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Cardiopulmonary Function in Healthy Individuals and in Patients After Hematopoietic Cell Transplantation

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Abstract

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Background: The cardiopulmonary exercise test (CPET) is the gold standard of clinical exercise tests, combining conventional stress testing with measurement of oxygen uptake and carbon dioxide production. In order to interpret CPET findings, adequate reference values are needed. Currently, no Swedish reference values exist.

Hematopoietic cell transplantation (HCT) is an established treatment for childhood leukemia, with a growing number of long-term survivors. This increases the importance of identifying and treating this therapy's late cardiac and pulmonary consequences.

Aims: The main aim of *Study I* was to compare the peak oxygen uptake ($V_{O_{2peak}}$) of healthy, 50-year-old Swedes with four commonly used international reference values. Secondary aims were to analyze peak workload and $V_{O_{2peak}}$ in regard to achieved respiratory exchange ratio (RER), and the significance of breathing reserve (BR) at peak exercise in healthy individuals.

The main aim of *Studies II–IV* was to investigate long-term cardiopulmonary effects in a group of patients, in median 18 years after HCT including preparative chemotherapy and total body irradiation.

Methods: A group of healthy, 50-year-old Swedes ($n = 181$; 91 females) were investigated in *Study I*, using CPET. The investigated subjects in *Studies II–IV* were aged 17–37 years and were compared with an age- and sex-matched control group. Cardiac function and pulmonary function were studied through echocardiography, spirometry and CPET at a single occasion.

Results: All reference values analyzed in *Study I* underestimated $V_{O_{2peak}}$ in women. $V_{O_{2peak}}$ was best predicted, for both men and women, using reference values by Jones *et al.* No evidence was found that $RER > 1.1$ would be better than $RER > 1.0$ as an indicator of good exercise performance in healthy individuals. In healthy individuals, lower BR is likely a response to higher workloads.

In *Studies II–IV*, all echocardiographic parameters were within normal range in patients after HCT. However, systolic and diastolic left ventricular function, and right ventricular function, were reduced in comparison with healthy controls. Exercise tests and CPET showed that long-term survivors after HCT, when compared with healthy individuals, had significantly decreased exercise capacity and reduced $V_{O_{2peak}}$ and other CPET parameters, reflecting effects on both the cardiac and the pulmonary functions.

Conclusions: All investigated reference values underestimated $V_{O_{2peak}}$ in 50-year-old Swedes, suggesting a need for Swedish reference values. HCT-treated leukemia patients displayed reduced exercise capacity and $V_{O_{2peak}}$. Regular follow-up of these patients with CPET could contribute to early detection of functional impairment.

Keywords: hematopoietic stem cell transplantation, echocardiography, cardiopulmonary exercise testing, stress testing, oncology, childhood leukemia, healthy adults, breathing reserve, oxygen uptake

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*To Andrei and Hans, without
You – nothing of this!
To Erik and Bertil, without
You – nothing at all!*

List of Papers

This thesis is based on the following papers, which are referred to in the text by the following Roman numerals.

- I Genberg M, Andrén B, Lind L, Hedenström H, Malinovsky A. Commonly used reference values underestimate oxygen uptake in healthy, 50-year-old Swedish women. *Clin Physiol Funct Imaging*. 2016 Jun 16. doi: 10.1111/cpf.12377
- II Öberg A, Genberg M, Malinovsky A, Hedenström H, Frisk P. Exercise capacity in young adults after hematopoietic cell transplantation in childhood. *Am J Transplant*. 2018. 18(2): p. 417–423.
- III Genberg M, Öberg A, Andrén B, Hedenström H, Frisk P, Flachskampf FA. Cardiac function after hematopoietic cell transplantation: An echocardiographic cross-sectional study in young adults treated in childhood. *Pediatric Blood Cancer*. 2015; 62:143–147.
- IV Genberg M, Öberg A, Hedenström H, Frisk P, Malinovsky A. Long-term effects on cardio-pulmonary exercise testing parameters in young adults treated with stem cell transplantation in childhood. *Manuscript*.

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Abbreviations, terminology and variables

ALL	Acute lymphoblastic leukemia
AT	Anaerobic threshold
AV	Atrioventricular
A wave	Peak velocity of atrial filling from the flow profile of the mitral valve
BF	Breathing frequency
BMI	Body mass index
BR	Breathing reserve
BR _{peak}	Breathing reserve at highest workload
BSA	Body surface area
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise testing
CR-10	Category Ratio, a subjective scale of symptoms, defined by Borg [1, 2]
DLCO	Diffusing capacity of the lungs for carbon monoxide
DLCOc	Diffusing capacity of the lungs for carbon monoxide corrected for hemoglobin
DLCOc/VA	Diffusing capacity of the lungs for carbon monoxide corrected for hemoglobin and alveolar volume
DT	Deceleration time from the flow profile of the mitral valve
DXA	Dual energy X-ray absorptiometry
E wave	Peak velocity of early filling from the flow profile of the mitral valve
E/A ratio	Ratio between E wave and A wave from the flow profile of the mitral valve
ECG	Electrocardiogram
FEV ₁	Forced expiratory volume in 1 second
FFM	Fat-free mass
FS	Fractional shortening
GH	Growth hormone
GH _{peak}	Peak value of spontaneous GH secretion
GVHD	Graft-versus-host disease
cGVHD	Chronic graft-versus-host disease
Hb	Hemoglobin
HCT	Hematopoietic cell transplantation

HW	Hansen-Wasserman's formula for reference values, used in Study I [3]
Jones 1	The first formula of Jones <i>et al.</i> for reference values, used in Study I [4]
Jones 2	The second formula of Jones <i>et al.</i> for reference values, used in Study I [4]
LBL	Lymphoblastic lymphoma
LV-EF	Left ventricular ejection fraction
LV-FS	Left ventricular fractional shortening
MAPSE	Mitral annular plane systolic excursion
mU/liter	Milliunit per liter
MVV	Maximum voluntary ventilation
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
O ₂ pulse	Oxygen pulse
Peak O ₂ pulse	Oxygen pulse at highest workload
POEM	Prospective investigation of Obesity, Energy and Metabolism
Pu-acc time	Acceleration time of pulmonary systolic flow velocity
RER	Respiratory exchange ratio
RER _{peak}	Respiratory exchange ratio at highest workload
RPE	Ratings of perceived exertion, defined by Borg [1, 2]
SBP	Systolic Blood Pressure
SHIP	Study of Health In Pomerania; reference values from the SHIP study are used in study I [5]
S _{pO₂}	Oxygen saturation
TAPSE	Tricuspid annular plane systolic excursion
TBI	Total body irradiation
TLC	Total lung capacity
TV _{peak}	Tidal volume at highest workload
VA	Alveolar volume
VC	Vital capacity
V _{CO₂}	Carbon dioxide production
V _{CO₂peak}	Carbon dioxide production at highest workload
V _{CO₂@AT}	Carbon dioxide production at anaerobic threshold
V _E	Minute ventilation
V _{Epeak}	Minute ventilation at highest workload
V _{O₂}	Oxygen uptake
V _{O₂peak}	Oxygen uptake at highest workload
W	Watt
W _{peak}	Highest (peak) workload
W _{peak} %	Highest (peak) workload in percent of predicted

Introduction

Long-term follow-up of patients with leukemia

Hematopoietic cell transplantation (HCT) has become an established treatment for childhood leukemia, with the number of long-term survivors increasing. Consequently, attention has become more focused on the late sequelae of this treatment. [6-8] Previous investigations have found reduced spirometric values in young adults treated with HCT in childhood, and there are also reports of impaired cardiac function in this patient group. [7-11] Strategies for detecting such complications are under discussion. [12-14]

Long-term survivors treated with HCT in childhood also show an increased risk of impaired physical performance, which may be due to deficits in pulmonary and musculoskeletal functions, as well as an increased risk of late cardiac complications. [7, 10, 15-21] This can be related to a number of causes, including chemotherapeutic drugs given in primary treatment, particularly anthracyclines, and the preparative regimen, which usually combines cyclophosphamide at high doses with total body irradiation (TBI). [9, 12, 16, 20, 22-28] Reduced physical performance can also be caused by, as well as lead to, physical inactivity. [7, 20]

From a cardiovascular perspective, comorbidities that may predispose to cardiac dysfunction are observed following HCT in childhood. These include chronic lung disease, renal impairment, hypertension, insulin resistance, and dyslipidemias. [9, 29-34] In addition, there are reports of a changed body composition after HCT, with high fat mass and low fat-free mass (FFM), [35, 36] which can also predispose to cardiovascular diseases. Such differences can contribute to methodological difficulties in comparing different study parameters between HCT-treated patients and healthy subjects with normal body composition.

As it may take many years for cardiac and respiratory dysfunctions to develop, it is essential to have a sufficiently long follow-up time. To date, there is only a limited number of studies of exercise capacity or cardiac function among subjects treated with HCT in childhood. [7, 10, 11, 15, 17-19, 37-39] Few of these studies have combined measurement of exercise capacity with simultaneous measurement of pulmonary or cardiac function. [7, 10, 11, 17-19] The reason to do this is to try to understand if the reduced exercise capacity is due to respiratory or cardiovascular limitation in this group of patients. Only one of these studies had a median follow-up time exceeding 10

years. [11] No studies have yet combined evaluation of exercise capacity with evaluation of both lung and heart function, at the same time, in long-term follow-up after HCT.

There are also reports of reduced peak oxygen uptake ($VO_{2\text{peak}}$) in survivors of pediatric cancer, especially childhood leukemia, compared with healthy controls. [7, 10, 11, 26, 27, 40] However, only limited investigation has been conducted regarding other parameters of gas exchange tests in long-term studies of childhood leukemia. A few studies have analyzed whether the decreased $VO_{2\text{peak}}$ can be attributed to pulmonary or cardiac limitations [7, 10, 11] but no study, as per the author's knowledge, has tried to correlate $VO_{2\text{peak}}$ with both echocardiographic and spirometric findings in the same patient group. Furthermore, most of the published studies have focused on $VO_{2\text{peak}}$ and have not reported other parameters from cardiopulmonary exercise tests (CPETs), such as oxygen pulse and ventilatory efficiency. [11, 26, 27]

In Sweden, there are requests for increased cooperation between different specialties, such as clinical physiology, cardiology and oncology, for functional assessment of patients who have undergone potentially cardiotoxic oncological treatment in childhood. [41] Internationally, for example at the Mayo Clinic in the United States, there is more structured follow-up of childhood cancer. [42]

Exercise capacity, exercise stress test and cardiopulmonary exercise test (CPET)

Evaluation of exercise capacity is of great importance in patients with chronic diseases, in order to grade disease severity, to follow up treatment given and to evaluate risk for disease-related events. Exercise capacity is usually assessed through exercise stress tests, 6-minute walk tests or cardiopulmonary exercise tests. These tests represent a combined evaluation of cardiopulmonary and neuromuscular function.

Exercise testing is a well-established procedure and has had widespread clinical use for many decades. [43, 44] Treadmill and cycle ergometers are the most commonly used exercise testing devices. Treadmill testing is generally favored in the United States, while clinical exercise testing on bicycle ergometers is commonly used in Sweden and other European countries, where bicycle use is more prevalent. The test protocol should be selected based on the purpose of testing and the individual patient. In Sweden, and in many other countries, ramp protocols are used, where the exercise load is increased continuously or in small steps. [44, 45] Symptom-limited testing is desirable for general evaluation. Reaching 85% of age-predicted maximal

heart rate is a commonly used indicator of sufficient subject effort during an exercise test.

