UNIVERSITY OF OSLO

FACULTY OF MEDICINE

THE IMPORTANCE OF PHYSICAL CAPACITY AND THE EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING IN HEART TRANSPLANT RECIPIENTS

Marianne Yardley

The Norwegian Health Association
&
Department of Cardiology,
Oslo University Hospital Rikshospitalet
&
Faculty of Medicine, University of Oslo

2017

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8377-024-7

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

TABLE OF CONTENTS

THIS THESIS – AT A GLANCE

This thesis is based upon studies of heart transplant recipients (HTx): previously heart failure (HF) patients with the worst possible prognosis. One of the most important prognostic factors in HF patients is physical capacity (estimated by VO_{2peak}). Patients with very poor physical performance (measured as VO_{2peak} values of $\leq$ 12 mL/kg/min) and otherwise eligible, may be listed as candidates for HTx. After such surgery, life-long immunosuppression therapy is needed to prevent rejection of the new heart. The dark side of immunosuppression is the increased risk of infections, kidney failure, cancer and advanced atherosclerosis (cardiac allograft vasculopathy (CAV)), with the two latter conditions as the main causes of later mortality. In a worldwide perspective, 50% of the HTx patients survive past 10 years.

Poor aerobic capacity prior to graft deterioration is not only limited to the failing heart, but also caused by peripheral factors, such as limited function in the skeletal muscles and in the blood vessels walls. Exercise rehabilitation after HTx is of major importance in order to improve physical capacity and prognosis. It is a crucial part of the recovery period and should be a life-long commitment thereafter. Surprisingly, little documentation exists on the importance of physical performance in relation to survival *after* HTx, although it is well documented in healthy subjects, and in patients with HF and coronary artery disease. Thus, in the first paper of this thesis, we wanted to study whether physical capacity was a similar dominant factor to estimate long-term prognosis in HTx recipients, as well as providing a solid basis to discuss the importance of different rehabilitation programs after HTx. To answer this scientific question, we performed a survival analysis on a group of HTx patients having retrievable physical health information.

The exercise modality improving physical capacity the best is repeatedly shown to be high-intensity interval training (HIT). In general, this has been well documented for years and more

recent research has showed that HIT is also feasible and efficient among HTx patients. One of these studies was carried out at Oslo University Hospital during 2009-2011; a randomized controlled trial (RCT) – the Transplant EXercise (TEX) study. The study concluded with a significant improvement in physical performance after HIT compared to a control group (usual care). In the second paper of this thesis, we present long-term results from this RCT. The follow-up study evaluates the long-term effects of HIT in the same HTx study population, five years after initial inclusion; the TEX 2 study.

The new knowledge about the positive effects of HIT after HTx was exhilarating and has initiated a change in the recommendations for exercise after HTx. The initial studies included maintenance HTx recipients only, and we found that a similar exercise intervention was needed among the newly transplanted (de novo) HTx patients; to get an evidence based rehabilitation program also in this group. This ongoing, multicenter RCT (the HITTS-study), with an inclusion period from Jan 2013 to Feb 2017 at our center is an important part of my PhD work. The design manuscript from the HITTS-study is the third thesis paper. The follow-up period is three years, and the results from this multicenter study have the potential to change current rehabilitation guidelines after HTx, also for de novo HTx patients.

Given the potential superior effects of HIT versus moderate intensity continuous training, we have investigated acute mechanisms that may trigger and possibly explain the difference between these two exercise modalities. In this explorative cross-over study we included 14 HTx patients and 5 healthy controls. We measured selected mediators of inflammation and blood vessel formation in blood samples drawn from the participants before, during and after the two exercise sessions. It constitutes the fourth and last paper of this thesis.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my three supervisors; Kari Nytrøen, Lars Gullestad and Bjørn Bendz.

Kari has been my main supervisor, my faithful supporter and cornerstone. Thank you for prompt and thorough response together with excellent guidance during my PhD-work. Your positive spirit, knowledge and encouragement have motivated me to always stretch for the next goal. I have enjoyed the time we have spent together, and I'm really glad for the coincidence that your home is close to my family's cabin, making it easy to meet up regularly in the future.

Lars Gullestad has shown me great enthusiasm throughout the project, always very interested in my research and initiates insightful discussion around the findings. You always have several new ideas to explore, which is very inspirational.

Bjørn Bendz has given me valuable opinions whenever I sought his advice. He has an expert eye for clarity and presentation, which has guided me to present my research with more precision and professionalism.

My main collaborating partner Katrine Rolid has been supportive, helpful and motivating. She has shown great interest in all my work, and for the studies where we have joint responsibility. She has also provided admirable care of both participants and patients.

The HTx nurses; Ingelin Grov, Anne Relboe, Marit Kuntz, the study nurses; Elisabeth Bjørkelund, Karianne Moss Hansen and the patient coordinator Hanne Gundhus, I am grateful for all your collaboration and support, and for the central role you all have taken part in, with the organization and control of patient flow.

The HTx doctors Einar Gude and Arne Andreassen, and the doctors in the angiographic laboratory; Ole Geir Solberg and Ketil Lunde. Thank you for the encouragement you show for my work, and for the pleasant collaboration in the past and ongoing clinical studies.

I would also like to thank my co-authors, especially Tor Ueland, Satish Arora, Annika Michelson and Pål Aukrust; you all have expert knowledge in your fields and I'm grateful for the contributions and novel insights.

Overall, being a Ph.D. candidate has been a positive experience, much thanks to my fellow working colleagues Andreas Auensen and Amjad Hussein. I was warmly welcomed into your office, and I enjoyed your company immediately. We began to take our lunches in fresh air as soon as the sun appeared, (mind you, we didn't have a choice since the neighbor offices kicked us out), anyhow this turned out to be a pleasant and peaceful tradition. Thank you for all the lunch breaks and hours joined in front of our screens. I will never forget you guys, and neither will Vegard, who had to listen to the same stories about you every week.

I want to thank my parents, for your never-ending kindness and generosity, and for all the interest you have showed during my research, presentations and publications. You have both given me loads of encouraging words up through, and I really appreciate it.

Vegard is my partner and also my best friend. I am grateful for his continuous support and love. Even with the past years being repeatedly about physical capacity and heart transplantation, you shoveled this nicely aside and asked me to be your wife. I am forever thankful to have you in my life, I know you never doubted my ability to complete this Ph.d. thesis, but I can't believe it, I did it!

ABBREVIATIONS

ANOVA analyses of variance

AT Anaerobic threshold

BIA bioelectrical impedance analysis

BDI Beck´s depression inventory

CAD coronary artery disease

CAV cardiac allograft vasculopathy

CO cardiac output

CPET Cardiopulmonary exercise test

CRI chronotropic response index

CRP C-reactive protein

DKK Dickkopf WNT signaling pathway inhibitor

ECG electrocardiogram

EEM external elastic membrane

EqO_2 Equivalent of O_2

GDF growth derived factor

HIT high-intensity interval training

HF heart failure

HR heart rate

HRQoL health related quality of life

HTx heart transplant, heart transplantation

IVUS intra vascular ultra sound

LV-EF left ventricle ejection fraction

MCS mental component sum-score

MI Myocardial infarction

MICT moderate intensity continuous training

OCT Optical coherence technology

RCT randomized controlled trial

RER respiratory exchange ratio

RPE rated perceived exertion

sCD40L soluble CD40 ligand

SD Standard deviation

SF-36 Short-form 36

SPARC secreted protein acidic and rich in cysteine

sTNFr soluble tumor necrosis factor receptor

PCS physical component sum-score

PF Physical function

PDGF Platelet-derived growth factor

VEGF vascular endothelial growth factor

VCAM vascular cell adhesion molecule

VO_{2peak} peak oxygen uptake

vWF Von Willebrand factor

LIST OF PUBLICATIONS

Paper I

Yardley M, Havik OE, Grov I, Relbo A, Gullestad L, Nytrøen K. Peak oxygen uptake and self-reported physical health are strong predictors of long-term survival after heart transplantation. Clin Transplant. 2016; 30:161-9. DOI: 10.1111/ctr.12672. Epub 2015 Dec 22

Paper II

Yardley M, Gullestad L, Bendz B, Bjørkelund E, Rolid K, Arora S, Nytrøen K. Long-term effects of high-intensity interval training in heart transplant recipients; a 5-year follow-up study of a randomized controlled trial. Clin Transplant. Accepted 2016 nov 10. Epub 2016 nov 12.

Paper III

Nytrøen K, Yardley M, Rolid K, Bjorkelund E, Karason K, Wigh JP, et al. Design and rationale of the HITTS randomized controlled trial: Effect of High-intensity Interval Training in de novo Heart Transplant Recipients in Scandinavia. Am Heart J. 2016; 172:96-105. DOI: 10.1016/j.ahj.2015.10.011. Epub 2015 Oct 21.

Paper IV

Yardley M, Ueland T, Aukrust P, Michelsen A, Bjørkelund E , Gullestad L, Nytrøen K. The immediate response in markers of inflammation and angiogenesis during exercise in heart transplant recipients. Transpl Int. Submitted Dec 2016.

1 INTRODUCTION

1.1 Heart transplantation

For patients with heart failure (HF) the 5-year mortality rates are 62% for women and 75% for men (1), with even higher rates in patients with end-stage HF (2). Heart transplantation (HTx) is an established treatment to improve survival in selected patients with end-stage HF. From 1983 and to date, nearly 900 HTx have been performed at Oslo University Hospital in Norway.

After HTx, the patients require lifelong immunosuppression to prevent rejection of the graft. These drugs have a potential to give adverse complications such as diabetes, gout, hypertension and osteoporosis, and serious side effects, such as higher risk of infections, renal failure and cancer. These side effects are the leading causes of death in the long-term, together with an advanced HTx-specific process of atherosclerosis, called coronary allograft vasculopathy (CAV) (3).

According to the 2012 ISHLT registry, the median survival for all HTx patients is 10 years, but if surviving the first year, the survival rates are higher and show a 63% survival past 10 years (3). Increased knowledge about CAV and immunosuppression has resulted in further improved survival. However, the HTx recipients still have a shorter estimated length of survival than the general population.

1.2 Physical capacity as a prognostic variable

The gold standard measurement of physical capacity is the peak oxygen uptake (VO_{2peak}), and is defined as "the maximum ability of the cardiovascular system to deliver oxygen to exercising muscles and of the exercising muscle to extract oxygen from the blood" (4). VO_{2peak} is shown to be a strong predictor of survival in general populations (5, 6), among patients with coronary artery disease (CAD) (7) and in patients with severe HF (8). Limited

exercise capacity is the cardinal symptom in HF. The HF patients with VO_{2peak} < 12 mL/kg/min are considered to have the worst prognosis, despite optimal medical therapy, and can be appropriate candidates listed for HTx (9). These patients are most likely men > 50 years of age (3). When evaluating younger patients and women, it is found reasonable to include age and gender adjusted levels of exercise capacity, and values ≤ 50% percent of predicted VO_{2peak} differentiate better in these populations (9).

However, studies addressing the relation between VO_{2peak} and survival *after* HTx are currently lacking, although a number of other predictors have been identified through register-data analyses. These predictors are: non-ischemic cardiomyopathy as the primary diagnosis, younger recipient age, younger donor-graft age and shorter allograft ischemic time; all associated with a better long-term prognosis (3, 10, 11). The mortality beyond one-year after HTx has remained relatively constant, and Stehlik et al. (3) predict that interventions resulting in a reduction of mortal events in the long-term are needed to achieve further improvements in survival after HTx.

1.3 Physical capacity after HTx

The dynamics of physical capacity after HTx is illustrated in Figure 1. Physical capacity does increase significantly after HTx as a result of therapy, as shown by measurements above 12 mL/kg/min in published studies (figure 1). Osada et al. (12) and figure 1 show that the highest rate of increase is found within the first years. In nearly 70% of the studies (figure 1), regardless of time after HTx, VO_{2peak} is below 20 mL/kg/min, also classified as Weber function class B-C (13). Patients within function class B and C are shown to be similar to CAD and HF patients referred to rehabilitation programs (14). VO_{2peak} is frequently used as the primary outcome measure in exercise intervention studies after HTx (15).

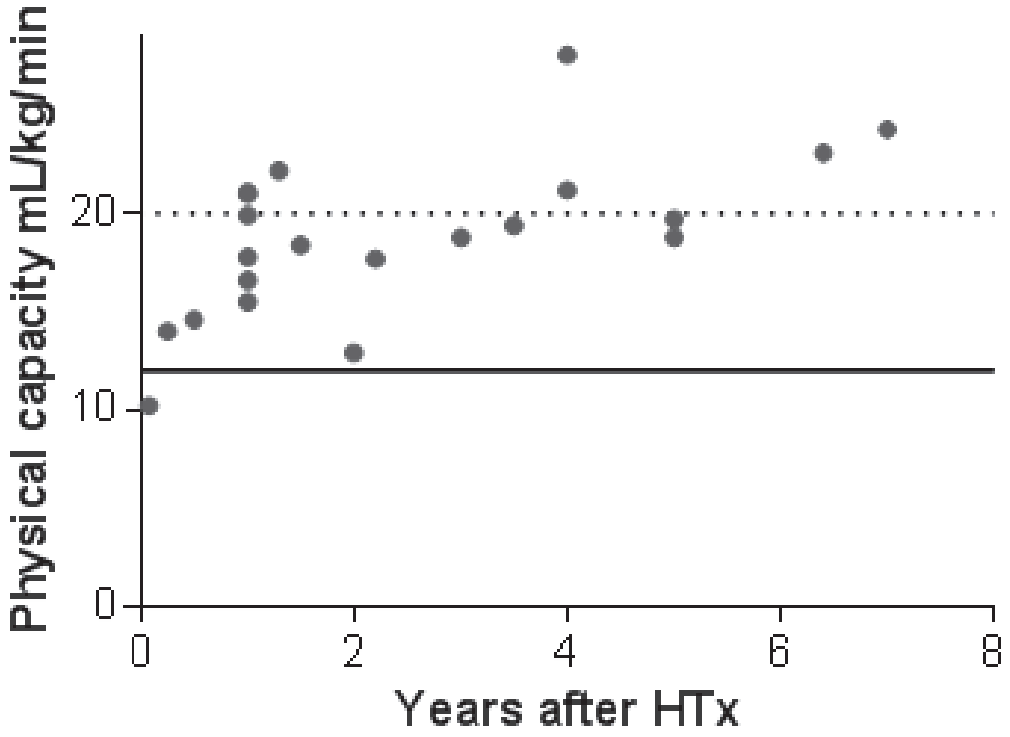

Figure 1: Physical capacity after HTx from published studies, illustrated by years after surgery. Black line at 12 mL/kg/min, show the threshold to be candidates for HTx, dottet line at 20 mL/min/kg, show the start of Weber function class A, representing good physical condition. The measurements are carried out from exercise tests from; Bernandi et al.(16), Carter et al.(17), Dall et al.(18), Ewert et al.(19), Givertz et al.(20), Gullestad et al.(21), Habedank et al.(22), Haykowski et al.(23), Hermann et al.(24), Hognestad et al.(25), Karpolat et al.(26), Kavanagh et al.(27), Kemp et al.(28), Kobashigawa et al.(29), Nytrøen et al.(30), Osada et al.(12), Renlund et al.(31), Schwaiblmair et al.(32), Squires et al.(33), Tegtbur et al.(34), Wu et al.(35).

