in practice or the resources to treat accordingly are insufficient, then the research is a waste of resources. The ultimate goal must be to improve quality of life and decrease mortality and morbidity. That is why it must also be considered what study design to use according to where we are in the process of understanding a phenomenon. In the case of the higher risk of stroke among women with atrial fibrillation, the reason has not yet been identified. Our study adds weight to the

idea of a biological difference but the study needs to be replicated. The same consideration should be made with regard to the physical activity paradox. Ultimately, it may make sense to conduct a randomized trial in terms of a preventive strategy but not currently.

Comparing Thesis study results to other studies concerning choice of rate controlling drug and optimal heart rate target

Currently, beta-blockers are first line therapy for rate control in atrial fibrillation, both for patients with heart failure and without, whereas calcium channel blockers are only for patients without heart failure.[1] Digoxin is second line therapy.[1] There are two questions that must be answered with regard to choice of rate controlling agent: Which drug is better for short term (and perhaps long term) exercise capacity, cardiac output and quality of life and which drug is better for long term hard outcomes such as heart failure and mortality. Achievement of 'optimal' heart rate control must consider both aspects. Consideration towards specific patient populations and comorbidity must also be considered.

Considering first the optimal drug in terms of heart failure and death, many physicians will prescribe beta-blockers to all patients with atrial fibrillation that need heart rate control as they are considered first-line therapy in heart failure improving survival.[58] However, Kotecha et al showed in an individual patient data meta-analysis including 18254 patients of which 3066 had atrial fibrillation at baseline that while there is a 27% reduction in all-cause mortality for patients in sinus rhythm, there is no prognostic benefit on mortality of beta-blockers versus placebo for patients with atrial fibrillation.[59] Important to note patients were not randomized to achieve heart rate control but rather for the indication of beta-blockers in heart failure to improve prognosis.

Calcium channel blockers may be considered in atrial fibrillation without heart failure with reduced ejection fraction. Calcium channel blockers are not recommended in atrial fibrillation patients with heart failure with reduced ejection fraction primarily because of the results from the Multicenter diltiazem postinfarction trial which found an increased risk of congestive heart failure compared with placebo in patients with peri-infarction left ventricular dysfunction especially in those with reduced ejection fraction.[1,204] Physiologically the negative inotropic action and perhaps neurohormonally induced changes have been suggested as a mechanism.[204] In observational studies, beta-blockers have been associated with the lowest risk compared with no rate control medication, and digoxin the highest.[205,206] However, it seems plausible that this was a result of residual confounding.[1]

One trial to highlight is the Rate AF trial randomizing 161 participants to either digoxin or bisoprolol found no difference on SF-36 physical component score.[69] The mean dose of digoxin was 161 ug per day whereas the mean dose of bisoprolol was 3.2 mg per day. The achieved resting heart rate was similar (75.4 for

digoxin and 74.3 for bisoprolol). There was also no difference on 6 minute walking distance, although the point estimate favored digoxin (geometric mean ratio 1.1 (0.9 – 1.3), P value 0.25).[69] There was a significant increased number of adverse events in the bisoprolol group compared with the digoxin group.[69] This may suggest that despite no effect on quality of life, there are real adverse effects associated with bisoprolol that may favor the use of digoxin over bisoprolol if lenient rate control can be achieved.

Taken together, direct evidence on hard outcomes for optimal rate controlling drug is limited although the results of the Rate-AF trial may suggest that digoxin should be consider in a wider group of patients. Study 2 confirms the lack of evidence for hard outcomes.

In terms of effect on exercise capacity, in line with the results of study 2, Palau et al showed in a randomized, cross-over trial that withdrawal of beta-blocker blockade in 52 patients with heart failure with preserved ejection fraction and atrial fibrillation lead to increased peak VO_2.[207] 19% of participants had atrial fibrillation. Careful consideration must be taken to generalize the results to atrial fibrillation and how the physiology fits atrial fibrillation and age-related changes in the myocardium, but this study could be taken as some additional indication that beta-blockers may reduce exercise capacity.[207]

Considering indirect evidence from the acute setting, Martindale et al performed a systematic review of beta-blockers versus calcium channel blockers for acute rate control in atrial fibrillation.[208] Including two studies comparing IV metoprolol with IV diltiazem with a total of 92 patients, they found a 1.8 (95% CI 1.2 – 2.6) higher chance of achieving rate control (either ventricular rate below 100 bpm or 20% reduction in ventricular rate), with IV diltiazem.[208] Although the acute setting is not directly comparable, it does perhaps hint at a place for calcium channel blockers if heart rate control is urgent. This could e.g. be the case, in a newly diagnosed atrial fibrillation patient seen in the outpatient clinic/emergency department that has a high ventricular rate without signs of heart failure, but who is distressed. Here it may be paramount to achieve fast heart rate control thereby avoiding hospitalization. However, an observational study by Atzema et al conducted in 24 emergency departments, found that beta-blockers were superior to calcium channel blockers in achieving heart rate control after 2 hours.[209]

Study 2 also showed that digoxin is inferior to beta-blockers and calcium channel blockers in terms of reducing maximal exertional heart rate. The reason behind this is a reduced ability to control the heart rate in the case of high sympathetic drive such as the case of exercise and may be considered reserved for sedentary people.[1] By extension, elderly patients are also included in this consideration.[210] This notion rest on the premise that ventricular rate should also be controlled during movement for optimal cardiac output and exercise capacity. However, the loss of ventricular control during exercise in study 2 did not result in reduced exercise capacity. This brings into question the role of heart rate on cardiac output and exercise

capacity. In an observational study by Song et al found that there was a U-shaped relationship between a composite outcome of hospitalizations and all-cause mortality and heart rate for patients with atrial fibrillation and preserved ejection fraction.[197] A possible physiological explanation was that loss of the atrial kick meant that ventricular filling is only dependent on the passive flow. By reducing the heart rate too much, the additional time for passive filling is not used and the reduced heart rate considering the formula cardiac output = stroke volume x heart rate means a reduction in cardiac output.[197] Likewise, She et al found that exercise capacity was not improved in the group achieving strict rate control during exercise (<110 bpm).[211] Lewis et al similarly did not find a reduced heart rate during exercise was associated with improved exercise capacity in six patients.[212] It may be difficult based on physiology to determine whether digoxin or beta-blockers/calcium channel blockers are better, when it is unclear what heart rate is optimal.

The above discussion illustrates the intimate relationship between atrial fibrillation, heart rate and choice of drug, strengthening the argument for a large trial using a factorial design randomizing both for type of drug and heart rate target if this discussion is to be settled. Likewise, a better documented understanding of the relationship between heart rate, echocardiographic parameters (such as EF, measures of diastolic dysfunction, etc), and atrial fibrillation on cardiac output and the relationship between cardiac output and exercise capacity may improve treatment and perhaps lead to individualized rate controlling plans.

Searching for evidence to avoid stroke and improving the 'C' part of the atrial fibrillation guidelines

Searching for additional evidence for the 'C' part of the atrial fibrillation management, the Thesis explored the evidence for the recommendation for physical activity in atrial fibrillation. As stated in the background section, the physical activity paradox based on epidemiological findings states that the benefit of physical activity depends on the context (and likely the underlying nature of the physical activity). Physical activity is recommended for patients with atrial fibrillation based on among other things expected effects on inflammation and positive remodeling of the heart. Physical activity is recommended both for prevention of atrial fibrillation and when atrial fibrillation develops.

In Study 4 which included a total of 5304 participants found that compared to low OPA, high OPA was associated with increased levels of hsCRP. In contrast, compared to high LTPA, low LTPA was also associated with a higher hsCRP. Both the magnitude and the robustness of the association seemed stronger for LTPA than OPA based on the point estimate and the sensitivity to analysis choice. There was no interaction between LPTA and OPA.

Before discussing the implications of this study, a brief discussion of the strength and limitations of the study is warranted. An important strength of the paper was the use of a job exposure matrix.[116] In the

CAMB cohort, patients were asked in detail about each of their employments during their career.[117,213] The combination of the job exposure matrix and the detail account of previous employments should improve the validity of assessment OPA compared with a self-reported assessment. Still, the exposure was not based on an "objective" measurement and could perhaps be improved further.

An important limitation was that OPA and LTPA were measured in two different ways. OPA was measured over the entirety of a participant's life-time whereas LTPA was current LTPA. A better capture of life-time LTPA would have been preferred.

This study does not explain why there was a difference depending on whether physical activity happened during ones occupation or during leisure time. It may be related to the type, duration and/or intensity of physical activity as well as the amount of rest.[77]

Comparing Thesis study results to other studies

The results of study 4 are compatible with the physical activity paradox being driven by differences in systemic inflammation.[77,213] Since there already is a relationship between atrial fibrillation and inflammation, this may be of particular interest in this population.[1,5] However, given the indirectness of the evidence and the hypothesis generating nature of the study, direct implications for current practice is very limited at this point but the context of physical activity may be important also for atrial fibrillation.

As described in the background section, epidemiological studies have described both as U-shaped, J-shaped, and no relationship at all between LTPA and development of atrial fibrillation.[40-42]

In contrast, the relationship between LTPA and inflammatory response has been described as inverse linear.[214] Possibly, although systemic inflammation is reduced with more LTPA, other mechanisms may come into play when the physical activity level becomes too high such as atrial dilation and a lower heart rate.[215]

Despite LTPA being associated with reduced CRP, direct evidence from randomized trials supporting physical activity remains elusive. In a systematic review from 2017, Risom et al assessed the effects of exercise-based cardiac rehabilitation for adults with atrial fibrillation.[216] They included six randomized trials including a total of 421 participants. They found no benefit on mortality, serious adverse events or quality of life. The evidence was graded a very low to low quality evidence.[216] They did, however, find an increase in exercise capacity.[216]

Much less literature has studied the relationship between OPA and atrial fibrillation. Frost et al assessed the relationship between work related physical activity and the risk of being discharged with a diagnosis of atrial fibrillation or flutter.[217] They used data from the Danish Diet, Cancer, and Health study.[217] They found

that in adjusted analysis compared to primarily sitting sedentary work activity, heavy work-load was not associated with an increased risk of developing atrial fibrillation (HR 1.15, CI 0.36 – 3.70). Work-load was self-reported via questionnaire.[217] Although this study suggests that OPA does not pose a direct risk factor for atrial fibrillation, the wide confidence intervals suggest that this may be due to lack of power.[217] Further, there are limitations to the design including that work exposure was self-reported and not intended to capture accumulated work exposure compared to Study 4.

In contrast, Skielboe et al using data from the Copenhagen City Heart study found that high OPA was associated with an increased risk of atrial fibrillation but there was no relationship between LTPA and risk of developing atrial fibrillation.[42] Differences between the two studies included a broader age group, longer follow-up and no exclusion of participants who were unemployed for more than one year.[42]

Altogether, there is evidence to suggest both that inflammation has a role to play in atrial fibrillation and epidemiological evidence linking differential effects to LTPA and OPA, respectively.

Conclusions

The DanAF trial attempts to help clarify the optimal heart rate target at rest for patients with atrial fibrillation on quality of life. So far, 75 patients from three sites have been recruited. In study 2, we found that there is very limited data on the best rate-controlling drug to prevent all-cause mortality, serious adverse events, or improve quality of life. Beta-blockers and calcium channel blockers seem superior to digoxin in reducing maximal exertional heart rate. It is uncertain if this translates to higher or lower exercise capacity, one of the main reason for lowering exertional heart rate. There seems to be no overall difference between beta-blockers and calcium channel blockers for heart rate control, but subgroup analysis suggest some beta-blockers may reduce heart rate more than calcium channel blockers and some beta-blockers less. In study 3 including participants with hypertension and left ventricular hypertrophy on ECG, only participants with new-onset atrial fibrillation had higher risk of stroke in women than in men, especially in older women. The same relationship was not seen in patients with a history of atrial fibrillation. Finally, in study 4 we found that hsCRP seems to depend on the context of the physical activity, and hence, a difference in systemic inflammation could be the mechanism behind the physical activity paradox.

Perspectives

There are many possible perspectives of this PhD thesis.

The DanAF trial is still progressing. It has brought together five cardiology departments to answer the important clinical question of the optimal heart rate target at rest in atrial fibrillation. This is an important

question for the patient as ensuring the highest quality of life seems unequivocally important. The trial will continue after this PhD. Bispebjerg-Frederiksberg hospital has begun recruitment and Amager-Hvidovre hospital has identified patients for inclusion. We expect more patients to be recruited directly from the general public through our advertisements, which have now been approved. We also expect additional sites from Region of Southern Denmark to begin recruitment. We hope to complete recruitment before June 2024.

Upon successful completion of the trial, it will be important that the results are implemented. It will be important to publish the results in a high impact journal to reach the broadest possible relevant crowd. This will include clinical personal in the cardiology departments but also departments such as geriatrics as well as general practitioners since a large portion of people with atrial fibrillation are only in contact with cardiologist in relation to complications. It will be insufficient just to publish the results. It will be important to reach out to important stakeholders – most notably national and international guidelines makers and make them aware of the publication.

Conducting the trial has made it clear to me that there are many structural barriers conducting clinical research: One barrier is the large heterogeneity between different departments on how to treat patients including the role of physiology vis-à-vis results from randomized clinical trials. I hope this trial will help facilitate future clinical trials within cardiology.

Another important structural barrier is the process of getting approval through the ethics committee and obtaining permission from the participating regional judicial offices. The presented abstract at the Danish conference for public health medicine highlights the difference in legal opinion on how to construct the legal framework to ensure that data is handled responsibly.[218] This difference in legal opinion, however, appears to have little to do with actually protecting the data of the participants, but instead is focused on legal interpretation of the specific language used in the GDPR law.[218] This PhD student can only encourage cooperation and a joint legal opinion from the different Regions and the Danish data protection agency as to not hinder research important for improving the lives of the patients.

The systematic review with meta-analysis showed that the evidence for the different rate controlling drugs is very limited especially for patient relevant outcomes and very heterogeneous regarding the drugs used, the dosage and co-interventions. This makes it prudent to conduct randomized trials such as the Rate-AF trial.[69] It may be worth considering to conduct it as a factorial design using both a lenient and strict rate control strategy since it is currently unclear which treatment strategy is superior with regard to quality of life.

The retrospective cohort study indicated that there seems to be “real” differences between men and women with atrial fibrillation and the differences are not only due to confounding. Ultimately, the goal of management of atrial fibrillation is to reduce the risk of adverse clinical events such as stroke. It will be important to further explore the mechanism behind this sex difference in stroke outcome. This may lead to identification of specific women (and men) who perhaps may benefit from more aggressive anticoagulation; either earlier or more potently. Together with the results of the EAST trial, early rhythm control should perhaps be considered more in elderly women. Or perhaps an enhanced understanding will lead to additional therapeutic targets whose merits will have to be tested in a randomized clinical trial.

The positive association between OPA and the levels of hsCRP is opposite to the negative association seen for LTPA supporting a connection with the physical activity paradox. The paper cannot stand alone as exploration of the other possible explanations to the physical activity paradox is essential.[77] Together with epidemiological studies, the results may impact on what is considered adequate physical activity in terms of prevention of cardiovascular complications for women and men with atrial fibrillation.

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Articles

Article 1 - Protocol for a randomized clinical trial, DanAF

BMJ Open

Lenient rate control versus strict rate control for atrial fibrillation: a protocol for the Danish Atrial Fibrillation (DanAF) randomised clinical trial

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To cite: Feinberg JB, Olsen MH, Brandes A, *et al*. Lenient rate control versus strict rate control for atrial fibrillation: a protocol for the Danish Atrial Fibrillation (DanAF) randomised clinical trial. *BMJ Open* 2021;**11**:e044744. doi:10.1136/bmjopen-2020-044744

► Prepublication history and additional material for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2020-044744).

Received 11 September 2020
Revised 18 February 2021
Accepted 28 February 2021

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ABSTRACT

Introduction Atrial fibrillation is the most common heart arrhythmia with a prevalence of approximately 2% in the western world. Atrial fibrillation is associated with an increased risk of death and morbidity. In many patients, a rate control strategy is recommended. The optimal heart rate target is disputed despite the results of the the RAte Control Efficacy in permanent atrial fibrillation: a comparison between lenient vs strict rate control II (RACE II) trial.

Our primary objective will be to investigate the effect of lenient rate control strategy (<110 beats per minute (bpm) at rest) compared with strict rate control strategy (<80 bpm at rest) on quality of life in patients with persistent or permanent atrial fibrillation.

Methods and analysis We plan a two-group, superiority randomised clinical trial. 350 outpatients with persistent or permanent atrial fibrillation will be recruited from four hospitals, across three regions in Denmark. Participants will be randomised 1:1 to a lenient medical rate control strategy (<110 bpm at rest) or a strict medical rate control strategy (<80 bpm at rest). The recruitment phase is planned to be 2 years with 3 years of follow-up. Recruitment is expected to start in January 2021. The primary outcome will be quality of life using the Short Form-36 (SF-36) questionnaire (physical component score). Secondary outcomes will be days alive outside hospital, symptom control using the Atrial Fibrillation Effect on Quality of Life, quality of life using the SF-36 questionnaire (mental component score) and serious adverse events. The primary assessment time point for all outcomes will be 1 year after randomisation.

Ethics and dissemination Ethics approval was obtained through the ethics committee in Region Zealand. The design and findings will be published in peer-reviewed journals as well as be made available on ClinicalTrials.gov.

Trial registration number NCT04542785.

Strengths and limitations of this study

- First trial assessing lenient versus strict rate control in patients who upon inclusion are considered as having persistent atrial fibrillation. Hence, this trial is expected to provide data on patients who upon inclusion have a relatively short duration of atrial fibrillation.
- First superiority trial with quality of life as primary outcome in patients with both permanent atrial fibrillation and persistent atrial fibrillation on inclusion.
- Pragmatic trial with multiple sites ensuring high external validity.
- Treatment providers are not blinded in a trial that is otherwise expected to have low risk of bias regarding blinding of other domains.
- Trial will not have enough power to assess 'hard outcomes' such as mortality and serious adverse events.

INTRODUCTION

Atrial fibrillation is the most common arrhythmia of the heart with a prevalence of approximately 2% in the western world.[1 2] Atrial fibrillation is associated with an increased risk of death and a number of morbidities.[3–9] The risks of both cerebral stroke and heart failure are increased nearly fivefold in patients with atrial fibrillation, and about 20% of all strokes may be due to atrial fibrillation.[3–8] Atrial fibrillation also has a significant impact on healthcare costs and accounts for approximately 1% of the National Health Service budget in the UK and approximately $26 dollars of annual expenses in the USA.[10 11]

Two different overall intervention strategies may be used for atrial fibrillation: a rhythm control strategy or a rate control strategy.[12–14]

We have previously shown in a systematic review with meta-analysis and trial sequential analysis that rhythm control strategies compared with rate control strategies seem to significantly increase the risk of serious adverse events in

patients with atrial fibrillation.[13 14] Based on current evidence as well as guidelines, it seems that most patients with atrial fibrillation should be treated with a rate control strategy unless there are specific reasons justifying a rhythm control strategy.[13 14]

The resting heart rate target for rate control has recently changed from below 80 beats per minute (bpm) to below 100–110 bpm at rest depending on the guideline.[12 14 15] This change was a result of the the RAte Control Efficacy in permanent atrial fibrillation: a comparison between lenient vs strict rate control II (RACE II) trial, which randomised 614 participants to a lenient rate control strategy (<110 bpm at rest) versus a strict rate control strategy (<80 bpm at rest).[16] The participants were outpatients with permanent atrial fibrillation. The RACE II trial showed that the lenient rate control strategy was non-inferior compared with the strict rate control strategy on the risk of a composite outcome of mortality, stroke, cardiac arrest, arrhythmic events, systematic emboli or major bleeding. Furthermore, the HR of 0.84 (90% CI 0.58 to 1.21) suggested that the lenient rate control group might decrease the risk of the composite outcome. The RACE II trial also showed no difference of the two strategies on quality life, but this analysis has questionable validity.[17]

A theoretical concern when using a lenient control strategy is that patients may develop heart failure if the heart rate is too fast.[18–20] The RACE II trial found that the lenient strategy was also non-inferior for heart failure patients but the majority of the participants had preserved EF at baseline.[21]

We searched the Cochrane Central Register of Controlled Trials, MEDLINE and ClinicalTrials.gov on 26 September 2019. Our literature search identified only the RACE II trial assessing the effect of lenient rate control versus strict rate control in atrial fibrillation. We found no systematic reviews or meta-analyses on the topic.

Trial rationale

Currently, lenient rate control is the guideline recommended initial rate control strategy.[14] However, this recommendation is primarily based on the RACE II trial, which had two major limitations. First, the validity of the RACE II trial results when assessing symptoms and quality of life were questionable mainly because of substantial problems with missing data. Regarding quality of life and symptom severity, only 437/614 (71%) participants had data available at maximum follow-up.[17] Furthermore, the authors did not use multiple imputation or other valid methods to handle the missing data.[22] Second, the RACE II trial only showed a lenient rate control strategy was non-inferior but could not answer if a lenient rate control strategy is superior to a strict rate control strategy. The RACE II trial was not adequately powered to confirm or reject minimal important differences between the two strategies. Conducting a superiority randomised clinical trial and afterwards performing a systematic review with meta-analysis will give us the possibility of confirming or rejecting that there is a difference in effect between the two strategies, at least on quality of life.

Health-related quality of life as an outcome

There are many definitions of health-related quality of life.[23 24] In general, quality of life questionnaires can be designed in two ways.[23] Generic questionnaires assess multiple domains applicable to a variety of health domains.[23] They more readily permit comparison across different disease and seem to have unquestionable patient relevance.[23 25] Generic quality of life scales are often criticised for being less sensitive to change than disease-specific quality of life scales, but when outcome results show no difference, it is most often unknown whether the lack of difference is caused by non-sensitive outcome scales or if the results demonstrate that there is no 'true' difference between the compared interventions when assessing 'generic' quality of life.[23 25] The opposite holds true for disease-specific questions, which in general are thought to be more responsive to change in the clinical condition than generic disease questionnaires but may be less patient relevant. The disease-specific questionnaires tend to focus more narrowly on the disease. Any increase in quality of life as a result of a treatment for a specific disease may be off set by unforeseen negative consequences of the treatment that the questionnaire by design will not capture.

We will therefore supplement the general assessment using Short Form-36 (SF-36) with a disease-specific questionnaire. Currently, there seems to be no optimal questionnaire.[25 26] The Atrial Fibrillation Effect on Quality of Life (AFEQT) is a validated, disease-specific questionnaire, which aims to capture the objective and subjective burden of disease.[27] It contains 20 items that aim to assess four domains: symptoms, activities, treatment concern and treatment satisfaction. It also includes a summary score that summarises the first three domains. It assesses the burden of the atrial fibrillation symptoms.[27 28]

When assessing quality of life, it is important to focus on a minimally important difference, which typically can be done using an anchor-based method or a distribution-based method, or a mix of the two.[29 30] To interpret the clinical significance of future trial results, we will carefully define minimal important differences for all primary and secondary outcomes (see 'Statistical plan and data analyses').[31]

Objectives

Our primary objective will be to investigate the effect of a lenient rate control strategy (<110 bpm at rest) compared with a strict rate control strategy (<80 bpm at rest) on quality of life in patients with persistent or permanent atrial fibrillation.

METHODS AND ANALYSIS

Trial design

The design of the Danish Atrial Fibrillation (DanAF) trial will be a randomised, two-group, superiority trial of lenient rate control versus strict rate control in patients with persistent or permanent atrial fibrillation at inclusion who accept rate control as the main strategy. Treatment providers responsible for the rate control treatment will not be blinded. Any other treatment providers (i.e. those

managing co-morbidities) will be attempted blinded as well as participants.

Three hundred and fifty outpatients will be recruited from four university hospitals in Denmark: Holbaek University Hospital, Hvidovre University Hospital, Region Zealand University Hospital – Roskilde and Odense University Hospital.

The present protocol follows the recommendation in the Standard Protocol Items: Recommendations for Interventional Trials guideline including all items from the WHO Trial Registration Data Set (online supplemental files 1 and 2).

Trial conduct

This trial will be conducted according to good clinical research practice and the latest Declaration of Helsinki.[32 33]

Randomisation

Participants will be randomised 1:1 to a lenient or a strict medical rate control strategy. The trial will use centralised randomisation at OPEN. Prior to the trial, a computer will generate randomisation sequences with varying block sizes between 6 and 10 that are unknown to the investigators. An internet-based randomisation system will be set up conducting randomisation stratified according to site, type of atrial fibrillation at inclusion (persistent vs permanent) and left ventricular ejection fraction (LVEF) (ejection fraction (EF) ≥40% and EF <40%). The randomising investigator will get access to the internet site through a personal password. The randomising investigator will not be an outcome assessor.

Blinding

The investigator prescribing the rate control medication (treatment provider) will not be blinded, as the treatment requires knowledge of the group the participant is randomised to. All other treatment providers, outcome assessors, data managers, statisticians and participants will be sought blinded (the participants will neither be informed of their rate control target nor their allocated intervention group). Blinded data will be sent to OPEN for blinded data management. Statistical analyses will be performed with the two intervention groups coded as 'A' and 'B' by two independent blinded statisticians. Two blinded conclusions will be drawn by the steering group: one assuming 'A' is the experimental group and 'B' is the control group—and one assuming the opposite. Based on these two blinded conclusions, two abstracts will be written (will be published as a supplement to the main publication). When the blinding is broken, the 'correct' abstract will be chosen, and the conclusions in this abstract will not be revised.

As all medical procedures are available to any treatment provider, we cannot foresee any reason for unblinding participants. If, however, any medical personnel deem it necessary to unblind a participant, the participant will be unblinded.

Selection of participants

Inclusion criteria

1. Participants withatrial fibrillation (ECG confirmed and diagnosed by the treatment provider) who at inclusion have either persistent (defined as atrial fibrillation for more than 7 days) or permanent atrial fibrillation (only rate control is considered going forward).
2. Rate control must be accepted as being the primary management strategy going forward. Consideration towards whether rhythm control is more appropriate must be considered, especially given the results of the Early treatment of Atrial fibrillation for Stroke prevention Trial (EAST) .[34]
3. Informed consent.
4. Adult (18 years or older).

Exclusion criteria

1. No informed consent.
2. Initial heart rate under 80 bpm at rest (assessed via ECG before randomisation).
3. Less than 3 weeks of anticoagulation with new oral anticoagulants or 4 weeks with efficient warfarin.
4. Participants dependent on a high ventricular rate to maintain a sufficient cardiac output. This will be based on an individual assessment of the possible participant. Such participants could be participants with heart failure, participants with a haemodynamically significant valve dysfunction or severely dehydrated participants. Other factors such as echocardiographic assessments, stability of the disease and similar will be factored in when judging if a participant is dependent on a high ventricular rate. Such a decision will be made before randomisation by the treatment provider.
5. Participants who are haemodynamically unstable and therefore require immediate electrical cardioversion.

Participant withdrawal

Participants can withdraw his or her consent at any time point for any reason but will be invited to still participate in the follow-up assessments.

Interventions

Lenient rate control

The heart rate will be assessed on a 12-lead resting ECG measured over 1 min after 5 min of rest. The treatment provider will target the highest tolerable resting heart rate <110 bpm. Treatment providers are encouraged not to attempt to lower the heart rate if already below 110 unless symptoms or other reasons necessitates this. If the heart rate is below 90, the treatment provider is encouraged to reduce rate limiting treatment. If the patient remains symptomatic due to atrial fibrillation after achieving this definition of heart rate control, Holter monitoring or exercise tests may be deemed necessary by the treatment provider.

These evaluations may be followed by adjustment of rate control drugs, rhythm control (electrical cardioversion, arrhythmia surgery and rhythm control medications)

or atrioventricular node ablation. In case of the need for rhythm control or atrioventricular node ablation, the allocated heart rate target is no longer relevant in management.

Strict rate control

Strict rate control achieved by using rate control medication (see further) will be defined as a mean resting heart rate <80 bpm with a general recommendation of targeting 70 bpm on a 12-lead resting ECG measured over 1 min after 5 min of rest. Exercise test to determine activity heart rates or Holter monitoring will only be performed if the treatment provider believes this is indicated. These evaluations may also be followed by adjustment of rate control medications, electrical cardioversion, arrhythmia surgery or atrioventricular node ablation (treatment provider's choice).

Rate control medications

Treatment will be provided according to current guidelines, and as such, the algorithm for treatment will be differentiated based on the status of left ventricular ejection fraction.[14] For participants with reduced LVEF, beta-blockers (metoprolol and bisoprolol) will be the primary therapy. Secondary therapies may include digoxin or amiodarone. For participants with preserved LVEF, the primary therapy will be beta-blockers (metoprolol and bisoprolol) or non-dihydropyridine calcium-channel blockers (verapamil) with secondary therapy consisting of digoxin or amiodarone.

We briefly summarise the pharmacological treatment in the DanAF trial (table 1).

Concomitant medication

Besides rate control, the treatment provider will be free to prescribe any other standard medical cointervention such as the need for anticoagulation (based on the CHA_2DS_2-VASc score and comorbidity,[14] hypertension management, heart failure management or lipid lowering drugs as long as the prescriptions adhere to guidelines.[14] This also includes recommendations regarding modifiable risk factors that may have adverse effects on atrial fibrillation management (excess alcohol, smoking and sleep apnoea).[14 35] A brief description of what is considered standard management of comorbidities and risk factors are given in online supplemental file 3. All other interventions are allowed if they are administered evenly in all intervention arms.

Table 1 Suggested daily doses for rate control agents

Metoprolol	50–200 mg
Bisoprolol	2.5–10 mg
Digoxin	62.5–250 µg maintenance dose according to weight, age and renal function; loading is usually required for 3–7 days
Verapamil	120–240 mg – no loading dose required

Follow-up and outcome events

All participants will attend a minimum of two follow-up visits within 2 months after randomisation. Further visits are possible with 2-week intervals until adequate titration of rate control therapy is as required or for other reasons such as participants having inadequate symptom control, management of comorbidities and so on. Treatment providers may plan a visit sooner or later if clinically indicated. To assess if the ECG guided heart rate target is representative of the heart rate under normal conditions, we will perform 24-hour Holter monitoring at the end of the titration phase and after 1 year of follow-up for documentation purposes.

After the initial adequate titration of rate control, participants are to follow the normal referral system in the Danish healthcare system. A hotline will be established where treatment providers may call and ask for the participant's rate control target. If treatment providers themselves do not contact the trial treatment provider, participants are encouraged to contact the trial treatment provider. If possible, a treatment provider involved in the trial will be the managing treatment provider of the referral, if the referral is to a participating department.

Primary outcome

- Quality of life using the SF-36 questionnaire (physical component score), continuous outcome.[36]

Secondary outcomes

- Days alive outside hospital, count outcome.
- Symptoms due to atrial fibrillation using the AFEQT, continuous outcome.[27]
- Quality of life using the SF-36 questionnaire (mental component score), continuous outcome.[36]
- Serious adverse events, dichotomous outcome. We will define a serious adverse event as any untoward medical occurrence that resulted in death, was life-threatening, required hospitalisation or prolongation of existing hospitalisation and resulted in persistent or significant disability or jeopardised the patient.[33]

Exploratory outcomes

- All-cause mortality, dichotomous outcome.
- Composite of all-cause mortality, stroke, myocardial infarction and cardiac arrest, dichotomous outcome.
- Cardiac mortality, dichotomous outcome.
- Stroke, dichotomous outcome.
- Hospitalisation for worsening of heart failure, dichotomous outcome.
- Number of hospital admissions, count outcome.
- Six-minute walking distance, continuous outcome.
- Healthcare costs.
- Various biomarkers (N-terminal pro-brain natriuretic peptide (nt-proBNP), high-sensitivity C reactive protein (hsCRP), high-sensitivity troponin I (hsTnI), growth differentiation factor-15 (GDF-15), interleukin 6 (IL6), cystatin-C, YKL40, soluble urokinase plasminogen activator receptor (suPAR) and fibulin-1).

- Switch to rhythm control strategy (such as rhythm control medication, DC-conversion, pulmonary vein isolation or arrhythmia surgery), dichotomous outcome.
- Implantation of a pacemaker or cardioverter–defibrillator with or without AV node ablation, dichotomous outcome.

Echocardiographic outcomes

- Size of left atrium (Left atrial volume index)).
- Size of left ventricle.
- Cardiac index (cardiac output/body surface area).
- Left ventricular ejection fraction.
- Tricuspid annular plane systolic excursion (TAPSE).[37]
- Midwall fractional shortening.
- Global longitudinal strain.
- Circumferential end-systolic stress.
- Diastolic dysfunction estimated by the relationship between left ventricular filling and the interval between two successive R waves on ECG (R-R interval) for the individual patient.
- Pulmonary pressure.

All secondary, exploratory and echocardiographic outcomes will only be hypothesis generating.

Adverse events

Participants will be asked during visits to the clinic if they had experienced any undesirable medical events.

Suspected unexpected serious adverse reactions (SUSAR) will be reported to the ethics committee within 7 days of investigators being aware of the event. Once a year, a report of all serious adverse events and serious adverse reaction will be submitted to the ethics committee.

Assessment time point

The primary assessment time point for all outcomes will be 1 year after randomisation.

Procedures for screening

Potential participants according to inclusion and exclusion criteria at Holbaek University Hospital, Hvidovre University Hospital, Region Zealand University Hospital – Roskilde and Odense University Hospital will receive an invitation to participate in the trial on a routine visit in the clinic or hospitalisation for atrial fibrillation. Possible participants will be identified by trial staff employed at the site.

Procedures for informed consent

Participants will receive printed material containing details of each study visit, the design and rational of the trial, participant rights (such as the right to withdraw), possible adverse reactions of medication and more. The printed material will be given either immediately after being identified as a possible candidate or during a private, information session where verbal information is given and the participants can ask any questions they may have. The information session will take place in an undisturbed environment. The information will be given by the project coordinator on site or medical personnel with equivalent prerequisites for conveying the project. Potential participants will be informed that they can bring a third party if they wish so. The participants will be given up to 3 weeks to consider participation depending on when they choose to schedule the information session. There will be a minimum of 48 hours from the information session to the obtaining of informed consent.

Data collection

Data will be attempted to be collected from all participants regardless of protocol adherence. Study plan and data will be as shown in table 2.

Echocardiography will be performed according to current international guidelines.[38] A detailed plan for the echocardiographic examination and recordings has been developed. The echocardiograms will be sent to a core echocardiographic reading centre at Holbaek Hospital to be assessed by one of two assessors that will be blinded.

Biobank

We will collect blood samples for a research biobank and measure: Nt-proBNP, hsCRP, hsTnI, GDF-15, IL6, Cystatin-C, YKL40, suPAR and fibulin-1. In addition to the above blood samples, we will collect the following three types of blood samples: 5 mL serum, 5 mL plasma and 5 mL citrat plasma to be stored for future research. Participants will be given separate information on this blood collection as well as be required to give a separate informed consent (online supplemental file 4).

Data management

All data will be sent encrypted to OPEN for management. All data on paper will be securely stored, and a copy will be sent to a computerised database.

The computerised database will be continuously checked for missing values and errors at 1-month intervals. Before a trial site begins recruitment, an internal monitoring of the following procedures will be checked: validation of inclusion and exclusion criteria, informed consent procedure, randomisation procedure and data entry into REDcap.

Statistical plan and data analyses

Sample size: quality of life using the SF-36 questionnaire (physical component score)

Using a minimal important difference of 3 points on the physical component score, an SD of 10, power of 80% and a significance level of 5% and a total of 350 participants will be needed.[17 39 40] Based on this sample size, we have estimated the power of all remaining outcomes (see online supplemental file 5).

Recruitment plans

We will involve key medical personnel at the different departments as well as hold sessions at the different departments informing of the trial.

Table 2 - Study schedule

Schedule	Visit 0 baseline	Visit 1	Visit 2	Visit 3	Visits 4, 5 and 6
Investigations	0 months	1 month±2 week	2 months±2 weeks	6 months±2 weeks	12 months/24 months/36 months ±2 weeks
Medical history	x	×			×
Clinical events (hospital, tests and so on)		×	×		×
CHA_2DS_2VASc score	×				×
EHRA SC	×	×	×		×
SF-36 and AFEQT	×				×
Physical examination	×				×
Vital signs (BP and HR)	×	×	×		×
Treatment adjustment (both for atrial fibrillation and any comorbidities)	×	×	×		×
Informed consent, inclusion/ exclusion criteria	×				
Randomisation	×				
Clinical laboratory tests (as indicated)	×	×	×		×
Study laboratory tests	×			×	×
12-lead ECG	×	×	×		×
Holter monitoring. ()=as clinically indicated	(×)	(×)	×		×
Echocardiography	×				×
Six-minute walking test	×				×

AFEQT, the atrial fibrillation effect on quality of life; BP, blood pressure; CHA_2DS_2VASc score, Score for determining the risk of stroke. Points are given for congestive heart failure (1), hypertension (1), age 75 or above (2), diabetes (1) previous stroke (2), vascular disease (1), age 65-75 (1) and female sex (1); EHRA SC, European heart rhythm association symptom classification; HR, heart rate; SF-36, Short Form-36.

Statistical analyses

A detailed statistical analysis plan will be published around 1 month after the trial has been launched. In short, our primary conclusions will be based on the results of our single primary outcome. Hence, we will consider a p value of 0.05 as our threshold for statistical significance.[31] The results of secondary outcomes, exploratory outcomes, subgroup analyses and possible per protocol analyses will be hypothesis generating only. We will assess whether the thresholds for statistical and clinical significance are crossed according to the five-step procedure proposed by Jakobsen *et al*.[31] The analyses of the outcomes will be based on the 'intention to treat' principle, that is, all randomised participants will be included in the analysis regardless of how much treatment they have received. In case of more than 5% not receiving the allocated heart rate target, we will secondarily analyse all outcomes according to the actual heart rate achieved (per protocol analysis) defined as the average heart rate on ECG after 5 min of rest. Participants who receive a rhythm control strategy (assessed by the treating physician) at our primary assessment time point will be excluded from this analysis. If outcomes are not present due to retraction of informed consent or dropout, the pattern of the missing data will be investigated. Missing data will be handled according to the recommendations proposed by Jakobsen *et al*.[22] In short, we will conduct a worst-best and best-worst case scenario, testing the potential impact of missing data.[22] If the pattern of missing data allows it, we will also conduct multiple imputations.[22]

Analysis methods

Continuous outcomes will be presented as means and SD with 95% CIs. Count outcomes will be presented as medians and IQRs. We will analyse continuous outcomes using mixed effects linear regression with 'site' as a random intercept using an exchangeable covariance matrix and type of atrial fibrillation at inclusion (persistent vs permanent) and LVEF (EF ≥40% and EF <40%) as a fixed effect.[41] We will analyse count data using the van Elteren's test stratifying for 'site'.[42] Dichotomous outcomes will be presented as proportions of participants in each group with the event, as well as risk ratios with 95% CIs. Dichotomous outcomes will be analysed using mixed effects generalised linear models using a log link function with 'site' as a random intercept using an exchangeable covariance matrix, and type of atrial fibrillation will be included as a fixed effect.[42] All outcomes will be analysed according to final value.

Subgroup analyses

All subgroup analyses will be regarded as hypothesis generating only, and we will not base any conclusions on these. We will in the planned statistical analysis plan (see 'Statistical analysis') in detail describe each planned subgroup analysis.