Exercise capacity, assessed through exercise stress testing, has also been shown to be a predictor of mortality in general. It has been shown that peak exercise capacity is the strongest predictor of risk of death among both healthy subjects and those with cardiovascular disease, in both younger and elderly patients. [46, 47] Exercise stress testing is noninvasive, inexpensive and provides clinically relevant diagnostic and prognostic information. [43, 44, 46]

The cardiopulmonary exercise test (CPET) is considered to be the gold standard, as it provides the clinician with a significantly higher amount of pathophysiological information than ordinary cardiac stress testing. [3, 48] CPET entails a conventional stress test, where ECG, blood pressure and symptoms are observed under increasing exercise load, with simultaneous measurement of oxygen uptake (V_{O_2}) and carbon dioxide production (V_{CO_2}). This makes it a sensitive method for identifying limiting factors in physical exertion. CPET provides the possibility to evaluate the exercise response of both pulmonary, cardiovascular, hematopoietic, neurophysiological and skeletal muscle organ systems. [48]

One disadvantage of CPET is a lack of solid reference values for all parameters. As Paap *et al.* (2014) stated, there is no single ideal set of reference values for CPET, and women are relatively understudied. [49] This means that the interpretation of CPET can sometimes be difficult and the boundary between what is normal and what is pathological can be hard to determine.

International guidelines suggest, in addition to reaching 85% of age-predicted maximal heart rate, a respiratory exchange ratio ($RER = V_{CO_2}/V_{O_2}$) $> 1.1-1.15$ at maximal exercise as an indicator of an excellent effort when using CPET. [48-50] However, in the studies of normal values, it is rare to report RER and further studies are therefore required in order to determine its importance. [3-5, 51]

Lung function is not generally thought to limit exercise capacity or peak oxygen uptake in healthy individuals. [48] A reduced breathing reserve (BR) at peak exercise is characteristic for patients with pulmonary diseases, while an impact on the oxygen pulse (O_2 pulse) is rather a sign of a cardiac limitation. [3, 50] However, the recommendations for BR and RER are made based mainly on patients referred for CPET investigations and the importance of BR and its relation to V_{O_2} at peak load ($V_{O_{2peak}}$) in healthy individuals have not been studied.[3] Relevant cut-off values for healthy individuals are not well-investigated.

Need for new reference values

In Sweden, there has recently been an introduction of new reference values for standard exercise testing, derived from a Swedish population, [45] and there have been discussions regarding which set of reference values should be used for CPET. No Swedish reference material for CPET is available and the common sets of reference values used in Sweden have not been evaluated in a general Swedish population.

To establish new reference values from a Swedish population is both expensive and time-consuming. Thus, a relevant initial step is to test if the existing sets of reference values available internationally appear to reliably predict CPET variables.

Evaluation of lung function with spirometry

Spirometry measures respiratory volumes and flows as well as diffusion capacity between the lungs and blood. It is a cost-efficient and well-established method, which is widely available. Spirometry is a diagnostic instrument for measuring the effect of disease on pulmonary function, assessing therapeutic intervention and assessing prognosis of a reduced lung function. It is also used as a screening test for individuals at risk of having pulmonary disease, as well as for screening of general respiratory health. [52]

Evaluation of cardiac function with echocardiography

Ultrasound of the heart (echocardiography) is today one of the primary methods for examination of most heart diseases. With moving real-time imagery, it provides information about the dimensions and function of the heart, which is valuable in almost all heart diseases. Used in combination with Doppler echocardiography, including color flow mapping, it also enables visualization of blood flow through the valves, and grading of any leakage or stenosis.

Thus, echocardiography is a very powerful method of investigation, with the further advantage of having no side effects. This also makes it useful as a screening tool. [53-55]

Study aims

Study I

- To compare $V_{O_{2peak}}$ in a population of healthy, 50-year-old Swedes with four of the most commonly used international reference values. [3-5]
- To analyze peak workload and $V_{O_{2peak}}$ in relation to achieved RER (> 1.1), BR at peak load and self-reported physical activity.

Studies II–IV

The overall aim for Studies II–IV was to find a clinical physiological examination method which could be recommended as the best suited for the long-term follow-up regimen of patients undergoing HCT.

Study II

- To compare the exercise capacity in a group of young adults treated with HCT and TBI in childhood with a group of healthy individuals.
- To ascertain whether exercise capacity was associated with lung function, heart function and/or levels of growth hormone (GH).

Study III

- To study long-term consequences on cardiac function, assessed by means of echocardiography and levels of NT-proBNP, in the same group of long-term survivors after HCT and TBI as in Study II.

Study IV

- To study which CPET parameters were affected in a long-term follow-up of the same HCT patients as in Studies II and III, compared with healthy controls.
- To investigate the relationship between CPET parameters and spirometric and echocardiographic findings in this population.

Methods

Study populations

Prospective investigation of Obesity, Energy and Metabolism (POEM)

Study I is based on the first 345 subjects included in the POEM study, a study of 50-year-old inhabitants of Uppsala, Sweden. The purpose of the POEM study was to study pathophysiological links between obesity and vascular dysfunction, as well as future cardiovascular events. The participants were examined using several methods, including cardiopulmonary exercise testing. [56]

In this thesis' first study, subjects with diagnosis of or medication for cardiopulmonary diseases were excluded ($n = 73$). This included coronary heart disease, such as myocardial infarction, hypertension, stroke, heart failure, diabetes, asthma or chronic obstructive pulmonary disease (COPD). Subjects with pathological ECG at rest or during exercise, abnormally high blood pressure at rest or abnormal results in lung function testing were also excluded ($n = 48$).

Only subjects who had a $RER \geq 1.0$ and a heart rate of at least 85% of maximum heart rate (calculated as 220 minus subject age) at peak exercise were included. Furthermore, subjects for whom CPET or spirometry was not available were also excluded, leaving a final study population of 181 participants for Study I: 91 females and 90 males.

Long-term follow-up of HCT and controls (Studies II, III and IV)

Patients

Between October 1985 and June 1999, 45 patients with acute lymphoblastic leukemia (ALL) or lymphoblastic lymphoma (LBL) were treated with autologous ($n = 31$), syngeneic ($n = 1$) or allogeneic ($n = 14$) HCT. All patients except one were under the age of 18 years at the time of the treatment, with the remaining patient being 18.3 years old. Chemotherapy and TBI were also included in the treatment of all patients.

Of these subjects, 29 were still alive at the time of this study (autologous/syngeneic, $n = 22$; allogeneic, $n = 7$), which was conducted between

November 2006 and June 2009. Patients who had developed chronic graft-versus-host disease (cGVHD) and patients who had not reached their final height were excluded. [9]

The criteria were fulfilled by 25 patients, who were subsequently invited to participate in Studies II, III and IV (autologous/syngeneic, n = 22, allogeneic, n = 3). One female and six males declined to participate, leading to a final study group of fifteen autografted patients and three recipients of allogeneic grafts in Study III. [9] One of the female autografted patients failed to perform exercise tests, including CPET, due to avascular necrosis of the femoral head. This resulted in a final study group of 17 patients in Studies II and IV.

In their primary treatment, all patients were given anthracyclines with doses within the range of 225–600 mg/m². All patients also received pretransplant conditioning treatment with cyclophosphamide-based chemotherapy and TBI. Short-term methotrexate and cyclosporine, tapered over six months, was used as prophylaxis against GVHD.

Control group

Letters were sent to ten potential control subjects for each patient, to obtain one age- and sex-matched control subject for each. These control subjects were randomly selected from a digital register of the population in Uppsala County. Of those who, per mail, said they were willing to participate, the first one to reply to a phone call and who was eligible for the study, was selected for participation. Subjects were eligible if they did not report having any disease or use of regular medication (except contraceptives). Furthermore, subjects who were pregnant or currently smoking were excluded. If none of the ten potential subjects who fulfilled the inclusion criteria accepted the invitation, the process was repeated with a new set of ten potential control subjects.

Anthropometry and body composition data

Height and weight for the participants were measured in all four studies and body mass index (BMI) was calculated as body mass in kilograms divided by height in meters squared (kg/m²).

Dual energy X-ray absorptiometry (DXA) (Lunar Radiation, Madison, WI, USA) was used in Studies II and III to measure fat-free mass (FFM). FFM was presented in kilograms and fat mass was presented as percentage of body weight.

Exercise test, CPET (cardiopulmonary exercise test) and Holter monitor

In the exercise test in Study II, peak workload (W_{peak}) was measured using a symptom-limited incremental test on a bicycle ergometer (Case 8000 Exercise Testing System, GE Medical Systems, Milwaukee, WI, USA).

The protocol was chosen with a starting load of 50 watts (W) for all participants and, depending on the individual's self-rated exercise capacity, the load was increased by 10, 15 or 20 W every minute until exhaustion.

Electrocardiogram (ECG), heart rate and systolic blood pressure were recorded during the test and the participant's perceived exhaustion, dyspnea, and leg fatigue were recorded using standardized scales, the Borg RPE scale and the Borg CR-10 scale. [1, 2]

The absolute values of W_{peak} were presented, as well as the percentage of predicted ($W_{\text{peak}}\%$) in accordance with Nordenfelt *et al.* [57]

CPET in Study I was performed on a bicycle ergometer (Ergoline – Ergoselect 100/200) and gas exchange was assessed using JaegerOxycon Pro (Erich Jaeger GmbH, Hoechberg, Germany) for all participants in the study.

The subjects were instructed to cycle for as long as they could manage. The initial workload was set to 30 W for women and 50 W for men, with the load increased by 10 W each minute for both women and men.

During the test, the peak workload was recorded and ECG, minute ventilation (V_E), oxygen uptake (V_{O_2}) and carbon dioxide production (V_{CO_2}) were monitored continuously. Blood pressure was recorded automatically every minute.

Breathing reserve (BR) was calculated as $V_{E\text{peak}}$ subtracted from maximum voluntary ventilation (MVV), and expressed as percent of MVV. MVV was calculated as 40 times FEV_1 , i.e., forced expiratory volume during one second, as registered through spirometry. $V_{E\text{peak}}$ is the minute ventilation at the highest workload.

Respiratory exchange ratio (RER) was calculated as eliminated carbon dioxide divided by oxygen uptake (V_{CO_2}/V_{O_2}). Anaerobic threshold (AT) was defined as the point where RER passed 1.0. [3]

Measurements of $V_{O_2\text{peak}}$ were compared with four sets of reference values: Jones 1 and 2, SHIP and Hansen-Wasserman (HW). [3-5] In the prediction of $V_{O_2\text{peak}}$, all four sets of reference values are based on the subject's height, age and sex. The SHIP study and Jones 2 also take weight into account. [4, 5] The prediction formula of HW takes ideal weight into account, [3] whereas Jones 1 does not use weight as a parameter in the formula. [4]

The same exercise testing was used for both Study II and Study IV, with the sole difference that we, in Study IV, also used the measured value of the gas exchange, obtained through CPET.

After steady state measurements at rest were recorded, an incremental workload protocol was used with 50 W as the initial load and step increases by 10, 15 or 20 W every minute until exhaustion. The Borg RPE scale was used for subjective ratings of perceived exertion and the Borg CR-10 scale for dyspnea and leg fatigue. [1, 2]

Gas exchange was assessed using a mask with a turbine for gas exchange analysis (Oxycon Sigma, Jaeger, Germany). V_{O_2} , V_{CO_2} , V_E , and breathing frequency (BF) were measured with readings every 30 seconds.