1.4 Exercise after HTx - the past and the future

To increase physical capacity and prevent long-term complications such as hypertension and diabetes, aerobic exercise after HTx has a positive effect, but their capacity still remains subnormal in most studies (36). High-intensity interval training (HIT) is proven to be a more efficient exercise modality than moderate-intensity continuous training (MICT) in order to increase VO_{2peak}, shown in patients with HF (37), CAD (38), metabolic disease (39), as well as in healthy individuals (40). The new knowledge has had a great impact on how general cardiac rehabilitation programs are organized today. These two different exercise modalities are illustrated in figures 2A and 2B (paper 3). HIT corresponds to an intensity of 16-18 on Borg's rated perceived exertion (RPE) 6-20 scale (41, 42) , and MICT to Borg 12-15.

***HIT: H**igh-intensity **I**nterval **T**raining is an exercise strategy with alternating short periods of intense endurance exercise with less-intense recovery periods. A usual HIT session may include 4 x 4min periods with high intensity (85-95% of maximal capacity), with active recovery periods of 3min between each interval (with 60-70% of maximal capacity).*

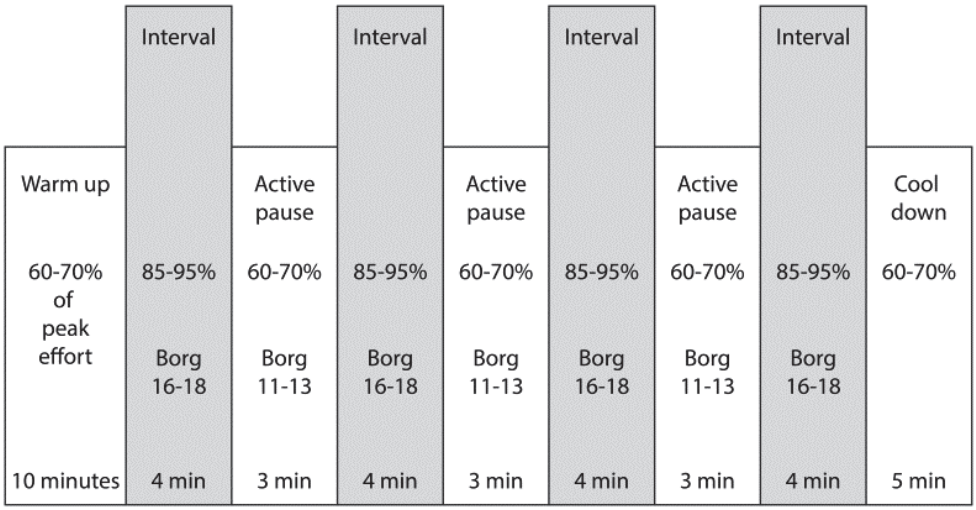

Figure 2A: Illustration of a session with High-intensity Interval Training, HIT.

***MICT: M**oderate-intensity **C**ontinuous **T**raining is an exercise strategy with moderate intensity (60-70% of maximal capacity) of endurance exercise in periods for usually 25- 30 minutes, with no recovery periods.*

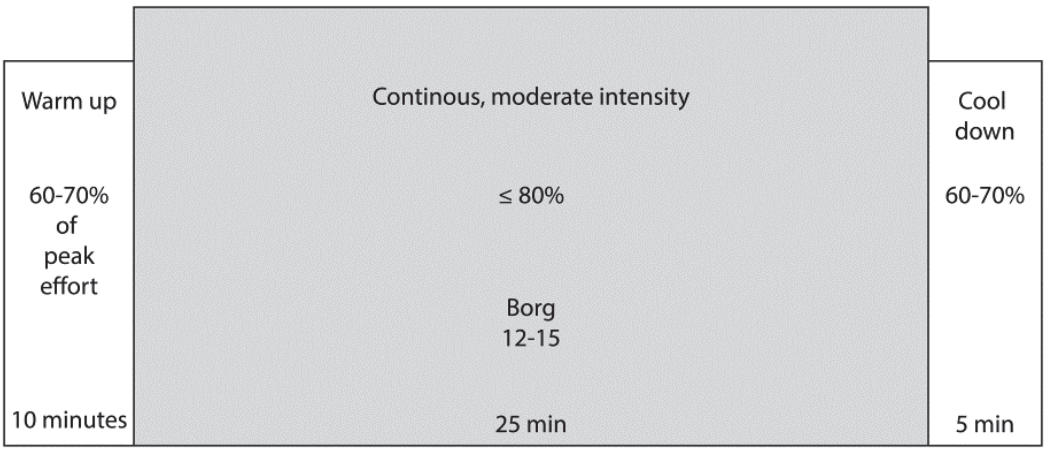

Figure 2B: Illustration of a session with Moderate Intensity Continuous Training, MICT.

Rehabilitation after HTx has traditionally had, and still has, a more conservative approach, with MICT as traditionally recommended, mainly due to uncertainty and concerns regarding denervation with consequently chronotropic incompetence and parasympathetic impairment (43). The heart rate (HR) will typically be higher at rest, with a slower increase during exercise, a lower maximum HR at peak exercise, and a slower HR decrease after exercise cessation (figure 3) (unpublished data).

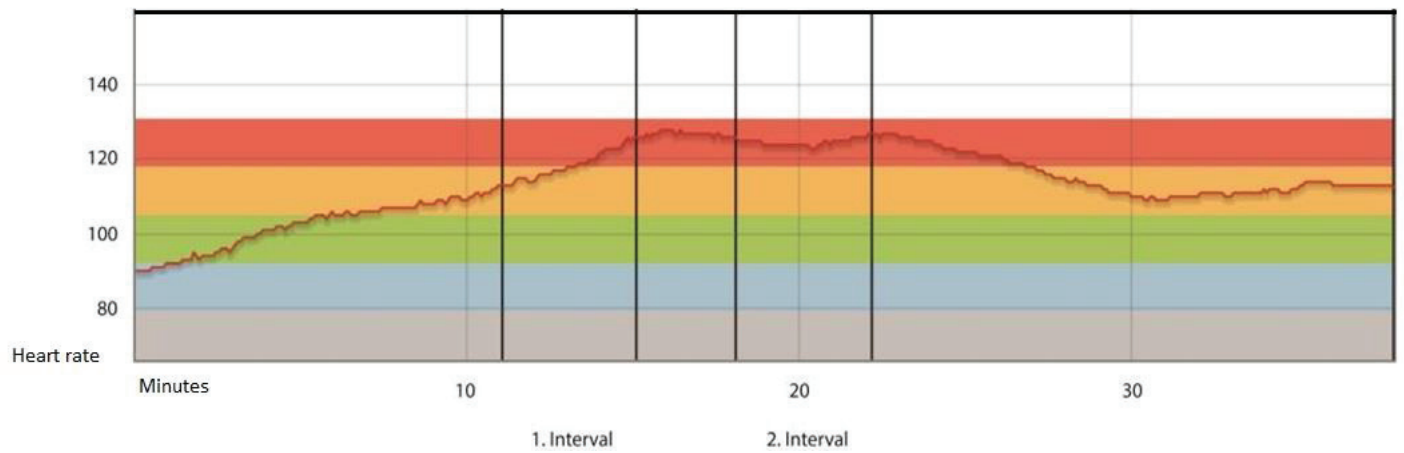

Figure 3: Patient from our hospital, 3 months post-HTx: HR curve during warm-up, two high-intensity intervals divided by one recovery period and cool-down. The curve shows a typical pattern of impaired HR responses in the early stage after HTx

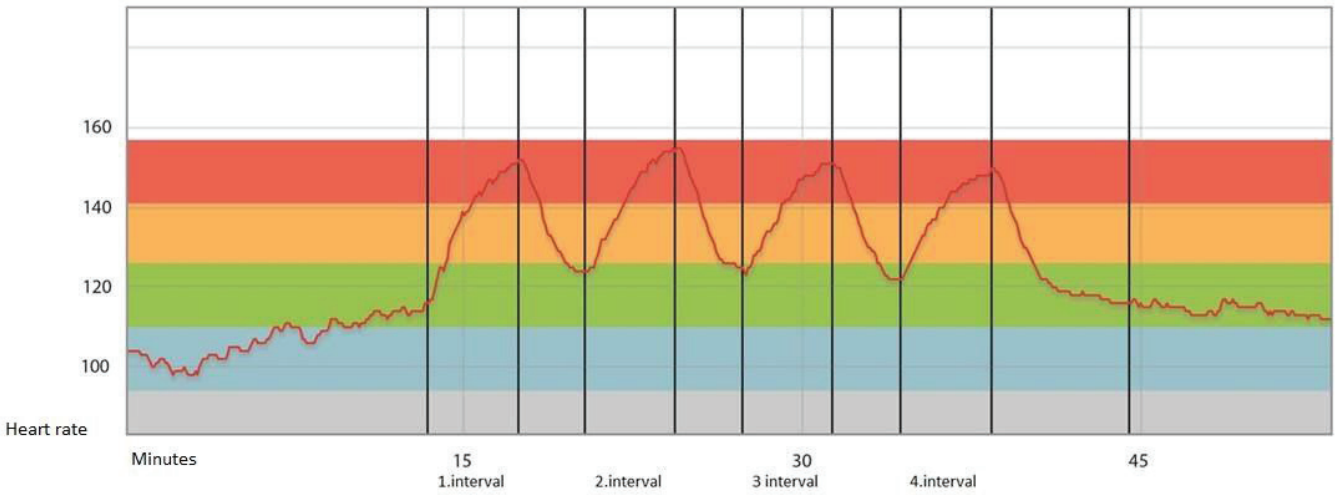

Figure 4: The same patient 12 months post-HTx: Heart rate curve during warm-up, four high-intensity intervals divided by 3 recovery periods and cool-down. The curve shows a largely normalized HR, with immediate HR adaptions to exercise intensity.

The chronotropic incompetence is most prominent the first months after HTx and tends to be largely normalized in the majority of patients after 12 months (44) as illustrated in figure 4 (unpublished data). Recent randomized controlled trials (RCT), have investigated the effect of HIT in the maintenance HTx recipients and have to a large extend overruled the traditional approach with MICT (18, 24, 30). These studies showed that HIT increased VO_{2peak} significantly compared to the control groups, and that a HIT intervention was safe and well tolerated. These results are recently mentioned in the updated recommendations from 2013 (45). As well as the increase in exercise capacity, the HIT group also improved their chronotropic response index (CRI) (18, 30), endothelial function (24) and had less CAV development after a long-term exercise intervention (46).

A similar HIT intervention in de-novo HTx patients is currently ongoing in Scandinavia, with our hospital as the core center (paper 3). One of the goals in this study is to update, optimize and implement new exercise prescriptions also in this group.

Knowledge about the long-term effects of HIT is still scarce, but an ongoing Cochrane review will be published shortly, on the effectiveness and safety of exercise-based rehabilitation and the effect on mortality and hospital admissions, especially in the HTx population (47). Two meta-analyses on exercise-based rehabilitation (MICT protocols) in HF populations are published (48, 49), showing a possible effect on survival and health related quality of life (HRQoL), but most importantly; a significantly decrease in re-hospitalization in the long-term.

1.5 Mechanisms behind the "HIT-effect"

The effects of a HIT intervention are mostly studied in healthy individuals, CAD and HF patients. The main mechanisms behind the increase in exercise capacity are shown to be through central factors, by a prominent improvement in cardiac output (CO) (37, 50).

However, the “HIT-effect” in the maintenance HTx recipients show different results, with peripheral factors as the main mechanisms; by improvement in skeletal muscle exercise capacity (30), endothelial function and vasodilatation (24), rather than an increased CO (51). The underlying triggers behind these peripheral effects are poorly understood, and the potential of inflammatory signaling pathways are not explored in detail. Markers of inflammation have been studied as an additional effect of exercise through long-term steady state levels (before and after exercise intervention), showing mostly neutral results (24, 46, 52). Investigation of immediate exercise effects in inflammatory signaling pathways during HIT could contribute to explain the “HIT-effect” in the HTx recipients further.

1.6 Cardiac allograft vasculopathy (CAV)

CAV is characterized by intimal thickening and a more diffuse narrowing of the coronary arteries´ lumen than conventional atherosclerosis (53). The mechanisms of development are described as both immunological and non-immunological, possibly modifiable factors (54). It can be detected by coronary angiography, but intravascular ultrasound (IVUS) is now more frequently used, and is a superior diagnostic tool to detect early changes in intimal thickening (early CAV) (55). The early CAV has been validated as a reliable surrogate marker for subsequent mortality, nonfatal major adverse cardiac events, and development of angiographic CAV following HTx (56, 57). CAV progression is a highly prioritized field of research among HTx clinicians and researchers, to further improve HTx prognosis. As a result, Kobashigawa at al. introduced statin therapy that showed to have beneficial effects on one-year survival and the incidence of CAV (58). Statins became routine therapy after HTx at our center from 1997. More recently, a Scandinavian multicenter RCT (The Schedule-study) has shown that early everolimus initiation with calcineurin inhibitor withdrawal reduce the progression of CAV in de-novo HTx recipients (59, 60). The effect of non-medical prevention

strategies has also been studied by IVUS, such as HIT interventions, and have shown less progression of arthrosclerosis in mice (61) and in patients with myocardial infarction (MI) (62), and we have also demonstrated less development of CAV the first year after initiation of HIT in HTx recipients (46).

1.7 Health related quality of life (HRQoL)

The quality of life after HTx has been reported to increase significantly, with high levels of satisfaction in overall HRQoL; also stable over a 5-year period (measured from 5 to 10 years after HTx) (63). Although, when HTx patients are compared with the general population the HRQoL remain beneath the normal values (64). To improve HRQoL, and especially physical health, exercise interventions have shown to be successful, this is in contrast to the more neutral results reported in control groups (35, 65). Research on HRQoL after HTx regarding the effect of HIT (compared to MICT) is very limited, and the existing studies show mixed results; some studies show similar effects on HRQoL (52), while others show a beneficial effect with a significant increase after HIT (30, 66).