In short, we will in each publication compare:

- Patients with heart failure compared with patients without heart failure (including subtypes).
- Men compared with women.
- Different durations of atrial fibrillation at randomisation.
  - Less than 1 year.
  - 1–2 years.
  - More than 2 years.
- Patients with age above compared with below 75 years.
- Patients according to the European Heart Rhythm Association symptoms score.

Data monitoring

A data safety monitoring committee (DSMC) independent from the sponsor and the investigators will be created. The DSMC will be free of conflicts of interest. The DSMC will be responsible for conducting an interim analysis after 50% of participants have been included and monitor if the trial still holds scientific merit. The DSMC will decide when/if a new interim analysis should be performed. The DSMC will make recommendations to the steering committee whether the trial should stop or continue (further details in online supplemental file 6).

Auditing

The trial can be audited by the regional ethics committee, which is independent from the investigators and sponsor.

Patient and public involvement

Patients were invited to a workshop after the initial draft was accepted by all participating departments. They were asked to give inputs to the chosen outcomes, the written material, the relevance of the objective of the trial and any other aspects they found relevant.

Patients are anticipated to work as ambassadors after the trial results are available. We will therefore perform a second workshop to involve patients in the best strategy for dissemination.

Ethics and dissemination

The management of patients is in accordance with standard care, and as such, patients are at no greater risk compared with receiving standard care outside the trial. It is therefore ethical for patients to be part of the trial. The potential benefit for future patients is that we may uncover a superior heart target to be the goal of future management of patients with atrial fibrillation.

The trial protocol has been approved by the regional ethics committee, which is a branch of the Danish ethics committee, the regulatory body approving research in Denmark. As such, the committees are independent from the trial. The committee reviewed the full protocol, the written material for the participants, the consent form and the administered questionnaires before giving approval. The ethics committee has the option of conducting an audit of the trial if it wishes to do so. The committee must be provided with a notification of any serious adverse events including Suspected unexpected serious adverse reactions within a week as well as a yearly report of serious adverse events. Any changes to the approved protocol will be submitted and approved before continuing the trial.

Site investigators or personnel with equivalent skills will obtain informed consent from possible participants (online supplemental file 7). Additional consent will be obtained in order to store blood samples for future research.

Before enrolment of participants, screening will be done by personnel employed at the study site using the local electronic journal system. Any information collected on potential and enrolled participants will be entered directly into REDcap, using a secure connection.

The project and its data have been registered at the Region Zealand, who is the data controller. Study investigators will have access to the full data set. OPEN, who is in charge of storing the data, will also have access to the full data set. Ethics review will also have access to data on request.

Participants, who suffer harm during the trial, are insured by the the Danish Patient Compensation Association.

Trial results will be sought published in a peer-reviewed journal. We will also communicate results directly to relevant patient advocacy groups, relevant medical associations and attempted presented at relevant congresses. Aggregate data analysis will be published in a clinical trial register no later than 3 years after trial results have been collected. Data sharing will be made available on request after approval from ethics committee.

Authorship will be granted according to the recommendations from the International Committee of Medical Journal Editors.[43]

DISCUSSION

Our trial has several strengths. It is a pragmatic trial assessing the benefits and harms of a lenient versus a strict rate control strategy on quality of life in patients with persistent or permanent atrial fibrillation. The number of inclusion and exclusion criteria is low, and hence, the external validity will be high. Participants will be recruited from more than one site, which will further increase the external validity. We have performed a sample size estimation based on previous evidence with realistic intervention effects, we will adjust the thresholds for statistical significance if the sample size is not reached, and we have chosen only one outcome we will base conclusion on. The remaining outcomes will be considered hypothesis generating only thereby taking into account problems with multiplicity. Furthermore, we have taken measures to reduce the risks of bias from the

allocation sequence generation, allocation concealment, blinding of outcome assessors and participants, selective outcome reporting, for-profit bias and missing outcome data. Hence, our trial will be conducted with a low risk of random errors ('play of chance') and with as low risk of systematic errors ('bias') as the trial design allows (see further).[31 44] In Denmark, a complete follow-up of all participants for death and hospitalisations is secured by an unique number given to all born in Denmark, Central Person Register.

Our trial also has limitations. The treatment providers responsible for the rate control intervention will not be blinded, which may bias our results. We will use 12-lead ECG to guide rate control therapy. Holter monitoring and measurement of the heart rate during exercise will only be used at the discretion of the investigator if deemed necessary. As such, there may be fluctuations in the heart rate we do not detect. Another limitation is that we do not have sufficient power to assess 'hard outcomes' such as mortality and serious adverse events. This will be explored in a future meta-analysis with individual patient data from the RACE II trial and other trials. The consequence may ultimately be that a superiority trial in terms of 'hard outcomes' is needed. Our results will only be generalisable to a population where rate control is considered appropriate as the main strategy going forward. The results of the EAST trial is expected to delay the initiation of rate control for many patients, and hence, our results will need to be interpreted in light of this. Yet another limitation is that participants presumably will receive different medications and procedures in the compared groups. If we show a difference (or lack of a difference) between the groups, it will be difficult to interpret what part of the treatment algorithm for reaching a certain rate target caused this difference.

We expect the results of this trial will play a part of future recommendations for rate control treatment in patients with both persistent and permanent atrial fibrillation.

Protocol version and amendments

This abbreviated version of the full protocol is based on V.2.0 of the protocol (January 2020). Any changes to the original protocol will be submitted to the regional ethics committee. After approval, changes will be conveyed to all investigators, participants and trial registries.

The findings will be published in a peer-reviewed journal as well as be made available on ClinicalTrials.gov.

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Acknowledgements The authors would like to thank the patient advisory committee at Holbaek Hospital. We would also like to thank Lise Pedersen and Bo Christensen from the Department of Clinical Biochemistry as well as Palle Lyngsie Pedersen from the Region of Zealand biobank for their help in planning the logistics surrounding the biobank.

Contributors JF, JCJ, AB, UD, UJOG, WB, MHO, ODP and IR participated integrally in the study design. CG provided vital advice on trial conduct. EEN and FS-H designed the echocardiography plan. MHO designed the plan for analysis of biomarkers. JF, JCJ and AB drafted the initial manuscript. All other authors provided critical revision and approved the final manuscript.

Funding The trial was initiated by clinicians at the participating hospitals. The research salary for research nurses is partly funded by the Region of Southern Denmark and Region Zealand joint research fund 2018 for year 1. The salary of the lead author for years 2 and 3 are provided by the Danish Heart foundation grant number 19-R134-A8959-22123. The salary for year 1 is granted by the University of Southern Denmark. The participating departments support the trial by dedicating work hours of the other investigators, supportive staff, logistical support and administrative support. The trial is investigator initiated. Holbaek Hospital is the sponsor, and the Region Zealand is the data controller. The study sponsors and funders had no influence on design; collection, management, analysis and interpretation of data; writing of the report; and the decision to submit the report for publication. The Danish Heart Foundation requires to be notified by email when a publication is accepted. Roles and responsibilities of additional parties are described in supplementary file 8.

Competing interests JBF (PI), IR, WB, EEN, FS-H, ODP, UG, CG and JCJ report no competing interests. MHO reports grants from Novo Nordic Foundation outside the submitted work. AB reports personal fees from Bayer, grants from Biotronik, personal fees from Boehringer Ingelheim, personal fees from Bristol-Myers Squibb, personal fees from MSD, grants from Theravance, outside the submitted work. UD reports a research grant from Bayer, personal fees from Pfizer, member of advisory board for Boehringer Ingelheim, member of advisory board for Merck, outside the submitted work.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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Supplemental appendix article 1 - Protocol for a randomized clinical trial, DanAF

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	Supplementary file 2
Protocol version	3	Date and version identifier	16
Funding	4	Sources and types of financial, material, and other support	17
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	17
	5b	Name and contact information for the trial sponsor	17
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	17
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	17

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-7
	6b	Explanation for choice of comparators	4-7
Objectives	7	Specific objectives or hypotheses	8
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9-10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10-12
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13-15
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	16-18

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	19 + supplementary file 5
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	16
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8-9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	18-19
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	16

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	19-20
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	20-21
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	20
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	21 + supplementary file 6
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	21 + supplementary file 6
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	22
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	22

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	22
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	22
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	22-23
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	26
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22-23
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	23
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	23
	31b	Authorship eligibility guidelines and any intended use of professional writers	23
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	supplementary file 7
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	supplementary file 4

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Data category	Trial information
1. Primary registry and trial identifying number	Clinicaltrials.gov (NCT04542785)
2. Date of Registration in Primary Registry	September 2020
3. Secondary Identifying Numbers	Region Zealand Ethics committee ID: SJ-797 Internal ID number Region Zealand: REG-078-2019
4. Source(s) of Monetary or Material Support	Holbaek University Hospital Odense University Hospital Hvidovre University Hospital Region Zealand University Hospital - Roskilde Region of Southern Denmark and Region Zealand joint research fund 2018 The Danish Heart foundation grant number 19-R134-A8959-22123 The University of Southern Denmark A.P. Moeller Foundation
5. Primary Sponsor	Holbaek Hospital Smedelundsgade 60, 4300 Holbaek Hospital Denmark
6. Secondary Sponsor(s)	
7. Contact for Public Queries	JBF
8. Contact for Scientific Queries	JBF
9. Public Title	Lenient rate control versus strict rate control for atrial fibrillation. The Danish Atrial Fibrillation (DanAF) randomised clinical trial
10. Scientific Title	Lenient rate control versus strict rate control for atrial fibrillation. The Danish Atrial Fibrillation (DanAF) randomised clinical trial
11. Countries of Recruitment	Denmark
12. Health Condition(s) or Problem(s) Studied	Atrial Fibrillation
13. Intervention(s)	Lenient rate control versus strict rate control
14. Key Inclusion and Exclusion Criteria	Inclusion criteria: 1. Atrial fibrillation (ECG-confirmed and diagnosed by the treating physician) persistent (defined as atrial fibrillation for more than 7 days) and permanent atrial fibrillation (only rate control is considered going forward); 2. Rate control must be accepted as being the primary management strategy going forward. 3.Informed consent; 4.Adult (18 years or older). Exclusion criteria: 1. No informed consent; 2.Initial heart rate under 80 bpm at rest (assessed via an electrocardiogram (ECG) before randomisation); 3. Less than 3 weeks of anticoagulation with NOAC or 4 weeks with efficient warfarin; 4. Participants dependent on a high ventricular rate to maintain a sufficient cardiac output. This will be based on an individual assessment of the possible

	participant. 5. Participants who are hemodynamic unstable and therefore require immediate conversion.
15. Study Type	1. Interventional study 2. Method of allocation: Randomised Masking: Participant and outcome assessors blinded Assignment: parallel Primary purpose: Comparing two strategies
16. Date of First Enrollment	Anticipated end of January 2021.
17. Sample Size	350 planned, 0 enrolled.
18. Recruitment Status	Pending
19. Primary Outcome(s)	Short Form-36 (SF-36) questionnaire (physical component score).
20. Key Secondary Outcomes	Secondary outcomes will be days alive outside hospital, symptom control using the Atrial Fibrillation Effect on Quality of Life, quality of life using the SF-36 questionnaire (mental component score), and serious adverse events.
21. Ethics Review	Approved on 30.10.2019 by The Ethics committee in Region Zealand. Alléen 15, 4180 Soroe. Telephone number: 57 87 52 83
22. Completion Date	Anticipated completion date January 2026
23. Summary Results	Not yet available
24. IPD Sharing Statement	Plan to Share IPD: Yes

Supplementary file 3 - Management of co-morbidities

Management of heart failure and hypertension

Management of heart failure will follow the recommendations of the European Society of Cardiology. Briefly, the table below summarizes the recommendations for medical therapy. Ultimately, any management is at the discretion of the treatment providers and participants.

	LVEF <40	LVEF ≥ 40
Step 1: All participants	ACEi (Ramipril 10 mg) or ARB (Losartan 150 mg x 1)	
Step 2: If still symptomatic	Spiron 50 mg x 1	
Step 3: If still symptomatic	ARNI 97/103 x 2 instead of ACEi/ARB	
Signs of congestion	Bendroflumethiazid 2.5 -10 mg/day or Furosemide 20-40 mg/day	Bendroflumethiazid 2.5 -10 mg or Furosemide 20-40 mg
Additional treatment if HomeBP > 130/80	Bendroflumethiazid 2.5 -10 mg or amlodipine 5-10 mg x 1 (or spiron 25-50 mg if not on step 2)	ACEi (Ramipril 10 mg) or ARB (Losartan 150 mg x 1) or Bendroflumethiazid 2.5 -10 mg or amlodipine 5-10 mg x 1 (Possibly spiron 25-50mg)

Sleep apnea

Participants will be systematically screen for signs of sleep apnea. If signs and symptoms of sleep apnea are discovered, participants will be referred to treatment if appropriate.

Obesity

Weight loss will be encouraged if BMI > 25. General advice will be provided and involvement of participants in local municipal programs will be discussed.

Smoking

Participants will be asked about their smoking habits as part of the initial work-up. Participants will be informed of the detrimental effects of smoking on health. Current smokers will be encouraged to quit and will be informed of available support programs through the municipals.

Alcohol

Participants will be asked about their alcohol habits as part of the initial work-up. Participants will be informed of current evidence regarding alcohol in atrial fibrillation and will be encouraged to abstain from alcohol or alternatively reduce their alcohol intake. Special emphasis will be put on participants who drink above 10 standard drinks/week.[1 2]

Physical activity

Participants will be asked about their physical activity and physical function. Based on an individual assessment, some participants may be offered exercised based cardiac rehabilitation, but it will not be systematically prescribed.[3] This will typically be participants who are limited in their daily activities or who have had a recent significant decline in their physical function. Participants with ischemic heart disease, heart failure or recent operation for valve disease will in general be referred to exercise-based cardiac rehabilitation.

1. Gillis AM. A Sober Reality? Alcohol, Abstinence, and Atrial Fibrillation. *N Engl J Med* 2020;382(1):83-84. doi: 10.1056/NEJMe1914981 [published Online First: 2020/01/02]
2. Voskoboinik A, Kalman JM, De Silva A, et al. Alcohol Abstinence in Drinkers with Atrial Fibrillation. *N Engl J Med* 2020;382(1):20-28. doi: 10.1056/NEJMoa1817591 [published Online First: 2020/01/02]
3. Risom SS, Zwisler AD, Johansen PP, et al. Exercise-based cardiac rehabilitation for adults with atrial fibrillation. *Cochrane Database Syst Rev* 2017;2:Cd011197. doi: 10.1002/14651858.CD011197.pub2 [published Online First: 2017/02/10]

Supplementary file 4 - biobank

We will further collect blood samples for a research biobank and measure: Nt-proBNP, hsCRP, hsTni, GDF-15, IL6, Cystatin-C, YKL40, suPAR and Fibulin-1. Due to the manner of which these analysis have to be analysed and the variations in the measurement depending on blood sample kit is used, blood samples will be collected at the first visit, after 6 months, and at follow-up after 1 year and analysed together. Follow up after two and three years will be analysed together. These analyses will require 10 mL of blood per collection. The blood samples are expected to be analysed either at a laboratory in Sweden or a laboratory in Denmark, but may end up being analysed in another EU country. The storage of data will abide by the Danish General Data Protection Regulation and the Danish Data Protection Act in Denmark.

Any spare blood that is collected will be stored in a biobank in Denmark for future unspecified research purposes. The storage of data will still abide by the Danish General Data Protection Regulation and the Danish Data Protection Act in Denmark.

In addition to the above blood samples, we will collect three different types of blood samples: 7 ml. serum, 7 ml plasma and 7 ml citrat plasma to be stored for future research. This will total approximately 31 mL of blood. The blood samples are expected to be analysed either at a laboratory in Sweden or a laboratory in Denmark, but may end up being analysed in another EU country. Participants will be given separate information on this blood collection as well as be required to give a separate informed consent.

The storage of data will abide by the Danish General Data Protection Regulation and the Danish Data Protection Act in Denmark.

Supplementary file 5 – Power estimations of secondary outcomes

The below power calculations are based on a sample size of 350 participants as specified in the main document.

Days alive outside hospital

Using a minimal important difference of 3 days, a standard deviation of 9, a risk of type I error of 5%, and accounting for the fact that the data is expected not to be normal distributed, we will be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) of 82.1%.[1]

The Atrial Fibrillation Effect on Quality-of-Life (AFEQT)

In previous trials the observed difference between groups was normally distributed with a standard deviation of 21.[2 3] Using a minimal important difference of 7, we will be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) of 87.5%. The Type I error probability associated with this test of this null hypothesis is 5%.

Quality of life using the SF-36 questionnaire (mental component score)

In previous trials the observed difference between groups was normally distributed with a standard deviation 10.[4-6] Using a minimal important difference of 4, we will be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) of 96%. The Type I error probability associated with this test of this null hypothesis is 5%.

Serious adverse events

We anticipate a failure rate among control of 20%. If we anticipate a relative risk reduction of 60%, we will be able to reject the null hypothesis with probability (power) of 90.2%. The Type I error probability associated with this test of this null hypothesis is 5%.

POWER ESTIMATIONS OF EXPLORATORY OUTCOMES

All-cause mortality

Prior data indicate that the mortality rate among controls is about 5%.[7] If we anticipate a relative risk reduction of 10%, we will be able to reject the null hypothesis with probability (power) of 5.7%. The Type I error probability associated with this test of this null hypothesis is 5%.

Composite of all-cause mortality, stroke, myocardial infarction and cardiac arrest

Prior data indicate that this outcome occurs in controls in about 8%.[7 8] If we anticipate a relative risk reduction of 10%, we will be able to reject the null hypothesis with probability (power) of 5.9%. The Type I error probability associated with this test of this null hypothesis is 5%.

Cardiac mortality

Prior data indicate that the failure rate among controls is 3.9%.[7] If we anticipate a relative risk reduction of 10%, we will be able to reject the null hypothesis with probability (power) of 5.4%. The Type I error probability associated with this test of this null hypothesis is 5%.

Stroke

Prior data indicate that cardiac mortality among controls is 3.9%.[7] If we anticipate a relative risk reduction of 10%, we will be able to reject the null hypothesis with probability (power) of 5.4%. The Type I error probability associated with this test of this null hypothesis is 5%.

Hospitalisation for worsening of heart failure

Prior data indicate that heart failure among controls is 27.4%.[7] If we anticipate a relative risk reduction of 10%, we will be able to reject the null hypothesis with probability (power) of 9.0%. The Type I error probability associated with this test of this null hypothesis is 5%.

Number of hospital admissions

Prior data indicate that number of participant who are hospitalised is 27.4%.[7] If we anticipate a relative risk reduction of 10%, we will be able to reject the null hypothesis with probability (power) of 9%. The Type I error probability associated with this test of this null hypothesis is 5%.

Six-minute walking distance

In previous trials the observed difference between groups was normally distributed with a standard deviation 75.[9-11] Using a minimal important difference of 40, we will be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) of 99.9%. The Type I error probability associated with this test of this null hypothesis is 5%.

Physical activity using trial accelerometer

Prior data indicates that the standard deviation among groups was 65 minutes pr. Day when measuring sedentary behaviour. Assuming a difference in groups of 20 minutes/day, we will be able to reject the null hypothesis with a probability of 81.9%. The type 1 error probability associated with this test of this null hypothesis is 5%.[12 13]

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2. Holmes DN, Piccini JP, Allen LA, et al. Defining Clinically Important Difference in the Atrial Fibrillation Effect on Quality-of-Life Score. *Circ Cardiovasc Qual Outcomes* 2019;12(5):e005358. doi: 10.1161/circoutcomes.118.005358 [published Online First: 2019/05/17]
3. Mark DB, Anstrom KJ, Sheng S, et al. Effect of Catheter Ablation vs Medical Therapy on Quality of Life Among Patients With Atrial Fibrillation: The CABANA Randomized Clinical Trial. *Jama* 2019;321(13):1275-85. doi: 10.1001/jama.2019.0692 [published Online First: 2019/03/16]
4. Wokhlu A, Monahan KH, Hodge DO, et al. Long-term quality of life after ablation of atrial fibrillation the impact of recurrence, symptom relief, and placebo effect. *J Am Coll Cardiol* 2010;55(21):2308-16. doi: 10.1016/j.jacc.2010.01.040 [published Online First: 2010/05/22]
5. Groenveld HF, Crijns HJ, Van den Berg MP, et al. The effect of rate control on quality of life in patients with permanent atrial fibrillation: data from the RACE II (Rate Control Efficacy in Permanent Atrial Fibrillation II) study. *J Am Coll Cardiol* 2011;58(17):1795-803. doi: 10.1016/j.jacc.2011.06.055 [published Online First: 2011/10/15]

6. Dorian P, Paquette M, Newman D, et al. Quality of life improves with treatment in the Canadian Trial of Atrial Fibrillation. *Am Heart J* 2002;143(6):984-90. [published Online First: 2002/06/21]
7. Van Gelder IC, Groenveld HF, Crijns HJGM, et al. Lenient versus Strict Rate Control in Patients with Atrial Fibrillation. *New England Journal of Medicine* 2010;362(15):1363-73. doi: 10.1056/NEJMoa1001337
8. Kirchhof P, Breithardt G, Camm AJ, et al. Improving outcomes in patients with atrial fibrillation: Rationale and design of the Early treatment of Atrial fibrillation for Stroke prevention Trial. *American Heart Journal* 2013;166(3):442-48. doi: https://doi.org/10.1016/j.ahj.2013.05.015
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10. Silvet H, Hawkins LA, Jacobson AK. Heart rate control in patients with chronic atrial fibrillation and heart failure. *Congest Heart Fail* 2013;19(1):25-8. doi: 10.1111/j.1751-7133.2012.00309.x [published Online First: 2012/09/11]
11. Ding L, Quan X-Q, Zhang S, et al. Correlation between impedance cardiography and 6 min walk distance in atrial fibrillation patients. *BMC Cardiovascular Disorders* 2016;16:133. doi: 10.1186/s12872-016-0297-0
12. Bellettiere J, LaMonte MJ, Evenson KR, et al. Sedentary behavior and cardiovascular disease in older women: The Objective Physical Activity and Cardiovascular Health (OPACH) Study. *Circulation* 2019;139(8):1036-46. doi: 10.1161/CIRCULATIONAHA.118.035312
13. Andersson C, Lyass A, Larson Martin G, et al. Physical Activity Measured by Accelerometry and its Associations With Cardiac Structure and Vascular Function in Young and Middle-Aged Adults. *Journal of the American Heart Association*;4(3):e001528. doi: 10.1161/JAHA.114.001528

Supplementary file 6. Short description of the independent Data Safety and Monitoring Committee (DSMC)

Introduction

This Charter defines the primary responsibilities for the independent Data safety and monitoring Committee (DSMC) of the randomised clinical trial DanAF. This includes the relationships with other aspects of the trial.

Primary responsibility of the DSMC

The DSMC will ensure the safety of trial participants. This will be achieved by the following tasks:

- Performing planned analyses of outcomes related to the safety of participants from the two rate control strategies during the trial.
- Continuously monitoring if the trial still holds scientific merit

Members of the DSMC

The exact composition of the DSMC will be specified later but is expected to consist of two clinicians and one person with adequate statistical knowledge to conduct the interim analysis. One member will be chosen as the committee chair.

Recommendations are recommended to be anonymous. However, in case of members not coming to an agreement, members will vote. The points of discussion will be part of the discussion of the DSMC report to the Steering Committee (SC). The members of the DSMC will be free of conflicts of interest. Assessment if members are free of conflict of interest will be decided by the SC.

Meetings

This is the initial DSMC charter. The final charter will be determined and signed as the last part of the first meeting of the DSMC (see below).

1. Meeting

The first meeting will be a finalization of the DSMC role during the trial. The following will be agreed on and finalized.

- How DSMC can request additional (unblinded) data
- How meetings will be held (virtually, physical meeting, phone)
- How many meetings are necessary.
- Decision on whether a test run is necessary.
- Finally, the charter will be finalised and signed.

2. meeting

The second meeting will take place as part of an interim analysis after 50% of the participants (n=175) have been recruited.

The DSMC will be allowed to conduct additional interim analyses independently of the SC. The following meeting may take place virtually, in person or by phone.

Communication

Different formats will be used in order to secure proper communication is established. The formats include open and closed reports as well as open and closed sessions.

Closed Sessions

These sessions will involve only DSMC members. Discussions will be based on a closed report that will be based on blinded data provided by the data manager. A single member will be in charge of preparing the report but may receive input from the other two members before finalizing the closed report.

If the DSMC deems it necessary, they may ask for unblinding of the data from the steering committee.

Data for review will be the composite outcome all-cause mortality, stroke, myocardial infarction and cardiac arrest mortality (and its individual components), serious adverse events including any serious adverse reactions.

Recommendations to the steering committee (open report)

The DSMC will report its recommendations to the SC based on safety considerations. If the DSMC recommends anything other than continuing the trial, there will be held a virtual meeting between the DSMC and the SC. The DSMC will here present the reasoning behind its recommendations.

The SC ultimately makes the decisions regarding all aspects of the trial.

Data

The DSMC will be provided with data on the following variables

1. Randomisation code (this will not reveal the allocated heart rate target)
2. The composite outcome of all-cause mortality, stroke, myocardial infarction and cardiac arrest and the individual components:
    a. All-cause mortality
    b. Stroke
    c. Myocardial infarction
    d. Cardiac arrest
3. Serious adverse events including subcategories of individual events
4. Numbers of participants lost to follow up

The DSMC will not be provided with data on site or any identifier the data is considered anonymized.

Analyses

The DSMC is recommended to use Lan-DeMets sequential monitoring boundaries.

Meta data

The DSMC will be provided with a detailed codebook that explains all the coding in the data set.

Supplementary file 7 – informed consent form

(S4)

Informed consent to participate in a health-related research project

Research project title: Lenient rate control versus strict rate control for atrial fibrillation. The Danish Atrial Fibrillation (DanAF) randomised clinical trial

Statement from trial participant:

I have received both written and verbal information and have received enough information regarding purpose, methods, harms and benefits to give informed consent.

I know that it is voluntary to participate and that I always have the right to withdraw my consent without losing my right to treatment now or in the future.

I give my consent to participate in the research project and that my biological material may be collected with the intention of storing it in a research biobank. I have received a copy of this consent form along with written information regarding the project for my personal use.

Participant name: _____

Date: _____ Signature: _____

If during the research project significant information regarding your health, you will be informed. If you would like not to be informed of any new information regarding your health that comes to our attention during the trial, we ask that you mark here: _____ (mark with an x)

Do you wish to be informed of the results of the trial and possible consequences for you?:

Yes _____ (mark with an x) No _____ (mark with an x)

Statement from the person providing information to the participant:

I declare that the participant has received written and verbal information about the trial.

To my knowledge there has been given enough information to make a decision to participate in the trial.

Printed name of the person, who has given the information:

Date: _____ Signature: _____

Regional ethics commitee project identification:

69694

Supplementary file 8 - Roles and responsibilities

Daily management team (including the Principal investigator (PI))

Conduct of DanAF

Preparation of protocol and revisions

Design of Redcap database

Organising steering committee meetings

Conceive manuscripts of results for review by the steering committee

In charge of supervising start-up of sites

Budget administration and contractual issues with individual centres

Organisation of central serum sample collection

Design of randomisation

Securing that the GDPR is complied with (by interaction with the Regional data controller)

Site investigators

Joshua Buron Feinberg (Holbaek University Hospital), Axel Brandes (Odense University Hospital), Ulrik Dixen (Hvidovre University Hospital) and Ole Dyg Pedersen (Region of Zealand University Hospital - Roskilde)

Responsible for the proper conduct at respective sites.

In charge of reporting Serious adverse events (SAE) including Suspected unexpected serious adverse reactions (SUSAR) to PI in a timely manner as well as reporting serious adverse events for annual review by the regional ethics committee.

Steering committee (SC)

All authors of the protocol will be invited to be part of the steering committee.

Agreement of final protocolReviewing progress of study and if necessary agreeing changes to the protocol.

In charge of reviewing proper conduct of the trial according to GCP, Helsinki-declaration and ethics review demands.

Providing advice to lead investigators and personnel.

Review of analyses provided by the blinded statistician

Review of manuscript prepared by daily management team

Assistance with international review

Data manager

Maintenance of trial IT system and data entry (OPEN).

Data verification (OPEN in collaboration with PI)

Providing data to the DSMC

Providing data to the blinded statistician

Outcome adjudication committee

Responsible for adjudicating serious adverse events.

Data safety monitoring committee

Responsible for the safety of trial participants and the continuous scientific merit for the trial. Will report findings to the SC.

Blinded statistician

Prepare analysis for the steering committee to review

Regional data controller (independent from trial)

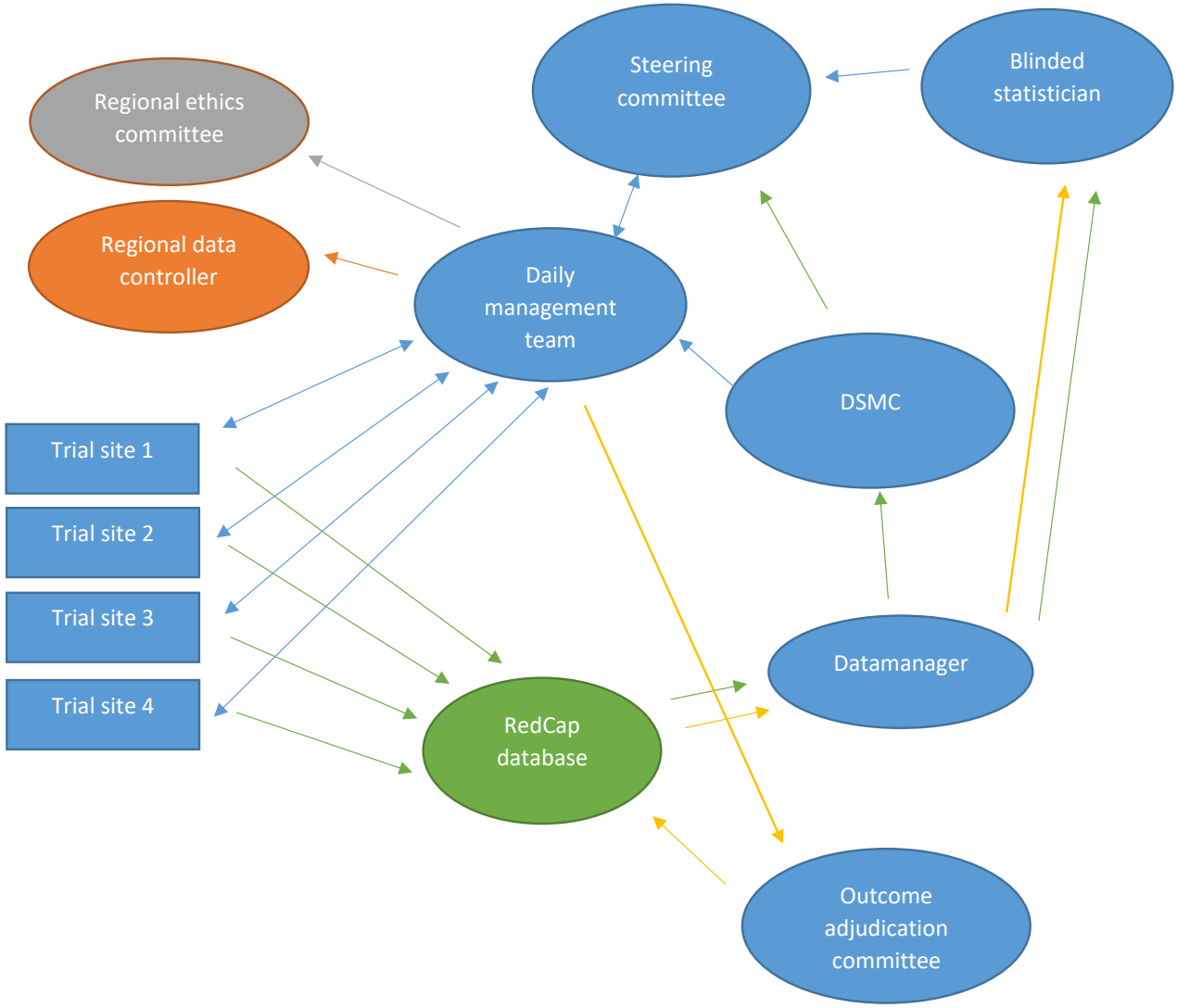
Data controller for the study hence must keep record of the type of data kept, data processor agreements and any other requirements needed to comply with GDPR

Regional ethics committee (independent from trial)

Approve the trial by review of protocol, written participant material, informed consent forms, etc.

Monitor trial through reports of SAE and SUSAR reported to them by the daily management team as well as the yearly report submitted by the PI.

Figure outlying the organisation



Grey arrow: Serious adverse events including SUSAR. Orange arrow: Information necessary to follow GDPR. Green arrow: Data. Yellow arrow: data for adjudication/adjudicated data.

Blue bubbles: Part of the trial organization. Green bubble: database. Orange/grey bubble: External regulatory body.

Article 2 - Systematic review of rate controlling drugs

The evidence for rate controlling drugs in outpatients with atrial fibrillation. A systematic review with meta-analysis and Trial Sequential Analysis

Joshua Buron Feinberg, Isak Mazanti Cold, Emil Eik Nielsen, Naqash Sethi, Kit Engedal Kristensen, Michael Hecht Olsen, Anne Merete Soja, Ole Dyg Pedersen, Ilan Raymond, Axel Brandes, Janus Christian Jakobsen

Abstract

Importance: Rate control attempts to improve symptoms and prognosis in atrial fibrillation. Only a few small trials are currently cited in guidelines. No previous systematic review has systematically compared the effects of the available rate controlling drugs.

Objective: To compare the different rate controlling drugs and if possible, rank them according to the available evidence.

Data sources: We searched for trials through searches of electronic databases up until September 2022 without language restriction.

Study selection: We included trials randomizing outpatients with atrial fibrillation to any rate control intervention.

Data extraction and synthesis: Our methodology was based on the Preferred Report Items of Systematic reviews with meta-analysis (PRISMA) and an eight-step assessment procedure. We assessed the risk of bias using Cochrane risk of Bias version 2. Meta-analysis was performed both with the fixed effect and random effects meta-analysis. Data extraction was performed by at least two persons independently.

Main outcomes and measures: The primary outcomes were all-cause mortality and serious adverse events. Our secondary outcomes were quality of life, symptom scores, and non-serious adverse events. Exploratory outcomes were resting heart rate, exertional heart rate, and exercise capacity, at maximum follow-up.

Results: We included 51 trials. All outcome results were at high risk of bias. There was no to very limited data on our primary and secondary outcomes.

Meta-analyses of 12 trials comparing different rate controlling drugs showed no difference between beta-blockers, calcium channel blockers, and digoxin on resting heart rate.

Meta-analyses of 11 trials comparing different rate controlling drugs showed that beta-blockers and calcium channel blockers reduced maximal exertional heart rate most. There was no difference between beta-blockers and calcium channel blockers. Test for subgroup differences indicated that atenolol, bisoprolol, and carvedilol may reduce maximal exertional heart more than calcium channel blockers.

Meta-analyses of 11 trials comparing different rate controlling drugs showed that calcium channel blockers and digoxin reduced exercise capacity least. We found indications that beta-blockers may reduce exercise capacity more than calcium channel blockers

Conclusion and relevance: The comparative effects of rate controlling drug for atrial fibrillation on patient important outcomes are unknown. Beta-blockers and calcium channel blockers seem to reduce maximal

exertional heart rate most. Beta-blockers may reduce exercise capacity compared with calcium channel blockers.

Registration: Prospero identifier CRD42022310938

Background

Description of the condition

Atrial fibrillation (AF) is the most prevalent arrhythmia in the world and associated with reduced quality of life and several complications, most notably stroke and heart failure.[1-3] Symptom management of AF usually consist of either a rate or rhythm control strategy, this choice depends primarily on the burden of symptoms, but changes in echocardiographic parameters, comorbidity and average resting heart rate also play key roles.[3]

The optimal heart rate when choosing rate control is unknown, but several guidelines suggest 60-110 beats per minute at rest.[3] Currently, lenient (<110 beats per minute (bpm)) rate control is accepted as the initial approach based on the RACE II trial.[3,4]

To achieve the target heart rate, current guidelines recommend several drugs.[3] Beta-blockers and non-dihydropryridine calcium channel blockers (CCB) are recommended as first line therapy with a few disease-specific considerations.[3]

If rate control is suboptimal (resting heart rate >110 bpm) or symptoms and quality of life are worsening, a combination of BB or a CCB with digoxin is usually recommended as second line.[3]

Why it is important to do the review

Rate control is a cornerstone in the management of AF. However, as stated in the current European guidelines, little robust evidence supports the best type and intensity of rate control.[3]

Recommendations for BBs and CCBs as first line therapy are primarily based on results from three small, randomised, primarily crossover trials (<30 participants in each group) and small retrospective observational studies.[5-8] These results indicate that digoxin is less effective in controlling heart rate during exercise and ineffective in patients with increased sympathetic drive.[5-8]

A ranking of potential drugs based on randomized clinical trials is important, as choice of rate controlling drug is a common clinical decision in AF management. To our knowledge, there exists no up-to-date systematic review with meta-analysis and network-meta-analysis, performing a comprehensive literature search, taking into account both the risk of random error (estimating the necessary number of participants and events) and evaluating the risk of systematic error using the latest risk of bias tools.

Objective

To compare the different rate controlling drugs and if possible, rank them according to all-cause mortality or serious adverse events. If not possible, present the available evidence from less patient relevant outcomes.

Methods

This systematic review has been developed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA).[9] The methodology was predefined and described on Prospero.[10]

In short, we included only randomized clinical trials comparing any drug which we considered rate control in adults (>18 years) with persistent/permanent AF in the outpatient setting.

An information specialist searched Cochrane Central Register of Controlled Trials (CENTRAL), Medical Literature Analysis and Retrieval System Online (MEDLINE), PubMed, Excerpta Medica database (EMBASE), Latin American and Caribbean Health Sciences Literature (LILACS; Bireme), Science Citation Index Expanded (SCI-EXPANDED; Web of Science), Conference Proceedings Citation Index—Science (CPCI-S; Web of Science). Additionally, we hand searched reference lists, major pharmaceutical companies and several databases for relevant publications. We did not make any restrictions based on language or year of publication.

Outcomes and subgroup analyses

Our primary outcomes were all-cause mortality and serious adverse events; secondary outcomes were health-related quality of life, AF symptom score, and non-serious adverse events. Planned exploratory outcomes were: achieved resting heart rate, successful achievement of resting heart rate target (as defined by trialist), exertional heart rate, exercise capacity, ejection fraction.

All outcomes were assessed at maximum follow-up.

Subgroup analyses

We had planned to perform several subgroup analyses. We performed the following subgroup analyses in one or more comparisons:

- Comparison of different rate controlling drugs within the class of drugs
- Trials from different time periods

We had not expected so many cross-over trials. To assess the impact of the trial design, we performed as post-hoc subgroup analysis comparing trials with a cross-over design with trials with a parallel design.

Data collection and bias

Three review authors (JBF, IMC, EEN) independently screened search results based initially on title and abstract, after that based on full-text review and provide reasons for exclusion of ineligible studies. All records were reviewed by at least two authors. Five review authors (JBF, IMC, EEN, NS, KEK) independently extracted characteristic, data and assessed risk of bias from the included trials. All records were reviewed by at least two authors. We contacted trial authors to request the missing data. We assessed risk of bias using the Cochrane Risk of Bias tool (version 2). Bias assessment was conducted on an outcome level. Disagreements were resolved through discussion.