In addition to the gas exchange parameters, ECG and heart rate were also monitored during the test.

BR, RER and AT were defined and calculated using the same methodology as in Study I. Ventilatory efficiency at anaerobic threshold was calculated as minute ventilation (V_E) at anaerobic threshold divided by carbon dioxide production at anaerobic threshold ($V_E/V_{CO_2@AT}$). [3]

When calculating predicted oxygen uptake at the highest workload, $V_{O_{2peak}}$, and predicted oxygen pulse at highest workload, peak O_2 pulse, Hansen-Wasserman's reference values were used. [3]

As a further investigation of heart rate and any arrhythmias, Holter monitoring, [58] i.e., long-term ECG recording, was performed during 24 hours in both patients and controls. The equipment used was MARS PC (GE Healthcare, Wauwatosa, WI, USA).

Spirometry

The spirometry in Study I was performed using the Oxycon Pro diagnostic system (Erich Jaeger GmbH, Hoechberg, Germany). For all subjects, the vital capacity (VC) and the forced expiratory volume in one second (FEV_1) were recorded. [52] For calculation of predicted values, Swedish reference values were used, in accordance with Hedenström *et al.* [59, 60]

As described under the section on the study population, participants with abnormal lung function were excluded from the study. Abnormal lung function was defined as FEV_1/VC ratio < 0.7 and/or VC $< 80\%$ of predicted and/or $FEV_1 < 80\%$ of predicted.

The pulmonary function in Studies II and IV was assessed for both the patients and the controls using a Jaeger MasterLab system (Erich Jaeger GmbH, Hoechberg, Germany) that enabled measurements of lung volumes and dynamic flow curves, as well as recording diffusing capacity for carbon monoxide (DLCO). [9] The detailed results from spirometry have been published previously [9] and are not included in this thesis.

The spirometry parameters included in these studies were total lung capacity (TLC), MVV, FEV_1 , DLCO and $DLCO_c$ (DLCO corrected for hemo-

globin (Hb)). The lung function variables were expressed as absolute values in Study IV. In Study II, they were expressed as percentages of predicted values, in accordance with Hedenström *et al.*, to facilitate comparisons [59, 60]

Study IV also included the diffusing capacity of lungs for carbon dioxide corrected for alveolar volume (DLCO_c/VA), which was based on the findings of Mathiesen *et al.* [7]

Echocardiography

Both patients and controls in Studies II–IV underwent standard two-dimensional echocardiographic investigations. The investigations were performed in accordance with international guidelines, at the same laboratory, and corrected for body surface area (BSA) when applicable. [61-63] Three different cardiac ultrasound units were used: Philips iE33, Philips Sonos 5500 (Philips Healthcare, Eindhoven, The Netherlands) and General Electric Vivid E9 (GE Healthcare, Wauwatosa, WI).

All echocardiographic measurements were made offline by one researcher (the author of this thesis), unaware of the clinical data of the subjects. All presented parameters were average values from three cardiac cycles.

Left atrial volume was measured, as well as interventricular septal thickness and left ventricular posterior wall thickness. The diameter of the left ventricle was measured, both in end diastole and end systole, and from that the left ventricular fractional shortening (LV-FS) was calculated. Left ventricular mass was calculated in accordance with Devereux *et al.* [64] As an estimation of after load, left ventricular end-systolic wall stress was calculated in accordance with Reichek *et al.* [65]

Left ventricular end-diastolic and end-systolic volumes were measured in accordance with the modified Simpson's biplane rule. [55] Both the stroke volume and the ejection fraction of the left ventricle were calculated based on these volumes. As a further assessment of left ventricular systolic function, mitral annular plane systolic excursion (MAPSE) was measured. [66]

Transmitral flow velocities were recorded, as a measurement of left ventricular diastolic function. Furthermore, the peak velocity of early rapid filling (E) and the peak velocity of atrial filling (A) were recorded. The E to A ratio (E/A) was calculated and the deceleration time (DT) was measured.

Right ventricular function was assessed through measurement of tricuspid annular plane systolic excursion (TAPSE). [62] The most commonly used estimate of pulmonary artery pressure uses the highest flow velocity in tricuspid regurgitation, [67] but as measurable leakage in the tricuspid valve was found in only a few subjects in this study, the tricuspid regurgitation could not be used to estimate the pulmonary systolic pressure.

Instead, the measure of the acceleration time of the pulmonary systolic flow velocity (Pu-acc time) was used to estimate the pulmonary systolic pressure. [68]

The detailed echocardiographic results have been presented in Study III.

Only the cardiac function variables with statistically significant differences between the patient and control groups were included in Studies II and IV. In Study II the exercise capacity was correlated to LV-FS, the E/A-ratio, MAPSE and TAPSE. In Study IV the CPET parameters V_{O2peak} , BR_{peak} , $V_E/V_{CO2@AT}$ and O_2 -pulse were correlated to LV-FS, MAPSE, TAPSE, A-wave, E/A-ratio and Pu-acc-time.

Smoking habits

In Study I, smoking history was self-reported and smoking history was measured in pack-years. The participants were classified as never, previous or current smokers.

Physical activity

In Study I, physical activity was graded using a previously used questionnaire. [69] This included questions on how many times per week the person exercised for a duration of at least 30 minutes at a low or medium/high intensity.

Based on the answers to the questions, the participants in Study I were divided in two groups: those who exercised at a medium or high intensity at least once a week and those performing only low intensity exercise.

Biochemical markers

Spontaneous growth hormone (GH) secretion was measured in Study II and Study III, using blood sampling every 30 minutes through the night (12 hours). This resulted in a total of 24 samples per participant.

GH was measured in mU/liter and the maximum peak value (GH_{peak}) was used for correlation analysis. Growth hormone deficiency was defined as $GH_{peak} < 9$ mU/liter. [70]

In Study III, the level of NT-proBNP in blood was measured as an indicator of cardiac function. [71]

Statistics

All continuous variables (sex, RER, BR and fitness level) in Study I were analyzed using unpaired t-tests.

Comparisons of categorical variables (BMI and smoking history) in Study I were carried out using chi-square tests.

Paired t-tests were used in the comparison of CPET parameters and different sets of reference values in Study I, and in all analyses of the study population and intergroup comparisons of continuous variables in Studies II, III and IV.

Comparisons between self-reported variables (physical exhaustion, breathlessness and leg fatigue) in Study II were performed using the Wilcoxon test.

In Study II, intergroup comparisons of categorical variables were performed using the McNemar test.

The relationships between pairs of variables in Studies II, III and IV were evaluated using Pearson's correlation coefficient.

All p values were two-sided and considered to be statistically significant if $p < 0.05$.

Ethics

All four studies were approved by the Regional Ethical Review Board at Uppsala University, approval numbers 2005:327 and 2009/057. All participants gave written informed consent before inclusion in the study.

Results

Study I

Population characteristics

Most of the participants in the study, 51.4%, were overweight (BMI > 25 kg/m²) or obese (BMI ≥ 30 kg/m²). [72] A significant difference between male and female subjects was found, with more of the male subjects being obese (19% vs. 10%). No participant was underweight (BMI < 18.5 kg/m²). [72]

A significant part of the participants, more than one third, were previous or current smokers and no sex differences were found with regard to smoking habits.

Most participants, both males (68%) and females (60%), exercised at medium or high intensity levels for at least 30 minutes per week.

Peak workload and specific parameters for CPET in relation to reference values

Peak workload (W_{peak}) and peak oxygen uptake ($V_{\text{O}_2\text{peak}}$) are presented in Table 1, in both absolute values and percent predicted, based on the most commonly used reference values.

Both the male and female participants achieved peak workload around 100% of predicted, based on the reference values of Brudin *et al.* [45]

Table 1. Peak workload (W_{peak}) and peak oxygen uptake (V_{O2peak}) in the study group of Study I and in comparison with the most commonly used reference values. All values expressed as means (SD). p values are given for comparisons between females and males and values < 0.05 are considered significant.

	<i>All</i> (<i>n</i> = 181)	<i>Females</i> (<i>n</i> = 91)	<i>Males</i> (<i>n</i> = 90)	<i>p</i> value
W_{peak} (W)	185.6 (50.4)	153.1 (30.3)	218.5 (45.1)	< 0.001
W_{peak} (% pred Brudin)	99.7 (18.3)	100.9 (18.2)	98.6 (18.6)	0.46
V_{O2peak} (ml/min)	2 242.2 (632.8)	1 797.0 (314.4)	2 692.4 (549.8)	< 0.001
V_{O2peak} (% pred HW)	107.9 (19.8)	115.3 (18.1)	103.4 (19.6)	< 0.001
V_{O2peak} (% pred SHIP)	107.7 (19.0)	111.4 (17.3)	104.1 (20.0)	< 0.001
V_{O2peak} (% pred Jones 1)	99.1 (20.8)	108.9 (20.2)	93.5 (17.6)	< 0.001
V_{O2peak} (% pred Jones 2)	100.5 (19.4)	107.8 (17.2)	96.1 (19.8)	< 0.001

Abbreviations: W_{peak} , peak workload in watts and in comparison with Brudin [45]; V_{O2peak} , peak oxygen uptake in ml/minute and in comparison with the reference values of HW (Hansen-Wasserman) [3], the SHIP study [5] and Jones 1 and 2 [4].

Significant differences in peak oxygen uptake between females and males were found when expressing the values as percent of predicted, based on all four reference value sets. For females, the predicted values for V_{O2peak} were underestimated ($> 100\%$ predicted) by all reference sets. This underestimation was most pronounced when using HW (Table 1).

The smallest difference between the predicted value and actual data for V_{O2peak} was found using the Jones 2 set of reference values (Table 1).

A visual comparison of the V_{O2peak} values between the studied subjects and the four reference values can be seen in Figure 1.

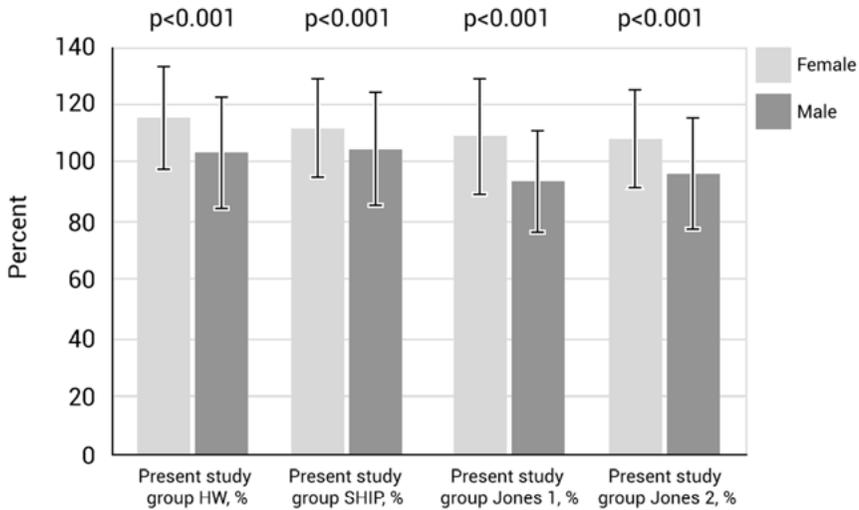


Figure 1. Peak oxygen uptake of the participants in Study I in comparison (%) with the reference values – HW = Hansen-Wasserman, SHIP, Jones 1 and 2.