1.8 Mental health; anxiety and depression

In the post-transplant stage the prevalence of significant depression and anxiety remains substantially above the general populations, and it tends to increase over time (67, 68). As it is found that depressed HTx recipients have a higher risk of mortality, screening for depressive symptoms during follow-up is recommended (69-71). As an approach to increase mental health, the effect of exercise and HIT has been studied. The results showed that exercise decrease the burden of depression and anxiety, with HIT showing significant positive effects compared with usual care (66). Additionally, the results align with the correlation between higher physical capacity and less depression rates (71, 72).

2 MAIN AIMS OF THE THESIS

The accumulating evidence that HIT is a safe and efficient modality of exercise also in HTx recipients has the past few years grown into a field of research which attracts worldwide focus and interest. The results published from our own center and others in this area, have generated an important base of evidence, from which new research questions frequently arise. My thesis contributes to this evidence base by addressing the following main questions:

I Do direct and indirect measures of physical capacity after HTx predict long-term survival?

II To what extend do patients continue with intensive training after an extensive HIT intervention, and do they sustain their improved physical capacity in the long-term?

III To describe the design and rationale of the randomized controlled trial; "Effect of High-intensity Interval Training in de-novo Heart Transplant Recipients in Scandinavia (the HITTS study)".

IV Does exercise in general trigger a release of vascular-, angiogenetic- and blood platelets- inflammatory markers in HTx recipients, and if so, is the response different between HIT and MICT sessions?

3 SUBJECTS & METHODS

3.1 HTx patients and routines in Norway

In Norway, HTx is only performed at Oslo University Hospital Rikshospitalet, and our HTx unit follows the patients closely throughout life. All patients are scheduled for annual follow-ups to assess their cardiovascular health, which includes clinical examination, blood samples, ultrasound and coronary left-sided (every second year) and right-sided catheterization (the first three years). The immunosuppression concentrations are determined locally every third month, communicated to the HTx unit, to evaluate if the dosages are adequate. All patients receive maintenance immunosuppressive therapy with Prednisolone (maintenance dose of 0.1mg/kg), Cyclosporine (as monotherapy or combined with Everolimus) or Tacrolimus, and Azathioprine or Mycophenolate mofetil. Especially HTx patients with renal failure are set to a low-dosage cyclosporine regime combined with Everolimus as it preserves kidney function.

3.2 Paper 1: Survival analysis

3.2.1 Patient population

This retrospective study investigated survival in two HTx populations; i) a cardiopulmonary exercise test cohort (CPET-cohort), who completed a VO_{2peak} test during their annual follow-up, and ii) a cohort who completed a HRQoL questionnaire, Short Form-36 version 1 (SF-36 v1) during their annual follow-up (the SF-36 cohort).

3.2.1.1 The CPET cohort

178 HTx patients with available data from a CPET performed in a previous study by Gullestad et al. (21) were included in this cohort. The test was completed during their annual follow-up between 1990 and 2003, and approximately 60% of the total HTx population

scheduled for annual appointment underwent the voluntary CPET (as it was not hospital routine at that time).

The CPET was performed with a stepwise protocol on an electrically braked bicycle ergometer with a starting load of 20-50 watt, increasing by 20-50 watt every second minute, individually determined to aim for an exercise session of 8-10 minutes. The patients' electrocardiogram (ECG) and HR were monitored continuously. Gas exchange was measured using the EOS/SPRINT system (E. Jaeger, GmbH CoKG; Wurzburg, Germany). When the patients were unable to keep the pedaling rate steady at 60 rounds per minute it was defined as peak exercise and the test was terminated. VO_{2peak} was defined as the highest VO_2 level (mean of 30 sec) achieved during peak exercise. Age predicted values for VO_{2peak} were calculated based on reference values presented by Åstrand et al (73).

3.2.1.2 The SF-36 cohort

133 HTx patients with available HRQoL data together with survival information were included in this cohort. The SF-36v1 questionnaire was completed once during their annual follow-up between 1998 and 2000 as a part of the previously conducted study by Havik et al. (69). 220 eligible participants came for their annual follow-up in this period, resulting in 60% participation rate. 82 patients in the SF-36 cohort were identical to patients in the CPET cohort.

Together with HRQoL, the absence or gradation of depression was also evaluated using the Beck´s depression inventory (BDI). In this questionnaire a sum-score under 10 indicates no depression (74). HRQoL was measured with the generic questionnaire SF-36v1, comprising eight subscales assessing self-reported health. In all scales, the raw sum score is linearly transformed to a 0-100 scale, with "0" indicating the least favorable health state and "100" the best possible health state (75). The subscales consists of; physical functioning (PF), role

limitations because of physical health problems, bodily pain, general health perceptions, vitality, social functioning, role limitations because of emotional problems, and mental health.

3.2.2 Background information and clinical data

Background information of the study participants and clinical variables were collected from the hospital's HTx-database. Blood samples, weight, blood pressure (BP), and smoking status were obtained at the actual time of the CPET (CPET cohort) or the completion of the SF-36v1 questionnaire (SF-36 cohort). The evaluation of CAV (assessed by Costanzo's classification (76)) and the left ventricular ejection fraction (LV-EF) were obtained from left-sided cardiac catheterization, whereas cardiac index was determined during the right-sided cardiac catheterization.

3.2.3 Statistical analysis

Data were analyzed using the SPSS version 22 (IBM Corporation, Armonk, NY, USA) in all papers if not stated otherwise. Comparisons between survivors and non-survivors were made using unpaired T-test or Mann-Whitney U-test for continuous data, as appropriate. For categorical data, Pearson´s Chi-square or Fischer´s Exact test were used. Survival rates were calculated using Kaplan-Meier survival analysis. Observation time starts at time of CPET and at time of SF-36 completion. Hard event was defined as death only. Based on the median VO_{2peak} value (CPET cohort) and median PF-score (SF-36 cohort), each cohort was divided into two groups, and compared using the Log-rank test.

Univariate cox proportional hazard regression analysis was performed to evaluate each predictor's effect on survival. The different exercise variables within the CPET-cohort were highly correlated, as were the different SF-36 variables within the SF-36 cohort. Thus, only VO_{2peak} and PF-score were selected for further multivariate analysis. To evaluate the adjusted effect of VO_{2peak} and self-reported physical health on long-term survival, we built an

explanatory model. Only variables assumed to be related both to the main predictor and to the survival time were defined as confounders. To maintain power in the multiple regression model, only confounders with significant P-values from univariate regression, together with VO_{2peak} (CPET-cohort) or PF-score (SF-36 cohort) were included. Finally, the predictors that did not add significant explanation to the model were removed step by step, and interactions and confounders (with near significant value (≤ 0.2) after univariate analyses) were checked one by one.

3.3 Paper 2: Long-term effects of HIT intervention in maintenance HTx patients: The TEX "Transplant Exercise" 2 study.

3.3.1 Patient population

Our study group carried out a RCT (2009-2011) to evaluate the effect and safety of HIT after HTx (the TEX 1 study). The primary outcome measure was VO_{2peak}, tested at baseline and after 12 months of exercise (30). The present work is a 5-year follow-up of this population (the TEX 2 study), evaluating long-term effects of the HIT intervention. All survivors in both groups were invited to participate. Inclusion criteria were; optimal medical therapy, stable clinical condition, ability to perform a maximal exercise test on the treadmill and provision of written consent. Exclusion criteria were; unstable clinical condition, infection, physical disability preventing exercise testing on the treadmill or other diseases/ injuries that were contraindicated with exercise at maximal capacity.

48 patients completed follow-up testing at the 1-year follow-up (30). From the time of completion of the TEX 1 study to the invitation to participate in the TEX 2 follow-up study, three patients died, leaving 45 eligible patients for inclusion. Forty-one of the 45 patients met the inclusion criteria, and they were all willing to participate (85% participation).

Reasons for exclusion were: Infection (n=1), ongoing treatment of cancer (n=1) and physical disabilities (n=2). The included patients underwent tests of physical performance and IVUS examination, in addition to the regular annual follow-up examinations.

3.3.2 The HIT intervention

The 12 month HIT intervention in TEX 1 was performed in close cooperation with each patient and the local physical therapist. The intervention was divided into three 8-week periods of exercise with three sessions every week. Each HIT session consisted of a 10 minutes warm-up period, followed by four intervals of four minutes (4 x 4) length. The intensity during the intervals should be 85-95% of their maximum HR, corresponding to Borg RPE scale of 16-18 (figures 2A and 2B). Depending upon each patient's fitness level, speed and/or inclination were individually adjusted as necessary during the intervention, always aiming towards the ability to perform full 4 x 4 sessions at the desired HR intensity zones. Each interval was followed by an active break (walking corresponding to Borg RPE scale of 11-13, lasting 75% of the length of the previous interval). All exercise sessions were carried out at a local training center, always guided by a physical therapist, with detailed instructions from our hospital.

The control group did not undergo a specific exercise intervention, but they were advised to continue their activities "as usual". The lack of a second intervention arm, an exercise group with supervised MICT protocol, was mainly due to limited resources.

During the following four years, the time after the 12 month intervention and to the 5-year follow-up, both groups followed normal routine for HTx patients, with no specific exercise program, but with regular advice regarding secondary prevention during their annual follow-up visits.

3.3.3 Activity monitoring

To ensure adequate intensity during the 12 month intervention, the patients in the HIT group were provided with a HR monitor (Polar FT1, Electro Oy, Finland) and taught in how to use it, along with information about the Borg scale. Each session was logged by the physical therapists according to frequency, duration and intensity; the HR was recorded towards the end of each HIT-interval and at the end of each active recovery period.

When patients came to their 5-year follow-up visit, they filled out a validated self-reported physical activity questionnaire (77), and their current daily physical activity was also measured. The physical activity was measured by activity frequency and intensity, monitored with SenseWear armband monitors (BodyMedia Inc, Pittsburgh, USA) worn for one week (approximately 23h/day). To define the intensity levels, the metabolic equivalent of task (MET)-scale was used (78). This scale is a physiological measure expressing the energy cost of activities in three categories: MET 1,5-2,9 = Light activity intensity, MET 3-5.9 = moderate activity intensity and MET >6 = high activity intensity.

3.3.4 Cardiopulmonary exercise test (CPET)

The CPET was performed with a modified test protocol from the European Society of Cardiology (79). Test termination criteria were respiratory exchange ratio (RER) > 1.05 and/or Borg RPE scale > 18. After termination of the test, the treadmill was stopped and the patient rested in sitting position for a recovery period of 2-4 minutes. Gas exchange was measured by "breath by breath", using Jaeger CPET systems. Blood pressure was measured automatically (Tango; Sun Tech Medical Instruments, NC, USA) before exercise, every second minute during exercise, and after exercise. ECG and HR were monitored continuously. HR_{peak} was set at peak exercise and the percentage of achieved age predicted HR_{peak} was estimated by HR_{peak} divided by (220–age). A HR_{peak} value <85% was considered as

pathologically low (80). $HR_{reserve}$ was measured as the difference between HR_{peak} and HR_{rest} (recorded during echocardiography). CRI was calculated as $HR_{reserve}$ divided by (the age predicted HR_{peak} - HR_{rest}). A CRI ratio of <0.80 was considered as abnormal (80). VO_{2peak} was calculated as the mean of the three highest 10 second measurements of VO_2 during peak exercise before volitional fatigue was reached. Age and gender predicted values for VO_{2peak} were calculated based on reference values presented by the American College of Sports Medicine 2014 guidelines (81). First anaerobic threshold (AT) was set using the ventilatory equivalent for oxygen (EqO_2), and the ventilatory efficiency was measured by the VE/VCO_2 slope, calculated from the start of exercise to the AT.

Figure 5: Study participant from the TEX 2 study, testing physical capacity on the treadmill. (Private photo. The image is reproduced with permission form the person in the picture.)

3.3.5 Muscle strength

The maximal muscle strength and muscular exercise capacity (endurance) were tested at each study visit. The test was performed in a sitting position on a Cybex 600 (Lumex, Ronkonkoma, NY, USA), testing quadriceps and hamstrings muscle strength, one leg at a time (figure 6). Five repetitions at an angular velocity of 60º/s were performed to estimate the maximal mean peak strength, measured in Newton meters. Muscular exercise capacity was measured through total work, in Joule, during 30 isokinetic contractions at 240º/s.

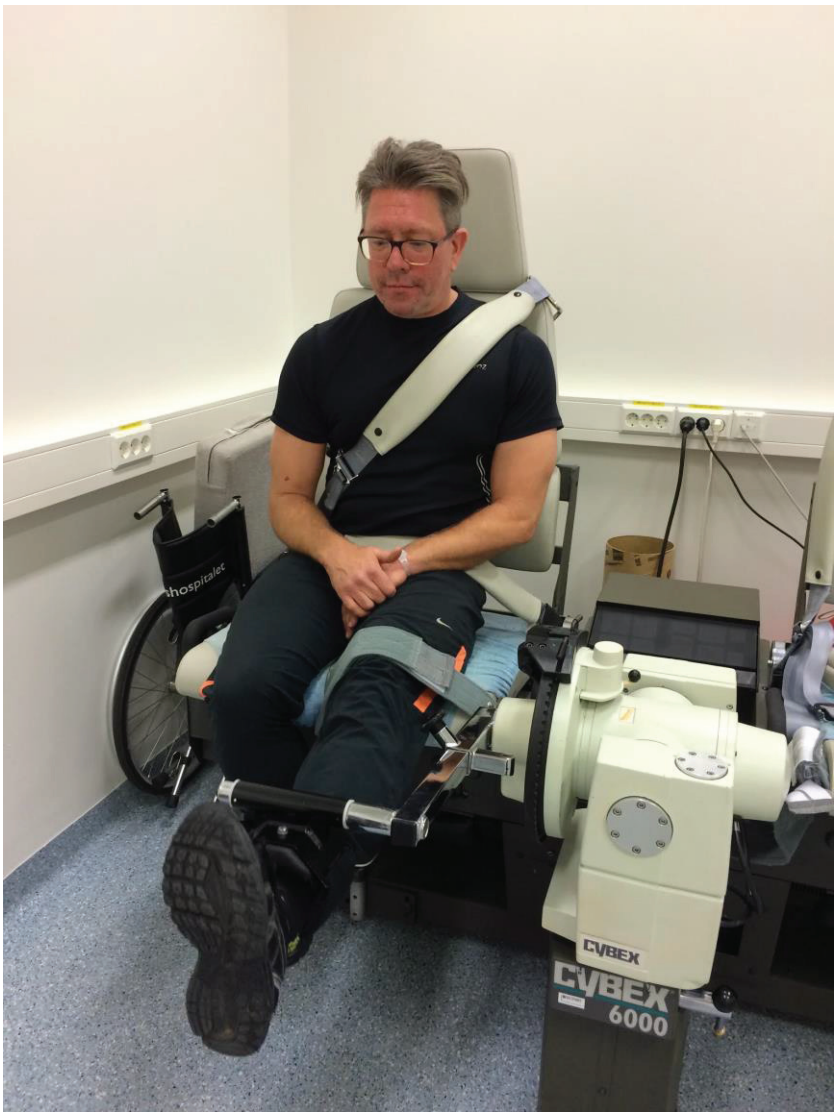

Figure 6: Patient included in the TEX 2 study performing the quadriceps and hamstring exercise test with the Cybex 600 machine. (Private photo. The image is reproduced with permission form the person in the picture.)