Data synthesis and assessment of significance

Results of each type of intervention were analyzed using intention-to-treat data. Stata version 17 (StataCorp LLC, College Station, TX, USA) was used for analyses. We conducted any meta-analysis according to the Cochrane Handbook of Systematic Reviews of Interventions, Keus et al, and Jakobsen et al.[11-13] Results of traditional meta-analysis were supplemented by Trial Sequential Analysis (TSA).[14] We conducted both a random-effects and fixed-effect meta-analysis for each comparison. For dichotomous outcomes we calculated risk ratios (RRs) with 95% confidence intervals (Cis). For continuous outcomes mean differences with 95% CIs were calculated. We assessed heterogeneity primarily by visual inspection of forest plots, and secondly by the I^2 statistic. Publication bias was assessed using funnel plots. We assessed two primary outcomes, and therefore, we considered a p value of 0.033 as the threshold for statistical significance.

Network meta-analysis

We had planned to conduct network meta-analysis. However, the trials used so many different drugs, doses of drugs, co-interventions that it was not possible to make a meaningful network meta-analysis.

Summary of findings table

We reported our two primary outcomes as well as resting heart rate, max exertional heart rate and exercise capacity in summary of findings tables. We used the five GRADE criteria (bias risk, consistency of the effect, imprecision, indirectness, and publication bias) to judge the certainty of evidence. We used Trial Sequential Analysis to judge imprecision.

Results

Results of the search for studies

The preliminary search was conducted on the 28th of January 2022. The formal screening began on the 15th of July 2022. The search was updated on 1st of September 2022. 22210 records were identified. In total

51 trials were included (figure 1). We identified 13 completed or ongoing trials without data. A detailed description of the trials are given in eTable 1a and eTable 1b. In short, all trials only included outpatients. 20 trials used a parallel design and 31 used a cross-over design. The trials were conducted in 18 different countries. The range of duration of AF went from 3 weeks to 8. years. The average age of participants across all 48 trials was 62.85 years. A total of 34.5% were female, 56% of participants had hypertension and the average baseline heart rate were 97.8 bpm. The length of follow-up ranged from 1 week to 12 months.

Beta-blockers versus CCB

9 trials assessed beta-blockers versus CCB.

Resting heart rate.

Meta-analysis of five trials showed no evidence of a difference between beta-blockers versus CCBs (MD 2.16, CI 95% -1.25 – 5.56, *P* value = 0.22) (eFigure 7). Visual inspection of the forest plot and the statistical test (I^2 = 22.72%) indicated low heterogeneity. The TSA Z-curve crossed the required information size to assess a 7.5 bpm difference, which was equal to SD/2. Hence, TSA confirmed that additional trials are futile. This outcome result was assessed at high risk of bias (eTable 2) and the certainty of the evidence was low (eTable 4).

Test for subgroup differences according to type of beta-blocker (*P* value = 0.16), CCBs used *(P* value = 0.63), parallel and cross-over trials (*P* value = 0.23), and year of publication (*P* value = 0.06) showed no evidence of a difference. The remaining preplanned subgroup analyses were not possible due to lack of relevant data

Maximal exertional heart rate

Meta-analysis of six trials showed no evidence of a difference between beta-blockers versus CCBs (MD -0.52, CI 95% -6.87 – 5.82, *P* value = 0.87). Visual inspection of the forest plot and the statistical test (I^2 = 33.07%) indicated moderate heterogeneity. The TSA Z-curve crossed the required information size to assess a 13 bpm difference, which was equal to SD/2. Hence, TSA confirmed that additional trials are futile. This outcome result was assessed at high risk of bias (eTable 2) and the certainty of the evidence was very low (eTable 4).

Test for subgroup differences according to type of beta-blocker (*P* value = 0.03) and year of publication (p=0.02) showed evidence of a difference

There were differences depending on which beta-blocker was used: Mix of Bisoprolol, atenolol or metoprolol: MD -15.00, CI 95% -32.59 – 2.59, *P* value = 0.09; propanolol: MD -1.00, CI 95% -20.15 – 18.15, *P* value = 0.92; xamoterol: MD 5.10, CI 95% -11.48 – 21.68, *P* value = 0.55; betaxolol MD -10.00, CI 95% -39.42 – 19.42, *P* value = 0.51; carvedilol: MD -10.00, CI 95% -20.47 – 0.47, *P* value = 0.06); metoprolol: MD 7.91, CI 95% 1.15 – 14.68, *P* value = 0.02. Results of the different subgroups are presented in eFigure 8.

Test for subgroup differences according to type of CCB (*P* value = 0.57), and parallel and cross-over trials (*P* value = 0.18) found no evidence of a difference. The remaining preplanned subgroup analyses were not possible due to lack of relevant data

Exercise capacity

Seven trials comparing beta-blockers versus CCBs assessed exercise capacity using different exercise tests and measuring units (eFigure 9).

Using standardized mean difference, a statistically significant difference was found (SMD -0.26, CI 95% -0.45 – -0.08, P value = 0.01; eFigure 10). The difference was not clinically significant (below SD/2).

This outcome result was assessed at high risk of bias (eTable 2) and the certainty of the evidence was very low. The preplanned subgroup analyses were not possible due to lack of relevant data.

Beta-blockers versus digoxin

Resting heart rate.

Meta-analysis of five trials did not show a statistically significant difference (MD -1.54, CI 95% -5.72 – 2.63, *P* value = 0.19). Visual inspection of the forest plot and statistical tests (I^2 = 34.24%) showed moderate heterogeneity which resulted in us presenting data for each individual beta-blocker alone (see below). TSA showed we had enough information to reject a difference of a 6.5 bpm difference, which was equal to SD/2.

This outcome result was assessed at high risk of bias (eTable 2) and the certainty of the evidence was very low (eTable 5). Tests for subgroup differences according to type of beta-blocker (*P* value = 0.19), cross-over design versus parallel group design (*P* value = 0.85), and year of publication (*P* value = 0.86) showed no evidence of a difference

However, since there was some statistical heterogeneity, we present results based on the type of beta-blocker:

Metoprolol: MD -8.20, CI 95% -15.91 – -0.49, *P* value = 0.037; sotalol: MD -3.00, CI 95% -15.85 – 9.85, *P* value = 0.647); bisoprolol MD -1.10, CI 95% -4.54 – 2.34, *P* value = 0.531; labetalol: MD 1.00, CI 95% -9.89 – 11.89, *P* value = 0.857; xamoterol: MD 10.00, CI 95% -3.30 – 23.30, *P* value = 0.141 (eFigure 11);

The remaining preplanned subgroup analyses were not possible due to lack of relevant data

Maximal exertional heart rate

Meta-analysis of four trials showed that beta-blocker versus digoxin reduced the maximal exertional heart rate (MD -33.50, CI 95% -5123 – -15.78, *P* value < 0.001). Visual inspection of the forest plot and statistical tests (I^2 = 91.58%) indicated substantial heterogeneity.

TSA showed we had enough information to reject a difference of a 9 bpm difference, which was equal to SD/2. This outcome result was assessed at high risk of bias (eTable 2) and the certainty of the evidence was low (eTable 5).

Tests for subgroup differences according to type of beta-blocker (*P* value < 0.001) and cross-over design versus parallel group design (*P* value < 0.001) showed evidence of a difference:

Sotalol: MD -59.00, CI 95% -71.92 – 46.08, *P* value < 0.001); metoprolol: MD -33.30, CI 95% -42.56– -24.04, *P* value < 0.001; labetalol: MD -21.00, CI 95% -30.05 – -11.95, *P* value < 0.001; xamoterol: MD -14.00, CI 95% -44.61 – 16.61, *P* value = 0.370 (eFigure 12).

The remaining preplanned subgroup analyses were not possible due to lack of relevant data

Exercise capacity

Six trials comparing beta-blockers versus digoxin assessed exercise capacity using different exercise tests and measuring units (eFigure 13). Meta-analysis using standardized mean difference, there was no statistically significant difference (SMD 0.37, CI 95% -0.01 - 0.74, *P* value = 0.05; eFigure 14).

This outcome result was assessed at high risk of bias (eTable 2) and the certainty of the evidence was very low (eTable 5).

The preplanned subgroup analyses were not possible due to lack of relevant data.

CCBs versus digoxin

8 trials assessed CCB and digoxin.

Resting heart rate

Meta-analysis of 5 trials showed that CCB reduced the resting heart rate (MD -6.46, CI 95% -12.16 – -0.77, P value = 0.03; eFigure 19). The difference was above our predefined minimal important difference (SD/2= 6). Visual inspection of the forest plot and statistical tests (I^2 = 55%) indicated moderate heterogeneity which could not be resolved. TSA showed we did not have enough information to confirm or reject a difference of 6 bpm. This outcome result was assessed at high risk of bias (eTable 2) and the certainty of the evidence was very low (eTable 7).

Tests for subgroup differences according to type of CCB (*P* value = 0.56), cross-over design versus parallel group design (*P* value = 0.72), and year of publication (*P* value = 0.72) showed no evidence of a difference. The remaining preplanned subgroup analyses were not possible due to lack of relevant data.

Exertional heart rate

Maximal exertional heart rate

Meta-analysis of four trials showed that CCB reduced maximal exertional heart rate (MD -21.74, CI 95% -36.61 – -6.87, *P* value = 0.0042). Visual inspection of the forest plot and statistical tests (I^2 = 76.95%) indicated substantial heterogeneity. When removing Ahuja et al the heterogeneity was resolved (eFigure 20). Meta-analysis still showed that CCB reduced the maximal exertional heart rate (MD -16.03, CI 95% -25.80 – -6.26, *P* value = 0.0013. TSA showed we did not have enough information to reject a difference of a 11 bpm difference. This outcome result was assessed at high risk of bias (eTable 2) and the certainty of the evidence was low (eTable 7).

Tests for subgroup differences according to type of CCB (*P* value = 0.56), and year of publication (*P* value = 0.84) showed no evidence of a difference. The remaining preplanned subgroup analyses were not possible due to lack of relevant data.

Exercise capacity

4 trials comparing CCB versus digoxin assessed exercise capacity using different exercise tests and measuring units (eFigure 22). Using standardized mean difference, there was no evidence of a difference (SMD 0.52, CI 95% -0.35 - 1.39, P value = 0.24). This outcome result was assessed at high risk of bias (eTable 2) and the certainty of the evidence was low (eTable 7). The preplanned subgroup analyses were not possible due to lack of relevant data.

Discussion

Main results

We included 51 trials. The trials used very different classes of drugs (i.e., beta-blockers, calcium channel blockers, digoxin etc.), different subclasses of drugs, and different doses. There was very limited data on all-cause mortality, serious adverse events, and quality of life, which should be considered the most important patient relevant outcomes. The largest included trial was RATE-AF trial.[15]

Beta-blockers, calcium channel blockers and digoxin all reduced resting heart rate (results not shown), but only beta-blockers and calcium channel blockers appeared to reduce maximal exertional heart rate. Digoxin did not appear to reduce exercise capacity compared with either beta-blocker or calcium channel blocker. Atenolol, bisoprolol, and carvedilol may reduce maximal exertional heart rate more than calcium channel blockers whereas metoprolol may reduce it less. Beta-blockers may reduce exercise capacity compared with calcium channel blockers, and metoprolol and carvedilol appear to reduce exercise capacity the most, but the evidence is uncertain.

There are several possible takeaways from our results to current clinical practice. Our results appear to indicate that better exertional heart rate control does not necessarily translate to better exercise capacity. This is one of the chief concerns with using digoxin for heart rate control and why it is reserved for sedentary patients.[3] Similarly, the recent Rate AF trial comparing bisoprolol to digoxin found no difference in six minute walking distance between digoxin and bisoprolol despite similar resting heart rate.[15] In contrast, the trial found an increased number of adverse events with bisoprolol compared with digoxin. If the results of the individual patient data meta-analysis comparing beta-blocker to placebo for heart failure in atrial fibrillation patients is considered valid, the argument for using beta-blockers over digoxin seems limited for atrial fibrillation.[16]

However, the results of the systematic review also suggest beta-blockers, specifically metoprolol and carvedilol, reduce exercise capacity compared with calcium channel blockers, while metoprolol reduce maximal exertional heart rate less than calcium channel blockers. Taken together, the results show a difficult relationship between exertional heart rate control, exercise capacity, and type of drug used.

Our systematic review has several strengths. Our methodology was predefined which limits the risk of data driven biased results. Our methodology will be based on the Preferred Reported Items for Systematic reviews and Meta-Analyses Protocol (PRISMA-P), Keus et al., an eight-step assessment as suggested by Jakobsen et al., Trial Sequential Analysis, and GRADE assessment.[1,12-14] Both beneficial and harmful effects were assessed.

Limitations

Our review also has several limitations. One major limitation was the way drugs were administered. Most trials did not have a target heart rate. Usually in clinical practice, the attending physician will have either a specific heart rate target in mind or adjusted until symptoms resolve. In most trials, drugs were administered at a fixed dose. The fixed doses were, however, often not comparable if the benchmark was current recommend maximum tolerable doses according to ESC.[3] There was lack of systematic descriptions of the included populations which makes it harder to assess the generalizability of the results. One important characteristic to note is the age of the participants: The participants included in the trials were younger and the implications of heart rate in younger persons may be markedly different than from those who are older.

Conclusion

The comparative effects of rate controlling drug for atrial fibrillation on patient important outcomes are unknown. Of the available drugs, beta-blockers and calcium channel blockers seem to reduce maximal exertional heart rate most. Beta-blockers may reduce exercise capacity compared with calcium channel blockers.

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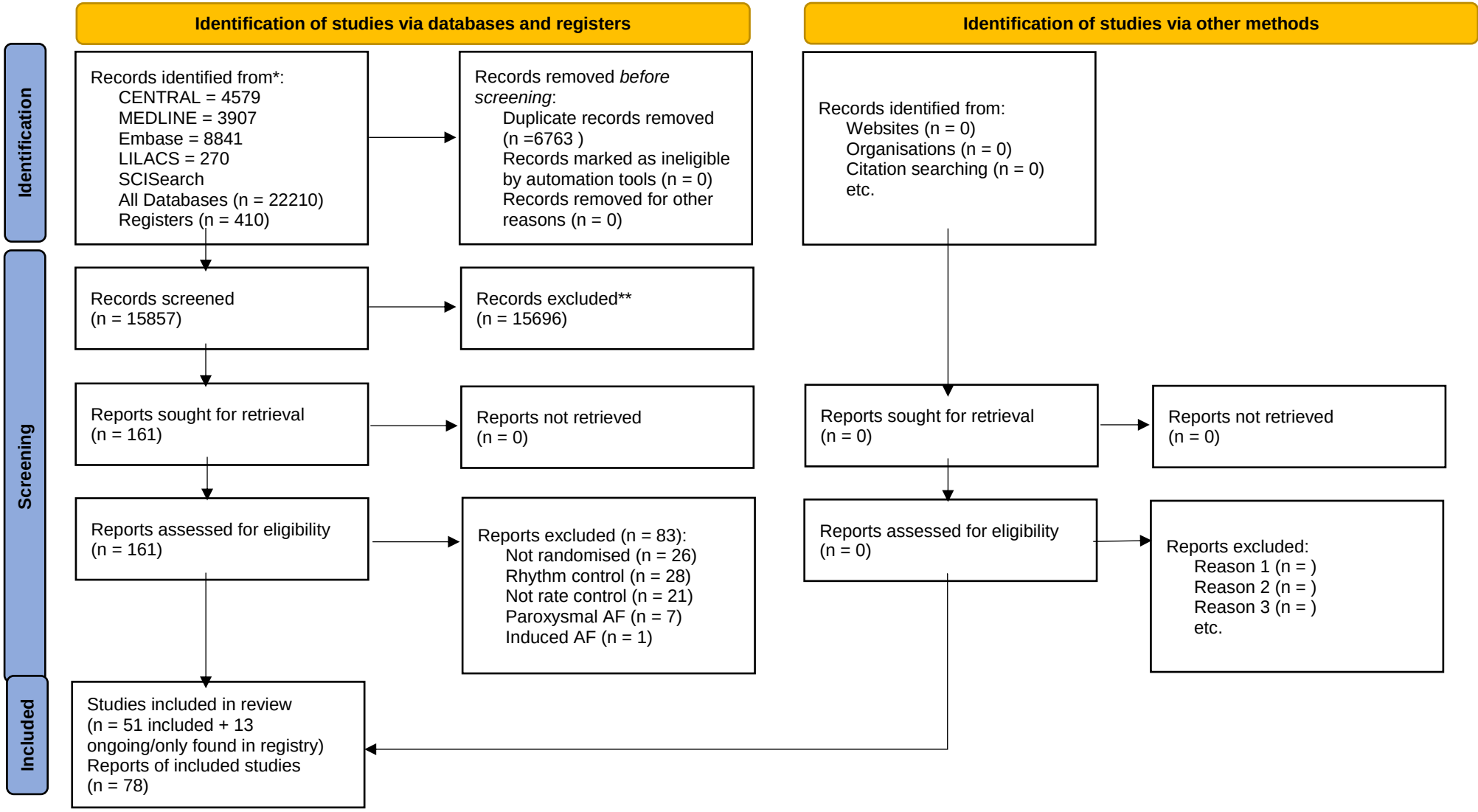
Figure 1 – Prisma 2020 flow chart



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	4-5
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	5
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	5
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5-6
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Appendix
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	6
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Appendix
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	6
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	7
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	6
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	6
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	6-7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	7
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	NA
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	7
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	7

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Figure 1
Study characteristics	17	Cite each included study and present its characteristics.	Table 1a and 1b
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Appendix
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	8-14 + eFigures,
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	8-14
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	8-14
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	8-14
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Appendix, SoF tables
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Appendix, SoF tables
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	15-16
	23b	Discuss any limitations of the evidence included in the review.	15-16
	23c	Discuss any limitations of the review processes used.	15-16
	23d	Discuss implications of the results for practice, policy, and future research.	15-16
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	1
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Appendix
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Appendix
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Article information section
Competing interests	26	Declare any competing interests of review authors.	Article information section
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Article information section

PRISMA 2020 Checklist

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Table of contents

eTables

eTable 1 – Characterstics of studies

eTable 1a – parallel studies							
Study ID	Country	Participants	Inclusion criteria	Exclusion criteria	Interventions	Outcomes used	Length of treatment /Length of follow-up including length of treatment
Afrodite 2011[1]	The Netherlands	Persistent AF	Persistent AF with HR >80 bpm at rest despite treatment with ≤2 rate control agents (i.e. beta blocker and/or calcium antagonist - subjects using digoxin were eligible), documented AF in the past 24 hours, age >45 years, predefined accepted conventional rate control treatment, and anticoagulant treatment in line with local guidelines	Main criteria for exclusion were paroxysmal or permanent AF, use of class I or III anti-arrhythmic drugs [AAD], scheduled for cardioversion or pulmonary vein ablation, unstable NYHA class III or all class IV heart failure, atrioventricular block grade 2 or 3, known severe renal or hepatic impairment, participation in a clinical drug study in the 3 months prior to inclusion, lactating women and those of childbearing potential who do not use adequate contraception.	**Arm 1:** Dronedarone 400 mg twice daily + conventional rate control **Arm 2:** Placebo + increase in conventional rate control.	Mean ventricular rate, mortality, SAE, AE.	12 week/12 weeks

Brodsky 1993[2]	USA	Chronic Afib	Resting heart rate >80 beats/min, a heart rate ~120 beats/min after 6 minutes of exercise with a modified Bruce exercise protocol, and an increase of ~50 beats/min in response to 6 minutes of the exercise protocol.	No Beta-blockers, calcium antagonists, or other antiarrhythmic agents. Patients with significant or unstable cardiac, renal, hepatic, endocrine, pulmonary, or neurologic disease, evidence of digitalis toxicity, hypokalemia (<3.5 mEq/L), hypomagnesemia (<1.5 mEq/L), or a corrected QT interval >450 msec	**Arm 1**: sotalol 80 mg /day **Arm 2**: sotalol 160 mg/day **Arm 3**: placebo. All arms received digoxin (below 0.375 mg/day)	Mortality, Heart rate (resting, exertional), change an average heart rate on Holter.	4 weeks/4 weeks
Capucci 2000[3]	Italy	Persistent, Pre direct current conversion	First episode of atrial fibrillation lasting longer than 2 weeks.	age >75 years, left atrial diameter >55 mm, thyrotoxicosis, pregnancy, acute myocarditis or pericarditis, acute myocardial infarction, uncompensated heart failure (New York Heart Association functional class III–IV), diastolic blood pressure >115 mmHg, history of pulmonary hypertension, unstable hepatic or renal function, amiodarone therapy within the last 12 months, or resting rate <90/min; also excluded were those affected by sick sinus syndrome, bundle-	**Arm 1**: Amiodaron 400 mg/day **Arm 2**: Diltizem, Starting dose of 60 mg 3 times daily, adjusted to achieve below 80 beats per minute	Mortality, non-serious adverse reactions, resting heart rate.	1 month/1 month

				branch block, and QT prolongation			
Connolly 2011[4]		Permanent atrial fibrillation (98%) or flutter (2%)	At least 65 years of age with at least one of the following risk factors: coronary artery disease; previous stroke or transient ischemic attack; symptomatic heart failure, which was defined as current New York Heart Association class II or III symptoms and admission to the hospital for heart failure in the previous year (but not in the most recent month); a left ventricular ejection fraction of 40% or less; peripheral arterial disease; or the combination of an age of 75 years or older, hypertension, and diabetes.	Major exclusion criteria were paroxysmal or persistent atrial fibrillation, use of an implantable cardioverter–defibrillator, sustained daytime bradycardia of less than 50 beats per minute, or a QT interval corrected for heart rate of more than 500 msec (or >530 msec for patients with a paced ventricular rhythm).	**Arm 1:** Dronedaron 400 mg twice daily **Arm 2**: placebo	Mortality, serious adverse events, adverse events, resting heart rate.	Treatment duration equal to median follow-up of 3.5 month.
Davy 2008[5]	9 European	Permanent atrial fibrillation	adult patients (≥21 years) with	Patients were excluded if they had a history of	**Arm 1:** Dronedarone 400 mg twice daily +	Mortality, SAE, AE, exertional	6 month/6 month.

	countries		documented, symptomatic permanent AF, for which cardioversion was not considered an option. To be eligible for inclusion, patients had to have a resting ventricular rate of ≥80 beat/min as measured on a 6-second rhythm strip.	unstable angina pectoris, a history of torsades de pointe, baseline (D0) plasma potassium b3.5 mmol/L, third-degree atrioventricular block or significant sinus node disease, New York Heart Association (NYHA) class III or IV congestive heart failure (CHF), or clinically relevant hematological, hepatic, gastrointestinal, renal, endocrinological, or psychiatric disease.	usual rate controlling drugs **Arm 2:** Placebo + usual rate controlling drugs	heart rate, average heart rate	
Dronedarone 2011[6]	Japan	181 Permanent atrial fibrillation	Duration more than 6 month, aged ≥20 years and resting ventricular heart rate ≥80 bpm	Unstable angina pectoris. History of torsades de pointes. Prolonged QT corrected interval (≥ 500 ms). Third degree atrioventricular block (AVB) on the screening ECG while in AF or, documentation on previous ECGs while in sinus rhythm of PR-interval > 0.28 sec or high degree AVB (2nd degree or higher) or, significant sinus node disease (documented pause ≥ 3 sec) - without a permanent pacemaker implanted.	**Arm 1:** Dronedarone 300 mg twice daily **Arm 2:** Dronedarone 400 mg twice daily **Arm 3:** Dronedarone 600 mg twice daily **Arm 4:** Placebo		

				Congestive Heart Failure (CHF) of New York Heart Association classification (NYHA) class IV or recent (within 1 month prior to randomization) unstable NYHA class III. Treatment with other class I or III anti-arrhythmic drugs. Patients treated with amiodarone during the 4 weeks preceding randomization. Clinically relevant haematologic, hepatic, gastro-intestinal, renal, pulmonary, endocrinologic (in particular thyroid) or psychiatric disease. Hypokalemia and hypomagnesemia must be corrected before randomization.			
Holming 2001[7]	Sweden	31 chronic atrial fibrillation participants	chronic afib not treated with any chronotropic drugs	None stated	**Arm 1:** Sotalol 80 mg 3 times daily **Arm 2:** Digoxin 0.13 – 0.25 mg daily **Arm 3:** Sotalol 80 mg 3 times daily + digoxin	Mortality, heart rate, (Resting +exertional) , exercise capacity.	1 month/1 month

					0.13 – 0.25 mg daily		
Inoue 2017[8]	Japan	Persistent or permanent AF	aged > 20 years 24 hour mean HR >80 beats per minute on Holter electrocardiogram	The study excluded patients who had received b-blockers, calcium channel blockers (diltiazem and verapamil), or antiarrhythmic drugs; or had been treated for heart failure (New York Heart Association, NYHA, class II–IV)	**Arm 1**: 5 mg carvedilol **Arm 2**: up to 10 mg carvedilol **Arm 3**: up to 20 mg carvedilol.	Resting heart rate/average heart rate.	6 weeks/10 weeks
Koh 1995a[9]	Korea	Chronic AF	>1 month duration	HR at rest of <60 beats/min, ejection fraction of the left ventricle <30%, diabetes mellitus, bronchial asthma, chronic obstructive lung disease, untreated thyrotoxicosis <2 months after myocardial infarction, and systolic blood pressure ~90 mm Hg.	**Arm 1:** diltiazem 90 mg twice daily + digoxin 0.125-0.5 mg **Arm 2**: No intervention **Arm 3**: Betaxolol 20 mg daily+ digoxin 0.125-0.5 mg **Arm 4**: Digoxin 0.125-0.5 mg	Resting heart rate, exertional heart rate, exercise capacity.	4 weeks/4 weeks
Ribeiro 1986[10]	Brazil	Chronic AF	None stated	None stated	**Arm 1:** Timolol 10 mg twice daily + digoxin **Arm 2:** placebo + digoxin	Only reports on timolol group.	10 weeks/10 weeks.
Simeonidou 2010[11]	Greece	Persistent or chronic AF	Preserved EF	None stated	**Arm 1:** Diltiazem titrated to resting HR<80 bpm	No outcomes used.	3 month/3 month

					and moderate exercise HR<100 bpm **Arm 2:** metoprolol titrated to resting HR<80 bpm and moderate exercise HR<100 bpm		
Tse 2001[12]	Hong Kong, China	Chronic AF	Previous failed attempt to restore and maintain sinus rhythm	(I) Intolerance of amiodarone or digoxin or contraindication to their therapy; (II) amiodarone therapy in the past 6 months; (III) clinically significant valvular heart disease; (IV) unstable angina or recent myocardial infarction in the past 6 months; (V) class III or IV heart failure; (VI) sick sinus syndrome; and (VII) implanted pacemaker	**Arm 1:** Digoxin, 0.25 mg daily or 0.125 mg daily if bodyweight < 50 kg or serum creatinine > 200 mmol/L **Arm 2:** Amiodaron, 600 mg daily for 1 week as a loading dose and then 100 mg daily	Mortality, non serious adverse reactions, QoL, symptom score, heart rate(averag e, exertional), exercise capacity	24 weeks/24 weeks
Tsuneda 2006[13]	Japan	Permanent AF	Resting HR between 60 and 80beats/min with digitalis for more than 6 months were selected	Patients with severe underlying cardiovascular diseases other than hypertension, New York Heart Association class III or IV symptoms, and contraindication for BB were excluded.	**Arm 1:** Bisoprolol, atenolol or metoprolol dosed to achieve HR 60-80 bpm **Arm 2:** Verapamil, dosed	Average heart rate, exertional heart rate, quality of life, exercise capacity	1 month/1 month

					to achieve HR 60-80 bpm		
Van Noord 2001[14]	The Netherlands	Pre DC participants	Persistent AF with a ventricular rate. 90 beats/min documented on resting ECG and planned electrocardioversion within 1 month	(1) history of second- or third-degree AV conduction block; (2) known sick sinus syndrome; (3) heart failure according to the New York Heart Association functional class III or IV; (4) unstable angina pectoris; (5) current treatment with calcium channel blockers or digoxin; (6) concomitant treatment with Class I or III antiarrhythmic drugs (amiodarone should not have been used during the last 3 months); (7) untreated hyperthyroidism or hypothyroidism; (8) serious pulmonary, hepatic, hematologic, metabolic, renal, gastrointestinal, central nervous system, or psychiatric disease; (9) pacemaker treatment; (10) contraindications for oral anticoagulant agents; and (11) age <18 or >85 years.	**Arm 1:** Verapamil 120 to 360 mg daily (depending on heart rate at inclusion and during 24-hour Holter monitoring 2 weeks after inclusion) **Arm 2:** Digoxin 0.125 mg – 0.25 mg daily dependent on age and renal function.	Mortality	1 month/1 month

Villani 2000[15]	Italy	Persistent AF, pre DC conversion.	stable circulatory conditions with chronic persistent AF (>2 weeks' duration) referred to our cardiac unit for the first electric cardioversion attempt	age >75 years, left atrial diameter >55 mm, thy- rotoxicosis, pregnancy, acute myocarditis or pericarditis, acute myocardial infarction, unstable severe heart failure (New York Heart Association functional class III to IV), diastolic blood pres- sure >115 mm Hg, history of pulmonary hypertension, unstable hepatic or renal function, amiodarone therapy within the last 12 months, or resting heart rate, without medication, of <90 beat/min. Also excluded were patients affected by sick sinus syndrome, bundle branch block, and/or QT prolongation (ie, corrected QT >0.45 s). All antiarrhythmic drugs administered before inclusion in the study were discontinued for at least >5 half-lives (included β-blockers and calcium channel blockers).	**Arm 1:** Diltiazem adjusted to resting HR < 80 bpm **Arm 2:** Amiodarone 400 mg/day **Arm 3**: digoxin 0.25 mg/day	Mortality, SAE, non-serious adverse events, resting heart rate.	1 month/1 month.
Wongcharoen 2016[16]	Thailand	Non-paroxysmal atrial fibrillation	Mean ventricular rate >70 beats/min.	None stated	**Arm 1:** Ivabradin 5 mg twice daily **Arm 2**: placebo	Mortality, adverse reactions, average heart rate,	1 month/1 month.

					Both arms: Other rate controlling drugs that the patient was stable on 3 month before randomisation	ejection fraction.	
Yamashita 2013[17]	Japan	Chronic AF	Outpatients with chronic (persistent or permanent) AF and whose resting heart rate was ≥80beats/min and systolic blood pressure ≥110 mmHg. To be randomised, Participants on 2.5 mg bisoprolol had to either have 1) resting HR in 12-lead ECG above 80 bpm, or (2) the resting HR in 12-lead ECG was 70–79 bpm and subjective symptoms did not resolve.	Heart failure, cardiomyopathy, cardiogenic shock, or myocarditis; patients with cardiac dysfunction, serious arrhythmia, severe aortic or mitral valve stenosis/regurgitation; patients with an implanted device previous myocardial infarction, who had undergone cardiovascular surgery unstable angina in the previous 6 months; and patients who had undergone electrical defibrillation or catheter ablation in the previous 3 months. contraindicated for beta-blockers; history of stroke patients con-traindicated for anticoagulant therapy. b-Blockers, diltiazem, verapamil, antiarrhythmics, and car-diotonic drugs (including digitalis) were not permitted to be	**Arm 1:** 5 mg for 2 weeks **Arm 2**: 2.5 mg bisoprolol for 2 weeks All participants had a 2 week run in period with 2.5 mg bisoprolol to assess if patients were eligible for the randomisation phase.	Mortality, SAE, resting heart rate, average heart rate.	2 weeks/4 weeks

				administered concomitantly with bisoprolol starting from 1 week prior to the start of treatment period 1 (6 months prior in the case of amiodarone).			
Yamashita 2018[18]	Japan	Persistent or permanent AF	Aged between 20 and 80 years with resting HR > 80 bpm	cardiogenic shock, heart failure (New York Heart Association functional class II–IV), cardiomyopathy, myocarditis, cardiac function deterioration (left ventricular ejection fraction <50%); severe arrhythmia, including atrioventricular block (II–III), sinoatrial block, and sick sinus syndrome; HR controlled by a pacemaker, implantable cardioverter defibrillator, or cardiac resynchronization therapy; patients who had undergone electrical defibrillation or catheter ablation; systolic blood pressure at randomization <110 mmHg; patients who had poor skin condition at the patch application site.	**Arm 1:** Bisoprolol 2.5 mg followed by 5 mg if the resting HR in 12-lead ECG was 80 bpm, or (2) the resting HR in 12-lead ECGwas 70–79 bpm and subjective symptoms did not resolve. **Arm 2**: Bisoprolol 2.5 mg daily **Arm 3**: Bisoprolol transdermal patch 8 mg **Arm 4**: Bisoprolol transdermal patch 4 mg	Mortality, SAE, non-SAE, resting heart rate, successful achievement of resting HR, average HR	1 month/1 month

Zoble 1987[19]	USA	Non paroxysmal AF	Ventricular rate at rest > 80/min or >_ 120/ min with exercise, and serum digoxin levels within the therapeutic range.	No renal, hepatic or thyroid disease, electrolyte disturbances or recent myocardial infarction (<3 months). Also excluded were patients with bronchospasm, severe chronic obstructive pulmonary disease, insulin-dependent diabetes, significant congestive heart failure, sick sinus syndrome or severe claudication. Excluded medications included tions included sodium channel blockers, calcium antagonists, other /Ladrenergic receptor blockers and sympathomimetic amines.	**Arm 1:** Nadolol up to 120 mg dependent on HR > 50 bpm + digoxin. **Arm 2**: Placebo + digoxin	Mortality, adverse reactions, resting HR, exertional heart rate, exercise capacity.	6 weeks/6 weeks

eTable 1b - Cross-over studies							
Study ID	Country	Participants	Additional inclusion criteria besides AF	Exclusion criteria	Interventions	Outcomes used	Length of treatment /total follow-up (including treatment period).
Ahuja 1989[20]	India	Persistent AF	Isolated reumatic stenosis of mitral valve, stable symptoms upon entry.	None described	**Arm 1**: 100 mg metoprolol twice daily **Arm 2**: digoxin 0.25 mg twice daily **Arm 3**: verapamil 80 mg daily All doses were ½ for the first 2 weeks.	Exercise capacity, heart rate (resting, exertional).	4 weeks/4 weeks
Ang 1990[21]	England	Chronic AF	Documented bradycardiac episode (<50 beats per minute).	None described	**Arm 1:** Xamoterol 200 mg twice daily **Arm 2:** Placebo **Arm 3:** Digoxin 0.065 – 0.125 mg (to achieve low therapeutic dose)	Exercise capacity, heart rate (resting, exertional).	2 weeks/2 weeks
Atwood 1987[22]	USA	Chronic AF	At least 6 month duration of AF	None had conges- tive heart failure at the time of the study and all were in New York Heart Association functional class I or II.	**Arm 1:** Celiprolol 600 mg daily + digoxin 0.25 mg daily **Arm 2:** Placebo + digoxin 0.25 mg	Exercise capacity, heart rate (resting).	1 week/1 week
Atwood 1989[23]	USA	chronic AF	At least 1 year's duration		**Arm 1:** Betaxolol 20 mg daily + digoxin unknown dose daily	Exercise capacity, heart	1 week/1 week

					Arm 2: Placebo + digoxin 0.25 mg	rate (resting, exertional).	
Bolognesi 1980[24]	Italy	Chronic AF	No additional inclusion criteria.	Subjects over 65 years of age, those with heart failure, marked bradycardia (<55 beats / min), and any grade A-V conduction disturbances were excluded.	**Arm 1**: Metoprolol 100 mg three times daily **Arm 2**: Metoprolol	Average heart rate (Holter)	1 week/1 week
Botto 1998[25]	Italy	Permanent AF (duration over 6 month).	Stable without significant structural heart disease, NYHA 1, HR >100 beats per minute Normal thyroid function	Renal failure, congestive heart failure, EF <40%, angina or recent MI (<6 months), preexcitation syndrome, electrolyte imbalance, uncontrolled hypertension, antiarrhythmic agents, necessity of digoxin therapy or contraindications to CCB.	**Arm 1**: Slow release gallopamil 100 mg twice daily **Arm 2**: Diltiazem 120 mg twice daily **Arm 3**: Verapamil 120 mg twice daily **Arm 4**: Digoxin therapeutic level (mean=0.250 mg/daily)	Heart rate (resting, exertional).	1 week/1 week
Channer 1987[26]	UK	Chronic AF	Symptoms of AF.	None stated	**Arm 1**: Verapamil 120 mg/daily + maintenance dose digoxin. **Arm 2**: Double maintenance dose digoxin (up to 0.5 mg/day)	Max exertional heart rate during 24 hour Holter, exercise capacity.	1 month/1 month
Channer 1994[27]	UK	Chronic AF	Inadequately controlled on digoxin.	None stated	**Arm 1:** Atenolol 50 mg daily **Arm 2**: Atenolol 100 mg daily **Arm 3**: Pindolol 10 mg daily **Arm 4**: Pindolol 30 mg daily	Resting heart rate, exertional heart rate, symptoms.	2 weeks/2 weeks

					All participants were kept on maintenance dose digoxin.		
Coburn 1979[28]	UK	AF	Uncontrolled (HR >100)	B-adrenoceptor blocking drugs, other anti-arrhythmic drugs or spironolactone	**Arm 1**: Digoxin 0.500 mg daily **Arm 2**: Medigoxin 0.300 mg daily.	Resting heart rate.	2 weeks/2 weeks.
Dahlström 1992[29]	Sweden	Chronic AF	AF of more than 6 months' duration of therapy, were treated with digoxin therapy, were males aged 30-75 years or postmenopausal females.	Criteria for exclusion were as follows: angina pectoris, decompensated heart disease NYHA classes III-IV, severe ventricular arrhythmias, untreated thyreotoxicosis, marked anemia, glaucoma, advanced pulmonary disease, systolic blood pressure c 95 or > 160195 mmHg (before or during the prestudy period), diabetes mellitus, severe hepatic or renal disease, inability to withdraw (a) other antiarrhythmic drugs, other than digoxin; (b) vasodilators, including calcium-entry blockers; (c) beta blockers; (d) tricyclic antidepressants, phenothiazins, and diazepam, and myocardial infarction within the preceding 6 months.	**Arm 1**: diltiazem 60 mg three times daily + propranolol 20 mg three times daily +digoxin **Arm 2**: diltiazem 60 mg three times daily + digoxin **Arm 3**: propranolol 20 mg three times daily +digoxin	Resting heart rate, exertional heart rate, exercise capacity.	4 weeks/4 weeks.
Dibianco 1984[30]	USA	Chronic AF	chronic (non-paroyxsmal Afib). Resting	Renal failure, congestive heart failure, angina, recent myocardial infarction,	**Arm 1**: Nadolol optitred to achieve < 80 bpm during	Resting heart rate, average heart rate,	4 weeks/4 weeks.