Reference values for the ventilatory efficiency at the anaerobic threshold, $V_E/V_{CO2@AT}$, were available in only one of the four studied sets of references, the SHIP study. [5] The values in the present study corresponded to 105% of the predicted values in both males and females.

Peak workload and V_{O2peak} in relation to RER and BR

More than half of the subjects in the study, 56%, reached a $RER \geq 1.1$. However, the W_{peak} and V_{O2peak} values reached showed no major differences between those with $RER \geq 1.1$ at peak workload and those with RER between 1.0 and 1.1, see Table 2. Also, the fitness degree was not significantly different between these groups (Table 2).

Approximately two-thirds of the subjects in the study group had $BR > 30\%$ at peak load. In the remainder of the study group ($BR \leq 30\%$), only six participants showed a $BR < 15\%$.

The group of participants with $BR \leq 30\%$ had notably higher W_{peak} and also notably higher V_{O2peak} than the participants with $BR > 30\%$. See also Table 2.

Table 2. W_{peak} and V_{O2peak} in relation to RER and BR in Study I.

All values expressed as means (SD) with exception of physical activity expressed as %. p values ≤ 0.05 are considered significant.

	<i>RER 1.0 – 1.1</i> (<i>n = 79</i>)	<i>RER ≥ 1.1</i> (<i>n = 102</i>)	<i>p value</i>
W_{peak}	191.5 (53.1)	181.2 (48.0)	0.17
W_{peak} (% pred Brudin)	98.6 (15.8)	100.5 (20.1)	0.49
V_{O2peak} (ml/min)	2 328.7 (649.5)	2 176.7 (615.0)	0.11
V_{O2peak} (ml/min/kg)	28.9 (6.9)	29.9 (6.4)	0.30
Medium-high intensity exercise (%)	68.0	59.8	0.26
	<i>BR ≤ 30 %</i> (<i>n = 57</i>)	<i>BR > 30 %</i> (<i>n = 124</i>)	<i>p-value</i>
W_{peak}	220.4 (51.8)	169.6 (40.9)	< 0.001
W_{peak} (% pred Brudin)	110.6 (18.5)	94.6 (16.9)	< 0.001
V_{O2peak} (ml/min)	2 709.5 (641.6)	2 027.4 (500.8)	< 0.001
V_{O2peak} (ml/min/kg)	34.3 (7.0)	27.2 (5.4)	< 0.001
Medium-high intensity exercise (%)	68.4	61.5	0.37

Abbreviations: W_{peak} , peak workload in watts and in % of predicted according to Brudin [45]; V_{O2peak} , peak oxygen uptake; RER, respiratory exchange ratio; BR, breathing reserve; Medium or high intensity exercise, the proportion of the study population who exercised at medium or high intensity levels for at least 30 minutes per week.

Studies II–IV

Population characteristics and pulmonary function

The population characteristics and spirometric data of both patients and controls included in Studies II and IV are showed in Table 3. It should be noted that Study III included an additional female patient and control.

Table 3. Basic data and spirometric data of the HCT-patients and the healthy age- and sex-matched controls.

Age at HCT and age at study expressed as median (range). Remaining results expressed as means (SD). p values ≤ 0.05 are considered significant.

	<i>Patients (SD)</i> <i>n = 17*</i> <i>(F = 7, M = 10)</i>	<i>Controls (SD)</i> <i>n = 17*</i> <i>(F = 7, M = 10)</i>	<i>p value</i>
<i>Age at HCT (years)</i>	9.8 (5.6-18.3)		
<i>Age at study (years)</i>	27.0 (17.3-37.1)	27.3 (18.9-38.6)	0.88
<i>Height (cm)</i>	168 (9.4)	175 (7.2)	0.001
<i>Weight (kg)</i>	64 (14.1)	74 (12.5)	0.01
<i>BMI (kg/m²)</i>	22.7 (4.1)	24.1 (3.2)	0.17
<i>Fat mass (% of total weigh)</i>	31.7 (11.3)	24.2 (10.7)	0.01
<i>FFM (kg)</i>	42.8 (8.2)	57.6 (11.5)	< 0.001
<i>Spirometry</i>			
<i>TLC (L)</i>	4.7 (1.2)	6.9 (1.1)	< 0.001
<i>TLC (% of predicted)</i>	77.9 (15.0)	104.0 (10.8)	< 0.001
<i>VC (L)</i>	3.42 (1.0)	5.24 (0.8)	< 0.001
<i>VC (% of predicted)</i>	75.0 (11.4)	101.0 (10.1)	< 0.001
<i>FEV₁ (L)</i>	2.9 (0.8)	4.1 (0.6)	< 0.001
<i>FEV₁ (% of predicted)</i>	76.7 (11.1)	100.3 (10.3)	< 0.001
<i>FEV₁/VC</i>	0.86 (0.07)	0.79 (0.08)	< 0.01
<i>DLCO_c (μmol/s/kPa)</i>	123.0 (25.6)	181.9 (34.8)	< 0.001
<i>DLCO_c (% of predicted)</i>	73.1 (11.2)	104.3 (13.9)	< 0.001
<i>DLCO_c/VA (μmol/s/kPa/l)</i>	28.6 (4.2)	29.3 (4.3)	0.63
<i>D LCO_c/VA (% of predict)</i>	93.9 (12.0)	102.1 (16.2)	0.27

Abbreviations: F, female; M, male; HCT, hematopoietic cell transplantation; BMI, body mass index; FFM, fat-free mass; TLC, total lung capacity; VC, vital capacity; FEV₁, forced expiratory volume in 1 s; DLCO_c, diffusing capacity of lungs for carbon dioxide corrected for Hb; DLCO/VA, diffusing capacity of lungs for carbon dioxide corrected for alveolar volume.

**) One additional female patient and control participated in the echocardiographic study.*

The patients and control group were matched for age and sex, but showed significant differences in body size and body composition.

The patients weighed less and were shorter than the controls. The patients also had less BSA than the controls ($1.72 \pm 0.20 \text{ m}^2$ vs. $1.90 \pm 0.17 \text{ m}^2$, $p < 0.05$), but there was no difference in body mass index (BMI) between the groups. The percentage of fat mass was significantly higher and FFM was significantly lower in the patients than in the controls.

Detailed spirometry data has been published previously. [9] Briefly, the patient group had reduced TLC, VC and FEV₁ both in absolute values and in percent of predicted [59, 60], see Table 3.

The patients also had reduced DLCO and DLCO_c compared with the controls, but no significant difference was found in DLCO_c/VA between the patients and controls, see Table 3.

Biochemical markers

For both the patients and controls, NT-proBNP was within the normal range, while the mean value for NT-proBNP was significantly higher for the patients than the controls ($77.9 \pm 55.0 \text{ ng/l}$ vs. $31.8 \pm 30.6 \text{ ng/l}$, $p < 0.01$).

A significantly lower GH_{peak} was measured in the patient group than in the control group ($9.7 \pm 7.7 \text{ mU/l}$ vs. $20.7 \pm 11.4 \text{ mU/l}$, $p < 0.01$).

Echocardiography

All measurements of echocardiographic variables were in the range of normal values, though a few of these measurements were close to the limits of the normal range.

Differences between patients and control were seen for some of the measurements; there was a difference in size of the left atrial volume (41.2 ml for patients vs. 48.8 ml for controls), and in the end-diastolic diameter of the left ventricle (4.4 cm in patients vs. 5.0 cm in controls). These differences were no longer found after correction for BSA.

The echocardiographic data, with significant differences between the patient and control group, are shown in Table 4.

Table 4. Echocardiographic data with significant difference between patients and healthy age- and sex-matched controls.

All values expressed as means (SD). p values ≤ 0.05 are considered significant.

	<i>Patients (SD)</i> <i>n = 18</i> <i>(F = 8, M = 10)</i>	<i>Controls (SD)</i> <i>n = 18</i> <i>(F = 8, M = 10)</i>	<i>p value</i>
<i>LV-FS</i>	0.30 (0.04)	0.34 (0.05)	< 0.05
<i>MAPSE (cm)</i>	1.14 (0.12)	1.37 (0.19)	< 0.001
<i>TAPSE (cm)</i>	2.3 (0.33)	2.49 (0.34)	< 0.01
<i>A (cm/s)</i>	50.4 (13.3)	31.6 (7.4)	< 0.01
<i>E/A</i>	1.4 (0.4)	2.2 (0.6)	< 0.001
<i>Pu-acc time (ms)</i>	117.4 (19.0)	135.9 (19.8)	< 0.05

Abbreviations: LV-FS, left ventricular fractional shortening; MAPSE, mitral annular plane systolic excursion; TAPSE, tricuspid annular plane systolic excursion; A, A wave from the flow profile of the mitral valve; E/A, the ratio between the E wave and the A wave from the flow profile of the mitral valve; Pu-acc time, acceleration time of the pulmonary valve flow.

Looking at the measurements that assess left ventricular systolic function, differences were seen in the fractional shortening (LV-FS) and MAPSE, where the measured values were lower in the patient group than in the control group. This can be seen in Table 4, and is represented visually in Figures 2 and 3.

No significant differences were seen between the patients and controls regarding stroke volume when corrected for BSA or EF (60.4% vs. 59.6% in mean). This also applied for the left ventricular end-systolic wall stress, where also no correlation with LV-FS was seen.

Among measurements characterizing right heart, shorter Pu-acc time and lower TAPSE was found in patients compared with controls (see Table 4 and Figure 3).

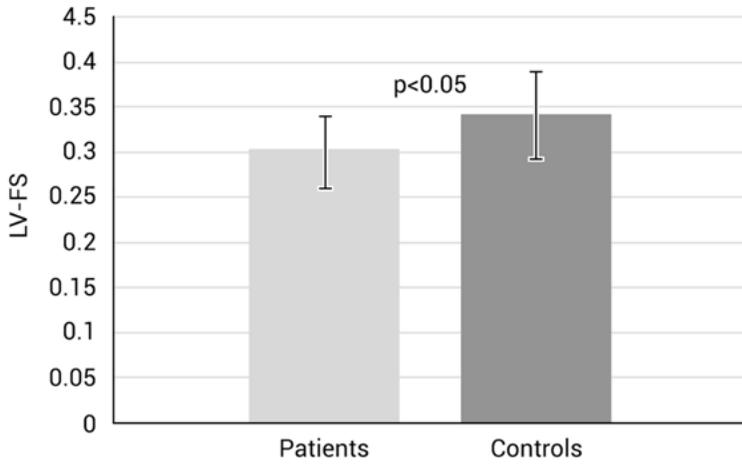


Figure 2. Left ventricular fractional shortening (LV-FS) in patients and controls.

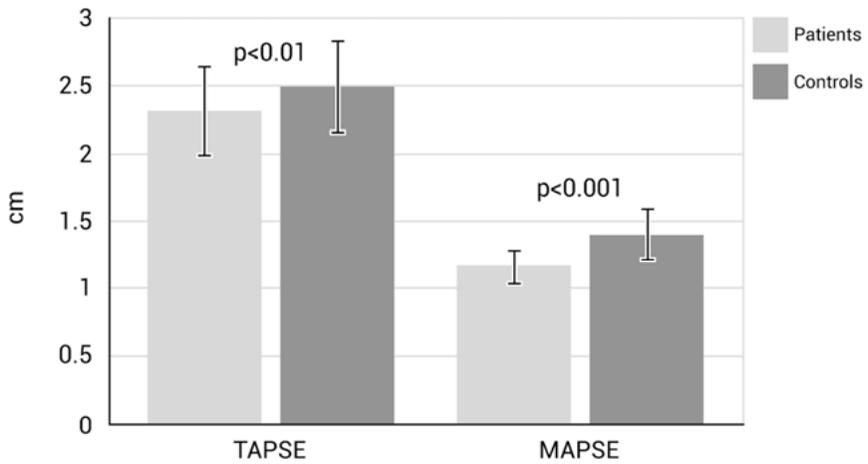


Figure 3. Mitral annular plane systolic excursion, MAPSE, and tricuspid annular plane systolic excursion, TAPSE, in patients and controls.