3.3.6 Bioelectrical impedance analysis

Body composition was measured, using bioelectrical impedance analysis (BIA) with Tanita InnerscanV model: BC-545N (Tanita, Arlington, Heights, IL, USA). The BIA is considered as a reliable method, used in several research fields and validated up against the gold standard dual-emission X-ray absorptiometry (82). Three BIA measurements per patient conducted in mean variables of amount; body fat, muscle mass, total body water, visceral fat, bone mass and basal metabolic rate measured at each study visit.

3.3.7 Coronary angiography and Intravascular ultrasound (IVUS)

To classify the CAV severity we graded the results from coronary angiography using the ISHLT CAV grading report (83). The coronary angiography was followed by a standard IVUS examination according to guidelines (84), performed with a dedicated catheter (Eagle Eye Platinum; Volcano Corp., Rancho Cordova, CA, USA). All IVUS imaging was performed in the same artery at baseline and follow-up for each patient, preferably the left anterior descending coronary artery. The recordings were performed with the same motorized pullback device (pullback speed 0.5 mm/s) with an image acquisition rate of 30 frames/sec and analyzed by a study-blinded, experienced IVUS technician using QIVUS software (version 2.1.11.0; Medis Medical Imaging, Leiden, The Netherlands). After manual contour detection of the lumen and external elastic membrane (EEM), lumen, vessel and intimal cross-sectional area were calculated for all patients and utilized to determine total atheroma volume and percent atheroma volume. Maximal intimal thickness was defined as the greatest distance from the intimal leading edge to the EEM. IVUS-virtual histology images was also analyzed using the QIVUS software to construct tissue maps with four major components; fibrous, fibrous fatty, dense calcified and necrotic core, all expressed as a percentage of the total intima area.

3.3.8 Health related quality of life (HRQoL)

The SF-36 version 2 questionnaire was used to measure HRQoL during the TEX study. This latest version of the generic questionnaire provides both a physical component sum-score (PCS) and a mental component sum-score (MCS). The questionnaire comprises the same eight subscales as the SF-36 v1, described in paragraph 3.2.1.2 (paper 1). For the SF-36 v2, the subscales and the two sum-scores, PCS and MCS, can be reported on a standardized scale based on the 1998 United States general population. The standardized score has a mean of 50 and a standard deviation (SD) of 10.

To evaluate the anxiety and depression rates the hospital anxiety and depression scale (HADS) (85) and BDI (74) were used. BDI is described in paragraph 3.2.1.2 (paper 1). HADS is a questionnaire expected to identify both anxiety and depression symptoms, using a cut-off score > 7.

3.3.9 Miscellaneous

Echocardiography was performed by experienced technicians as part of the annual routine follow-up, and results were described by blinded cardiologists. BP was examined manually in sitting position. Blood samples were drawn, and stored in a biobank, at each study visit.

3.3.10 Statistical methods

The baseline data was analyzed with unpaired T-test for between-group comparisons, and for categorical data, Pearson's Chi-square or Fischer's Exact tests were used as appropriate. 5-year data were compared with baseline data using the mean difference (mean [95%CI]) within groups, and for between group comparison the unpaired T-test or Mann-Whitney U-test was used. We also incorporated the 1-year follow-up data and compared groups with analyses of variance (ANOVA). P-values < 0.05 (two-sided) were considered statistically significant.

3.4 Paper 3: The HITTS trial; High-intensity Interval Training in de novo heart Transplant recipients in Scandinavia.

3.4.1 Patient population

This work is a currently ongoing Scandinavian RCT that investigates the effects of a HIT protocol in de novo HTx recipients, compared with a MICT protocol, which is the current guideline. The inclusion of participants started at Oslo University Hospital Rikshospitalet, in January 2013, with a planned enrollment of 120 patients from three University hospitals (Oslo N=60, Copenhagen N=30 and Gothenburg N=30), with the coordinating center in Oslo. The criteria for enrollment are:

- Clinically stable HTx recipient, approximately 8-12 weeks after HTx
- Above 18 years of age
- Received (with no major changes) immunosuppressive therapy as per local protocol
- Both willing and capable of giving written informed consent for study participation
- Positively evaluated to complete the study intervention

In the cases of rejection, the potential inclusion is postponed; for a grade one rejection at least one clean biopsy is mandatory, after a grade two rejection, at least two clean biopsies are mandatory, and for a grade 3 rejection, the patients are excluded from participation.

3.4.2 Study protocol

Figure 7 shows the timeline of the HITTS study. The study procedures are measured at baseline (10 weeks ± 2 weeks after HTx), after nine months of exercise in the HIT and MICT group (1 year after HTx), and again at the 3-year annual follow-up after HTx.

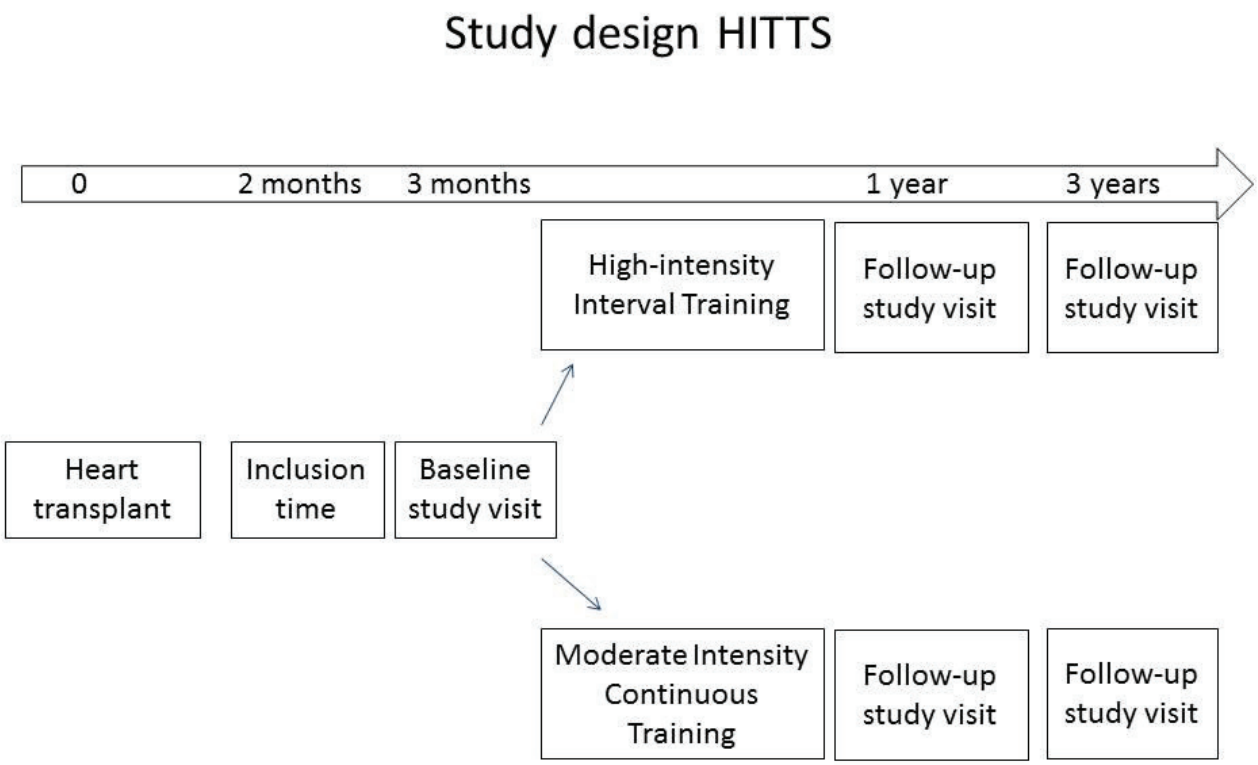

Figure 7: HITTS study protocol; de-novo heart transplanted recipients.

3.4.3 The HIT intervention

The HIT intervention is divided into three exercise periods as illustrated in figure 8 with three exercise sessions weekly through the intervention period. In the first exercise period the HTx patients are introduced to one HIT session per week with the other two sessions consisting of strengthening exercises and one combined aerobic and strength session. The training is always supervised by a local physical therapist, who is in close collaboration with the physical

therapist at our hospital. The next three months HIT is increased to two sessions per week (exercise period 2), and in the third and last exercise period, prior to the 1-year follow-up, it is increased to three weekly HIT sessions. The intensity during the intervals should, as in the TEX study, be at 85-95% of maximum exercise capacity.

0-3 months	3-6(7) months	7 months Included if closed fascilities during holidays	8-9 months
HIT allways supervised by a physical therapist 3 x weekly: **1 HIT-session** 1 strengthening exercise session 1 combined; aerobic & strenght exercise	HIT allways supervised by a physical therapist 3 x weekly: **2 HIT-sessions** 1 strength exercise	Non-supervised training period 2-3 x weekly: no HIT sessions 2-3 exercise sessions	HIT allways supervised by a physical therapist 3 x weekly: **3 HIT sessions**

Figure 8: A schematic presentation of the HIT intervention divided in three (four) exercise periods.

Depending on each patient's fitness level, interval duration and amount are individually adjusted and increased during the intervention. The aim being to perform full 4 x 4 sessions during the last exercise period. Resistance training is also included in the exercise protocol for the first six months. This is a necessity in de novo patients as the majority suffers from various levels of deconditioning and atrophy from their pre-transplant stage.

The HTx patients in the MICT group perform the same amount of physical activity as in the HIT group, but at a continuous, moderate intensity (approximately 60-80% of peak effort) similar to our traditional and current rehabilitation guidelines. As for the HIT group, the patients in the MICT group are also referred to a local physical therapist for nine months.

All the physical therapists, either committed to the HIT or the MICT protocol, return detailed logs to our hospital with information about type of exercise, duration, and the intensity of the preformed exercise sessions (including information of Borg RPE scale and measures of HR) as described in the TEX study (30).

3.4.4 Evaluation of CAV

In addition to IVUS and angiography, Optical coherence technology (OCT) and coronary physiology are measured at each study visit. OCT is a novel high-resolution intravascular imaging technique allowing characterization of the inner layer of the coronary arteries (figure 9) (unpublished data). Thus, in combination with IVUS, it will enable visualization of the blood vessel wall microstructure at an unprecedented level of detail to give a precise evaluation of the progression of CAV during the intervention (86). Recent research in CAD patients show that visual interpretation alone can be an unreliable method for evaluating the clinical physiological significance of coronary stenosis, and assessment of coronary microcirculation is suggested to improve differentiation (87). Accordingly, both visual and physiological examinations are included in our current study. Measures of coronary physiology include fractional flow reserve and coronary flow reserve, and measurement of the index of microcirculatory resistance.

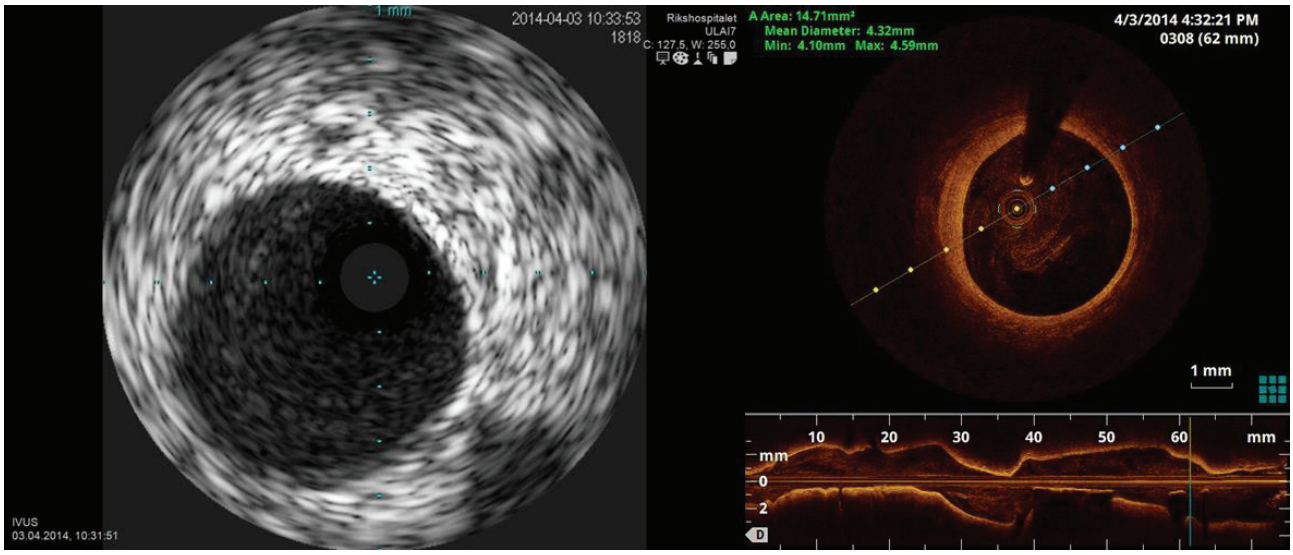

Figure 9: IVUS image (left) with corresponding coronary artery segment showed with OCT (right) (not equally rotated). The images are from recordings performed in our catheterization laboratory. The IVUS image show a diffuse intimal thickening (early CAV) that is much more visible with higher resolution by OCT.

3.4.5 Endothelial function

To evaluate the effects of exercise on vascular function, we measure endothelial function at baseline and at the 1-year follow-up. In this study we use peripheral arterial tonometry (EndoPAT-2000; Itamar Medical, Carcera, Israel), which is a novel method to evaluate endothelial function.