			heart rates >80 bpm or to show a rate of 120 bpm or an increment of greater than 50 bpm during mild treadmill exercise provocation (3 minutes, 1.75 mph, 10% grade)	preexcitation syndrome, electrolyte disturbances, or uncontrolled hypertension. Contraindications to beta-adrenergic blockade, bradycardia, insulin dependent diabetes mellitus, or congestive heart failure, agents.	rest/120 during exercise, average 87 mg + digoxin if taken before start of trial. **Arm 2**: placebo + digoxin if taken before start of trial.	exertional heart rate, physical capacity.	
Farshi 1999[31]	USA	Chronic AF	No additional criteria	The patients with echocardiographic LVEF less than 35%, heart rate less than 55 bpm, Wolff-Parkinson-White syndrome, clinically significant renal, thyroid or hepatic dysfunction, uncontrolled hypertension, sick sinus syndrome, implanted pacemaker, unstable angina or acute myocardial infarction or persistent systolic blood pressure less than 95 mm Hg were excluded from the study. Patients receiving other medications such as theophylline, clonidine or inhaled beta-agonists, which might affect	**Arm 1**: atenolol 50 mg+ digoxin 0.250 mg **Arm 2**: Digoxin 0.250 mg **Arm 3**: Atenolol 50 mg **Arm 4**: Diltiazem 240 mg **Arm 5**: Diltiazem 240 mg + digoxin 0.250 mg	Average heart rate, exertional heart rate, exercise capacity.	2 weeks/2 weeks

				ventricular response in AF, as well as those with previous exposure to amiodarone, were excluded. Subjects who recently used an investigational drug or those with a history of untoward reaction to any of the medications used in the present study were also excluded.			
Furniss 1989[32]	UK	Chronic AF	Impairment of ventricular function (EF < 35%).	None stated.	**Arm 1**: xamoterol unknown dose + digoxin **Arm 2**: placebo + digoxin.	Max exertional heart rate	Unknown
James 1989[33]	UK	Chronic AF	Symptoms	Symptomatic with palpations and/or breathlessness.	**Arm 1**: Pindolol 15 mg twice daily+ digoxin **Arm 2**: Verapamil 40 mg three times daily + digoxin	Exertional heart rate.	1 month/1 month
Kochiadakis 2001[34]	Greece	Chronic AF	>1 month duration	No history or signs of heart failure; absence of severe valvular heart disease; no evidence of ischaemic heart disease; no evidence of renal, hepatic, endocrine, pulmonary or neurological disease; no history of untoward reaction to any of the medications used in the	**Arm 1**: Sotalol 40 mg titrated up to HR target < 70 bpm (mean dose 206 mg) **Arm 2**: Metoprolol 50 mg titrated up to HR target < 70 bpm (mean dose 182 mg)	Resting heart rate, exertional heart rate, exercise capacity.	4 weeks/4 weeks.

				present study; and the ability to undergo a treadmill exercise test			
Lang 1982[35]	Israel	Chronic AF	>1 year duration.	Hepatic failure, moderate renal impairment (creatinine above 2 mg/dl), diabetes mellitus, electrolyte imbalance, and inability to undergo exercise testing on an ergometry bicycle were excluded from the study.	**Arm 1**: Verapamil individual dose assessed before randomisation resulting in 15% heart rate reduction + digoxin **Arm 2**: placebo + digoxin.	Resting heart rate, exertional heart rate, exercise capacity.	4 weeks/4 weeks
Laweson-Matthew 1995[36]	Unknown (prob UK).	Chronic AF	Chronic AF treated with digoxin for at least 3 month, had an abbreviated mental state score of >7/10 and were without contra-indications to B-adrenoceptor blockers.	Patients with mild heart failure (NYHA class I or II) were entered provided the requirement for loop diuretics was less than frusemide 80 mg or equivalent daily.	**Arm 1**: Xamoterol 200 mg twice daily + digoxin (average 200 ug). **Arm 2**: Xamoterol 200 mg twice daily + placebo	Symptom score (VAS), 24 hour Holtermonitoring.	1 month/1 month.
Lewis 1987[37]	Scotland	Chronic AF	No additional criteria.	None had uncontrolled cardiac failure, symptomatic ischaemic heart disease or a history of the sick sinus syndrome.	**Arm 1**: Verapamil increasing up to 120 mg three times daily **Arm 2**: Digoxin serum guided (1.3-2.6 nmol)	Resting heart rate, exertional heart rate.	6 weeks/6 weeks

Lewis 1988[38,39]	Scotland	Chronic AF	At least 1 years' duration	None stated.	**Arm 1**: Diltiazem 60 mg three times daily + Digoxin serum guided (1.3-2.6 nmol) **Arm 2**: Diltiazem 60 mg three times daily **Arm 3**: Digoxin serum guided (1.3-2.6 nmol)	Resting heart rate, average heart rate, exertional heart rate, exercise capacity, symptoms on VAS.	4 weeks/ 4 weeks
Lewis 1989[40]	Scotland	Chronic AF	At least 1 years' duration	No history of uncontrolled cardiac failure, "sick-sinus syndrome,", obstructive airways disease, insulin-dependent diabetes mellitus, or angina pectoris of a severity sufficient to limit exercise tolerance."	**Arm 1**: Atenolol 50 mg daily + digoxin. **Arm 2**: Verapamil 80 mg twice daily + digoxin **Arm 3**: Xamoterol 200 mg twice daily + digoxin **Arm 4**: Placebo + Digoxin	Resting heart rate, average heart rate, exertional heart rate, exercise capacity.	4 weeks/ 4 weeks
Lin 1986[41]	USA	Chronic AF	Stable, required to have a heart rate > 120 bpm or 30% increase in HR between minute 2 and 3 of exercise test.	None mentioned.	**Arm 1**: Celiprolol highest tolerated dose (most 600 mg) + digoxin therapeutic range. **Arm 2**: Placebo + digoxin therapeutic range	Exertional heart rate.	1 week/1 week
Lundström 1990[42]	Sweden	Chronic AF	>1 month	No exclusion criteria listed.	**Arm 1**: Diltiazem 270 mg daily + digoxin	Resting heart rate, average heart rate,	3 weeks/3 weeks

					Arm 2: Verapamil 240 mg daily + digoxin	exertional heart rate, exercise capacity.	
Lundström 1992[43]	Sweden	Chronic AF	No additional criteria.	Complete AV block, severe ventricular arrhytmias, bronchopulmonary disease, thyrotoxicosis, myocardial infarction that occurred less than 2 months before entry into the study, hepatic or renal disease, or any other disease that would be likely to interfere with the evaluation of drug effetcs were excluded, as were patients who required therapy with b-blockers, calcium channel blockers, or antiarrhythmic drugs.	**Arm 1**: Xamoterol 200 mg /daily + usual digoxin dosis (17/21 received digoxin). **Arm 2**: Xamoterol 400 mg /daily + usual digoxin dosis (17/21 received digoxin). **Arm 3**: Verapamil 240 mg daily + usual digoxin dosis (17/21 received digoxin). **Arm 4**: Placebo + usual digoxin dosis (17/21 received digoxin).	Resting heart rate, average heart rate, exertional heart rate, exercise capacity, symptoms on VAS scale.	2 weeks/2 weeks
Mitrovic 1981[44]	Germany	Chronic AF	All patients NYHA II/III	Exclusion criteria to beta-blocker.	**Arm 1**: 200 mg beta-blocker once daily + digoxin **Arm 2**: Placebo + digoxin	Average heart rate (missing SDs).	1 week/1 week
Molajo 1984[45]	UK	Chronic AF	>1 year duration.	None took any other antiarrhythmic agent apart from digoxin.	**Arm 1**: Xamoterol 200 mg daily +digoxin (0.25 – 0.325 mg) **Arm 2**: Placebo + digoxin (0.25 – 0.325 mg daily).	Heart rate (resting, exertional, exercise capacity)	2 weeks/2 weeks

Panidis 1983[46]	USA	Chronic AF or flutter	> maximal rate above 100 beats per minute in a exercise test between 2nd and 3rd minute.	Clinically overt congestive heart failure unstable angina, uncontrolled severe hypertension, Wolff-Parkinson-White Syndrome, renal or hepatic failure, insulin- dependent diabetes mellitus, or sick sinus syndrome without a functioning implanted pacemaker were excluded.	**Arm 1**: Verapamil individual dose assessed before randomisation resulting in 15% heart rate reduction **Arm 2**: Placebo	Resting heart rate, exertional heart rate.	2 weeks/2 weeks
Pomfret 1988[47]	New Zealand	Chronic AF	Greater than 6 month duration of chronic AF.	Clinical evidence of heart failure, were thyrotoxic or required other anti-arrhythmic therapy apart from digoxin or verapamil.	**Arm 1**: High dose digoxin (0.25-0.5 mg) **Arm 2**: 0.25 mg digoxin + 40 mg verapamil three times daily **Arm 3**: 40 mg verapamil three times daily **Arm 4**: 80 mg verapamil three times daily	Resting heart rate, exertional heart rate.	2 weeks/2 weeks
Scardi 1993[48]	Italy	Chronic AF	HR > 90, who were not controlled on digoxin.	Recent heart failure, systolic blood pressure below 100, myocardial infarction or any other disease that might interfere with the evaluation of drug effects.	**Arm 1**: Clonidin 0.075 mg twice daily +digoxin **Arm 2**: Placebo + digoxin.	Resting heart rate	3 days/3 days
Ulimoen 2013[49-51]	Norway	Permanent AF	Eligible patients were >18 years old, with permanent	The main exclusion criteria were congestive heart failure or ischemic heart disease with the need for	**Arm 1**: Metoprolol 100 mg daily **Arm 2**: Diltiazem 360 mg daily	Heart rate(resting, exertional), exercise	3 weeks/3 weeks.

			AF of 3 months' duration and had a heart rate at rest of 80 beats/min or an average heart rate of 100 beats/min during the day	concomitant treatment with b blockers, hypotension, treatment with class I or III antiarrhythmic drugs, severe renal or hepatic failure, and pregnancy	**Arm 3**: Verapamil 240 mg daily **Arm 4**: Carvedilol 25 mg daily	capacity, symptoms.	
Wong 1990[52]	Hong-Kong	11 participants with chronic AF. All were digitalised prior to randomisation and had a 2-3 week titration phase with beta-blocker.	Chronic, stable AF.	No Wolff-Parkinson-White or sick sinus syndrome.	**Arm 1:** Placebo **Arm 2:** Digoxin 0.25 mg daily **Arm 3:** Labetalol up to 200 mg twice daily + digoxin therapeutic dose **Arm 4**: Labetalol up to 400 mg twice daily	Exercise capacity, resting heart rate, moderate exertional heart rate, peak heart rate.	

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eTable 2 – Risk of bias table

Unique ID		D1	DS	D2	D3	D4	D5	Overall
Afrodite	1	!	!	!	-	-	+	-
Ahuja 1989	1	!	!	-	-	-	!	-
Ang 1990	1	!	!	!	+	+	!	!
Atwood 1987	1	!	!	+	+	+	!	!
Atwood 1989	1	!	!	!	+	-	!	-
Bolognesi 198	1	!	!	-	-	-	!	-
Botto 1998	1	!	-	!	+	-	-	-
Brodsky 1994-	1	!	-	!	+	+	!	-
Brodsky 1994-	1	!		!	+	-	!	-
Capucci 2000	1	!	!	!	+	-	!	-
CHANNER 198	1	!	-	!	+	-	!	-
CHANNER 199	1	!	-	!	+	-	!	-
Coburn 1976	1	!	!	!	+	-	!	-
Connolly 2011	1	+	-	+	+	+	+	+
Dahlstrom 19	1	!	-	!	-	+	!	-
Dahlstrom 19	1	!	-	!	-	-	!	-
Davy 2008	1	!	!	+	-	!	-	-
Dibianco 1984	1	!	!	+	+	+	!	!
Dronedaron 2	1	!		!	-	+	+	-
Frashi 1999	1	!	!	!	-	-	!	-
Holming 2001	1	!		!	+	-	!	-
inoue 2017	1	!	!	!	+	-	!	-
James 1989	1	!	!	-	+	-	!	-
Kochiadakis 2	1	!	!	!	+	-	!	-
Koh 1995-Cros	1	!	!	!	-	-	!	-
Koh 1995-M	1	!	!	!	-	+	!	-
Koh 1995-R	1	!		!	-	!	!	-
Kotecha-HR	1	+		-	+	-	!	-
Kotecha-M	1	+		!	+	+	!	!

+ Low risk

! Some concerns

- High risk

D1 Randomisation process

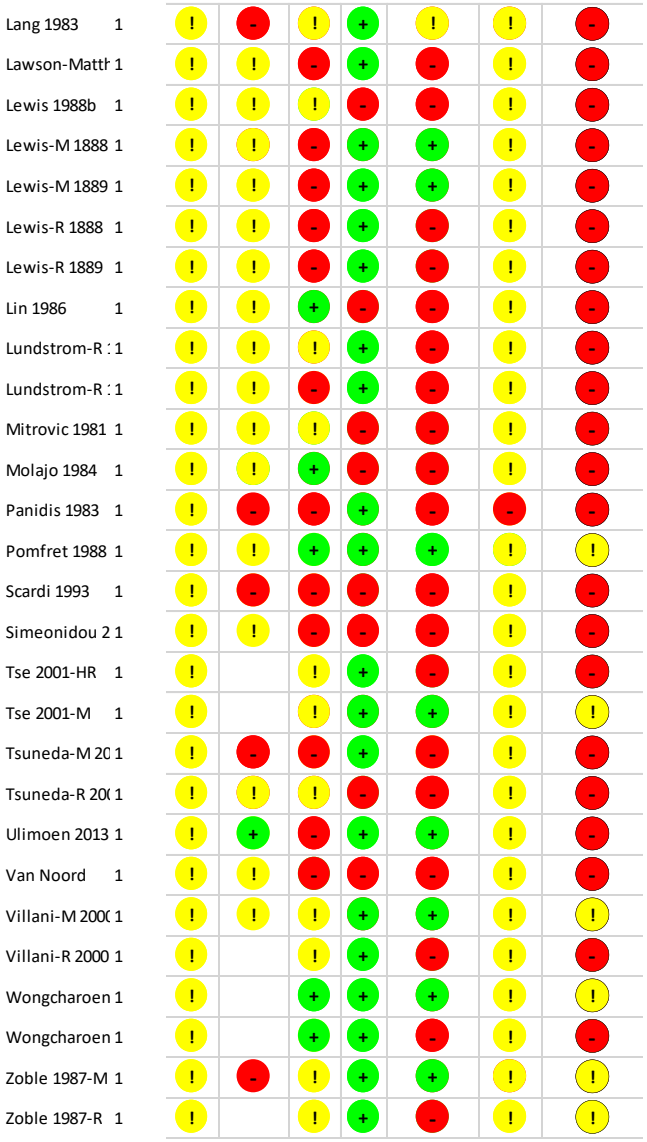
DS Bias arising from period and carryover effects

D2 Deviations from the intended interventions

D3 Missing outcome data

D4 Measurement of the outcome

D5 Selection of the reported result



eTable 3

Beta-blocker compared to placebo or in addition to another rate controlling drug for rate control in atrial fibrillation

Outcome № of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Certainty	What happens
				Difference		
All-cause mortality № of participants:	not estimable	0.0%	**0.0%** (0 to 0)	**0.0% fewer** (0 fewer to 0 fewer)	⨁◯◯◯ Very low[a,b]	Not possible to assess due to no events

132 (5 RCTs)						
Serious adverse events № of participants: (0 RCTs)	not estimable	0.0%	**0.0%** (0 to 0)	**0.0% fewer** (0 fewer to 0 fewer)	⨁◯◯◯ Very low[a,b]	Not possible to assess due to no events
Resting heart rate № of participants: 332 (11 RCTs)	-		-	MD **11.27 beats per minute lower** (14.97 lower to 7.57 lower)	⨁⨁◯◯ Low[a]	Beta-blocker may reduce resting heart rate but the risk of bias from the studies was large.
Maximal exertional heart rate № of participants: 340 (11 RCTs)	-		-	MD **34 bpm lower** (41.57 lower to 26.42 lower)	⨁⨁◯◯ Low[a]	Beta-blockers likely results in a large reduction in maximal exertional heart rate but the risk of bias is high. The magnitude of effect seems to depend on the beta-blocker used.
Exercise capacity № of participants: 291 (10 RCTs)	-	-	-	SMD **0.05 SD higher** (0.26 lower to 0.36 higher)	⨁◯◯◯ Very low[a,c]	The evidence is very uncertain about the effect of beta-blocker on exercise capacity.

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; **MD:** mean difference; **SMD:** standardised mean difference

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations
a. High risk of bias based on RoB2
b. No events
c. Very different ways of measuring exercise capacity

eTable 4

Beta-blocker compared to calcium channel blocker for rate control in atrial fibrillation

Outcome № of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Certainty	What happens
				Difference		
All-cause mortality № of participants: (1 RCT)	not estimable	0.0%	**0.0%** (0 to 0)	**0.0% fewer** (0 fewer to 0 fewer)	⨁◯◯◯ Very low[a,b]	Not possible to assess due to no events

Beta-blocker compared to calcium channel blocker for rate control in atrial fibrillation

Outcome № of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Certainty	What happens
				Difference		
Serious adverse events № of participants: (0 RCTs)	not estimable	0.0%	**0.0%** (0 to 0)	**0.0% fewer** (0 fewer to 0 fewer)	⨁◯◯◯ Very low[a,b]	Not possible to assess due to no events
Resting heart rate № of participants: 357 (5 RCTs)	-		-	MD **2.16 beats per minute higher** (1.25 lower to 5.56 higher)	⨁⨁◯◯ Low[a]	There seems to be no difference between beta-blockers and calcium channel blockers although the risk of bias was high. TSA confirmed that further trials are futile to find a difference of 7.5 bpm or higher.
Maximal exertional heart rate № of participants: 398 (6 RCTs)	-		-	MD **0.52 bpm lower** (6.87 lower to 5.82 higher)	⨁◯◯◯ Very low[a,c]	When pooling, there seems to be no difference between beta-blockers and calcium channel blockers although the risk of bias was high. TSA confirmed that further trials are futile to find a difference of 7.5 bpm or higher. However, different beta-blockers seemed to have different effects.
Exercise capacity № of participants: 446 (7 RCTs)	-	-	-	SMD **0.26 SD lower** (0.45 lower to 0.08 higher)	⨁◯◯◯ Very low[a,d]	The evidence is very uncertain about the effect of beta-blockers versus calcium channel blockers on exercise capacity.

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **MD:** mean difference; **SMD:** standardised mean difference

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations
a. High risk of bias based on RoB2
b. No events
c. Different results depending on the beta-blocker used
d. Very different ways of measuring exercise capacity

eTable 5

Beta-blocker compared to digoxin for rate control in atrial fibrillation

Outcome № of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Certainty	What happens
				Difference		
All-cause mortality № of participants: 160 (2 RCTs)	**RR 1.75** (0.53 to 5.75)	5.0%	**8.8%** (2.7 to 28.8)	**3.8% more** (2,4 fewer to 23,8 more)	⨁◯◯◯ Very low[a,b]	The evidence is very uncertain about the effect of beta-blockers on all-cause mortality.
Serious adverse events № of participants: 160 (1 RCT)	**RR 1.610** (0.870 to 2.998)	16.3%	**26.2%** (14.1 to 48.7)	**9.9% more** (2,1 fewer to 32,5 more)	⨁◯◯◯ Very low[a,b]	The evidence is very uncertain about the effect of beta-blockers on serious adverse events.
Resting heart rate № of participants: 232 (5 RCTs)	-		-	MD **1.54 beats per minute lower** (5.72 lower to 2.63 higher)	⨁◯◯◯ Very low[a,c]	The evidence is uncertain. The different beta-blockers seemed to have different effect on heart rate.
Maximal exertional heart rate № of participants: 85 (4 RCTs)	-		-	MD **33.5 bpm lower** (51.23 lower to 15.78 lower)	⨁⨁◯◯ Low[a]	The evidence suggests beta-blockers reduces maximal exertional heart rate. Different beta-blockers may reduce the heart rate differently.
Exercise capacity № of participants: 105 (10 RCTs)	-	-	-	SMD **0.37 SD higher** (0.01 lower to 0.74 higher)	⨁◯◯◯ Very low[a,d,e]	Beta-blockers may increase/have little to no effect on exercise capacity but the evidence is very uncertain.

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **MD:** mean difference; **RR:** risk ratio; **SMD:** standardised mean difference

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations
a. High risk of bias based on RoB2
b. TSA showed that we did not have enough data to reject or confirm 25% RRR
c. Different results for different beta-blockers
d. Very different ways of measuring exercise capacity
e. Confidence interval includes both no effect and important benefits

eTable 6

Calcium channel blocker compared to placebo or in addition to another rate controlling drug for rate control in atrial fibrillation

Outcome № of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Certainty	What happens
				Difference		
All-cause mortality № of participants: (1 RCT)	not estimable	0.0%	**0.0%** (0 to 0)	**0.0% fewer** (0 fewer to 0 fewer)	⨁◯◯◯ Very low[a,b]	Not possible to assess due to no events
Serious adverse events № of participants: (0 RCTs)	not estimable	0.0%	**0.0%** (0 to 0)	**0.0% fewer** (0 fewer to 0 fewer)	⨁◯◯◯ Very low[a,b]	Not possible to assess due to no events
Resting heart rate № of participants: 195 (6 RCTs)	-		-	MD **17.37 beats per minute lower** (22.22 lower to 12.53 lower)	⨁⨁◯◯ Low[a]	Calcium channel blockers may result in a large reduction in resting heart rate, but the risk of bias was high.
Maximal exertional heart rate № of participants: 195 (6 RCTs)	-		-	MD **29.83 bpm lower** (36.49 lower to 23.18 lower)	⨁⨁◯◯ Low[a]	Calcium channel blockers may result in a large reduction in resting heart rate, but the risk of bias was high.
Exercise capacity № of participants: 163 (6 RCTs)	-	-	-	SMD **0.37 SD higher** (0.01 higher to 0.74 higher)	⨁◯◯◯ Very low[a,c]	The evidence is very uncertain about the effect of calcium channel blockers on exercise capacity.

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **MD:** mean difference; **SMD:** standardised mean difference

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations
a. High risk of bias based on RoB2
b. No events
c. Very different ways of measuring exercise capacity

eTable 7

Calcium channel blocker compared to digoxin for rate control in atrial fibrillation

Outcome № of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Certainty	What happens
				Difference		
All-cause mortality № of participants: 173 (2 RCTs)	**RR 3.06** (0.33 to 28.42)	1.3%	**3.9%** (0.4 to 36)	**2.6% more** (0,8 fewer to 34,7 more)	⨁◯◯◯ Very low[a,b]	Not possible to assess due to no events
Serious adverse events № of participants: (0 RCTs)	not estimable	0.0%	**0.0%** (0 to 0)	**0.0% fewer** (0 fewer to 0 fewer)	⨁◯◯◯ Very low[a,c]	Not possible to assess due to no events
Resting heart rate № of participants: 158 (5 RCTs)	-		-	MD **6.46 beats per minute lower** (12.16 lower to 0.77 lower)	⨁◯◯◯ Very low[a,d]	Calcium channel blockers may reduce/have little to no effect on resting heart rate but the evidence is very uncertain.
Maximal exertional heart rate № of participants: 110 (4 RCTs)	-		-	MD **21.74 bpm lower** (36.61 lower to 6.87 lower)	⨁⨁◯◯ Low[a]	Calcium channel blockers likely results in a large reduction in maximal exertional heart rate but the risk of bias is high.
Exercise capacity № of participants: 62 (4 RCTs)	-	-	-	SMD **0.52 SD higher** (0.35 lower to 1.39 higher)	⨁◯◯◯ Very low[a,e]	The evidence is very uncertain about the effect of calcium channel blockers on exercise capacity.

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **MD:** mean difference; **RR:** risk ratio; **SMD:** standardised mean difference

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations

a. High risk of bias based on RoB2
b. TSA showed we did not have enough data to confirm or reject a 25% RRR
c. No events
d. Statistical heterogeneity that could not be resolved
e. Very different ways of measuring exercise capacity

eTable 8

Digoxin compared to placebo or in addition to another rate controlling drug for rate control in atrial fibrillation

Outcome № of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Certainty	What happens
				Difference		
All-cause mortality № of participants: 94 (2 RCTs)	not estimable	0.0%	**0.0%** (0 to 0)	**0.0% fewer** (0 fewer to 0 fewer)	⨁◯◯◯ Very low[a,b]	Not possible to assess due to no events
Serious adverse events № of participants: (0 RCTs)	not estimable	0.0%	**0.0%** (0 to 0)	**0.0% fewer** (0 fewer to 0 fewer)	⨁◯◯◯ Very low[a,c]	Not possible to assess due to no events
Resting heart rate № of participants: 132 (5 RCTs)	-		-	MD **8.4 beats per minute lower** (13.49 lower to 3.31 lower)	⨁⨁◯◯ Low[a]	The evidence suggests digoxin reduces resting heart rate but the risk of bias was high.
Maximal exertional heart rate № of participants: 146 (6 RCTs)	-		-	MD **6.72 bpm lower** (16.16 lower to 2.72 higher)	⨁◯◯◯ Very low[a,d,e]	Digoxin may reduce/have little to no effect on maximal exertional heart rate but the evidence is very uncertain.
Exercise capacity № of participants: 209 (7 RCTs)	-	-	-	SMD **0.11 SD lower** (0.38 lower to 0.15 higher)	⨁◯◯◯ Very low[a,f]	The evidence is very uncertain about the effect of digoxin on exercise capacity.

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

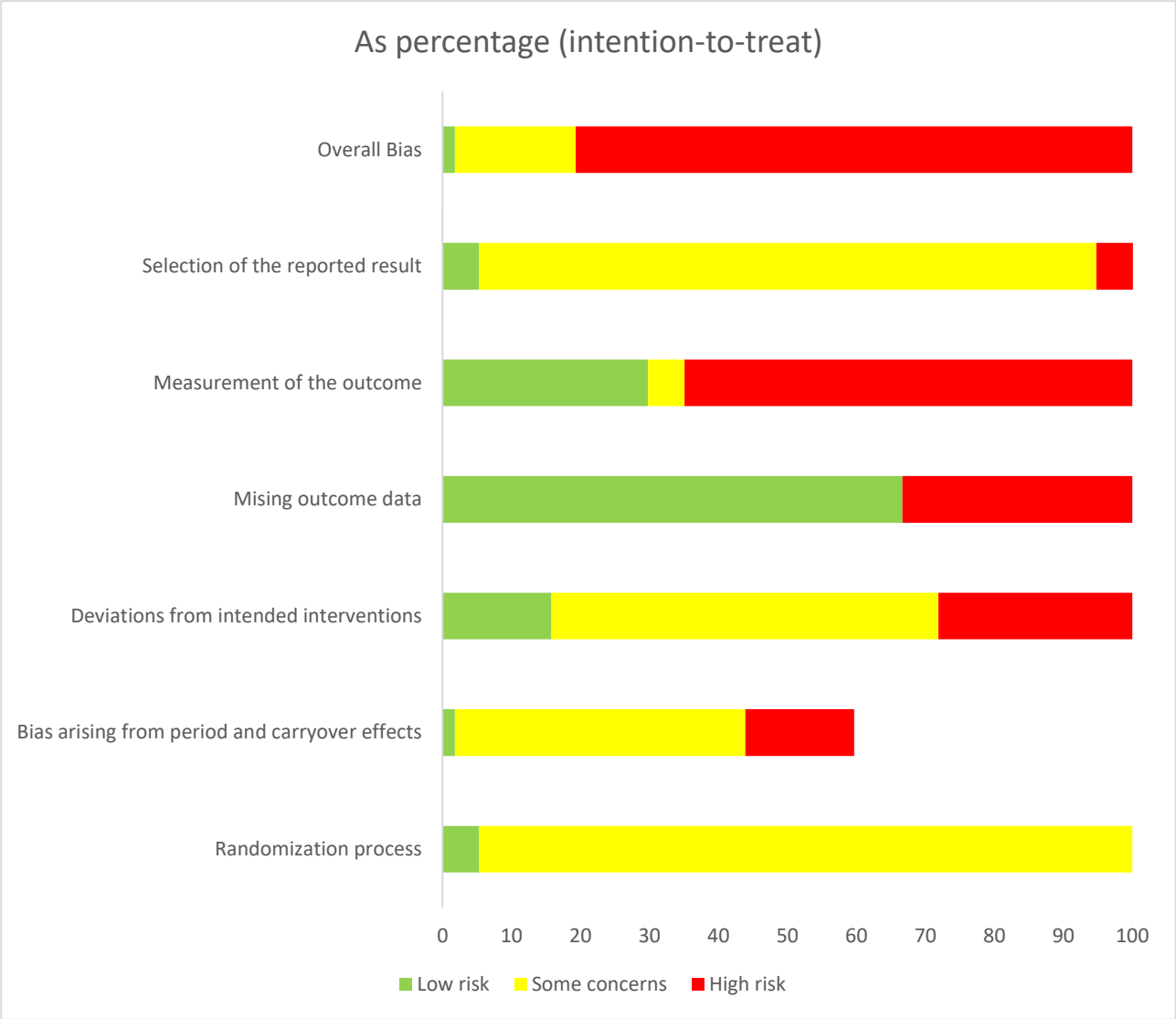
CI: confidence interval; **MD:** mean difference; **RR:** risk ratio; **SMD:** standardised mean difference

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations

a. High risk of bias based on RoB2
b. TSA showed we did not have enough data to confirm or reject a 25% RRR
c. No events
d. Statistical heterogeneity that could not be resolved
e. TSA showed we did not have enough information to assess a 11 beat per minute difference
f. Very different ways of measuring exercise capacity

Risk of bias distribution

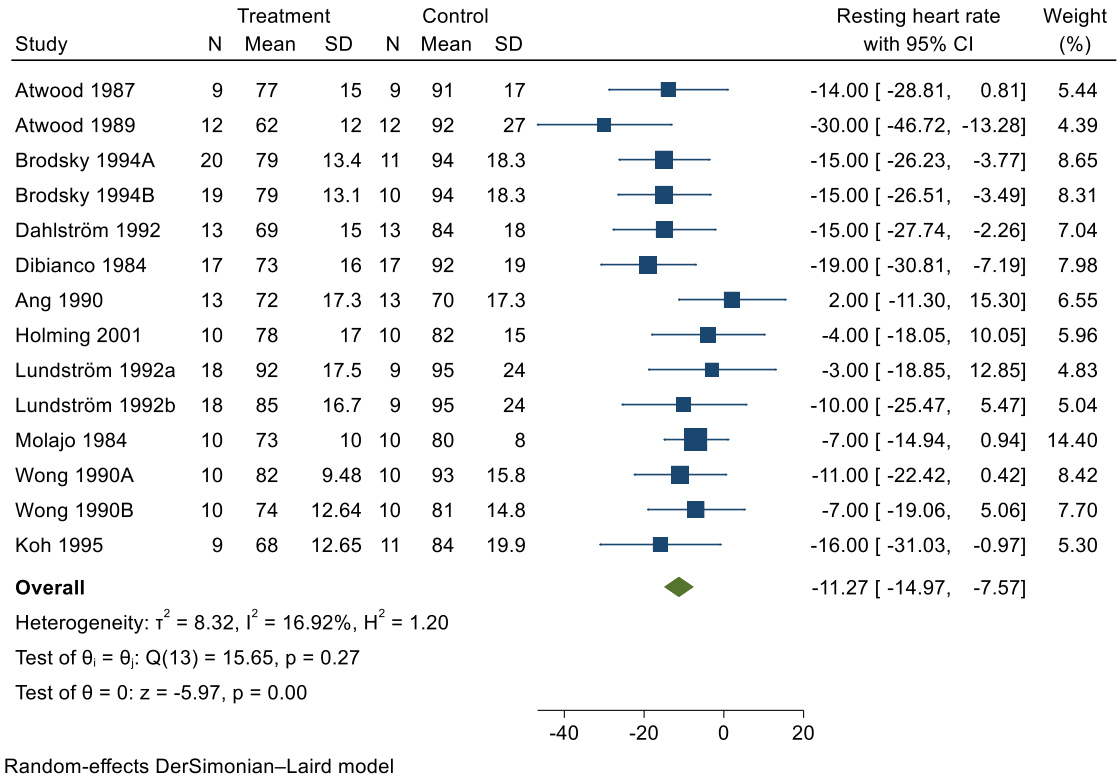


Beta-blocker versus placebo or beta-blocker in addition to another rate controlling drug versus the same rate controlling drug

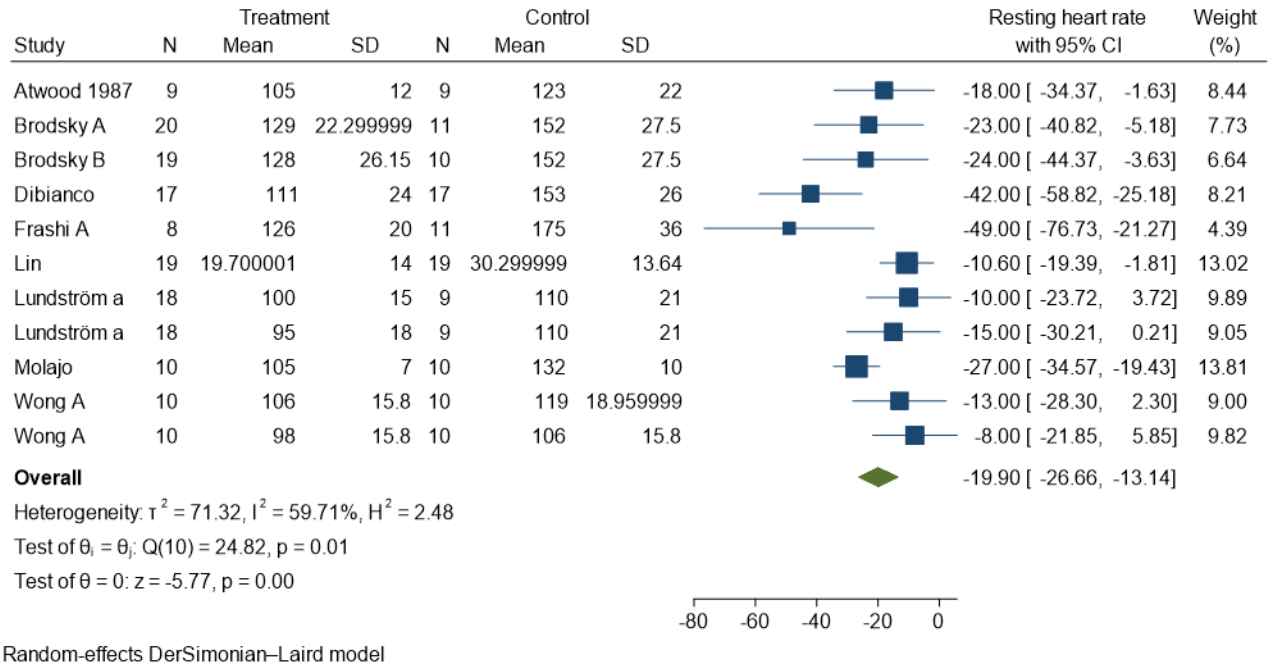
eFigure 1 – All-cause mortality

Study	Treatment Yes	Treatment No	Control Yes	Control No
Brodsky 1994A	0	20	0	11
Brodsky 1994B	0	19	0	10
Holming 2001	0	10	0	10
Zoble 1987	0	16	0	16
Koh 1995	0	9	0	11