The E/A ratio, a diastolic measurement of the left ventricle, was found to be notably lower in the patient group when compared with the control group, see Figure 4. This was mainly due to a higher A wave in the patients.

The E/A ratio showed a positive correlation with GH_{peak} ($r = 0.48$, $p < 0.05$) and a negative correlation with NT-proBNP ($r = -0.48$, $p < 0.05$).

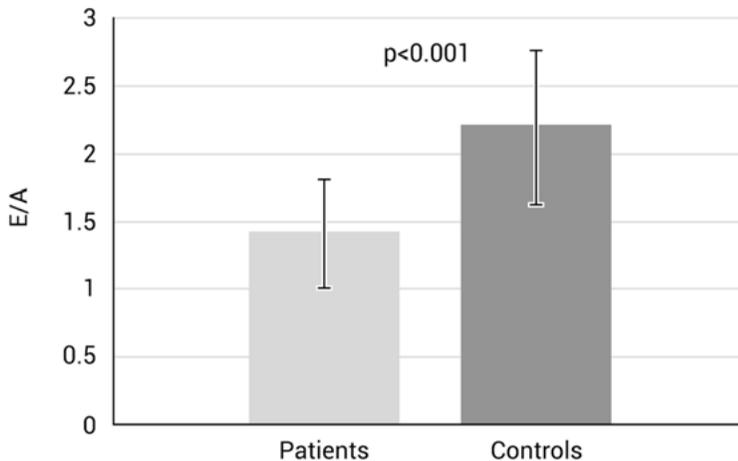


Figure 4. The ratio between the E wave and the A wave from the flow profile of the mitral valve, E/A, in patients and controls.

No major valvular heart disease was found, either when using color flow mapping or when using spectral Doppler.

In this group of patients, no echocardiographic correlations with the doses of anthracyclines were seen.

Exercise test and CPET

The exercise capacity and CPET data are presented in Table 5, for both the patients and the healthy control group.

Table 5. Exercise capacity and CPET data of patients and healthy age- and sex-matched controls.

All results expressed as means (SD), p values ≤ 0.05 were considered significant.

	<i>Patients (SD)</i> <i>n = 17</i> <i>(F = 7, M = 10)</i>	<i>Controls (SD)</i> <i>n = 17</i> <i>(F = 7, M = 10)</i>	<i>p value</i>
W_{peak} (watt)	147.1 (45.1)	244.7 (54.7)	< 0.001
W_{peak}/kg (watt)	2.3 (0.7)	3.3 (0.6)	< 0.001
W_{peak}/kg FFM (watt)	3.3 (0.6)	4.3 (0.4)	< 0.001
W_{peak} (% predicted)	63.2 (10.5)	96.1 (9.5)	< 0.001
HR_{rest} (beats/min)	75.7 (8.9)	67.1 (13.9)	0.04
HR_{peak} (beats/min)	182.4 (9.2)	183.8 (10.2)	0.66
ΔSBP (mmHg)	59.7 (16.1)	70.0 (19.0)	0.10
VO_{2peak} (ml/min)	1 929.2 (672.6)	3 002.4 (664.6)	< 0.001
VO_{2peak} (% predicted)	75 (21.8)	101 (17.2)	< 0.001
Peak O_2 pulse (ml/min/beat)	10.6 (3.8)	16.4 (4.0)	< 0.001
Peak O_2 pulse (% predicted)	80.2 (24.8)	106.2 (18.6)	< 0.001
BF_{peak} (breaths/min)	40.4 (11.3)	37.4 (6.1)	0.33
TV_{peak} (L)	1.91 (0.54)	2.72 (0.55)	< 0.001
VE_{peak} (L/min)	74.8 (22.1)	100.7 (21.7)	0.001
BR_{peak} (L/min)	42.4 (24.6)	63.5 (24.2)	0.017
BR_{peak} % of MVV	35.3 (14.1)	38.2 (12.1)	0.54
RER_{peak}	1.2 (0.2)	1.2 (0.1)	0.171
$V_E/VCO_{2@AT}$	27.4 (2.3)	25.5 (3.0)	0.048
$VO_{2@AT}/pred VO_{2peak}$ (%)	51.8 (14.5)	71.3 (20.7)	< 0.001

Abbreviations: W_{peak} , peak workload; FFM, fat free mass; HR_{rest} , heart rate at rest; HR_{peak} , heart rate at peak workload; ΔSBP , systolic blood pressure increase during work; VO_{2peak} , peak oxygen uptake; O_2 pulse, oxygen pulse; BF_{peak} , breath frequency at peak workload; TV_{peak} , tidal volume at peak workload; VE_{peak} , ventilation at peak workload; BR_{peak} , breath reserve at peak workload; MVV, maximal voluntary ventilation; RER, respiratory exchange ratio; $V_E/VCO_{2@AT}$, ventilatory efficiency at anaerobic threshold; $VO_{2@AT}/pred VO_{2peak}$ (%), oxygen uptake at anaerobic threshold in % of predicted peak oxygen uptake.

The mean peak workload was nearly 100 W lower for the patients compared with the controls (Table 5 and Figure 5). The difference regarding peak workload was consistent also when taking into account weight (Table 5) and fat-free mass ($W_{\text{peak}}/\text{kg FFM}$) (Table 5 and Figure 6). For both sexes, patients achieved significantly lower peak workload adjusted for fat-free mass than their respective controls (male 3.6 vs. 4.3 watt/kg, $p < 0.01$; female 3.0 vs. 4.2 watt/kg, $p < 0.01$).

Also in the analyses of W_{peak} , expressed as a percentage of the predicted value, the patients achieved significantly worse values than the controls, a difference that could be seen in both sexes (male 65.1% vs. 96.1%, $p < 0.01$; female 60.6% vs. 96.0%, $p < 0.01$).

The measured W_{peak} value was below 80% of the predicted value for all the studied patients, whereas only one of the controls displayed a similarly low value (100% vs. 6%, $p < 0.001$).

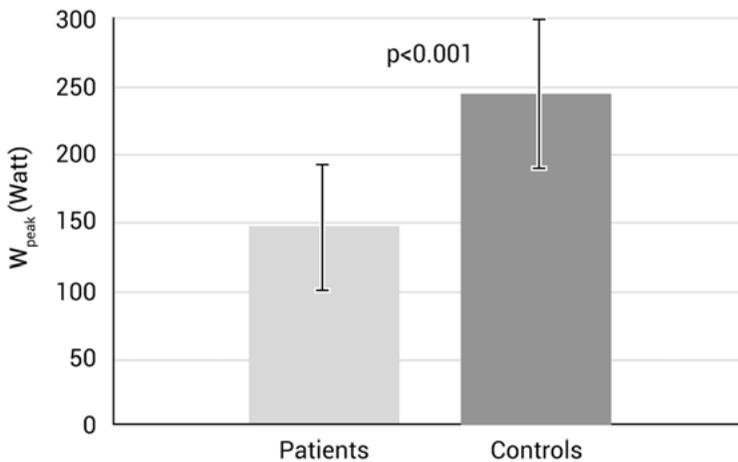


Figure 5. Peak exercise capacity (W_{peak}) in patients and controls.

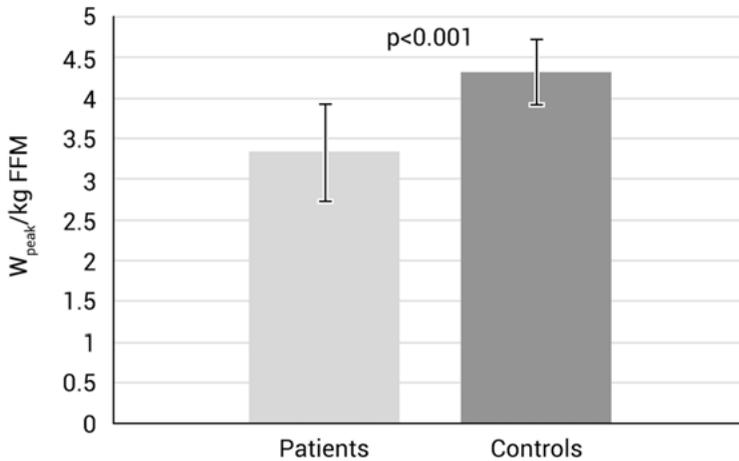


Figure 6. Peak exercise capacity adjusted for fat-free mass ($W_{\text{peak}}/\text{kg FFM}$) in patients and controls.

The resting heart rate was notably higher in the patients compared with the control group. This difference was also observed by means of 24-hour ECG monitoring, which provided further verification. No other significant differences between patients and controls were found through the long-term ECG-monitoring.

At peak workload, no significant difference was observed between the patients and the control group, either in terms of heart rate (both in absolute values and as percentage of maximum heart rate) or in self-reported physical exhaustion or breathlessness.

However, the participants in the control group reported a higher level of leg fatigue than the patients. On the Borg CR-10 scale (scale 1–10; none at all to extremely strong) [1], the value in the patient group was 6.5 ± 3 vs. 9 ± 1 in the control group, $p < 0.05$.

Patients were found to have significantly lower peak oxygen uptake ($\text{VO}_{2\text{peak}}$) both in absolute values and in percent of predicted [3] compared with controls (Table 5 and Figure 7).

Also when adjusted for body weight, $\text{VO}_{2\text{peak}}$ was lower in the patient group than in the control group (mean 31.0 mL/kg/min vs. 40.8 mL/kg/min , $p = 0.02$).

Oxygen uptake at anaerobic threshold in percent of predicted peak oxygen uptake ($\text{VO}_{2@AT}/\text{pred } \text{VO}_{2\text{peak}} \%$) was also reduced in the patient group compared with the control group (Table 5).

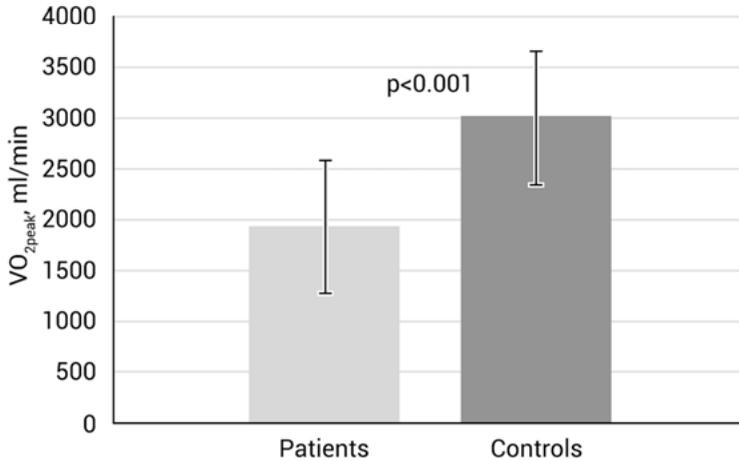


Figure 7. Peak oxygen uptake ($VO_{2\text{peak}}$) in patients and controls.