3.4.6 Miscellaneous

Echocardiography, blood samples, measurement of body composition, muscle capacity, HRQoL, depression and anxiety are performed as described in paragraph 3.3.8 (the TEX study) at each study visit. BP is measured over 24 hours and is reported as mean BP during the day, night and overall time.

3.4.7 Statistical methods

The primary end point is the mean change in VO_{2peak} from baseline (8-12 weeks after HTx) to follow-up (1 year after HTx). Based on previous studies (in maintenance HTx patients) we assumed that the HIT intervention can increase VO_{2peak} with approximately 5-7 mL/kg/min

(24, 30). Power calculation was performed with an alfa of 5% and power of 80%. Based on an expected mean difference between groups after intervention of 3 mL/kg/min and a SD of 5 mL/kg/min we needed 44 patients in each group. To compensate for drop-outs the planned enrollment was set to 120 patients.

3.5 Paper 4: The immediate effect of HIT on markers of inflammation and angiogenesis: The BIT study; Blood samples during High-intensity Interval Training.

3.5.1 Patient population

All eligible HTx patients in the Oslo region were invited to participate in the BIT study (n=26). The Oslo region was chosen in order to reduce travel expenses due to limited financial resources. Fifteen of the 26 HTx patients met the inclusion criteria and were willing to participate. Inclusion criteria were; above 18 years of age, 1-10 years since HTx, stable medical condition, no recent changes in immunosuppression treatment and acceptable travel distances for day trips. In addition to the 15 HTx patients, five participants with no history of heart disease were enrolled as a reference group. Just prior to study commencement, one HTx patient was injured, preventing participation. The final population completing the study was therefore 14 HTx patients and 5 controls.

3.5.2 Study design and procedures

The BIT study was designed as a cross-over study to explore the immediate responses in markers of inflammation and angiogenesis of a HIT session, compared with a MICT session.

Each study participant had three study visits with one week wash-out between each visit. The first study visit consisted of baseline blood samples and a maximal CPET. The next two visits consisted of one HIT session and one MICT session, in a randomized order. The study was

carried out in our test lab at the Department of Cardiology, Oslo University Hospital Rikshospitalet.

The CPET results defined each patient's intensity zones. The moderate intensity zone was defined as actual HR between 60-70% of their VO_{2peak}, and the high intensity zone was defined as actual HR between 85-95% of their VO_{2peak}.

3.5.3 Exercise sessions with blood samples

The sessions were monitored with HR sensors to ensure that each patient exercised in his/her individually defined intensity zone. Additionally, an intravenous line into the forearm was attached. For both HIT and MICT exercise sessions, the patients had four blood samples taken; halfway into the exercise session, at the end of exercise, and after one and two hours of recovery. Each blood sample started with one collection tube destroyed, followed by two EDTA glasses (chilled on ice) and one collection tube with serum. The blood sample taken during exercise was drawn from the patient while standing on the treadmill, causing a 30-60 seconds interruption of the exercise session.

Blood was centrifuged immediately (plasma), or allowed to clot (serum) for one to two hours before centrifugation. Plasma was centrifuged at 2100g for 20 minutes to obtained platelet-poor plasma and serum at 1900g for 15 minutes after clotting, both at 4˚C. Plasma and serum were frozen in aliquots at -80˚C until analysis and thawed less than three times.

The MICT session consisted of a 30 minutes exercise at 60-70% of peak effort (figure 10) (paper 4). Blood samples were drawn midway through the session and at the end of session.

The HIT session consisted of four blocks of four minutes intervals with high intensity, corresponding to 85-95% of peak effort, intermittent by a three minutes active pause between the intervals (figure 11) (paper 4). The first blood sample was taken immediately after the

second interval and the second blood sample was taken immediately after the last interval. For both sessions, the third and fourth blood samples were drawn after one and two hours of recovery. To evaluate whether the sessions were completed according to protocol, the main investigator (M. Yardley) went through each patient's continuous HR-graph using the online Polar software (polarpersonaltraining.com, Polar Electro Oy 2015, Finland).

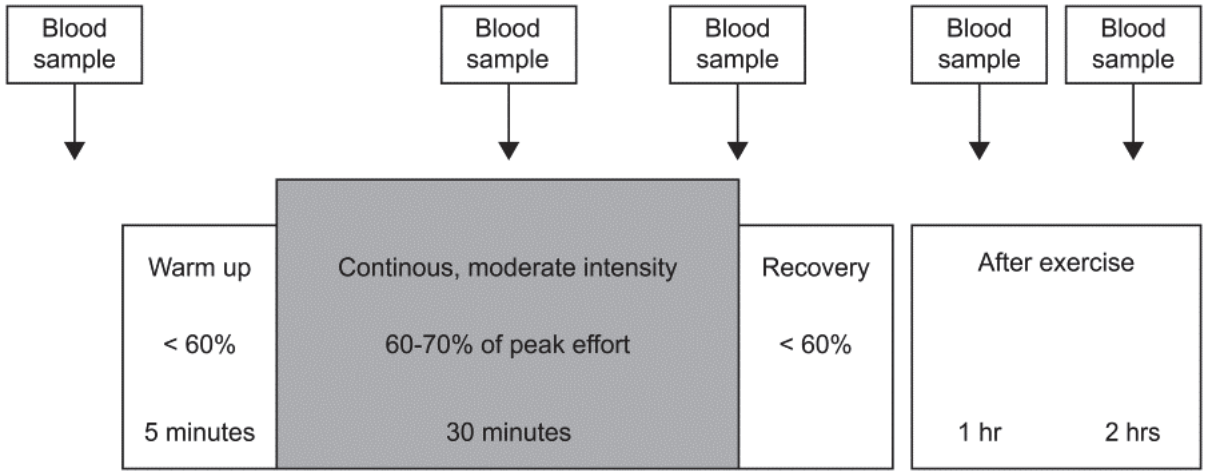

Figure 10: Illustration of the MICT-session, with timing of all blood samples.

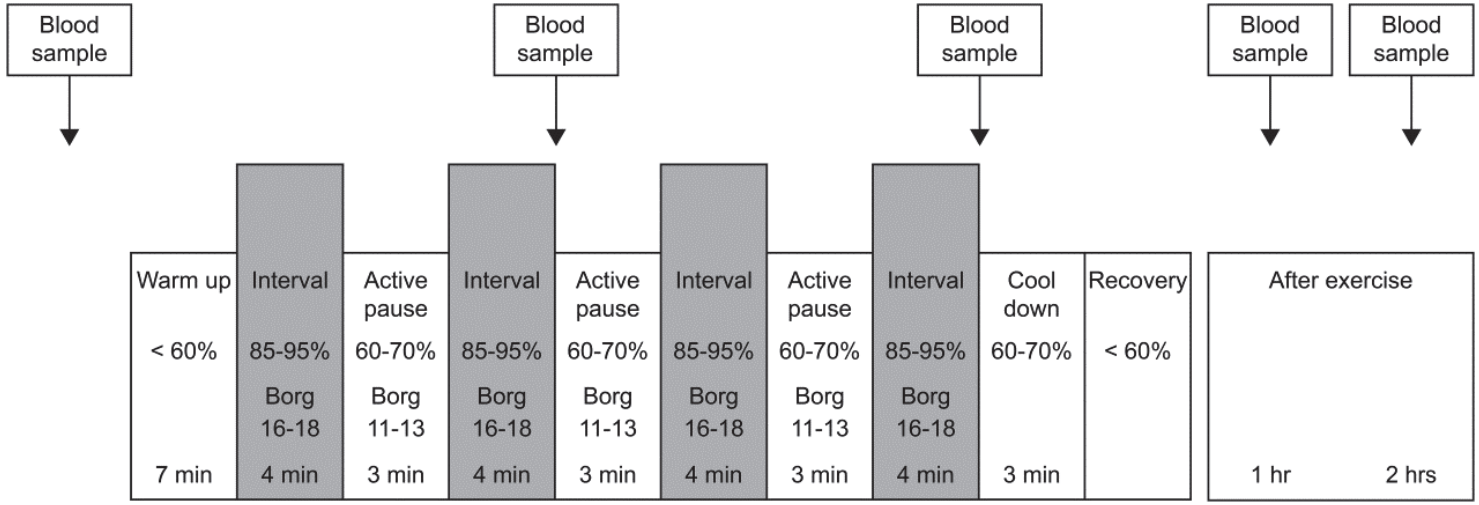

Figure 11: Illustration of the HIT-session, with timing of all blood samples.

3.5.4 Inflammatory biomarkers

To explore the inflammatory response to exercise, selected biomarkers were analyzed. For general inflammation, C-reactive protein (CRP) and soluble tumor necrosis factor receptor (sTNFr)-1, was measured. For vascular inflammation, we analyzed vascular cell adhesion molecule (VCAM)-1 and Von Willebrand factor (vWF). To explore blood platelet activation, we analyzed Platelet-derived growth factor (PDGF), soluble CD40 ligand (sCD40L) and Dickkopf WNT signaling pathway inhibitor (DKK-1). To explore angiogenetic activation, we analyzed vascular endothelial growth factor (VEGF)-1, endostatin, Angiopoetin-2 and its receptor. The levels of growth derived factor (GDF)-15, secreted protein acidic and rich in cysteine (SPARC) and ST2, member of the Interleukin 1 receptor family, where measured as selected cardio- and myokines.

The levels of VCAM-1, CRP, GDF-15 and vWF were measured in plasma, and all remaining biomarkers were analyzed in serum. Plasma or serum levels of inflammatory biomarkers were determined in duplicate by EIA (DuoSets, R&D systems, Minneapolis, MN, USA) in 384 microtiter plates. The intra-assay coefficient of variation was < 10%.

3.5.5 Statistical analyses

Descriptive statistics was presented as mean±SD or median (1.quartile, 3. quartile) for continuous variables, and in percentages for categorical variables. To compare the demographic variables and inflammatory markers between the control group and the HTx recipients at baseline, T-test or Mann-Whitney U test was used as appropriate. Categorical variables were compared with Pearson's Chi-square or Fisher's Exact test. The level of significance was set to 0.05. To evaluate the inflammatory biomarker's response during and after a HIT and MICT session, a two-way repeated ANOVA was used. The variables with

skew distribution were log transformed in advance. For evaluation of the interaction effect, the P-value of significance was set to 0.10.

4 ETHICAL CONSIDERATIONS

4.1 The RCT studies

The RCTs (papers 2-4) were all approved by the South-East Regional Committee for Medical and Health Research Ethics in Norway, and by the Department of Privacy and Data security at our hospital. The same studies were registered in the Clinical Trials Registration with ID numbers: NCT02213770 (paper 2), NCT01796379 (paper 3), and NCT02602834 (paper 4). All participants were given oral and written information about the studies in advance, and were included after they had provided their written consent. Pictures with study participants are only used after oral and written confirmation.

4.2 Ethical considerations - The HITTS trial

The blunted HR adaptation after HTx has been the main reason to refrain from HIT in the traditional rehabilitation programs as earlier described (page 17). More recent research has investigated HIT in maintenance HTx patients and showed it as feasible, safe and efficient, and thus, HIT is recently mentioned in the new recommendations, but applies so far only to maintenance HTx recipients (45). The rehabilitation in de novo HTx patients still has a traditional approach, seemingly based on precautions rather than scientific evidence. Because of this void of research and based on the positive effects of HIT found in the maintenance HTx patients, we considered it to be more unethical *not* to explore both exercise modalities (HIT and MICT) also in de novo HTx patients, even with the present CI. Optimal safety is established in the HIT group to preserve the ethical concerns due to the chronotropic response: All patients are tested to maximal capacity in our hospital before the first HIT session. The patients wear HR monitors, and are only allowed to perform HIT together with a dedicated local physical therapist in a one-to-one session. All local physiotherapists get thorough guidance from our specialized study-physiotherapist before the first HIT session and

stay in regular contact with our hospital during the entire intervention, and last, the specialized physiotherapist has a low threshold in involving transplant nurses and/or cardiologists if any medical concerns should appear during the exercise intervention.

5 SUMMARY OF RESULTS

5.1 Paper 1 - Survival analysis

By September 2014, 42 of the 178 patients in the CPET cohort were still alive and without a new transplant. The median survival time after the transplantation, for the entire group, was 12 years, while the HTx patients with exercise capacity above the median value of 19.6 mL/kg/min lived for a median of 16 years after HTx. In the adjusted cox-regression multivariate model, the survivors were characterized by a higher physical capacity, younger age and less development of CAV.

In the SF-36 cohort, 46 of the 133 HTx patients were still alive by September 2014. Median survival for the entire group was 10 years, while the patients with PF-score above median value of 90 lived for a median of 14 years after HTx. In the adjusted cox-regression multivariate model the survivors were characterized by higher levels of self-reported physical health, younger age, non-smokers and less development of CAV.

Other well-known predictors of HTx survival such as diagnosis prior to HTx, ischemic time, donor age, measurements of cardiac output and kidney function by creatinine did not add any additional explanation to the models.

5.2 Paper 2 - The TEX 2 trial

In the TEX 2 study, the results at the 5-year follow-up showed that the exercise group and the control group had a similar daily activity level with moderate intensity. The mean± SD daily activity (with METS $\geq$ 3.0) was measured to be 1.5 ± 1.0 hours daily for the total population. The initial significant increase in physical capacity after the HIT intervention at the 1-year follow-up was lost during the next 4 years in the exercise group. Within the control group, the physical performance showed a significant decrease from baseline to the 5-year follow-up, but

no significant differences between the two groups were found at the 5-year follow-up (figure 12). However, the development of anxiety symptoms was significantly different between the exercise and the control group at the 5-year follow-up; the exercise group showed a decreased in symptoms of anxiety, while the control group increased in anxiety symptom score.