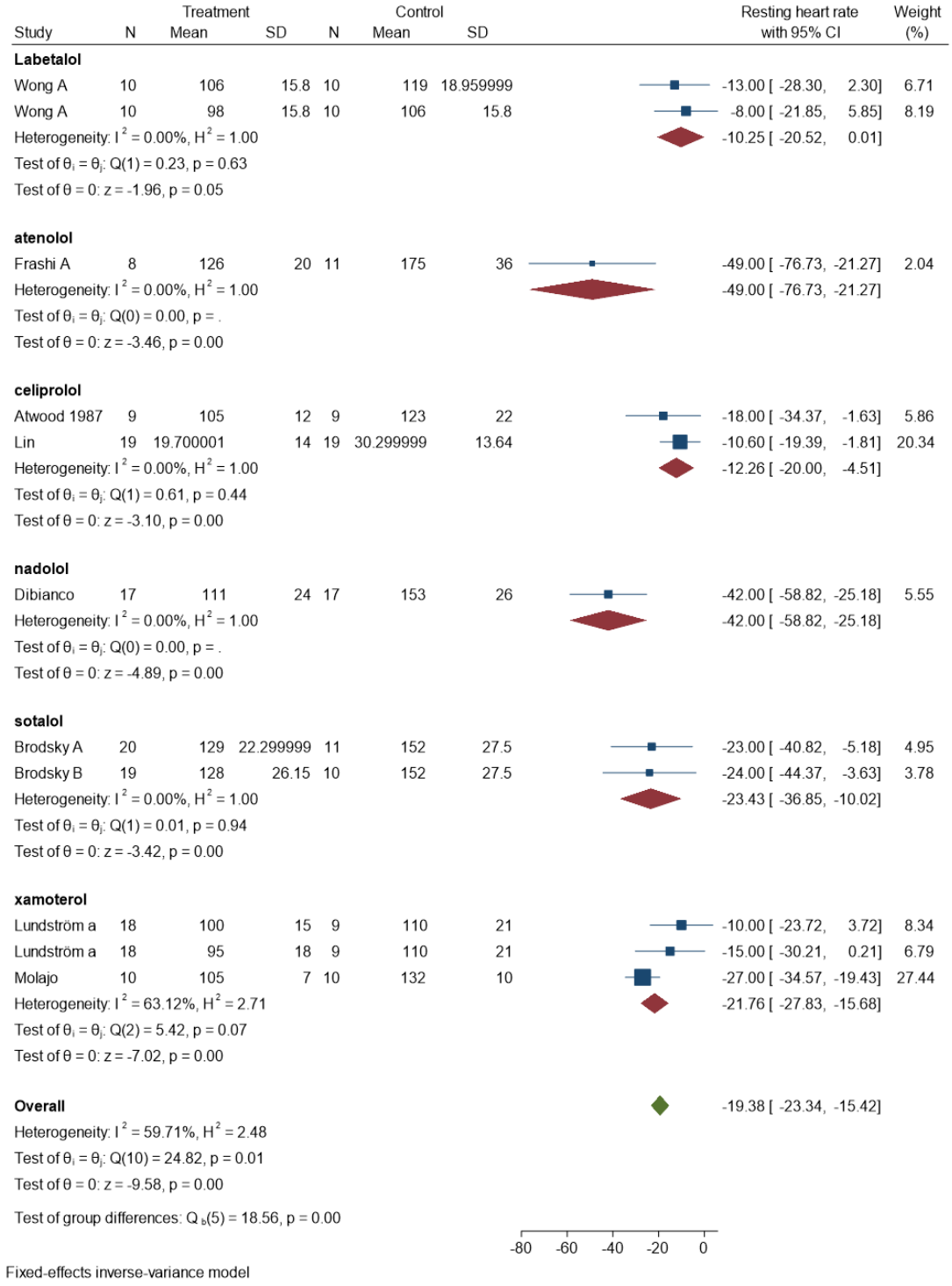
eFigure 2 – Resting heart rate



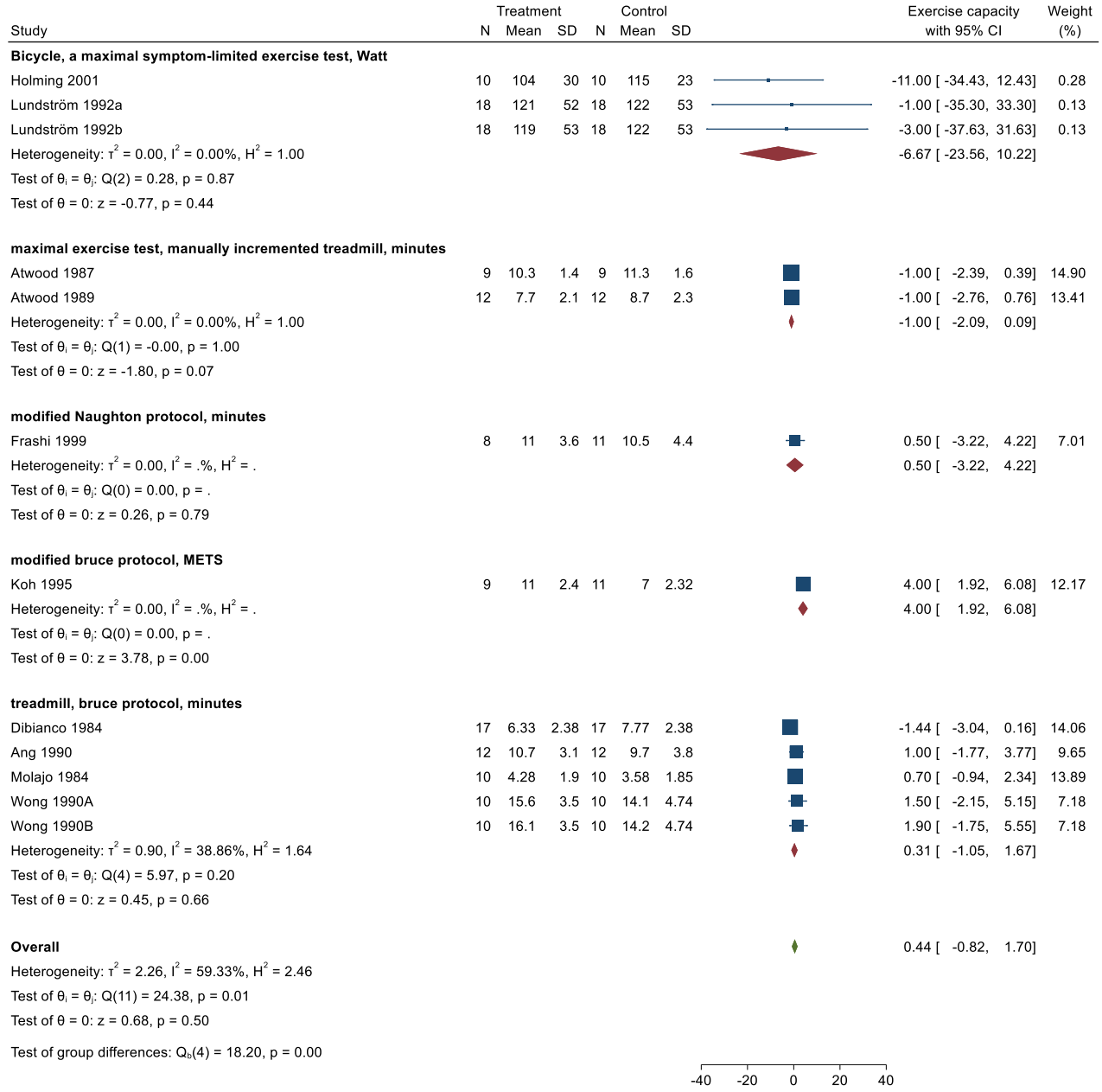
eFigure 3 – Submax HR



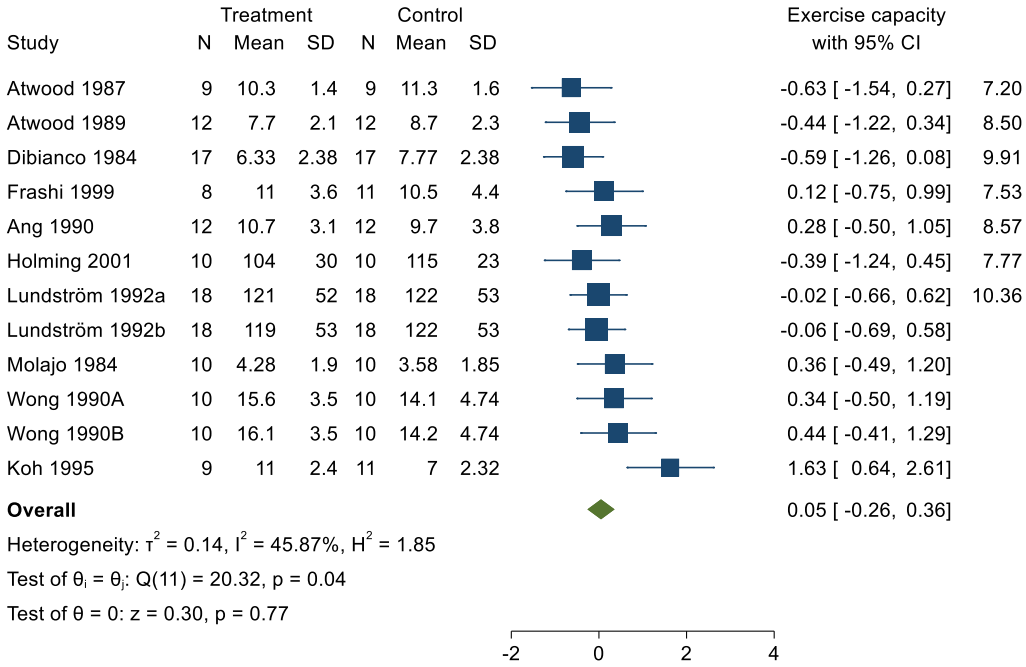
eFigure 4 – Submax HR by subgroup



eFigure 5 – Exercise capacity

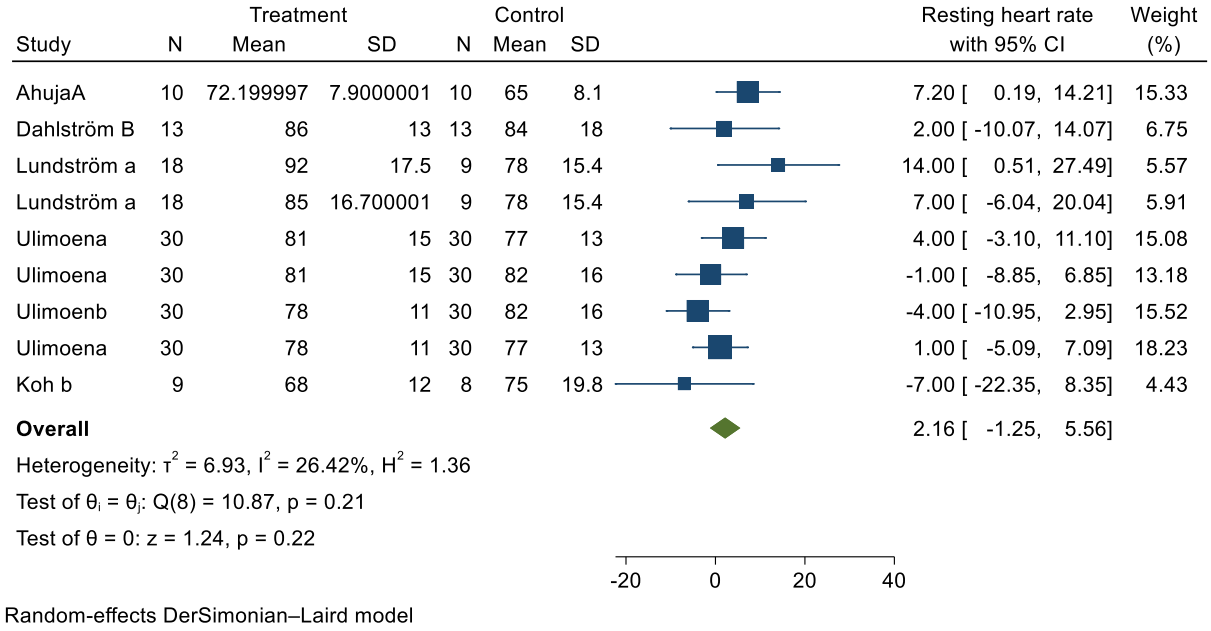


eFigure 6 – Exercise capacity standardized mean difference

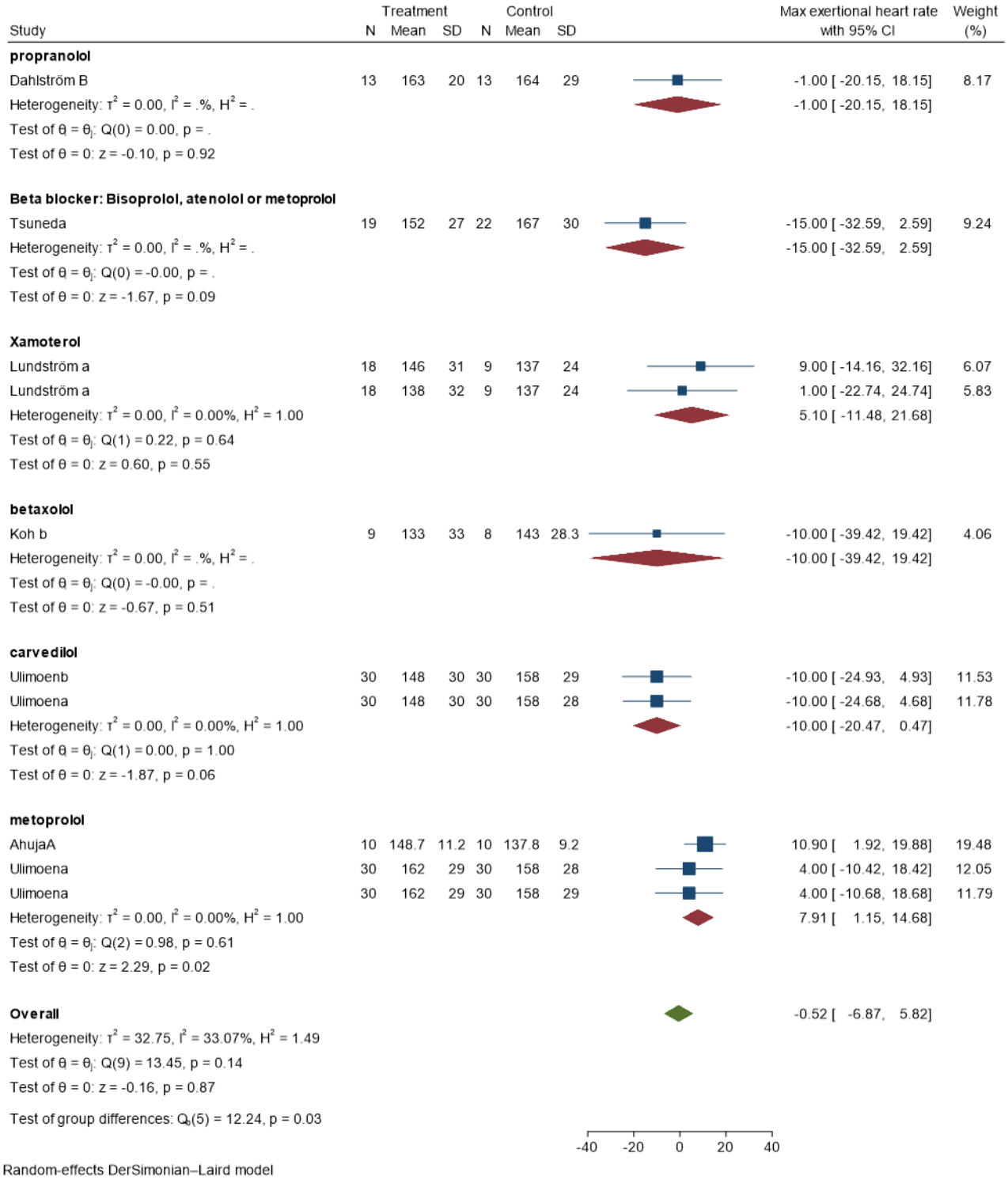


Beta-blocker versus calcium channel blocker

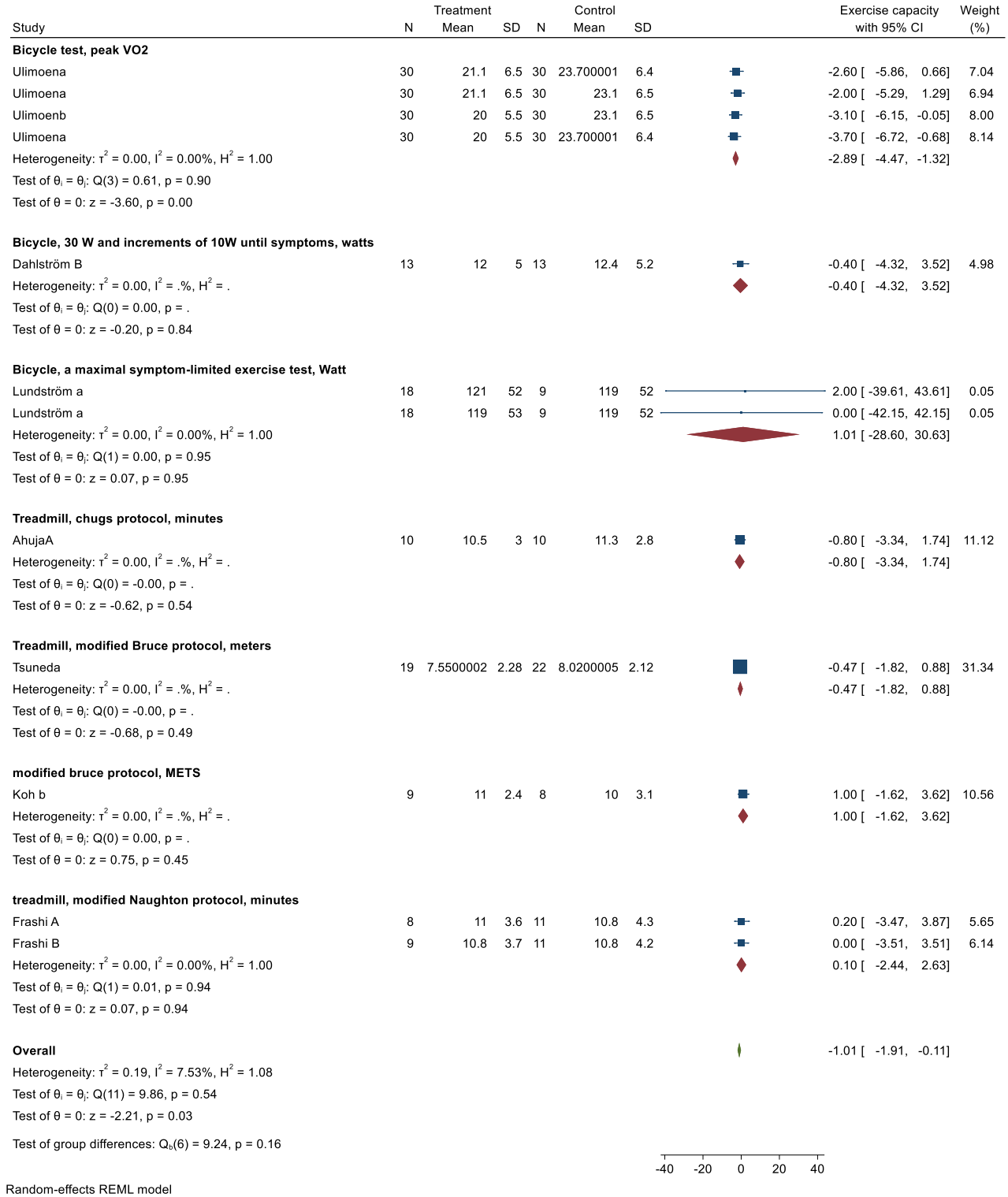
eFigure 7 – Resting heart rate



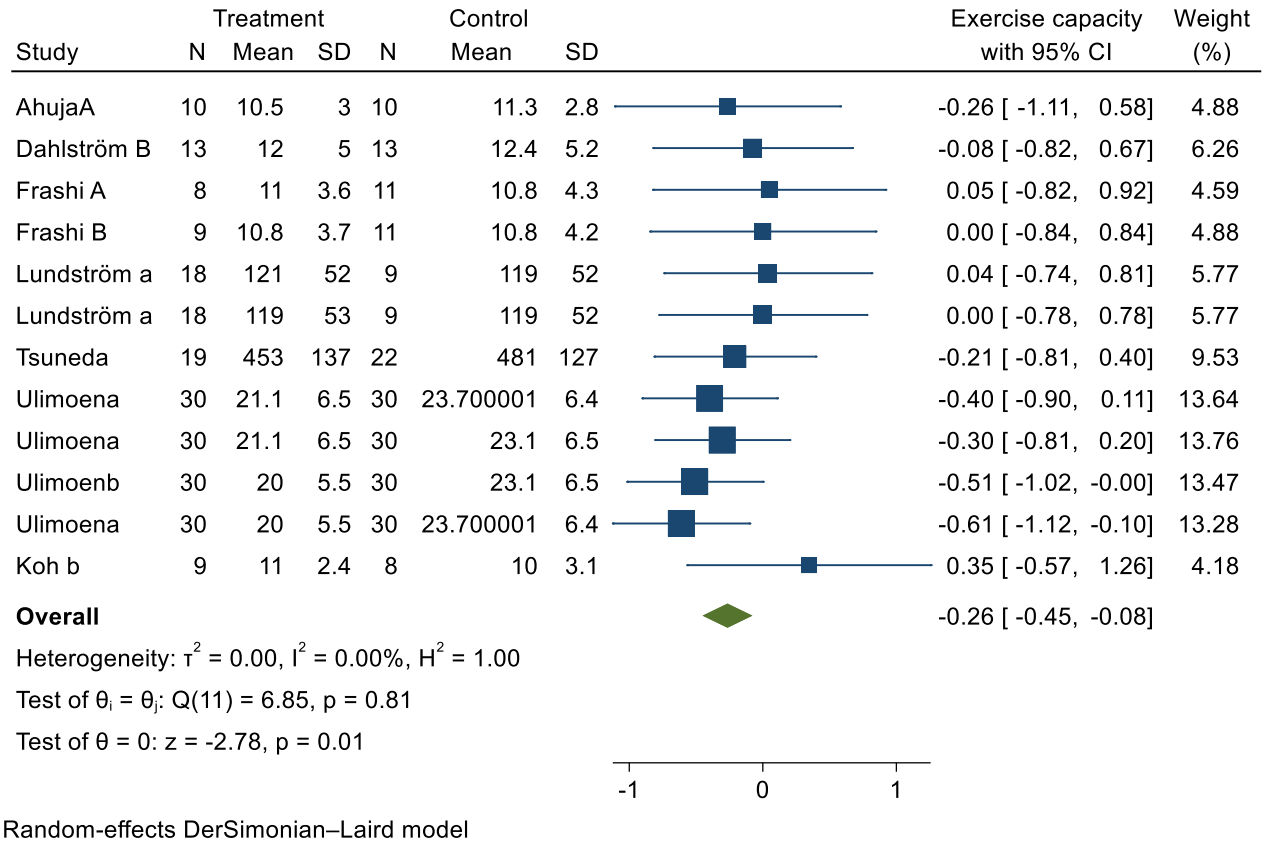
eFigure 8 – Max exertional heart rate



eFigure 9 – Exercise capacity

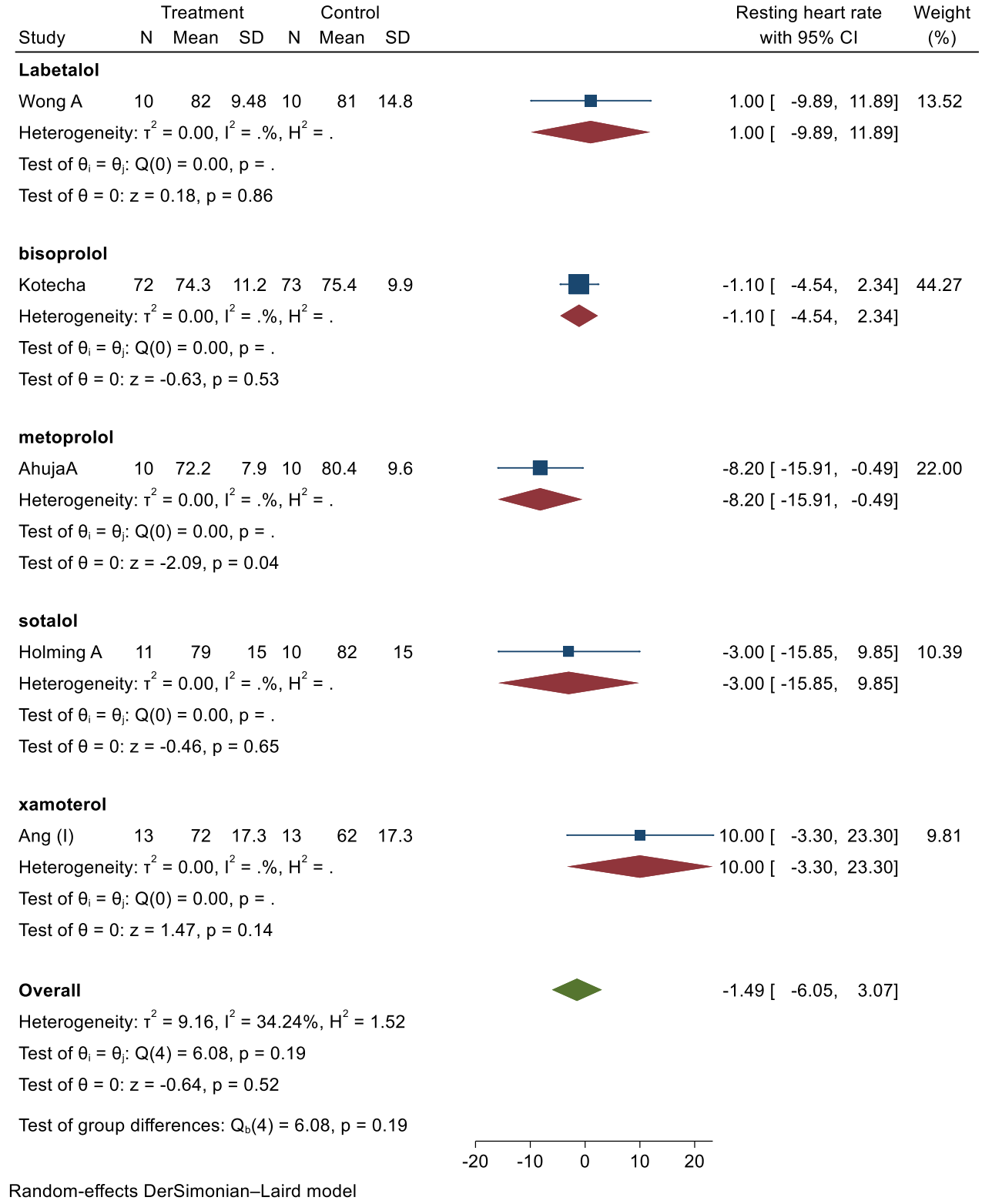


eFigure 10 – Exercise capacity standardized mean difference

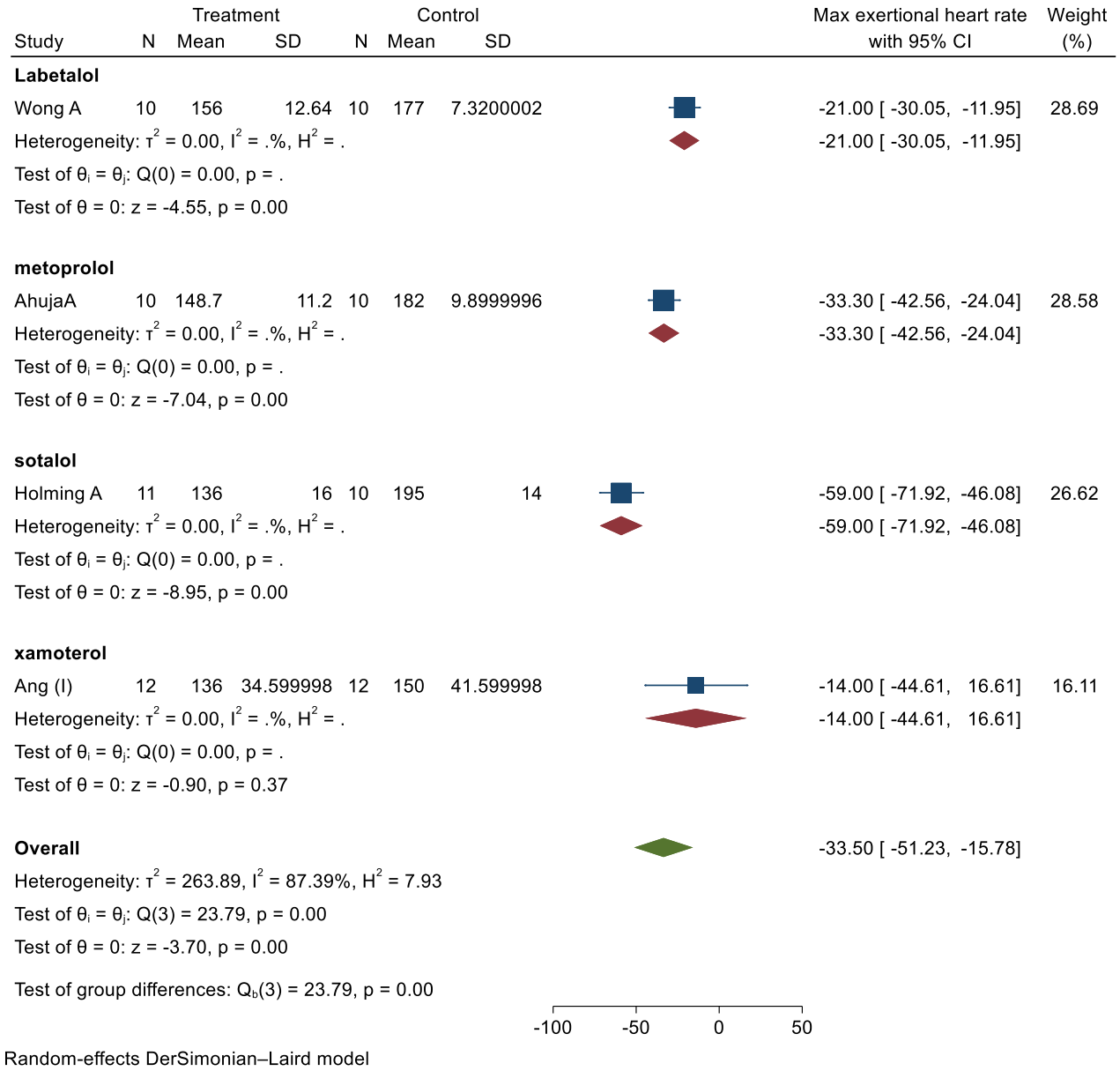


Beta-blocker v. digoxin

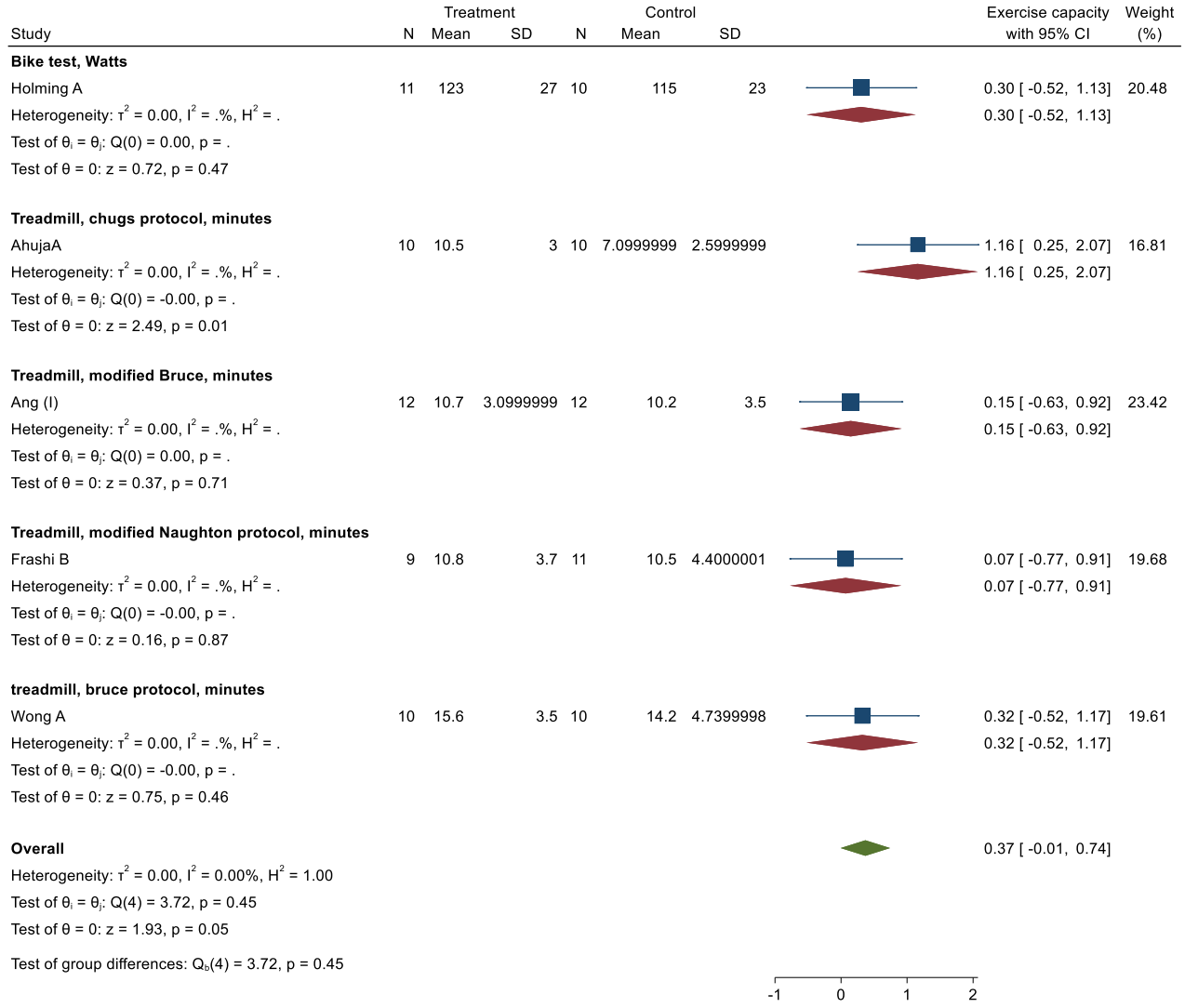
eFigure 11 – Resting heart rate



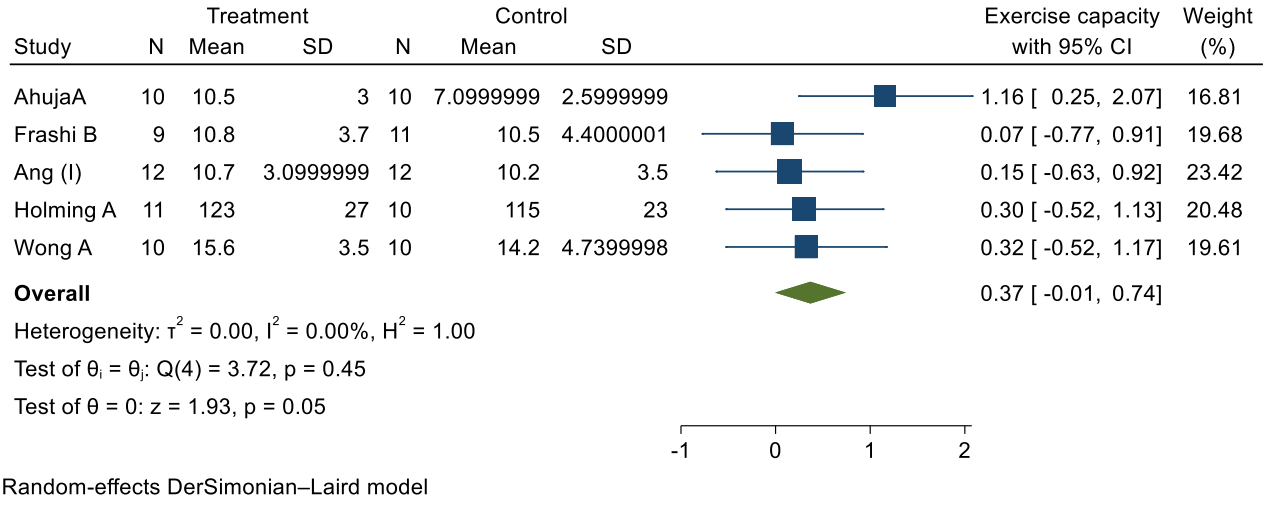
eFigure 12 – Maximal exertional heart rate



eFigure 13 – Exercise capacity

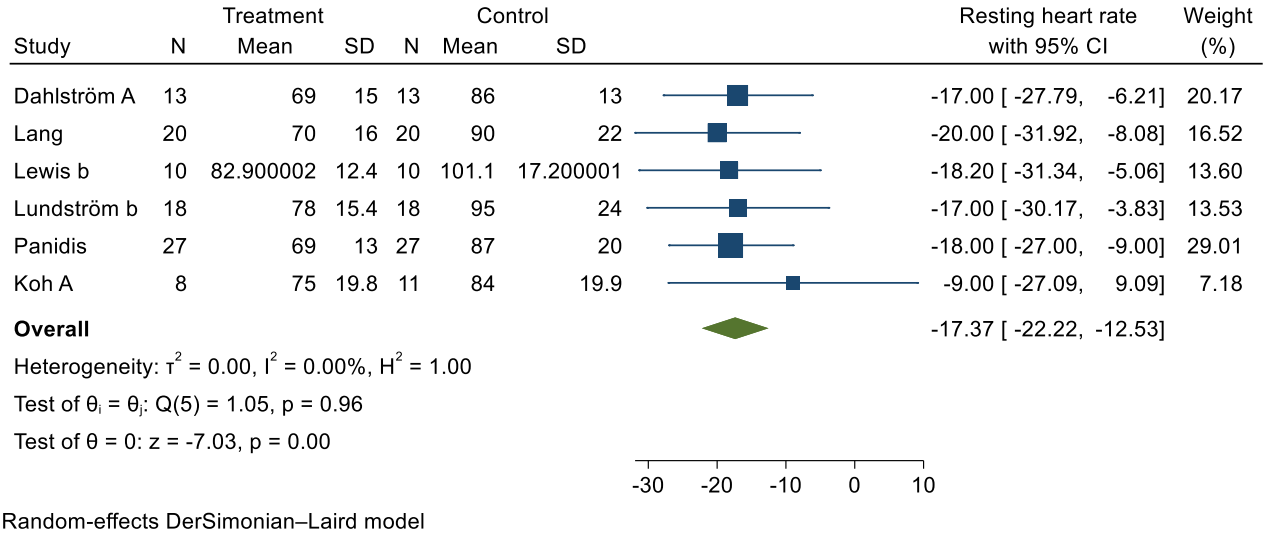


eFigure 14 – Exercise capacity using standardized mean difference

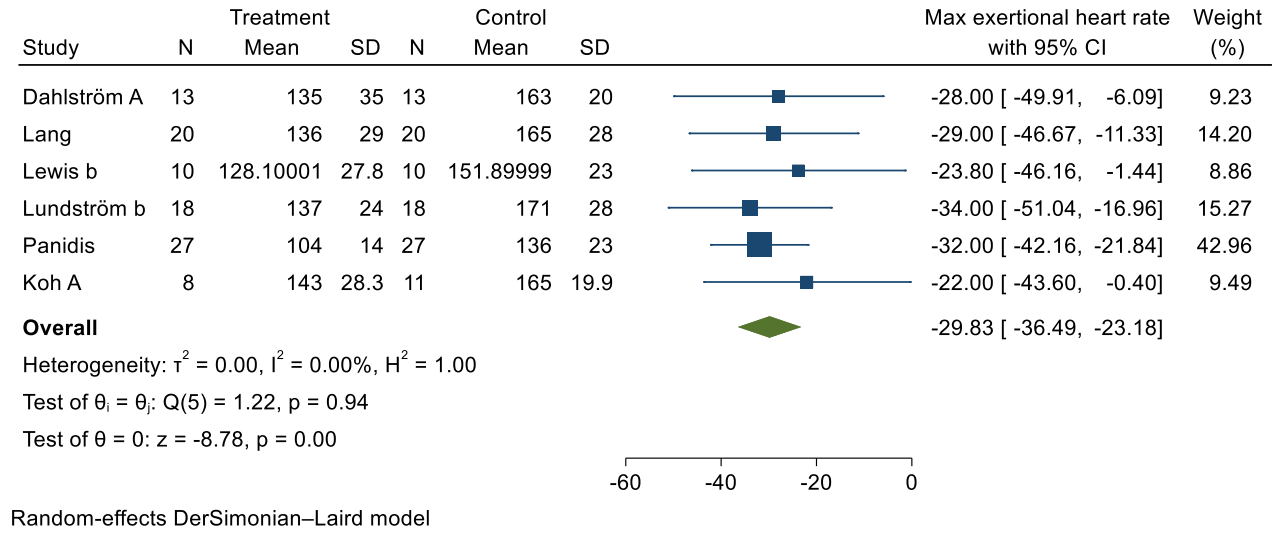


Calcium channel blocker versus placebo or in addition to another rate controlling drug