The patients had significantly reduced peak oxygen pulse (O_2 pulse) compared with the controls, both in absolute values and in percent of predicted [3] (Table 5 and Figure 8).

Already at the anaerobic threshold, the O_2 pulse was significantly reduced for the patients when compared with the controls (mean 9.2 ml/beat vs. 14.1 ml/beat, $p < 0.001$).

At rest, prior to the exercise test, no difference in O_2 pulse was observed between patients and controls (mean 4.7 ml/beat in patients vs. 5.3 ml/beat in controls, $p = 0.19$).

In comparison with the control group, the patients also showed significantly reduced peak tidal volume (TV_{peak}), peak ventilation (VE_{peak}) and peak breathing reserve (BR_{peak}) (Table 5).

The ventilation efficiency at the anaerobic threshold ($V_E/VCO_{2@AT}$) was also reduced in the patient group compared with the control group (Table 5 and Figure 9).

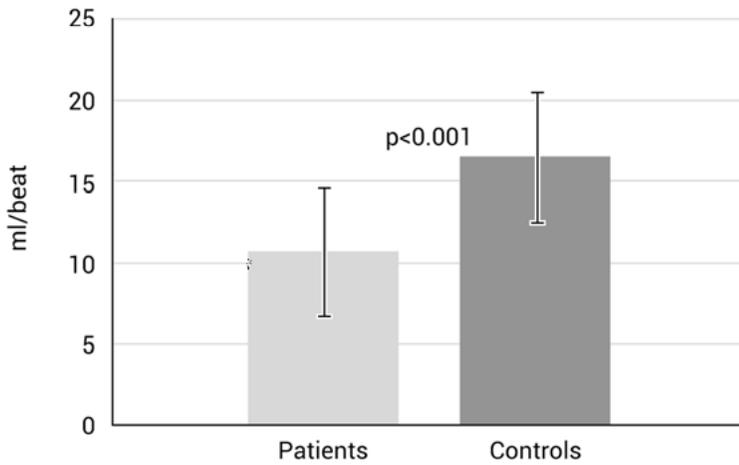


Figure 8. Peak oxygen pulse (O_2 pulse) in patients and controls.

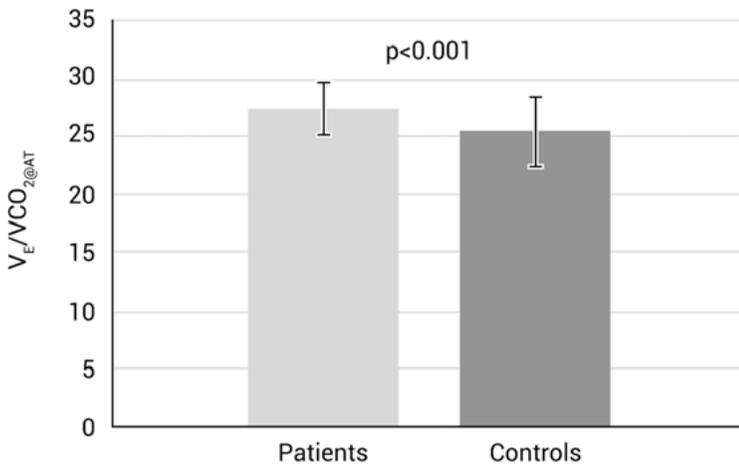


Figure 9. Ventilation efficiency at anaerobic threshold ($V_E/V_{CO_2@AT}$) in patients and controls

There were no significant differences found regarding breathing frequency at peak workload (BF_{peak}), breathing reserve in percent of maximal voluntary ventilation ($BR\%$ of MVV), or respiratory exchange ratio at peak workload (RER_{peak}) when comparing patients and controls, see Table 5.

Assessment of CPET data and exercise capacity in relation to pulmonary and cardiac function.

The correlations between VO_{2peak} and BR_{peak} and the spirometric and echocardiographic data are presented in Table 6 for all participants in the study (n = 34).

Similarly, the correlations between $V_E/VCO_{2@AT}$, and O_2 pulse and the spirometric and echocardiographic parameters for all participants (n = 34) are presented in Table 7.

Table 6. Peak oxygen uptake (VO_{2peak}) and breathing reserve at peak load (BR_{peak}) in relation to spirometric and echocardiographic parameters for all participants in the study (n = 34).

*p values between 0.01 and 0.05, **p values between 0.01 and 0.001, ***p values < 0.001

	VO_{2peak}		BR_{peak}	
	<i>rho</i>	<i>p value</i>	<i>rho</i>	<i>p value</i>
<i>LV-FS</i>	0.13	0.53	-0.07	0.75
<i>MAPSE</i>	0.65***	< 0.0001	0.08	0.70
<i>TAPSE</i>	0.65***	< 0.0001	-0.01	0.95
<i>A</i>	-0.47*	0.014	-0.17	0.40
<i>E/A</i>	0.53**	0.004	0.10	0.61
<i>Pu-acc time</i>	0.40*	0.038	0.10	0.61
<i>TLC</i>	0.74***	< 0.0001	0.63***	< 0.0001
<i>VC</i>	0.74***	< 0.0001	0.67***	< 0.0001
<i>FEV₁</i>	0.71***	< 0.0001	0.73***	< 0.0001
<i>FEV₁/VC</i>	-0.40*	0.039	-0.17	0.39
<i>DLCO_c</i>	0.87***	< 0.0001	0.34	0.08
<i>DLCO_c/VA</i>	0.32	0.10	-0.36	0.07

Abbreviations: LV-FS, left ventricular fractional shortening; MAPSE, mitral annular plane systolic excursion; TAPSE, tricuspid annular plane systolic excursion; A, A wave from the flow of the mitral valve; E/A, the ratio between the E wave and the A wave from the flow profile of the mitral valve; Pu-acc time, acceleration time of the pulmonary valve flow; TLC, total lung capacity; VC, vital capacity; FEV₁, forced expiratory volume in 1 s; DLCO_c, diffusing capacity of lungs for carbon dioxide corrected for Hb; DLCO_c/VA, diffusing capacity of lungs for carbon dioxide corrected for alveolar volume.

Table 7. Ventilatory efficiency at anaerobic threshold ($V_E/VCO_{2@AT}$) and oxygen pulse (O_2 pulse) in relation to spirometric and echocardiographic parameters for all participants in the study (n = 34).

*p values between 0.01 and 0.05, **p values between 0.01 and 0.001, ***p values < 0.001

	$V_E/VCO_{2@AT}$		O_2 pulse	
	<i>rho</i>	<i>p value</i>	<i>rho</i>	<i>p value</i>
<i>LV-FS</i>	-0.09	0.65	0.09	0.65
<i>MAPSE</i>	-0.24	0.24	0.64***	< 0.0001
<i>TAPSE</i>	-0.25	0.21	0.66***	< 0.0001
<i>A</i>	0.37	0.059	-0.42*	0.031
<i>E/A</i>	-0.41*	0.03	0.49**	0.009
<i>Pu-acc time</i>	-0.08	0.70	0.39*	0.047
<i>TLC</i>	-0.59**	0.001	0.73***	< 0.0001
<i>VC</i>	-0.60**	0.001	0.72***	< 0.0001
<i>FEV₁</i>	-0.56**	0.003	0.69***	< 0.0001
<i>FEV₁/VC</i>	0.39*	0.047	-0.40*	0.040
<i>DLCO_c</i>	-0.75***	<0.0001	0.84***	< 0.0001
<i>DLCO_c/VA</i>	-0.33	0.09	0.28	0.16

Abbreviations: LV-FS, left ventricular fractional shortening; MAPSE, mitral annular plane systolic excursion; TAPSE, tricuspid annular plane systolic excursion; A, A wave from the flow of the mitral valve; E/A, the ratio between the E wave and the A wave from the flow profile of the mitral valve; Pu-acc time, acceleration time of the pulmonary valve flow; TLC, total lung capacity; VC, vital capacity; FEV₁, forced expiratory volume in 1 s; DLCO_c, diffusing capacity of lungs for carbon dioxide corrected for Hb; DLCO_c/VA, diffusing capacity of lungs for carbon dioxide corrected for alveolar volume.

In all participants (n = 34), VO_{2peak} and O_2 pulse correlated significantly with both the echocardiographic values of MAPSE, TAPSE, A wave, E/A ratio and Pu-acc time and the spirometric values of TLC, VC, FEV₁, FEV₁/VC and DCLO_c.

Significant correlation was also found in all participants between BR_{peak} and $V_E/VCO_{2@AT}$, on one hand, and TLC, VC, and FEV₁ on the other hand.

Additionally, significant correlations were found between $V_E/VCO_{2@AT}$ and the spirometric parameters FEV₁/VC and DLCO_c.

When the patients and controls were analyzed separately ($n = 17$ in each group), significant correlations were found, in both the patient and control groups, between V_{O2peak} , $V_E/V_{CO2@AT}$ and O_2 pulse on one hand and $DLCO_c$ on the other hand ($p < 0.05$).

In both the patients and controls, respectively, there was also a significant correlation between BR_{peak} and FEV_1 ($p < 0.05$).

In the same separate analysis, the correlations between V_{O2peak} and MAPSE ($\rho = 0.62$ and $p = 0.02$) and between O_2 pulse and MAPSE ($\rho = 0.61$ and $p = 0.02$) were significant in the patient group only ($n = 17$).

Significant correlation was also found in the patient group only between BR_{peak} and TLC ($\rho = 0.69$ and $p = 0.007$), between BR_{peak} and VC ($\rho = 0.78$ and $p = 0.001$), between BR_{peak} and TAPSE ($\rho = -0.59$ and $p = 0.03$), and between BR_{peak} and $DLCO_c/VA$ ($\rho = -0.66$ and $p = 0.01$).

For the patients, peak exercise capacity in percentage of predicted values [57], $W_{peak\%}$, displayed a significant correlation with TLC ($r = 0.54$, $p = 0.03$) and with MVV ($r = 0.54$, $p = 0.03$), both also expressed as a percentage of the predicted values [59, 60].

However, W_{peak} correlated neither with FEV_1 or $DLCO$ nor with any of the echocardiographic variables.

Inverse correlations were found between the percentage of fat mass and both W_{peak}/kg FFM and W_{peak}/kg body weight ($r = -0.58$, $p = 0.02$ and $r = -0.84$, $p < 0.001$, respectively).

Discussion

$V_{O_{2peak}}$ in relation to reference values

In Study I, CPET variables in a population of healthy, 50-year-old Swedes, were compared with four of the most commonly used international reference values: Jones 1 and 2, the SHIP study and Hansen-Wasserman. [3-5] The main finding was that $V_{O_{2peak}}$ was underestimated in all subjects with both the SHIP and Hansen-Wasserman equations, two of the most commonly used reference equations.[3, 5] In comparison with Jones 1 and 2 [4], the measured differences to the references were minor, although an underestimation of $V_{O_{2peak}}$ in females was found here too. Further differences for Jones 1 and 2, as compared with the measured values, included a slight overestimation for males. This difference was slightly larger for Jones 1 than Jones 2.

In many of the CPET variables, including V_{O_2} , there is a variation with sex, age, body size, degree of physical fitness and the type of physical activity performed. This is also accounted for, to a varying extent, within the reference formulas. [3-5] The best prediction of $V_{O_{2peak}}$ was obtained with the oldest of the four references, Jones 2, which takes height, weight, sex and age into account. [4]

The results might in part be affected by the populations included in the studies used to set the reference values. On a general note, the subjects in the Jones *et al.* study, which was used as a basis for the Jones 1 and Jones 2 reference values, seems to be the most similar to the population in the present study. [4]

The SHIP study, which used the largest sample size, included patients with hypertension and patients on beta blocker medication. [5] This might have been a key difference compared with the population in Study I, where such patients were excluded in order to achieve the healthiest study population possible. This, in turn, might be one of the explanations for the underestimation of $V_{O_{2peak}}$ when using the reference values from the SHIP study.