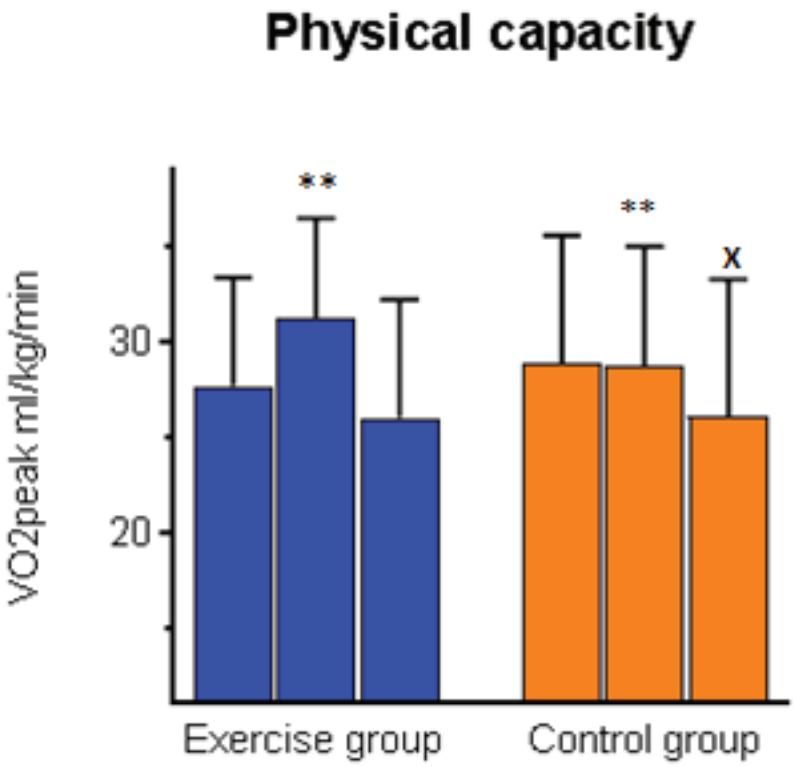

Figure 12: Measurements of VO_{2peak} at baseline, 1-year and 5-year follow-up.
***show significant changes between groups*
^{X} show significant changes from baseline to 5-year follow-up within group

We also studied the relationship between measured VO_{2peak} and self-reported physical health (PF-score) and found a positive correlation, as illustrated in figure 13. This result strengthens the prognostic effect we found both of VO_{2peak} and PF-score in the survival analysis.

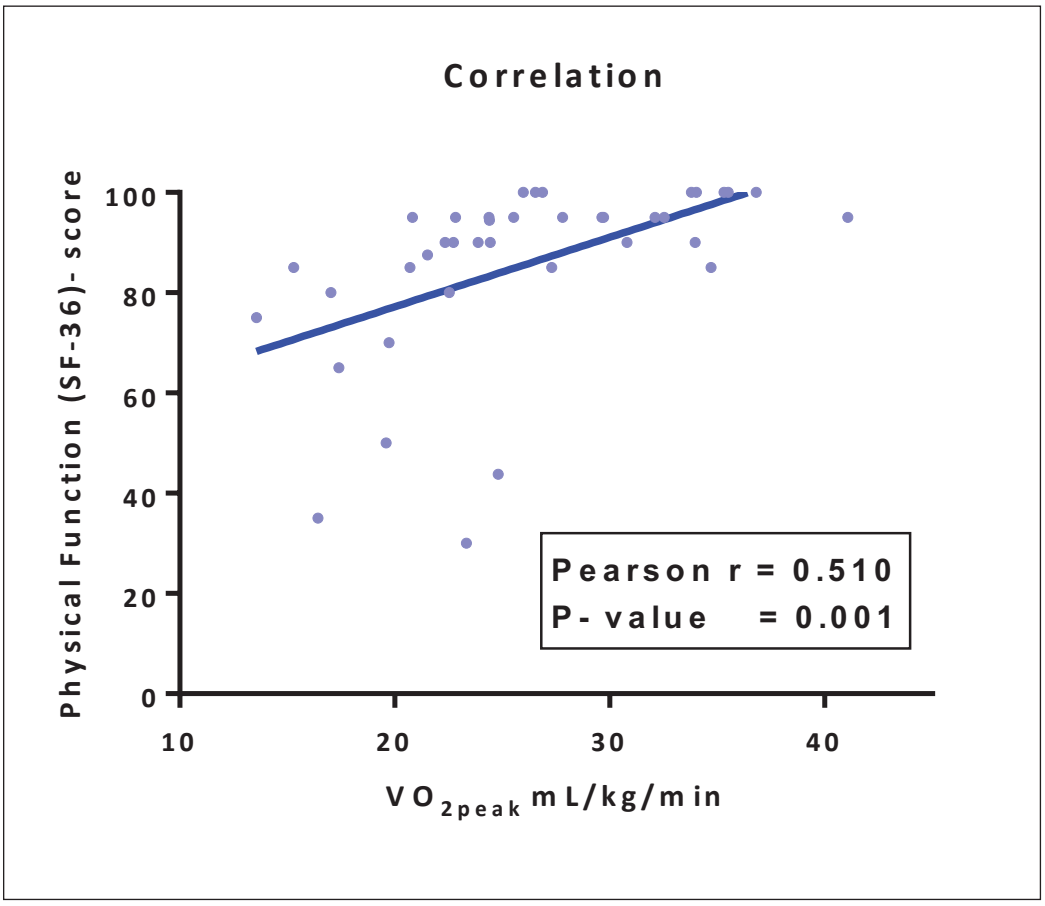

Figure 13: Correlation between Physical capacity (VO_{2peak}) and Physical Function- score in the TEX 2 population.

5.3 Paper 3 - The HITTS study

In this ongoing study, we aim to test whether systematic HIT is feasible also in newly transplanted HTx recipients, and whether the effect of HIT on VO_{2peak} is superior to the effect of MICT. So far, 117 adult HTx have been performed during the HITTS inclusion period (from our site). Thirteen patients died during the time from the transplant and to inclusion time at 6-8 weeks post-transplant, leaving 104 available HTx patients, of which 75 met the inclusion criteria and were positive to participate, resulting in a participation rate of 72%.

Fifty-seven patients have completed the one year intervention period, and 18 have also completed the long-term 3-year follow-up. So far, the HIT-intervention is well tolerated, patients are motivated and no safety issues have been observed. We believe this project will provide new knowledge regarding both short and long-term beneficial effects of exercise, and possible underlying mechanisms. There is a void of research in this field, especially regarding RCT's investigating long-term effects in de novo HTx recipients.

5.4 Paper 4 - The BIT study

The HTx patients (n=14) in the BIT study were 86% men with mean±SD age of 53±13 years and time since HTx of 3±2 years. The mean±SD VO_{2peak} value was 31.0±6.8 mL/kg/min, corresponding to 85% of expected age and gender matched values. The participants were found to be exercising in the correct intensity zones; at 65±4% of their peak capacity during the MICT session, and at 89±3% of their peak capacity during the interval in the HIT session. The main results from the enzyme immunoassays analyses were that exercise, regardless of intensity, induced a significant immediate response in several vascular, angiogenetic and particularly in platelet derived inflammatory mediators in HTx recipients shown in figure 14. With HIT we found an increased response in vWF, VEGF-1 and Angiopoetin-2, and a decreased response in GDF-15, significantly different from MICT (figure 14).

	MICT	HIT
General inflammation		
CRP	→	→
sTNFr-1	↑	↑
Vascular inflammation		
vWFd	↓	↑
VCAM	→	→
Blood platelets		
PDGF	↑	↑
sCD40L	↑	↑
DKK-1	↑	↑
Angiogenesis		
VEGF-1	↑	↑↑
Ang2	↑	↑↑
Tie -2	→	→
Endostatin	→	→
Cardiokine/ myokine		
GDF-15	↑	↓ *
ST2	→	→
SPARC	↑	↑

Figure 14: A simplified illustration of the ANOVA results: the response in markers of inflammation and angiogenesis during HIT and MICT sessions. Horizontal arrows illustrate non-significant response during exercise. Arrows pointing up illustrate a significant increase with exercise, regardless of intensity, and two arrows illustrate a significant increase by increasing intensity (HIT). An arrow pointing down, illustrates a significant decrease in response during exercise.

**The decrease is found in the recovery period (0-2h) after the exercise-session.*

6 DISCUSSION

6.1 The relationship between physical health and long-term survival

Earlier studies addressing survival, have estimated how physical performance *pre* HTx is related to survival *after* HTx. Physical capacity (measured by VO_{2peak}) in this population is well known to predict survival and supports the clinicians in the selection of HTx candidates (9). Our study documents that also VO_{2peak} measured *after* HTx is a strong predictor for long-term survival (paper 1). Our results are in line with the only study we found that demonstrated a relationship between physical performance (measured by VE/VCO_2 slope) and survival in a small sample of 49 HTx patients (88). Other related studies on this topic describe how VO_{2peak} is related to soft end-points; how a beneficial VO_{2peak} correlates with NYHA class 1-2 after HTx (89) and how the pre-transplant $VO_{2peak,}$ together with age, predict the gain in physical capacity post HTx (12). Succeeding our study on survival, Rosenbaum et al. (90) published new knowledge in this field, with a study investigating the effect of early rehabilitation on survival: they concluded that early cardiac rehabilitation participation after HTx could predict survival time.

The measurement of physical capacity requires CPET equipment and test personnel, and thus, is quite costly. Although VO_{2peak} is the gold standard to examine exercise performance, there are other physical tests with limited costs that can be useful in the follow-up, found to correlate with CPET results. Such physical tests are the 6-minute walk test and the shuttle walking test (91). If resources are limited, we found also that the self-reported physical health (PF-score) showed similar effect on long-term survival in the HTx population (paper 1). Research in general populations underscore the importance of physical activity and report a dose-response effect on survival rates (92, 93), as well as an strong dose-response relation on self-reported health (94). As shown in the TEX 2 study (figure 13, page 49) physical

performance measured as VO_{2peak} is highly correlated with PF-scores, and both were found to be highly associated with prognosis in our survival analysis (paper 1). Accordingly, we suggest that such measures should be more frequently used *after* HTx to identify patients at higher risk for complications.

6.2 HIT intervention and the long-term effects

The lack of research regarding possible long-term benefits of exercise was pointed out in the published meeting report from 2014: "Consensus recommendations for a research agenda in exercise in solid organ transplantation" (95). We investigated this matter in the TEX 2 study, showing that patients who followed a 12 month HIT intervention were not able to maintain their high physical capacity in the long-term. These findings were explained by the similar amount of daily (moderate) activity in both the HIT and the control group, measured at the 5-year follow-up. Our results differ from the study by Moholdt et al. (96) who investigated long-term effects of a HIT intervention after MI. These MI-patients still had a significantly higher aerobic performance at the 30 months follow-up compared to the control group, explained by more frequent exercise in the HIT group. We have now reported the long-term effects of HIT after HTx (TEX 2 study). Although the initial 1-year gain in physical capacity in the HIT group was not sustained, they showed a less marked decline than the control group. Only the control group showed a significant decrease within group from baseline to the 5-year follow-up. This significant decrease, corresponding to a 9% decline in mL/kg/min, could mostly be explained by an expected age-related decrease in VO_{2peak}. Healthy young adults show a decline of 3-6% each decade, and this decline is shown to accelerate with age; a decline of 15% is found normal and corresponds to the age group of the TEX population (97). This age related VO_2-decline is related to decreasing maximal stroke volume, decreasing blood flow to skeletal muscles and mitochondrial dysfunction (98). As for the HIT group, the

decrease from baseline to the 5-year follow-up in VO_{2peak} was less pronounced (-6%), and could indicate a hidden long-term effect of the intervention.

As well as measures of physical capacity, we measured physical and mental health at each study visit, and the results showed less development of anxiety symptoms in the HIT group, significantly different from the control group at the 5-year follow-up. This beneficial trend in anxiety development together with no negative trends in other secondary end points, support the statements of HIT as a safe exercise modality in HF patients (99), and in maintenance HTx patients (18, 24, 30). The long-term difference in anxiety between the HIT group and the control group is considered a valuable finding, as anxiety is a frequent health issue after HTx, especially in the long-term follow-up (67). This might suggest that a 1-year "heavy" exercise intervention has a long-term value when it comes to self-confidence and trust regarding what your heart (and body) actually can tolerate of exertion, strain and physical work.

6.3 HIT intervention in de novo HTx recipients

While HIT already is an established exercise modality in patients with HF (37) and CAD (38), and more recently in maintenance HTx (18, 24, 30), the upcoming results from the HITTS study will contribute to fill the gap of knowledge related to the effect of HIT among de novo HTx recipients. In addition to exercise capacity measurement, other important secondary outcomes are; development of CAV, improvements in chronotropic response and changes in cardiac and endothelial function. The results from the HITTS study will make a strong contribution to improve and increase the knowledge-base about how early HTx-rehabilitation should be organized to get the most optimal results. The study is followed closely by our dedicated HTx-staff at our hospital, and one of our main goals is to document knowledge about safety and effects of HIT, and thereby initiate an update of the current guidelines. If HIT is found to be safe (and with potentially beneficial effects) also among de novo HTx

patients, the patients will have the possibility to participate in established cardiac rehabilitation programs, which usually combines both MICT and HIT exercise. These rehabilitation programs are usually group based, rather than only consistent individual physiotherapy, thus demanding less government resources.

6.4 HIT and the effect on CAV

HIT is shown to have a positive effect on CAV progression in mice (61) and also in patients who have experienced MI (62). We found the same trend in maintenance HTx recipients after the HIT intervention in the TEX 1 study (46), but the positive effects were not sustained in the long-term as shown in the TEX 2 study (paper 2). Furthermore, exercise is shown to have a positive influence on the endothelium through increased nitric oxide production, and by reduction of inflammation (100, 101). This effect could possibly be enhanced through higher shear stress triggered with higher exercise intensity. A gain in endothelial function following a HIT intervention is found in CAD patients (102). However, a relatively small sample size in the TEX study limits our conclusion in the HTx population, and the effect of HIT on CAV should be examined in a larger sample and include a second intervention arm with MICT. It has been explored how early medical therapy can influence CAV progression in the long-term, and studies with Everolimus are found to have positive impact on CAV severity in de novo HTx patients, whereas no effect is seen if Everolimus is introduced later on (103). The effect on CAV severity by an early initiation was also sustained in the long-term (59, 60). This illustrates an "opportunity window" during the first year after HTx. Knowing that the CAV development is most pronounced the first year after HTx, we anticipate that similar mechanisms may be seen with an early initiation of HIT. Results from the HITTS study will contribute to a better understanding of the relationship between exercise and CAV development.