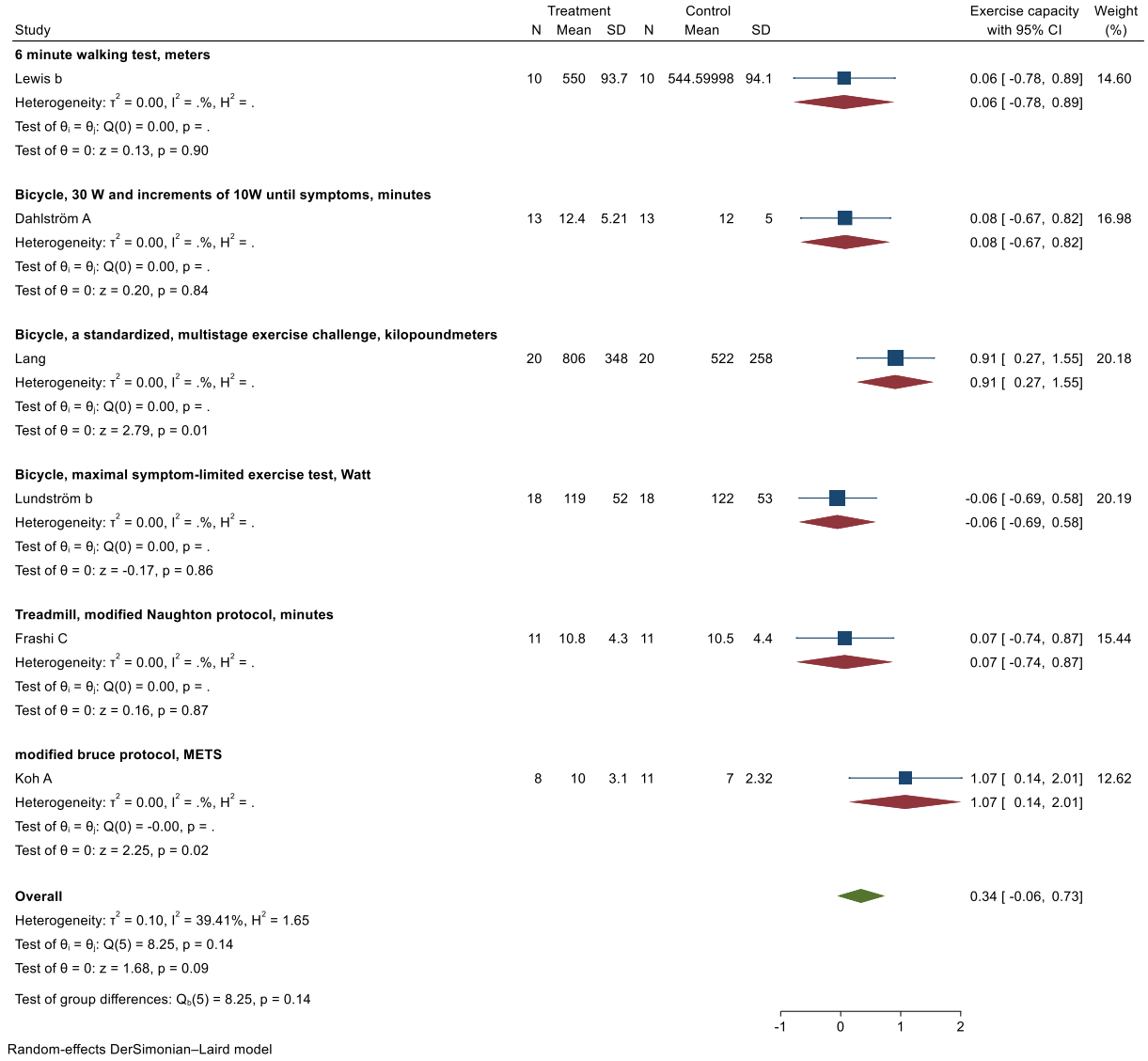
eFigure 15 – Resting heart rate



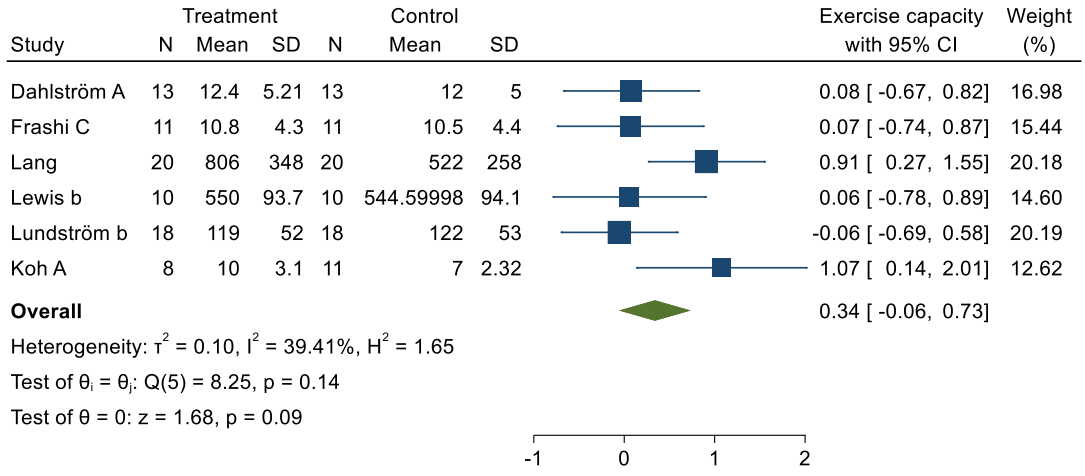
eFigure 16 – Maximal exertional heart rate



eFigure 17 – Exercise capacity



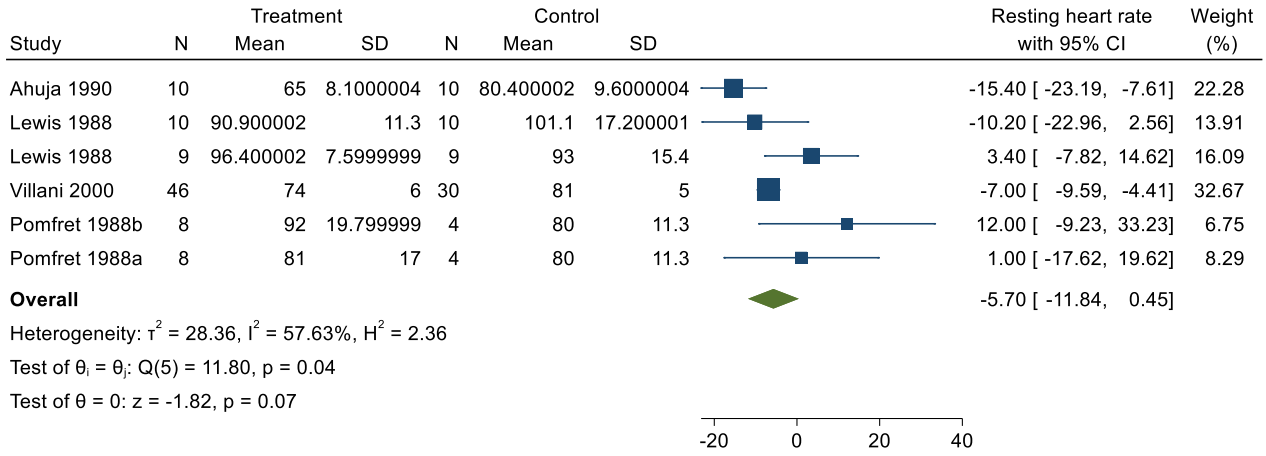
eFigure 18 – Exercise capacity standardized mean difference



Random-effects DerSimonian–Laird model

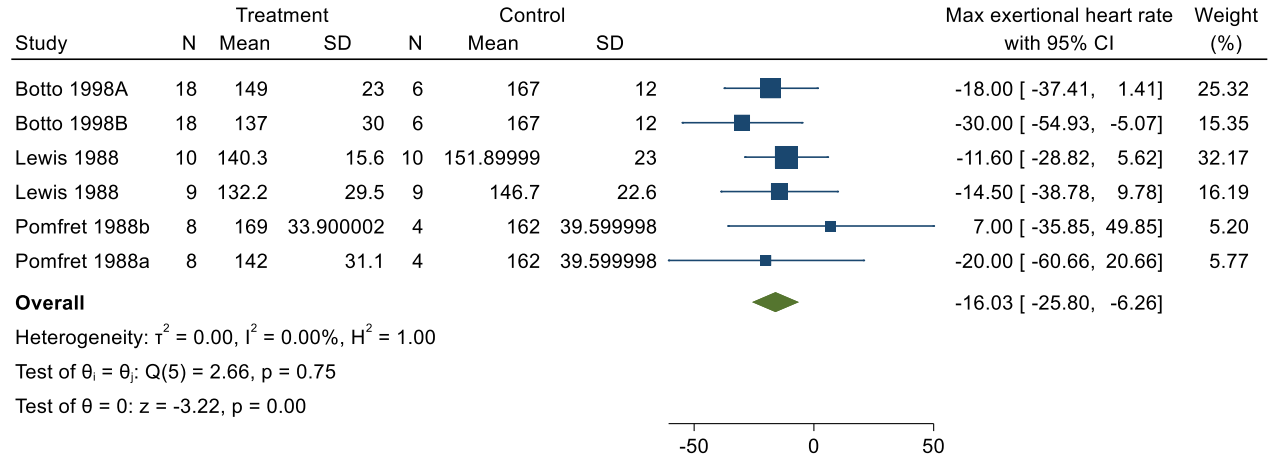
Calcium channel blocker versus digoxin

eFigure 19 – Resting heart rate



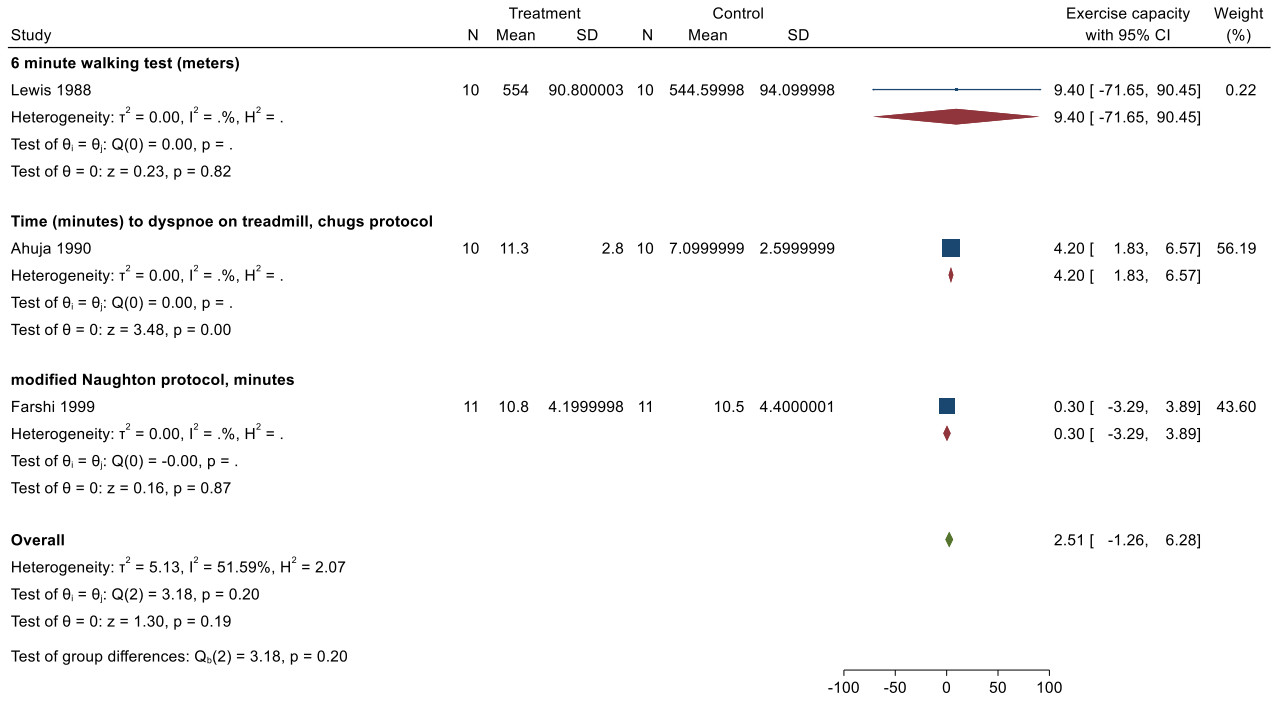
Random-effects DerSimonian–Laird model

eFigure 20 – Maximal exertional heart rate



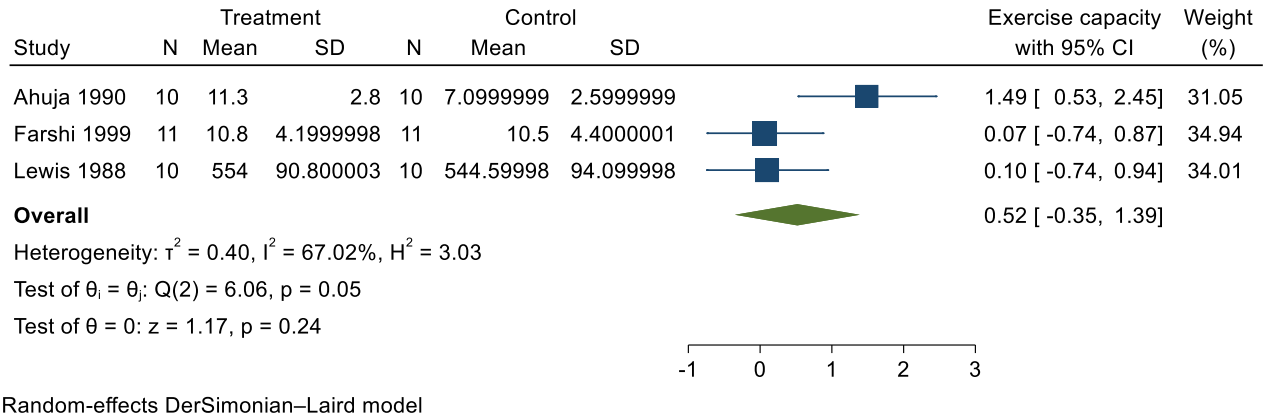
Random-effects DerSimonian–Laird model

eFigure 21 – Exercise capacity



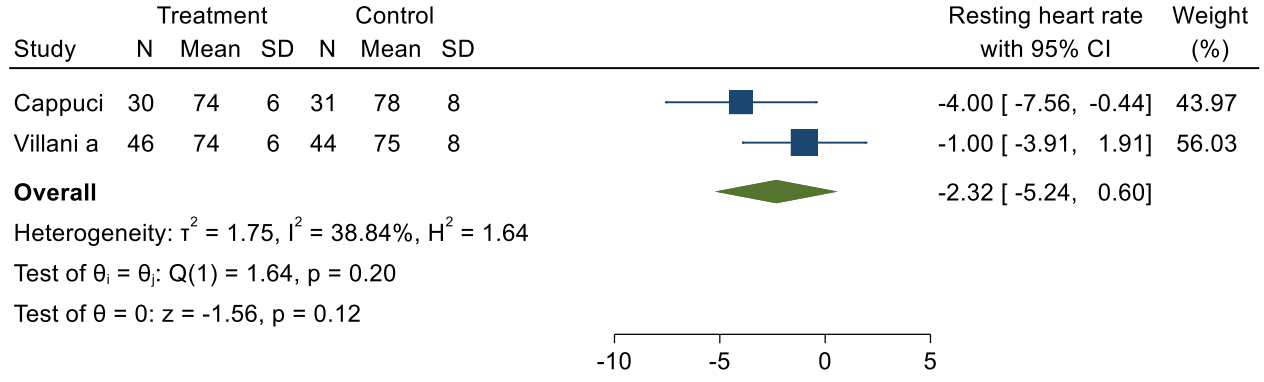
Random-effects REML model

eFigure 22 – Exercise capacity standardized mean difference



Calcium channel blocker versus amiodarone

eFigure 23 – Resting heart rate



Digoxin versus placebo or digoxin in addition to another rate controlling drug

eFigure 24 – Resting heart rate

Study	Treatment N	Treatment Mean	Treatment SD	Control N	Control Mean	Control SD	Resting heart rate with 95% CI	Weight (%)
Ang (II)	13	62	17.3	13	70	17.3	-8.00 [-21.30, 5.30]	14.65
Holming B	10	78	17	11	79	15	-1.00 [-14.68, 12.68]	13.84
Lewis b	10	82.900002	12.4	10	90.900002	11.3	-8.00 [-18.40, 2.40]	23.97
Wong A	10	74	12.64	10	82	9.48	-8.00 [-17.79, 1.79]	27.02
Wong B	10	81	14.8	10	93	15.8	-12.00 [-25.42, 1.42]	14.39
Koh b	11	84	19.9	14	105	29.9	-21.00 [-41.55, -0.45]	6.13
Overall							-8.40 [-13.49, -3.31]	

Heterogeneity: τ^2 = 0.00, I^2 = 0.00%, H^2 = 1.00

Test of $\theta_i = \theta_j$: Q(5) = 2.86, p = 0.72

Test of θ = 0: z = -3.24, p = 0.00

Random-effects DerSimonian–Laird model

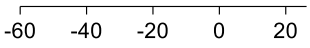
eFigure 25 – Maximal exertional heart rate

Study	Treatment N	Treatment Mean	Treatment SD	Control N	Control Mean	Control SD	Max exertional heart rate with 95% CI	Weight (%)
Ang (II)	12	150	41.6	12	159	34.6	-9.00 [-39.61, 21.61]	7.21
Holming B	10	144	26	11	136	16	8.00 [-10.27, 26.27]	14.08
Lewis b	10	128.10001	27.8	10	140.3	15.6	-12.20 [-31.96, 7.56]	12.92
Wong A	10	154	12.64	10	156	12.64	-2.00 [-13.08, 9.08]	21.10
Wong B	10	177	7.32	10	175	9.48	2.00 [-5.42, 9.42]	25.13
Koh b	11	165	19.9	14	196	29.9	-31.00 [-51.55, -10.45]	12.34
Pomfret 1988a	8	143	28.2	8	169	33.9	-26.00 [-56.56, 4.56]	7.23
Overall							-6.72 [-16.16, 2.72]	

Heterogeneity: τ^2 = 78.00, I^2 = 55.36%, H^2 = 2.24

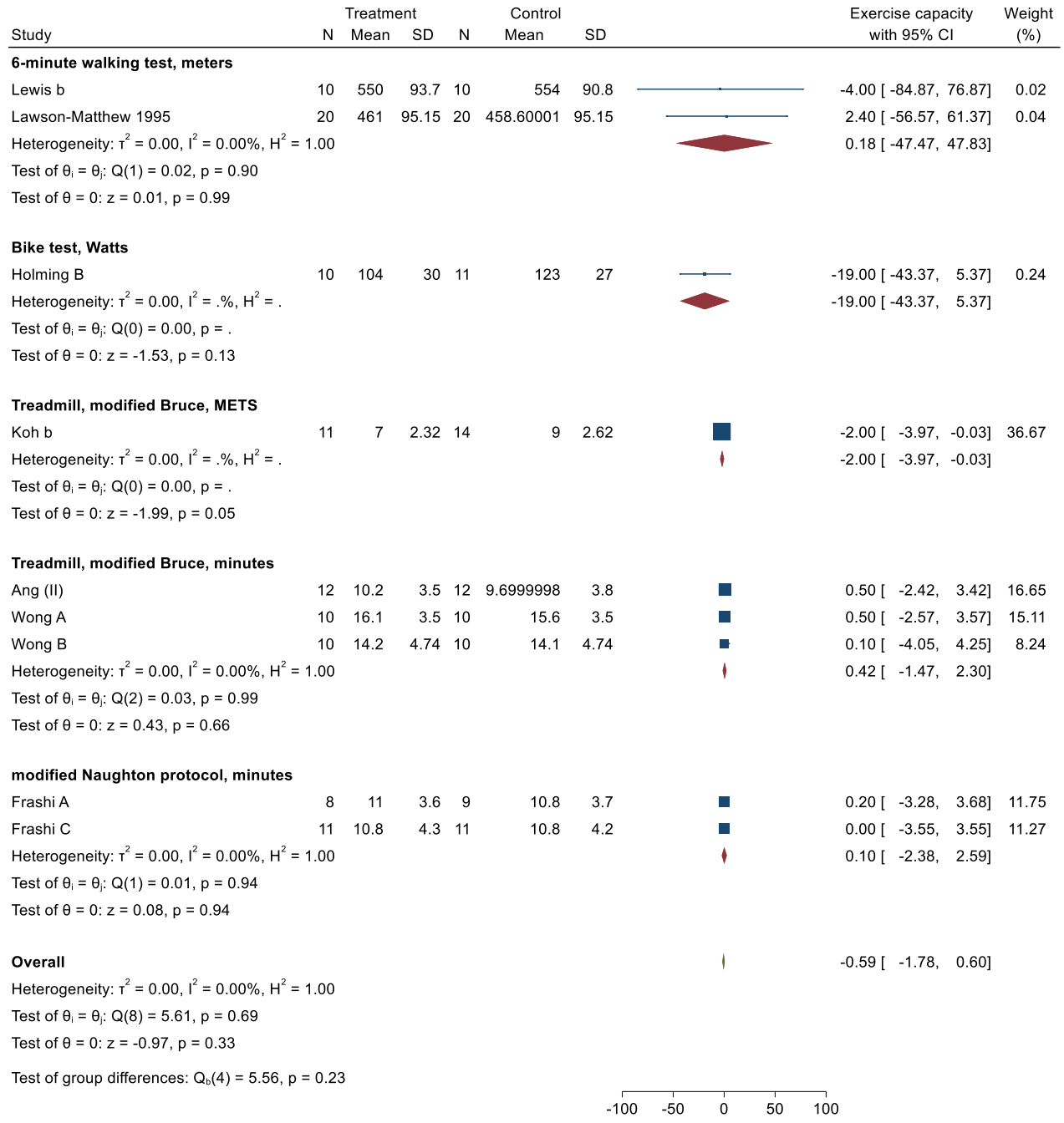
Test of $\theta_i = \theta_j$: Q(6) = 13.44, p = 0.04

Test of θ = 0: z = -1.40, p = 0.16

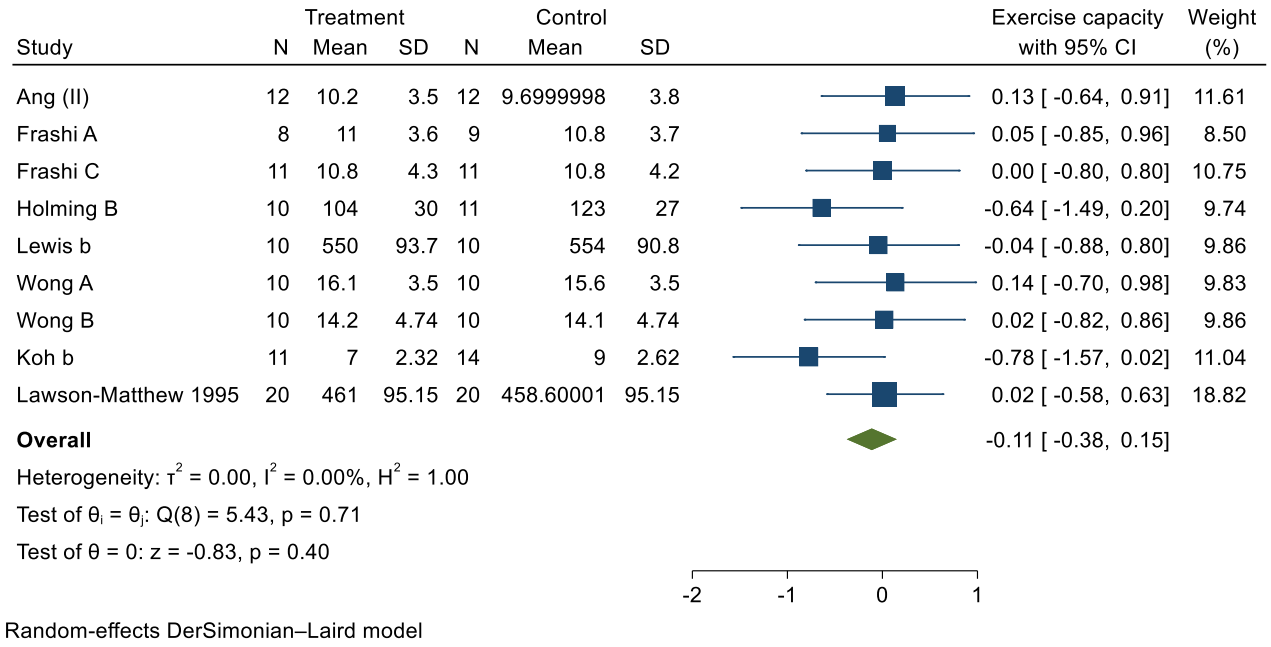


Random-effects DerSimonian–Laird model

eFigure 26 – Exercise capacity



eFigure 27 – Exercise capacity standardized mean difference



Additional results of comparisons

Beta-blocker versus placebo

Primary outcomes

All-cause mortality

Five trials assessed mortality but did not report any events (eFigure 1). Hence, no meta-analysis was performed.

Serious adverse events

No trials reported serious adverse events other than death.

Secondary outcomes

Health-related quality of life (any validated continuous scale). These may be both generic and disease-specific questionnaires.

One cross-over trial (Lundström et al) randomizing a total of 21 participants reported on quality of life using a 10-point VAS scale from "Much worse than usual" to "Much better than usual".[43] T--test was not statistically significant (MD -0.3, CI 95% -1.015 – 0.415, P value 0.40.

Arial fibrillation symptom score such as European Heart Rhythm Association (EHRA), New York Heart Association (NYHA).

No trial reported symptoms scores.

Non serious adverse events.

No trial reported non-serious adverse events.

Exploratory outcomes

Submaximal exertional heart rate

8 trials (7 cross-over and 1 parallel trial) assessed submaximal exertional heart rate. Meta-analysis showed that beta-blockers reduced the submaximal exertional heart rate (MD -19.90, CI 95% -26.66 – -13.14, *P* value < 0.0001; eFigure 3). The difference was above our predefined minimal important difference (SD/2 = 11). Visual inspection of the forest plot and statistical tests (I^2 = 59.7%) indicated moderate heterogeneity. Presenting the results for each beta-blocker separately resolved most of the heterogeneity (see below). TSA confirmed the meta-analysis result. This outcome result was assessed at high risk of bias.

Test for subgroup differences according to the specific beta-blocker was significant (*P* value = 0.001). All types of beta-blockers reduced the heart rate but with different effect sizes: atenolol: MD -49.00, CI 95% -76.73 – -21.27, *P* value = 0.001; celiprolol: MD -12.26, CI 95% -20.00 – -4.151, *P* value = 0.002; nadolol: MD -42.00, CI 95% -58.82 – -25.18, *P* value < 0.001; xamoterol: MD -18.66, CI 95% -30.01 – -7.31, *P* value =

0.001; sotalol: MD -23.43, CI 95% -36.85 – -10.02, *P* value = 0.001; labetalol: MD -10.25, CI 95% -20.52 – 0.01, *P* value = 0.050). Results of the different subgroups are presented in eFigure 4.

Tests for subgroup differences found no difference between trials with a cross-over design and trials with a parallel design (*P* =0.61) and year of trial publication (*P* = 0.86) were not significant. The remaining preplanned subgroup analyses were not possible due to lack of relevant data.

Exercise capacity

They used very different types Four trials used a treadmill with either a modified or original Bruce protocol measured in minutes (MD 0.31 CI 95% -1.05 – 1.67, P value = 0.66), 2 trials used their own treadmill protocols measured in minutes (MD -1.00 CI 95% -2.09 – 0.09, *P* value = 0.07), 1 trial used a treadmill following the Bruce protocol measured in METS (MD 4.00 CI 95% 1.92 – 6.08, *P* value < 0.01), 1 trial used a treadmill using the modified Naughton protocol measured in minutes (MD 0.50 CI 95% -3.22 – 4.22, *P* value = 0.79, 2 trials used a bike test with different protocols measured in watt (MD -6.67 CI 95% -23.56 – 10.22, *P* value = 0.44).

Successful achievement of resting heart rate target (as defined by trialist)

No trial assessed successful achievement of resting heart rate target.

Ejection Fraction

0 trials reported ejection fraction.

Beta-blocker versus calcium channel blocker

Primary outcomes

All-cause mortality

One trial (Koh 1995) with 0 events in both arms reported on mortality.[5] Hence, no meta-analysis was performed.

Serious adverse events

No trials assessed serious adverse events other than death.

Secondary outcomes

Health-related quality of life (any validated continuous scale). These may be both generic and disease-specific questionnaires.

Two trials reported on quality of life.[13,43] Meta-analysis was not considered appropriate due to the difference in scale. Lundström et al used a 10-point VAS scale from "Much worse than usual" to "Much better than usual".[43] Unpaired t-test was not statistically significant (MD 0.40 CI 95% -0.42 – 1.225, P value 0.3351.

Tsuneda et al reported quality of life using SF-36 and AFQLQ.[13] Unpaired t-test for the physical component score of SF-36 was not statistically significant ((MD 0.5, CI 95% -3.875 – 4.875, P value 0.8184).

Unpaired t-test for the mental component score of SF-36 was not statistically significant (MD -0.7, CI 95% -4.81 – 3.41, P value 0.7321).

Tsuneda et al also reported a disease-specific questionnaire developed for Japan, AFQLQ.[53] No statistically significant difference in either of the three subscales were found.[13]

Arial fibrillation symptom score such as European Heart Rhythm Association (EHRA), New York Heart Association (NYHA).

No trial reported on symptom score.

Non-serious adverse events.

No trial reported non-serious adverse events.

Exploratory outcomes

Submaximal exertional heart rate

Two trials assessed submaximal exertional heart rate.[43,54] Beta-blocker versus CCB did not result in a statistically significant difference on the maximal exertional heart rate (MD -1.63, CI 95% -15.74 – 12.47, *P* value = 0.82). There was moderate heterogeneity when inspecting the forest plot and from the statistical test (I^2 = 54.03%). TSA showed we did not have enough information to assess a 13-bpm difference, which was equal to SD/2.

This outcome result was assessed at high risk of bias.

Tests for subgroup differences according to the specific beta-blocker and CCB were statistically significant (*P* value = 0.01). Farshi et al comparing among other treatments atenolol and diltiazem, found atenolol reduced submaximal exercise heart rate (MD -20.00, CI 95% -40.59 – -0.53, *P* value = 0.044).[54] Lundstrom et al comparing xamoterol with verapamil, found no difference (MD 7, CI 95% -1.79 – 17.37, *P* value = 0.111)

Exercise capacity

Seven trials assessed exercise capacity. 2 trials used a bicycle test; 1 trial measured peak VO2 (MD -2.89 CI 95% -4.47 – -1.32, P value < 0.01), 1 trial measured in watts (MD 1.01 CI 95% -28.60 – 30.63, P value = 0.95). 4 trials used a treadmill; 1 using Chugs protocol measured in minutes (MD -0.80 CI 95% -3.34 – 1.74, P value = 0.54), 1 trial with a modified Bruce protocol measured in METS(MD 1.00 CI 95% -1.62 – 3.62, P value = 0.45), 1 trial using Bruce's protocol measured in meters (MD -0.47 CI 95% -1.82 – 0.88, P value = 0.49), and 1 trial used a modified Naughton protocol in minutes (MD 0.10 CI 95% -2.44 – 2.63, P value = 0.94).

Successful achievement of resting heart rate target (as defined by trialist)

One trial reported on successful achievement of resting heart rate taget (Ulimoen).[55,56] All 60 participants achieve lenient rate control during all 4 interventions. For strict rate control out of 60 participants, 34 achieved strict rate control with diltiazem, 29 with verapamil, 34 with metoprolol and 35 with carvedilol.

Ejection Fraction

0 trials reported ejection fraction.

Beta-blocker versus digoxin

All-cause mortality

Two trials assessed all-cause mortality. One trial had zero events in both arms. Another trial, Kotecha 2020 randomised 160 participants to bisoprolol versus digoxin. 7/80 in the bisoprolol arm and 4/80 in the digoxin arm died at 12 months. The result using Fischer's exact test did not indicate any difference between treatment groups (RR 1.75, CI 0.53 – 5.75; *P* value = 0.53).

TSA showed we did not have enough data to confirm or reject a 25% difference in mortality.

This outcome result was assessed at high risk of bias etable 2. No subgroup analyses were performed.

Serious adverse events

One trial, Kotecha 2020 assessed on serious adverse events.[7] 21/80 participants in the bisoprolol arm and 13/80 participants in the digoxin arm reported one or more serious adverse events. The result was not statistically significant RR 1.61 (0.870 – 2.998, *P* value = 0.13). TSA showed we did not have enough data to confirm or reject a 25% difference in mortality.

This outcome result was assessed at high risk of bias. No subgroup analyses were performed.

Secondary outcomes

Health-related quality of life (any validated continuous scale). These may be both generic and disease-specific questionnaires.

Kotecha et al reported quality of life using SF-36, AFEQT and EQ-5D-5L.[57]

The trial analysed SF-36 at one year using linear regression adjusted for baseline score, sex and modified EHRA symptom classification at baseline, age at randomization, and baseline LVEF.[57] Kotecha et al found no statistically significant difference on the SF-36 physical component score (MD -1.6, CI 95% -4.7 – 1.4, *P* value = 0.29) or the mental component score (MD -1.4, CI 95% -4.2 – 1.5, *P* value = 0.34). The confidence interval for both results contained clinically significant differences. The VAS scale as part of the EQ-5D-5L was statistically significantly lower in the bisoprolol group (MD -5.5 CI 95% -0.3 – -10.6, P value 0.04). The summary part of the 5Q-5D-5L was not statistically significantly higher.

The overall score for AFEQT was not statistically significantly lower in the bisoprolol group compared with the digoxin group (MD -4.1 CI 95% -8.7 – 0.5, P value 0.08). The confidence intervals contained clinically important differences.

Arial fibrillation symptom score such as European Heart Rhythm Association (EHRA), New York Heart Association (NYHA)

Kotecha et al reported on symptoms scores using the modified EHRA score.[57] Using a ordinal logistic regression, digoxin improved EHRA score over bisoprolol at 12 month (OR 0.16, 95% CI 0.08 – 0.33, P value < 0.001).

Non serious adverse events.

Kotecha et al reported adverse events (seemed to include only non-serious).[57] 20/80 participants treated with digoxin and 51/80 participants treated with bisoprolol developed an adverse events, (χ^2 = 24.91, P value < .001).

Exploratory outcomes

Submaximal exertional heart rate

Two trials (2 cross-over trials) assessed submaximal exertional heart rate. Beta-blocker versus digoxin did not result in a statistically significant difference on the submaximal exertional heart rate (MD -20.29, CI 95% -64.17 – 23.60, *P* value = 0.36). There was substantial heterogeneity when inspecting the forest plot and from the statistical test (I^2 = 85.23%). Hence, we present results for the individual beta-blockers below.

TSA showed could not be generated due to too little information.

This outcome result was assessed at high risk of bias.

Test for subgroup differences according to the specific beta-blocker and year of publication (complete overlap) was statistically significant (*P* value = 0.01).

Labetalol: MD 0.00, CI 95% -13.85 – 13.85, *P* value = 1.00; atenolol MD -45.00, CI 95% -75.94 – 14.06, *P* value = 0.004).

Exercise capacity

6 trials assessed exercise capacity with one trial only reporting median and IQR. 4 trials used a treadmill; 1 using Chugs protocol measured in minutes (MD 1.16 CI 95% 0.25 – 2.07, *P* value = 0.01), 1 trial using modified Bruce protocol measured in minutes (MD 0.15 CI 95% -0.63 – 0.92, *P* value = 0.71), 1 trial using Bruce protocol measured in minutes (MD 0.32 CI 95% -0.52 – 1.17, *P* value = 0.46) and 1 trial used a modified Naughton protocol in minutes (MD 0.07 CI 95% -0.77 – 0.91), *P* value = 0.87). 1 trial used a bike test measured in watts (MD 0.30 CI 95% -0.52 – 1.13, *P* value = 0.47). 1 trial used 6-minute walking distance measured in minutes. The trial reported a mean ratio of 1.1 (0.9 to 1.3, P = 0.25) due to skewness of data. The point estimate favors digoxin over bisoprolol.

Successful achievement of resting heart rate target (as defined by trialist)

No trial reported this successful achievement of resting heart rate target.

Ejection Fraction

Kotecha et al assessed ejection fraction. They found no difference (adjusted mean difference was 0.8%, 95% CI -1.3 to 3.0, P value 0.45).[57]

Calcium channel blocker versus placebo

Primary outcomes

All-cause mortality

One trial assessed zero events in both treatment arms. Hence, no meta-analysis or test of a single trial was performed.

Serious adverse events

No trials assessed serious adverse events other than death. Hence, no meta-analysis was performed.

Secondary outcomes

Health-related quality of life (any validated continuous scale). These may be both generic and disease-specific questionnaires

Two trials reported quality of life.[37] Lundström et al used a 10-point VAS scale from "Much worse than usual" to "Much better than usual". Unpaired t-test was not statistically significant (MD -0.50 CI 95% -1.49 – 0.49, P value 0.3102. The standard deviation was 1-1.8. Considering a 0.5 difference the minimally clinically significant difference, the confidence intervals contained clinically relevant differences.

Lewis et al reported a VAS scale across multiple, individual symptoms but there were missing standard deviations and the lack of description of the VAS scale made us refrain from reporting the results.

Arial fibrillation symptom score such as European Heart Rhythm Association (EHRA), New York Heart Association (NYHA).

No trial reported on symptom score.

Non-serious adverse events.

No trial reported on non-serious adverse events.

Exploratory outcomes

Submaximal exertional heart rate

Four trials (4 cross-over trials) assessed submaximal exertional heart rate. Meta-analysis showed that CCB reduced the maximal exertional heart rate (MD -27.95, CI 95% -34.87 – -21.02, *P* value < 0.0001). The difference was above our predefined minimal important difference (SD/2 = 14). TSA confirmed the meta-

analysis result We found no signs heterogeneity when inspecting the forest plot and from the statistical test (I^2 = 0.00%).

This outcome result was assessed at high risk of bias. Test for subgroup differences according to which CCB was used (*P* = 0.94). All trials were cross-over trials and were published in the same time period.

Exercise capacity

6 trials assessed exercise capacity. 2 trials used a treadmill; 1 trial using modified Bruce protocol measured in METS (MD 1.07 CI 95% 0.14 – 2.01), *P* value = 0.02), and 1 trial used a modified Naughton protocol in minutes (MD 0.07 CI 95% -0.74 – 0.87), *P* value = 0.87). 3 trials used three different bike tests; 1 measured in watts (MD -0.06 CI 95% -0.69 – 0.58), *P* value = 0.85), 1 measured in kilopoundmeters (MD 0.91 CI 95% 0.27 – 1.55), *P* value = 0.01), and 1 measured in minutes (MD 0.08 CI 95% -0.67 – 0.82), *P* value = 0.84). 1 trial used 6-minute walking distance measured in meters (MD 0.06 CI 95% -0.76 – 0.89), *P* value = 0.90).

Successful achievement of resting heart rate target (as defined by trialist)

No trial reported on successful achievement of resting heart rate.

Ejection Fraction

0 trials reported ejection fraction.

Calcium channel blocker versus digoxin

Primary outcomes

All-cause mortality

Two trials assessed all-cause mortality.[8,9] Villani et al had zero events in both arms. Van Noord et al reported in their trial randomizing 97 participants to diltiazem or digoxin that 3/48 and 1/49 participants died. The result did not indicate any difference between treatment groups (RR 3.06, CI 0.33 – 28.42; p – value = 0.36).

Serious adverse events

No trials assessed serious adverse events other than death. Hence, no meta-analysis was performed.

Secondary outcomes

Health-related quality of life (any validated continuous scale). These may be both generic and disease-specific questionnaires

One trial reported quality of life.[37] Lewis et al reported a VAS scale across multiple, individual symptoms but there were missing standard deviations and the lack of description of the VAS scale made us refrain from reporting the results.

Arial fibrillation symptom score such as European Heart Rhythm Association (EHRA), New York Heart Association (NYHA).

No trial reported on symptom score.

Non serious adverse events.

No trial reported on Non serious adverse events.

Exploratory outcomes

Submaximal exertional heart rate

Two cross-over trial assessed submaximal exertional heart rate. Meta-analysis showed that CCB versus digoxin did not statistically significantly reduce the submaximal exertional heart rate (MD -10.27, CI 95% -27.58 – 7.03, *P* value = 0.24). There were no signs of statistical heterogeneity when inspecting the forest plot. The statistical test showed possible statistical heterogeneity (I^2 = 23.88%).

TSA showed we did not have enough information to reject a difference of a 13 bpm difference, which was equal to SD/2.

This outcome result was assessed at high risk of bias

Test for subgroup differences according to which CCB was used (*P* = 0.34), cross-over design versus parallel group design (*P* = 0.45), and year of publication (*P* = 0.86) were not statistically significant.

Exercise capacity

4 trials assessed exercise capacity. 2 trials used six-minute walking distance; one reported as median + range (see further below), and one reported as mean in meters (MD 9.40 CI 95% -71.65 – 90.45, *P* value = 0.82). Two trials used a treadmill; 1 using Chugs protocol measured in minutes (MD 4.20 CI 95% 1.83 to 6.57), *P* value < 0.01, 1 trial using modified Naughton protocol measured in minutes (MD 0.30 CI 95% -3.29 – 3.89), *P* value = 0.87). Results are presented in eFigure 21.

Channer et al reported a median six-minute walking distance 454 meters, range 335-629 meters in the verapamil + digoxin arm versus 461 range 324-637 in the digoxin only arm. The difference was not statistically significant.