Compared with the studied population, the participants in the SHIP study also had a higher BMI value, which might further contribute to the underestimation of $V_{O_{2peak}}$.

An underestimation of $V_{O_{2peak}}$ in females was found for all four reference value sets. This value can be affected by the level of physical fitness. While it is difficult to assess, it might be the case that the degree of physical fitness among 50-year-old women varies between the studied population and the studies underlying the reference values.

This might have changed with time. Study I is more recent than the reference studies, especially Jones *et al.*, and since knowledge of the importance of physical activity might be higher today than at the time of the older studies, particularly among women, this could lead to an underestimation when using the older reference value sets.

Patients using beta blockers were included in the SHIP study. This use was slightly higher among the female subjects than the male subjects (7% vs. 3%), which might also contribute to the more pronounced underestimation of $V_{O_{2peak}}$ for females, when using the SHIP study reference values.

After the publication of the first study in this thesis, a working group within the Swedish Society for Clinical Physiology conducted a validation of different sets of reference values in a larger age range. [73] Based on this, the current recommendation in Sweden is to use references from the SHIP study (Gläser 2010) for oxygen uptake ($V_{O_{2peak}}$) and ventilation slope (V_E/V_{CO_2} slope). [5]

O_{2peak} in relation to achieved RER and BR at peak exercise

According to guidelines, a RER > 1.1 at peak load is an indicator of an excellent effort. [48, 50]

However, no notable difference in either W_{peak} or $V_{O_{2peak}}$ was found between those with RER 1.0–1.1 and those with RER ≥ 1.1 . Nor was there any difference between the two groups when $V_{O_{2peak}}$ was adjusted for body weight. This indicates that a high degree of effort, subjectively estimated by the tested person, together with a chronotropic response and RER > 1.0, is sufficient for the CPET test to be valid.

A low BR is often used as a sign of ventilatory limitation. However, in this study of healthy subjects, better $V_{O_{2peak}}$, both adjusted and unadjusted for body weight, and higher W_{peak} were found in those participants who used a higher degree of the breathing reserve (BR $\leq 30\%$).

This suggests that a lower BR at peak load indicates a higher exercise capacity in healthy individuals.

Only six participants in the study could be classified as ventilatory limited, with a BR < 15%. [3, 48, 50, 74] Thus, most of the participants' BRs were within the range of normal values, although close to the lower limit of this range.

Long-term follow-up of cardiopulmonary function in leukemia patients after HCT

Survivors of leukemia have increased risk of both respiratory and cardiovascular dysfunction, as well as increased risk of impaired physical performance. [6] Such dysfunctions may take many years to develop, but there are few long-term follow-ups reported in these patients and no study, to the author's knowledge, includes evaluation of both cardiac and pulmonary function both at rest and during exercise.

These studies were unique in two ways: Firstly, the follow-up time was nearly two decades after HCT, and, secondly, the participants underwent spirometry, echocardiography, exercise test, CPET as well as Holter ECG at the same occasion.

This enabled a correlation of findings related to both cardiac and pulmonary function, using the results of both the exercise tests and the CPET investigations. Previous studies in the literature have not had all these test results available for a single population.

Lung function assessed using spirometry

The spirometry showed signs of restrictive lung disease, as reduced lung volumes were found in the patients when compared with healthy controls. The patients also had reduced diffusion capacity. However, this normalized after correction for the lung volumes.

The spirometry parameters that differed between the patient and control groups were included in the models used to assess the limitation in exercise capacity and CPET parameters.

Cardiac function assessed using echocardiography

In the long-term follow-up of patients, in median 18.2 years after treatment with HCT and TBI in childhood, the echocardiographic parameters were within the normal range with regard to both left ventricular dimensions and left ventricular systolic and diastolic function. [61, 62, 66, 75, 76]

Despite this, there was a significant difference between the patients and controls in certain parameters. The patient group had significantly reduced FS, MAPSE, E/A ratio and TAPSE, suggesting a relatively impaired both systolic and diastolic left ventricular function and a reduced right ventricular function in the patients compared with the controls.

In addition, the pulmonary flow acceleration time was significantly reduced in the patients compared with the controls, which might be due to the restrictive lung damage seen in the patient group. [9]

Vandekerckhove *et al.* found higher left ventricular end-systolic diameter and reduced septal wall thickness in a group of young teenagers investigated with a mean follow-up time of 6.6 years after HCT, when compared with normal values from literature. [10]

We could not find any such difference, although a size difference in left atrial volume and in left ventricular end-diastolic diameter was seen between the patients and controls. However, after indexing for BSA, these differences disappeared.

No correction for body size is currently recommended in the clinical praxis when measuring fractional shortening, variables reflecting diastolic function or AV plane systolic excursions. Despite this, it could not be excluded that a variance in body size may have had an effect on the results for these parameters as well.

There was a significant difference between the patient group and the control group in resting heart rate. Since E, A and E/A ratio are easily affected by differences in heart rate, this difference might have contributed to the lower E/A ratio in the patient group.

As a counterargument to this, it should be mentioned that the E/A ratio was shown to inversely correlate with NT-proBNP. This suggests that the lowered E/A ratio is rather a sign of impaired left ventricular diastolic function in the patient group.

Measured NT-proBNP values have not previously been presented in long-term follow-ups after HCT in childhood. The study showed that the levels of NT-proBNP were within the normal limits, for both the patient group and the control group. Still, it could be seen that patient group displayed a notably higher mean level, which in turn could increase the risk for developing a cardiovascular disease. [77]

An elevated NT-proBNP can also be seen in patients with impaired glomerular function, which has been observed after HCT. [29, 34] However, in this study, no differences in mean glomerular filtration rate were found between the patient group and the control group. [34]

Exercise test and CPET

Exercise capacity in long-term follow-up, approximately 17 years after patients were treated with HCT and TBI in childhood, was decreased to approximately 60% of that in gender- and age-matched controls.

In CPET, the patients also showed significantly reduced oxygen uptake, oxygen pulse, ventilation, and breathing reserve, as well as reduced ventilatory efficiency at anaerobic threshold compared with the controls.

There was a significant difference between the patients and controls in both body size and body composition. The patients were shorter, had a lower weight and had a higher proportion of fat mass than the controls.

This can be a contributing factor to the lower exercise capacity and oxygen uptake in the patient group compared with the controls. However, after correction for body weight, both in absolute value and in fat-free mass, the exercise peak level was still significantly reduced in the patients compared with the controls.

Also when comparing the predicted values of both peak load (in W) and $VO_{2\text{peak}}$, where body size is taken into account, reductions by approximately 33% and 25%, respectively, were seen in the patient group when compared with the controls.

The higher proportion of fat mass and higher heart rate at rest in the patients indicate a reduced level of habitual physical activity in the patients as compared with the controls. Nevertheless, the subjects in the patient group achieved nearly the same degree of self-rated physical exhaustion and peak heart rate, with a slightly, but significantly, lower degree of leg fatigue.

Three previously published studies that presented exercise capacity in long-term survivors after childhood HCT, including a follow-up period exceeding 5 years after HCT, could be identified. [7, 10, 15]

Vandekerckhove *et al.* reported a peak workload in a patient group of approximately 88% that in the healthy control group, in a study conducted 6.6 years after HCT (97.9 W vs. 111.6 W). [10]

Hogarty *et al.* reported that peak workload decreased to 77% of the predicted value, 5 years after HCT. [15] The corresponding percentage in our study, 17 years after HCT, was found to be 63%.

Mathiesen *et al.* reported that the $W_{\text{peak}}/\text{kg}$ in patients was 78% of the same value in a healthy control group (3.1 vs. 4.0 W/kg), after a mean follow-up time of 7 years. [7] The corresponding percentage in this study, 17 years after HCT, was 70%.

In our study, the peak oxygen uptake was decreased to approximately 64% of that of the controls.

Reduced $V_{O_{2peak}}$ in survivors of childhood leukemia has been shown in several previous studies. [7, 10, 11, 26, 27, 40] In many of these studies, the patients who underwent HCT were excluded or not specified. [26, 27, 40] Christiansen *et al.* performed a study using a different method for exercise testing, with a treadmill ergometer instead of a bicycle ergometer, making the results difficult to compare directly with those of this study. [11]

The studies of Vandekerckhove *et al.* [10] and of Mathiesen *et al.* [7] had study designs that were relatively similar to this study, but with shorter follow-up times. Compared with the reported $V_{O_{2peak}}$ in these two studies, the value was slightly lower in the patient group in our study.

In line with this, the relative difference compared with the healthy control group is more pronounced in this study than in theirs. This is especially the case when compared with the results in the study of Mathiesen *et al.* [7] where the follow-up time was 3–10 years, i.e., significantly shorter than in this study.

This indicates that the time that has passed since treatment with HCT may act as a factor further increasing impairment of both work performance and oxygen uptake.

Using CPET, reduced VE_{peak} was found in the patient group compared with the controls. This reduction was about 25%, roughly the same level of restriction as was seen in the lung volumes at rest. The reduced VE_{peak} is most likely due to reduced tidal volume at peak workload, as no differences in breathing frequency at peak load were seen between the patients and the controls. However, with breathing frequency and breathing reserve within normal values at peak load, [3, 48] the patients had no unequivocal pulmonary limitation at CPET.

Compared with the controls, the patients showed slight, but significantly, increased $V_E/V_{CO_{2@AT}}$. The V_E/V_{CO_2} value reflects the ventilatory efficiency, and the value at anaerobic threshold has been shown to be in good agreement with the ventilation during work, up to the respiratory compensation point, assessed by means of V_E/V_{CO_2} slope. [3, 78] Even though a slightly impaired ventilation was seen in the patients, it is an unspecific finding, as an increased V_E/V_{CO_2} may indicate either a pulmonary or a cardiac limitation, or both. [3]

Oxygen pulse is considered to be a measure of cardiac function and to reflect the stroke volume of the heart. [3] In the patient group of this study, a significantly lower O_2 pulse was seen already at the anaerobic threshold, as compared with the controls. With rising load, the value of the O_2 pulse rose in both groups, but the difference between the patients and controls remained up to peak workload.

In all participants of this study, both the patients and controls, significant positive associations were found between peak oxygen uptake and oxygen pulse on one hand and several of the CPET parameters that reflect both the cardiac and pulmonary function on the other hand.

In a separate analysis of only the patient group, a significant positive association between peak workload and the total lung capacity in rest was also seen, but no significant correlations between the W_{peak} and any of the echocardiographic parameters were found.

This indicates that the restrictive lung component could be a main cause of the reduced exercise capacity in the patients.

At rest, there was also a greater impact on the lung volumes compared with the echocardiographic variables, all of which were within the normal range, although some with significant differences when compared with the controls.

In a separate analysis of the CPET data for the patients, correlations between BR and the lung volumes were noted, which could also indicate pulmonary limitations.

Furthermore, correlations between $VO_{2\text{peak}}$ and O_2 pulse on one hand and MAPSE and $DLCO_c$ on the other hand were consistent in the patient group. This might indicate an additional cardiac limitation.

Thus, looking at the CPET variables, signs of both a cardiac and a pulmonary limitation could be seen in the patients in the study.