6.5 HIT and the immediate responses in markers of inflammation and angiogenesis

Exercise training, regardless of intensity, led to an increase in multiple systemic, angiogenetic and platelet derived inflammatory mediators (the BIT study, paper 4). These results are in line with published research showing the pro-coagulation state during exercise, with blood platelet activation potentially reflecting the increase in catecholamines and shear stress (104), promotion of NO production from activated endothelial cells (105, 106), and regulation of the growth and repair of blood vessels (107). The activation of the endothelium and thereby induction of capillary growth in skeletal muscle through pro-angiogenetic mechanisms may play an important role in the beneficial effects of HIT. When we compared the response in inflammatory mediators during the HIT and MICT sessions, we observed a higher response in both Angiopoetin-2 and VEGF-1 with increased intensity. Kilian et al. (108) have previously shown an increase in mRNA for VEGF in whole blood during HIT in healthy children. VEGF is dominantly secreted by working skeletal muscles, an essential factor to increase capillary density, oxygen delivery and thereby exercise performance (109-111). Based on our previous results showing improved muscular exercise capacity after HIT (30), and now the finding of an increased VEGF response, we suggest that this mechanism is of high importance also in the HTx recipients. The fact that HIT markedly increased mediators of angiogenesis and neovascularization, provide new knowledge about potential mechanisms behind the HIT-effect in HTx recipients.

6.6 Limitations

6.6.1 Paper 1 - Survival analysis

In survival analysis, the aim is to find all information that could contribute to explain the survival, but this is usually limited because the data is collected retrospectively. In this paper, we included several well-established predictors in the analysis, but some factors were not

available such as; the need of circulation support, the early postoperative period, data on the donor's health and HLA match/mismatch. However, these variables have shown to predict survival only in the early stage after HTx, and if they had been available in our study, they would probably not have had a strong impact on the results (112). As for other limitations, the development in HF and HTx treatment has changed over time, particularly in the case of bridge to transplant and new immunosuppression therapies, and this limits our possibility to conclude. Future studies should strive to include measures of physical health in their survival analysis to update our findings.

6.6.2 Paper 2- The TEX 2 study

The included patients in the TEX 2 study were only 41, and the neutral difference between groups found at the 5-year follow-up can be a result of a type II error. The physical capacity measured in the entire group at the 5-year follow-up was in the upper limit of the age matched values seen after HTx, and that is documented in international publications. These high VO_{2peak} values could possibly influence and camouflage any potential long-term effects of HIT, and it could be a result of selection bias. However, we know there are differences in measured VO_{2peak} levels when comparing general populations from different nations. For instance are Norwegian VO_{2peak} levels measured to be 20% higher than reported in the United States (113, 114). These "above normal" VO_{2peak} values measured in the TEX study can therefore be considered as representative for our HTx population, and might not be a result of selection bias. As for the investigation of CAV in the TEX 2 study, thirty patients had available IVUS recordings from all three time-points, corresponding to 80% of the study participants. The positive finding of less CAV development measured at the 1-year follow-up, which was lost in the long-term, might be due to the low sample size and the great variance in

the data at 1-year follow-up. Thus, the effect of HIT on CAV should be examined in a larger sample before any definite conclusions are made in this matter.

6.6.3 Paper 3 - the HITTS study

Incidence of selection bias is common in exercise intervention studies. Participating patients are likely to be motivated for regular exercise and might be potentially healthier than the patients who decline to participate. Accordingly, we have tried to minimize such bias in the HITTS study. For example, only four patients were unwilling to participate. The Norwegian part of the inclusion in the HIITS study will be completed in February 2017, and our preliminary results show that we have succeeded with high patient participation.

6.6.4 Paper 4 - The BIT study

The number of included patients in the BIT study was rather low, especially the number of healthy controls, and the number of mediators analyzed was relatively high. Thus, some of the findings (both negative and positive) could be a result of chance. Moreover, correlation between different responses does not necessarily imply a causal relationship, and more mechanistic studies are needed to substantiate if the beneficial effects of HIT are mediated through angiogenetic factors in HTx recipients.

7 CONCLUDING REMARKS

Our findings suggest that measures of physical health should be included frequently also *after* HTx, as they predict prognosis and survival in the long-term. A dose-response effect of physical capacity on survival was also found in the HTx population.

HIT is a feasible and efficient modality of exercise among maintenance HTx recipients, but the mechanisms behind this effect is poorly understood. Our findings suggest that the beneficial effects seen in HTx recipients differ from CAD and HF patients, with more prominent peripheral effects from HIT exercise, rather than central adaptations with increased CO. We showed that HIT significantly increased levels of inflammatory mediators of angiogenesis, suggesting that HIT can regulate and stimulate blood vessel formation in skeletal muscles and thus increase physical capacity.

Considering exercise prescription and future guidelines, our findings suggest that moderate levels of exercise and intensity are insufficient to maintain the improved VO_{2peak} achieved after a HIT intervention. Thus, intermittent periods of HIT are likely to be necessary. Also, the number and length of HIT intervals needed in a HIT session should be further investigated. If a modified HIT protocol with shorter and fewer intervals has comparable effect to a 4 x 4 protocol, it could probably increase the patients' motivation and adherence to exercise in the long-term. When considering other long-term effects, the benefit from a tough and intense HIT-intervention showed a positive effect in the development of anxiety symptoms. The exercise prescription in de novo HTx recipients is still conservative, consisting mainly of MICT exercise, but this traditional guideline might change when the ongoing HITTS study is completed. The results from the HITTS study will have the potential to update, optimize and possibly include HIT as a safe exercise modality in future guidelines.

8 REFERENCE LIST

1. Ho KK, Anderson KM, Kannel WB, Grossman W, Levy D. Survival after the onset of congestive heart failure in Framingham Heart Study subjects. Circulation. 1993:88:107-15.

2. Zannad F, Mebazaa A, Juilliere Y, Cohen-Solal A, Guize L, Alla F, et al. Clinical profile, contemporary management and one-year mortality in patients with severe acute heart failure syndromes: The EFICA study. Eur J Heart Fail. 2006:8:697-705.

3. Stehlik J, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th Official Adult Heart Transplant Report—2012. J Heart Lung Transplant. 2012:31:1052-64.

4. C D. Rehabilitation of patients with coronary artery disease. In: Heart Disease, a Textbook of Cardiovascular Medicine, 4th ed. E B, editor. WB Saunders, Philadelphia1992.

5. Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. JAMA. 2009:301:2024-35.

6. Arena R, Myers J, Guazzi M. The future of aerobic exercise testing in clinical practice: is it the ultimate vital sign? Future Cardiol. 2010:6:325-42.

7. Terence Kavanagh M, FRCP(C); Donald J. Mertens, MD, MSc; Larry F. Hamm, PhD;, Joseph Beyene PJK, RN; Paul Corey, PhD; Roy J. Shephard, MD, PhD. Prediction of Long-Term Prognosis in 12 169 Men Referred for Cardiac Rehabilitation. Circulation. 2002:106:666-71.

8. Cahalin LP, Chase P, Arena R, Myers J, Bensimhon D, Peberdy MA, et al. A meta-analysis of the prognostic significance of cardiopulmonary exercise testing in patients with heart failure. Heart Fail rev. 2013:18:79-94.

9. Mehra MR, Kobashigawa J, Starling R, Russell S, Uber PA, Parameshwar J, et al. Listing criteria for heart transplantation: International Society for Heart and Lung

Transplantation guidelines for the care of cardiac transplant candidates--2006. J Heart Lung Transplant. 2006:25:1024-42.

10. Jaramillo N, Segovia J, Gomez-Bueno M, Garcia-Cosio D, Castedo E, Serrano S, et al. Characteristics of patients with survival longer than 20 years following heart transplantation. Rev Esp Cardiol. 2013:66:797-802.

11. Tallaj JA, Pamboukian SV, George JF, Kirklin JK, Brown RN, McGiffin DC, et al. Have risk factors for mortality after heart transplantation changed over time? Insights from 19 years of Cardiac Transplant Research Database study. J Heart Lung Transplant. 2014:33:1304-11.

12. Osada N, Chaitman BR, Donohue TJ, Wolford TL, Stelken AM, Miller LW. Long-term cardiopulmonary exercise performance after heart transplantation. Am J Cardiol. 1997:79:451-6.

13. Weber KT, Kinasewitz GT, Janicki JS, Fishman AP. Oxygen utilization and ventilation during exercise in patients with chronic cardiac failure. Circulation. 1982:65:1213-23.

14. Soumagne D. Weber classification in cardiac rehabilitation. Acta Cardiol. 2012:67:285-90.

15. Janaudis-Ferreira T, Mathur S, Konidis S, Tansey CM, Beaurepaire C. Outcomes in randomized controlled trials of exercise interventions in solid organ transplant. World J Transplant. 2016:6:774-89.

16. Bernardi L, Radaelli A, Passino C, Falcone C, Auguadro C, Martinelli L, et al. Effects of physical training on cardiovascular control after heart transplantation. Int J Cardiol. 2007:118:356-62.

17. Carter R A-RO, Stevenson A, et al. Exercise responses following heart tranplantation; 5 years follow up. Scott Med J. 2006:51:6-14.

18. Dall CH, Snoer M, Christensen S, Monk-Hansen T, Frederiksen M, Gustafsson F, et al. Effect of High-Intensity Training Versus Moderate Training on Peak Oxygen Uptake and Chronotropic Response in Heart Transplant Recipients: A Randomized Crossover Trial. Am J Transplant. 2014:14:2391-9.

19. Ewert R, Wensel R, Bruch L, Mutze S, Bauer U, Plauth M, et al. Relationship between impaired pulmonary diffusion and cardiopulmonary exercise capacity after heart transplantation. Chest. 2000:117:968-75.

20. Givertz MM, Hartley LH, Colucci WS. Long-term sequential changes in exercise capacity and chronotropic responsiveness after cardiac transplantation. Circulation. 1997:96:232-7.

21. Gullestad L, Myers J, Edvardsen T, Kjekshus J, Geiran O, Simonsen S. Predictors of exercise capacity and the impact of angiographic coronary artery disease in heart transplant recipients. Am Heart J. 2004:147:49-54.

22. Habedank D, Ewert R, Hummel M, Wensel R, Hetzer R, Anker SD. Changes in exercise capacity, ventilation, and body weight following heart transplantation. Eur J Heart Fail. 2007:9:310-6.

23. Haykowsky M TD, Kim D, Tymchak W. Exercise training improves aerobic capacity and skeletal function in heart transplant recipients. Am J Transplant. 2009:9:734-9.

24. Hermann TS, Dall CH, Christensen SB, Goetze JP, Prescott E, Gustafsson F. Effect of high intensity exercise on peak oxygen uptake and endothelial function in long-term heart transplant recipients. Am J Transplant. 2011:11:536-41.

25. Hognestad A, Holm T, Simonsen S, Kjekshus J, Andreassen AK. Serial measurements of peripheral vascular reactivity and exercise capacity in congestive heart failure and after heart transplantation. J Card Fail. 2005:11:447-54.

26. Karapolat H, Eyigor S, Zoghi M, Yagdi T, Nalbantgil S, Durmaz B, et al. Effects of cardiac rehabilitation program on exercise capacity and chronotropic variables in patients with orthotopic heart transplant. Clin Res Cardiol. 2008:97:449-56.

27. Kavanagh T, Mertens DJ, Shephard RJ, Beyene J, Kennedy J, Campbell R, et al. Long-term cardiorespiratory results of exercise training following cardiac transplantation. The Am J Cardiol. 2003:91:190-4.

28. Kemp DL, Jennison SH, Stelken AM, Younis LT, Miller LW. Association of resting heart rate and chronotropic response. The American journal of cardiology. 1995:75:751-2.

29. Kobashigawa J A LD, Lee N. A controlled trial of exercise rehabilitation after heart transplantation. N Engl J Med 1999:340:272-7.

30. Nytroen K, Rustad LA, Aukrust P, Ueland T, Hallen J, Holm I, et al. High-intensity interval training improves peak oxygen uptake and muscular exercise capacity in heart transplant recipients. Am J Transplant. 2012:12:3134-42.

31. Renlund DG, Taylor DO, Ensley RD, O'Connell JB, Gilbert EM, Bristow MR, et al. Exercise capacity after heart transplantation: influence of donor and recipient characteristics. J Heart Lung Transplant. 1996:15:16-24.

32. Schwaiblmair M, von Scheidt W, Uberfuhr P, Reichart B, Vogelmeier C. Lung function and cardiopulmonary exercise performance after heart transplantation: influence of cardiac allograft vasculopathy. Chest. 1999:116:332-9.

33. Squires RW. Exercise therapy for cardiac transplant recipients. Prog Cardiovasc Dis. 2011:53:429-36.

34. Tegtbur U, Busse MW, Jung K, Pethig K, Haverich A. Time course of physical reconditioning during exercise rehabilitation late after heart transplantation. J Heart Lung Transplant. 2005:24:270-4.

35. Wu YT CC, Chou NK et al. Efficacy of a home-based exercise program for orthotopic heart transplant recipients. Cardiology. 2008:111:87-93.

36. Hsieh PL, Wu YT, Chao WJ. Effects of exercise training in heart transplant recipients: a meta-analysis. Cardiology. 2010-2011:120:27-35.

37. Wisloff U, Stoylen A, Loennechen JP, Bruvold M, Rognmo O, Haram PM, et al. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. Circulation. 2007:115:3086-94.

38. Elliott AD, Rajopadhyaya K, Bentley DJ, Beltrame JF, Aromataris EC. Interval Training Versus Continuous Exercise in Patients with Coronary Artery Disease: A Meta-Analysis. Heart, Lung and Circulation. 2015:24:149-57.

39. Tjonna AE, Lee SJ, Rognmo O, Stolen TO, Bye A, Haram PM, et al. Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. Circulation. 2008:118:346-54.

40. Wisloff U, Ellingsen O, Kemi OJ. High-intensity interval training to maximize cardiac benefits of exercise training? Exerc Sport Sci rev. 2009:37:139-46.

41. Borg G. Perceived exertion as an indicator of somatic stress. Scand J Rehabil Med. 1970:2:92-8.

42. Anderssen SA, Stromme SB. [Physical activity and health--recommendations]. Tidsskrift for den Norske legeforening. 2001:121:2037-41.

43. Pina IL, Apstein CS, Balady GJ, Belardinelli R, Chaitman BR, Duscha BD, et al. Exercise and heart failure: A statement from the American Heart Association Committee on exercise, rehabilitation, and prevention. Circulation. 2003:107:1210-25.

44. Nytroen K, Myers J, Chan KN, Geiran OR, Gullestad L. Chronotropic responses to exercise in heart transplant recipients: 1-yr follow-up. Am J Phys Med Rehabil. 2011:90:579-88.

45. Mezzani A, Hamm LF, Jones AM, McBride PE, Moholdt T, Stone JA, et al. Aerobic exercise intensity assessment and prescription in cardiac rehabilitation: a joint position statement of the European Association for Cardiovascular Prevention and Rehabilitation, the American Association of Cardiovascular and Pulmonary Rehabilitation and the Canadian Association of Cardiac Rehabilitation. Eur J Prev Cardiology. 2013:20:442-67.