Successful achievement of resting heart rate target (as defined by trialist)

No trial reported on successful achievement of resting heart rate target.

*Ej*ection Fraction

0 trials reported ejection fraction.

Calcium channel blocker versus amiodarone

Two trials assessed calcium channel blockers with amiodarone.

Primary outcomes

All-cause mortality

Two trials assessed zero events in both treatment arms. Hence, no meta-analysis was performed.

Serious adverse events

No trials reported serious adverse events other than death. Hence, no meta-analysis was performed.

Secondary outcomes

Health-related quality of life (any validated continuous scale). These may be both generic and disease-specific questionnaires.

No trials reported quality of life.

Arial fibrillation symptom score such as European Heart Rhythm Association (EHRA), New York Heart Association (NYHA).

No trial reported on symptom score.

Non serious adverse events.

No trial reported on non-serious adverse events.

Exploratory outcomes

Exploratory outcomes

Achieved resting heart rate

Two trials (2 parallel trials) assessed resting heart rate.[8,10] Meta-analysis showed no statistically significant difference (MD -2.32, CI 95% -5.24– 0.60, P value = 0.12; eFigure 23). There were no signs of statistical heterogeneity when inspecting the forest plot. The statistical test showed some heterogeneity (I^2 = 38.84%).

TSA showed we did not have enough information to reject a difference of a 4 bpm difference, which was equal to SD/2.

This outcome result was assessed at high risk of bias.

The remaining subgroup analyses were not possible due to lack of relevant data

Exertional heart rate

No trials reported submaximal exertional heart rate nor maximal exertional heart rate.

Exercise capacity

No trials reported exercise capacity.

Successful achievement of resting heart rate target (as defined by trialist)

No trial reported on successful achievement of resting heart rate target.

Ejection Fraction

0 trials reported ejection fraction.

Digoxin versus placebo or digoxin in addition to another rate controlling drug

Primary outcomes

All-cause mortality

Two trials reported zero events in both treatment arms. Hence, no meta-analysis was performed.

Serious adverse events

No trials assessed serious adverse events other than death. Hence, no meta-analysis was performed.

Secondary outcomes

Health-related quality of life (any validated continuous scale). These may be both generic and disease-specific questionnaires

One trial reported quality of life.[37] Lewis et al reported a VAS scale across multiple, individual symptoms but there were missing standard deviations and the lack of description of the VAS scale made us refrain from reporting the results.

Arial fibrillation symptom score such as European Heart Rhythm Association (EHRA), New York Heart Association (NYHA).

No trial reported on symptom score.

Non serious adverse events.

No trial reported on non-serious adverse events.

Exploratory outcomes

Submaximal exertional heart rate

Three trials (3 cross-over trials) assessed submaximal exertional heart rate. Meta-analysis showed that digoxin reduced the maximal exertional heart rate (MD -10.77, CI 95% -19.10 – -2.42, *P* value = 0.01). The difference was below our predefined minimal important difference (SD/2 = 12). There was no signs of statistical heterogeneity when inspecting the forest plot and from the statistical test (I^2 = 0.00%).

TSA confirmed the meta-analysis result. This outcome result was assessed at high risk of bias.

Test for subgroup differences comparing year of publication (*P* = 0.61) was not statistically significant.

Exercise capacity

7 trials assessed exercise capacity. 2 trials did a 6-minute walking test measured in meters (MD 0.18 CI 95% -47.47 – 47.83, *P* value = 0.99), 4 trials used a treadmill; 2 trials used the modified Bruce protocol measured in minutes (MD 0.42 CI 95% -1.47 – 2.30, *P* value = 0.66), 1 trial used the modified Naughton protocol in minutes (MD 0.10 CI 95% -2.38 – 2.59, *P* value = 0.94), and 1 trial used the modified Bruce protocol measured in METS (MD -2.00 CI 95% -3.97 – -0.03, *P* value = 0.05). 1 trial used a Bike test measured in watts (MD -19.00 CI 95% -43.37 – 5.37, *P* value = 0.13). Results are presented in eFigure 26.

Successful achievement of resting heart rate target (as defined by trialist)

No trial reported on successful achievement of resting heart rate target

Ejection Fraction

0 trials reported ejection fraction.

Digoxin versus amiodarone

Two trials assessed on digoxin versus amiodarone.

Primary outcomes

All-cause mortality

Two trials reported zero events in both treatment arms. Hence, no meta-analysis was performed.

Serious adverse events

No trials assessed serious adverse events other than death. Hence, no meta-analysis was performed.

Secondary outcomes

Health-related quality of life (any validated continuous scale). These may be both generic and disease-specific questionnaires

One trial assessed quality of life using SF-36.[58] Using student's t-test, they found no statistically significant difference across the 8 subdomains of SF-36.[58]

Arial fibrillation symptom score such as European Heart Rhythm Association (EHRA), New York Heart Association (NYHA).

No trial reported on symptom score.

Non serious adverse events.

No trial reported on non-serious adverse events.

Exploratory outcomes

Achieved resting heart rate

One parallel trial assessed resting heart rate.[11] Two sample ttest showed a statistically significant higher heart rate with digoxin (MD 6, CI 95% 2.72 – 9.28, *P* value = 0.0005). The difference was not clinically significant.

Exertional heart rate

One parallel trial assessed maximal heart rate.[11] Unpaired ttest showed no statistically significant difference (MD -7.00, CI 95% -41.39 – 27.39, *P* value = 0.6691).

No trials assessed submaximal heart rate.

Exercise capacity

One trial assessed on exercise capacity and found no difference (MD -1.4, CI 95% -3.275 – 0.475, *P* value = 0.1316).[11]

Successful achievement of resting heart rate target (as defined by trialist)

No trial reported on successful achievement of resting heart rate target

Ejection Fraction

One trial reported on ejection fraction and found no difference (MD 0.02, CI 95% -0.0978 – 0.1378, P value = 0.7211).[12]

Beta-blocker versus amiodarone

No trials assessed beta-blockers versus amiodarone

Risk of bias assessment

Bias arising from the randomization process

Low risk of bias: Adequately concealed allocation (e.g. central randomization or independent unit such as a pharmacy) and if there are no baseline imbalances or baseline imbalances are compatible with chance, and random or unpredictable method (e.g. computer generated sequence) to generate the allocation sequence.

Some concerns: 1) Adequately concealed allocation and a problem with the method of sequence generation or baseline imbalances that suggest a problematic randomization process, or 2) if no information is provided about concealment of allocation and baseline imbalances appear to be compatible with chance, or 3) if no information to answer any of the signaling questions.

High risk of bias: 1) Allocation sequence not adequately concealed, or 2) there is no information about concealment of the allocation sequence and baseline imbalances that suggest a problem with the randomization process.

Bias due to deviation from intended interventions

Low risk of bias: 1) If participants, health care professionals, and people delivering the interventions were unaware of randomization groups during the trial, or 2) they were aware of intervention groups during the trial but deviations from the intended was usual practice, or unlikely to impact the outcome and no participants were analyzed in a group that the participant was not assigned to.

Some concerns: Participants, health care professionals, and people were aware of intervention groups and 1) there was no information on whether there were deviations from the intended interventions, or 2) there were deviations from the interventions, but the deviations were not likely to have affected outcome or were balanced between the groups.

High risk of bias: Participants, health care professionals or people were aware of the intervention groups during the trial and there were deviations from the intended interventions that were unbalanced between the groups and likely to have affected the outcome, or some participants were analyzed in the wrong intervention group, and there was potential for substantial impact on the estimated effect size.

Bias due to missing outcome data

Low risk of bias: Data were available for all, or nearly (around 5%) all randomized participants or there is evidence that the result was not biased by missing data or that missingness in the outcome could not depend on its true value.

Some concerns: An unclear degree of missing data and there is no evidence that the effect estimate is robust to missing data.

High risk of bias: High degree of missing data, differential missing data, and no evidence that the effect estimate is robust to missing data.

Bias in measurement of outcomes

Low risk of bias: Outcome assessors were unaware of the intervention received by study participants, or aware but were unlikely to be influenced by this knowledge.

Some concerns: No information available to determine if the outcome is likely influenced by knowledge of the intervention received.

High risk of bias: The outcome assessment was likely to be influenced by knowledge of the intervention received.

Bias arising from selective reporting of results

Low risk of bias: Reported outcome data was unlikely to have been selected on the basis on the results from multiple outcome measurements.

Some concern: Insufficient information available to rule out the possibility of selective outcome reporting based on the results from multiple outcome measurements.

High risk of bias: Reported data is likely to have been selected based on the results from multiple outcome measurements or analyses.

Overall assessment of risk of bias

Low risk of bias: If the study is judged as low risk across all domains.

High risk of bias: If the study is judged as some concerns or high risk of bias in at least one domain. If a trial is sponsored by the industry and or if just one author has affiliation to the industry, the publication will be judged as having some concern or high risk of for-profit bias. The domains 3, 4, and 5 will be assessed for each outcome result.

Plan for making network meta-analysis

The synthesis comparator will consist of all the interventions as well as possible control interventions described in the methods section as well as any other drugs, which intend to reduce the rate in atrial fibrillation. We will group drugs according to the classes of drugs described in the method section. The characteristics of the trials and their populations will be described by frequencies and percentages for dichotomous data and means with SD for continuous data. Each outcome dataset will be presented in a separate network diagram, where the size of the nodes is proportional to the total number of participants, and the width of each line corresponds to the number of studies comparing the connected treatments. Furthermore, the connecting lines will be marked according to the average risk of bias per treatment comparison, using green for low, yellow for moderate, and red for high risk of bias. It is assumed that any participant who meets inclusion criteria is equally likely to be randomized to each intervention in the comparator set.

Network meta-analysis will only be conducted if the ranking of the interventions is unclear and if a connected network of trials can be constructed. If conducted, the assumptions of transitivity and consistency will be assessed prior to analysis. The network meta-analysis was planned to follow the recommendations of Shim et al.[3]

Article 3 - Retrospective cohort study on sex, atrial fibrillation and risk of stroke

Word count text: 2508
Word count abstract: 240
References: 21
Tables: 3, supplemental tables: 5, figure 1

Original Article

Sex differences in atrial fibrillation and associated complications in hypertensive patients with left ventricular hypertrophy: The LIFE study

Sex differences in AF and associated complications

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All author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

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Keywords: Blood Pressure, hypertension, atrial fibrillation, left ventricular hypertrophy, sex characteristics, stroke

Funding: JB Feinberg is supported by a grant from the Danish Heart Foundation (grant number 19-R134-A8959-22123).

Conflict of interests SEK reports ad hoc lecture honoraria from Getz, Merck Healthcare KGaA, Sanofi, and Vector-Intas. All other authors declare no conflicts of interest.

Abbreviations: AF = Atrial fibrillation, AFBHN = Variable name for the 3-level categorical variable ANOVA = Analysis of variance, BMI = Body Mass Index, CHA_2DS_2VASc = A score for calculating stroke risk for patients with atrial fibrillation, CI = confidence interval, COPD= Chronic Obstructive Pulmonary Disease, LIFE = Losartan Intervention for Endpoint, MI = Myocardial Infarction SHR= Sub hazard ratio, TIA = Transient Ischemic Attack.

Abstract

Background: There is no consensus on whether biological differences account for the higher risk of stroke seen in females compared to males with atrial fibrillation.

Methods: Capitalising on The Losartan Intervention for Endpoint study, a multicenter randomized clinical trial randomizing 9,193 patients and followed for at least four years, we aimed to identify sex differences in the risk of stroke in the presence of atrial fibrillation in patients with hypertension and left ventricular hypertrophy (LVH).

Results: 342 patients had a history of atrial fibrillation, and 669 developed new-onset atrial fibrillation. History of atrial fibrillation and new-onset atrial fibrillation were more prevalent among males (5.0% vs. 2.9% and 3.0% vs. 0.9%) in patients aged 55-63 years, but the relative difference decreased with age. Females with new-onset atrial fibrillation tended to have a higher risk of stroke than males (HR 1.52 [95% CI 0.95-2.43]). However, females with a history of atrial fibrillation did not have a higher risk than males (HR 0.88 [95% CI 0.5-1.6]). In patients with new-onset atrial fibrillation, the relative higher stroke risk in females increased with age. Among patients with a history of atrial fibrillation, stroke risk was comparable and increased with age in both sexes.

Conclusions: Among patients with hypertension and LVH, females with new-onset atrial fibrillation had a higher risk of stroke than males, especially in patients above 64 years. However, the risk did not differ between the sexes among patients with a history of atrial fibrillation.

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia. Both the incidence and prevalence of AF are generally lower in women than in men, with large regional and study variance.[1-3] The risks of both cerebral stroke and heart failure are increased nearly fivefold in patients with AF, and about 20% of strokes may be due to AF.[4,5] The risk of stroke and mortality is higher in females with AF.[1] A systematic review with meta-analysis, including 30 studies and 4,371,714 patients, assessed whether AF was a stronger predictor of cardiovascular disease and death in females than males.[6] They found that AF was a stronger predictor in females than males for all-cause mortality (RR 1.12, 95% confidence interval (CI) 1.07 to 1.17), stroke (RR 1.99, 95% CI 1.46 to 2.71), and cardiovascular mortality (RR 1.93, 95% CI 1.44 to 2.60).[6] Ultimately, these results have led to the inclusion of sex in risk schemes for stroke, such as CHA_2DS_2VASc[7], but the sex-specific threshold for initiation of anticoagulation therapy (as female sex is seen as an effect modifier and not a risk factor) means that being female alone does not result in anticoagulant therapy.[7]

It remains unclear whether the difference in risk of stroke observed between females and males with AF is due to biological differences (smaller size, thrombogenesis, genetics, and effects of sex hormones) or is related to differences in risk factors between sexes.[8,9] If there exists a true difference in the risk of complications (such as stroke) due to sex, this may require special attention to females regarding screening and risk management. On the other hand, if the difference in AF-associated risk results from age and comorbidities, emphasis should not be placed on sex but rather on age and comorbidities. Accordingly, the present study was undertaken to investigate whether the greater risk of stroke in females with AF in a high-risk hypertensive population was due to true differences in risk between females and males.

2. Methods

2.1 Study design and patient population

The Losartan Intervention for Endpoint Reduction (LIFE) study, a multicentre randomized controlled trial, randomized 9,193 patients in 7 countries from June 1995 to May 1997 with essential hypertension and left ventricular hypertrophy to either losartan-based or atenolol-based therapy. [10,11] Patients aged 55-80 years, with primary hypertension defined as a sitting systolic and diastolic blood pressure of 160-200 mmHg or 95-115 mmHg, respectively, and left ventricular hypertrophy based on electrocardiogram were included. Patients with myocardial infarction or stroke within the past 6 months or heart failure with known reduced ejection fraction were excluded.

2.2 Data collection

Information was collected on demographic, clinical characteristics, medical history, and blood samples at baseline. The patients were followed yearly for at least four years for the primary outcome defined as a composite of cardiovascular death, stroke, or myocardial infarction.

2.3 Statistical methods

Data analyses were done using STATA 17.[12] All outcomes were analyzed according to the intention-to-treat principle, and all randomized patients were included in the analyses. Descriptive statistics identified frequencies and percentages. We conducted chi-square tests for categorical variables. For continuous variables with a two-level independent variable, we used an unpaired t-test. For continuous dependent variables with a three-level independent variable(s), we used ANOVA with Tukey's post-hoc test. We used logistic regression to identify factors independently associated with having a history of AF and developing new-onset AF. We used Cox

regression to assess the impact on the risk of variables of interest (sex, AF, and age). We supplemented the Cox regression with the Fine-Gray regression for the overall analysis. In the full model, we included treatment allocation (atenolol/losartan), AF, age, systolic blood pressure, body mass index, smoking status, diabetes, previous cerebrovascular disease, previous myocardial infarction, and previous heart failure at baseline. We supplemented our primary analysis with an analysis considering new-onset AF as a time-varying covariate for the primary outcome of interest, stroke. We reported hazard ratios from the models without interaction terms and p-values from the Wald test for our interaction terms. We performed stratified analyses according to sex and age based on tertiles. The two upper age groups were further combined to achieve enough events. The assumptions of independent observations were true for our analyses. We tested the assumption of proportional hazards using the Schoenfield residuals for both the adjusted and unadjusted Cox regressions. A P-value < 0.05 was considered statistically significant in all analyses.

3. Results

3.1 Patient characteristics

The characteristics of the population are given in **Supplemental table 1**. Patients with a history of AF tended to be older and had more comorbidities at baseline than patients without AF. Prevalences of a history of AF and new-onset AF were higher among males than females across all tertiles, but the relative difference was lower in the older age groups (**Figure 1**). History of AF and developing new-onset AF were associated with older age and male sex (**Supplemental table 2**).

Drug treatment of AF was similar between males and females at baseline and at the end of the study, except that more females received digoxin than males at the end of the study (**Supplemental table 3**).

3.2 Clinical outcomes

Using Cox regression analyses without interaction terms, patients with a history of AF had a higher risk of stroke (HR 2.64, CI 95% 1.95-3.58), all-cause mortality (HR 1.96, CI 95% 1.52-2.52), and the composite cardiovascular outcome (HR 2.16, CI 95% 1.73 – 2.70) than those without AF (**Table 1**). Using Fine-Gray regression, patients with a history of AF had a higher risk of stroke (Sub hazard ratio [SHR]2.63, 95% CI (1.92 – 3.60). Patients with new-onset AF had a 2.31-fold higher risk of stroke (CI 95% 1.81 – 2.95), 1.33-fold higher all-cause mortality (CI 95% 1.05 – 1.67), and 1.86-fold higher composite cardiovascular outcome (CI 95% 1.55 – 2.23) than those without AF, but lower risks than those with a history of AF (**Table 1)**. Using Fine-Gray regression, patients with a new-onset AF had a higher risk of stroke (SHR 2.31, CI 95% 1.80 – 2.96). Using Cox regression, analyzing new-onset atrial fibrillation as a time-varying covariate, patients with a history of AF had higher rates of stroke (HR 2.55, CI 95% 1.89 – 3.45) than those without AF. Similarly, patients with new-onset AF had a higher risk of stroke (HR 3.06, CI 95% 2.27 – 4.14) than those without AF.

We tested for prognostic interactions between AF, age, and sex for the risk of stroke using the Wald test and found significant interactions between new-onset AF and age (P = 0.018) as well as new-onset AF and sex (P = 0.003) but not between a history of AF, age and sex for the risk of stroke. An overview of the results of the statistical tests for interaction is provided in a table in **Supplemental table 4a, 4b, 4c**. The prognostic interaction between new-onset AF and age seemed stronger and was negative in males.

In stratified analysis, where participants with new-onset AF and a history of AF were analyzed separately, no evidence of a difference was found between females and males with a history of AF on stroke, all-cause mortality, and the composite cardiovascular endpoint (**Table 2**).

For those with new-onset AF, the point estimate suggested a higher risk of stroke for females than males, although this was not statically significant (**Table 2**). In stratified analysis according to sex and age, the risk of stroke associated with new-onset AF increased with age in females above/below 64 years (from HR 0.87, 95% CIs 0.12-6.18 to HR 3.05, 95% CIs 2.17 – 4.29) and decreased with age in males above/below 64 years (from HR 2.63 95% CIs 1.19 – 5.86 to HR 1.48, 95% CIs 0.98 – 2.25). There was a statistically not significantly lower stroke risk in females compared with males with new-onset AF younger than 64 years (HR 0.16, 0.01 – 2.01) and higher stroke risk in females than males with new-onset AF older than 64 years (HR 1.6, 95% CIs 0.95 – 2.70) (**Table 3**). Similar results were found for the composite cardiovascular outcome in patients with new-onset AF (**Supplemental table 5**). However, in patients with a history of AF, the risk of stroke (from HR 1.71 95% CIs 0.53 – 5.89 to HR 2.65 95% CIs 1.43 – 4.92) and the composite cardiovascular outcome (from HR 1.19 (0.52 – 2.74) to HR 2.5 95% CIs 1.64 – 3.84) increased with age in males (**Table 3** and **Supplemental table 5**). A tendency toward similar results was found in females, but the results were imprecise due to few events and lack of statistical power (**Table 3** and **Supplemental table 5**).

4. Discussion

Main findings

In our study of patients with hypertension and left ventricular hypertrophy we found that 1) the prevalences of AF and incidence of new-onset AF increased more with age in females,

and 2) higher event rates in patients with a history of AF were independent of sex and age, where as the stroke risk in females with new-onset AF was lower than in males in patients younger than 64 years but higher in patients older than 64 years. The age-sex interactions seen for stroke were not seen for all-cause mortality, strengthening support for a biological interaction between sex and new-onset AF for the risk of stroke.

Mechanisms behind the findings

There may be many reasons for the difference in risk of stroke between males and females with new-onset AF. In LIFE, women had both smaller stroke volumes and cardiac output than men (although similar stroke volume index and cardiac index) and thus lower flow through the left atrium.[9,13,14] In addition, losing the atrial component packing the left ventricle during atrial fibrillation may, in fact, result in even lower flow and a higher risk of thrombus formation.

Given the timing of the increased risk, it seems less likely that sex hormones directly play a role in the increased risk of stroke.[2,8] However, genetics and sex hormones may play a role in the development of atrial fibrillation through a difference in electrophysiological properties, such as differences in calcium channels of the myocardium.[8] There may also be an interaction with testosterone and estrogen regarding the expression and excitability of calcium, potassium, and sodium channels in pre-menopausal females.[2,8]

The alternative hypothesis to real sex differences, is a a difference in risk factors, including hypertension, diabetes, heart failure, LV diameter, and a high body mass index.[2,3,14] This difference in etiology of atrial fibrillation and response may be one of the mechanisms behind the alternative hypothesis where the difference in risk of stroke is attributable to a difference in risk factors.[8] Age is a strong predictor of AF and its complications, and since females live longer than

males, this survival bias may also contribute to the perceived difference in risk among males and females However, since the association was present in new-onset AF and not in patients with a history of AF, this explanation seems unlikely. Further, in agreement with previous findings, we also did not find the prevalence of comorbidities uniformly higher in females compared with males. Females tend to have a higher incidence of valvular heart disease, whereas males have more coronary artery disease.[2,15]

Another theory suggests the difference in risk of complications is due to a difference in treatment. In an observational study, males were more often treated with a rhythm control strategy than females despite females having more symptoms.[16] However, in our study, we found that treatment was similar among males and females (**Supplemental table 3**), and this did not provide a basis for an explanation.

Clinical implications

The results of our study makes it more likely that the sex differences found are not the result of residual confounding. Hence, focus should be on the mechanism behind the increased risk associated with atrial fibrillation itself and biological sex differences, and not solely on improving risk factors. In the EAST trial, the point estimate for the primary composite outcome (including stroke) for the participants randomised to an early aggressive rhythm control strategy was lower for the oldest population (>74 of age) compared with participants younger than 74 of age as well as lower for women compared with men.[17] These results taken together support that the increased risk in older women may be prevented by a more aggressive rhythm control strategy early on.

Our results are compatible with previously reported results.[18,19] Currently, there is no evidence to support sex-specific recommendations regarding anticoagulation, as the risk is also dependent on age.

Limitations

Our study had some limitations. The analysis was not pre-specified in the LIFE study analysis plan. As such, the risk of a type I error with post-hoc analyses increases and must be considered. The patients included in the study were taken from the LIFE cohort consisting of patients with hypertension and ECG-confirmed left ventricular hypertrophy. The patients were not recruited or stratified for AF. Although echocardiography was not used systematically to confirm the diagnosis, in 13% of participants an echocardiography was performed an confirmed that most of the LIFE study patients also had structural left ventricular hypertrophy.[20] In any case, patients with ECG-confirmed hypertrophy, although perhaps distinct from participants with only structural hypertrophy, are at larger risk of complications.[21]

We defined three categories concerning AF: Those who never had AF, those who were diagnosed with new-onset AF during the study, and those with a history of AF upon entry. In some cases, the diagnosis of new-onset AF was first made after the diagnosis of stroke (11%). This may question the direction of a possible causal relationship. However, ECGs was only taken yearly, and therefore paroxysmal AF may have preceded the stroke. A supplementary analysis using new-onset AF was a time-varying covariate resulted in slightly higher HR for new-onset atrial fibrillaiton supporting our primary analysis. However, some patients who died may have developed unidentified AF before dying, which would have biased the result in the opposite direction.

The included patients were all included between 1995-1997. A tool for calculating the risk of stroke and guiding anticoagulation therapy was first introduced with the first CHAD2s

score in 2001, with several subsequent updates, lowering the age limit from 75 to 65 for lifelong anticoagulation therapy. Anticoagulation therapy treatment is crucial to reducing the risk of stroke in patients with AF.[7] In LIFE, around 20% of AF patients received anticoagulation therapy at baseline and 43% at the end of the study. By modern standards, this would be considered low in a population with an average age of 67.

In conclusion, in patients with hypertension and left ventricular hypertrophy on ECG, new-onset AF was associated with higher risk of stroke in females than in males, particularly in those older than 64 years. In patients with hypertension and left ventricular hypertrophy, a history of AF was associated with the same risk of stroke in males and females. This may suggest that the sex-difference observed are a result of real sex differences, and not residual confounding.

Acknowledgements

We thank all the original investigators, supporting staff, and patients involved in the LIFE study.

5. Disclosures

SEK reports ad hoc lecture honoraria from Getz, Merck Healthcare KGaA, Sanofi, and Vector-Intas. All other authors declare no conflicts of interest.

Data availability

The data supporting the findings are available upon reasonable request.

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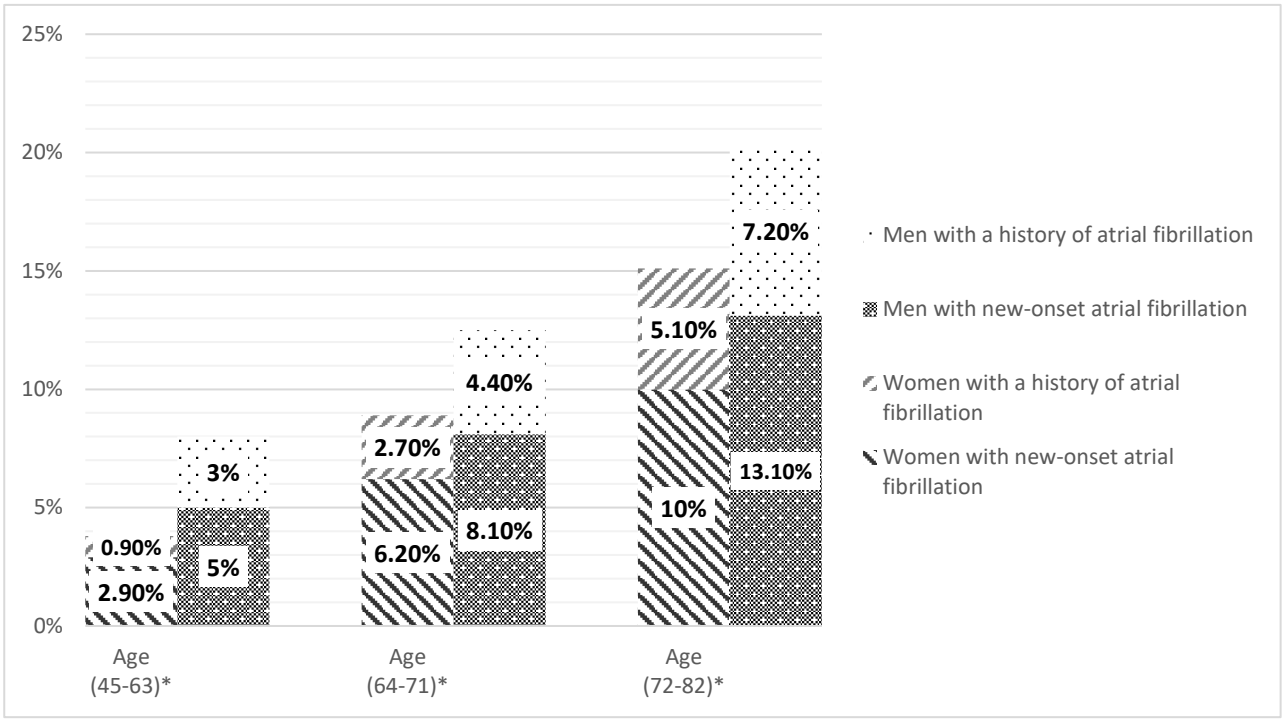
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13.10%
Age (45-63)*
Age (64-71)*
Age (72-82)*
Men with a history of atrial fibrillation
Men with new-onset atrial fibrillation
Women with a history of atrial fibrillation
Women with new-onset atrial fibrillation

Figure 1. Comparison of atrial fibrillation distribution by sex across age tertiles. Distribution compared in each age group with chi squared. P-value = 0.002 or less for all three comparisons.

Table 1. Hazard ratios comparing a history of atrial fibrillation/new-onset atrial fibrillation with no atrial fibrillation.

End point	No AF (n = 8182)		History of AF (n = 342)		New-onset AF (n = 669)		Unadjusted History of AF	Adjusted history of AF	unadjusted new-onset AF	Adjusted new-onset AF	P value for HR new-onset AF versus a history of AF
	Rate (per 1000 years)	n (%)	Rate (per 1000 years)	n (%)	Rate (per 1000 years)	n (%)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	
Stroke	9.7	391 (4.8%)	34.5	54 (15.8%)	29.6	94 (14.1%)	3.54 (2.67-4.71)	2.64 (1.95 – 3.58)	3.05 (2.43 – 3.82)	2.31 (1.81 – 2.95)	P = 0.456
All-cause mortality	16.0	639 (7.8%)	50.2	79 (23.1%)	29.6	96 (14.3%)	3.19 (2.52-4.03)	1.96 (1.52 – 2.52)	1.86 (1.50 - 2.30)	1.33 (1.05 – 1.67)	P = 0.016
Composite of death, MI, and stroke	20.8	833 (10.2%)	64.5	101 (29.5%)	50.4	160 (23.9%)	3.13 (2.55 – 3.85)	2.16 (1.73 – 2.70)	2.45 (2.07 – 2.90)	1.86 (1.55 – 2.23)	P = 0.270

Hazard ratios for stroke, all-cause mortality and the composite outcome comparing 1) a history of atrial fibrillation with no atrial fibrillation, and 2) new-onset atrial fibrillation with no atrial fibrillation. The multivariate analysis was adjusted for treatment allocation, age, sex, systolic blood pressure, cholesterol, body mass index, smoking, diabetes, history of transient ischemic attack/stroke, previous MI, and history of heart failure. There was no interaction term.

AF = atrial fibrillation. MI = Myocardial infarction.

Table 2. Hazard ratios for females versus males with history of atrial fibrillation or new-onset atrial fibrillation

End point	Males with a history of atrial fibrillation (n = 195)		Females with a history of atrial fibrillation (n = 147)		Unadjusted	Adjusted	Interaction between sex and atrial fibrillation
	Raw incidence rate (per 1000 years)	n (%)	Raw incidence rate (per 1000 years)	n (%)	HR (95%CI)	HR (95%CI)	P – value
Stroke	30.0	27 (13.8%)	40.5	27 (18.3%)	1.35 (0.8 – 2.3)	0.88 (0.5 – 1.6)	0.117
All-cause mortality	47.9	43 (22.1%)	53.3	36 (24.4%)	1.1 (0.7 – 1.7)	0.91 (0.6 – 1.5)	0.166
Composite of death, myocardial infarction, and stroke	69.1	57 (29.2%)	50.6	44 (29.9%)	0.97 (0.66 – 1.45)	0.69 (0.44 – 1.06)	0.232
End point	**Males with new-onset atrial fibrillation (n = 346)**		**Females with new-onset atrial fibrillation (n = 323)**		**Unadjusted**	**Adjusted**	
	Rate (per 1000 years)	**n (%)**	**Rate (per 1000 years)**	**n (%)**	**HR (95%CI)**	**HR (95%CI)**	**P – value**
Stroke	24.3	40 (11.6%)	35.2	54 (16.7%)	1.44 (0.96 – 2.17)	1.52 (0.95 – 2.43)	0.003
All-cause mortality	29.0	48 (13.9%)	30.3	48 (14.9%)	1.03 (0.69 – 1.54)	0.90 (0.57 – 1.44)	0.040
Composite of death, myocardial infarction, and stroke	53.4	83 (24.0%)	51.4	77 (23.8%)	0.87 (0.64 – 1.20)	0.86 (0.60 – 1.23)	0.009

Hazard ratios for stroke, all-cause mortality and the composite outcome stratified according to type of atrial fibrillation (a history of atrial fibrillation or new-onset atrial fibrillation. Multivariate analysis was adjusted for treatment allocation, age, sex, systolic blood pressure, cholesterol, body mass index, smoking, diabetes, history of Transient ischemic attack/stroke, previous myocardial infarction, and history of heart failure. There was no interaction term.	

Table 3. Age stratified adjusted hazard ratios comparing incidence rates of stroke in females and in males with new-onset atrial fibrillation or history of atrial fibrillation

Age tertiles	**Adjusted risk of stroke associated with a new-onset atrial fibrillation in males**	**Adjusted risk of stroke associated with a new-onset atrial fibrillation in females**	**Adjusted risk of stroke for females versus males with new-onset atrial fibrillation**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Age 55*– 63	2.63 (1.19 – 5.86)	0.87 (0.12 – 6.18)	0.16 (0.01 – 2.01)
Age 64 – 71	1.94 (1.12 – 3.35)	5.28 (3.01 – 9.08)	1.54 (0.73 – 3.22)
Age 72 – 82	1.10 (0.58 – 2.10)	2.39 (1.55 – 3.69)	1.86 (0.84 – 4.10)
Age tertiles	**Adjusted risk of stroke associated with a history of atrial fibrillation in males**	**Adjusted risk of stroke associated with a history of atrial fibrillation in females**	**Adjusted risk of stroke for females versus males with a history of atrial fibrillation**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Age 55* – 63	1.71 (0.53 – 5.89)	Too few events	-
Age 64 – 71	1.89 (0.96 – 3.73)	1.18 (0.35 – 4.00)	-
Age 72 – 82	2.65 (1.43 – 4.92)	4.14 (2.53 – 6.79)	-

Hazard ratios for stroke stratified according to age. Multivariate analysis was adjusted for treatment allocation, systolic blood pressure, cholesterol, body mass index, smoking, diabetes, history of transient ischemic attack/stroke, previous myocardial infarction, and history of heart failure. *There were under 100 participants between 45-55 who were protocol violators and were included.

Supplementary appendix

Feinberg et al. Sex differences in atrial fibrillation and associated complications in hypertensive patients with left ventricular hypertrophy: The LIFE study

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Supplemental table 1. Baseline characteristics

Baseline characteristics	Non-AF (n= 8.851)	New-onset AF (n = 669)	History of AF (n = 342)	Females new-onset AF (n = 323)	Males new-onset AF (n = 346)	Females with a history of AF (n = 147)	Males with a history of AF (n = 195)
Age (years)	66.6 ± 7.0	69.8 ± 6.6	70.3 ± 6.5*	71.0 ± 6.2	68.7 ± 6.7*	72.0 ± 5.8	69.0 ± 6.7*
White	7544 (92.4%)	636 (95.1%)	323 (94.7%)NS	309 (95.7%)	327 (94.1%)NS	140 (95.2%)	183 (93.8%)NS
Losartan	4136 (50.3%)	312 (46.6%)	157 (46.0%)*	152 (47.1%)	160 (46.2%)NS	64 (43.5%)	93 (47.7%)NS
Systolic blood pressure (mmHg)	174 ± 14	177±14	176 ± 14*	178.5	175.5*	176 ± 14	175 ± 14 NS
Diastolic blood pressure (mmHg)	98 ± 10	97 ± 9	96 ± 10*	96.4	97.3 NS	94 ± 10	98 ± 10*
Total cholesterol	6.1 ± 1.1	5.9 ± 1.1	5.7 ± 1.1*	6.2±1.1	5.6±1.1*	5.9 ± 1.1	5.5 ± 1.1NS
Hdl	1.5 ± 0.4	1.5 ± 0.4	1.5 ± 0.4NS	1.6 ± 0.4	1.4 ± 0.4*	1.5 ± 1.4	1.3 ± 3.8*
Creatinine	86.6 ± 19.9	87.7 ± 21.6	94.6 ± 21.9*	81.4 ± 24.1	93.4 ± 17.0*	85.8 ± 18.8	101.1 ± 21.9*
Glucose	6.0 ± 2.2	6.1 ± 2.1	6.4 ± 2.4¤	6.0 ± 2.3	6.1 ± 1.9 NS	6.5 ± 2.8	6.4 ± 2.3NS
BMI (kg/m²)	28.0 ± 4.8	28.1 (5.0%)	27.4 ± 4.6NS	28.4 ± 5.5	27.8 ± 4.4NS	27.4 ± 5.5	27.4 ± 3.7NS
Current smoker	1349 (16.5%)	101 (15.1%)	49 (14.3%)NS	42 (13.0%)	59 (17.1%)NS	15 (10.2%)	34 (17.4%)NS
Ischaemic heart disease	1215 (14.9%)	146 (21.82%)	108 (31.6%)*	55 (17.0%)	91 (26.3%)*	42 (28.6%)	66 (33.8%)NS
Previous MI	472 (5.8%)	57 (8.5%)	40 (11.7%)*	18 (5.6%)	39 (11.2%)*	8 (5.4%)	32 (16.4%)*
Heart failure	108 (1.3%)	21 (3.14%)	37 (3.1%)*	10 (3.1%)	11 (3.2%)NS	21 (14.3%)	16 (8.2%)NS
Stroke/TIA	615 (7.5%)	64 (9.6%)	49 (14.3) *	29 (9.0%)	35 (10.1%)NS	24 (16.3%)	25 (12.8%)NS
COPD	328 (4.0%)	32 (4.78%)	25 (7.3%) ¤	14 (4.3%)	18 (5.2%)NS	6 (4.1%)	19 (9.7%)*
Diabetes	1016 (12.6%)	95 (14.2%)	84 (24.6%) ¤	45 (13.9%)	50 (14.5%)NS	41 (27.9%)	43 (22.1%)NS

In the latter four columns, males versus females for new-onset atrial fibrillation and patients with a history of atrial fibrillation, respectively. * $P < 0.05$ for a difference between both patients with a history of atrial fibrillation and with new-onset atrial fibrillation compared with patients with no atrial fibrillation. ¤ $P < 0.05$ for a difference between patients with a history of atrial fibrillation compared with patients with no atrial fibrillation. Latter four columns use t-test/chi squared test.

AF = Atrial Fibrillation. BMI = Body Mass Index, COPD= Chronic Obstructive Pulmonary Disease, MI = Myocardial Infarction, TIA = Transient Ischemic Attack

Supplemental table 2. Logistic regression of factors associated with history of atrial fibrillation or new-onset atrial fibrillation.