Strengths and weaknesses

Oxygen in healthy 50-year-olds

Major strengths include the large sample size, and the results showing that all studied reference values underestimated oxygen uptake in women. All of the participants also reached normal exercise performance based on the reference values of Brudin *et al.*, [45] indicating that the study group is representative of the Swedish population in the studied age group.

A notable weakness of the study is that all the participants are 50 years old. This makes it too limited to establish a Swedish reference material for CPET, without further additions. A reference material based on the results of this study must be supplemented with other studies, in order to cover all age groups.

Long-term follow-up after HCT

The main strengths include the long follow-up time and the analysis of data covering both cardiac and pulmonary function, both at rest and during exercise, at the same occasion. Furthermore, the study group was homogeneous and the control group was well-defined.

The major weakness is the small size of the study population. Only 18 of the 25 patients invited opted to participate in the study, which may have caused selection bias. The small sample size also makes it impossible to draw any certain conclusions, especially from the correlations in the specific analyses for the patient and control group, respectively. Another shortcoming is that we did not investigate the patients' physical activity levels, a variable which has formerly been reported to be associated with the measured peak oxygen uptake. [7] An additional weakness is that more modern assessment methods of cardiac function, such as tissue Doppler and deformation imaging, were not available at the time of the examinations.

Conclusions

Oxygen uptake, RER and BR in healthy 50-year-old Swedes

- In our material, $V_{O_{2peak}}$ was best predicted using the reference formulas of Jones *et al.*, which take sex, age, weight and height into account
- All commonly used reference values underestimated $V_{O_{2peak}}$ in females.
- No difference was found in the relation between peak oxygen uptake or peak workload and $RER > 1.1$ and that for $RER > 1.0$.
- Lower BR was related to higher peak oxygen uptake.

Long-term follow-up of cardiopulmonary function in leukemia patients after HCT

- Young adult HCT survivors had significantly decreased exercise capacity compared with healthy individuals.
- The decreased exercise capacity in HCT survivors was related to smaller lung volumes (TLC), but not to echocardiographic parameters.
- Echocardiographic parameters were within normal range for the patients at the time of follow-up.
- Small, but statistically significant, differences were found in the patients regarding systolic longitudinal and diastolic left ventricular function; both were reduced compared with in the controls. Right ventricular function was also reduced.

- $VO_{2\text{peak}}$, oxygen pulse and ventilator efficiency were impaired in the patients compared with the controls.
- The decreased $VO_{2\text{peak}}$ and O_2 pulse seemed to depend on limitations in both lung function and cardiac function.
- The impairment of $V_{O_{2\text{peak}}}$ was larger than earlier reported in the literature, possibly due to the longer time of follow-up.

Clinical/practical implications and future perspectives

Oxygen uptake, RER and BR in healthy 50-year-old Swedes

In Sweden, and other countries, there is a need for updated reference values of CPET variables, especially for women.

The clinical importance of reliable reference values is high. Incorrect reference values, that underestimate the abilities of patients, can lead to erroneous diagnoses and thus mean that patients do not get proper treatment. Following these studies, there is still a need to evaluate existing reference values for CPET in age groups other than 50-year-olds. Ideally, new reference values would be developed in a representative Swedish population.

Our findings regarding RER and BR have practical significance in both performance and interpretation of CPET.

The current recommendations regarding achieving $RER > 1.1$ in healthy individuals is not related to achieving a higher workload; it is sufficient to reach a $RER > 1.0$ in healthy individuals.

A low BR can be found also in healthy individuals with normal lung function, as a reaction to a high level of physical exertion.

Long-term follow-up of cardiopulmonary function in leukemia patients after HCT

Long-term effects on many organ system, including the cardiac and pulmonary systems, are known after HCT, and there are also many potential causes of late effects after HCT. [6]

This makes it important to follow HCT patients over a longer period of time, preferably the entire life span, since organ dysfunction may become manifest many years after the treatment. Current clinical routine (at the Uppsala University Hospital) is to perform echocardiography and spirometry regularly following HCT, until 10 years after treatment. Isotope angiography is also performed at 5 and 10 years after the HCT treatment. Thereafter, only screening tests are performed with pro-BNP.

Although echocardiographic parameters were within the normal range in the patients in this study, parameters of systolic and diastolic function of left ventricle and parameters of systolic function of right ventricle were lower than in controls. Further studies should investigate whether these changes could predict who would have further deterioration of the cardiac function.

Exercise stress increases the demands on both lung function and cardiac function. This means that the onset of dysfunction appears earlier during exertion compared with during rest. Using exercise tests and CPET, the studies also showed that reduced working capacity and oxygen uptake, in the patient group, seemingly depended on both the pulmonary and cardiac function. Unfortunately, the sample size was too limited to draw certain conclusions from the sub-analysis of the patient group, and thus a study with an equally long follow-up time, but a larger studied population, would be desirable.

At the time of this thesis' publication, the tests on the patients in these studies were conducted about 10 years ago. If possible, it would be desirable to conduct the same assessments with the same study population again today, about 30 years after given treatment.

Finally, a further clinical recommendation is to introduce regular screening of HCT patients with CPET, where both exercise capacity and gas exchange are evaluated. This will show potential exercise impairments and provide an indication to the cause of these possible limitations: pulmonary and/or cardiac. Depending on which of the parameters is affected, the appropriate method for further investigation can be selected. This would be the most physiological way to detect early limitations of the heart function and lung function, and to be able to offer appropriate treatment early.

Sammanfattning på svenska

Bakgrund, syfte och metod

Hematopoietisk celltransplantation (HCT) är numera en vedertagen behandlingsmetod vid barnleukemi, och ett ökande antal patienter överlever lång tid efter given behandling. Därför ökar också betydelsen av att identifiera och behandla sena komplikationer av denna behandlingsmetod. Det har även rapporterats att patienter som genomgått HCT har ökad risk för nedsatt arbets- och syreupptagningsförmåga lång tid efter behandlingen. Det är dock oklart vad detta beror på, och de eventuella hjärt-/lungbiverkningarna är inte helt klarlagda.

Huvudsyftet med denna avhandling (*Studie II–IV*) var att undersöka långtidseffekterna hos en grupp nu vuxna patienter som behandlades med HCT i barndomen. I den förberedande behandlingen ingick även cellgifter och helkroppsstrålbehandling. Åldersspannet på patienterna var 17–37 år och resultatet jämfördes med resultat från lika många ålders- och könsmatchade friska kontroller. Uppföljningstiden var ungefär två decennier (i median 18 år sedan given behandling), vilket också är den längsta kända.

I dessa studier har framför allt hjärtfunktion, arbetsförmåga och lungornas gasutbyte studerats. Korrelationer mellan resultat från studier av arbetsförmågan och gasutbytet under ansträngning och hjärt- och lungfunktionen i vila analyserades.

Ergospiometri, det vill säga arbetsprov kombinerat med samtidig mätning av gasutbyte, utfördes i en grupp om 17 patienter. Vid samma undersökningstillfälle utfördes även hjärtultraljud och lungfunktionsmätning i vila. I dessa båda undersökningar deltog ytterligare en patient som tyvärr inte kunde medverka i ergospiometrin, på grund av en utvecklad nekros i höftbenet.

Ergospiometri är en känslig metod för att bedöma arbetsförmåga och kartlägga vilken organfunktion som eventuellt är begränsande (t.ex. hjärt- eller lungfunktion). Det finns hittills inget referensmaterial baserat på en svensk normalpopulation, och därför är det av intresse att utvärdera om befintliga internationella referensvärden är applicerbara på en svensk population.

Huvudsyftet i denna avhandlings *Studie I* var att jämföra högsta syreupptaget under arbete ($V_{O_2\text{peak}}$) hos en grupp friska svenska 50-åringar ($n = 181$, varav 91 kvinnor och 90 män), som deltog i den populationsbaserade

POEM-studien (The Prospective Investigation of Obesity, Energy and Metabolism), med fyra av de mest frekvent använda internationella referensvärdena.

Hos de friska 50-åringarna analyserades även högsta belastningen och V_{O2peak} , i förhållande till uppnådd respiratorisk utbyteskvot (RER), liksom betydelsen av andningsreserven (BR) vid högsta arbetsbelastningen. RER definieras som kvoten mellan koldioxidproduktionen och syreupptaget (V_{CO2}/V_{O2}) och ett värde större än 1,1–1,15 på högsta belastningen vid ergospirometri anses vara ett mått på utmärkt ansträngningsgrad. En sänkt BR på högsta belastningen är karakteristiskt för personer med en begränsande lungfunktion.

Resultat och slutsatser

Alla studerade referensvärden i *Studie I* sågs underskatta V_{O2peak} hos kvinnor. Bästa prediktionen av syreupptaget sågs i de äldsta referensvärdena, definierade av Jones *et al.*

Den kliniska betydelsen av tillförlitliga referensvärden är stor. Ett felaktigt referensvärde, som underskattar en patients förmåga, kan leda till felaktig diagnos eller en fördröjning i diagnostiken, och således medföra att patienten inte får eventuell behandling i rätt tid.

Som en förlängning av detta resultat fordras framgent även en utvärdering av befintliga referensvärden i andra åldersgrupper än 50-åringar. Allra bäst vore om ett nytt referensmaterial utarbetades med grund i en representativ svensk population.

Vid analysen av RER och BR på högsta arbetsbelastningen hos den friska populationen av 50-åringar, sågs ingen skillnad, varken i uppnådd belastning eller i V_{O2peak} , mellan deltagare med ett RER-värde på 1,0–1,1 och deltagare med $RER \geq 1,1$ på högsta belastningen. Således kan ett uppnått $RER > 1,0$ vara tillräckligt som en markör för god arbetsprestation hos friska.

I denna friska population sågs ett högt utnyttjande av andningsreserven (lågt BR-värde) vara relaterat till en högre V_{O2peak} . Ett lågt BR-värde kan alltså även ses hos friska personer med normal lungfunktion, som en reaktion på hög fysisk ansträngning.

Studie II–IV fokuserade på patienter som behandlats med HCT för knappt 20 år sedan. Hos dessa sågs, med hjärtultraljud i vila, en hjärtfunktion inom normalområdet. I jämförelse med den friska kontrollgruppen sågs dock både en relativt sänkt pumpförmåga och en relativt lägre fyllnad av hjärtats vänstra kammare, liksom en relativ försämring av högerkammarfunktionen, hos patienterna.

Lungfunktionsmätningarna (som tidigare har presenterats och inte primärt ingår i denna avhandling) av dessa patienter visade en ökning av restriktiv lungfunktionsbegränsning och en sänkt diffusionskapacitet i lungorna jämfört med hos den friska kontrollgruppen.

Vid ergospirometri sågs en betydligt sänkt arbetsförmåga (147 watt hos patienterna jämfört med 245 watt hos personerna i kontrollgruppen) liksom en reducerad V_{O2peak} (75 % av förväntat värde, jämfört med 101 %). Vid analys av övriga variabler med ergospirometri verkade den reducerade kapaciteten bero på begränsningar i både hjärt- och lungfunktion.

Sänkningen av V_{O2peak} hos dessa patienter, som genomgått HCT, var också större än vad som setts i andra, tidigare studier, vilket möjligen kan bero på den längre uppföljningstiden.

En klinisk rekommendation är att införa regelbunden screening med ergospirometri av patienter som genomgått HCT, som det mest fysiologiska sättet att tidigt upptäcka begränsningar av lung- och/eller hjärtfunktion.

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