46. Nytroen K, Rustad LA, Erikstad I, Aukrust P, Ueland T, Lekva T, et al. Effect of high-intensity interval training on progression of cardiac allograft vasculopathy. J Heart Lung Transplant. 2013:32:1073-80.

47. Anderson L, Dall CH, Nguyen TT, Burgess L, Taylor RS. Exercise-based cardiac rehabilitation in heart transplant recipients. Cochrane Database of Systematic Reviews. 2016.

48. Piepoli MF, Davos C, Francis DP, Coats AJ. Exercise training meta-analysis of trials in patients with chronic heart failure (ExTraMATCH). BMJ. 2004:328:189.

49. Sagar VA, Davies EJ, Briscoe S, Coats AJS, Dalal HM, Lough F, et al. Exercise-based rehabilitation for heart failure: systematic review and meta-analysis. Open Heart. 2015:2.

50. Kemi OJ, Wisloff U. High-intensity aerobic exercise training improves the heart in health and disease. J Cardiopulm Rehabil Prev. 2010:30:2-11.

51. Rustad LA, Nytroen K, Amundsen BH, Gullestad L, Aakhus S. One year of high-intensity interval training improves exercise capacity, but not left ventricular function in stable heart transplant recipients: a randomised controlled trial. Eur J Prev Cardiol. 2014:21:181-91.

52. Dall CH, Gustafsson F, Christensen SB, Dela F, Langberg H, Prescott E. Effect of moderate- versus high-intensity exercise on vascular function, biomarkers and quality of life in heart transplant recipients: A randomized, crossover trial. J Heart Lung Transplant. 2015:34:1033-41.

53. Avery RK. Cardiac-allograft vasculopathy. N Engl J Med. 2003:349:829-30.

54. Jansen MA, Otten HG, de Weger RA, Huibers MM. Immunological and Fibrotic Mechanisms in Cardiac Allograft Vasculopathy. Transplantation. 2015:99:2467-75.

55. Tuzcu EM, Kapadia SR, Sachar R, Ziada KM, Crowe TD, Feng J, et al. Intravascular ultrasound evidence of angiographically silent progression in coronary atherosclerosis predicts long-term morbidity and mortality after cardiac transplantation. J Am Coll Cardiol. 2005:45:1538-42.

56. Kobashigawa JA, Tobis JM, Starling RC, Tuzcu EM, Smith AL, Valantine HA, et al. Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. J Am Coll Cardiol. 2005:45:1532-7.

57. Potena L, Masetti M, Sabatino M, Bacchi-Reggiani ML, Pece V, Prestinenzi P, et al. Interplay of coronary angiography and intravascular ultrasound in predicting long-term outcomes after heart transplantation. J Heart Lung Transplant. 2015:34:1146-53.

58. Kobashigawa JA, Katznelson S, Laks H, Johnson JA, Yeatman L, Wang XM, et al. Effect of pravastatin on outcomes after cardiac transplantation. N Engl J Med. 1995:333:621-7.

59. Arora S, Andreassen AK, Andersson B, Gustafsson F, Eiskjaer H, Botker HE, et al. The Effect of Everolimus Initiation and Calcineurin Inhibitor Elimination on Cardiac Allograft Vasculopathy in De Novo Recipients: One-Year Results of a Scandinavian Randomized Trial. Am J Transplant. 2015:15:1967-75.

60. Andreassen AK, Andersson B, Gustafsson F, Eiskjaer H, Radegran G, Gude E, et al. Everolimus Initiation With Early Calcineurin Inhibitor Withdrawal in De Novo Heart Transplant Recipients: Three-Year Results From the Randomized SCHEDULE Study. Am J Transplant . 2016:16:1238-47.

61. DeCampli WM. Of mice and men ... does exercise decrease progression of transplant coronary vasculopathy? J Thorac Cardiovasc Surg. 2015:149:337-9.

62. Madssen E, Moholdt T, Videm V, Wisloff U, Hegbom K, Wiseth R. Coronary atheroma regression and plaque characteristics assessed by grayscale and radiofrequency intravascular ultrasound after aerobic exercise. Am J Cardiol. 2014:114:1504-11.

63. Grady KL, Naftel DC, Kobashigawa J, Chait J, Young JB, Pelegrin D, et al. Patterns and predictors of quality of life at 5 to 10 years after heart transplantation. J Heart Lung Transplant. 2007:26:535-43.

64. Karam VH, Gasquet I, Delvart V, Hiesse C, Dorent R, Danet C, et al. Quality of life in adult survivors beyond 10 years after liver, kidney, and heart transplantation. Transplantation. 2003:76:1699-704.

65. Karapolat H, Eyigor S, Durmaz B, Nalbantgil S, Yagdi T, Zoghi M. The effect of functional performance, respiratory function and osteopenia on the quality of life after heart transplantation. Int J Cardiol. 2008:124:381-3.

66. Christensen SB, Dall CH, Prescott E, Pedersen SS, Gustafsson F. A high-intensity exercise program improves exercise capacity, self-perceived health, anxiety and depression in heart transplant recipients: a randomized, controlled trial. J Heart Lung Transplant.2012:31:106-7.

67. Dew MA, DiMartini AF. Psychological disorders and distress after adult cardiothoracic transplantation. J Cardiovasc Nurs. 2005:20:S51-66.

68. Sivertsen B, Relbo A, Gullestad L, Hellesvik M, Grov I, Andreassen A, et al. [Self-assessed health and psychological symptoms after heart transplantation]. Tidsskrift for den Norske legeforening. 2007:127:3198-201.

69. Havik OE, Sivertsen B, Relbo A, Hellesvik M, Grov I, Geiran O, et al. Depressive symptoms and all-cause mortality after heart transplantation. Transplantation. 2007:84:97-103.

70. Dew MA, Rosenberger EM, Myaskovsky L, DiMartini AF, DeVito Dabbs AJ, Posluszny DM, et al. Depression and Anxiety as Risk Factors for Morbidity and Mortality After Organ Transplantation: A Systematic Review and Meta-Analysis. Transplantation. 2015:100:988-1003.

71. Karapolat H, Eyigor S, Durmaz B, Yagdi T, Nalbantgil S, Karakula S. The relationship between depressive symptoms and anxiety and quality of life and functional capacity in heart transplant patients. Clin Res Cardiol. 2007:96:593-9.

72. Ulubay G, Ulasli SS, Sezgin A, Haberal M. Assessing exercise performance after heart transplantation. Clin Transpl. 2007:21:398-404.

73. Astrand I. Aerobic work capacity in men and women with special reference to age. Acta Phys Scand Suppl. 1960:49:1-92.

74. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961:4:561-71.

75. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992:30:473-83.

76. Costanzo MR, Naftel DC, Pritzker MR, Heilman JK, 3rd, Boehmer JP, Brozena SC, et al. Heart transplant coronary artery disease detected by coronary angiography: a multiinstitutional study of preoperative donor and recipient risk factors. Cardiac Transplant Research Database. J Heart Lung Transpl. 1998:17:744-53.

77. Kurtze N, Rangul V, Hustvedt BE, Flanders WD. Reliability and validity of self-reported physical activity in the Nord-Trondelag Health Study: HUNT 1. Scand J Public Health. 2008:36:52-61.

78. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Jr., Tudor-Locke C, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. Med Sci Sports Exerc. 2011:43:1575-81.

79. Working Group on Cardiac Rehabilitation & Excercise Physiology and Working Group on Heart Failure of the European Society of Cardiology. Recommendations for exercise testing in chronic heart failure patients. Eur Heart J. 2001:22:37-45.

80. Freeman JV, Dewey FE, Hadley DM, Myers J, Froelicher VF. Autonomic nervous system interaction with the cardiovascular system during exercise. Prog Cardiovasc Dis. 2006:48:342-62.

81. American College of Sports Medicine. ACSM's guidelines for exercise testing and prescription, 9th edn. Baltimore: Wolters Kluwer, Lippincott, Williams & Wilkins; 2014.

82. Jaffrin MY. Body composition determination by bioimpedance: an update. Curr Opin in Clin Nutr Metab Care. 2009:12:482-6.

83. Mehra MR, Crespo-Leiro MG, Dipchand A, Ensminger SM, Hiemann NE, Kobashigawa JA, et al. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. J Heart Lung Transplant. 2010:29:717-27.

84. Mintz GS, Garcia-Garcia HM, Nicholls SJ, Weissman NJ, Bruining N, Crowe T, et al. Clinical expert consensus document on standards for acquisition, measurement and reporting of intravascular ultrasound regression/progression studies. EuroIntervention. 2011:6:1123-30, 9.

85. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand. 1983:67:361-70.

86. Tearney GJ, Regar E, Akasaka T, Adriaenssens T, Barlis P, Bezerra HG, et al. Consensus standards for acquisition, measurement, and reporting of intravascular optical coherence tomography studies: a report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation. J Am Coll Cardiol. 2012:59:1058-72.

87. Berry C, Corcoran D, Hennigan B, Watkins S, Layland J, Oldroyd KG. Fractional flow reserve-guided management in stable coronary disease and acute myocardial infarction: recent developments. Eur Heart J. 2015:36:3155-64.

88. Grigioni F, Specchia S, Maietta P, Potena L, Bacchi-Reggiani ML, Ghetti G, et al. Changes in exercise capacity induced by heart transplantation: prognostic and therapeutic implications. Scand J Med Sci Sports. 2011:21:519-25.

89. Chang AC, Shyr Y, Groves J, Chomsky DB, Davis SF, Wilson JR, et al. The Utility of Exercise Testing after Cardiac Transplantation in Older Patients. J Surg Res. 1999:81:48-54.

90. Rosenbaum AN, Kremers WK, Schirger JA, Thomas RJ, Squires RW, Allison TG, et al. Association Between Early Cardiac Rehabilitation and Long-term Survival in Cardiac Transplant Recipients. Mayo Clin Proc. 2016:91:149-56.

91. ATS committee on Proficiency standards for clinical pulmonary function laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002:166:111-7.

92. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. 2012:380:219-29.

93. Holme I, Anderssen SA. [Physical activity, smoking and mortality among men who participated in the Oslo studies of 1972 and 2000]. Tidsskrift for den Norske legeforening. 2014:134:1743-8.

94. Eriksen L, Curtis T, Gronbaek M, Helge JW, Tolstrup JS. The association between physical activity, cardiorespiratory fitness and self-rated health. Prev Med. 2013:57:900-2.

95. Mathur S, Janaudis-Ferreira T, Wickerson L, Singer LG, Patcai J, Rozenberg D, et al. Meeting report: consensus recommendations for a research agenda in exercise in solid organ transplantation. Am J Transplant. 2014:14:2235-45.

96. Moholdt T, Aamot IL, Granoien I, Gjerde L, Myklebust G, Walderhaug L, et al. Long-term follow-up after cardiac rehabilitation: a randomized study of usual care exercise training versus aerobic interval training after myocardial infarction. Int J Cardiol. 2011:152:388-90.

97. Fleg JL, Morrell CH, Bos AG, Brant LJ, Talbot LA, Wright JG, et al. Accelerated longitudinal decline of aerobic capacity in healthy older adults. Circulation. 2005:112:674-82.

98. Betik AC, Hepple RT. Determinants of VO2 max decline with aging: an integrated perspective. Appl Physiol Nutr Metab. 2008:33:130-40.

99. Ismail H, McFarlane JR, Nojoumian AH, Dieberg G, Smart NA. Clinical outcomes and cardiovascular responses to different exercise training intensities in patients with heart failure: a systematic review and meta-analysis. JACC Heart Fail. 2013:1:514-22.

100. Lavie CJ, Arena R, Swift DL, Johannsen NM, Sui X, Lee D-c, et al. Exercise and the Cardiovascular System. Clinical science and cardiovascular outcomes. Circ Res. 2015:117:207-19.

101. Lavie CJ, Church TS, Milani RV, Earnest CP. Impact of physical activity, cardiorespiratory fitness, and exercise training on markers of inflammation. J Cardiopulm Rehabil Prev. 2011:31:137-45.

102. Cornish AK, Broadbent S, Cheema BS. Interval training for patients with coronary artery disease: a systematic review. Eur J Appl Physiol. 2011:111:579-89.

103. Arora S, Erikstad I, Ueland T, Sigurdardottir V, Ekmehag B, Jansson K, et al. Virtual histology assessment of cardiac allograft vasculopathy following introduction of everolimus--results of a multicenter trial. Am J Transplant. 2012:12:2700-9.

104. Wallen NH, Goodall AH, Li N, Hjemdahl P. Activation of haemostasis by exercise, mental stress and adrenaline: effects on platelet sensitivity to thrombin and thrombin generation. Clin Sci. 1999:97:27-35.

105. Crimi E, Ignarro LJ, Cacciatore F, Napoli C. Mechanisms by which exercise training benefits patients with heart failure. Nat rev Cardiol. 2009:6:292-300.

106. Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, Shu Y, et al. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. Circulation. 2003:107:3152-8.

107. Wang Y, Li M, Dong F, Zhang J, Zhang F. Physical exercise-induced protection on ischemic cardiovascular and cerebrovascular diseases. Int J Clin Exp Med. 2015:8:19859-66.

108. Kilian Y, Wehmeier UF, Wahl P, Mester J, Hilberg T, Sperlich B. Acute Response of Circulating Vascular Regulating MicroRNAs during and after High-Intensity and High-Volume Cycling in Children. Front Physiol. 2016:7:92.

109. Delavar H, Nogueira L, Wagner PD, Hogan MC, Metzger D, Breen EC. Skeletal myofiber VEGF is essential for the exercise training response in adult mice. Am J Physiol Regul Integr Comp Physiol. 2014:306:R586-95.

110. Olfert IM, Baum O, Hellsten Y, Egginton S. Advances and challenges in skeletal muscle angiogenesis. Am J Physiol Heart Circ Physiol. 2016:310:H326-36.

111. Hoier B, Hellsten Y. Exercise-induced capillary growth in human skeletal muscle and the dynamics of VEGF. Microcirculation. 2014:21:301-14.

112. Stehlik J, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dobbels F, et al. The Registry of the International Society for Heart and Lung Transplantation: Twenty-eighth Adult Heart Transplant Report--2011. J Heart Lung Transplant. 2011:30:1078-94.

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

114. Kaminsky LA, Arena R, Myers J. Reference Standards for Cardiorespiratory Fitness Measured With Cardiopulmonary Exercise Testing: Data From the Fitness Registry and the Importance of Exercise National Database. Mayo Clin Proc. 2015:90:1515-23.

Papers 1- 4