	History of atrial fibrillation				New-onset atrial fibrillation			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	Odds ratio (CI)	P - Value	Odds ratio (CI)	P - Value	Odds ratio (CI)	P - Value	Odds ratio (CI)	P - Value
Age	1.08 (1.06 – 1.10)	<0.001	1.07 (1.04 – 1.09)	<0.001	1.07 (1.06 – 1.08)	<0.001	1.07 (1.06 – 1.09)	<0.001
Females	0.63 (0.51 – 0.79)	<0.001	0.83 (0.64 – 1.09)	0.186	0.78 (0.67 – 0.91)	0.002	0.62 (0.51 – 0.76)	<0.001
Atenolol treatment	1.19 (0.96 – 1.48)	0.115	1.24 (0.98 – 1.57)	0.088	1.16 (0.99 – 1.36)	0.064	1.11 (0.94 – 1.31)	0.234
Systolic blood pressure	1.01 (1.00 – 1.01)	0.090	1.00 (0.99 – 1.01)	0.647	1.01 (1.00 – 1.02)	<0.001	1.01 (1.00 – 1.02)	0.001
BMI	0.97 (0.95 – 1.00)	0.021	0.97 (0.94 – 0.99)	0.016	1.00 (0.99 – 1.02)	0.634	1.03 (1.01 – 1.05)	0.003
Stroke/TIA	2.01 (1.47 – 2.75)	<0.001	1.47 (1.05 – 2.08)	0.0026	1.25 (0.95 – 1-64)	0.102	1.06 (0.79 – 1.42)	0.703
Diabetes	2.27 (1.76 – 2.92)	<0.001	1.85 (1.30 – 2.64)	0.001	1.12 (0.89 – 1.40)	0.338	0.96 (0.71 – 1.32)	0.821
Current smoker	0.85 (0.63 – 1.16)	0.311	1.01 (0.73 – 1.41)	0.003	0.91 (0.73 – 1.13)	0.386	1.02 (0.80 – 1.29)	0.870
Cholesterol	0.73 (0.66 – 0.82)	<0.001	0.81 (0.73 – 0.91)	<0.001	0.91 (0.84 – 0.98)	0.011	0.91 (0.84 – 0.99)	0.001
HDL	0.44 (0.33 – 0.59)	0.001	0.58 (0.42 – 0.81)	0.001	0.97 (0.81 – 1.18)	0.782	1.18 (0.94 – 1.47)	0.128
Creatinine	1.01 (1.01 – 1.02)	<0.001	1.01 (1.00 – 1.01)	0.035	1.00 (1.00-1.01)	0.305	1.00 (0.99 – 1.00)	0.050
Glucose	1.07 (1.03 – 1.11)	0.001	0.99 (0.93 – 1.05)	0.723	1.01 (0.97 – 1.05)	0.627	1.32 (0.78 – 2.23)	0.306
Heart failure	8.20 (5.59 – 12.03)	<0.001	5.34 (3.43 – 8.30)	<0.001	1.87 (1.18 – 2.98)	0.008	1.23 (0.72 – 2.08)	0.446
Previous MI	2.08 (1.48 – 2.91)	<0.001	1.07 (0.72 – 1.59)	0.733	1.45 (1.10 – 1.94)	0.010	1.21 (0.89 – 1.65)	0.222

Odds ratios for a history of atrial fibrillation. There was no interaction term. BMI = Body Mass Index, CI = confidence interval, HDL = high-density

lipoprotein MI = myocardial infarction, TIA = Transient Ischemic Attack

Supplementary table 3. Concomitant therapies

Concomitant therapy	Men with atrial a history fibrillation n = 195		Women with a history atrial fibrillation n = 147		Men with new-onset atrial fibrillation n = 346		Women with new-onset atrial atrial fibrillation n = 323	
	Previous treatment	At study end	Previous treatment[a]	At study end[b]	Previous treatment	At study end	Previous treatment	At study end[b]
K-vitamin antagonist	39 (20.0%)	85 (43.6%)	30 (20.4%)[NS]	64 (43.5%)[NS]	14 (4.1%)	149 (43.1%)	7 (2.2%)[NS]	134 (41.5%)[NS]
Aspirin, clopiodgrel, dipyridamole, ticlide	4 (2.1%)	13 (6.7%)	1 (0.68%)[NS]	10 (6.8%)[NS]	1 (0.3%)	22 (6.4%)	1 (0.3%)[NS]	21 (6.5%)[NS]
Beta-blocker	63 (32.3%)	52 (26.7%)	48 (32.6%)[NS]	50 (34.0%)[NS]	112 (32.4%)	150 (43.4%)	101 (31.3%)[NS]	146 (45.2%)[NS]
Digoxin	98 (50.3%)	104 (53.3%)	88 (59.9%)[NS]	92 (62.6%)[NS]	15 (4.3%)	138 (39.9%)	24(7.4%)[NS]	158 (48.9%)[¤]
Verapamil	14 (7.2%)	9 (4.6%)	9 (6.1%)[NS]	17 (11.6%)[¤]	7 (2.0%)	28 (8.1%)	9 (2.8%)[NS]	28 (8.7%)[NS]
Diltiazem	6 (3.1%)	18 (9.2%)	9 (6.1%)[NS]	11 (7.5%) [NS]	25 (7.2%)	32 (9.3%)	13 (4.0%)[NS]	33 (10.2%)[NS]
Class IA antiarrhythmic drug	9 (4.6%)	10 (5.1%)	9 (6.1%)[NS]	12 (8.2%)[NS]	2 (0.6%)	7 (2.0%)	0 (0%)[NS]	5 (1.6%)[NS]
Class IC antiarrhythmic drug	7 (3.6%)	6 (3.1%)	5 (3.4%)[NS]	6 (4.1%)[NS]	1 (0.3%)	16 (4.6%)	1 (0.3%)[NS]	15 (4.6%)[NS]
Class III antiarrhythmic drug	2 (1.0%)	12 (6.2%)	2 (1.4%)[NS]	8 (5.4%)[NS]	1 (0.3%)	25 (7.2%)	0 (0%)[NS]	16 (5.0%) [NS]

[a] Statistical significance compared with previous treatment in men

[b] Statistical significance compared with treatment at study end in men

[¤] P <0.05

Supplementary table 4a. Interactions between AF (history or new-onset), age and sex for predicting stroke

Population	Interaction term	Unadjusted model - History of AF	Adjusted model - History of AF	Unadjusted model - New-onset AF	Adjusted model - New-onset AF
Men and women	Sex#AFBHN	P = 0.026	P = 0.117	P = 0.002	P = 0.003
Men and women	Age#AFBHN	P = 0.235	P = 0.243	P = 0.040	P = 0.018
Only men	Age#AFBHN	P = 0.514	P = 0.087	P = 0.019	P = 0.019
Only women	Age#AFBHN	P = 0.03	P = 0.022	P = 0.173	P = 0.072

The reported p-values are the results of the Wald test. AF = Atrial Fibrillation. AFBHN = Variable name for the 3-level categorical variable with the categories being no atrial fibrillation, a history of atrial fibrillation and new-onset atrial fibrillation.

Supplementary table 4b. Interactions between AF (history or new-onset), age and sex for predicting ACM

Population	Interaction term	Unadjusted model - History of AF	Adjusted model - History of AF	Unadjusted model - New-onset AF	Adjusted model - New-onset AF
Men and women	Sex#AFBHN	P = 0.018	P = 0.166	P = 0.021	P = 0.040
Men and women	Age#AFBHN	P = 0.700	P = 0.667	P = 0.839	P = 0.707
Only men	Age#AFBHN	P = 0.584	P = 0.489	P = 0.902	P = 0.982
Only women	Age#AFBHN	P = 0.824	P = 0.715	P = 0.468	P = 0.353

The reported p-values are the results of the Wald test. AF = Atrial Fibrillation. AFBHN = Variable name for the 3-level categorical variable with the categories being no atrial fibrillation, a history of atrial fibrillation and new-onset atrial fibrillation.

Supplementary table 4c. Interactions between AF (history or new-onset), age and sex for predicting the composite outcome of cardiovascular death, myocardial infarction, and stroke.

Population	Interaction term	Unadjusted model - History of AF	Adjusted model - History of AF	Unadjusted model - New-onset AF	Adjusted model - New-onset AF
Men and women	Sex#AFBHN	P = 0.015	P = 0.232	P = 0.004	P = 0.009
Men and women	Age#AFBHN	P = 0.228	P = 0.181	P = 0.014	P = 0.006
Only men	Age#AFBHN	P = 0.726	P = 0.546	P = 0.022	P = 0.013
Only women	Age#AFBHN	P = 0.112	P = 0.118	P = 0.087	P = 0.045

The reported p-values are the results of the Wald test. AF = Atrial Fibrillation. AFBHN = Variable name for the 3-level categorical variable with the categories being no atrial fibrillation, a history of atrial fibrillation and new-onset atrial fibrillation.

Supplementary table 5. Age stratified hazard ratios comparing incidence rates of composite cardiovascular outcome in women and in men with new-onset AF

Age tertiles	**Adjusted risk of composite cardiovascular outcome associated with new-onset AF in men**	**Adjusted risk of composite cardiovascular outcome associated with new-onset AF in women**	**Adjusted risk of composite cardiovascular outcome for women versus men with new-onset AF**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Age 45-63	1.79 (1.01 – 3.17)	1.38 (0.35 – 19.36)	0.40 (0.11 – 1.52)
Age 64-71	1.76 (1.19 – 2.61)	3.92 (2.49 – 6.17)	0.91 (0.51 – 1.62)
Age 72-82	1.29 (0.86 – 1.93)	1.74 (1.23 – 2.45)	1.11 (0.64 – 1.93)
Age tertiles	**Adjusted risk of composite cardiovascular outcome associated with a history of AF in men**	**Adjusted risk of composite cardiovascular outcome associated with a history of AF in women**	**Adjusted risk of composite cardiovascular outcome for women versus men with a history of AF**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Age 45-63	1.19 (0.52 – 2.74)	2.59 (0.35 – 19.35)	-
Age 64-71	1.80 (1.12 – 2.90)	1.65 (0.70 – 3.88)	-
Age 72-82	2.52 (1.64 – 3.84)	3.05 (2.05 – 4.55)	-

Hazard ratios for composite cardiovascular outcome stratified according to age. Multivariate analysis was adjusted for treatment allocation, systolic blood pressure, cholesterol, body mass index, smoking, diabetes, history of transient ischemic attack/stroke, previous myocardial infarction, and history of heart failure.
AF = Atrial fibrillation.

Article 4 - Cross-sectional study on the physical activity paradox

Physical activity paradox: could inflammation be a key factor?

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bjsports-2022-105429).

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Accepted 28 July 2022
Published Online First 12 August 2022

To cite: Feinberg JB, Møller A, Siersma V, *et al*. *Br J Sports Med* 2022;**56**:1224–1229.

ABSTRACT

Objective The aim of this study was to test the extent to which physical activity performed during work and leisure is associated with systemic inflammation.

Methods Data regarding job history and high-sensitivity C reactive protein (hs-CRP) levels, as well as potential confounders, came from the Copenhagen Aging and Midlife Biobank. The participants' self-reported job history was combined with a job exposure matrix to give a more valid assessment of cumulated occupational physical activity compared with conventional self-reported activity. Occupational physical activity was measured as cumulative ton-years (lifting 1000 kg each day for a year). Current leisure time physical activity was self-reported into four different categories. We analysed the association between occupational physical activity, current leisure time physical activity and hs-CRP level in a multivariable linear regression model with adjustment for age, sex, smoking history, number of chronic diseases, body mass index and alcohol.

Results In unadjusted analysis, higher occupational physical activity was associated with increased hs-CRP levels, while higher leisure time physical activity was associated with lower hs-CRP levels. In adjusted analysis, lower leisure time physical activity resulted in 12% higher hs-CRP levels while higher occupational physical activities showed a 6% increase in hs-CRP. When we analysed occupational and leisure time physical activity as continuous variables, only leisure time physical activity affected hs-CRP.

Conclusion This study indicates that the relationship between physical activity and hs-CRP depends on the setting of physical activity, with lower hs-CRP related to leisure time physical activity and higher hs-CRP related to occupational physical activity. The results suggest that systemic inflammation may explain the physical activity paradox.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The benefits of physical activity appear to depend on the context: whether it happens during ones occupation or leisure time. Occupational physical activity has been associated with an increased risk of coronary heart disease and mortality. Multiple hypotheses have been proposed to explain the mechanisms behind this physical activity paradox.

WHAT THIS STUDY ADDS

⇒ Lower leisure time physical activity and higher occupational physical activity are associated with increased high-sensitivity C reactive protein levels. This study supports that systemic inflammation may be one of the mechanisms behind the physical activity paradox.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study makes it prudent to further study the role of systemic inflammation in the context of the physical activity paradox.

INTRODUCTION

High occupational physical activity has been shown to be associated with as much as a 25% increase in risk for coronary heart disease and mortality compared with low occupational physical activity, even after adjustments for confounders (most commonly smoking, alcohol drinking, body mass index (BMI) and education level).[1–7] The opposite holds true for leisure time physical activity where both moderate and high leisure time physical activity are associated with a lower risk of coronary heart disease.[2 5] The literature, however, is not in agreement with regard to the relative importance of leisure time physical activity and occupational time physical activity for the development of cardiovascular disease and mortality, which may also depend on how the activity is measured.[1 2] Still, the fact that the health benefits of physical activity seem to depend on whether activity happens in connection with work or leisure is called 'the physical activity paradox'.[2 8]

The physical activity paradox may be explained by six mechanisms (with some overlap) that each can be tested and possibly refuted as the possible explanations for the physical activity paradox.[8] (1) Occupational physical activity is of too low intensity/too long duration, not granting the cardiopulmonary fitness benefits seen with leisure time physical activity; (2) occupational physical activity increases the average 24-hour heart rate which is known to be an independent risk factor for developing heart disease; (3) occupational physical activity includes more heavy and static activity than leisure time physical activity which elevates the average 24-hour blood pressure, which in turn increases the risk of cardiovascular disease; (4) occupational physical activity does not leave enough time for recovery; (5) occupational physical activity is less worker-controlled leading to scenarios that are detrimental to the worker's health, such as improper clothing with respect to the environment, dehydration, injuries and mental stress; (6) occupational physical activity increases the levels of inflammation. This

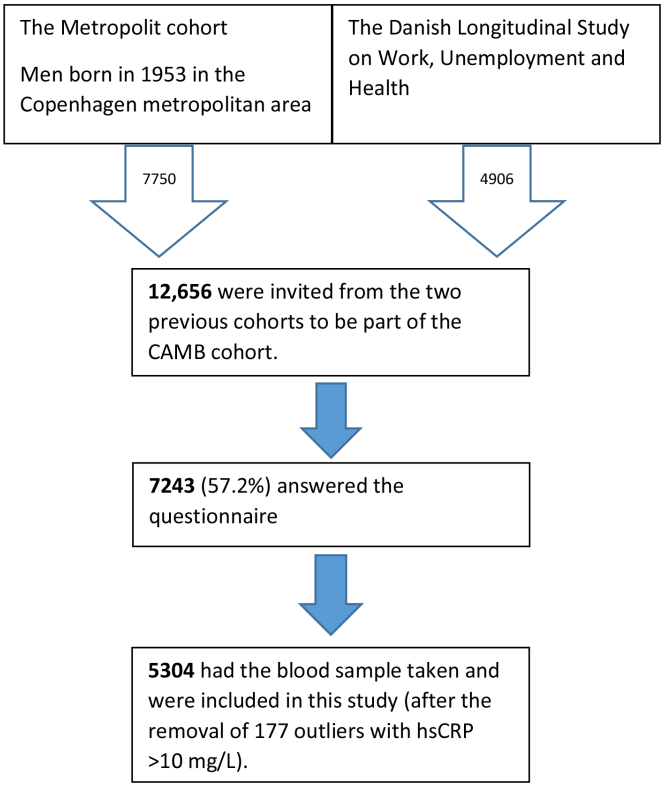

Figure 1. Flow chart of participants and cohorts used from the Copenhagen Aging and Midlife Biobank. CAMB, Copenhagen Aging and Midlife Biobank; hsCRP, high-sensitivity C reactive protein.

last suggested mechanism is the focus of the current study and is further explained below.

It seems plausible that high occupational physical activity many days in a row does not allow for sufficient recovery time to initiate the proper cellular response that would lower the resting inflammation.[9 10] Instead, occupational physical activity may lead to higher sustained levels of inflammation increasing the risk of atherosclerosis and other cardiovascular diseases.[1 8] Mechanisms 4 and 6 are somewhat overlapping, but mechanism 6 focuses on the inflammation being the driver of the paradox.

Systemic inflammation is conventionally thought of as occurring in the setting of acute disease, where it activates the immune system to fight off infection.[11] However, sustained systemic inflammation also appears to play a key part in the development of several diseases such as diabetes, atrial fibrillation and atherosclerosis.[11 12] An especially well-documented biomarker for this association is C reactive protein (CRP), which serves as a downstream marker of the inflammatory response which may, for example, lead to the formation of atherosclerotic plaques.[13] Other more upstream key biomarkers in the inflammatory response are tumour necrosis factor (TNF)-alpha, interleukin (IL)-6 and IL-1.[13]

The way the inflammatory response may be activated appears to depend on the type of event that activates the inflammatory response.[11] In the setting of acute disease, several proinflammatory cytokines are released, some of the most well-known are TNF-alpha, IL-6 and IL-1.[11] In contrast, it appears that TNF-alpha is not released as part of a non-classical inflammatory response after leisure time physical activity while IL-6 is released in both kinds of inflammatory responses. Leisure time physical activity also appears to act by activating an anti-inflammatory response both directly through IL-6, IL-10 and indirectly by having an effect on fat distribution and endothelial function.[14] Leisure time physical activity may also have a modulating effect on toll-like receptors, which normally are thought to play a role in the acute inflammatory response.[14]

If a difference in inflammatory response precipitates the physical activity paradox, it remains to be seen whether the adverse effects of high occupational physical activity can be mitigated by higher levels of leisure time physical activity.[9 10]

The aim of this study is to test the extent to which physical activity performed during work and leisure is associated with systemic inflammation.

METHODS

Participants

The current cross-sectional study uses data from the Copenhagen Aging and Midlife Biobank (CAMB) cohort.[15] CAMB was established in 2009 and was based on inviting participants from three existing Danish cohorts to answer questionnaires and perform tests. We only used data from two of the cohorts: 'The Danish Longitudinal Study on Work, Unemployment and Health' (DALWUH) and the 'the Metropolit Cohort' (MP).[15–17] The DALWUH cohort originally consisted of 7125 men and women who were randomly selected with an age between 40 and 50 years before 1 October 1999. The response rate was 69%. The MP originally comprised of 11.532 boys born in 1953 in the Copenhagen metropolitan area. Ninety-four per cent of the boys in the Copenhagen metropolitan area in the year of 1953 were included. The third cohort, The Copenhagen Perinatal Cohort, included mostly information on the prenatal, natal and postnatal period. The data collection in CAMB took place between April 2009 and March 2011. In total, 12 656 middle-aged men and women from DALWUH, and men from MP, were invited to take part in the CAMB cohort (figure 1—flow chart). Of the 12 656 invited, a total of 7243 participants answered the questionnaire (40%). Five thousand five hundred and seventy-six attended the physical examination, and 5304 had blood sample taken including the inflammatory marker: high-sensitivity CRP (hs-CRP).[15]

An attrition analysis showed that those who completed both the questionnaire and tests were more employed (90.0% vs 75.3%), and had a higher education level (40.2% had tertiary education vs 23.8% of non-responders).[15]

Exposure and outcome

Occupational physical activity was based on self-reported job history combined with data from a job exposure matrix.[18] The job exposure matrix was constructed based on expert opinion from five experts. Experts were presented a job group, which contained multiple job titles assumed to have the same exposure pattern. Experts were instructed to give their opinion on the amount of heavy lifting pr. day and the variation in lifting across the job group. Any disagreement on mean exposure was resolved by discussion. The CAMB questionnaire contained information on the length of service for the five longest held occupations held by each participant. The job history was coded according to the 1988 revision of the Danish Version of the International Standard Classification of Occupations register. We used the codes to retrieve information from a job exposure matrix.[18 19] The choice was made to combine the self-reported job history with a job matrix due to low reliability of self-reported occupational physical activity in a study by Møller *et al*.[19] Occupational physical activity was measured by heavy lifting reported as ton-years (lifting a 100 kg/day for a year).

We retrieved information on current leisure time physical activity from the CAMB questionnaire as self-reported physical activity per week (7 days). The questionnaire did not specify a time period for participants to consider so this was up for each participants' own interpretation. Participants were on average 54.4 years old and in the later part of their working career. Participants reported one of four different levels of leisure time physical activity: competitive sport regularly and several times a week; physical training or heavy house or garden work at least 4 hours per week; go for walks, biking or other kinds of light exercise at least 4 hours per week or; read, watch television or have other sedentary activities.[20–22]

We reconfigured the level of both occupational physical activity and leisure time physical activity into two categories (high and low), making it possible to define four groups of varying occupational and leisure time physical activity. The divisional line for occupational physical activity was 10 ton years, hence the low group had less than 10 ton years and the high group had more. For leisure time physical activity participants who answered they were sedentary or did light physical activity were combined, and the participants who did medium or hard leisure time physical activity were combined.

Outcome was hs-CRP measured in mg/L as a surrogate measure for systemic chronic inflammation.

Blood samples were collected without participants fasting, and stored at −80°C. Within 2 years, hs-CRP was analysed with a high sensitive assay (Tina quant, Roche Diagnostics GmbH, Mannheim, Germany) using latex-entrenched immune-turbidimetry analysis (Roche/Hitachi automatic instrument COBAS).[23 24]

CRP outliers (>10 mg/L) were excluded to account for high CRP values that could be related to prevalent disease. A total of 177 outliers were removed. The outliers had a similar age (54.0 years vs 54.5 years), similar alcohol consumption (12.0 units vs 11.9 units), higher BMI (28.3 vs 25.9), had smoked more (20.8 pack years vs 15.6 pack years), had more occupational physical activity (14.0 ton years vs 9.5 ton years) and more sedentary leisure time physical activity (21% vs 9%).

Covariates

We hypothesised that the inflammatory response measured as hs-CRP is dependent on whether physical activity happens during work or leisure. We considered the following potential confounders: age as a continuous variable, sex as a binary variable, smoking history measured as pack-years (1 pack year=20 cigarettes/day in a year) as a continuous variable, alcohol consumption measured as the number of units (1 unit=8 g of pure alcohol) of alcohol per week as a continuous variable; and BMI measured in kg/m^2 as a continuous variable. Chronic diseases were categorised in 0, 1 or ≥2 number of chronic diseases. The self-reported chronic diseases we considered of relevance were asthma, diabetes, hypertension, angina pectoris, stroke, myocardial infarction, bronchitis, emphysema, rheumatoid arthritis, osteoarthritis, cancer, anxiety, depression/other psychiatric diseases and back pain. These diseases were chosen, as the diseases or their treatment were specifically registered for the cohorts and were judged to potentially influence the CRP levels.

The categorisation of social class into six groups in table 1 was based on the article by Christensen *et al*.[25] We here briefly summarise the different classes: social class I: 4 years of university training, for example, government advisor; social class II: 3 years of theoretical training such as nurse, primary school teacher; social class III: 1.5 years of theoretical training, for example, accountant; social class IV: up to 1 year of theoretical training, for example, sales assistant; social class V: manual jobs without much training, for example, construction worker; social class VI: economically inactive such as the unemployed.

Statistical analysis

The association between each of the two types of physical activity and the average level of hs-CRP was assessed in multivariable linear regression models in which hs-CRP was log10-transformed. This transformation gives multiplicative effects between the categories of physical activity—that is, how many times the hs-CRP increases on average if the physical activity changes from low to high—if the regression coefficients are transformed back to original hs-CRP scale; it is these back-transformed coefficients that are reported in text and tables. We analysed each of the two types of physical activity separately (performing additional analyses where the other type was used as extra adjustment). The analyses were performed unadjusted and adjusted for potential confounders: age, sex, BMI, units of alcohol consumed each week, number of chronic diseases and smoking history.

We conducted all analyses using SAS software (Statistical Analysis Software 9.4, SAS Institute, Cary, North Carolina, USA).

We performed two post-hoc analyses. We performed a stratified analysis according to social class as well as performed our linear regression treating both occupational physical activity and leisure time physical activity as a continuous outcome instead of a dichotomous outcome. They are presented in online supplemental file 1.

RESULTS

A summary of the main characteristics of the population included in the study is presented in table 1. The average age of the participants was 54 years and 68.7% of participants were men. The average duration of working life was 29.3 years and the average lifting measured as ton years was 9.46 (SD 19.16, min 0 max 174.8). Most participants did light physical leisure exercise (57.8%) followed by medium/hard (32.9%) and sedentary (9.3%). The mean BMI was 25.9 kg/m^2 (SD 4.01, min 14.28, max 56.61), the mean cumulative smoking burden was 15.65 pack years (SD 22.41, min 0 max 525), the mean amount of alcohol pr. week was 11.95 units (SD 12.37, min 0 max 160).

In unadjusted analysis, hs-CRP increased with higher levels of occupational physical activity, with hs-CRP increasing with 23% when going from low occupational physical activity to high occupational physical activity (table 2). We found the opposite was true for leisure time physical activity where comparing high leisure time physical activity to low leisure time physical activity resulted in a 27% increase in hs-CRP.

In adjusted analyses, the increase in hs-CRP attributable to lower leisure-time physical activity was 12%. Higher occupational physical activity increased hs-CRP with 6%. The 6% increase with higher occupational physical activity was not statistically significant when the model also contained leisure time physical activity (p=0.0657), but the magnitude of effect was similar (column 3 of table 2).

An interaction between occupational and leisure time physical activity on hs-CRP was not statistically significant (p=0.98). The estimated effect of the combined types of physical activity also shows that this may be accurately calculated as the product of the individual effects.

We also performed the above analyses treating both occupational physical activity and leisure time physical activity

Table 1 Characteristics of the study population

	Whole population	High occupational physical activity	Low occupational physical activity	P value	High leisure time physical activity	Low leisure time physical activity	P value
Age (years), mean (SD)	54.4 (3.9)	54.8 (3.8)	54.3 (3.9)	<0.0001	54.2 (3.8)	54.5 (3.9)	=0.01
Men	3644 (68.7%)	1077 (79.13%)	2479 (64.83%)		1284 (74.74%)	2301 (65.69%)	
Women	1660 (31.3%)	284 (20.87%)	1345 (35.17%)	<0.0001	434 (25.26%)	1202 (34.31%)	<0.0001
Social Class 1	823 (16.0%)	28 (2.08%)	795 (20.97%)		328 (19.29%)	496 (14.31%)	
Social Class 2	1354 (26.35%)	160 (11.89%)	1194 (31.49%)		482 (28.35%)	871 (25.14%)	
Social Class 3	1220 (23.74%)	362 (26.89%)	858 (22.63%)		420 (24.71%)	799 (23.06%)	
Social Class 4	835 (16.25%)	338 (25.11%)	497 (13.11%)		266 (15.65%)	575 (16.59%)	
Social Class 5	434 (8.45%)	278 (20.65%)	156 (4.11%)		115 (6.76%)	318 (9.18%)	
Social Class 6	472 (9.19%)	180 (13.37%)	292 (7.70%)	<0.0001	89 (5.24%)	406 (11.72%)	<0.0001
BMI (<18.5)	47 (0.92%)	11 (0.82%)	36 (0.95%)		10 (0.59%)	38 (1.10%)	
BMI (18.5–25)	2241 (43.77%)	445 (33%)	1796 (47.51%)		856 (50.15%)	1409 (40.88%)	
BMI (25–<30)	2166 (42.30%)	644 (48.06%)	1522 (40.26%)		696 (40.77%)	1477 (42.85%)	
BMI (>30)	666 (13.01%)	240 (17.91%)	426 (11.27%)	<0.0001	145 (8.49%)	523 (15.17%)	<0.0001
0 units alcohol/week	563 (10.97%)	188 (13.99%)	375 (9.90%)		136 (7.99%)	435 (12.56%)	
1–14/21 units alcohol/week	3652 (71.16%)	874 (65.03%)	2778 (73.34%)		1316 (77.32%)	2357 (68.06%)	
14/21–35 units alcohol/week	694 (13.53%)	185 (13.76%)	509 (13.44%)		209 (12.28%)	485 (14.01%)	
>35 units alcohol/week	223 (4.35%)	97 (7.22%)	126 (3.33%)	<0.0001	41 (2.41%)	186 (5.37%)	<0.0001
Smokers*	1164 (22.48%)	434 (31.94%)	730 (19.11%)		257 (14.97%)	924 (26.41%)	
Non-smokers	4015 (77.52%)	925 (68.06%)	3090 (80.89%)	<0.0001	1460 (85.03%)	2575 (73.59%)	<0.0001
0 chronic disease	1792 (34.2%)	378 (27.77%)	1400 (36.63%)		1098 (31.36%)	684 (39.81%)	
1 chronic disease	1792 (34.2%)	448 (32.92%)	1323 (34.62%)		1165 (33.28%)	620 (36.09%)	
2+ chronic disease	1663 (31.7%)	535 (39.31%)	1099 (28.75%)	<0.0001	1238 (35.36%)	414 (24.10%)	<0.0001
Armed forces occupations	52 (1%)	–	–		–	–	
Managers	472 (9%)	–	–		–	–	
Professionals	630 (12%)	–	–		–	–	
Technicians and associate professionals	1050 (20%)	–	–		–	–	
Clerical support workers	787 (15%)	–	–		–	–	
Service and sales workers	630 (12%)	–	–		–	–	
Skilled agricultural, forestry and fishery workers	52 (1%)	–	–		–	–	
Craft and related trades workers	892 (17%)	–	–		–	–	
Plant and machine operators, and assemblers	157 (3%)	–	–		–	–	
Elementary occupations	367 (7%)	–	–		–	–	
No stated occupation	157 (3%)	–	–		–	–	

*Smokers were grouped into currently active smokers and non-active smokers, including previous smokers.
BMI, body mass index.

as continuous variables. The results were somewhat similar, although occupational physical activity seemed to influence hs-CRP less in this analyses (online supplemental sTable 1). R^2 was the same whether occupational physical activity and leisure time physical activity were considered as continuous variables or were transformed into a dichotomous variable.

We also performed the analyses stratified for social class. Results were similar for each strata (online supplemental sTable 2).

DISCUSSION

Main results

In this study, we observed that high leisure time physical activity was associated with a lower inflammatory response, that is, lower hs-CRP levels, and high occupational physical activity was associated with high inflammatory response, that is, higher hs-CRP levels; the latter association was weaker after adjusting for known confounders. When analysing both occupational physical activity and leisure time physical activity as continuous variableeisure time physical activity seemed to be more strongly associated with hs-CRP whereas occupational physical activity did not seem to influence hs-CRP.

Our results strengthen one of the six hypotheses that occupational physical activity generates a different physiological response compared with leisure physical activity.[8] The impact on hs-CRP for occupational physical activity seems to be weaker compared with leisure physical activity. Our results do not explain why there is this difference in the hs-CRP response depending on whether one is physical active in leisure time or during work time, but our study supports the theory that systemic chronic inflammation could ultimately lead to this difference in cardiovascular risk.[2]

We tested one of the six possible explanations previously hypothesised to explain the physical activity paradox.[8] The results of this study should be reviewed together with any studies examining the other hypotheses. We suggest that such an

Table 2 Results of analyses

	Hs-CRP (mg/L)		Unadjusted		Adjusted for potential confounders		Adjusted for potential confounders and the other PA	
	Median (IQR)	Mean (SD)	Factor increase in average hs-CRP (95% CI)*	P value	Factor increase in average hs-CRP (95% CI)	P value	Factor increase in average hs-CRP (95% CI)	P value
Model for occupational physical activity								
Low occupational physical activity	1.0 (0.5–2.1)	1.7 (1.8)	Ref		Ref		Ref	
High occupational physical activity	1.3 (0.7–2.6)	2.0 (2.0)	1.23 (1.16 to 1.31)	<0.0001	1.06 (1.00 to 1.12)	0.0477	1.06 (1.00 to 1.12)	0.0657
Model for leisure time physical activity								
Low leisure time physical activity	1.2 (0.6–2.4)	1.9 (1.9)	1.27 (1.21 to 1.35)	<0.0001	1.12 (1.06 to 1.18)	<0.0001	1.12 (1.06 to 1.18)	<0.0001
High leisure time physical activity	0.9 (0.5–1.8)	1.5 (1.6)	Ref		Ref		Ref	

The four top rows with results show results with leisure time physical activity and occupational physical activity as individual variables in a linear regression.
*Because we log transformed the hs-CRP, the results are measured as factor increase which can be transformed into a percentage increase. For example, going from high leisure time physical activity to low leisure time physical activity in the unadjusted analysis resulted in a factor increase of 1.27 which means a 27% increase in hs-CRP level with this change of leisure time physical activity level. Since the change is relative this means the change is dependent on the initial level of hs-CRP.
hs-CRP, high-sensitivity C reactive protein; PA, physical activity.

article should set up a framework to further guide the research concerning the paradox moving forward ultimately leading to interventions that can improve health. Future studies may look for the molecular mechanisms acting to induce an elevated CRP response. Factors to consider may be the length of the exposure and the work–rest cycle. It may, for example, be that the normal work–rest cycle of a normal working week results in too short a resting period which leads to a sustained inflammatory response.[26] It may also be worth considering whether the type of activity plays a role: occupational physical activity is more static whereas leisure time physical activity is more dynamic. Further subjects of interest are the technical aspects of measurement issues including considerations on how to make the measurement of physical exposure more objective.[27 28]

Strength and limitations

In this present study, we used a job exposure matrix instead of self-reported exposure, which should increase the validity of this assessment. Combining this with a detailed, self-reported job history used in CAMB, the accuracy of the exposure variable for occupational physical activity should have greatly improved. However, there is still a risk of misclassification of the occupational physical activity, as exposure is based on job title, but we expect this on average will have little influence. The agreement in the job exposure matrix was moderate (kappa=0.49) for heavy lifting, hence it could still be improved.[18] Heavy lifting was used as surrogate for occupational physical activity; however, one could use other measures of occupational physical activity as well. Analyses of the Job Exposure Matrix have shown that job types normally defined as jobs with high physical activity include heavy lifting.[18] However, although perhaps better than self-reported exposure, ideally an objective way of assessing exposure would have been preferred. The use of a job exposure matrix also introduced another possible bias: the job matrix assumes a homogeneous exposure according to job title and this assumption may be false.[18] Choosing ton-years as our exposure variable as the surrogate for physical activity during work, we captured both intensity and duration of physical exposure in one outcome. This, however, is ultimately also a weakness of the study, as the study may consider intensive physical exposure over a short period and less intensive physical exposure over a longer period as the same exposure.

Leisure physical activity was self-reported and sought to capture the leisure physical activity during 1 week at the time of answering which was on average in the later part of their working career. The questionnaire did not specify the time period for this activity, for example, over the last 3 months. In contrast, the occupational physical activity questions sought to capture the cumulative exposure. This is a limitation when interpreting our results. A better measure of cumulative leisure physical activity would have been preferred, but previous work has shown low validity of self-reports of cumulative exposures according to occupational physical activity. Therefore, a cohort study as mentioned above, including data on leisure time physical activity would be preferred in future studies.

We chose to categorise physical activity in groups to increase the understanding of the study. However, through the review process, we were suggested to study the effect also with continuous variables. There are similarities in the findings, but also discrepancies. This discrepancies must be considered when interpreting the study.

Our study only assessed hs-CRP as a surrogate measure of systemic chronic inflammation. Our study may therefore not provide a complete picture of the inflammatory response. Future studies may want to assess other markers of systemic inflammation. Furthermore, we did not have access to data about the use of medication such as non-steroidal anti-inflammatory drugs that could influence the level of hs-CRP and we did not adjust for acute inflammatory events. This may lead to some residual confounding despite removing outliers with hs-CRP >10 mg/L.

We considered also adjusting for social class in our analyses, but were concerned that this would eliminate part of the effect that we were to assess. Social class is often for a large part defined from one's occupation; low social class typically implies manual work, that is, an occupation with high physical activity. Including social class would adjust out a potential pathway from occupational physical activity to systemic inflammation which is part of the association of interest.

Our study included a large number of participants, which was a strength. However, our study was at risk of attrition bias

from two sources. First, attrition analysis from the CAMB-study have shown that non-responders differed from responders with respect to health and social factors. This may impact the generalisability of our results. Second, some participants who answered the questionnaire did not complete the measurement of hs-CRP.[15 29] It may be that the participants without a blood sample taken would have higher hs-CRP.

Conclusion

This study indicates that the relationship between physical activity and hs-CRP depends on the setting of physical activity, with lower hs-CRP associated with leisure time physical activity and higher hs-CRP associated with occupational physical activity. The results suggest that systemic inflammation may explain the physical activity paradox.

Acknowledgements The authors thank the staff at the Department of Public Health and the National Research Center for the Working Environment, who undertook the data collection. Further thanks to Kirsten Avlund, Nils-Erik Fiehn, Poul Holm-Pedersen, Erik Lykke Mortensen and Merete Osler, who initiated and established CAMB from 2009 to 2011 together with Helle Bruunsgaard, Åse Marie Hansen and Rikke Lund. The authors acknowledge the crucial role of the initiators and steering groups of the Metropolit Cohort, the CPC, and the Danish Longitudinal Study on Work Unemployment and Health.

Contributors OSM and AM conceived the study. JBF wrote the initial draft. AM and JBF performed the analysis with assistance from VS. VS provided overall statistical advice. All authors revised the manuscript and approved the final version. JBF serves as the guarantor.

Funding Salary for first author Joshua Buron Feinberg paid by the Danish Heart Foundation (grant number 19-R134-A8959-22123) and the University of Southern Denmark.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Request are made to the PI of CAMB. More information is available from https://camb.ku.dk/.

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Supplementary material

sTable 1 - analyses treating occupational physical activity and leisure time physical activity as continuous variables.

	Unadjusted		Adjusted	
	Factor increase (95% CI)*	P - value	Factor increase (95% CI)	P-value
Leisure time physical activity (pr. decrease in activity level)	1.23 (1.18 – 1.28)	<0.0001	1.09 (1.04 – 1.13)	<0.0001
Occupational physical activity (pr. increase in ton year)	1.004 (1.002 – 1.005)	<0.0001	1.001 (0.999 – 1.002)	0.26

*Because we log transformed the dependent variable, the results are measured as factor increase. E.g. a factor increase of 1.2 translates to a 20% increase in hsCRP level with a 1 unit decrease in leisure time physical activity.

sTable 2 – stratified analysis according to social class treating occupational physical activity and leisure time physical activity as continuous variables.

		Adjusted	
	variable	Factor increase (95% CI)*	P-value
Social class I (n = 798)	Leisure time physical activity (pr. decrease in activity level)	1.09 (1.02-1.21)	0.127
	Occupational physical activity (pr. increase in ton year)	0.99 (0.94-1.04)	0.722
Social class II (n=1298)	Leisure time physical activity (pr. decrease in activity level)	1.06 (0.98-1.16)	0.156
	Occupational physical activity (pr. increase in ton year)	0.98 (0.96-1.00)	0.05
Social class III (n=1158)	Leisure time physical activity (pr. decrease in activity level)	1.05 (0.96-1.16)	0.26
	Occupational physical activity (pr. increase in ton year)	1.00 (0.99-1.02)	0.53

Social class IV (n= 811)	Leisure time physical activity (pr. decrease in activity level)	1.16 (1.01-1.32)	0.032
	Occupational physical activity (pr. increase in ton year)	1.00 (0.99-1.02)	0.658
Social class V (n= 419)	Leisure time physical activity (pr. decrease in activity level)	1.00 (0.81-1.25)	0.95
	Occupational physical activity (pr. increase in ton year)	1.00 (0.99-1.02)	0.93
*Because we log transformed the dependent variable, the results are measured as factor increase. E.g. a factor increase of 1.2 translates to a 20% increase in hsCRP level with a 1 unit decrease in leisure time physical activity.			